Screening And Characterization Of Bacterial Strains That Can Affectively Remove Fluoride – An Attempt To Biosorption

Dr. V.D.S. Kumari Perumalla[1]*, Dr. A. Veerabrahmam[2], Dr. B. Archana[3], Gopi.G[4]

[1]*Assistant Professor, M.V.R Degree College, Gajuwaka, Visakhapatnam – 26
[2] Assistant Professor, St.Joseph's College for women(A), VSP – 4
[3,4]Lecturer, M.V.R Degree College, Gajuwaka, Visakhapatnam – 26

***Corresponding Author:** Dr. V. D. S. Kumari Perumalla
*Assistant Professor, M.V.R Degree College, Gajuwaka, Visakhapatnam – 26. Email ID – pvdsk16@gmail.com, Phone No: 7981671945

Abstract:
Fluoride is beneficial when its concentration is in permissible limit. Excess fluoride concentration in drinking water leads to various health problems like fluorosis. Various conventional techniques are employed for defluorination which have both advantages and disadvantages. In this regard an attempt of Biosorption techniques was done in the present study by using bacterial strains isolated from Baratang Island of Andaman &Nicobar. A total of 16 strains were screened from primary screening of 200 bacterial strains. Secondary screening of these 16 strains with four media, different physical conditions and incubation periods revealed five potential strains. Further analysis and field application of five potential strains found that the strains were capable of removing 50-70% of Fluoride under laboratory conditions. The strains were identified and characterized. The studies conclusively suggest that the five bacterial strains has the ability to reduce fluoride contamination and provide opportunities for further investigations that may lead to the development of a new Biosorption technique for addressing the high concentrations of fluoride in ground waters. The present study has also given much scope for further studies to consider the methodology for commercial exploitation with certain modifications depending on the location.

1. Introduction

Fluoride exists in the Earth's crust and is rated 13th in abundance. Fluoride can enter groundwater by natural processes; the soil at the foot of mountains is particularly likely to be high in fluoride content derived from the weathering and leaching of bedrock.Fluoride can also enter into the environment through Runoff and infiltration of chemical fertilizers in agricultural areas,septic and sewage treatment system discharges in communities with fluoridated water supplies and liquid waste from industrial sources(Singh, Lataye, and Wasewar, 2017).

Fluoride is considered beneficial in drinking water at levels of about 0.7 mg / L but is harmful once it exceeds 1.5 mg / L which is the permissible limit, being followed in most of the Nations (WHO, 1985, Smet, 1990 and NHMRC, 2004). The difference between desirable doses and toxic doses of fluoride is ill – defined, and fluoride may therefore be considered as an essential mineral with a narrow margin of safety (WHO, 1984). Many studies relating to Reverse Osmosis in the purification of water are well documented (Schneiter and Middlebrook, 1983; Fu *et al.,* 1995; Arora *et al.,* 2004 and Ndiaye *et al.,* 2005). Majority of the experimental and theoretical research studies are devoted on the use of Nanofiltration technique (Diawara, 2008; Hu and Dickson, 2006; Banson*et al.,* 2006; Szymczyk*et al.,* 2006; Szymczyk and Fievet, 2005). Water and waste water demineralization was carried out with Nanofiltration (Lhassani*et al.,* 2001; Tahaikt*et al.,* 2007). Fluoride removal operations in underground water using a Nanofiltration influenced of various experimental parameters such as initial fluoride concentration, pressure and volume reduction factors (Tahaikt*et al.,* 2008).

Various other approaches such as membrane separation technique (Ndiaye, 20005), electro-coagulation method (Sandoval, 2014), Reverse Osmosis(Colla, 2016), adsorption (Ali, 2014), ion exchange process (Markovski,, 2016), Nalgonda method, particle trade (Tirkey,2018)etc., have been employed for defluoridation of potable water (Gwala, 2011). However, the above methods for treatment of drinking water and wastewater have some disadvantages like high costs, energy consumption, and secondary contaminants after treatment and inefficiency in eliminating of all contaminants present in water and wastewater (Gentili&Fick, 2017).

Microorganisms have the ability to establish Biosorption to various contaminants(Chouhan, 2012), as the bacterial cell wall contains binding groups of toxic pollutants like sulfhynyl, phosphates, carboxylates and amines that aid in metal ion interaction (Kleinubing, 2011). Microorganisms are playing a key role in Biosorption of polluted water contaminants over the last few decades (Igiri, *et al.,* 2018; Mondal *et al.,* 2017). *Azolla filiculoides*(Zazoul*et al.,* 2014) *Providencia vermicola*(Mukherjee, *et al.,* 2017) *Cyanobacteria*, *Aspergillus niger* (Annadurai, *et al.,* 2019)are few

microorganisms reported to successfully remove fluoride from aqueous solution. The advantages of using microorganisms over other treatments are, ease of operation and lower sludge production.

The present research therefore aims to test and evaluate the defluorination ability of five microorganisms S_{13} - *Enterococcus fecalis*,S_{35} - *Streptococcus spp.*,S_{54} and S_{55} - *Enterobacter spp.*andS_{56} - *Pseudomonas aeuriginosa*, isolated from Baratang Island of Andaman &Nicobar. The research was carried out in laboratory at Microbiology Department, M.V.R Degree College, affiliated to Andhra University, Gajuwaka, Visakhapatnam District, Andhra Pradesh, India, followed by its characterization and description.

In the present investigation an attempt has been made to study the fluoride bioremedial efficiency of bacterial strains in relation to physicochemical factors. Characterization studies performed to find out the group of the biosorbents.

2. Materials and methods

2.1. Water Analysis:

Assessment of ground water quality and the resulting information could be useful to develop appropriate solutions for water related problems and to find the water quality. With this consideration the areas of Sagar nagar, MVP colony, Seethammadhara, Isukathota, Venkojipalem, Vepagunta, Ramnagar, Old Gajuwaka, New Gajuwaka and Vadalapudi which are part and parcel of GVMC were chosen for the present study.

All the study locations are densely populated with high degree of urban activities. The study locations are included in Image 2.1. The ground water quality of the study locations is carried out using APHA (2005) methodology.

Image 2.1: The study locations of the present study.

The collected samples were analyzed for physical and chemical parameters as per the method of assessment for ground water quality described in standard methods for the examination of water and waste water by American Public Health Association (APHA, 2005).

Physical parameters: Color, Turbidity, Electrical conductivity, Temperature.
Chemical parameters: pH, Total Alkalinity, Total Hardness, Total Dissolved Solids (TDS).
Organic Constituents: Dissolved oxygen (DO), Biological oxygen demand (BOD), Chemical oxygen demand (COD).
Inorganic Constituents: Ammonia, Sulfates, Sodium, Potassium, Nitrate, Nitrite, Phenols, Chlorides, Fluoride (SPADNS method) (Bellack and Schouboe 1958).

2.2 Isolation of Bacterial Strains:

Soil samples for the present study were collected from **Baratang** island of **Andaman.** Soil samples were collected in sterilized polythene bags with a sterilized spatula and transported under controlled conditions to laboratory for further analysis. Isolation of microbes was done using serial – dilution agar plate procedure and Enrichment culture technique. The Actinomycetes and Fungal media were supplemented with Chlorotetracycline to inhibit the growth of bacteria (Cappuccino, 2005 and Aneja, 2003). The isolated Bacterial strains were used for Biosorption studies.

2.3Primary Screening:
Fresh Basal Medium (appendix I - 32) having concentration of fluoride (10 mg / L) was prepared. Overnight fresh cultures were transferred as inoculums and incubated at 37^0C for 24hrs. Biosorption activity was measured by SPADNS method (Monica Bhatnagar, 2002). The positively responded strains were subjected to secondary screening.

2.4 Secondary Screening:
The conditions like Temperature, pH, and Oxygen content permit microbial growth and activity that influences the Biosorption process. Hence, optimization study was carried out in order to find the effect of Nutrients, pH, Temperature, Duration of incubation, availability of molecular Oxygen and concentration of fluoride on percent defluorination.

2.5Field samples analysis:
The Biosoprtion potential of the best performing strains was tested by applying the same to the ground water samples collected from Vadlapudi (S10) and Vepagunta (S6) since the ground waters of the two locations recorded maximum concentration of fluoride among the test locations.

The application of the present methodology has been carried out with 2 types of samples designated as A and B; sample – A: representing only water while sample – B contains water with Basal medium. The Biosorption of the ground waters of the two locations was carried out at 37^0C incubation temperature and 7.0 pH in 3 incubation periods (24hrs, 48hrs& 72hrs). After each incubation period, the sample was centrifuged and the fluoride content was estimated by SPADNS method.

2.6 Identification of strains:
Strains that are exhibiting high performance were identified by observing Growth on selective media, Cultural and Biochemical Characteristics.

3. Results and Discussion
3.1 Water Analysis:
The physicochemical parameters of 50 water samples collected from different locations revealed that two locations Vepagunta and Vadlapudi with high Fluoride Content - 3.3 and 3.0 mg / L.
Table 3.1

Table 3.1: Ground water Quality of the Sampling Sites

S. No	SS	C	T	EC	Temp. ^{O}C	pH	TA	TH	TDS	DO	COD	BOD	NH_3	SO_4^{2-}	Na^+	K^+	NO_2^-	NO_3^-	P	Cl^-	F^-
1.	S1	CL	TL	1000	30.6	7.4	548	296	680	8.24	220	2.50	BDL	45	13.68	25.42	Tr	5.0	BDL	100	1.7
2.	S2	CL	TL	1552	29.8	7.6	311	470	670	7.67	250	2.20	BDL	53	15.32	27.82	0.10	20.0	BDL	155	2.5
3.	S3	CL	TL	1222	28.9	7.0	373	223	1000	7.54	280	1.20	BDL	35	17.18	30.04	0.07	25.6	BDL	75	1.5
4.	S4	CL	TL	1000	29.6	8.2	894	736	1200	7.41	200	1.20	BDL	30	11.08	31.42	0.08	21.3	BDL	90	1.8
5.	S5	CL	TL	1348	28.5	7.2	336	560	960	7.28	260	1.50	BDL	40	13.52	35.63	0.10	29.0	BDL	85	1.2
6.	S6	CL	TL	1000	28.2	7.6	311	378	1460	7.16	310	1.28	BDL	56	12.82	30.54	Tr	21.0	BDL	120	3.3
7.	S7	CL	TL	1278	29.8	7.4	453	480	890	7.81	350	2.32	BDL	45	23.12	36.32	0.03	20.5	BDL	160	1.6
8.	S8	CL	TL	1000	29.2	7.8	407	433	1848	7.95	400	2.32	BDL	48	18.86	26.16	Tr	25.4	BDL	200	1.5
9.	S9	CL	TL	900	29.4	7.4	850	552	1240	7.54	300	1.20	BDL	50	22.13	26.62	Tr	28.5	BDL	180	1.2
10.	S10	CL	TL	1000	28.5	7.6	350	600	980	7.41	300	1.20	BDL	40	21.63	38.62	0.02	25.2	BDL	180	3.0

All the values were an average of 5 determinants. All the parameters are expressed in mg / L except pH, EC, Tr- Traces, BDL-Below Detectable Limit CL- Color Less; TL – Turbidity Less. SS – Sampling Station

Legend of the Table:

C- Conductivity	T-Turbidity	EC- Electrical Conductivity
TH-Total Hardness TDS – Total Dissolved Solids.	TA-Total Alkalinity	Temp-Temperature
DO- Dissolved Oxygen	COD- Chemical Oxygen Demand	BOD- Bio Chemical Oxygen Demand
NH_3- Ammonia	SO_4^{2-}Sulphate	Na^+- Sodium K^+-Potassium
NO_2^--Nitrite NO_3^--Ntrate	P- Phosphorous	Cl^- - Chloride F^- - Fluoride,

3.2Isolation of Bacterial Strains:A total of 200 Bacterial strains were isolated from the soil (10 g) of Baratang Island of Andaman. The bacterial strains were purified by repeated streaking onto Nutrient agar plates and the purified colonies were stored at 4^0C temperature for further use. The isolated 200 strains were subjected to Primary screening.

Table 3.2 Enumeration of Microbial Flora from thesoil of Baratang Island

Organism	Dilution	Dilution factor	Number of colonies/ plate			Average number of Colonies/ dilution
			I	II	III	
Bacteria	10^{-4}	10^{4}	69	51	63	$183 / 3 = 61 \times 10^{3}$
	10^{-5}	10^{5}	61	46	52	$159 / 3 = 53 \times 10^{4}$
	10^{-6}	10^{6}	52	43	49	$144 / 3 = 48 \times 10^{5}$
	10^{-7}	10^{7}	43	32	39	$114 / 3 = 38 \times 10^{6}$
Actino-mycetes	10^{-3}	10^{3}	18	15	15	$48 / 3 = 16 \times 10^{2}$
	10^{-4}	10^{4}	11	9	7	$27 / 3 = 9 \times 10^{3}$
	10^{-5}	10^{5}	9	5	4	$18 / 3 = 6 \times 10^{4}$
	10^{-6}	10^{6}	7	5	3	$15 / 3 = 5 \times 10^{5}$
Fungi	10^{-2}	10^{2}	8	6	7	$21 / 3 = 7 \times 10^{1}$
	10^{-3}	10^{3}	5	3	4	$12 / 3 = 4 \times 10^{2}$
	10^{-4}	10^{4}	5	2	2	$9 / 3 = 3 \times 10^{3}$
	10^{-5}	10^{5}	4	2	2	$9 / 3 = 3 \times 10^{4}$

3.3 Primary Screening:

The isolated 200 bacterial strains were screened and the results of first 10 strains ($S_1 – S_{10}$) were presented in Table 3.3.

Table 3.3 Primary screening of the first ten bacterial strains.

S. No	Strain. No	Vol. of B.M (ml)	*Vol. of NaF^-(ml)	Vol. of Inoculum (ml)	% of Biosorption
1	S_1	2.0	8.0	1.0	90
2	S_2	2.0	8.0	1.0	90
3	S_3	2.0	8.0	1.0	-
4	S_4	2.0	8.0	1.0	-
5	S_5	2.0	8.0	1.0	-
6	S_6	2.0	8.0	1.0	-
7	S_7	2.0	8.0	1.0	-
8	S_8	2.0	8.0	1.0	-
9	S_9	2.0	8.0	1.0	-
10	S_{10}	2.0	8.0	1.0	-

BM = Basal Medium, pH = 7.0, Temperature = 37^0C, *10mg/L,
Incubation period – 24hrs.

Similar pattern was observed when the remaining 190 strains were subjected to screening. Among the 200 bacterial strains that were subjected to primary screening, a total of 16 bacterial strains were identified as potential biosorbents. These strains were designated as S_1, S_2, S_{13}, S_{16}, S_{24}, S_{25}, S_{26}, S_{29}, S_{32}, S_{35}, S_{47}, S_{52}, S_{54}, S_{55}, S_{56} and S_{57} and were subjected to secondary screening.

3.4Secondary Screening:

Media: Biosorption studies were performed at different incubation periods, incubation temperatures and pH in 4 media.
LB broth > Basal medium > Nutrient broth > Peptone water

pH: Based on the above studies the performance of Biosorption in the 3 pH levels followed the following order in all the 4 different media at 37^0C incubation temperature.
pH 7.0 > pH 4.0 > pH 10.0

Incubation temperature:

Based on the above studies the performance of Biosorption at the three incubation temperatures followed the following order in the 4 different media and at 3 pH conditions.
37^0C > 60^0 C > 10^0 C.

Incubation period:

The Biosorption studies by the potential biosorbents were carried out at three incubation periods; 24hrs, 48hrs and 72hrs. Among the three incubation periods studied, the one that affected maximum Biosorption was selected as the optimum incubation period. The optimum period required for maximum Biosorption at 37^0C temperature and pH 7.0 is presented in Table.6.1.

Table 3.4 Incubation period for maximum Biosorption at different F^- conc.

Fluoride concentration mg / L									
	10			**20**			**30**		
Incubation Period (hrs)									
Medium	24	48	72	24	48	72	24	48	72
	Period required for maximum sorption								
NB		✓				✓			
PW		✓				✓			
BM	✓				✓				✓
LB	✓				✓				✓

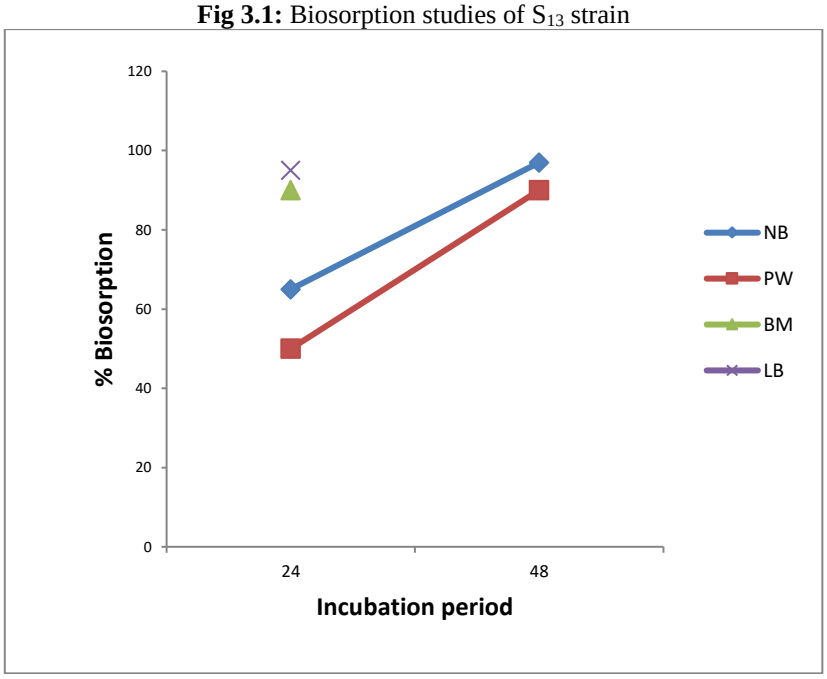
NB = Nutrient Broth; PW = Peptone Water; BM = Basal Medium; LB = Luria Bertani broth.

The five bacterial strains; S_{13}, S_{35}, S_{54}, S_{55} and S_{56} identified from the 16 designated bacterial strains showed potential for Biosorption of Fluoride in ground waters.The Biosorption capacity of these 5 in 4 different media at 37^0C temperature of incubation, pH 7.0 in 3 incubation periods was evaluatedat 10mg / L, 20mg / L and 30 mg / L concentration of fluoride.

Table 3.5 (a) Biosorption studies of S_{13} strain.

Incubation period	24 hrs	48hrs
Medium	% Biosorption	
Nutrient Broth	65	97
Peptone Water	50	90
Basal Medium	90	CS
LB broth	95	CS

Fig 3.1: Biosorption studies of S_{13} strain



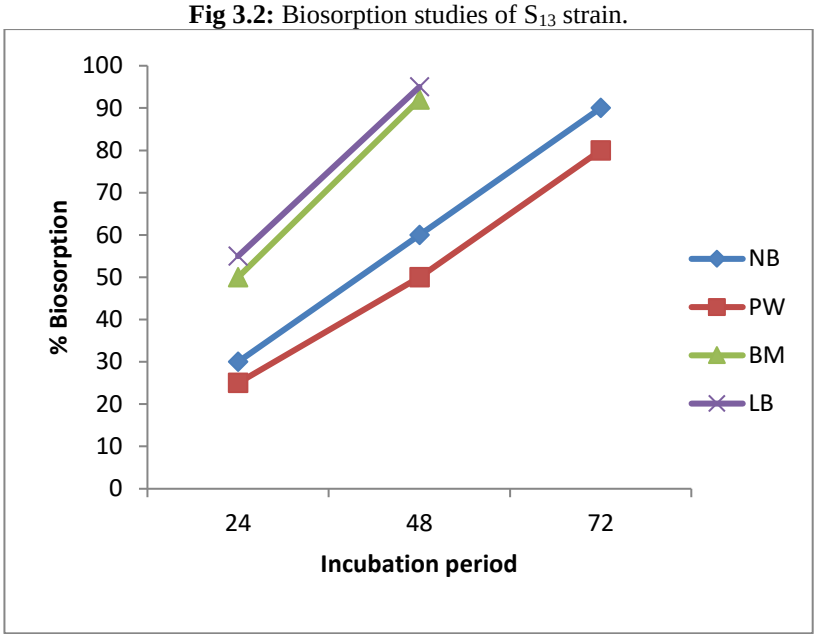
NB = Nutrient Broth; PW = Peptone Water; BM = Basal Medium; LB = Luria Bertani broth.

Conditions: Temperature of incubation: 37^0C. Fluoride concentration: 10 mg / L. pH :7.0, CS= complete sorption

Table 3.5(b) Biosorption studies of S_{13} strain.

Incubation period	24hrs	48hrs	72hrs
Medium	% Biosorption		
Nutrient Broth	30	60	90
Peptone Water	25	50	80
Basal Medium	50	92	CS
LB broth	55	95	CS

Fig 3.2: Biosorption studies of S_{13} strain.

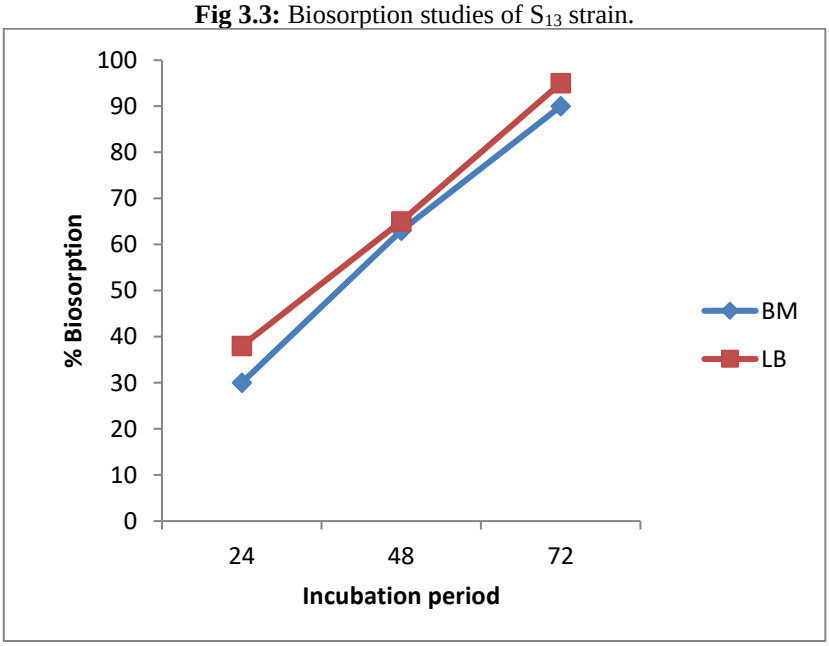


NB = Nutrient Broth; PW = Peptone Water; BM = Basal Medium; LB = Luria Bertani broth.
Conditions: Temperature of incubation: 37^0C. Fluoride concentration: 20 mg / L. pH :7.0, CS= complete sorption

Table 3.5(c) Biosorption studies of S_{13} strain.

Incubation period	24hrs	48hrs	72hrs
Medium	% Biosorption		
Basal Medium	30	63	90
LB broth	38	65	95

Fig 3.3: Biosorption studies of S_{13} strain.

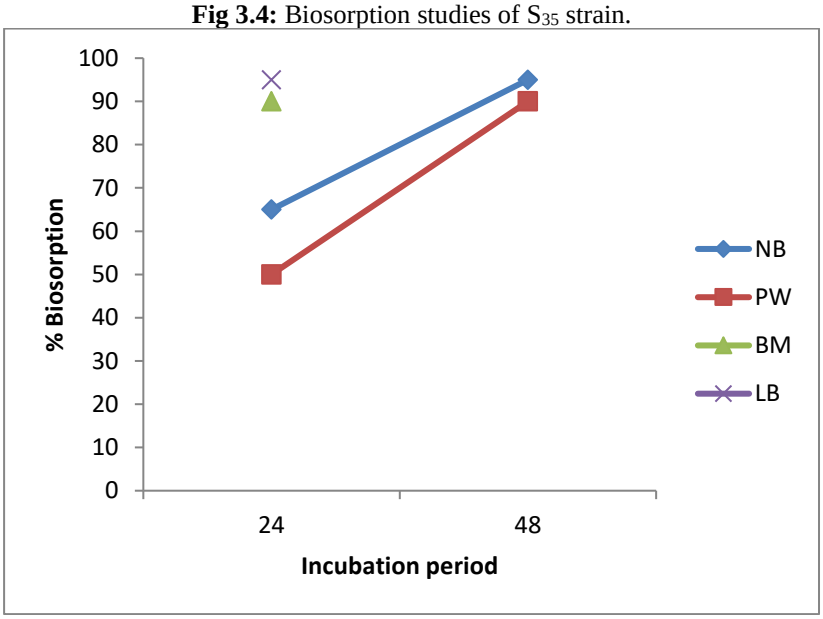


BM = Basal Medium; LB = Luria Bertani broth
Conditions: Temperature of incubation: 37^0C. Fluoride concentration: 30 mg / L. pH :7.0,

Table 3.6 (a) Biosorption studies of S_{35} strain.

Incubation period	24 hrs	72hrs
Medium	% Biosorption	
Nutrient Broth	65	95
Peptone Water	50	90
Basal Medium	90	CS
LB broth	95	CS

Fig 3.4: Biosorption studies of S_{35} strain.

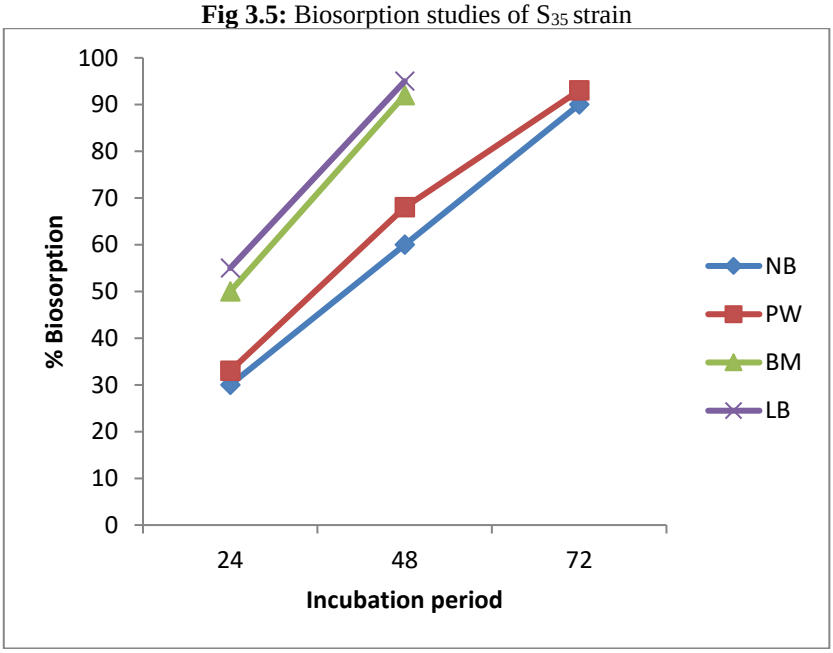


NB = Nutrient Broth; PW = Peptone Water; BM = Basal Medium; LB = Luria Bertani broth.
Conditions: Temperature of incubation: 37^0C. Fluoride concentration: 10 mg / L. pH :7.0, CS= complete sorption

Table 3.6 (b) Biosorption studies of S_{35} strain.

Incubation period	24hrs	48hrs	72hrs
Medium	% Biosorption		
Nutrient Broth	30	60	90
Peptone Water	33	68	93
Basal Medium	50	92	CS
LB broth	55	95	CS

Fig 3.5: Biosorption studies of S_{35} strain

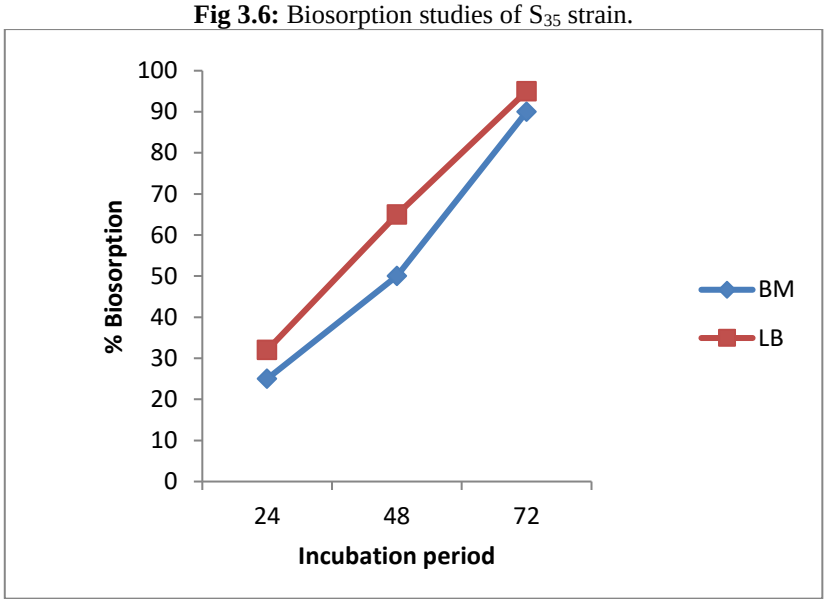


NB = Nutrient Broth; PW = Peptone Water; BM = Basal Medium; LB = Luria Bertanibroth
Conditions: Temperature of incubation: 37^0C. Fluoride concentration: 20 mg / L. pH :7.0,

Table 3.6(c) Biosorption studies of S_{35} strain.

Incubation period	24hrs	48hrs	72hrs
Medium	% Biosorption		
Basal Medium	25	50	90
LB broth	32	65	95

Fig 3.6: Biosorption studies of S_{35} strain.



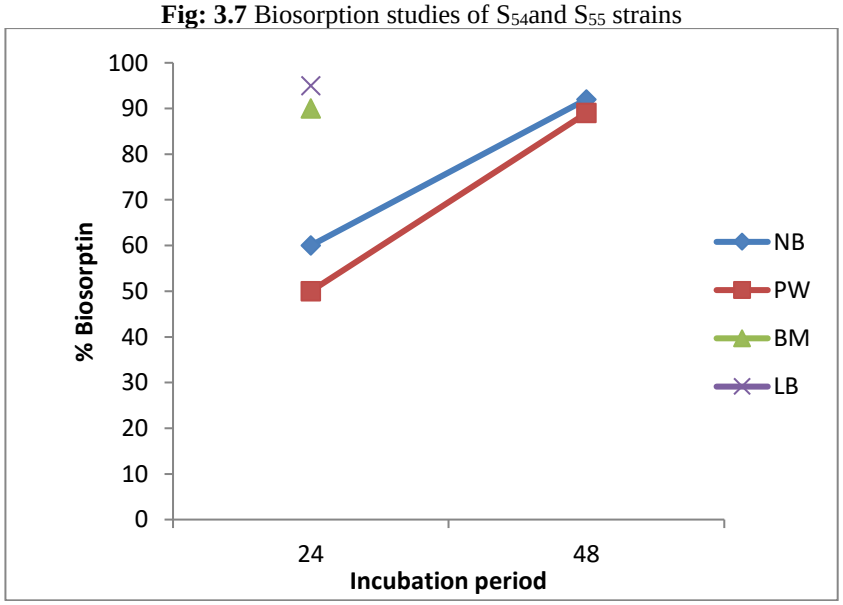
BM = Basal Medium; LB = Luria Bertani broth.

Conditions: Temperature of incubation: 37^0C. Fluoride concentration: 30 mg / L. pH :7.0, CS= complete sorption

Table 3.7(a) Biosorption studies of $S_{54}$and S_{55} strains

Incubation period	24 hrs	72hrs
Medium	% biosorption	
Nutrient Broth	60	92
Peptone Water	50	89
Basal medium	90	CS
LB broth	95	CS

Fig: 3.7 Biosorption studies of $S_{54}$and S_{55} strains



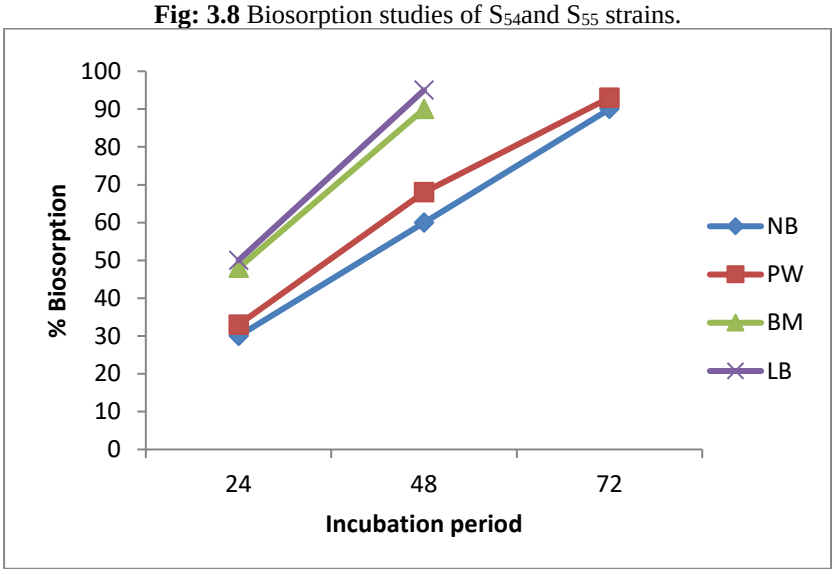
NB = Nutrient Broth; PW = Peptone Water; BM = Basal Medium; LB = Luria Bertanibroth

Conditions: Temperature of incubation: 37^0C. Fluoride concentration: 10 mg / L. pH :7.0, CS = Complete Sorption.

Table 3.7(b) Biosorption studies of $S_{54}$and S_{55} strains.

Incubation period	24hrs	48hrs	72hrs
Medium	% Biosorption		
Nutrient broth	30	60	90
Peptone water	33	68	93
Basal medium	48	90	CS
LB broth	50	95	CS

Fig: 3.8 Biosorption studies of $S_{54}$and S_{55} strains.

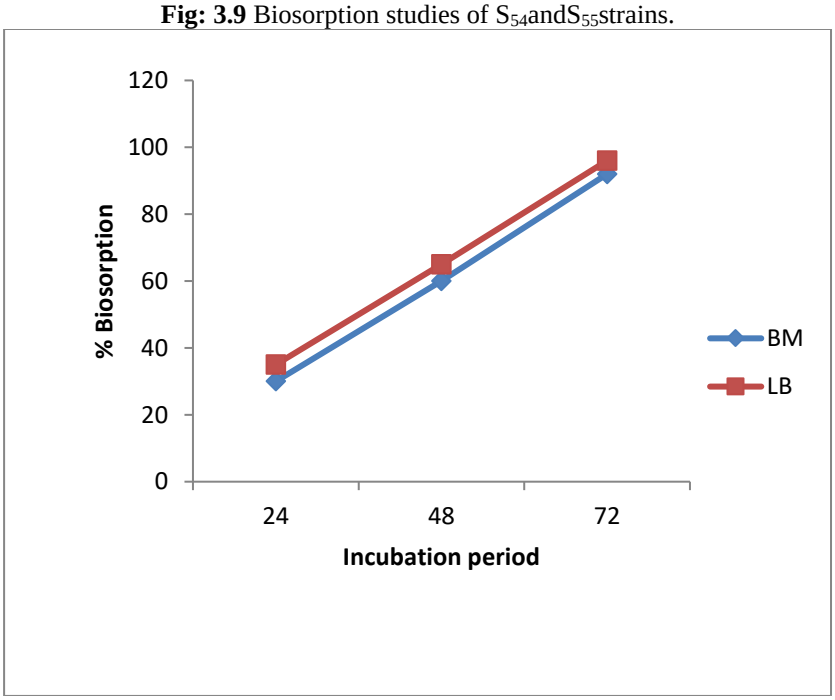


NB = Nutrient Broth; PW = Peptone Water; BM = Basal Medium; LB = Luria Bertani broth.
Conditions: Temperature of incubation: 37^0C. Fluoride concentration: 20 mg / L. pH :7.0, CS= complete sorption

Table 3.7(c) Biosorption studies of $S_{54}$and S_{55} strains.

Incubation period	24hrs	48hrs	72hrs
Medium	% Biosorption		
Basal medium	30	60	92
LB broth	35	65	96

Fig: 3.9 Biosorption studies of S_{54}andS_{55}strains.

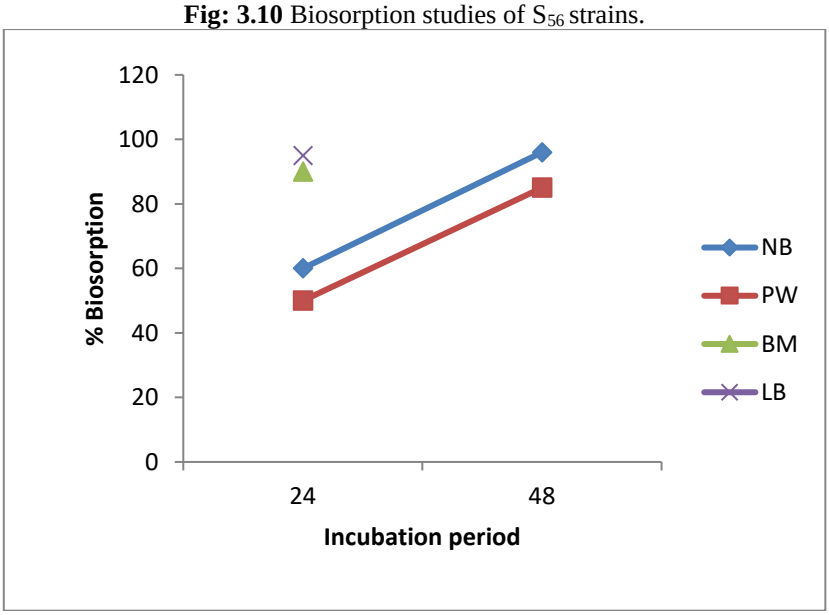


BM = Basal Medium; LB = Luria Bertani broth.
Conditions: Temperature of incubation: 37^0C. Fluoride concentration: 30 mg / L. pH :7.0,

Table 3.8(a) Biosorption studies of S_{56} strain.

Incubation period	24 hrs	72hrs
Medium	% biosorption	
Nutrient Broth	60	96
Peptone Water	50	85
Basal Medium	90	CS
LB broth	95	CS

Fig: 3.10 Biosorption studies of S_{56} strains.

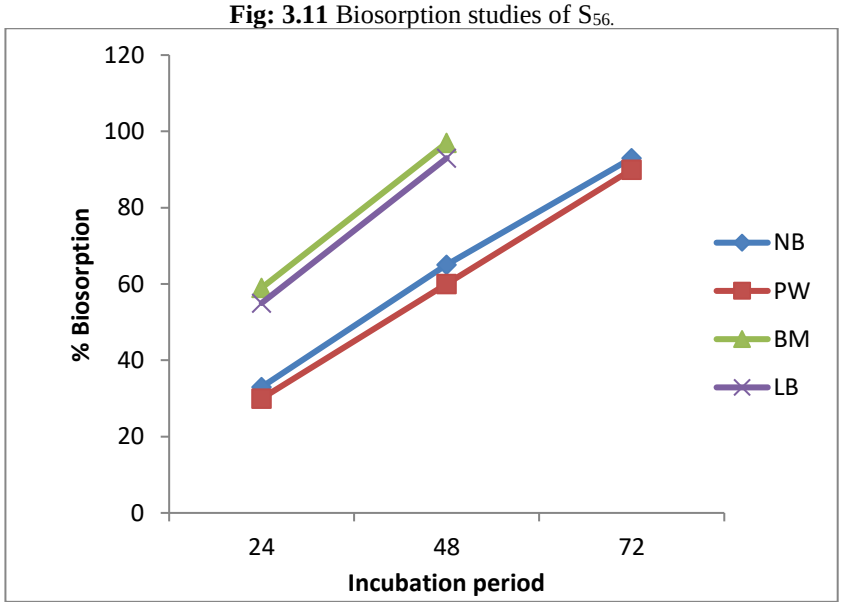


NB = Nutrient Broth; PW = Peptone Water; BM = Basal Medium; LB = Luria Bertani
Conditions: Temperature of incubation: 37^0C. Fluoride concentration: 10 mg / L. pH :7.0, CS= complete sorption

Table 3.8(b) Biosorption studies of S_{56} strain.

Incubation period	24hrs	48hrs	72hrs
Medium	% Biosorption		
Nutrient broth	33	65	93
Peptone water	30	60	90
Basal medium	59	97	CS
LB broth	55	93	CS

Fig: 3.11 Biosorption studies of S_{56}.

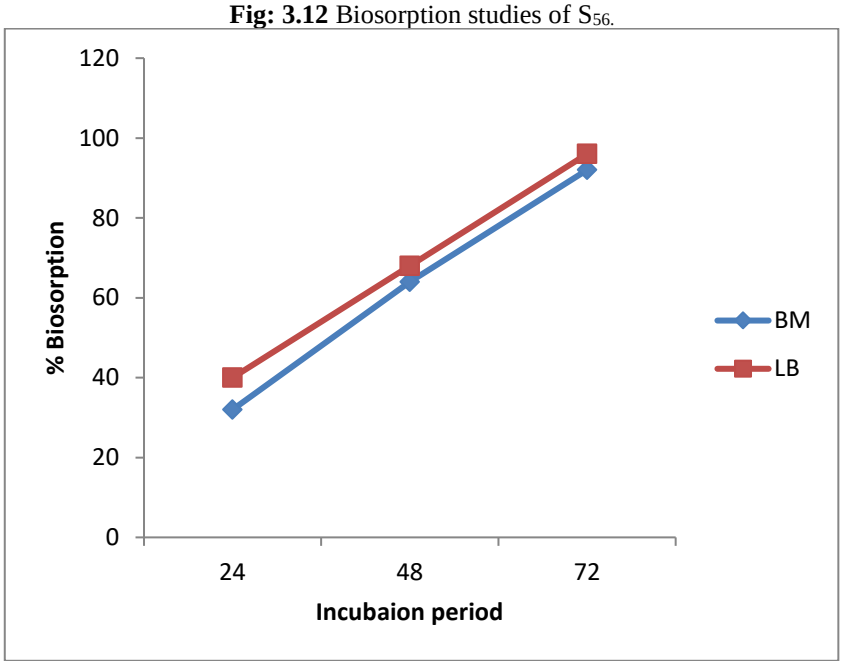


NB = Nutrient Broth; PW = Peptone Water; BM = Basal Medium; LB = Luria Bertanibroth
Conditions: Temperature of incubation: 37^0C. Fluoride concentration: 20 mg / L. pH :7.0, CS = Complete Sorption.

Table 3.8 (c) Biosorption studies of S_{56} strain.

Incubation period	24hrs	48hrs	72hrs
Medium	% Biosorption		
Basal medium	32	64	92
LB broth	40	68	96

Fig: 3.12 Biosorption studies of $S_{56.}$



BM = Basal Medium; LB = Luria Bertani broth.
Conditions: Temperature of incubation: 37^0C. Fluoride concentration: 30 mg / L.pH :7.0

3.4Analysis of Field Samples

Table 3.9 Biosorption data relating to the field water samples.

I. P*	Strain. No	Vadlapudi (S10)		Vepagunta (S6)	
		% of Biosorption			
		A	B	A	B
24hrs	S_{13}	Nil	Nil	Nil	Nil
	S_{35}	Nil	Nil	Nil	Nil
	S_{54}	Nil	30	Nil	30
	S_{55}	Nil	30	Nil	30
	S_{56}	30	50	30	50
48hrs	S_{13}	Nil	30	Nil	30
	S_{35}	Nil	30	Nil	30
	S_{54}	30	50	30	50
	S_{55}	30	50	30	50
	S_{56}	50	75	50	75
72hrs	S_{13}	30	50	30	50
	S_{35}	30	50	30	50
	S_{54}	50	70	50	70
	S_{55}	50	70	50	70
	S_{56}	75	90	75	90

Sample - A: Only water.
Sample - B: Water (8mL) with Basal Medium (2mL).
Temperature of incubation: 37^0C; pH – 7.0.
Inoculums volume: 1mL.
* I.P = Incubation Period.

The Biosorption studies on the ground waters of the two study locations; Vepagunta and Vadlamudi with the highest concentration of Fluoride indicate that $S_{56,}$ S_{55}& S_{54} strains showed 50% and more Fluoride reduction from the ground waters at 48 and 72 hrs of incubation period and in neutral pH.The present methodology has shown much promise in the case of field samples and has reduced more than 50% of the original fluoride concentration (3ppm) in the ground waters.

3.5 Identification of strains

Table 3.10: Growth characteristics on selective media.

Strain. no	Bile Esculin Agar	Brain Heart Infusion Agar	Cetrimide Agar	EMB agar	Endo Agar	Lactose Agar	Mac Conkey Agar	Mannital Salt Agar	Phenyl Alanine Agar	*Pseudomonas* Agar	TCBS Agar	Violet Red Bile Agar
S_{13}	Black colonies	+	–	+ Pale pink	–	+	–	-	–	–	–	–
S_{35}	Black colonies	+	–	–	–	-	–	-	–	–	–	–
S_{54}	pale colonies	+	–	+	Pale pink (24hr) Deep pink (48hr)	+	Pale pink	-	–	–	–	+
S_{55}	pale colonies	+	–	+	Pale pink (24hr) Deep pink (48hr)	+	Pale pink	-	–	–	–	+
S_{56}	pale colonies	+	+	–	Pale pink (24hr) Deep pink (48hr)	–	Pale pink	-	–	+	–	+

+ = presence of growth; - = absence of growth; Incubation period = 24hrs; Temperature of incubation – 37^0C

The characterization studies indicated that the five Biosorbents (S_{13}, S_{35}, S_{54}, S_{55} and S_{56}) may be categorized as
S_{13} -*Enterococcus fecalis*, S_{35} - *.Streptococcus spp*.,
S_{54} and S_{55} - *Enterobacter spp.* And S_{56} - *Pseudomonas aeuriginosa*.

4. Conclusion:

Fluoride is one of the very few chemicals that have been shown to cause significant effects on living systems in general and human beings in particular through drinking-water. Application of Defluoridation techniques to remove fluoride from groundwater is vital to the health and well-being of people and livestock in areas endemic to fluorosis. The physico chemical characteristics of the ground waters of the ten study locations indicate that two locations (Vepagunta and Vadlamudi) have shown the highest concentrations of Fluoride. A total of 200 Bacterial colonies were isolated from Baratang Island of Andaman &Nicobar, subjected to primary screening and out of which 16 strains were identified for effective defluoridation. The16 strains were isolated, separated and designated as S_1, S_2, S_{13}, S_{16}, S_{24}, S_{25}, S_{26}, S_{29}, S_{32}, S_{35}, S_{47}, S_{52}, S_{54}, S_{55}, S_{56} and S_{57}. These 16 strains were subjected to secondary screening under various conditions and out of which 5 strains were identified - S_{13}, S_{35}, S_{54}, S_{55} and S_{56}.

The characterization studies indicated that the five biosorbents (S_{13}, S_{35}, S_{54}, S_{55} and S_{56}) may be categorized as
S_{13} -*Enterococcus fecalis*,
S_{35} - *.Streptococcus spp*.,
S_{54} and S_{55} - *Enterobacter spp.* and
S_{56} - *Pseudomonas aeuriginosa*

The application of the microbial biosorbents to the field samples revealed that the designated five bacterial strains (S_{13}, S_{35}, S_{54}, S_{55} and S_{56}) for Biosorption of Fluoride rich ground waters follow the following order.
$S_{56} > S_{55} = S_{54} > S_{13} = S_{35}$

The experimental results indicate that the identified bacterial strains have reduced more than 50% of the initial concentration of fluoride in all the four media at 48 and 72 hrs of incubation period in neutral pH under laboratory conditions.

References

1. Anija, K.R. (2005). Experiment in Microbiology, plant pathology and Biotechnology, 4thEditon. New Age International publishers., 220 – 227.
2. APHA. (2005). Standard methods for the examination of water and waste water – 21st Edition, published by American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF).
3. APHA. (2005). Standard methods for the examination of water and waste water – 21st Edition, published by American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF).
4. Arora, M., Maheswari, R.C., Jain, S.K., Gupta, A. (2004). Use of membrane technology for potable water production. Desalination., 170, 105- 112.

5. B.E. Igiri, S.I.R. Okoduwa, G.O. Idoko, E.P. Akabuogu, A.O. Adeyi, I.K. Ejiogu, **(2018)**, Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a review, **Journal of Toxicology** pp. 1-16.
6. Banson, S., Ben- David, A., Oren, Y., Fregger, V. (2006). Characterization of ion transport in the active layer of RO and NF Polyamide Membranes. Desalination., 199, 31- 33.
7. Bellack, E., and Schouboe, P.J. (1958). Rapid photometric determination of fluoride in water. Anal. Chem., 30, 2032 – 2034.
8. Bhatnagar. M., Bhatnagar, A., Sapnajha. (2002). Interactive biosorption by micro Algal Biomass as a tool for fluoride removal. Biotechnology Letteres., 24, 1079 – 1081.
9. Cappuccino Laboratory, James. G., Nataliesherman. (2005). Microbiology: A Manual 6th Edition. Pearson Publication. 115, 119.
10. Diawara, C.K. (2008).Nanofiltration process efficiency in water desalination. Sep.purif. Rev., 37, 303- 325.
11. F.G. Gentili, J. Fick, (2016). Algal cultivation in urban wastewater: an efficient way to reduce pharmaceutical pollutants, J. Appl. Phycol. **Fluoride**, 47 (4) pp. 349-358.
12. Fu, P., Ruiz, H., Lozier, J., Thompson, K., Spangenberg, C. (1995). A pilot study on ground water natural organics removal by low pressure membranes. Desalination., 102,47-56.
13. Hu, K., Dickson, J.M. (2006). Nanofiltration membrane performance on fluoride removal from water. J. Member Sci., 279, 529- 538.
14. Ali, **(2014).** Water treatment by adsorption columns: evaluation at ground levelSep. Purif. Rev., 43 (3) pp. 175-205.
15. J. Markovski, J. Garcia, K.D. Hristovski, P. Westerhoff, **(2016).** Nano-enabling of strong-base ion-exchange media via a room temperature aluminum hydroxide synthesis method to simultaneously remove nitrate and fluorideSci. Total Environ., 599–600 pp. 1848-1855
16. K. Singh, D.H. Lataye, K.L. Wasewar, **(2017).** Removal of fluoride from aqueous solution by using bael (Aegle marmelos) shell activated carbon: kinetic, equilibrium and thermodynamic study, J. Fluor. Chem., 194, pp. 23-32.
17. Lhassani, A., Rumeau, M., Benjelloun, D., Pontie, M. (2001). Selective demineralization of water by nanofiltration: application to the defluorination of brackish water. Water. Res., 35, 3260 -3264.
18. M.A. Sandoval, R. Fuentes, J.L. Nava, I. Rodríguez, (2014). Fluoride removal from drinking water by electrocoagulation in a continuous filter press reactor coupled to a flocculator and clarifierSep. Purif. Technol., 134 pp. 163-170.
19. M.A. Zazouli, A.H. Mahvi, S. Dobaradaran, M. Barafrashtehpour, Y. Mahdavi, D. Balarak, (2014), Adsorption of fluoride from aqueous solution by modified Azolla filiculoides,
20. N.K. Mondal, A. Samanta, S. Dutta, S. Chattoraj, **(2017),** Optimization of Cr (VI) biosorption onto Aspergillus niger using 3-level Box-Behnken design: equilibrium, kinetic, hermodynamic and regeneration studies, Journal of Genetic Engineering and Biotechnology, 15 (1) pp. 151-160.
21. Ndiaye, P.I., Moulin, P., Dominguez, L., Millet, J.C., Charbit, F. (2005). Removal of fluoride from electrc industrial effluent by RO membrane separation. Desalination., 173, 25 – 32.
22. NHMRC, (National Health and Medical Research Council). (2004). Australian Drinking water Guidelines.
23. P. Gwala, S. Andey, V. Mhaisalkar, S. Labhasetwar, C. Kshirsagar, (2011). Lab scale study on electrocoagulation defluoridationprocess optimization along with aluminium leaching in theprocess and comparison with full-scale plant operation, Water Sci. Technol., 63 p. 2788
24. P. Tirkey, T. Bhattacharya, S. Chakraborty, (2018). Optimization of fluoride removal from aqueous solution using Jamun (Syzygiumcumini) leaf ash, Process Saf. Environ. Prot., 115 pp. 125-138
25. P.I. Ndiaye, P. Moulin, L. Dominguez, J.C. Millet, F. Charbit, **(2005)**. Removal of fluoride from electronic industrial effluentby RO membrane separationDesalination, 173 (1) pp. 25-32.
26. S. Chouhan, U. Tuteja, S.J.S. Flora, **(2012).** Isolation, identification and characterization of fluoride resistant bacteria: possible role in bioremediation, Appl. Biochem. Microbiol., 48 pp. 43-50
27. S. Mukherjee, P. Sahu, G. Halder, **(2017),** Microbial remediation of fluoride-contaminated water via a novel bacterium Providencia vermicola (KX926492), J. Environ. Manag., 204 pp. 413-423
28. S.J. Kleinubing, E.A. da Silva, M.G.C. da Silva, E. Guibal, **(2011).** Equilibrium of Cu (II) and Ni (II) biosorption by marine algae Sargassum filipendula in dynamic system: competitiveness and selectivity, Bioresour. Technol., 102 (7) pp. 4610-4617.
29. S.T. Annadurai, P. Arivalagan, R. Sundaram, R. Mariappan, A.P. Munusamy, **(2019),** Batch and column approach on biosorption of fluoride from aqueous medium using live, dead and various pretreated Aspergillus niger (FS18) biomass, Surfaces and Interfaces, 15 pp. 60-69.
30. Schneiter, R.W., Middlebrooks, E.J. (1983). Arsenic and fluoride removal from ground water by reverse osmosis. Environ. Int., 9, 289- 291.
31. Smet, J. (1990). Fluoride in drinking water In: Frencken, LE (Ed), Endemic fluorosis in developing countries – cause Effects and possible solution: Report of a symposium Held in Delft, The Netherlands. Nether lands Organization for Applied scientific Research.
32. Szymczyk, A., Fievet, P. (2005). Investigation transport properties of Nanofiltration membranes by means of a sterict, electric and dielectric exclusive model. J. Member. Sci., 252, 77 – 88.
33. Szymczyk, A., Sbai, M., Fievet , P., Vidonne, A. (2006). Transport Properties and electro kinetic characterization of an amphoteric nanofilter. Langmuir., 22, 3910 -3919.

34. Tahaikt, M, EL.Habbani, R., AitHandou, A., Achary, I., Amor, Z., Taku, M., Alma, A., Boughriba, A., Hafsi, M., Elmidaoui, A. (2007). Fluoride removal from ground water by nanofiltration. Desalination., 212, 46 -53.
35. Tahaikt, M., AitHaddou, A., El Habana R., Amor, Z, Elhannouni, F., Taky, M., Kharif, M., Boughriba, A., Hafsi, M., Elmidoui, A. (2008). Comparison of the performance of three commercial membranes in fluoride removal by nanofiltration. Continuous operation. Desalination., 225, 209- 219.
36. V. Colla, T.A. Branca, F. Rosito, C. Lucca, B.P. Vivas, V.M. Delmiro ,(2016).Sustainable reverse osmosis application for wastewater treatment in the steel industryJ. Clean. Prod., 130 pp. 103-115.
37. WHO (World Health Organization, Geneva, Switzerland). (1984). Fluoride and fluorides Geneva world Health Organization Environmental Health Criteria 36.
38. WHO, (1985) Guidelines for Drinking water quality vol, 3. World Health Organization. Geneva, 1-2.