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BIOSORPTION OF FLUORIDE IN GROUND WATERS BY BACTERIAL STRAINS – A REVIEW OF RESEARCH WORK CARRIED AT ANDHRA UNIVERSITY, VISAKHAPATNAM

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ABSTRACT:

Fluoride the anionic form of fluorine is widely distributed in nature. The number of Fluorosis afflicted countries has been steadily increasing. The problem of high fluoride content in drinking water resources leads consequently a higher incidence of fluorosis. In addition to conventional methods, an attempt of Biosorption was done in this research work for effective removal of Fluoride from Ground waters. The physicochemical analysis of groundwater samples were carried out to identify the fluoride rich water bodies. A total of 200 bacterial strains were isolated from soil sample of Baratang Island, Andaman and were subjected to primary screening where 16 strains were showed good absorption. The effects of nutrients, physical factors were studied with these strains and finally a total of 5 strains were identified as best absorbers and subjected field sample analysis. The experimental results indicate that the identified bacterial strains have reduced more than 50% of the initial concentration of fluoride in all the studied conditions and they may be considered for Defluoridation of drinking water after careful evaluation of the methodology under various field conditions.

1. INTRODUCTION

The quality of water is of vital concern for human race since it is directly linked with the human welfare. Both surface and ground waters are involved in the sustenance of the biosphere in general and human race in particular.

Ground water is the main source of drinking water for majority of the people around the World (http://edugreen.teri.res.in). Ground water constitutes 97% of global fresh water and is the major preferred source of drinking water in rural as well as urban areas, particularly in the developing countries like India because treatment of the same, including disinfection is often not required (WHO and UNISEF, 2004).

Chemical composition of surface or subsurface is one of the prime factors on which the suitability of water for domestic, industrial, or agricultural purpose depends. Due to various ecological factors either natural or anthropogenic, the ground water is getting polluted (Kass *et al.,* 2005, Amina *et al.,* 2004, Oren *et al.,* 2004 and Anwar 2003).

The ground water quality is also being impaired by many natural constituents, of which **Fluoride** stands first as a pollutant of geogenic origin in many countries of the World. The fluoride research in the past few decades suggests that concentrations above **1.5 ppm** in drinking water increase the severity of the incurable disease **Fluorosis** (Ayoob and Gupta, 2006). As of now, **Fluorosis** is

playing havoc in more than **25** Nations across the World and in many countries; the number of people suffering from fluoride poisoning is staggering. The most recent proclamation that more than 200 million people across the globe are "at risk" of fluorosis, raises global alarm and anguish (Taiyuan declarations, 2004).

2. FLUORIDE CONSEQUENCES:

In India the Fluoride is endemic to **36,988** habitations, depicting its dominance (first report of DDWS 2004) and over the past seven decades, the prevalence and severity of Fluorosis has increased quite radically in India, reaching almost epidemic proportions. At present, 20 out of 35 States and Union Territories are under fluoride attack. Due to severity of impacts with excess fluoride in ground water, the WHO permissible limit of fluoride in India has been reduced from 1.5 to 1.0 ppm in 1998 (UNICEF, 1999).

Presence of Fluoride in water goes on accumulating in bones up to 55 years of age. At high doses fluoride can interfere with carbohydrate, lipid, protein, vitamin, enzyme and mineral metabolism (WHO, 1985 and Susheela, *et al.,* 1993). Fluorosis can manifest in:

a) Dental fluorosis b) Skeletal fluorosis,
c) Non–skeletal manifestations and d) Genu valgum.

Since Fluoride possess significant health problems to human beings in particular through drinking-water, Defluoridation of drinking water is the only practicable option to overcome the problem of excessive fluoride in drinking water, where alternate source is not available. Application of Defluoridation techniques to remove fluoride from groundwater is crucial to the health and wellbeing of people and livestock in areas endemic to fluorosis.

While various Defluoridation techniques have been explored, each one has its limitations. The conventional techniques include 1. Membrane Techniques - Reverse osmosis, Nanofiltration, Dialysis, Electro dialysis and 2. Adsorption Techniques - Alumina and aluminum based adsorbents, Calcium, Carbon, Zeolites, Synthetic resins, Layered double hydroxides, Clay, Soil which are often too costly, not easily adoptable and environmentally not acceptable (Meenakshi and Maheswari, 2006)**.**

3. BIOSORPTION:

These problems can be addressed by developing alternative methods of Defluoridation with emphasis on cost, adoptability and acceptability. In this context Biosorption is being considered as a viable option for Defluoridation of drinking water since it is cost effective, easily adoptable and environmentally compatible. **Biosorption** is a physiochemical process that occurs naturally in certain biomasses which allows it to passively concentrate and bind contaminants onto its cellular structure (Volesky, 1990).

Living and nonliving cells of Prokaryotic or Eukaryotic species of Algae, Fungi and Bacteria are employed for the adsorption of Fluoride (Ajmal *et al.,* 1998). The plant cells are preferred over animal cells as they contain cell walls. These cell walls contain polysaccharides, xylem, and chitin. The proteins present in the cell walls represent a potential biosorbents (Gadd, 1990 and Volesky, 1990).

Mechanism of Biosorption involves 2 paths. A slower phase where there is independent fluoride binding *i.e* surface adsorption and active or intracellular uptake of fluoride ions *i.e* passive diffusion. Biosorption is based on the availability of both the ionic charge and covalent binding. The extent of fluoride Biosorption varies from species to species. Recently considerable interest has been

generated on the application of biosorbent materials for the removal of various pollutants (Gadd *et al.*, 1988, Maca Skie and Dean 1989, Mc Hale and Mc Hale 1994, Williams and Edyvean 1997, Gupta *et al.*, 2000, Illami *et al.*, 2005,).

4. RESEARCH WORK CARRIED

In this context, a research work was carried on "**Studies on Biosorption of fluoride in Ground Waters by Bacterial strains**" which is highly relevant to the Fluorosis afflicted areas in general and country like India in particular; since it is the second most populous country in the World.

The primary objective of the research is to evaluate new biosorbents of microbial origin so as to develop Biosorption methods.

4.1 Physicochemical analysis of water

The physico chemical characteristics of the ground waters of the ten studied Locations in and around areas of Visakhapatnam, indicate that the ground waters of the study area contain low to high levels of pollution. Among the ten study locations, two locations (Vepagunta and Vadlamudi) have shown the highest concentrations of Fluoride among the ground waters of the Study area (APHA 2005).

Table 4.1 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,

4.2 Isolation of microbial flora

The microbial flora isolated from the study soils of the Baratang Island of Andaman & Nicobar Islands has obtained a total of 200 Bacterial colonies, 36 Actinomycetes colonies and 17 Fungal colonies. The soil sample was serially diluted and transferred on to media; Nutrient agar for isolation of Bacteria, Glycerol Yeast extract for isolation of Actinomycetes and Sabourauds Dextrose agar for isolation of Fungi (Toratora, Funke and Case, 1995, Cappuccino, 2005; Aneja, 2005; Prescott, Harley and Klein, 2005 and).

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Table 4.2 Enumeration of Microbial Flora from the soil 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 X 10^{3}
	10^{-5}	10^{5}	61	46	52	159 / 3 = 53 X 10^{4}
	10^{-6}	10^{6}	52	43	49	144 / 3 = 48 X 10^{5}
	10^{-7}	10^{7}	43	32	39	114 / 3 = 38 X 10^{6}
Actino- mycetes	10^{-3}	10^{3}	18	15	15	48 / 3 = 16 X 10^{2}
	10^{-4}	10^{4}	11	9	7	27 / 3 = 9 X 10^{3}
	10^{-5}	10^{5}	9	5	4	18 / 3 = 6 X 10^{4}
	10^{-6}	10^{6}	7	5	3	15 / 3 = 5 X 10^{5}
Fungi	10^{-2}	10^{2}	8	6	7	21 / 3 = 7 X 10^{1}
	10^{-3}	10^{3}	5	3	4	12 / 3 = 4 X 10^{2}
	10^{-4}	10^{4}	5	2	2	9 / 3 = 3 X 10^{3}
	10^{-5}	10^{5}	4	2	2	9 / 3 = 3 X 10^{4}

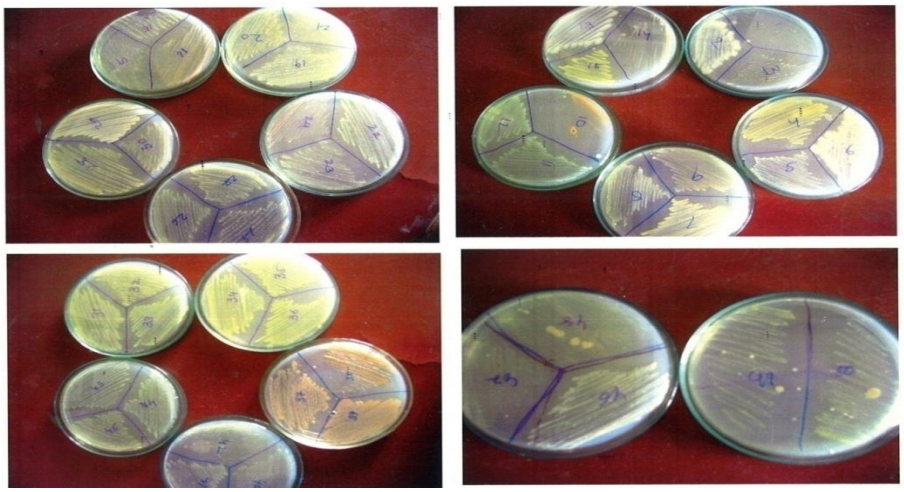

Image 4.1 Few Bacterial and Actinomycetes colonies isolated from soil of Baratang Island

4.3 Primary Screening:

The primary screening of the 200 bacterial colonies isolated from the study soils revealed that 16 out of 200 bacterial colonies showed more affinity towards Biosorption of Fluoride. 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} from Baratang Island

Table 4.3 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.

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4.4 Biosorption Studies (Secondary Screening):
These 16 bacterial strains were subjected to Biosorption of Fluoride in 4 different media; Nutrient broth, Peptone water, Basal medium and LB (Luria Bertani) broth at different pH 4.0, 7.0 and 10.0 under various Incubation periods; 24 hrs, 48 hrs and 72 hrs.

Medium: Biosorption studies were performed at different incubation periods, incubation temperatures and pH in 4 media. The studies indicate that 72 hrs of incubation period is required to obtain maximum adsorption in 10 mg / L of fluoride concentration in the four media at pH 4.0 and 10.0 in all the 4 media while at pH 7.0 the maximum sorption for 10 mg / L fluoride concentration was realized at 48 hrs incubation in Nutrient broth and Peptone water and at 24 hrs in Basal medium and LB broth media. In Basal medium and LB broth the 5 potential strains S_{13}, S_{35}, S_{54}, S_{55} and S_{56} have exhibited maximum (90% – 100%). Biosorption in, 20 mg / L and 30 mg / L of fluoride concentration at 7.0 pH for 48 hrs and 72 hrs of incubation periods respectively. Basing on the studies the performance of Biosorption in the 4 different media followed the bellow order at 37^0C incubation temperature and 7.0 pH.

LB broth > Basal medium > Nutrient broth > Peptone water

pH: - Maximum Biosorption of Fluoride was evaluated at three pH levels (acidic – 4.0, neutral – 7.0 and alkaline – 10.0) and for the three incubation temperatures (10^0C, 37^0C & 60^0C). The results of these tests indicated that all the strains considered in the present study are Neutrophiles but have the ability to withstand slight pH variations. 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:
Maximum sorption was observed at 37^0C temperature under the experimental conditions (different media, pH and incubation periods) indicating its dominance over the other two temperatures. 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

Table 4.4 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.
Among the 16 designated bacterial strains, 5 strains designated as $S_{13,}$ $S_{35,}$ $S_{54,}$ S_{55} and S_{56} have shown the potential for Biosorption of Fluoride. The Biosorption capacity of these 5 strains ($S_{13,}$ $S_{35,}$ $S_{54,}$ S_{55} and S_{56}) in 4 different media (Nutrient Broth, Peptone Water, Basal Medium and LB Broth) at 37^0C temperature of incubation, pH 7.0 in 3 incubation periods (24 hrs, 48 hrs and 72 hrs) was evaluated. The Biosorption of all the five strains ($S_{13,}$ $S_{35,}$ $S_{54,}$ S_{55} and S_{56}) were studied at 10mg / L, 20mg / L and 30 mg / L concentration of fluoride.

4.5 Analysis of field samples

The efficacy of the five biosorbents ($S_{13,}$ $S_{35,}$ $S_{54,}$ S_{55} and S_{56}) identified through the present investigation was tested with Fluoride rich ground waters collected from Visakhapatnam urban region. 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.

Table 4. 5 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.

4.6 Identification bacterial strains

The designated cultures were isolated into pure forms and it is usually identified by a combination of information derived from microscopic observations like morphology and arrangement of cells and cultural (growth) characteristics on both agar media as well as broth; the gram staining reactions; the occurrence of motility and biochemical characteristics.

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Table 4.15: Biochemical characteristics

Strain No	Extracellular Enzyme Activities				Intracellular Enzyme Activities								IMViC Series of tests			
	Gelatin hydrolysis	Starch hydrolysis	Lipid hydrolysis	Casein hydrolysis	Fermentation			H_2S Production	NO_3 reduction	Urease	Catalase	Oxidase	Idnole	MR	VP	Citrate utilization
					Lactose	Dextrose	Sucrose									
S_{13}	-	-	-	-	A	A	A	-	-	-	+	-	-	+	-	-
S_{35}	-	-	-	-	A	A	A	-	-	-	+	-	-	+	-	-
S_{54}	-	-	-	-	AG	AG	AG	-	+	-	+	-	-	-	+	+
S_{55}	-	-	-	-	AG	AG	AG	-	+	-	+	-	-	-	+	+
S_{56}	+ (rapid)	-	+	-	-	-	-	-	+	-	+	+	-	-	-	+

+ = Positive; - = Negative; A = Acid positive; AG = Acid and Gas Positive; MR = Methyl Red Test; VP = Voges Proskauer Test Incubation temperature - 37^0C; Incubation period – 24hrs.

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.

5. CONCLUSION

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. The above information reveal that the biosorbents evaluated through the present study have the potential to remove 50 % of the initial concentration of fluoride under laboratory conditions and 50 % of the initial concentration (3 ppm) of fluoride in field samples, hence they may be considered for Defluoridation of drinking waters after careful evaluation of the methodology under various field conditions.
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.

Recommendations:

- ✓ Microbial Defluoridation technique may prove to be effective and deserves field trials.
- ✓ The Defluoridation of ground water with the microbial biosorbents should follow disinfection before human consumption.

REFERENCES:

1. WHO. (2004). World Health Organization, Geneva, Switzerland. Fluoride in drinking water, Background Document for Preparation of WHO guidelines for drinking water quality. (Www. Who. int/ water. Sanitation - health /dwq/guidelines/en)

2. Lansing M. John P. Harley, Donad A. Klein (2005). 6th Edition. Mc Grow Hill Publication, 118 – 126 and 104 -108.

3. Ajmal, M., Khan, A.H., Ahmad S., Ahmad, A., Water Res., 32, (10), 305-(1998).

4. Amina, C., Lhadi, L.K., Younsi, A., Murdy, J. (2004). Environmental Impact of an Urban Landfill on a coastal aquifer. J. Afr. Earth. Sic., 39, (3-5), 509-516.

5. Anwar, F. (2003). Assessment and analysis of industrial liquid waste and study disposal at Unlined landfill sites in arid climate. Waste Manage., 23, (9), 817-824.

6. 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).

7. Ayoobo S., Gupta, A.K., (2006). Fluoride in drinking water: A Review on the status and stress Effects. Critical reviews in Environment Science and Technology., 36: 433-487.

8. Cappuccino Laboratory, James. G., Nataliesherman. (2005). Microbiology: A Manual 6th Edition. Pearson Publication. 115, 119.

9. First Report of DDWS (Development of Drinking water supply). (2004). Standing Committee on Rural Development, Ministry of Rural Development Presented to the 14th hook Sabah Secretariat, New Delhi, India.

10. Gadd, G.M. (1990). Biosorption chemistry and Industry., 13, 421.

11. Gadd, G.M., Rehum. H.J., Reed G. (Eds). (1988). Biotechnology A-comprehensive Treatise special Microbial Process, Vol, 6b, VCH, Veriagsgesell schoft, Weinheim, Germany, 401-433.

12. Gupta, R., Ahuja, P., Khan S., Saxena, R.K., Mohapatra, H. (2000). Microbial biosorbents: meeting challenges of Heavy metal Pollution in aqueous solutions. Cur. Sci., 78, 967-973.

13. Illami, T., Gulay. B., Emine, Y., Gokben, B. (2005). Equilibrium and kinetic studies on biosorption of Hg (II) Cd (II) and Pb (II) ions onto micro algae Chlamydomonos reinhardtil. J. Environ Manag., 77, 85-92.

14. Kass, A., Yechini Gavrieli, Y., Vengosh, A., Starinsky. (2005). The impact of fresh water and waste water irrigation on the chemistry of shallow ground water: A case study from the Israeli Coastal aquifer. J. Hydro., 300, (1-4), 314-331.

15. Maca Skie. L.E., Dean, A.C.R. (1989). Biological water treatment. Asian. R. Lis.s, New York. 159-201.

16. Mc Hale. A.P., Mc Hale.S. (1994). Microbial Biosorption of metals, potential in the treatment of metal pollution. Biotechnology. Adv., 12, 647-652.

17. Menakshi, Maheshwari, R.C. (2006). Fluoride in drinking water and its removal. Center for rural development and Technology. India Institute of technology Delhi, Hauz Khas, New Delhi India.

18. Oren, O., Yechieli, Y., Bohlke, J. K., Dody, A. (2004). Contamination of ground water under cultivated fields in an arid environment, Central Arava valley Israel. J. Hydrol., 290, (3 / 4), 312-328.

19. Susheela, A.K., Kumar, A., Bhavnagar, M., and Abrader R. (1993). Prevalence of endemic fluorosis with gastro –intestinal manifestation in people living in some North Indian villages. Fluoride., 26, 97- 104.

20. Taiyuan Declaration on water quality and Arsenic. (2004). Inter Regional Conference on water quality and Arsenic Mitigation Organized in Taiyuan, China, November 23- 26.

21. Tortora, G.J., Funke, B.R., Case C.L. (1995). Microbiology An Introduction 5th Edition The Benjamin / Cummings Publishing, Co., Inc., Red Wood City, CA, 255 – 272.

22. UNICEF. (1999) Fluoride in water: An over view. P.11, UNICEF Program Division, WES, Section, New York, 13.

23. Volesky B., Holan Z.R. (1995). Biosoption of Heavy metals. Biotechnology Prog., 11, 235-250.

24. Volesky, Bohumil. (1990). Biosorption of Heavy metals. Fluorida, C R C Press, Inc., Boca Raton.

25. WHO, (1985) Guidelines for Drinking water quality vol, 3. World Health Organization. Geneva, 1-2.

26. Williams, C.J., Edyvean, R.G.J. (1997). Ion exchange in nickel Biosorption by sea weed materials. Biotechnology. Prog., 13, 424-428.

27. http://edugreen.teri.res.in