CIBTech Journal of Microbiology ISSN: 2319-3867 (Online)
An Online International Journal Available at http://www.cibtech.org/cjm.htm
2015 Vol. 4 (1) January-March, pp.78-84/Tamilvani et al.
Research Article

BIODEGRADATION ON FLUORIDE CONTAMINATED SOIL AND WATER IN DHARMAPURI DISTRICT OF TAMILNADU INDIA

Tamilvani T., King Solomon E. and *Rajesh Kannan V.
[1]*Department of Microbiology, Bharathidasan University, Tiruchirappalli - 620024, Tamil Nadu, India*
[2]*Department of Toxicology, Bioscience Research Foundation, Kandamangalam, Chennai- 602002, Tamilnadu, India*
**Author for Correspondence*

ABSTRACT
Fluorides are important industrial chemicals with a number of applications like aluminium production, drinking water fluoridation and the manufacture of fluoridated dental preparations. The present study was undertaken to explore and understand the level of fluoride and occurrence of endemic fluorosis. Fluoride contaminated water and soil samples were collected from Dharmapuri district situated in the northern part of Tamil Nadu. The efficiency of fluoride degradation by the fungal isolates from fluoride contaminated samples was found out by growing them in selective enrichment medium amended with sodium fluoride. Results revealed that the fluoride degradation was maximum in *Aspergillus* sp.5 from the soil sample of Dharmapuri and minimum in *Rhizopus* sp. from the water sample of Pennagaram. From this, it was concluded that the use of fluoride degradation agent to fluoride fungi isolated from fluoride contaminated water and soil area, which can be implemented for fluoride degradation in an environmentally safe mode.

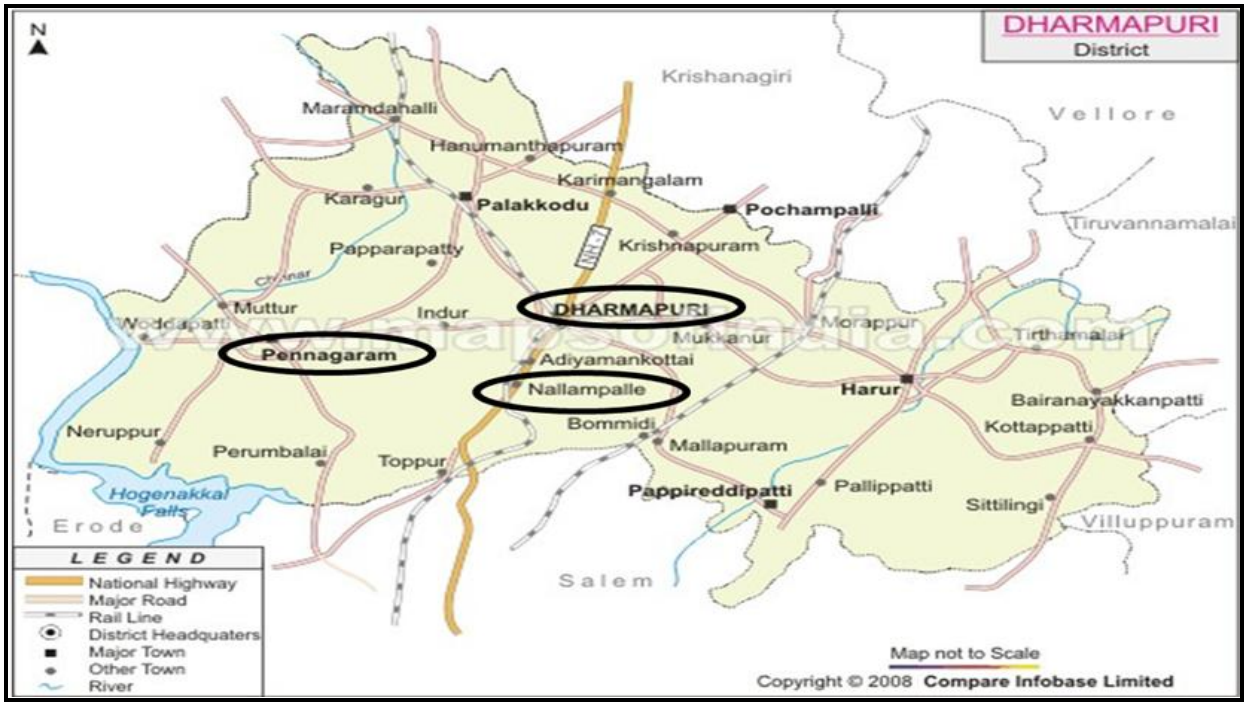
Keywords: *Biodegradation, Sodium Fluoride, Aspergillus sp., Rhizopus sp.*

INTRODUCTION
During the past few decades, widespread application of fluoroaromatic compounds as agrochemicals and pharmaceuticals has lead to an increased occurrence of environmental contaminants containing fluorine (Key *et al.,* 1997). Pollution of groundwater resources occurs through point and diffuses sources. Point source pollutions are effluents from industries and sewage-treatment plants. Diffuse pollution sources are agricultural runoffs due to inorganic fertilizers, pesticides and natural contamination of groundwater by fluoride, arsenic and dissolved salts due to geochemical activities. Most of the fluoride found in groundwater is naturally occurring from the breakdown of rocks and soils or weathering and deposition of atmospheric volcanic particles. Fluorinated compounds are rare in nature (Harper *et al.,* 1999). The stability of the carbon-fluorine bond (116 kcal/mol in CH_3F, compared to 81 kcal/mol for the carbon-chlorine bond in CH_3Cl) makes most fluorine containing compounds much more resistant to biodegradation than their unsubstituted analogs. The safe limit of fluoride in drinking water is 1.0 mg/L (WHO (1984). The endemic fluorosis in India is largely of hydro geochemical origin. It has been observed that low calcium and high bicarbonate alkalinity favors high fluoride content in groundwater (Bulusu and Nawlakhe, 1988). Water with high fluoride content is generally soft, has high pH and contains large amount of silica and this leads to fluorosis the problem is severe in India as almost 80% of the rural population depends on untreated ground water for potable water supplies (Sudhakar and Mamatha, 2004). Depending in the presence of some chemicals in the parent rocks, water may have excessive amount of fluoride. Moderate concentration of fluoride act as skeletal stabilizer, however, high fluoride concentration may result in endemic conditions known as fluorosis. Non-skeletal fluoride is whereby soft tissues are affected due to prolonged intake of fluoride in high concentrations. One way of avoid health problems were associated with drinking high fluoride containing water is to avoid domestic use of such fluoride water. Though the alternative sources of water are unavailable, defluoridation may offer practical solution to the problem. Therefore is needed to reduce excessive fluoride from drinking water. In the present study, is to describe the isolation and characterization of fungal species growing on sodium fluoride as the sole source of carbon and energy. Based on the identification of several intermediates for the degradation of sodium fluoride by this fungal species is proposed.

MATERIALS AND METHODS

Study Area

Fluoride contaminated water and soil samples were collected from Dharmapuri district (Pennagaram, Dharmapuri town, Nallampalle) is situated in the Northern part of Tamil Nadu. The study area lies at a geographical extent covering an area of 4497.77 Sq. km, which is 3.46% of Tamil Nadu state. It lies between 11° 47' and 12° 33'North latitudes and 77° 02' and 78° 40' East longitudes (Figure 1).



Analysis of Physico-chemical Properties

The physico-chemical properties such as pH, electrical conductivity (EC), total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), biological oxygen demand (BOD) and chemical oxygen demand (COD) was analyzed in collected water samples. The pH of the water and soil samples was found out using the pH and EC meter. TS, TDS, TSS, BOD, COD and fluoride of the water were investigated by the procedures of standard methods (APHA, 1998). Total nitrogen (N), available phosphorus (P) and exchangeable potassium (K) was analyzed in soil testing laboratory, Tamil Nadu Agricultural Department, Mannarpuram, Tiruchirappalli, Tamil Nadu, India

Isolation and Enrichment of Fluoride Degrading Fungi from Fluoride Contaminated Water and Soil

Fungal isolates were isolated by selective enrichment from various water and soil samples from the fluoride contaminated area. The fluoride degrading fungi were isolated and identified by examining microscopically for cell morphology with wet mount preparation using lacto phenol cotton blue stain.

A soil sample (100 g) was added to 300 ml of mineral salts medium (Dorn *et al.*, 1974) containing 2.5 mM sodium fluoride (0.35 g/liter) and 2.5 mM succinic acid (0.3 g/liter) in closed Erlenmeyer flasks. Alternatively, one volume of a water sample was mixed with one volume of medium containing 5.0 mM sodium fluoride and 5.0 mM succinic acid. After being shaken for 4 days at 30°C, the substrate concentration decreased; subcultures were transferred every second day for five passages into fresh mineral salts medium containing 2.5 mM sodium fluoride. The cultures were plated on agar plates containing mineral salts medium with or without 2.5 mM sodium fluoride. After 7 days, fungal colonies appeared on the plates with sodium fluoride, whereas no colonies were observed on plates without the substrate. After several transfers on solid medium with sodium fluoride as the only growth substrate, the fungi were isolated and inoculated in mineral salt broth and placed in the shaker for 7 days incubation

then the broth was centrifuged. After centrifugation, the required amount of sample was taken to measure optical density and to estimate the fluoride degradation.

SPADNS Method

In the SPADNS (sodium 2-(parasulfophenylazo)-1, 8-dihydroxy-3, 6-naphthalene disulfonate) colorimetric method was used to estimate the fluoride degradation. Fluoride reacts with the lake, dissociating a proportion into a colorless complex anion (ZrF_6^{2-}) and the dye. As the amount of fluoride increases, the product becomes progressively lighter. After preliminary distillation, the distillate was reacted with the zirconium-dye lake and measured calorimetrically at 570 nm (Clesceri *et al.,* 1998).

RESULTS AND DISCUSSION

Results

Physico-chemical Properties

The pH of the fluoride contaminated samples were showed at high pH of 10.6 at Nallampalle, next to Pennagram water sample of 10.3 and soil sample was slightly reduced at 9.5.But all the samples were found to be as alkaline condition (Figure 2). The electrical conductivity of the fluoride contaminated water and soil samples were found be high ranging from 100 µS/cm to 119 µS/cm in water and 25.8 µS/cm in soil (Figure 3). The EC of Pennagaram water sample was found to lower than the other two places.

The total solid, total dissolved solids and total suspended solids present in the fluoride contaminated water was investigated (Figure 4). The total dissolved solid was found to be 916.49, 888.30 and 899.80 mg/L. In the water sample was studied the COD values ranges from 17.4 to 11.2 mg/L, which are below the permissible level (Table 1).

Analysis of Soil Properties and Water Fluoride Permissible Levels

The texture of soil was sandy clay loam. The soil macronutrients of NPK content were found to be 105, 4.5 and 143 kg/acre respectively in the fluoride contaminated soil (Table 2). And fluoride contents were showed high in Pennagaram as 1.8mg/L than other water samples collected from different sites, but both the soil and water were exceed the WHO permissible level limit as 1.5mg/L.

Isolation of Fungi from Fluoride Contaminated Water and Soil

Three fungal isolates from water and five from soil samples, which could grow on sodium fluoride as their sole source of carbon and energy, were isolated by using selective enrichment technique. The isolated fungal cultures were identified microscopically using lactophenol cotton blue. The identified fungal species were *Aspergillus* sp.1, *Aspergillus* sp.2, *Rhizopus* sp., *Aspergillus* sp.3, *Aspergillus* sp.4, *Aspergillus* sp.5, *Aspergillus* sp.6, *Aspergillus* sp.7 (Table 3).

Degradation of Sodium Fluoride

After an incubation period of 7 days, the fluoride degradation was estimated by SPADNS method the maximum fluoride degradation (0.65 mg/L) was showed by *Aspergillus* sp.5, which was isolated from the soil sample of Dharmapuri town and a minimum of degradation (0.36 mg/L) was observed by *Rhizopus* sp. isolated from the water sample of Pennagaram (Table 5).

Discussion

According to WHO (1997) and ISO (1992), permissible limit for fluoride in drinking water is 1.5 mg/L. The fluoride levels in collected water and soil samples were high and exceed the permissible limit (Figure 5). In the present study, the alkaline condition of the water and soil reveals the presence of salts in water and soil and higher than the recommended limit and EC of water and soil and samples were found to be than the recommended limit (WHO (1992). TDS values were very high in the wells and exceed the maximum permissible limit. Its values in the range of 50 to 150 mg/L make the water unfit for any use (Ranu *et al.,* 1991). The identified BOD level is lower than the permissible limit (0.02 mg/L), an indication of the contamination (Meenakshi *et al.,* 2004).

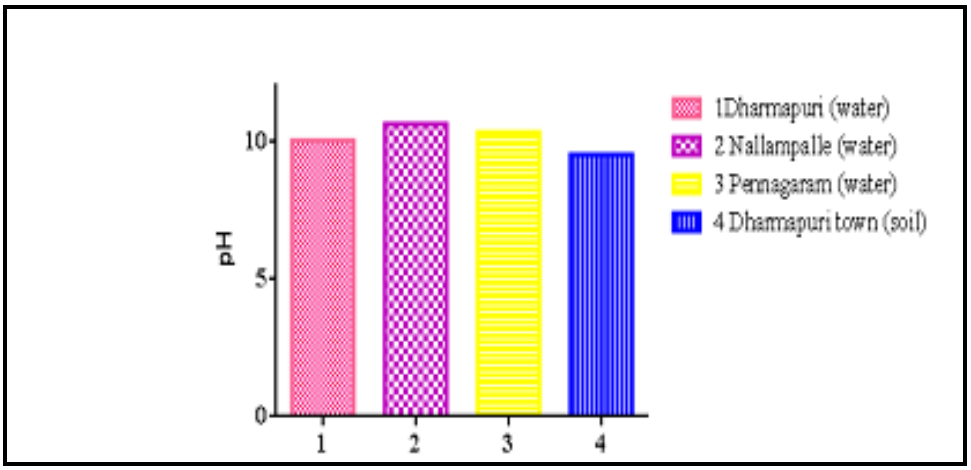


Figure 2: pH of the fluoride contaminated water and soil samples

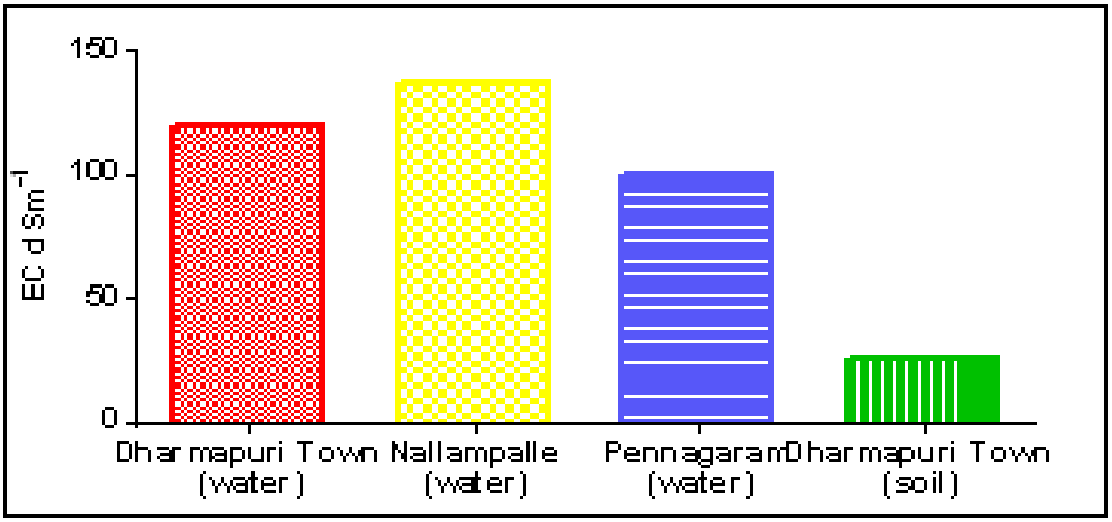


Figure 3: Fluoride levels in water and soil sample

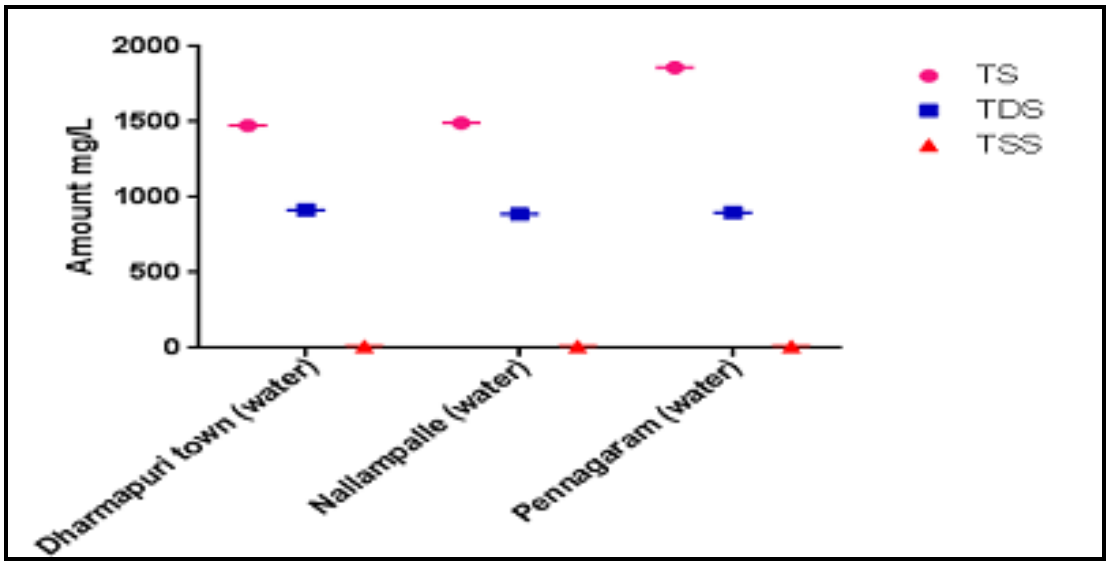


Figure 4: Variation of TS, TDS and TSS of the fluoride contaminated water samples

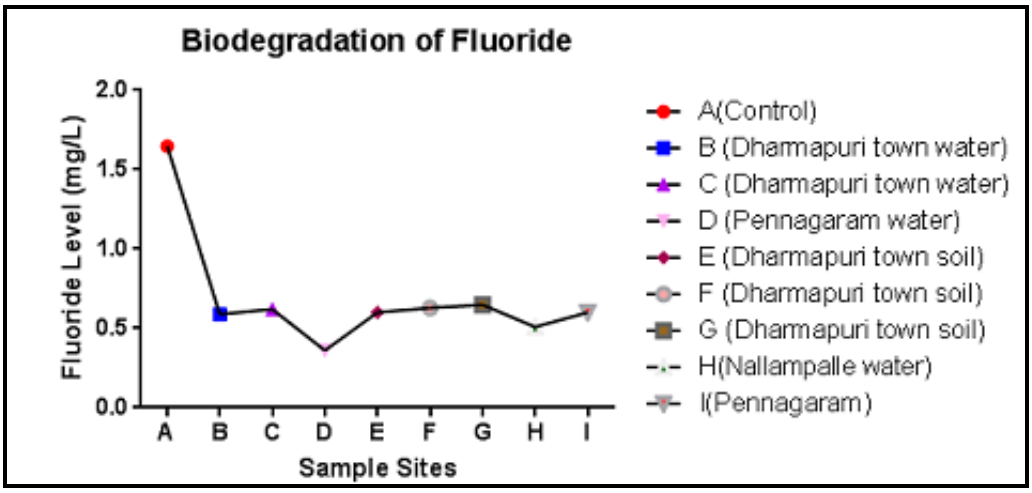


Figure 5: Biodegradation of sodium fluoride by different isolates obtained from Dharmapuri district. The carbon source used is 5.0mM sodium fluoride

Table 1: BOD and COD level of the fluoride contaminated water samples

Source	COD (mg/L)	BOD (mg/L)
Dharmapuri town (Water)	17.4	0.02
Nallampalle (Water)	14.2	0.02
Pennagaram (Water)	11.2	0.02

Table 2: Properties of soil and water fluoride permissible levels

S.No.	Samples	Result
1	Dharmapuri town soil Texture	Sandy clay loam
2	Dharmapuri town soil Total nitrogen	105 Kg/acre
3	Dharmapuri town soil available phosphorus	4.5 Kg/acre
4	Dharmapuri town soil exchangeable potassium	143 Kg/acre
5	Dharmapuri town water	1.75mg/L
6	Nallampallae town water	1.71mg/L
7	Pennagaram town water	1.8mg/L

Table 3: Fungal species used for fluoride degradation

S .No.	Sources	Fungal species used for fluoride degradation
1	Dharmapuri town (Water)	*Aspergillus* sp.1
2	Dharmapuri town (Water)	*Aspergillus* sp.2
3	Pennagaram (Water)	*Rhizopus* sp.
4	Dharmapuri town (Soil)	*Aspergillus* sp.3
5	Dharmapuri town (Soil)	*Aspergillus* sp.4
6	Dharmapuri town (Soil)	*Aspergillus* sp.5
7	Nallampalle (Water)	*Aspergillus* sp.6
8	Pennagaram (Water)	*Aspergillus* sp.7
9	Control	---

The presence of fluoride contents in the water and soil samples can be attributed as geological deposit, geochemistry of location and extensive application of fertilizers like rock phosphates (Karthikeyan *et al.*, 2010). Extensive application of phosphate fertilizers in agriculture land and phosphate rocks and minerals present in soil undergoes dissolutions due to heavy rainfall in the monsoon seasons increases fluoride ion

in soil and water sources gradually. Prolong use of fluoride containing water may lead to reason for dental fluorosis (Baruah *et al.*, 2011).

Gloeophyllum striatum is involved in 2-fluorophenol degradation (Kramer *et al.*, 2004). Fluoride as florophenol is degraded by several kinds of microorganisms. In bacteria, yeasts and imperfect fungi, fluorophenols are initially oxidized by intracellular NAD(P)H-dependent phenol hydroxylases, which may attack different positions of the phenolic ring. In *Rhodococcus opacus*, 2-fluorophenol is concomitantly hydroxylated at the C2 and C6 position at a ratio of 80:20 (Bondar *et al.*, 1999), thus leading to partial oxidative defluorination. The mold *Penicillium simplicissimum* has produced fluoride from 2-fluorophenol at 9% of the amount that theoretically could be achieved upon complete dehalogenation (Marr *et al.*, 1996). In contrast, defluorination of 2-fluorophenol was not observed with *Penicillium frequentans*, where 3-fluorocatechol, the only hydroxylated aromatic product detected, was found to accumulate (Hofrichter *et al.*, 1994) In the present study, the maximum fluoride degradation (0.65 mg/L) was by *Aspergillus* sp.5 isolated from the soil sample of Dharmapuri town and a minimum of degradation (0.36 mg/L) was by *Rhizopus* sp. isolated from the water sample of Pennagaram.

ACKNOWLEDGMENT

The authors thank Rhizosphere Biology Group, Department of Microbiology, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India for valuable help to execute this research and magnificent comments in the manuscript.

REFERENCES

APHA (1998). *Standard Methods for Examination of Water and Wastewater*, 20th edition. American Public Health Association, Washington, DC.

Baruah BK, Haque A, Das B, Misra H, Medhi C and Misra AK (2011). Fluoride ion in drinking water and prevalence of dental fluorosis among the population in some tea garden belt villages in Golaghat district of Assam, India. *Archives of Applied Science Research* **3**(1) 175-179.

Bondar VS, Boersma MG, Van Berkel WJ, Finkelstein ZI, Golovlev EL, Baskunov BP, Vervoort J, Golovleva LA and Rietjens IM (1999). Preferential oxidative dehalogenation upon conversion of 2-halophenols by Rhodococcus opacus 1G. *FEMS Microbiology Letters* **181** 73–82.

Bulusu KR and Nawlakhe WG (1988). Defluoridation of water with activated alumina batch operations. *Indian Journal of Environmental Health* **30**(3) 262-299

Bulusu Kr and Nawlakhe WG (1990). Defluoridation of water with activated alumina continuous con-tacfing system. *Indian Journal of Environmental Health* **32** 197-218.

Clesceri LS, Greenberg AE and Eaton AD (1998). *Standard Methods for the Examination of Water and Wastewater*, 20th edition. American Public Health Association, Washington 1325.

Dorn E, Hellwig M, Reineke W and Knackmuss HJ (1974). Isolation and characterization of a 3-chlorobenzoate degrading pseudomonad. *Archives of Microbiology* **99** 61-70

Harper DB and Hagan OD (1999). Fluorine-containing natural products. *Fluorine Chemistry* **100** 127–133.

Hem JD (1959). Study and interpretation of the chemical characteristic of natural water. Water Supply Paper, 2254, USGS, USA.

Hofrichter M, Bublitz B and Fritsche W (1994). Unspecific degradation of halogenated phenols by the soil fungus Penicillium frequentans. *Journal of Basic Microbiology* **34** 163–172.

ISO (1992). Water quality – determination of dissolved fluoride, chloride, nitrite, orthophosphate, bromide, nitrate and sulfate ions, using liquid chromatography of ions- part 1 method for water with low contamination.

Karthikeyan K, Nanthakumar K, Velmurugan P, Tamilarasi S and Lakshmanaperumalsamy P (2010). Prevalence of certain inorganic constituents in groundwater samples of Erode district, Tamilnadu, India, with special emphasis on fluoride, fluorosis and its remedial measures. *Environmental Monitoring and Assessment* **160** 141–155.

Key BD, Howell RD and Criddle CS (1997). Fluorinated organics in the biosphere. *Environmental Science & Technology* **31** 2445–2454.
Kramer C, Kreisel G, Fahr K, Kassbohrer J and Schlosser D (2004). Degradation of 2-fluorophenol by the brown-rot fungus Gloeophyllum striatum: evidence for the involvement of extracellular Fenton chemistry. *Applied Microbiology and Biotechnology* **64** 387–395.
Marr J, Kremer S, Sterner O and Anke H (1996). Transformation and mineralization of halophenols by Penicillium simplicissimum SK9117. *Biodegradation* **7** 165–171.
Meenakshi VK, Garg Kavita and Renuka A Malik (2004). Ground water quality in some villages of Haryana, India: focus on fluoride and fluorosis. *Journal of Hazardous Materials* **106B** 85–97.
Ranu G, Singh OU, Tandon SN and Mathur (1991). A study of water quality and metal speciation of Yamuna River. *Asia Environment* **13** 3-10.
Sudhakar Rao SM and Mamatha PR (2004). De-fluoridation of water by IISc method. Provisional Indian Patent (application under consideration).
WHO (1984). *Drinking Water Guidelines and Standards.*
WHO (1992). *A Guide to the Development of on-site Sanitation.*
WHO (1997). *Guidelines for Drinking-Water Quality*, 2nd edition **3**.