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Fluoride Delineation and Bacterial Diversity of Potable Water in Mandal Parishads of Kurnool District, Andhra Pradesh, India

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Abstract: An attempt was made to delineate fluoride and identify bacterial diversity in the drinking water sources of 27 Mandal Parishads of Kurnool district, Andhra Pradesh, India. Fourteen water samples of groundwater and thirteen samples of surface water were collected and analysed for fluoride concentration as well as bacterial diversity. Results revealed that 57.14% of the groundwater samples had a fluoride concentration over the permissible limit, whereas 15.38% of surface water samples crossed the permissible limit. Bacterial species predominantly found in the groundwater samples are *Bacillus subtilis* whose presence seemed more in Dornipadu (DOR), Pamulapadu (PPU), Nandavaram (NDV) and Kosigi (KOS). *Exiguobacterium* sp. prevailed much in Chagalamarri (CHA), Midthur (MID), Jupadu Bungalow (JBW) and Gonegandla (GON). *Acinetobacter* sp. is rich in Allagadda (ALG) and Atmakur (ATK). *Chryseobacterium* sp. dominated in Orvakal (ORV); Pagidyala (PGA) had more concentrations of *Staphylococcus warneri* and Kolimigundla (KOL) had reported high levels of *Aeromonas punctate*. Surface water samples possess predominant bacterial species, *Bacillus subtilis* dominated in Uyyalawada (UYY), Sanjamala (SAN), Banaganapalle (BPL) and Mantralayam (MAN). *Exiguobacterium* sp was abundant in Koilkuntla (KOI), Kowthalam (KOW) and Pattikonda (PAT). *Bergeyella* sp. reported high in Gadivemula (GAD). *Pantoea* sp. was more in Yemmiganur (YMG); Aspari (ASP) was rich in *Kocuria marina*; *Acinetobacter junii* dominant in Devanakonda (DKD). *Paracoccus sigandrium* prevailed more in Owk.

Key words: Bacterial diversity, fluoride delineation, groundwater, surface water.

Introduction

India is amongst the 23 nations of the world where people are dependent on groundwater with fluoride levels above those prescribed by the World Health Organization (WHO, 1993). Fluoride contamination of groundwater is a severe problem in India due to which people are affected by fluorosis (Susheela, 1999). In India, affected states with fluorosis are Rajasthan, Gujarat, Telangana, Andhra Pradesh, Maharashtra, Bihar, Madhya Pradesh, Kerala, Uttar Pradesh, Orissa, Punjab, Haryana, Karnataka and Tamilnadu. Kurnool district of Andhra Pradesh is drought affected region and with a socially and economically backward population. The primary sources of potable water for the community are surface and sub-surface water bodies. The surface water sources get depleted post rainy season. Therefore, the only alternative source of drinking water here is sub-surface water. Several cases of deformities in bones and teeth were seen, which were found to be the clinical complications of fluoride water consumption. Challenges were continuous, leading to fluorosis.

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Bacteria are natural inhabitants of all aqueous environments. The freshwater source of drinking is surface water in many countries. Contamination of pipelines due to low pressure in the distribution network, intermittent supply, the inadequacy of wastewater collection systems, and leaking pipes are common problems in developing countries (Jurdi et al., 2002). Many factors influence the quality of water in the distribution system and can lead to chemical and microbial contamination of drinking water. Culture-dependent methods can perform microbial contamination studies. Cultivable bacteria represent a small part of the water. Proteobacteria (mainly Alpha-, Beta- and Gammaproteobacteria) were frequently observed as the dominant phylum in treated drinking waters, as noted by researchers Eichler et al. (2006); Poitelon et al. (2009); Revetta et al. (2010). Among the most common cultivable bacteria in drinking water are members of the phyla Proteobacteria, as reported by Eichler et al. (2006) and Revetta et al. (2010). A comprehensive understanding of bacterial biology and ecology requires a culture-dependent practice reported by Alain and Querellou (2009). Municipal water requires continuous monitoring to meet the water need of communities in the Kurnool district.

Despite regular practices by the government agencies for decades, a considerable range of fluoride-affected clinical complications is evident. Therefore, an alternative scientific remediation method that is cost-effective, eco-friendly and easily practiced by the local population is the grave concern of the day. The objectives of the current research are fluoride delineation and its local remedial measures of water in Mandal headquarters of Kurnool district and also to identify dominating bacterial groups in potable water of the same locality.

Materials and Methods

Water Sampling

UV sterilised one-litre bottles were used to collect surface water from Mandal Parishads Uyyalawada, Koilakuntla, Sanjamala, Owk, Banaganapalle, Gadivemula, Yemmiganur, Mantralayam, Kowthalam, Adoni, Aspari, Pattikonda, Devanakonda and Gadivemula Mandal Parishads of Kurnool district, Andhra Pradesh, India. These Mandal Parishads gave sample codes as UYY, KOI, SAN, OWK, BPL, GAD, YMG, MAN, KOW, ADN, ASP, PAT, DKD and GDV, respectively.

UV sterilised 1-L bottles were used to collect groundwater from Chagalamarri, Allagadda, Dornipadu, Orvakal, Midthur, Nandikotkur, Jupadu Bungalow, Pamulapadu, Pagidyala, Atmakur, Gonegandla, Nandavaram, Kosigi, Kolimigundla Mandal Parishads in Kurnool district, Andhra Pradesh, India. These Mandal Parishads gave sample codes as CHA, ALG, DOR, ORV, MID, NDK, JBW, PPU, PGA, ATK, GON, NDV, KOS and KOL, respectively (Figure 1).

Table 1: Isolates obtained from surface water

Name of strain(s) (Accession number)	*Similarity with*	*Homology (%)*
Bacillus subtilis BPL, SAN, MAN MT623564	*Bacillus subtilis spizizenii* NR_112686.1	98.76%
Paracoccus sigandrium OWK MT623394	*Paracoccus acridae* SCU-M53 NR_151998.1/ *Paracoccus speluncae* NR_149253.1	100% / 98.40%
Chryseobacterium sp. GAD	*Chryseobacterium taklimakanense* X-65 NR_044561.1	97.86%
Exiguobacterium aurantiacum PAT MT614322	*Exiguobacterium mexicanum* NR_042424.1	98.66%
Kocuria marina ASP MT614251	*Kocuria marina* KMM NR_025723.1	91.24%
Exiguobacterium aestuarii KOW	*Exiguobacterium aestuarii* TF-16 NR_043005.1/ *Exiguobacterium profundum* 10C NR_043204.1	99.38% / 99.25%
Exiguobacterium sp. KOI MT613878	*Exiguobacterium mexicanum* NR_042424.1	95.42%
Bacillus subtilis UYY MT613862	*B. subtilis inaquosorum* NR_104873.1	99.80%
Acinetobacter junii DKD MT613873	*Acinetobacter junii* NR_117623.1 & NR_026208.1/ *Acinetobacter modestus* NR_148845.1	100% / 100% / 98.99%
Pantoea sp. YMG MT613877	*Erwinia mallotivora* NR_041974.1 *Pantoea stewartii* NR_104928.1	91.19% / 91.05%

Physico-Chemical Analysis

These water samples were analysed for fluoride by following the Standard Method (APHA 2012).

Bacterial Diversity Analysis

Bacterial colonies were isolated by serial dilution method from water samples by spreading 100 µL of the water samples on a nutrient agar medium and incubated at 37°C for one day to screen the bacterial populations. The bacterial isolate's molecular identification is done using 16S rRNA sequencing. DNA was isolated from the bacterial isolates using the Microbial DNA Isolation Kit (Mo Bio Laboratories). PCR was used to amplify the 16S rRNA gene and sequenced following Reddy et al., 2000. The 16S rRNA gene sequence of the isolate was subjected to a BLAST sequence similarity search to identify the nearest taxa by using the study of Altschul (1990).

Bioremediation

Coriandrum sativum was used for the defluoridation of water samples of the study area. *Coriandrum sativum* is readily available and cost-effective and the samples were subjected to biosorption experiments following Srinivasulu et al. (2018).

Results and Discussion

Surface Water Samples

The sample of ASP and PAT was above the Permissible limit of BIS (2012). Fluoride concentration of surface water samples ranges from 0.30 to 1.64. Out of 13 surface water samples, only 02 (15.38%) have crossed the permissible limit and 11 (84.62%) are below the permissible limit, as illustrated in Table 2. In general, the abundance of fluorine in freshwater varies from 0.25 to 1 mg/L (Hawkes & Webb, 1962). The fluoride content of Surface water also varies from 0 to 6.4 mg/L, depending on the groundwater fluoride content feeding a given stream (Fleischer and Robinson, 1963). The fluoride content is mainly from the granitic rocks of the area (Zuane, 1990).

Physical Parameters of Surface Water Samples in Mandal Parishads of Kurnool District, Andhra Pradesh, India

The pH values of surface water samples of UYY, KOI, SAN, OWK, BPL, GAD, YMG, MAN, KOW, ADN, ASP, PAT and DKD ranged between 6.38 and 7.96 and have a role in solubility calculations or geochemical equilibrium (Todd, 1980). The colour of surface water samples of UYY, KOI, SAN, OWK, BPL, GAD, YMG, MAN, KOW, ADN, ASP, PAT and DKD varied between > 1.0 and 3.0. The electrical conductivity of the water varied from 263 – 1427 µs/cm. The samples were classified into type I (EC < 1500 µs/cm) and type II (EC: 1500 µs/cm and 3000 µs/cm) water-based on EC. As per the classification surface water samples of UYY, KOI, SAN, OWK, BPL, GAD, YMG, MAN, KOW, ADN, ASP, PAT and DKD come under type I. The odour of the surface water samples of UYY, KOI, SAN, OWK, BPL, GAD, YMG, MAN, KOW, ADN, ASP, PAT and DKD were agreeable. The turbidity of the surface water samples of UYY, KOI, SAN, OWK, BPL, GAD, YMG, ADN, ASP and DKD were within desirable limits of BIS, 2012 and the surface water samples of MAN, KOW, PAT exceeded the desirable range of BIS Standard.

Chemical Parameters of Surface Water Samples in Mandal Parishads of Kurnool District, Andhra Pradesh, India

The total hardness of water samples was found between 42 and 437 mg/L. Four samples of BPL, MAN, ASP and DKD crossed the acceptable limit of Total hardness (200 mg/l) and the remaining UYY, KOI, SAN, OWK, BPL, GAD, YMG, MAN, KOW, ADN, ASP and PAT found below the acceptable limit of BIS, 2012. The study area's water quality falls under Soft, Moderately

Table 2: Fluoride in groundwater samples of Kurnool district, Andhra Pradesh, India

Fluoride content (mg/L)	*No of samples*	*Percentage*
0-0.50	-	-
0.6-1.0	-	-
1.0-1.5	06	42.85
1.6-2.0	07	50.00
2.1-2.5	01	07.14

Table 3: Fluoride in surface water samples of Kurnool district, Andhra Pradesh, India

S. No	*Fluoride content (mg/L)*	*No of samples*	*Percentage*
1	0-0.50	02	15.38
2	0.6-1.0	01	07.69
3	1.0-1.5	08	61.53
4	1.6-2.0	02	15.38
5	2.1-2.5	-	-

Hard, Hard class and Very Hard of Sawyer and Mc Carty classification, as shown below in Table 4.

Table 4: Classification of surface water samples of Mandal Parishads in Kurnool district, Andhra Pradesh, India centered on hardness by Sawyer and Mc Cartly (1967)

Hardness (mg/L)	*(Class)*	*Samples of water*
0-75	Soft	KOW, ADN, YMG
75-150	Moderately Hard	PAT, UYY, KOI, OWK
150-300	Hard	ASP, DKD, SAN, GAD
>300	Very Hard	MAN, BPL

The magnesium values were found between 2.56 - 53.96 mg/L. Excluding surface water samples of BPL, MAN, DKD all other remaining surface water samples of UYY, KOI, SAN, OWK, GAD, YMG, KOW, ADN ASP and PAT were as per the acceptable limit of magnesium (30 mg/L). The value of Calcium was from 11 - 98 mg/L. Excluding surface water samples of BPL and MAN all other remaining surface water samples of UYY, KOI, SAN, OWK, GAD, YMG, KOW, ADN, ASP, PAT and DKD found within the desirable limit of the acceptable limit of Calcium (75 mg/L), according to BIS, 2012. Surface water samples of UYY, KOI, SAN, OWK, BPL, GAD, YMG, MAN, KOW, ADN, ASP, PAT and DKD had sodium and potassium concentration within the desirable limit of BIS, 2012. The sodium was found between 32 - 122.60 mg/L. Potassium value ranged from 3.60 - 25.59 mg/L.

Total dissolved solids were found between 152 and 924 mg/L. Surface water samples of BPL, MAN and DKD crossed the acceptable limit of TDS (500 mg/L) according to BIS, 2012 and all other surface water samples of UYY, KOI, SAN, OWK, GAD, YMG, KOW, ADN, ASP and PAT were as per the desirable limit of BIS, 2012. High Total dissolved solids in water may form Kidney stones (Garg et al., 2009).

The sources of sulfate in water are septic wastage, animal sewage, and industrial waste (Anderson and Johnson, 1979; Jalali, 2008). The sulfate concentration ranged between 10.90 and 96 mg/L, which indicates all samples were below the acceptable limit of BIS, 2012. Surface water samples of UYY, KOI, SAN, OWK, BPL, GAD, YMG, MAN, KOW, ADN, ASP, PAT and DKD had chloride and sulphate concentration within the desirable limit of BIS, 2012. Chloride content was found between 31 and 164 mg/L and sulfate concentration was found between 10.90 and 96 mg/L.

Total alkalinity was found between 56 - 355 mg/L. Surface water samples of BPL, MAN and DKD samples crossed the acceptable limit of Total Alkalinity (200 mg/L) and remaining surface water samples of UYY, KOI, SAN, OWK, GAD, YMG, KOW, ADN, ASP and PAT were as per the desirable limit of BIS (2012). Nitrate was found between 4.5 and 35.30 mg/L. Surface water samples of UYY, KOI, SAN, OWK, BPL, GAD, YMG, MAN, KOW, ADN, ASP, PAT, and DKD are within the desirable limit of BIS (2012).

Trace Metals of Surface Water in Mandal Parishads of Kurnool district, Andhra Pradesh, India

Iron in samples was found to be between 0.12 and 0.34 mg/L. Surface water samples PAT had crossed the desirable limit of Iron and surface water samples of UYY, KOI, SAN, OWK, BPL, GAD, YMG, MAN, KOW, ADN, ASP and DKD were found within the desirable limit of BIS, 2012. The trace metals manganese, copper, zinc and nickel concentrations of samples of UYY, KOI, SAN, OWK, BPL, GAD, YMG, MAN, KOW, ADN, ASP, PAT and DKD were found within desirable limits of BIS, 2012 and their ranges are as follows <0.001-0.030, <0.001-0.040, <0.001-0.030 and <0.001-0.030, respectively. Surface water samples of BPL, GAD, ASP, PAT and DKD exceeded the desirable limit. They can be due to the flow of the micronutrients added to the crops into the surface bodies and other samples of UYY, KOI, SAN, OWK, YMG, MAN, KOW & ADN were within the desirable limit of BIS, 2012. The lead values of surface water samples ranged between <0.001 and 0.020 mg/L. The relative dominance of the trace metals in surface water was observed in the following sequence Pb > Fe > Zn. Physico-Chemical parameters in surface water samples of Mandal Parishads in Kurnool district, Andhra Pradesh, India, compared with the Bureau of Indian Standards: 10500 – 2012 (BIS, 2012), as mentioned in Table 5.

The heterotrophic plate count is used as an indicator of microbiological quality, and the count has to be below 100 colony forming units (CFU) per milliliter (mL) of water (Anonymous, 1998). The heterotrophic plate count of the water samples of UYY, KOI, SAN, OWK, BPL, GAD, YMG, MAN, KOW, ASP, PAT and DKD exceeded the BIS (2012) and water sample ADN was within the desirable limit of BIS (2012). The high heterotrophic plate count may be due to stagnation of water, and long residence times in drinking water distribution systems (Lautenschalger et al., 2010). Increased bacterial abundance in water samples might

Table 5: Comparison of physico-chemical parameters in surface water samples of Mandal Parishads in Kurnool district, Andhra Pradesh, India with Bureau of Indian Standards: 10500–2012

Constituents	*Water samples Range*	*Desirable limit*	*Permissible limit*
Colour	>1.0-3.0	5 Hazen units	25 Hazen units
Turbidity	0.30-6.90	5.0 NTU	10 NTU
pH	6.38-7.96	6.5-8.5	NR
Electrical conductivity	263-1427	NS	NS
Total alkalinity	56-355	200	600
Total dissolved solids	152-924	500	2000
Chloride	31-164	250	1000
Total hardness	42-437	200	600
Nitrate	4.5-35.30	45	NR
Sulphate	10.90-96	200	400
Sodium	32-122.60	NS	NS
Potassium	3.60-25.59	NS	NS
Calcium	11-98	75	200
Magnesium	2.56-53.96	30	100
Fluoride	0.30-1.64	1.0	1.5
Iron	0.120-0.340	0.3	NR
Manganese	<0.001-0.030	0.1	0.3
Copper	<0.001-0.040	0.05	1.5
Zinc	<0.001-0.030	5.0	15
Lead	<0.001-0.020	0.01	NR
Nickel	<0.001-0.030	0.02	NR

NR: No Relaxation; NS: Not specified; Except pH, Electrical conductivity, Turbidity all other parameters units are expressed in mg/L.

be due to the depletion of disinfectants (Nescerecka et al., 2014).

The surface water samples of Uyyalawada (UYY), Sanjamala (SAN), Banaganapalle (BPL) and Mantralayam (MAN) were found to be dominated by *Bacillus subtilis*. Koilkuntla (KOI), Kowthalam (KOW) and Pattikonda (PAT) contain Exiguobacterium sp. Gadivemula (GAD) was rich in Bergeyella sp. Pantoea sp. was found more in Yemmiganur (YMG); Aspari (ASP) was rich in Kocuria marina; Devanakonda (DKD) was dominated by Acinetobacterjunii; and Paracoccus sigandrium was located in Owk as determined by 16S rRNA sequencing mentioned in Table 1.

Groundwater Samples

Fluoride Analysis

Groundwater samples of CHA, DOR, ORV, NDK, PPU, ATK, GON, NDV and KOL exceeded the permissible limit of fluoride (BIS, 2012). Most of the groundwater samples were found beyond the acceptable limit of fluoride. From the results presented in Figure 2, it is found that the fluoride content ranges from 1.08 to 2.06 mg/L, with an average of 1.48 mg/L in groundwater samples. Among 14 groundwater samples, only 06 (42.86%) have the fluoride content within the permissible limit (1.5 mg/L) and 08 (57.14%) samples above permissible limits, as shown in Table 3. The problem is considered to be more acute with the groundwater. Groundwater samples showed high fluoride due to the weathering and leaching of rocks (Rao et al., 2003). Weathering of granite fluorine leached out in the initial stages itself; hence, in granitic areas, even the water at standard depth has a high F-content (Zuane, 1990). In groundwater, fluoride is most elevated for granitic rocks followed by alkali rocks, limestone, dolomite, shale, clay and least for basaltic rocks (Lloyd & Heathcote, 1985). The geology of the district has added a variable quantity of fluoride to

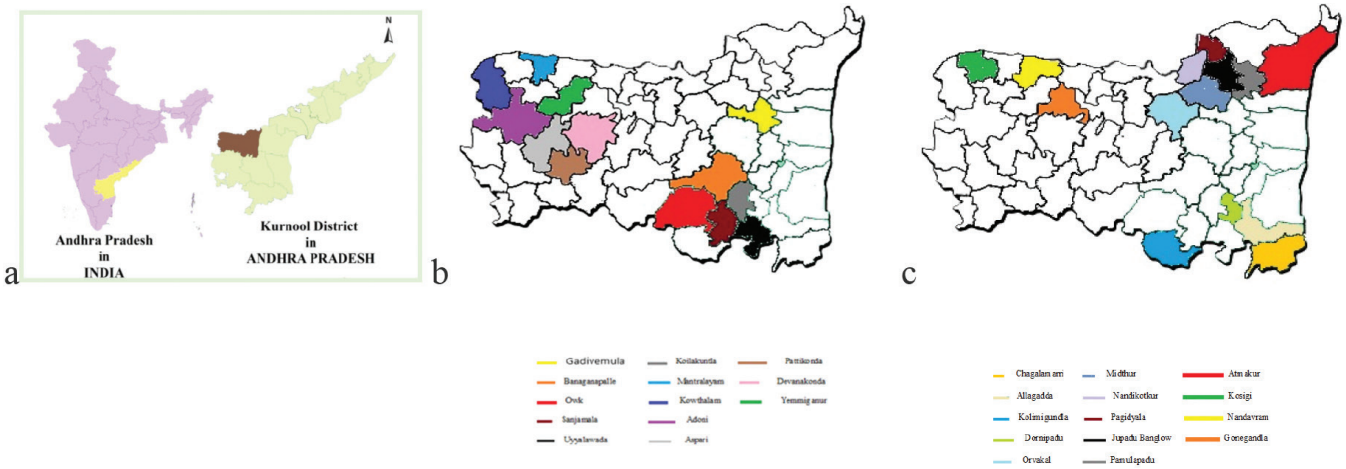

Figure 1: Location map of study area a. Kurnool, Andhra Pradesh, India b. Surface water samples c. Ground water samples.

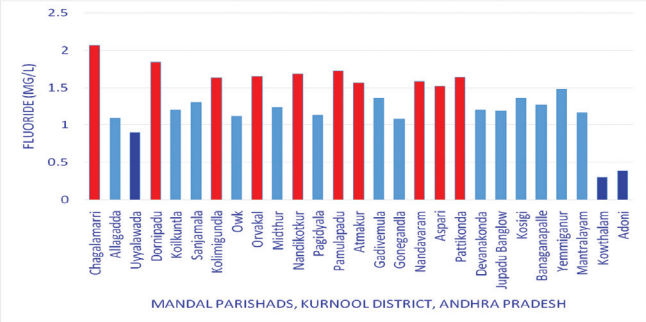

Figure 2: The fluoride concentration of 27 Mandal Parishads, Kurnool district, Andhra Pradesh.

the groundwater, leading to a considerable threat of fluorosis.

Most of the physicochemical parameters of groundwater samples were within the BIS, 2012, and groundwater samples of Dornpadu (DOR), Pamulapadu (PPU), Nandavaram (NDV) and Kosigi (KOS) were found to be dominated by Bacillus subtilis. Chagalamarri (CHA), Midthur (MID), Jupadu Bungalow (JBW) and Gonegandla (GON) were abundant in *Exiguobacterium* sp. Allagadda (ALG) and Atmakur (ATK) have shown abundance in *Acinetobacter* sp. Orvakal (ORV) was found to be dominated by *Chryseobacterium* sp.; Pagidyala (PGA) sample had more concentrations of *Staphylococcus* warneri, and Kolimigundla (KOL) had reported high levels of Aeromonas punctate (Yadav & Pindi, 2018), as mentioned in Table 6.

The opportunistic pathogens and their possible pathogenesis of 1. *Acinetobacter* sp.: Emerging nosocomial pathogens; 2. *Acinetobacter junii*: Bacteremia in preterm infants and pediatric oncologic patients; 3. *Staphylococcus warneri*: Osteomyelitis; 4. *Kocuria* sp.: Bacteremia, Peritonitis; 5. Aeromonas sp: Sepsis, meningitis, cellulitis, ear, nose, throat infections, endocarditis, urinary tract infection, and conjunctivitis.

Bioremediation

The *Coriandrum sativum* leaves biomass was tested with water samples. The sorbent reduced the level of

Table 6: Isolates obtained from ground water

Name of strain(s) (Accession number)	*Similarity with*	*Homology*
Exiguobacterium sp. CHA; MID; GON. MT626714	*Exiguobacterium mexicanum* NR_042424.1	98.47%
Bacillus subtilis DOR; PPU; NDV; KOS. MT626711	*Bacillus subtilis* NR_112686.1	99.66
Exiguibacterium mexicanum JBW. MT626708	*Exiguobacterium aurantiacum* NR_043478.1	98.69%
Staphylococcus warneri PGA. MT626676	*Staphylococcus warneri* NR_025922.1 *Staphylococcus pasteuri* NR_114435.1	97.11% / 97.00%
Acinetobacter sp. ALG; ATK. MT626671	*Acinetobacter modestus* NR_148845.1	97.01 %
Chryseobacterium sp. ORV. MT624338	*Chryseobacterium cucumeris* NR_156145.1	98.81%
Aeromonas caviae	*Aeromonas cavaiae/ Aeromonas tecta* NR118043.1	98.12% / 97.98 %

Table 7: Application of biosorbent in real field water samples

Sample location	*Sample Code*	*Initial Fluoride concentration C_i (mg/L)*	*Final Fluoride concentration C_e (mg/L)*	*Removal efficiency (%)*
Chagalamarri	CHA	2.06	0.93	0.55
Dornipadu	DOR	1.84	0.85	0.54
Orvakal	ORV	1.65	0.73	0.56
Nandikotkur	NDK	1.68	0.75	0.55
Pamulapadu	PPU	1.72	0.76	0.56
Atmakur	ATK	1.56	0.69	0.58
Nandavaram	NDV	1.58	0.70	0.56
Kolimigundla	KOL	1.63	0.72	0.56

fluoride concentration with an average of 42% removal efficiency by using a 0.35 mg dose for 50 ml of sample, pH=6.5 for a contact time of 70 minutes, as shown in Table 7.

Conclusion

The fluoride toxicity was identified to be more acute with the groundwater samples compared to that of surface water samples. The fluoride delineation in the Kurnool district would help the Government to draft and issue health policies. The opportunistic bacteria found may cause diseases in neonates and immune-compromised humans. Hence necessary action is to be taken in these respects; *Coriandrum sativum* can act as a biosorbent for the remediation of fluoride from water.

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