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Phosphate Solubilizing Bacteria (PSB) and their Role in De-fluoridation of Consumption Waters by means of Leaching and as well with Conclusion through Spectrophotometric Evaluate

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Abstract
It is estimated the concentrations of fluoride in groundwaters used for constumption purpose. Samples from different mineral water suppliers (bore well sources) and municipal and/or panchayat drinking water taps were collected and analyzed for fluoride using spectrophotometer. The caliculation of fluoride concentrations against absorption (optical density at 542nm) in consumption waters collected are suitable for human or not, were determined by the standard curve of ammonium fluoride solution ranged from 1 to 10 mM. In addition, the leached fluoride estimation from water was perfomed in small 15 liters plastic buckets using phosphate solubilizing bacteria (PSB). The PSB was rooted in plastic buckets mixing with calcium (white cement) which was painted with a brush on inner surface of the bucket.
Key words: *bio-leaching, phosphate solubilizing bacteria, fluoride, consumption waters*

1. Introduction
The Phosphate Solubilising Bacteria have been isolated for the phosphate-enrichment in green plants and it has been used as a "familiar microbial bio-fertilizer" for the utilization of inorganic phosphate of soil that converted by them as to the form of organic phosphate (Agrawal *et al*., 2014). The example for a mixture of such kind of organisms that have been isolated and characterized as prokaryotic microbial oraganisms and cetain fungi by different laboratories (Agrawal *et al*., 2014; Zhao and Lin., 2001) included for enrichment of phosphate in plants.

Several problems are faced in many parts of our globe due to the presence of high concentration of fluoride in consumption water that cause fluorosis (the lieasons caused in dental and skeletal systems of human populations). In India many states have been affected with fluorosis including Andhra Pradesh and Telengana where several districts with many regions are reported being contaminated with high concentration of fluoride in ground waters (Banerjee, 2015). The halogens cannot be found in nature in their elemental form. They are invariably found as salts of the halide ions (F-, Cl-, Br-, and I-). In addition, after consuming the water, it was also found that fluoride ions *in vitro* do not inhibit homicidic action of antibiotics like amoxicillin and erythromycin on *Staphylococcus aureus* in the blood of human beings (Anderson *et al*., 1986). it was also failed to reproduce these results in same bacteria at the concentrations of fluoride ranged between 1.25 µM and 1.25 mM, greatly below those normally used to stimulate granulocytes that are nothing but immune cells (Gabler and Leong, 1979). It is interesting to note, however, that granulocytes of human lost their phagocytic (cell eating) activity against influence of fluoride at a low fluoride concentration of 1.25 mM (Gabler and Hunter, 1987). It was also found that fluoride ions inhibit phagocytosis and intracellular killing of yeast cells (Gabler and Hunter, 1987).

In the present study, a correlation has been made by using phosphate-solubilizing microbial mixture for the defluoridation purpose from the consumption waters of different places of both mineral water plants and public drinking water supplies of west Krishna district of Andhra Pradesh, India. Since there were no major studies in the past and in the recent past (Kumar *et.al.*, 2012), the present study was carried out to understand the present status of ground water quality in Jaggayyapeta and its surrounding areas near Vijayawada with random water samples of public water supplies and registered mineral water plants of Veerallapadu-521170, Kanchikacherala-521180, Nawabpeta-521185, and Jaggayyapeta-521175, in a total of four rural areas.

2. Materials and Methods
2.1 WATER TESTING FOR FLUORIDE - Using silver nitrate solution
This test was performed in solutions only. Since it does not work for a solid, for the standard fluoride assay ammonium fluoride (obtained from Chemspure, Chennai-600098, India) salt is dissolved in aqua-guard with reverse osmosis purified water. The solution is acidified first by adding dilute nitric acid {it is to be remembered that the addition of 1ml each silver nitrate (0.02N from Finar Chemicals Limited, Ahemdbad-380006, India) + concentrated nitric acid (from Chemspure, Chennai-600098, India, and final concentration maintained is 25%) in a series (i.e., one by one with a gap time between them for addition is 2 to 4 minute) to the 1ml water sample that has to be analyzed}. The principle is that the nitric acid reacts with, and removes, other ions that might also give a confusing precipitate with silver nitrate. The addition of silver nitrate solution is to support '+ve (forming)' or '–ve (not forming)' precipitation. If fluoride (F^- ion) is present means it will be observed no precipitation in the solution. If other halogens like chloride (Cl^- ion), bromide (Br^- ion), and iodide (I^- ion) are there means it will be observed more or less white precipitation. The chloride precipitate is obviously white, but the other two aren't really very different from each other. The precipitates

change colour if they are exposed to light that may turn on grey or purplish tints. Total absence of a precipitate shows that it doesn't have chloride, bromide or iodide ions. The chemistry of the test says that the precipitates are the insoluble silver halides like silver chloride, silver bromide or silver iodide. However, silver fluoride is soluble, and so there will not be precipitation. The addition of 1 ml of concentrated sulphuric acid (from Molychem, Mumbai-400002, India, and final concentration maintained is 25%) has given the variations in the spectrophotometric (Spectrophotometer model 304 used is the made of Electronics India, India-173220) values (Table.1 and Table.2). The addition gap of 2 to 4 minutes is also maintained for H_2SO_4. The observed variations or inconsistent results of optical density as shown in Table.1 and Table.2 by keeping the reaction mixture with prolonged time at room temperature is because of the formation of HF^- (HF^- generally gives the fumes of which are mixed with air and gives the conformation test with the glass silica to form silicon acid, silicon oxy-fluoride, silicon fluoride) (Clark, 2002).

$$F^- + H_2SO_4 \Longrightarrow HSO_4^- + \mathbf{HF}$$

Table 1. Estimation of fluoride from both mineral and public tap consumption waters. The description/conclusions were explained in one of the columns of the table.

S.NO	SOURCE OF WATER FROM VILLAGE/TOWN	OPTICAL DENSITY at 542 nm		DESCRIPTION /CONCLUSION
		MINERAL	TAP	
1	KANCHIIKACHERLA-521180	0.156	0.303	The mineral water has given less precipitation showing the level of fluoride is average to high. However, the public tap water has given maximum precipitation showing the level of fluoride is very less to nil. The reason could be because of chlorination by public supplying authorities which can replace the fluorine. The liberated fluorine forms HF which is volatile at low pHs
2	VEERALLAPADU-521185	0.130	0.430	Here in this town the mineral water content showed average to high level of fluoride. The public tap water got nil with the possible reason which could be because of the chlorination of the water by public supplying authorities.
3	MIET, VIDYA NAGAR, JAGGAYYAPETA-521175	0.210	0.050	The bore well source (the tap water supply from overhead tank) of MIET crude water is having considerable towards high level of fluoride. We are safe because of chlorination of utility water. For consumption we are with aqua guard + reverse osmosis process where the fluoride content in consumption water is weak. In addition the liberated fluorine from water is volatile at low pH in the form of HF.
4	NAWABPET -521170	0.0295	NOT COLLECTED	The mineral water supply is noticed as high level of fluoride content, overcoming the volatile HF at low pH, is also supporting the survey, which says the public of Nawabpet are suffering with little bit muscular symptoms.

Table 2. Estimation of Fluoride from the consumption waters of different sources after leaching of fluoride by PSB which were seeded in paint ($CaCl_2$), the optical density was measured after 24 Hrs. And 48 Hrs. of exposure to PSB for leaching

S.NO	SAMPLE	OPTICAL DENSITY at 542nm		DESCRIPTION/CONCLUSION
		24 Hrs exposure to PSB seeded in white cement	48 Hrs exposure to PSB seeded in white cement	
1	Only water	0.05	0.00	The optical density is absolutely low. It indicated the presence of high concentration of fluoride content which is overcoming the HF volatility at low pH by the addition of nitric and sulphuric acid during the process of estimation.
2	Only water leached by PSB	0.062	0.07	The optical density is absolutely low and a very little bit (low percentage) conformation of leaching by PSB indicating the possibility of fluoride leaching by PSB (microbial) organisms.
3	NH_4F (10mM) leached by PSB	0.05	1.0	To conform in terms of standard chemical compound solution, after the 48 hrs bio-leaching, the indication of precipitation conformed the possibility of control of fluoride concentration by bio-leaching
4	Sample from NAVABPET	0.08	NOT PERFORMED, however, the survey reported us as the public are normal and healthy by consuming tap water.	When compared to average initial concentration of fluoride as 0.0295 there indicated chances of leaching by PSB of mineral water for the controlling the contamination.

2.2 Bioleaching

The main fluoride (in general which may be applicable to all most all of the halide ions) leaching studies by PSB were performed in 15 litres plastic buckets. The small scale studies that are by painting (with the materials mixed with 25 grams of fine commercial calcium powder from a local retail paint merchant + 25 grams of PSB dried powder in mineral water) on the inner surface of the plastic buckets are performed. The permanent fix of the paint on to the inner surface was observed when the paint is mixed with paint commercial gum (obtained from local retail merchant, Nandi bond gum from Lakshmi Surya Chemicals, Piduguralla-522413, A.P., India) (Figure.1)

Figure.1. Paint commercial Calcium powder and commercial gum was mixed with PSB and painted on the inner surface of the buckets. The buckets were loaded with ¾ the volumes of different samples for bioleaching of fluoride where the concentration remained was determined by the spectrophotometric analysis at 542 nm.

The preliminary bacterial standardization of leaching (approximate titration of weight of dried powder of bacterial mixture versus water) was performed with 10 ml water in test tubes. These tubes are containing 10 ml of aqua guard purified bore-well water of MIET, Vidyanagar, Jaggayyapeta-521175 in Krishna District of the Andhra Pradesh state, India. The microbial mixture of 0.25 gr., 0.5 gr., 1 gr., and 2 grams was added to the separate tubes containing aqua guard purified water (along with standard ammonium fluoride solution in the concentration range of 1 mM to 10 mM made in aqua guard purified water as shown in Figure. 2) that used in the leaching by PSB, a mixture around 21 harmless

microbial strains was obtained from Sneha Biotech Laboratory (where the group is marketing this PSB as one of the bio-fertilizers), Vijayawada-520010, India.

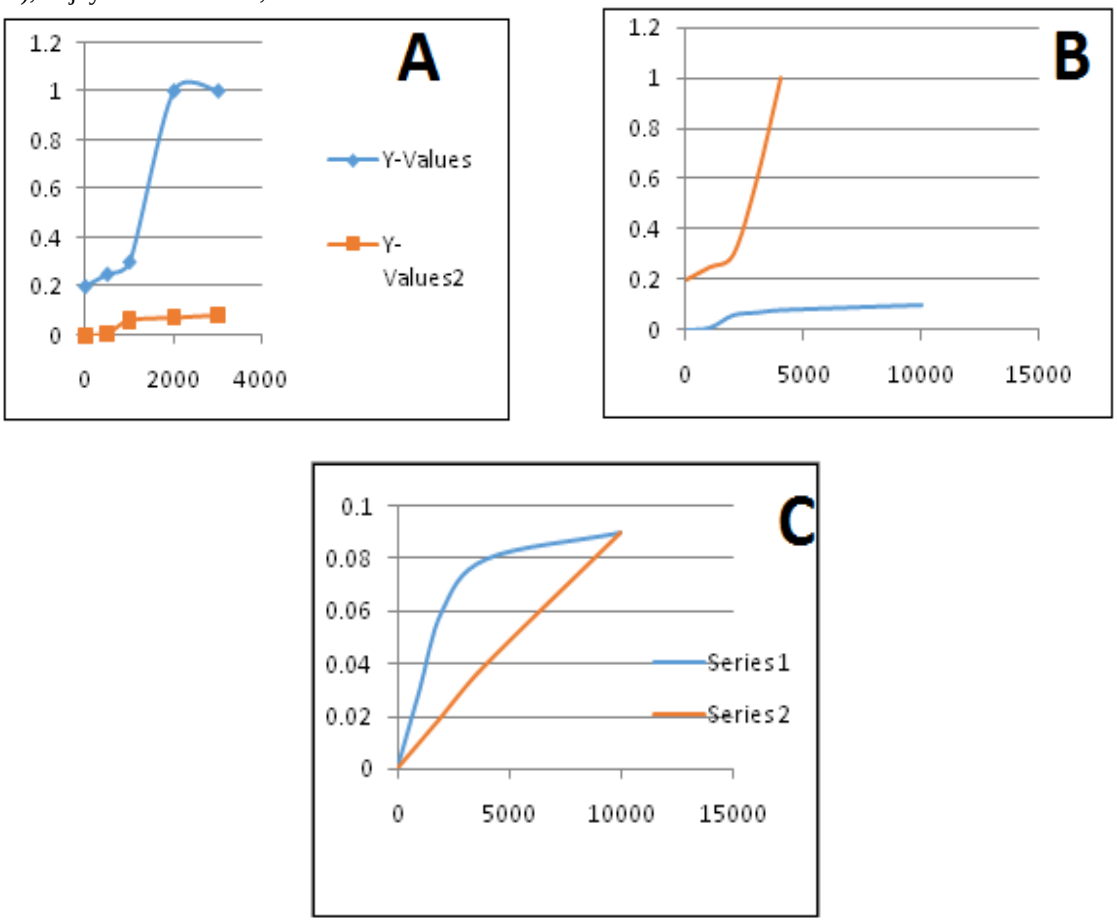

Figure 2. A, B, C are the Standardization experiments for the estimation and leaching of Ammonium fluoride standard solution. 1% Ammonium fluoride solution was prepared diluted to get standards of 1000 micro- grams, 2000 micro-grams,4000 micro-grams, and 10000 micro-grams fluoride as represented on the 'x' axis of figure. 2B and 2C.

Figure 2A shows the initial titre experiment with PSB leaching for the qualitative observation. As the weight concentration of PSB increases the percentage of precipitate formation with rise in optical density shows the fluoride has been leached by PSB. Blue line is O.D. of more precipitate shows less fluoride concentration. Orange line shows least O.D. (lesser than O.1) shows the high concentration of fluoride. The experiment proved that fluoride is leached by PSB. Figure 2B & 2C shows the estimation of fluoride in order to get standard curve. For the sensitivity findings the O.D. was determined at spectrophotometric level of sensitivity factor 1 and sensitivity factor 2 while determining the optical density. The optical density was measured at sensitivity factor 1 (Figure. 2B) and at sensitivity factor 2 (Figure.2C) and plotted the graph.

3. Results and Discussion

In the large scale studies by using the techniques of 'environmental biology for water treatment', the precipitation with fluoride ions is one of the options for the selective removal of calcium (as shown in the following equation) and magnesium that are causing hardness to water (Nanda and Biswal, 2011). One of the eradication of hard substances from the soil by the addition of fluoride ions may bond readily with several alkaline earth-metals by using hydrometallurgical operations resulting in the formation of insoluble species. These precipitates can be separated from the leach liquor and recycled to regenerate reagents or saleable products in hydrometallurgical processes (Sharma, 1990; Booster, van Sandwijk, and Reuter, 2000, 2001).

$$2\ F^-_{(aq)} + Ca\ (NO_3)_{2(aq)} \rightarrow CaF_{2\ (s)} + 2\ NO_3^-{}_{(aq)}$$

$$CaF_2(s) + H_2SO_4(aq) \rightarrow 2\ HF(aq) + CaSO_4(aq)$$

It has also been observed for the removal of fluoride from water by means of chemical processes is by making bonds with other cations and/or also by the several cationic precipitation methods (Nanda and Biswal, 2011). However, several chemical studies have shown that these processes may leave residual fluoride concentrations as high as 1000 mg/L of the earth minerals (Edmunds and Smedley, 1996) that was observed as well in ground waters of Kenya (Nair *et al.*, 1984). In order to avoid the presence of high concentrations of fluoride (in hydrometallurgical studies and also in consumption water suppliers), recently studies have directed for removal of fluoride with the mechanisms of 'bioleaching' by exhibiting toxic effects on the bacteria (Torrisi, 2001). The research now has been dedicated to develop methods for fluoride removal in this process for the consumption waters (Ma *et al.*, 2013; www.epa.gov/research:2014). The main idea behind the experimentations of leaching by PSB mixing with calcium chloride (refined and processed pure commercial calcium paint that is used for painting houses and water tanks) is for the large scale application for public drinking water supply tanks to eradicate the fluoride contamination and to formulate and supply of pure fluoride free drinking water. The final reason is that most of the microbial organisms of PSB used are may be showing the tolerance

of fluoride for their growth metabolism. This fact was also proved at the gene level in microbial organisms by a group of scientists (Ma *et al., 2013*) studying the gene expression and/or the synthesis of gene categories closely related to fluoride tolerance include cell membrane, energy metabolism, transport and binding functions of proteins, DNA metabolism, cell processing, synthesis and transportation of protein and other functional and metabolic pathways.

Generally, the gums are synthesized as synthetic polymers (containing maximum as 'C' and 'H') and/or the natural sources of vegetative plants (e.g., *Acacia* i.e., Gum Arabic from *Acacia* gum bio-polymer is used to preserve microorganisms during sampling). The natural gum products are specifically having carbohydrate source (i.e., Gum Arabic from *Acacia* tree for research generally believed to be a branched bio-polymer of galactose, rhamnose, arabinose etc., and a point to be noted here that the 'gum Arabic' is common name of *Acacia arabica* which is called 'nalla tumma' in telugu) which may help for the metabolic activity of PSB that are seeded in the paint. Of course, the water, carbon, and a little bit nitrogen with exposure of air may help in the metabolic activities of PSB that are located in paint fix.

Fluoride ions are found in minerals such as fluorite (CaF_2) and cryolite (Na_3AlF_6) etc. Fluorine is also used in the manufacture of Teflon, a synthetic polymer, i.e., poly tetra-fluoroethylene (C_2F_4) n, which is used for everything from linings for pots and pans to gaskets that are inert to chemical reactions. So, it is safe to use paint of calcium or emulsion paints seeded with harmless microbes (the best example is probiotic micro-organisms) to tanks and as well as pots for fluoride free potted (drinking) water. In separate experiments it is revealed that uptake of oil emulsion particles in the presence of fluoride were inhibited by human as well as rabbit cultured cells (Bober *et al.* 2000). By considered all the literature links one may come to a conclusion that the painting the public and/or private drinking water supplies tanks with calcium or with oil emulsions which are seeded with microbial organisms utilized for the bioleaching of fluoride. In addition, lot of the bacteria and yeast are used as probiotics. If the mixtures of probiotics are seeded with paints for fluoride leaching that may give relief to the human beings even after a little bit of probiotic microbial organisms contamination is there during the consumption, where low concentrations of fluoride may stimulate the immune system towards the phagocytises.

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