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Toxicity of fluoride to microbial activity and population in soil

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Abstract

The fluoride contamination in the soil imposes a serious impact on the soil characteristics as it affects the microbial activity and diversity in soil owing to its antimicrobial action. The antimicrobial activity of fluoride is mainly mediated by, (1) enzyme inhibition, (2) alteration of proton transfer via cell membranes, and (3) inhibition of biochemical processes in the microorganisms. The main sources of fluoride contamination in the soil are industrial discharge, weathering of rocks, and atmospheric deposition. Soil microorganisms play a very important role in the mineralization process and thus help in nutrient recycling during biogeochemical cycles. The mineralization of organic matter to nutrients by microorganisms is metabolism-dependent so the alteration of the activity of key enzymes by the fluoride changes the microbial diversity of soil. The microbial enzyme activity is highly susceptible to change in environmental factors and is therefore considered the most used parameter for soil pollution studies.

Keywords: fluoride pollution, microflora, enzyme activity, soil fertility, phosphatases, biogeochemical cycles.

1. Introduction

Soil microorganisms are critical in nutrient recycling during biogeochemical cycles[1,2]. The mineralization of organic matter by microorganisms to an inorganic nutrient in the soil is primarily controlled by the soil's physiochemical properties and biological composition. The soil characteristics are key factors for determining plant ecosystem type and the holding capacity of the land for human and animal livelihood. Any factor that alters the characteristics of soil can degrade the soil quality and hence hinder the microorganisms, human, and plant populations[3].

Fluoride is one of the most abundant elements in the natural environment[4]. The natural sources through which fluoride moves into the environment include volcanic eruptions and weathering of fluoride-containing rocks[5,6]. However, anthropogenic sources are majorly responsible for the increased level of fluoride in the environment the sources include, phosphate fertilizers, industrialization, burning of fossil fuels, etc.[7,8]. The increased level of fluoride toxicity in soil imposes a direct threat to the soil system.

Fluorides are usually present in the complex form with minerals or clay and hence immobile in soil. The immobility of fluoride can be useful for groundwater resources but shows a contrasting effect on the soil microbial population[9]. Several studies showed a negative correlation between increased fluoride concentration and microbial activity and population in soil[10, 11]. Tscherko and Kandelar[12] reported an up to 80% decrease in microbial biomass due to severe contamination of fluorine in soil. Also, the decreased microbial population can result in an increased accumulation of organic matter in the soil[13, 14].

The antimicrobial activity of fluoride is mainly responsible for the shift in microbial activity and composition in soil[15, 16]. Fluoride interacts with microbial cells via three modes, namely, enzyme inhibition, alteration in proton transfer through the cell membrane, and inhibiting of several important biochemical pathways of microbes[17]. Wilke[14] reported the inhibition of enzymes alkaline phosphatases and arylsulfatase as well as the nitrification process in soil contaminated with fluoride. Similarly, Reddy and Kaur[18] and Yadu *et al*.[19] showed a negative impact of fluoride on the activity of enzymes soil peroxidases, and ATPase. Rao and Pal[13] evaluated that the concentration of fluoride ranging from 380-1803 mg/g soil can inhibit microbial growth and the organic matter decomposition process. Later, Cronin *et al*.[20] reported that a fluoride concentration below 200 mg/g can cause inhibition of soil respiration and dehydrogenase activity, and a concentration ranging from 200-2000 mg/g can inhibit the denitrification process in soil.

3. Distribution of Fluoride in Soil

In soil, more than 90% of fluoride in insoluble forms occurs in the form of mineral complexes or adsorbed to soil particles, and only a few percent are in dissolved form in the soil solution[21]. About 330 ppm of fluorine is present in soil which varies from 150-400 ppm depending upon the location of the soil. However, for contaminated soils, the value ranges between 1000-3500 ppm. A higher level of fluoride is usually reported in soil treated with phosphate fertilizers, or soil in the vicinity of fluoride-emitting industries, coal-fired power plants, and hazardous waste sites[22].

4. Sources of Fluoride in Soil

The sources of fluoride contamination in the soil can be broadly categorized into two groups namely, natural, and anthropogenic sources. Both sources contribute extensively to fluoride contamination in the air, water, soil, flora, and fauna. The natural sources include weathering of fluoride-containing minerals (e.g., Cryolite, fluorapatite, fluorspar), volcanic eruptions, and fluoride-rich agricultural residues (grasses and forages). Anthropogenic fluoride contamination includes human activities such as industrialization, motorization, fluoride-containing pesticides, and fertilizers, and fluoridation of drinking water, dental products, and fire extinguishers[23].

5. Microorganism-Soil Interactions

Soil is a complex ecosystem containing a diverse range of bacteria, fungi, protists, and animals forming <1% of the total mass of soil[24]. The microorganisms generally reside in pores between soil particles and are sometimes associated with plants. The availability of water and the exchange of gases in pore space, make it an ideal habitat for the diverse range of microorganisms [25].

In a natural ecosystem, these soil microorganisms have a key role in several biogeochemical cycles[26]. They help by transferring and recycling nutrients between several reservoirs through the mineralization process. During the mineralization process, the organic nutrients are converted into inorganic forms such as ammonium, nitrate, phosphate, and sulfate that can be easily utilized by plants[24].

Almost every chemical transformation reaction that occurs in soil requires the active involvement of microorganisms[27]. The chemical transformation of soil is a metabolism-dependent process and hence involves various enzymes that play a key role in this transformation[28, 29]. The activity of microorganisms is largely dependent on the soil's physical properties, and ecological interactions[30].

Thus, the change in the properties of soil due to pollution may cause a shift in the microbial population.

6. Effect of Fluoride on Soil Microorganisms

Exposure of contaminants to soil microbial communities led to variations in their abundance, diversity, and activities[31]. The community structure and activity of soil microbes are significantly correlated with soil fertility and ecosystem productivity[32]. Fluoride has an adverse effect on the function of the microbial communities in soil. The fluoride can alter the microbial community in the following ways, (1) Enzyme Inhibition: binds to the active site and inhibits enzyme activity, (2) Acting as Phosphate analogs (aluminofluoride or beryllium fluoride complexes): alters biochemical pathways involved in signal transduction and cell growth, (3) inhibition of biochemical processes in the microorganisms (glycolytic cycle): proton transfer alteration (Figure 1).

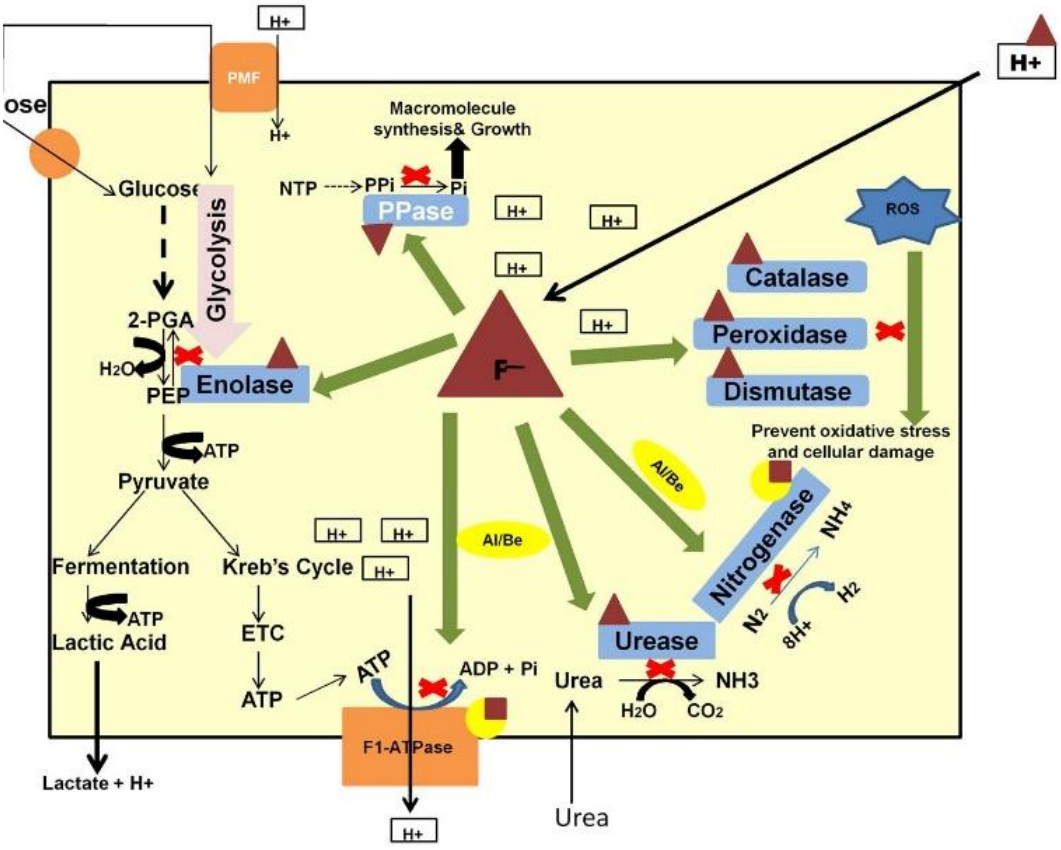

Figure 1: Effect of fluoride on the microbial cell

7. Enzyme inhibition

The fluoride in F^- or HF can also directly bind to the active site of enzymes or proteins and inhibit their activity. In general, F^- would substitute OH^- groups on the active site of the enzymes and thus can be utilized to understand the role of the hydroxyl group or water molecules in the catalytic mechanism of an enzyme[15]. The inhibition of enzyme activity by fluoride has been reported to affect one or more physiological activities in microorganisms.

Warburg and Christian[33] first reported the inhibitory action of fluoride on the glycolysis pathways of microbial cells. The inhibition of this pathway is associated with the inhibition of enzyme enolase[34, 35]. In the glycolysis pathway,

the enzyme enolase causes the conversion of 2-phosphoglycerate (PGA) into phosphoenolpyruvate (PEP). It contains two Mg^{2+} ions, one at each subunit (dimeric enzyme). Qin *et al*.[36] reported that the formation of the Pi–F_2–Mg_2 complex is responsible for enzyme inhibition and consequently leads to inhibition of the glycolysis pathway. The fluoride alone at low concentration can cause inhibition of enzymes and does not require complexation with metal (Al or Be). Kustrzeba-Wojcicka and Golczak[37] used the enzyme enolase extracted from the fungus *Candida albicans* and studied the inhibitory effect of fluoride on it. They have demonstrated that the addition of phosphate in assay mixture results in competitive inhibition whereas noncompetitive inhibition occurs when no phosphate was added. Since fluoride-inhibited enolase can be reactivated by an excess amount of substrate 2-phosphoglycerate thus described as quasi-irreversible inhibition[38]. Additionally, the inhibition of glycolytic enzymes (e.g., enolase) in microbial cells occurs by two modes, i.e., direct binding of fluoride to the enolase and secondly by fluoride-mediated acidification of cytoplasm in acidic conditions thereby inhibiting the glycolytic enzymes. However, later contributes more to the fluoride inhibition of enolase than binding directly to the active site of the enzyme[15].

Todd and Hausinger[39] reported a similarity in fluoride inhibition of enolase and urease as in both cases inhibitions increased with time causing a complete loss of enzymatic activity. Similarly, as enolase, it is also a metalloenzyme containing two nickels in the active site of enzymes (Ni-1 and Ni-2). They proposed that fluoride bind primarily to the activated complex to form a urease-substrate-F complex or urease-carbamate-F complex. Also, the inhibition is pH-dependent and increases in acidic conditions.

Several reports suggest the inhibition of heme-based peroxidases and catalases in a pH-sensitive manner and tend to increase in an acidic environment[15, 40]. Both enzymes are necessary for microorganisms to provide defence against oxidative damage. Phan *et al*.[41] reported that the fluoride-induced inhibition of catalase influences the adaptation of bacteria in acidic conditions due to oxidative damage. Similarly, Mn/Fe superoxide dismutases and copper-based enzymes such as galactose oxidase or Cu/Zn superoxide dismutases have been reported to show fluoride inhibition[42, 43].

Chen *et al*.[44] studied the fluoride inhibition of aminopeptidases of *Aeromonas proteolytica* and reported non-competitive inhibition occurs at a pH range of 6-9. A similar result was obtained with the Zn-activated aminopeptidase of *Streptomyces griseus*. Moreover, it was found that fluoride inhibition involves binding with water molecules in the active site of enzymes[45].

For the evaluation of soil health, the measurement of phosphatase activity in soil is needed. Fluoride pollution tends to reduce the phosphatase activity of soil thereby inhibiting the enzyme activity. Phosphatase enzymes have been categorized into two groups, group I phosphatases (acid phosphatases, bacterial alkaline phosphatases, and protein tyrosine phosphatases) contain nucleophiles at the active site (His, Ser, and Cys, respectively) and involve intermediate state formation. Moreover, the mechanism of action of enzyme alkaline phosphatases involves metal cofactors whereas absent in acid phosphatase and tyrosine phosphatases. Group II phosphatase (protein phosphatases and purple acid phosphatases) involves a direct attack of water molecules without intermediate formation. The group II phosphatases contain metallic centers and are inhibited uncompetitively by fluoride. However, several studies have shown the inhibitory effect of fluoride on the phosphatases without metal cofactors but the mechanism of inhibition is still unknown[46, 47].

8. Acting as phosphate analogs

Fluoride tends to form strong complexes with metals such as Al (Aluminium) and Be (Beryllium) which can imitate phosphate groups and eventually cause inhibition of several phosphate-transferring enzymes for example phosphohydrolases. GTPases phosphatases, and

ATPases. The role of these metals as modulators of enzyme activities was first reported by Sternweis and Gilman[48]. They observed that the presence of a trace amount of aluminium in the fluoride salts was mainly responsible for the activation of the enzyme adenylate cyclase which has key regulatory roles in all cells.

Due to the resemblance of AlF_4^- and $BeF_3^-.H_2O$ complexes with phosphate molecules (bond length of P-O, Be-F, and Al-F ≈ 1.55Å; both contain electronegative atoms, F and O can exhibit hydrogen bonding) can easily make a way into several metabolic pathways and function as phosphate analogs and can modulate the activity of a range of phosphoryl transfer enzymes[49]. These enzymes have key roles in several fundamental biochemical pathways needed for signalling energy transduction and regulation of cell growth. The effect of fluoride on the F1-ATPases of microorganisms has been known for several years, but the actual reason behind this came into light after the discovery of Sternweis and Gilman[48] which states that the inhibition of F1-ATPase is due to micromolar concentration of aluminum in fluoride salts. Similarly, the report suggests that the activity of F1-ATPases on both mitochondrial and bacterial membranes has been inhibited by the micromolar concentration of the aluminium fluoride complex together with ADP. Also, the beryllium ions showed the same inhibitory effect as aluminium ions. Further, proposed that because of structural similarities between AlF_4^- and PO_4^{3-}, AlF_4^- would mimic γ- phosphate of ATP binding to the active site and form a fluoro aluminate-ADP-F1 complex, subsequently imitating the intermediate of catalytic cycle operating in F1 particle[50].

Braig *et al.* [51] used an X-ray crystallography technique for the structural analysis of a complex that mimics the transition state of the enzyme. The structural analysis of bovine mitochondrial F1 ATPase inhibited by the complex (Mg^{2+}ADP and aluminium trifluoride) has established that aluminium fluoride binds instead of γ- phosphate of ATP. Also, the study showed that the Mg^{2+}ADP-AlF_3 complex is a transition state analog. Further study showed that out of three active sites of F1-ATPase enzyme from bovine mitochondria, two were occupied by ADP-alumino-fluoride and a third by ADP and sulfate. This complex containing all bounded active sites resembles the stage at which energy transfer primarily occurs during ATP synthesis[52]. Clarke *et al.* [53] reported that the complex of BeF_3 and MgADP inhibits the activity of enzyme nitrogenases of the bacteria *Klebsiella pneumonia*. The inhibitory complex contains two BeF_3 ions bound to each Mo-Fe protein and the major complex also involved the Fe protein. However, this is a completely reversible type of inhibition but occurs at a slow rate.

Besides, these metal complexes (Al or Be) have been widely used as a tool to study enzymology and regulatory physiology to understand the molecular mechanism of action of enzymes as well as entrap the regulatory proteins in their active state and evaluate their structure and function[15, 54]. Datta *et al.* [55] have determined the structure of the Rec A protein from *Mycobacterium tuberculosis* by using the ADP-AlF4 complex. This complex acts as an analog and interacts with the ATP binding site contained in the P-loop of the RecA protein. Similarly, Cho *et al.* [56] used BeF3 to study the structure of CheY (Chemotaxis protein) in an active state through NMR (nuclear magnetic resonance) technique. It is involved in the transmission of sensory signals membrane chemoreceptor to the flagellar motor and helps in flagellar rotation. The active form of CheY is Phosphorylated at aspartate-57 but exists in this form for a short period of time. The beryllofluoride is used to induce a persistent activation state and thus allows detailed structural analysis of CheY in its active form.

a. Alteration in proton transport across membranes

Being smaller in size HF can easily move through water channels (aquaporins) present in biological membranes[15]. HF act as a weak acid in dilute

solution whereas strong acid in concentrated solution[57]. Several reports have demonstrated the role of HF in proton transport across the membranes of bacterial cells and thus reducing the pH gradient (ΔpH) across the cell membrane[39, 58]. Sutton *et al.* [59] demonstrated the permeability coefficient of HF and F^- using an artificial membrane and found that HF showed nearly 10^7 times higher value than later. Thus, predominately fluoride in the form of HF in an acidic environment can move across the cell. Inside the cell cytoplasm having higher pH causes HF to further dissociate into H^+ resulting in cytoplasm acidification and decreasing the electrochemical potential across the membrane, and F^- acts as an enzyme inhibitor. The functioning of HF in an acidic environment and F-ATPase is contradictory, as the former transports protons into the cells whereas the converse is true for later. Thus, HF acts as a decoupler of oxidative phosphorylation[15, 60].

However, F^- (fluoride ion) is not capable of suppressing the F-ATPase enzyme activity completely. The optimum pH for the enzyme of low acid-tolerant (*S. sanguis*) and high acid-tolerant (*L. casei*) organisms has been reported as >7 and 5.5 respectively[60].

The inhibitory action of fluoride on biochemical pathways such as glycolysis occurring in microorganisms is highly pH-dependent. For complete inhibition of glycolysis, a concentration of fluoride above 10mM is required at neutral pH, whereas at pH 4 just micromoles of fluoride are sufficient. The increase in proton permeability of bacterial cells due to fluoride is approximately proportional to the ability of fluoride to decrease the acid tolerance of glycolyzing cells[15, 61]. This increase in permeability can subsequently cause an increase in demand for ATP to transfer the proton out of the cell via F-ATPase and thus results in lower growth efficiency of the microbial cells. For example, bacteria, *S. mutans* (GS-5) cultured on glucose limited-media in a chemostat at different pH have demonstrated variation in growth efficiency with pH. The microbial culture at pH 7 has shown no effect on microbial growth with 0.1 mM of NaF. However, with a decrease in growth pH, the efficiency of fluoride in reducing the growth yield of the microbial cells has increased gradually. The microbial culture containing 0.1 mM NaF was flushed out of the reactor at a pH value of 5.8. This decline in growth yield occurred as ATP formed during glycolysis was utilized to maintain ΔpH across the membrane and became unavailable for the growth processes. Also, at a pH value of 5.8, unfluoridated cultures have shown about a one-third reduction in growth yield and complete wash-out from chemostat at a pH value below five[15].

Sensitization to acidification can influence various systems in microorganisms such as physiology and their capacity to adapt to an acidic environment. For example, fluoride-induced inhibition of alkali production from arginine in acidic conditions[62] or of respiration[63]. The inhibition of alkali production further enhances sensitization to the acidification of cells and thus weakens their adaptation capacities. However, the inhibition of respiration in microbial cells would decrease the oxidative stress in the cell. Phan *et al.* [63] reported that sensitization to acidification can result also in the death of bacteria. Furthermore, at lethal low pH values, the ΔpH across the cell membranes is maintained to some extent but can be declined by fluoride.

Fluoride (NaF) has been reported as a powerful inhibitor of solute-transport systems in the cytoplasm of microbial cells. They generally interfere with the energy transduction in the transport process by decreasing the pH gradient across the membrane or by providing less ATP than needed as most of ATP is utilized to fulfill the increased demand for movement of proton out of the cell. However, this inhibition is a reversible type and is not fatal in short-term exposure but in the long term, it can be lethal[64].

Besides, fluoride also tends to inhibit the synthesis and export of macromolecules across microbial membranes. This effect of fluoride might be correlated with the ability to reduce the pH gradient across the membrane which in turn may induce activation of various hydrolytic enzymes

(e.g., autolysin) of cell membranes and cell walls as well as may cause inhibition of export protein[65, 66]. Also, the fluoride-metal complexes have been reported to affect export protein because of their inhibitory action on the ATPase component of transport protein (e.g., ABC transport systems) [48].

9. Conclusion

Fluoride contamination in soil plays an important role in the modulation of microbial activity and population due to its antimicrobial activity. As soil microorganism has a very critical role in the recycling of nutrients through mineralization, the alteration in microbial biomass results in decreased decomposition of organic matter and ultimately loss in soil fertility. Moreover, soil enzymes are closely related to soil physical properties and microbial activities or biomass. Soil enzymes (phosphatases, urease, dehydrogenase, etc.) have great potential in assessing the health of soil biota. The fluorides negatively impact the activity of these enzymes and thus reduce soil health. Further, soil health deterioration negatively impacts animal, human, and plant health as the surrounding (air, surface water, groundwater) gets adversely affected by contaminated and mismanaged soil.

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Conflicts of interest

Not Applicable.

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