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RESEARCH ARTICLE

EFFECT OF FLUORIDE CONTAMINATION ON MICROBIAL ENZYMATIC ACTIVITY IN SOIL

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Abstract

The accumulation of environmental contaminant such as fluoride in wetlands especially in rice fields is increasing drastically by the action of fertilizers, pesticides, mining, waste disposal, and atmospheric pollution of which Kuttanad, the unique tropical wetland agro ecosystem is not an exception. Accumulation of excess F^- in the environment poses serious burden to all organisms. Since soil microbial enzymes respond more quickly to various environmental conditions and soil management practices than any other soil quality parameters and thus used to detect early the changes in the soil health. In view of this, the activities of various enzymes involved in the biogeochemical cycles such as urease, protease, dehydrogenase, arylsulphatase, acid and alkaline phosphatase were investigated. Result showed thatF^- had an inhibitory effect on the activity of all the enzymes studied possibly due to the adverse effect of F^- on microorganisms.

Introduction:-

Fluorine, derived from Latin word "fluere" meaning "to flow" is a naturally occurring and abundant element in the halogen family. Fluorine is highly reactive and most electronegative of all chemical elements (Greenwood and Earnshaw, 1984; Gillespie et al., 1989) and is therefore never encountered in nature in the elemental form. It is commonly found in its ionized form, fluoride (F^-) or reacted with another element. Fluoride accounts for about 0.06 to 0.09 per cent of the Earth's crust (Koritnig, 1951).

Major sources of F^- are mostly natural, i.e., F^- containing mineral rock like fluorspar or fluorite (CaF_2) cryolite (Na_3AlF_6), fluorapatite ($Ca_5(PO_4)_3F$) villiaumite (NaF) and topaz ($Al_2(SiO_4)F_2$). Fluoride containing rocks are considered as the reservoir of F^- (WHO, 1984). Anthropogenic activities also increase the fluoride level in soil which include application of phosphate fertilizers, plant protection chemicals and pesticides. Soils exposed to large emission of fluoride tend to accumulate it, which eventually has an adverse effect on agricultural production.

Most studies have reported that higher concentration of F^- in the soil poses serious threat to soil microorganisms which in turn causes inhibition of some of the soil enzymes (Nowak et al., 2005; Evdokimova and Korneykova, 2010); while few studies contrasts these findings that the enzyme activity increased in response to certain dose/level of F^- availability (Telesiński et al., 2008; Smolik et al., 2009). Marquis et al. (2003) showed that the activity of catalase and urease in soil was inhibited by increasing levels of F^-. Similar inhibition in the activity of soil protease (Garcia-Gil et al., 2013), urease (Pati and Sahu, 1998; Langer and Günther, 2001), dehydrogenase (Sinclair et al., 1997; Pati and Sahu, 1998; Langer and Günther, 2001), arylsulphatase (Tscherko and Kandeler, 1997; Langer and

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Günther, 2001) and phosphatases (Walker, 2010; Poulsen, 2011; Nowak et al., 2005) consequent to increase in soil F^- has been reported.

Analysis of the enzymatic activity of soil is one of the most popular 'soil fertility indicators' that support soil quality assessment (Klikocka et al.,2012). Thus, measurement of soil enzyme activities may provide a useful index of ecosystem status (Dick, 1992; Klikocka et al., 2012; Utobo and Tewari, 2013). Protease has been considered as a sensitive indicator of soil contamination and/or improvement in Andosols (Shahriari et al., 2010) Banu et al. (2010) demonstrated the use of protease enzyme as an indicator of contamination in soils polluted with dairy waste water. Activity of dehydrogenase enzyme was made use in the assessment of contamination of the environment with herbicides (Kucharski et al., 2009) and petroleum products (Kaczyńska et al., 2015). The potential use of enzymes such as dehydrogenase, phosphatases and urease as indicators of soil pollution by pesticides has been demonstrated by Cycoń et al. (2005). Activity of arylsulphatase was used to monitor soil contamination with polycyclic aromatic hydrocarbons (Lipińska et al., 2014). In view of this, the activities of various enzymes involved in the biogeochemical cycles were investigated

Materials And Methods:-

Site description

There are some places which unlike others are filled with ambience in their own.Truly enjoying their beauty is another thing.One such destination is Kuttanad. It is a unique low lying wetland nick named as "Rice Bowls of Kerala", contribute nearly 20% of the total rice production of the Kerala state of India. In Kuttanad, water is the main source of F^- contamination. The high F^- concentration in Kuttanad water samples could be due to the dissolution of fluorapatite which is a common mineral in the Tertiary sediments of the area (Raj and Shaji, 2017) or because of the interaction of the fertilizers, pesticides and other chemicals related with agricultural activities (Annadurai et al., 2014).

Soil collection

15 locations of Kuttanad rice fields were selected for soil sampling during the fallow period. Samples were withdrawn from a depth of 20 cm from the surface layer using a soil auger. For studying microbial enzyme activities, soils from three locations which had a low, medium and high F- concentration (Roshni and Harikumar, 2021) were chosen.Five replicate samples (ca 500 g) taken from each location were collected, air-dried and stored at 4^0C till analysis.

Assay Of Soil Enzymes

Dehydrogenase

Dehydrogenase activity in soil was determined following the method ofCasida et al. (1964) by reduction of 2, 3, 5-triphenyl tetrazolium chloride (TTC). Soil sample (5 g) was treated with $CaCO_3$ (50 mg), 3% (w/v) 2, 3, 5-triphenyltetrazolium chloride (1ml) and incubated for 24 h at 37^0C. The triphenylformazan formed was extracted from the reaction mixture with methanol and assayed at 485 nm in a Shimadzu UV-1207 UV-Vis spectrophotometer

Urease

Urease activity in soil was assayed by the method of Kandeler and Gerber (1988). Briefly, 5 g soil were placed in a 100 ml Erlenmeyer flask and wetted with 2.5 ml 0.08 M aqueous urea solution and incubated at 37^0C. After 2 h 50 ml of 1N KCl to 0.01N HCl were added to the mixture and shaken for 30 min. The resulting suspensions were filtered. The filtrate (1 ml) was diluted to 10 ml with distilled water and successively, 5 ml Na salicylate (100 ml 0.12% Na nitropursside, 100 ml 17% Na salicylate, 100 ml distilled water) and 2 ml 0.1% Na dichlorisocyanuriate were added. The OD was determined at 690 nm after 30 min incubation at room temperature.

Protease

Protease activity was determined by the method of Ladd and Butler (1972) as modified by Kandeler et al. (1999). One g of soil was incubated with 10 ml of a buffered (Tris buffer 0.2 M, pH 8.1) sodium caseinate solution (2% w/v in Tris) for 2 h at 50^0C in a shaking water bath. The tyrosine formed was extracted with 0.92 M trichloroacetic acid and measured spectrophotometrically with Folin-Ciocalteu reagent at 700 nm.

Phosphatases

Acid phosphatase

Acid phosphomonoesteraseactivity was assayed by mixing 1 g soil with 4 ml of 0.1 M Modified Universal Buffer (MUB) (pH 6.5), 0.25 ml toluene and 1 ml of 25 mMp-nitrophenylphosphate solution. After incubation of 1 h at 37^0C, the enzyme reaction was stopped by adding 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH. After shaking the mixture for a few seconds, the suspension was filtered through Whatman No 2 filter paper. The yellow colour intensity of the filtrate was measured spectrophotometrically at 420 nm (Tabatabai and Bremner, 1969; Tabatabai, 1994).

Alkaline phosphatase

For the assay of alkaline phosphomonoesterase, the procedure was the same as the one used for acid phosphatase, but using MUB (pH 11) instead of MUB (pH 6.5).

Arylsulphatase

The soil arylsulphatase activity was determined by the method of Tabatabai and Bremner (1970). Briefly, 1 g air-dried soil was mixed with 4 ml of 0.5 M acetate buffer, 0.25 ml toluene and 1 ml of 0.05 M p-nitrophenylsulphate solution. Samples were shaken and incubated at 37^0C for 1 h. After adding 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH, the concentration of the formed yellow nitrophenol was determined from the absorbance at 400 nm.

Statistical analysis

Analysis of variance (ANOVA) and multivariate ANOVA (MANOVA) were used to determine the effect of each treatment and the interaction between them. If the ANOVA/MANOVA detected significant differences, means separation was accomplished through the Tukey's Honestly Significant Difference (HSD) test at $P<0.05$. Pearson two tailed correlation analysis was performed to study the relationship between the different variables studied.

Result And Discussion:-

Protease activity in soil was significantly ($P<0.05$) inhibited by increasing F^- concentration which decreased to 93% in high F^- soils. Same was the case with soil urease as well with a low activity in soils of high F^- concentration. However, the difference in activity of this enzyme between low and high F^- is narrow (Table 1).

Table 1:- Protease and urease activity as influenced by soil F.

Location	F concentration	Soil Protease (μ_g tyrosine g^{-1} soil h^{-1})	Soil Urease (μ_g N g^{-1} soil h^{-1})
Thalavady	Low	1.14 ± 0.03^b	12.45 ± 0.26^b
Mankompu	Medium	0.12 ± 0.01^a	11.41 ± 0.36^a
Muttar	High	0.08 ± 0.01^a	9.87 ± 0.20^a
Mean values within the column followed by the same letter(s) are not significantly different at $P<0.05$ according to Tukey's HSD			

In general, the activity of soluble phosphatases in soil showed a higher value in low F^- soil which was found to be negatively affected by high levels of F^- in soil (Table 2). Acid phosphatase activity was inhibited by 14.28% and 28.57% in medium and high levels of F^- respectively. Whereas, it was 48% and 66% in the case of alkaline phosphatase.

Table 2:- Soluble phosphatase (acid and alkaline) activity as influenced by soil F.

Location	F concentration	Phosphatase	
		Acid (μ_g p-NP g^{-1} soil h^{-1})	Alkaline (μ_g p-NP g^{-1} soil h^{-1})
Thalavady	Low	0.35 ± 0.01^b	0.48 ± 0.04^b
Mankompu	Medium	0.30 ± 0.03^{ab}	0.25 ± 0.01^a
Muttar	High	0.25 ± 0.02^a	0.16 ± 0.01^a
Mean values within the column followed by the same letter(s) are not significantly different at $P<0.05$ according to Tukey's HSD			

The activity of arylsulphatase in relation to soil F^- concentration is presented in table 3. The activity of the enzyme significantly varied with F^- levels which was relatively high in low F^- soil followed by medium F^- soil. The lowest value was noted in soils of high F^-.

Table 3:- Aryl sulphatase activity as influenced by soil F.

Location	F concentration	Aryl sulphatase (μ_g p-NP g^{-1} soil h^{-1})
Thalavady	Low	6.27 ± 0.18^b
Mankompu	Medium	4.22 ± 0.13^a
Muttar	High	3.76 ± 0.27^a
Mean values within the column followed by the same letter(s) are not significantly different at P<0.05 according to Tukey's HSD		

Dehydrogenase activity was significantly (P<0.05) high in low F^-soil compared to other soil F^- levels (Table 4). The activity of the enzyme decreased to 46.04% in medium F^- soil. Maximum decrease of 67.15% was noted in high F^- soil.

Table 4:- Soil dehydrogenase activity as influenced by soil F.

Location	F concentration	Soil Dehydrogenase (μ_g TPF g^{-1} soil h^{-1})
Thalavady	Low	2.71 ± 0.09^c
Mankompu	Medium	1.43 ± 0.14^b
Muttar	High	0.89 ± 0.07^a
Mean values within the column followed by the same letter(s) are not significantly different at P<0.05 according to Tukey's HSD		

It is clear from the corresponding slope of regression analysis that the activity of these enzymes was negatively affected by increasing concentration of F^- in soil (Fig. 1).

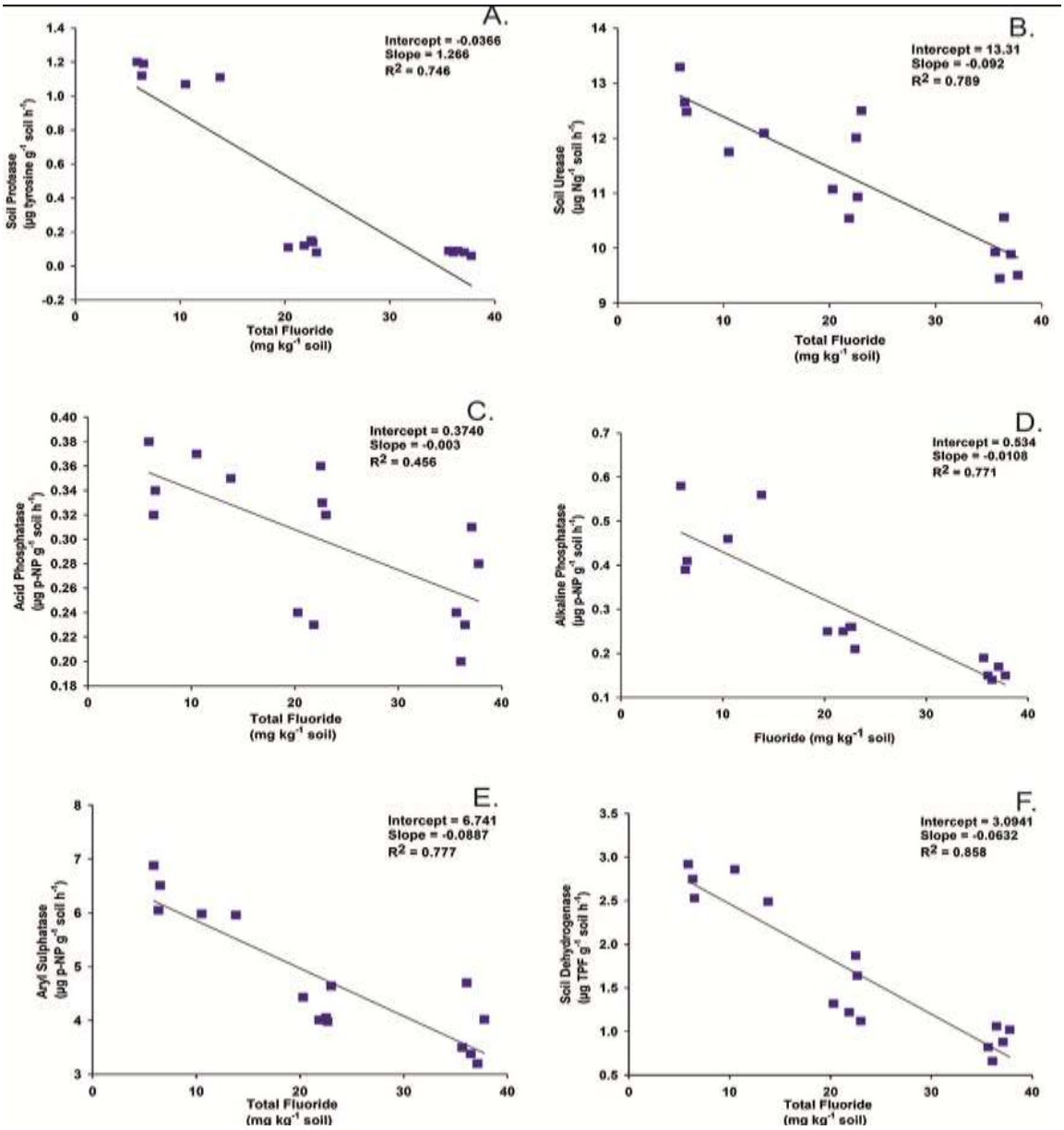

Fig. 1:- Relationship between soil enzyme activity and F.

There is contradictory opinion regarding the activity of soil enzymes with increasing F^- concentration in soil. For example, Telesiński et al. (2008) observed an increased soil enzyme activity in response to certain dose/level of F^- availability. Inhibition of soil enzymes with increasing F^- concentration has been reported elsewhere by other scientists (Langer and Günther 2001; Poulsen 2011; Garcia-Gil et al., 2013). However, in the present study, F^- had an inhibitory effect on the activity of all the enzymes studied possibly due to the adverse effect of F^- on microorganisms. However, among the enzymes, protease, alkaline phosphatase and dehydrogenase were most affected indicating the impediment in the hydrolysis of protein components, release of inorganic P and oxidation of organic matter in F^- affected soils of Kuttanad.

Conclusion:-

There is contradictory opinion regarding the activity of soil enzymes with increasing F^- concentration in soil.In the present study, F^- had an inhibitory effect on the activity of all the enzymes studied possibly due to the adverse effect of F^- on microorganisms. However, among the enzymes, protease, alkaline phosphatase and dehydrogenase were most affected.

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