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# Soil microbial activity, yield and grain quality of rice (*Oryza sativa* L.) as influenced by graded doses of fluoride in Alfisols

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## ABSTRACT

*A field experiment was conducted at Regional Research and Technology Transfer Station (RRTTS),Chiplima, OUAT, during 2014-15 and 2015-16 to study the impact of graded doses of fluoride on soil microbial activity, yield and grain quality of rice. Six doses of fluoride i.e., No fluoride, 100, 150, 200, 250 and 300 ppm F in form of hydrofluoric acid (HF) were applied as foliar spray. Highest grain (36.73 q/ha) and straw (47.34 q/ha) yield of rice was recorded with $T_1$ (No fluoride) where as lowest grain (32.90 q/ha) and straw (40.09 q/ha) yield was observed with $T_6$ (foliar spray of F @ 300 ppm as HF). The grain yield was decreased significantly with increasing doses of fluoride. The decrease in grain yield was highest (10.42 %) with $T_6$ (foliar spray of F @ 300 ppm as HF) over control ($T_1$ = No fluoride) where a lowest value of 3.02 % was observed Significantly highest total chlorophyll content (4.53 mg/ g fresh leaf) was observed with $T_1$ which was decreased to lowest value of 2.26 mg/ g fresh leaf ($T_6$) due to increasing doses of fluoride. The lowest value with respect to leaf area index (4.18), grain length (4.38 mm), kernel elongation (6.18 mm), plant height (123.78 cm ) and panicle length (22.28 cm ) was recorded with $T_6$ (foliar spray of F @ 300 ppm as HF). Howeverhighest F uptake (24.71 kg/ha) was recorded with foliar spray of F @ 300 ppm as HF ($T_6$).The variation in grain quality parameters such as protein content ($R^2$=0.982), amylose content ($R^2$=0.981) and alkali value ($R^2$=0.839) were significant due to increase in F doses. Soil microbial biomass carbon (MBC), soil dehydrogenase activity (DHA) and soil bacterial population were decreased with increased doses of F irrespective of crop growth stages.*

***Key words:*** *Fluoride, rice yield, grain quality, MBC, dehydrogenase, soil bacterial population*

## INTRODUCTION

Fluoride (F) is not an essential plant nutrient element, but it interferes with the plant metabolism for which plant undergoes some physiological changes leading to chlorosis, necrosis and leaf burning as well as decline in yield. In soil environment F concentration more than the threshold limitalters microbial activity (Qin et al., 2006). F is a necessary element for human health with lower concentrations. Excessive F intake through drinking water results in dental fluorosis and skeletal fluorosis (Li Hai-rong et al., 2009; WHO, 2004).On the other hand, F also enters the human body through foods and beverages (Malde et al., 2011; Battaleb-Looieet al., 2013). It was observed that Fuptake by plant tissues from uncontaminated soils rarely exceed 30 mg $kg^{-1}$of dry mass, still many plants are quite sensitive to F uptake (Gupta and Banerjee, 2009). The plants get affected not only by anthropogenic fluoride sources such as emission from brick kiln industries, aluminium smelters, phosphatic fertilizer but also affected by higher content of F insoil and groundwater (Jha et al., 2013). Kumar et al., 2010 reported higher concentration of F in groundwater samples of Mirzapur district of Uttar Pradesh, India. The fluoride uptake by plants is dependent on various parameters such as pH, activity of F in soil solution and plant species (Pickering, 1985). After the foliar application of F to the plant, it is translocated to different plant parts through xylem. The influence of fluorides in food depends on itsconcentration in soil, water or ambient air where the crop is grown.

Rice (*Oryza sativa* L.) is the staple food of more than 60 per cent people of South Eastern Asia. To sustain rice production and productivity, soil management approach by application of integrated nutrient management (Nayak et al., 2012) and modern scientific cropping sequences (Roy et al., 2011; Kumar et al., 2016) are the possible options.It has been reported (Elloumi et al., 2005) that plant suffers from some physiological changes such as stunted growth, chlorosis, leaf necrosis and leaf tip burning on exposure to fluoride. The objective of this study was to examine the effect of different doses of F on rice growth, yield, grain quality and post harvest soil microbial quality.

## MATERIALS AND METHODS

A field experiment was conducted at Regional Research and Technology Transfer Station (RRTTS), OUAT, Chiplima (22°33' N latitude and 81°21' E longitude and with analtitude of 178.8 m above mean sea level) in the year 2014-15 and 2015-16. The experiment was conducted in a randomized block design (RBD) with four replications and six treatments.The treatmentscomprised of $T_1$-Control (No fluoride); $T_2$- Foliar spray of F = 100 ppm as HF; $T_3$- Foliar spray of F = 150 ppm as HF; $T_4$- Foliar spray of F = 200 ppm as HF; $T_5$- Foliar spray of F = 250 ppm as HF; $T_6$- Foliar spray of F = 300 ppm as HF. 21 days old seedlings of rice cv. Swarna (MTU-7092) were transplanted in different plots with a spacing of 25 cm x 15 cm and fertilizer was applied @80 kg N, 40 kg $P_2O_5$ and 40 kg $K_2O$ per ha. Full dose of $P_2O_5$ and $K_2O$ and 50% of recommended dose of N were applied as basal and rest 50% N was applied in two splits after 30 days of transplanting. The graded doses of fluoride were prepared from analytical reagent grade of HF. F was applied (@150, 200, 250 and 300 ppm) four times in entire crop growth periodas foliar sprayi.e, first at 7DAP, second at 15 DAP, third at 30 DAP and fourth at 45 DAP. Agronomic and yield parameters were recorded as per the procedure described in Kumar et al., 2017. Different grain quality parameters like protein content (%), amylose content (%) and alkali value were studied. The protein content was calculated by multiplying the N content of the grain (estimated by Kjeldahl digestion method) by a factor of 5.95 (Page et al., 1982). The amylose content of milled rice was estimated by using colorimetric iodine assay method (Using UV-VIS Spectrophotometer) and dilute KOH was used to estimate alkali value of milled rice grains (Juliano, 1971, Little et. al, 1958). Alkali value of rice was studied as a post harvest rice quality parameter. Amylose along with glucose is a linear polymer of starch. In starch amylose varies from 15-35 %.After completion of experiments surfacesoil samples were collected from different plots, processed and different physico-chemical properties were determined as per the standard procedures (Page et al., 1982). Microbial biomass carbon (MBC), dehydrogenase activity (DHA) and soil bacterial population at different crop growth stages were determined as described by Wu et al. (1990), Tabatabai, (1994) and Pelczar et al. (1978) respectively. The total F in grain of rice was determined by extractingthe dried ground and sieved samples with 0.1 N perchloricacid and measuring F concentrationwith the help of ORION 5-star series ion analyzer, using Ion Selective Electrode (ISE). The collected experimental data were analysed statistically (Panse and Sukhatme, 1985).

**Table 1.** Initial soil parameters of the experimental site.

| Parameters | |
|---|---|
| Sand (%) | 72 |
| Silt (%) | 16 |
| Clay (%) | 12 |
| Texture | Sandy loam |
| B.D. (Mg $m^{-3}$) | 1.38 |
| P.D. (Mg $m^{-3}$) | 2.17 |
| Porosity (%) | 36.41 |
| pH | 5.6 |
| EC (dS $m^{-1}$) | 0.12 |
| O.C. (g $kg^{-1}$) | 5.7 |
| Mineralizable N (kg $ha^{-1}$) | 195 |
| Bray's P (kg $ha^{-1}$) | 13.6 |
| $NH_4OAc$ extractable K (kg $ha^{-1}$) | 173 |
| $CaCl_2$ extractable S (kg $ha^{-1}$) | 58.8 |
| Available Fluoride (ppm) | 14.8 |
| Dehydrogenase "(mg TPF $g^{-1}$ soil $day^{-1}$) | 14.12 |
| MBC (mg $g^{-1}$ dry soil) | 138.45 |

## RESULTS AND DISCUSSION

The soil of the experimental site was sandy loam (Sand 72%, silt 16% and clay 12%) acidic in reaction (pH 5.6), low in mineralizable N (195 kg $ha^{-1}$) and available Bray's P (13.6 kg $ha^{-1}$) where as medium in $NH_4OAc$ extractable K (173 kg $ha^{-1}$) with non-hazardous salt content (EC 0.12 dS/m). It is medium inorganic carbon

**Table 2.** Effect of graded doses of F on growth, yield and F-uptake by rice (mean value of two years).

| Treatment Details | Plant height (cm) at 60 DAT | Effective Tillers/ hill | Panicle length (cm) | Grain Length (mm) | Kernel Elong-ation (mm) | LAI | Chlor-ophyll content (mg/g fresh tissue) | F Uptake by Fruit (kg/ha) | Grain yield (q/ha) | Straw yield (q/ha) |
|---|---|---|---|---|---|---|---|---|---|---|
| $T_1$: Control (No Fluoride) | 136.18 | 13.04 | 25.95 | 5.19 | 6.88 | 4.93 | 4.53 | 16.53 | 36.73 | 47.34 |
| $T_2$: Foliar spray of F @100 ppm as HF | 134.78 | 12.14 | 24.65 | 4.84 | 6.53 | 4.55 | 3.38 | 21.89 | 35.65 | 46.34 |
| $T_3$: Foliar spray of F @150 ppm as HF | 132.95 | 12.16 | 24.48 | 4.60 | 6.35 | 4.48 | 3.14 | 22.76 | 35.15 | 45.18 |
| $T_4$: Foliar spray of F @200 ppm as HF | 130.48 | 12.28 | 23.55 | 4.81 | 6.43 | 4.40 | 2.79 | 23.30 | 34.53 | 44.84 |
| $T_5$: Foliar spray of F@250 ppm as HF | 128.68 | 10.52 | 22.53 | 4.45 | 6.38 | 4.25 | 2.30 | 24.10 | 33.30 | 43.96 |
| $T_6$: Foliar spray of F @300 ppm as HF | 123.78 | 9.58 | 22.28 | 4.38 | 6.18 | 4.18 | 2.26 | 24.71 | 32.90 | 40.09 |
| Sem (±) | 2.52 | 0.98 | 0.81 | 0.11 | 0.15 | 0.11 | 0.12 | 0.68 | 1.07 | 1.01 |
| CD (p = 0.05) | 7.60 | 2.78 | 2.43 | 0.31 | 0.44 | 0.34 | 0.37 | 2.06 | 3.22 | 2.01 |

(5.7 g $kg^{-1}$) and low in available fluoride (14.8 ppm).

## Yield and yield attributing characters

The result of F application on yield and yield attributing characters ofriceindicated that the variation in plant height (136 to 124 cm ) of rice was not significantly differed due to application of different doses of F (table2). Maximum reduction in plant height (9.1%) was recorded with $T_6$ (foliar spray of F @ 300 ppm as HF). Effective tillers per hill (13.04 to 9.58 ) due to application of graded doses of F were statistically at par with each other. Panicle length (25.95 to 22.28 cm) and grain length (5.19 to 4.38 mm) varied significantly due to application of different doses of F. Kernel elongation was measured by picking randomly ten cooked and cooled kernels. It was observed that kernel elongation (mm) of rice varied significantly from 6.88 to 6.18 mm while leaf area index (LAI) varied from 4.93 to 4.18 with graded doses of F application in different treatments. Significantly highest chlorophyll content of 4.53 mg/g fresh tissuewas observed with $T_1$ (control plot-No Fluoride applied). With the application of graded doses of F, chlorophyll content was affected significantly and it was much suppressed at $T_6$ (2.26 mg/g fresh tissue). F uptake by rice grain at different treatments varied non significantly from 16.53 to 24.71 kg/hawith graded doses of F application. Significantly highest grain yieldof rice (36.73 q $ha^{-1}$) was observed with $T_1$ (control plot-No Fluoride applied) where as lowest value of 32.90 q $ha^{-1}$ was observed with $T_6$ (foliar spray of F @ 300 ppm as HF). A decrease in grain yield of rice was recorded from 36.73 to 32.90 q/ha due to application of graded doses of F in different plots. A maximum of 10.42 % reductionin grain yield was observed with $T_6$ (foliar spray of F @ 300 ppm as HF) over control. Similar trend was also observed with straw yield of rice. Straw yieldwas significantly decreased from 47.34 to 40.09 q/ha with increased doses of fluoride. Similar results were also reported by Jha et. al., 2013b and Bisrate Wongel et al., 2013. Inhibition of glycolysis pathways by inhibiting the enzyme activities due F application may the possible reason of yield reduction in rice (Qin et al., 2006).

## Grain quality parameters

Cooking quality of grain is an important parameter to evaluate the rice grain quality. In this study different quality parameters like protein content (%), amylose content (%) and alkali value were studied (Fig. 1). Protein content ofmatured rice grain was significantly correlated ($R^2$=0.982). It was observed that the mean protein content of rice grain was decreased from 7.40 to 5.28 (%) with increased doses of fluoride. The highest amylose content (21.4 %) in rice grain was observed with $T_1$ (No Fluoride applied)where as lowest value (18.53 %) was recorded in $T_6$ (foliar spray of F @ 300 ppm as HF). From this study it is indicatedthat amylase content ranged between low to medium in rice grains.From the graph (Fig. 1) it was observed that, amylose content was strongly correlated with different doses of F($R^2$=0.981). The reason for low to intermediate amylose content in rice might be due to

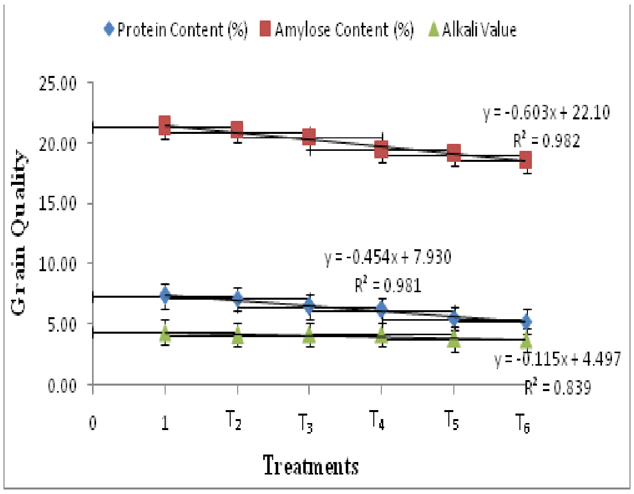


**Fig. 1.** Grain quality parameters of rice as influenced by graded doses of fluoride (mean value of two years).

increase in F concentration from 100 to 300 ppm. Application of dilute KOH to rice grains degraded the starch molecules and the shape of the grain was changed to a dispersed grain which resulted in the alkali value of rice grain varying from 3.78 to 4.40. A weak correlation was established between different doses of F applied and alkali value of rice grain ($R^2$=0.839) as compared to protein and amylose content.

## Soil microbial activities

Influence of F application on microbial biomass carbon, dehydrogenase enzyme activity and soil bacterial population were studied by collecting soil samples from different F treated plots within 24 hours of F application to estimate the microbial activities. From the result (Fig. 2) it was indicated that microbial biomass carbon (MBC) content was increased with increase in crop growth period. MBC content was varied between 147.89 to 168.45 mg $g^{-1}$. The highest value was recorded at 45 days after planting (DAP) and whereas lowest value was obtainedat 7 DAP. However, with the increase in F concentration from 100 ppm to 300 ppm both stimulation and inhibition effect was noticed to MBC.The inhibition was much observed at $T_6$ (300 ppm F was applied as foliar spray) which corroborated the result indicated by Panda et al., 2017.

## Dehydrogenase enzyme activity

Dehydrogenase enzymes are involved in the oxidation and reduction reactions in soil which reflects the metabolic activity of the soil (Salazar et al. 2011).

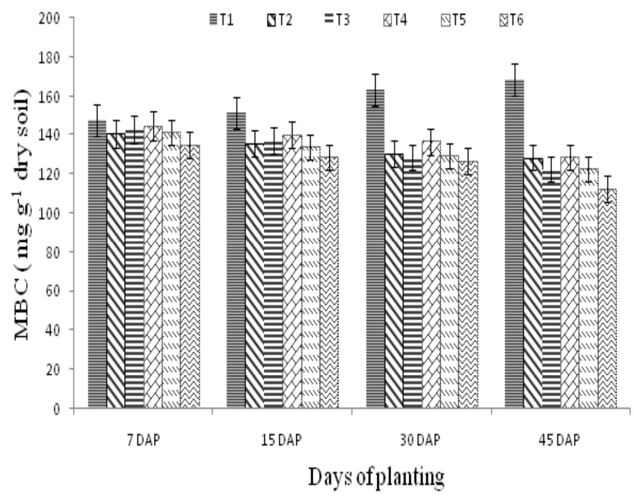


**Fig. 2.** Effect of graded doses of fluoride on soil microbial activity (MBC) at different growth stages of rice.

Highest dehydrogenase activity (DHA) was recorded with $T_1$ (where no F was applied) and it increased with increase in crop growth (Fig. 3) upto 45 DAP. However an inhibition effect was recorded with the application of graded doses of F in soil. The inhibition of dehydrogenase activity is expressed in terms of mgtriphenylformazan(TPF) form edg$^{-1}$ soil day$^{-1}$ in different treatments. In F treated plots, with the increase in crop growth period the DHA was gradually inhibited and the rate of inhibition was more pronouncedat $T_6$. The inhibition effect may be due to suppression of microbes by F application. However in some treated plots slight stimulation effect was noticed which may

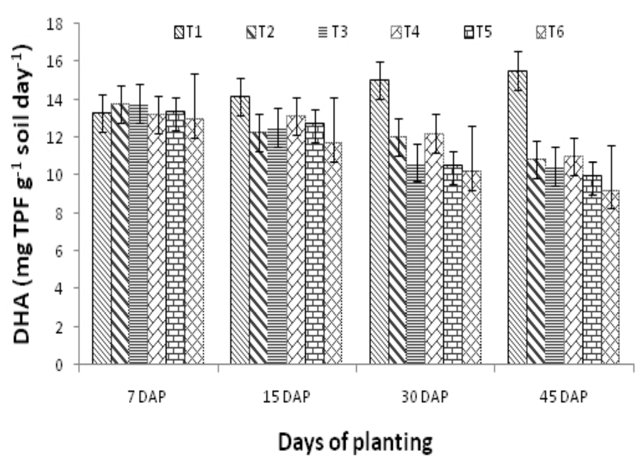


**Fig. 3.** Effect of graded doses of fluoride on soil dehydrogenase activity (DHA) at different growth stages of rice.

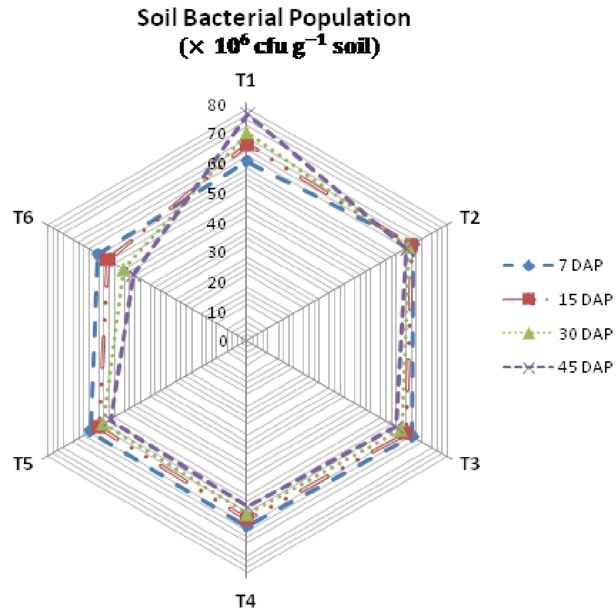


**Fig. 4.** Effect of graded doses of fluoride on soil bacterial populationat different growth stages of rice.

be due to release of more enzymes from the dead and living microbes, as soil enzymes are both extracellular and intracellular in nature (Casida et al., 1964). Similar findings were also reported by Tscherko and Kandeler, 1997. Soil enzymatic activities were also influenced by addition of elements through fly ash (Raja et al., 2015).

## Bacterial population in soil

In soil ecosystem the bacterial population was highest at surface soil and nutrient transformation is accelerated by soil bacteria (Tabatabai, 1994). From this experimenthighest bacteria population (Fig. 4) was recordedfrom the plots receiving no F ($T_1$ = No. F application). Interestingly with increase in growth period of rice the bacterial population was increased from 7 to 45 DAP (60.5 × $10^6$ cf ug$^{-1}$ soil to 76.5 ×$10^6$ cf ug$^{-1}$ soil). However, with the application of F the bacterial population was suppressed in soil at different F treated plots. With increased doses of F from 100 to 300 ppm a corresponding decrease in bacterial population was recorded (65 × $10^6$ cf ug$^{-1}$ soil to 44.5 × $10^6$ cf ug$^{-1}$ soil ). At $T_6$ (Foliar spray of F@ 300 ppm as HF) the bacterial population was recorded lowest as compared to other F treated plots.

From the present investigation it was concluded that graded doses of fluoride decreased the crop growth, grain and straw yield of rice and microbial activity in soil. Increased doses of F @ 100 to 300 ppm as HF reduced the rice grain quality to lower and intermediate class. Fluoride uptake by grain was also increased due to increased doses of F whichindirectly affects the cooking quality of rice grains. Inhibition effect was recorded to MBC, DHA and soil bacteria population with slight stimulation in some of the treatments. Microbial inhibitionwas gradually increased with an increase in F application from 100 to 300 ppm and highestinhibition was recorded at $T_6$ (Foliar spray of F@ 300 ppm as HF).

## REFERENCES

Battaleb-Looie S, Moore F, Malde MK and Jacks G (2013). Fluoride levels in commercially available rice in Ethiopia. Journal of Food Composition Analysis 29: 94

BisrateWonge T, Chandravanshi BS and Feleke Z (2013). Fluoride levels in commercially available rice in Ethiopia. Bulletin of Chemical Society of Ethiopia 27(2): 179-189

Casida LE, Jr Klein DL and Santaro T (1964). Soil dehydrogenase activity. Soil Science 96: 371-376

Elloumi N, Abdallah FB, Mezghani I, Rhouma A and Boukhrisb M (2005). Effect of fluoride on almond seedlings in culture solution. Fluoride 38(3):193-198

Gupta S and Banerjee S (2009). Fluoride accumulation in paddy (*Oryza sativa* L.) irrigated with fluoride-contaminated groundwater in an endemic area of the Birbhum district, West Bengal. Fluoride 42(3): 224-227

Jha SK, Sharma YK, Damodaran T, Mishra VK and Sharma DK (2013a). Bio-concentration of fluoride in Lady's finger (*Abelmoschusesculentus*) grown in contaminated alkaline soil and evaluation of exposure risk in human. Toxicological & Environmental Chemistry 95(1):138-149

Jha SK, Damodaran T, Verma CL, Mishra VK, Sharma DK, Sah V, Rai RB and Dhama K (2013b). Fluoride partitioning in rice (*Oryza sativa*) and wheat (*Triticum aestivum*) upon irrigation with fluoride-contaminated water and its risk assessment. South Asian Journal of Experimental Biology 3(3): 137-144

Juliano BO (1971). A simplified assay for milled rice amylose. Cereal Science Today16: 334-338

Kumar A ,Nath T and Raha P (2010). Status of Fluoride in Groundwater and its Suitability Assessment for Drinking Purpose in Mirzapur District of India. International Journal of Tropical Agriculture 28(1-2): 297-301

Kumar A, Nayak AK, Pani DR and Das BS (2017). Physiological and morphological responses of four different rice cultivars to soil water potential based deficit irrigation management strategies. Field Crops Research 205: 78-94

Kumar M, Kumar R, Meena KL, Rajkhowa DJ and Kumar A (2016). Productivity enhancement of rice through crop establishment techniques for livelihood improvement in Eastern Himalayas. Oryza 53(3): 300-308

Li Hai-rong, Qing-bin Liub, Wu-yiWanga, Lin-sheng Yanga, Yong-hua Li a, Fu-jianFenga, Xiao-yuZhaoa, Kun Houc and Ge Wang B (2009). Fluoride in drinking water, brick tea infusion and human urine in two counties in Inner Mongolia, China Journal of Hazardous Materials167: 892

Little RR, Hilder GB and DawsonEH (1958). Differential effect of dilute alkali on 25 varieties of mild white rice. Cereal Chemistry 35: 111-126

Malde MK, Scheidegger R, Julshamn K and Bader and HP (2011). Substance Flow Analysis: A Case Study of Fluoride Exposure through Food and Beverages in Young Children Living in Ethiopia. Environmental Health Perspectives 119: 579

Nayak AK, Gangwar B, Shukla AK, Mazumdar SP, Kumar A, RajaR, Kumar A , Kumar V, Rai PK and Mohan U (2012). Long-term effect of different integrated nutrient management on soil organic carbon and its fractions and sustainability of rice-wheat system in Indo Gangetic Plains of India. Field Crops Research 127: 129-139

Page AL, Millar RH and Keeney DR (1982). Methods of soil analysis : Part 2, chemical and microbiological properties, 2nd Ed. No. 9, In the series of Agronomy, American Society of Agronomy, Inc. Soil Science Society of America, Inc. Medison Wisconsin, USA

Panda N, Dash AK, Sarangi N, Purohit D and Prusty M (2017). Effect of graded doses of fluoride on soil microbial activity, growth and yield of okra (*Abelmoschuse sculentus* L.) grown in Alfisols. Journal of the Coastal Agricultural Research 35(2): 10-14

Panse VG and Sukhatme PV (1985). Statistical Methods for Agricultural Workers, ICAR Publication, New Delhi

Pelczar MJJr, Reid RD and Chan ECS (1978). Microbiology.4th Edition. Tata Mcgraw- Hill publishing House, New Delhi

Pickering WF (1985). The mobility of soluble fluoride in soils. Environmental Pollution, series B, Chemical and Physical 9(4): 281-308

Qin J, Chai G, Brewer JM, Lovelace LL and Lebioda L (2006). Fluoride inhibition of enolase: Crystal Structure and Thermodynamics. Biochemistry 45(3): 793-800

Roy DK, Kumar R and Kumar A (2011). Production potentiality and sustainability of rice-based cropping sequences in flood prone lowlands of North Bihar. Oryza 48(1): 47-51

R Raja, Nayak AK, Shukla AK, Rao KS, Gautam P, Lal B, Tripathi R, Shahid M, Panda BB, Kumar A, Bhattacharyya P, Bardhan G, Gupta S and Patra DK (2015). Impairment of soil health due to fly ash-fugitive dust deposition from coal-fired thermal power plants.Environmental Monitoring and Assessment.187: 679

Salazar S, Sanchez L, Alvarez J, Valverde A, Galindo P, Igual, J, Peix, I and Regina S (2011). Correlation among soil enzyme activities under different forest system management practices. Ecosystem Engineering 37: 1123-1131

Tabatabai MA (1994). Soil enzymes.In "methods of soil analysis. Part 2. Microbiological and Biochemical Properties" (R.W. Weaver, S. Angle and P.S. Bottomly (Eds.), pp. 775-826. SSSA, Madison, WI

Tscherko D and Kandeler E (1997). Ecotoxicological effects of fluorine deposits on microbial biomass and enzyme activity in grassland. European Journal of Soil Science 48: 329-335

World Health Organization (2004). Fluoride in Drinking Water, World Health Organization, Geneva.

Wu J, JoergensenRG, Pommerening B, ChaussodR and Brookes PC (1990). Measurement of soil microbial biomass C by fumigation extraction an automated procedure. Soil Biology and Biochemistry 22: 1167-1169