# Effect of different types of sugarcane bagasse biochar on soil enzymatic activities under spinach crop grown in an experimentally fluoride contaminated soil




## ABSTRACT

**Aims:** To elucidate the impacts of fluorine contamination in soil at various levels on soil enzymatic activities, as well as the effects of adding biochar made from sugarcane bagasse and modified with alum and phosphoric acid to the substrate to reduce the deleterious effects of fluorine.

**Study design:**The pot experiment was carried under factorial CRD (complete randomized design).

**Place and Duration of Study:**The pot experiment followed by laboratory analysis was conducted in the Net house of Centre for Environment Assessment & Climate Change, G.B. Pant National Institute of Himalayan environment, Kosi-Katarmal, Almora (UK), India during Rabi season (January to March) 2022.

**Methodology:**The present study was undertaken with 2 levels of fluoride (Factor A) and 3 levels of each three types of biochar viz. non activated biochar, phosphoric acid activated biochar and alum treated biochar with a control (no biochar) i.e. 10 treatments of biochar (Factor B). The combination of Factor A and Factor B comprises 20 treatment combinations with three replicates. The soil enzymes such as urease, dehydrogenase, acid phosphatase and alkalinephosphatase, were examined in the postharvest soil sample.

**Results:**The outcomes demonstrated that the soil enzymatic activity of post-harvest soil under spinach cropwas greatly improved with the application of fluoride in case of soil dehydrogenase, acid phosphatase and alkaline phosphatase except soil urease. Application of alum modified biochar and phosphoric acid modified biochars at different application rates significantly all the 4 studied soil enzymatic activities. Compared to the control, $PAMB_{40}$ increases the urease, dehydrogenase, acid phosphatase and alkaline phosphatase by 32.71, 58.83, 142.60 and 31.10%, respectively.

**Conclusion:**This study suggested that biochar treated with phosphoric acid and alum might be utilised as

a long term improvement to enhance quality of soil, the importance of which would be vital for agriculture.

***Keywords: Biochar, phytoremediation, bioremediation, sugarcane bagasse, soil enzymes***

## 1. INTRODUCTION

Fluorine (F), being themost electronegative and the lightest element of halogen family, it naturally forms the dissolved complexes that are found as fluoride (F) ions in the Earth's crust. Geological conditions such as hydrothermal solutions cause the natural occurrenceof F due tosolubilization of alkali and ferromagnesian rocks causes its presence in groundwater. [1]. Due to the negative effects of fluoride on human health, it is crucial to understand the processes by which cryolite ($Na_3AlF_6$), fluorapatite ($Ca_{10}F_2(PO_4)_6$), and fluorospar ($CaF_2$) minerals in volcanic eruptions, ferromagnesian rocks and the primary aerosols are weathered and dissolved. Additionally, a significant amount of F pollution occurs during disinfectants, insecticides, phosphate-fertilisers, glassware, preservatives, and metalsprocessing such as steel, aluminium, dyeing of textile, TV cathode ray tubes, plastics, and brick productions. [2].

Fluoride is not a necessary element for plants, unlike sulphur, nitrogen, and chlorine, and its background concentration is typically quite low (sometimes as low as 1 and typically less than 10 g F/g dry weight in most species). This is due to the fact that F naturally occurs in air at levels close to detection limit, and that plants only absorb a little amount of F from the soil [3]. When compared to other chemicals ($O_3$, $SO_2$, PAN, $Cl_2$, or HCl), fluoride compounds like HF and $SiF_4$ are between one and three orders more harmful, therefore even minute amounts of fluoride emissions into the air can cause tremendous injury to plants [4,5]. Since F is the most harmful air pollutant that affects forests, crops, and regular vegetation, the researchers' primary tasks should be to biomonitor for fluoride contamination, develop fluoride-appropriate air quality criteria, and determine the levels of fluoride in the air, soil, vegetation, and water [6].

The main cause of soil fluoride contamination is the usage of phosphorous fertilisers, which typically contain less than 1 to more than 1.5 percent fluorine. The pH of the soil and the amount of clay minerals depend on how it behaves in the soil [7]. In normal circumstances, soil has a total fluoride level of 150–400 mg/kg. Values over 1000 mg/kg have been observed in thick clay soils. Human health is impacted by contaminated soil in several ways, including direct contact with the soil, inhalation of soil toxins that have evaporated, consumption of contaminated food products, and penetration of contaminated soil into groundwater used for human consumption [8, 9, 10].

High levels of fluorine and related compounds are recognized to be harmful to all living things, including humans. Fluorine is present in high concentrations in the soil environment, which puts soil-dwelling microorganisms at considerable risk [11] and inhibits some soil enzymes that link biogenous elements (P, Ca, Mg, etc.) and disturbs their balance in the organism [12]. One of the most common "soil fertility indicators" that aid in soil quality assessment is an examination of the enzymatic activity of the soil. Soil enzymes can be thought of as indicators that point to how much a soil environment has degraded. Numerous research have shown that analyzing the activity of specific soil enzymes can give us accurate information for assessing the quality of the soil [13, 14, 15, 16, 17].

This study aims to assess the impacts of fluorine contamination in soil at various levels on some enzymatic activities of soil, as well as the effects of adding biochar made from sugarcane bagasse and modified with alum and phosphoric acid to the substrate to lessen the deleterious effects of fluorine.

## 2. Material and Methods

### 2.1 Preparation of the biochar

Sugarcane bagasse (SB) were used as the feedstock to synthesize biochar. The feedstock SB was obtained from a nearby juice facility in New Delhi, and it was first washed under running water to get rid of any impurities and extra sugar prior to being dried for 24 hours at 105°C in a hot-air oven to achieve constant weight and then kept in a container for processing. It was then broken into smaller pieces before it was pyrolyzed. For 24 hours, the sugarcane bagasse sample was activated by being treated with phosphoric acid and alum in a 10 percent (w/v) solution. The sample that had been impregnated was then dried at 105°C before being pyrolyzed in a 200-liter empty oil barrel that had been placed inside a fire brick enclosure for pyrolysis.After pyrolysis, the samples were repeatedly washed in distilled water to bring the pH of the washing solution to a stable level. The activated samples were then subjected to drying at 70°C for 2 hours and stored in an airtight plastic bottle for further study. The pH for the phosphoric acid treated biochar and the alum treated biochar reached constant at 4.50-4.99 while the normal biochar (not activated) was having pH of 6.5-7.0.

### 2.2 Experimentaldetails

The current study's objective was to look at how several types of modified sugarcane bagasse biochar impacted soil enzymatic activities under spinach crop grown in an experimentally fluoride contaminated soil". The pot experiment (Table 1) was conducted in the Net house of Centre for Environment Assessment & Climate Change, G.B. Pant National Institute of Himalayan environment, Kosi-Katarmal, Almora (UK), India during Rabi season (January to March) 2022.

**Table 1. Experimental details of pot experiment**

| ***S. No.*** | ***Particulars*** | |
|---|---|---|
| ***1.*** | Year of experimentation | 2021-22 |
| ***2.*** | No. of treatments | |
| | ***2.1*** Factor A (fluoride levels) | 02 |
| | ***2.2*** Factor B (biochar levels) | 10 |
| ***3.*** | No. of replications | 03 |
| ***4.*** | Total no. of pots | 02*10*03= 60 |
| ***5.*** | Test crop | Spinach |
| ***6.*** | Experimental Design | Factorial complete randomized design (FCRD) |

### 2.3 TREATMENT DETAILS OF POT EXPERIMENT

The present study was undertaken with 2 levels of fluoride (Factor A) and 3 levels of each three types of biochar viz. non activated biochar, phosphoric acid activated biochar and alum treated biochar with a control (no biochar) i.e. 10 treatments of biochar (Factor B). The combination of Factor A and Factor B comprises 20 treatment combinations as below:

**Table 2. Details of Factor A (Fluoride levels) and Factor B (Biochar levels) as treatments for the pot experiment**

| **Factor A (Fluoride levels)** | **Factor B (Biochar levels)** |
|---|---|
| $F_{250}$ =250 mg F $kg^{-1}$ of soil<br>$F_{1000}$ =1000 mg F $kg^{-1}$ of soil | $B_0$ =0 g Modified SB biochar $kg^{-1}$ of soil (Control – No Modified SB biochar)<br>$NAB_{10}$ = 10 g Modified SB biochar $kg^{-1}$ of soil |

| | |
|---|---|
| | $NAB_{20}$ = 20 g Modified SB biochar $kg^{-1}$ of soil<br>$NAB_{40}$ = 40 g Modified SB biochar $kg^{-1}$ of soil<br>$PAB_{10}$ = 10 g Modified SB biochar $kg^{-1}$ of soil<br>$PAB_{20}$ = 20 g Modified SB biochar $kg^{-1}$ of soil<br>$PAB_{40}$ = 40 g Modified SB biochar $kg^{-1}$ of soil<br>$AAB_{10}$ = 10 g Modified SB biochar $kg^{-1}$ of soil<br>$AAB_{20}$ = 20 g Modified SB biochar $kg^{-1}$ of soil<br>$AAB_{40}$ = 40 g Modified SB biochar $kg^{-1}$ of soil |

Where, NAB, PAB and AAB depict not activated biochar, phosphoric acid activated biochar and alum activated biochar, respectively.

### 2.4 Enzymatic assay

Soil enzymatic activities were carried out within a week of soil sampling. Using standard procedures as reported by Casida et al. [18], dehydrogenase was evaluated in soil samples from pot trialsand urease, acid and alkaline phosphatase activities were assessed by the process as described by Tabatabai and Bremner [19].

### 2.5 Statistical analyses

Statistical analysis of the experimentally generated data was performed using SPSS and the Analysis of Variance approach as outlined by Gomez et al. [20]. With the use of the variance ratio test, the significance of the treatment effect was assessed. To compare the treatment means, a critical difference (CD) at a 5% level of significance was calculated.

## 3. Results and discussions

### 3.1 Soil urease

The soil urease enzyme activity has been employed most frequently since it is intimately connected to the normal activities of the microbial communities, as a biological indicator of soil quality.The urease enzyme activity was measured in the post-harvest soil as the data has been shown in table 3. Data analysis showed that fluoride and other biochar applications at different amounts have a substantial impact on urease enzyme activity in the post-harvest soil.

It is evident that the urease enzyme activity was non significantly affected by fluoride application. In general, the urease activity significantly increased by the biochar application. Phosphoric acid modified biochar has the significant effect on dehydrogenase activity over the alum modified biochar and normal biochar. The PAMB40 has the highest activity (53.72) among all the biochar at all the levels and the lowest were reported in control (no biochar). PAMB40 were found to increase the dehydrogenase activity the highest, followed by AMB40 when compared to the control (no biochar). PAMB20, AMB10, AMB20 and AMB40 were statistically at par with each other to increase the soil urease activity which were found to increase the activity by 28.9, 21.4, 22.2 and28.15%, respectively over the control (no biochar). The interaction of fluoride with the biochar application on the urease enzyme activity in the post-harvest-soil was found to be non-significant.

In an pot experiment with the rapeseed plant, the addition of biochar to the soil was observed to improve the urease activity [13]. Jiang et al. [14] revealed that the concentration of biochar applied had a significant impact on the urease activity, which was shown to be higher with biochar addition than with the control.The outcomes corroborated with those of Anuradha et al. [15], who also presented related similar results.In contrast to corn cob biochars, which suppress activity of urease, swine dung biochars have more nutrients than plant-based biochars [16], which was found to boost not only the microbial but also supported the urease activity in soil. [17].

### 3.2 Soil acid phosphatase

Phosphatase is an enzyme that hydrolyzes organic and inorganic P molecules so that the plants can use them. Phosphatases are substrate-induced, and the amount of orthophosphate that roots and microorganisms excrete depends on how much of it they need. The application of biochar at various levels and the growing amount of soil pollution with fluorine both considerably and dramatically changed the activity of acid phosphatase in the examined soils. A soil examined following the harvest of spinach crop was generally increasing alongside increasing fluoride contamination. It has been also observed that acid phosphatase activity is increasing with the all the biochar application at increasing levels. Perusal of the table 4 clearly revealed that the acid phosphatase activity is increased in general. Specifically, the increase in the acid phosphatase activity is over 109% when fluoride is applied @ 1000 ppm over 250 ppm at control (no biochar)condition. The mean values of acid phosphatase activity in the post-harvest soil showed that the highest activity in the PAMB40 followed by PAMB20 at the 142.6 and 125.5%, respectively over control (no biochar). Whereas, AMB20, AMB40 and PAMB10 were statistically at par with each other to increase the acid phosphatase activity in the soil by 91.8, 92.7 and 98.3%, respectively over the control. The interaction of fluoride with the biochar application on the acid phosphatase enzyme activity in the post-harvest-soil was determined to be the most important and highest acid phosphatase activity was observed in the combination with F1000 × PAMB40 followed by F250 × PAMB40, having increase of 105.8% and 219.5%, respectively over their respective control.

According to Kotroczó et al. [21], root interactions enhance soil enzyme activity. Fresh biochar's surface can act as a catalyst for enzymatic reactions and encourage plant roots to secrete enzymes into the soil, which may be related to soil temperature [22]. However, more research is needed to confirm this possibility.

### 3.3Soil alkaline phosphatase

It has been observed that alkaline phosphatase activity is increasing with the biochar application and increasing levels of fluoride. However, perusal of the table 4 clearly revealed that the alkaline phosphatase activity is depressed with increasing level of fluoride in control (no biochar).The application of biochar, in general, increases the alkaline phosphatase activity of the post-harvest soil. The mean values of alkaline phosphatase activity in the post-harvest soil exhibited the highest alkaline phosphatase activity was observed in the PAMB40 followed by PAMB20, PAMB10 AMB40which increases the activity by31.1, 28.2, 26.0 and 24.8%, respectively over control (no biochar). PAMB40, PAMB20, PAMB10 and AMB40 were statistically at par with each other to increase the alkaline phosphatase activity in the soil when compared with their averaged values. The interaction of fluoride with the biochar application on the alkaline phosphatase enzyme activity in the post-harvest-soil was determined to be significant, and the combination showed the highest alkaline phosphatase activitywith F1000 × PAMB40. The normal biochar has also significant increase in the activity over control (no biochar). The alum modified biochar increases the alkaline phosphatase activity but statistically, they were determined to be on par with one another.The identical outcomes of statistically equal are also have been observed in the case of phosphoric acid modified biochar.

### 3.4 Soil dehydrogenase

The soil dehydrogenase enzyme activity has been utilized as a biological measure of soil quality since it is strongly related to the average microbial population activity. The dehydrogenase enzyme activity was assessed in the post-harvest soil and is shown in Table 6 expressed in µg Tri Phenyl Formazan (TPF) generated per gram soil per day. The analysis of the results showed that the dehydrogenase enzyme activity in the post-harvest soil is significantly impacted by the application of fluoride and different types of biochar at various amounts.

It is evident that the dehydrogenase enzyme activity was significantly lower with the fluoride level applied @ 1000 ppm (F1000) over the fluoride level applied @ 250 ppm (F250). The highest dehydrogenase activity was observed in the PAMB40 both in F250 and F1000 having an increase of 26.52 and 28.21%, respectively over control.In general, the dehydrogenase activity increases with the biochar application. Phosphoric acid modified biochar has the significant effect on dehydrogenase activity over the alum modified biochar and normal biochar. The PAMB40 has the highest activity among all the biochar at all the levels. PAMB10, PAMB20 and PAMB40 were found to increase the dehydrogenase activity by 40.5, 46.2 and 58.8%, respectively when compared to the control (no biochar). Dehydrogenase activity increased over control by 30.1 and 34.7 percent with AMB20 and AMB40, respectively. However, AMB20 and AMB 40 were statistically found at par when compared with each other. The interaction of fluoride with the biochar application on the dehydrogenase enzyme activity in the post-harvest-soil was found to be non-significant.

In response to a certain dose/level of F availability, Telesiski et al. [23] detected an increase in soil enzyme activity. Other researchers have reported that increasing F concentrations inhibit soil enzymes [24, 25, 26].

**Table 3. Influence of different levels of fluoride contamination and biochar application on soil urease**

| ***Biochar doses*** | ***Fluoride levels*** | | |
|---|---|---|---|
| | **250** | **1000** | **Mean** |
| *Control* | 39.10 | 41.82 | 40.46 |
| *NB@10 g/kg* | 41.14 | 43.18 | 42.16 |
| *NB@20 g/kg* | 43.18 | 44.54 | 43.86 |
| *NB@40 g/kg* | 45.22 | 46.58 | 45.90 |
| *AMB@10 g/kg* | 48.28 | 49.98 | 49.13 |
| *AMB@20 g/kg* | 49.64 | 49.30 | 49.47 |
| *AMB@40 g/kg* | 51.00 | 52.70 | 51.85 |
| *PAMB@10 g/kg* | 44.54 | 46.92 | 45.73 |
| *PAMB@20 g/kg* | 48.96 | 51.34 | 50.15 |
| *PAMB@40 g/kg* | 52.36 | 55.08 | 53.72 |
| ***Mean*** | 46.34 | 48.14 | |
| ***CD*** | F | B | F X B |
| | NS | 5.33 | NS |

*NB= Normal biochar, AMB= Alum modified biochar, PAMB= Phosphoric acid modified biochar

**Table 4. Influence of different levels of fluoride contamination and biochar application on soil alkaline phosphatase**

| ***Biochar doses*** | ***Fluoride levels*** | | |
|---|---|---|---|
| | **250** | **1000** | **Mean** |
| *Control* | 79.91 | 72.30 | 76.10 |
| *NB@10 g/kg* | 81.82 | 90.36 | 86.09 |
| *NB@20 g/kg* | 83.68 | 94.75 | 89.21 |
| *NB@40 g/kg* | 84.28 | 96.98 | 90.63 |
| *AMB@10 g/kg* | 84.69 | 101.44 | 93.07 |
| *AMB@20 g/kg* | 85.16 | 103.64 | 94.40 |
| *AMB@40 g/kg* | 85.72 | 104.34 | 95.03 |
| *PAMB@10 g/kg* | 87.16 | 104.76 | 95.96 |
| *PAMB@20 g/kg* | 89.16 | 106.00 | 97.58 |
| *PAMB@40 g/kg* | 91.98 | 107.59 | 99.78 |
| ***Mean*** | 85.36 | 98.22 | |
| ***CD*** | F | B | F X B |
| | 1.50 | 3.36 | 4.75 |

*NB= Normal biochar, AMB= Alum modified biochar, PAMB= Phosphoric acid modified biochar

**Table 5. Influence of different levels of fluoride contamination and biochar application on soil acid phosphatase**

| *Biochar doses* | *Fluoride levels* | | |
|---|---|---|---|
| | **250** | **1000** | **Mean** |
| *Control* | 96.58 | 202.21 | 149.39 |
| *NB@10 g/kg* | 158.48 | 248.85 | 203.67 |
| *NB@20 g/kg* | 171.34 | 266.09 | 218.72 |
| *NB@40 g/kg* | 174.94 | 271.92 | 223.43 |
| *AMB@10 g/kg* | 191.36 | 292.80 | 242.08 |
| *AMB@20 g/kg* | 234.83 | 338.47 | 286.65 |
| *AMB@40 g/kg* | 235.72 | 340.06 | 287.89 |
| *PAMB@10 g/kg* | 243.83 | 348.58 | 296.21 |
| *PAMB@20 g/kg* | 283.83 | 389.83 | 336.83 |
| *PAMB@40 g/kg* | 308.64 | 416.23 | 362.44 |
| ***Mean*** | 209.96 | 311.50 | |
| ***CD*** | F | B | F X B |
| | 11.70 | 26.16 | NS |

*NB= Normal biochar, AMB= Alum modified biochar, PAMB= Phosphoric acid modified biochar

**Table 6. Influence of different levels of fluoride contamination and biochar application on soil dehydrogenase**

| *Biochar doses* | *Fluoride levels* | | |
|---|---|---|---|
| | **250** | **1000** | **Mean** |
| *Control* | 12.93 | 12.19 | 12.56 |
| *NB@10 g/kg* | 13.93 | 13.05 | 13.49 |
| *NB@20 g/kg* | 14.67 | 13.80 | 14.24 |
| *NB@40 g/kg* | 15.32 | 14.50 | 14.91 |
| *AMB@10 g/kg* | 16.08 | 14.98 | 15.53 |
| *AMB@20 g/kg* | 16.38 | 16.32 | 16.35 |
| *AMB@40 g/kg* | 16.93 | 16.94 | 16.93 |
| *PAMB@10 g/kg* | 17.59 | 17.71 | 17.65 |
| *PAMB@20 g/kg* | 18.65 | 18.07 | 18.36 |
| *PAMB@40 g/kg* | 21.17 | 18.73 | 19.95 |
| ***Mean*** | 16.36 | 15.63 | |
| ***CD*** | F | B | F X B |
| | 0.47 | 1.05 | NS |

*NB= Normal biochar, AMB= Alum modified biochar, PAMB= Phosphoric acid modified biochar

## 4. CONCLUSION

Fluorine addition to soil boosts its enzymatic activity, which is advantageous for microorganisms' high utilisation of soil nitrogen and carbon. This would result in favourable microbe proliferation, which would enhance the microbial' enzymatic processes and activities. The addition of alum and phosphoric acid modified biochar significantly increased the activities of urease, dehydrogenase, and phosphatase (acid and alkaline), demonstrating that the application of modified biochar to soil proved beneficial to promote enzyme activity in fluoride-contaminated soil and increase the use efficiency of nutrients in the soil.. According to this study, biochar that has been treated with phosphoric acid and alum could be used to improve soil quality, which is essential for agriculture, in a sustainable way.