

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 shed light on the impact of soil contamination with fluorine at different levels on some enzymatic activities of soil, and the influence of non-treated and alum and phosphoric acid treated biochars prepared from sugarcane bagasse added to the substrate in order to control the toxic effect of fluorine.

Study design: The pot experiment was conducted 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 postharvest soil sample were analysed for soil enzymes viz. urease, dehydrogenase, acid- and alkaline phosphatase.

Results: The results showed that the soil enzymatic activities of post-harvest soil under spinach crop was significantly increased 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, PAMB₄₀ increases the urease, dehydrogenase, acid phosphatase and alkaline phosphatase by 32.71, 58.83, 142.60 and 31.10%, respectively.

Conclusion: This study indicated that phosphoric acid and alum modified biochar could be used as a

sustainable amendment for improvement of soil quality, which would take a vital role in agriculture.

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

1. INTRODUCTION

Fluorine (F) is the lightest halogen and the most electronegative chemical element. It occurs naturally in the Earth's crust as fluoride (F⁻) ions and its respective dissolved complexes in aqueous solutions. Its presence in groundwater is due to geological conditions as it exists naturally in mafic minerals, alkali rocks, and hydrothermal solutions [1]. Fluoride impact on human health makes its hydrogeochemistry an important as it is released naturally through weathering and dissolution of fluorospars (CaF₂), fluorapatite (Ca₁₀F₂(PO₄)₆), and cryolite (Na₃AlF₆) minerals in volcanic emissions and in marine aerosols. Furthermore, emissions occur during the manufacturing of pesticides, disinfectants, wood preservatives, metals, glasses, steel, aluminum processing, TV picture tubes, phosphate fertilizers, glass, brick, plastics, and textile dyeing [2].

Fluoride, unlike sulphur, nitrogen and chlorine, is not an essential element for plants, its background concentration is generally quite low (often as low as 1 and usually less than 10 µg F/g dry weight in most species). This is a result of the natural occurrence of F in air in close to detection limit, furthermore plants take up little F from the soil [3]. Since the fluoride compounds like HF and SiF₄ are more dangerous (between 1 and 3 orders) when contrasted with different compounds (O₃, SO₂, PAN, Cl₂, or HCl), even little arrivals of fluorides into the air can bring about extraordinary harms to plants [4,5]. Consequently, F is most critical and damaging air pollutants influencing backwoods, yields and regular vegetation, so biomonitoring for fluoride contamination; building up fitting air quality criteria for fluoride; and deciding the measures of fluoride in air, soil, vegetation and water are basic assignments for the researchers [6].

Fluoride contamination of soil is fundamentally because of utilization of phosphorous fertilizers which contain less than 1 to more than 1.5% Fluorine. Its conduct in soil relies on soil pH and content of clay minerals in the soil [7]. The normal total fluoride content of soil ranges from 150-400 mg/kg. In heavy clay soils, values surpassing 1000 mg/kg have been enlisted. Polluted soil influences human wellbeing through direct contact with soil, by means of inhalation of soil contaminants which have vapourized,

through ingestion of polluted sustenance items and by penetration of soil defilement into groundwater utilized for human utilization [8, 9, 10].

It is known, that high concentrations of fluorine and its compounds are dangerous for living organisms, including humans being. The high concentration of fluorine in the soil environment, poses a serious threat to soil-dwelling microorganisms [11] and causes inhibition of some of the soil enzymes, to the linking of biogenous elements (P, Ca, Mg etc.) and the disturbance of their balance in the organism [12]. Analysis of the enzymatic activity of soil is one of the most popular 'soil fertility indicators' that support soil quality assessment. Thus, soil enzymes can be treated as indicators suggesting the degree of degradation of a soil environment. Numerous studies have demonstrated that analysis of the activity of certain soil enzymes can provide us with reliable data for an evaluation of soil quality [13, 14, 15, 16, 17].

The aim of this study has been to shed light on the impact of soil contamination with fluorine at different levels on some enzymatic activities of soil, and the influence of non-treated and alum and phosphoric acid treated biochars prepared from sugarcane bagasse added to the substrate in order to control the toxic effect 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 before being dried in a dry oven at 105°C for 24 hours to attain constant weight and being stored 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 Study design

The present investigation was carried out to study the “effect of different types of sugarcane bagasse biochar on soil enzymatic activities of 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	B ₀ = 0 g biochar kg ⁻¹ of soil (Control – No biochar)
F ₁₀₀₀ = 1000 mg F kg ⁻¹ of soil	NAB ₁₀ = 10 g biochar kg ⁻¹ of soil

	NAB ₂₀ = 20 g biochar kg ⁻¹ of soil
	NAB ₄₀ = 40 g biochar kg ⁻¹ of soil
	PAB ₁₀ = 10 g biochar kg ⁻¹ of soil
	PAB ₂₀ = 20 g biochar kg ⁻¹ of soil
	PAB ₄₀ = 40 g biochar kg ⁻¹ of soil
	AAB ₁₀ = 10 g biochar kg ⁻¹ of soil
	AAB ₂₀ = 20 g biochar kg ⁻¹ of soil
	AAB ₄₀ = 40 g 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. Dehydrogenase was assessed in soil samples of pot experiments using standard procedures as described by Casida et al. [18], and urease, acid and alkaline phosphatase activities were assessed by the process as described by Tabatabai and Bremner [19].

2.5 Statistical analyses

Data generated as a result of experimentation were analysed statistically by the method of Analysis of Variance as described by Gomez et al. [20] using SPSS. The significance of treatment effect was judged with the help of variance ratio test. Critical difference (CD) at 5 percent level of significance was worked out to determine the difference between treatment means.

3. Results and discussions

3.1 Soil urease

The soil urease enzyme activity is closely associated with the average activity of microbial populations and has most commonly used as a biological indicator of soil quality. The urease enzyme activity was measured in the post-harvest soil and the data is presented in table 3. Perusal of the data revealed that the effect of fluoride and different biochar application at varied levels have significant effect on activity of urease enzyme 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 and 28.15%, 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.

The addition of biochars in soil improving the urease activities has been reported in potted rape and other plants [13]. Jiang et al [14] showed that the addition of biochars increases the urease activity with biochar added in soil were higher than that of the control, and the activities increased as the amount of biochar added elevated, which showed the same results as reported by Anuradha et al. [15]. Differing from the inhibition of urease activity caused by corn cob biochars [16], the biochars derived from swine-manure contained more nutrients than plant based biochars [17], which would enhance the microorganism activities and the urease activities.

3.2 Soil acid phosphatase

Phosphatase is an enzyme responsible for hydrolyzing inorganic and organic P compounds so that they become available to the plants. Phosphatases are the substrate inducible and the intensity of excretion by roots and microorganisms is determined by their requirement for orthophosphate. The activity of acid phosphatase in the analyzed soils was highly and significantly varied by the increasing degree of soil pollution with fluorine and varied levels of biochar application. In the soil sampled after 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 found to be significant and the 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.

Kotroczó et al. [21] observed that soil enzyme activity is stimulated by root reactions. The surface of fresh biochar can provide substrates for enzymatic reactions and stimulate plant roots to exude enzymes into the soil [22] which might be possibly related to the soil temperature; however, further verification of this possibility is required.

3.3 Soil 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 showed that the highest alkaline phosphatase activity was observed in the PAMB40 followed by PAMB20, PAMB10 and AMB40 which increases the activity by 31.1, 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 found to be significant and the highest alkaline phosphatase activity was observed in the combination with 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 they were found statistically at par with each other. The same results of statistically at par is also have been observed in the case of phosphoric acid modified biochar.

3.4 Soil dehydrogenase

The soil dehydrogenase enzyme activity is closely associated with the average activity of microbial populations and has most commonly used as a biological indicator of soil quality. The dehydrogenase enzyme activity was measured in the post-harvest soil and expressed in μg Tri Phenyl Formazan (TPF) produced per gram soil per day, and is presented in table 6. Perusal of the data revealed that the effect of fluoride and different biochar application at varied levels have significant effect on activity of dehydrogenase enzyme in the post-harvest soil.

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). AMB20 and AMB40 was also increases the dehydrogenase activity by 30.1 and 34.7%, respectively over control. 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.

Telesiński et al. [23] 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 [24, 25, 26]

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

<i>Biochar doses</i>	<i>Fluoride levels</i>		
	250	1000	Mean
<i>Control</i>	39.10	41.82	40.46
<i>NB@10 g/kg</i>	41.14	43.18	42.16
<i>NB@20 g/kg</i>	43.18	44.54	43.86
<i>NB@40 g/kg</i>	45.22	46.58	45.90
<i>AMB@10 g/kg</i>	48.28	49.98	49.13
<i>AMB@20 g/kg</i>	49.64	49.30	49.47
<i>AMB@40 g/kg</i>	51.00	52.70	51.85
<i>PAMB@10 g/kg</i>	44.54	46.92	45.73
<i>PAMB@20 g/kg</i>	48.96	51.34	50.15
<i>PAMB@40 g/kg</i>	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 AlKl

Phosphotase

Biochar doses	Fluoride levels		
	250	1000	Mean
<i>Control</i>	79.91	72.30	76.10
<i>NB@10 g/kg</i>	81.82	90.36	86.09
<i>NB@20 g/kg</i>	83.68	94.75	89.21
<i>NB@40 g/kg</i>	84.28	96.98	90.63
<i>AMB@10 g/kg</i>	84.69	101.44	93.07
<i>AMB@20 g/kg</i>	85.16	103.64	94.40
<i>AMB@40 g/kg</i>	85.72	104.34	95.03
<i>PAMB@10 g/kg</i>	87.16	104.76	95.96
<i>PAMB@20 g/kg</i>	89.16	106.00	97.58
<i>PAMB@40 g/kg</i>	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

Phosphotase

Biochar doses	Fluoride levels		
	250	1000	Mean
<i>Control</i>	96.58	202.21	149.39
<i>NB@10 g/kg</i>	158.48	248.85	203.67
<i>NB@20 g/kg</i>	171.34	266.09	218.72
<i>NB@40 g/kg</i>	174.94	271.92	223.43
<i>AMB@10 g/kg</i>	191.36	292.80	242.08
<i>AMB@20 g/kg</i>	234.83	338.47	286.65
<i>AMB@40 g/kg</i>	235.72	340.06	287.89
<i>PAMB@10 g/kg</i>	243.83	348.58	296.21
<i>PAMB@20 g/kg</i>	283.83	389.83	336.83
<i>PAMB@40 g/kg</i>	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
<i>Control</i>	12.93	12.19	12.56
<i>NB@10 g/kg</i>	13.93	13.05	13.49
<i>NB@20 g/kg</i>	14.67	13.80	14.24
<i>NB@40 g/kg</i>	15.32	14.50	14.91
<i>AMB@10 g/kg</i>	16.08	14.98	15.53
<i>AMB@20 g/kg</i>	16.38	16.32	16.35
<i>AMB@40 g/kg</i>	16.93	16.94	16.93
<i>PAMB@10 g/kg</i>	17.59	17.71	17.65
<i>PAMB@20 g/kg</i>	18.65	18.07	18.36
<i>PAMB@40 g/kg</i>	21.17	18.73	19.95
Mean	16.36	15.63	
CD	F	B	F X B

	0.47	1.05	NS
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*NB= Normal biochar, AMB= Alum modified biochar, PAMB= Phosphoric acid modified biochar

4. CONCLUSION

The addition of fluorine in the soil increases the enzymatic activities of soil which would be beneficial for the high use efficiency of soil carbon and nitrogen by microorganisms. This would result in favourable microorganism proliferation, which increased the microorganism activities and enzymatic reactions. This study also showed that the addition of alum and phosphoric acid modified biochar significantly improved the activities of urease, dehydrogenase and phosphatase (acid and alkaline), indicating that the application of modified biochar in soil was beneficial to improve the effectiveness of nutrients in the soil and stimulate the increase of enzyme activities in fluoride contaminated soil. This study indicated that phosphoric acid and alum modified biochar could be used as a sustainable amendment for improvement of soil quality, which would take a vital role in agriculture.

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