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Effect of Different Arsenic and Biochar Levels on Soil Microbial Population and Enzymatic Activity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Arsenic (As) poses a pervasive environmental contamination problem on a global scale. Human activities have significantly contributed to the extensive presence of arsenic (As) in soils. Recently, there has been growing interest in exploring the potential of biochar in addressing the issue of As-contaminated soils. This study focused on evaluating the effects of two types of biochar, namely straw biochar and iron-modified biochar, on the composition of soil microbial communities and enzymatic activity in soil contaminated with arsenic. After conducting a pot experiment for a duration of 9 months, the microbial communities and enzymatic activity were analyzed. Biochar refers to carbon-rich porous solids that are produced by heating biomasses under low oxygen

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conditions. These biochars are regarded as environmentally friendly sorbents that can be employed for the treatment of different types of arsenic contamination. The increased abundance of soil microbial populations and the enhanced enzymatic properties suggest that biochar fosters the richness and diversity of bacterial communities. Consequently, these improvements in the soil environment and biological quality highlight the potential of iron-modified biochar as an alternative agent for remediating arsenic-contaminated soils.

Keywords: Soil biological property; arsenic contamination; biochar; MTU-7029.

1. INTRODUCTION

Arsenic (As) is one of the most dangerous metals found in agricultural soils due to its toxicity to the growth and development of plants, animals, and microbes [1]. It poses a risk to human health through the food chain as well [2]. Currently, the contamination of soil and water with arsenic has become a global problem [3,4]. There is a growing focus on finding ways to remove arsenic from agricultural soils and water or to reduce its availability [5,6]. The structure of bacterial communities, which reflects soil's physical and chemical processes, is considered a crucial indicator of soil function and fertility [7]. Changes in microbial communities can be associated with a decrease in available Arsenic (As) after the addition of biochar. Microorganisms can serve as indicators of heavy metal toxicity, such as Arsenic, while also reflecting soil function and fertility. Biochar has garnered research interest as a potential solution for the sorption of aqueous arsenic, as well as other metal and metalloid contaminants, due to its low production costs and environmentally friendly nature as it is typically derived from organic waste products [8]. However, further research is required to develop economically viable biochar treatment processes that enhance its sorption efficiency compared to untreated biomass (Zhu et al., 2016). Unmodified biochar, with its negatively charged surface, may not be an effective sorbent for arsenic oxyanions due to static repulsion [5]. Iron-reducing bacteria can cause the release of a significant amount of arsenic into the soil solution by reducing As (V) that is adsorbed onto iron oxides to As (III), which is less adsorbed [9]. Increased levels of dissolved organic matter resulting from biochar addition promote the reductive dissolution of Fe (III) minerals mediated by microbes, thereby facilitating the release of arsenic and affecting its speciation and mobilization in soils [10,11]. Studies have demonstrated that iron has a strong affinity for arsenic and can be utilized for soil remediation [12,8,13]. It has also been shown that poorly crystalline Fe-oxyhydroxy sulfate effectively removes arsenic from soils [14]. While numerous studies have investigated the toxicity of arsenic to rice plants, only a limited number have focused on microorganisms, which play a crucial role as decomposers in the soil ecosystem [15,13,16,17]. In this initial phase of studying the effects of arsenic on soil microorganisms, the present research aimed to identify differences in microbial populations between arsenic-polluted and unpolluted soils.

2. MATERIALS AND METHODS

2.1 Experimental Details

Prior to transplanting, the field was thoroughly ploughed and flooded for puddling and levelling, with the initial soil conditions showing a pH of 7.75, electrical conductivity of 0.26 dS m^{-1}, organic carbon content of 0.49%, and available nitrogen, phosphorus, and potassium levels of 162 kg ha^{-1}, 16.4 kg ha^{-1}, and 216 kg ha^{-1} respectively. The dehydrogenase and alkaline phosphatase activity in the soil were measured at 53 µgTPF g^{-1} soil day^{-1} and 38 µg pNP g^{-1} soil h^{-1} respectively. After thorough mixing, the soil was filled into pots.

To create a stock solution of $Na_2HAsO_4.7H_2O$, 2.08 grams of sodium arsenate salt were dissolved in a small amount of water, and the volume was raised to 1000 ml using milli Q water, resulting in a concentration of 1000 mg L^{-1}. Different concentrations of arsenic (As), namely 50 and 100 mg kg^{-1}, were prepared from the stock solution and applied to the soil in the evening using a burette. The treated soil was mixed thoroughly and incubated for a month. The recommended fertilizer dose for nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) were 120, 60, and 60 kg ha^{-1} respectively, which was calculated accordingly for 10 kg of soil. Wheat straw obtained from the Institute of Agricultural Sciences farm was harvested, dried, and crushed using a cutting machine with a pore size of 2 mm. The crushed samples were then subjected to a temperature of 550 °C in a drum for 3 hours to produce biochar. The wheat straw

biochar was immersed in a $FeCl_3$ solution. A quantity of 10 g of biochar was mixed with 100 ml of $FeCl_3$ solution (0.75 mol L^{-1}) for 24 hours, filtered, and dried at room temperature. Subsequently, it was oven-dried at 80°C for 24 hours. The biochar was applied one week before transplanting.

- For the biological analysis, soil samples were collected from the rice field at 40, 80, and 120 days to determine the activity of dehydrogenase and alkaline phosphatase. The dehydrogenase activity (DHA) was measured using the assay described by Casida et al. [18], while alkaline phosphatase and urease activity were determined following the procedures of Tabatabai and Bremner [19]. The population counts of bacteria, fungi, and actinomycetes were determined using the dilution plate technique suggested by Subba Rao [20] with nutrient agar (NA), potato dextrose agar medium (PDA), and Kenknight's media respectively. The rice variety used in the pot experiment was MTU-7029, a commonly grown variety in Uttar Pradesh.

2.2 Treatment Details

T1: Recommended dose of fertilizer; T2: RDF + As @ 50 mg kg^{-1} , T3: RDF + As @ 100 mg kg^{-1} , T4: RDF + simple biochar @ 7.5 t ha^{-1} , T5 : RDF + simple biochar @ 10 t ha^{-1} , T6: RDF + Fe enriched biochar @ 7.5 t ha^{-1} , T7: RDF + Fe enriched biochar @ 10 t ha^{-1} , T8: RDF + As @ 50 mg kg^{-1} + simple biochar 7.5 t ha^{-1} , T9: RDF + As @ 50 mg kg^{-1} + simple biochar 10 t ha^{-1}, T10: RDF + As @ 100 mg kg^{-1} + simple biochar 7.5 t ha^{-1} , T11: RDF + As @ 100 mg kg^{-1} + simple biochar 10 t ha^{-1}, T12: RDF + As @ 50 mg kg^{-1} + Fe enriched biochar @ 7.5 t ha^{-1} , T13: RDF + As @ 50 mg kg^{-1} + Fe enriched biochar @ 10 t ha^{-1} , T14: RDF + As @ 100 mg kg^{-1} + Fe enriched biochar @ 7.5 t ha^{-1} , T15: RDF + As @ 100 mg kg^{-1} + Fe enriched biochar @ 10 t ha^{-1}

3. RESULTS AND DISCUSSION

3.1 Microbial Population

Results depicted in Table 1 show that the bacterial population significantly varies in application of biochar (simple and iron enriched) and arsenic in pot soil. Highest bacterial population was found in treatment T5 (RDF + simple biochar @ 10 t ha^{-1}) followed by T7 (RDF + Fe enriched biochar @ 10 t ha^{-1}) and T4 (RDF + Simple biochar 7.5 t ha^{-1}) whereas, the minimum bacterial population was found with T3 (RDF + As @ 100 mg kg^{-1}). It was observed that the application of simple biochar as well as Fe enriched improved the bacterial population significantly as compared to arsenic-contaminated soil. A similar trend was reported by Ghosh et al., [21], Pan et al., [22] and Pathak et al., [23]. The fungal population in the study show that it significantly varies with biochar and arsenic-treated pot soil. The highest fungal population was observed in the treatment T5 which was statistically at par with T7, T4 and T1. The lowest value of fungal population was recorded in treatment T3, where only As was applied a high dose. So it was observed that the effect of arsenic toxicity decreases the fungal population [24].

The same result recorded by the actinomycetes population shown in Table 1 and Fig. 4. Results revealed that the maximum actinomycetes population was recorded by treatment T4 which is at par with T6, T5 and T7 whereas, the minimum actinomycetes population was recorded in T3 followed by T2. It shows that the application of biochar (simple and Fe enriched) leads to the significant increase in the actinomycetes population found in arsenic contain soil. But the combination of biochar with As, the significantly higher actinomycetes population were recorded by treatment T13 at par with T12 and T9 at par with T8.

3.2 Enzyme Activity

In this section, we will discuss the effect of different doses of simple and iron-enriched biochar and arsenic toxicity on soil enzymatic properties.

Dehydrogenase activity in the soil was observed in the pot experiment and shown in Table 1 and depicted in Fig. 4 revealed that the highest dehydrogenase activity was found in the treatment T7 which is at par with T5 and the lowest value of dehydrogenase activity was found in treatment T3 followed by T2, T10 and T14. Application of biochar with arsenic also increases dehydrogenase activity significantly viz. treatment T15 at par with T11 and T12 followed by T8. It shows that dehydrogenase activity was reduced in arsenic with biochar-applied soil. In the case of urease enzyme activity, the highest value was reported in T5 which is statistically at par with treatments T6

and T4, where, the simple and Fe-enriched biochar was applied with a high dose. Whereas, the minimum urease activity was found in treatment T3 (only As was applied with a high dose). Combinedeffects of biochar and As applied also show significant results of higher urease activity were reported with the treatments T9 which is statistically at par with treatments T12 and T13.

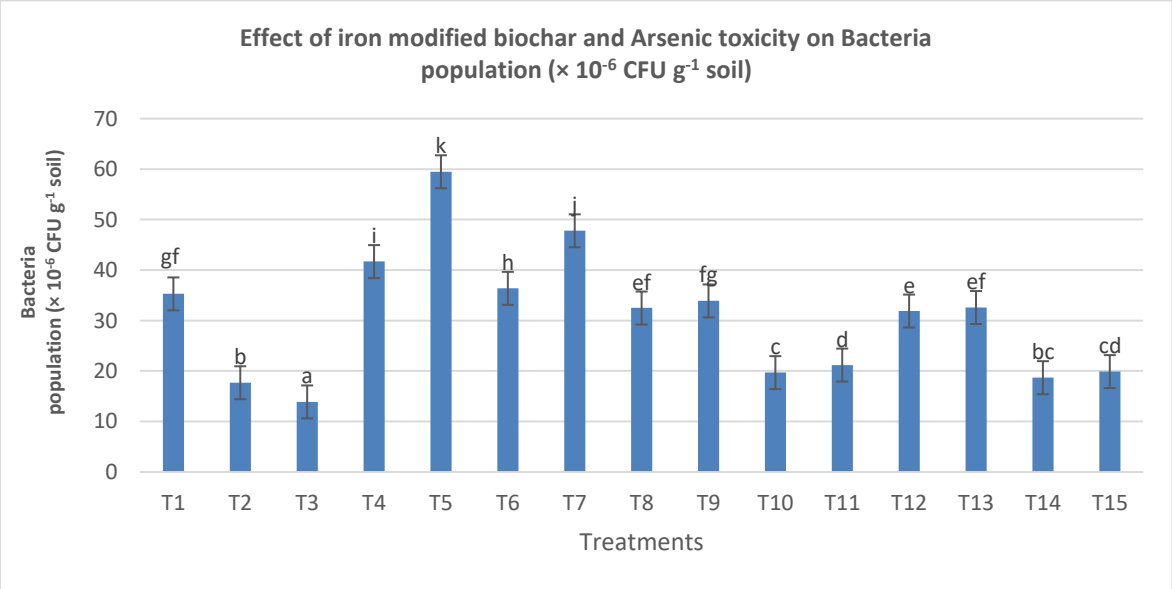

Fig. 1. Impact of modified biochar on soil bacterial population. Within each column, means that are followed by comparable lowercase letters are not significantly different (p≤ 0.05, Duncan's multiple range tests). Vertical bars show the ± standard error of the mean

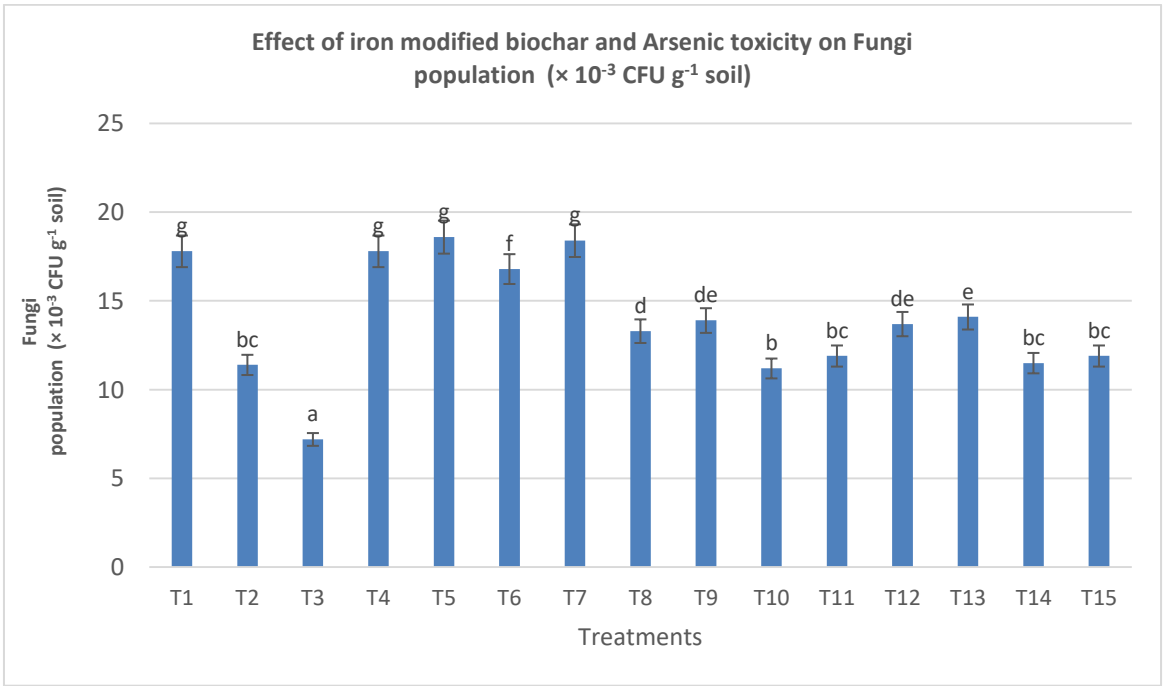

Fig. 2. Effect of modified biochar on soil fungal population. Within each column, means that are followed by comparable lowercase letters are not significantly different (p≤ 0.05, Duncan's multiple range tests). Vertical bars show the ± standard error of the mean

Table 1. Biological properties influenced by different treatments

Treatments	Bacteria (× 106 CFU g^{-1} soil)	Fungi (×103 CFU g^{-1} soil)	Actinomycetes (× 105CFU g^{-1} soil)	Dehydrogenase (µg TPFg^{-1} soil day^{-1})	Urease Activity (µg Urea Hydrolyzed g^{-1} Soil h^{-1})	Alkaline Phosphatase (µg p-NP formed g^{-1} soil h^{-1})
T1	35.3gh	17.8g	22.4f	62.5g	111e	67.6f
T2	17.7b	11.4bc	15.8b	32.2b	82.6c	47.6b
T3	13.9a	7.20a	11.9a	21.7a	50.6a	42.4a
T4	41.7i	17.8g	23.2g	68.8i	127f	68.3f
T5	59.5k	18.6g	22.3fg	75.4j	135g	71.6g
T6	36.4h	16.8f	23.7g	66.3h	128f	67.6f
T7	47.8j	18.4g	22.1fg	75.7j	126f	72.9g
T8	32.5ef	13.3d	21.4def	46.7e	98.9d	60.1de
T9	33.9fg	13.9de	21.5f	55.6f	111e	62.8e
T10	19.7c	11.2b	20.6c	35.7c	71.7b	56.1c
T11	21.2d	11.9bc	20.8c	38.4d	71.5b	61.8e
T12	31.9e	13.7de	21.6ef	46.6e	102d	60.6de
T13	32.6ef	14.1e	21.9ef	60.7g	102d	62.5e
T14	18.7bc	11.5bc	20.9cd	37.6d	74.5b	56.4c
T15	19.9cd	11.9bc	20.8cde	38.2d	79.8c	58.3cd
SEM	0.50	0.25	0.32	0.67	1.57	1.04
CD 5%	1.45	0.71	0.93	1.93	4.54	3.00

Means followed by similar lowercase letters within each column are not statistically different (P≤0.05, Duncan's multiple range test

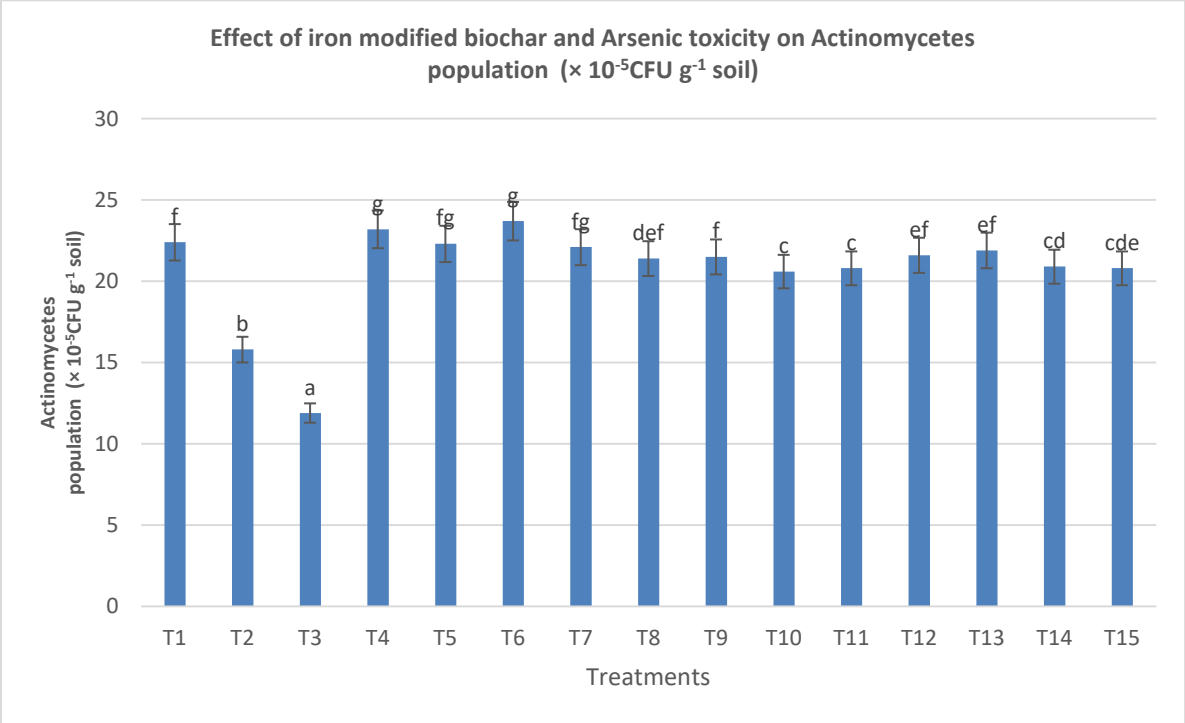

Fig. 3. Effect of modified biochar on the number of actinomycetes in soil. Within each column, means that are followed by comparable lowercase letters are not significantly different (p≤ 0.05, Duncan's multiple range tests). Vertical bars show the ± standard error of the mean

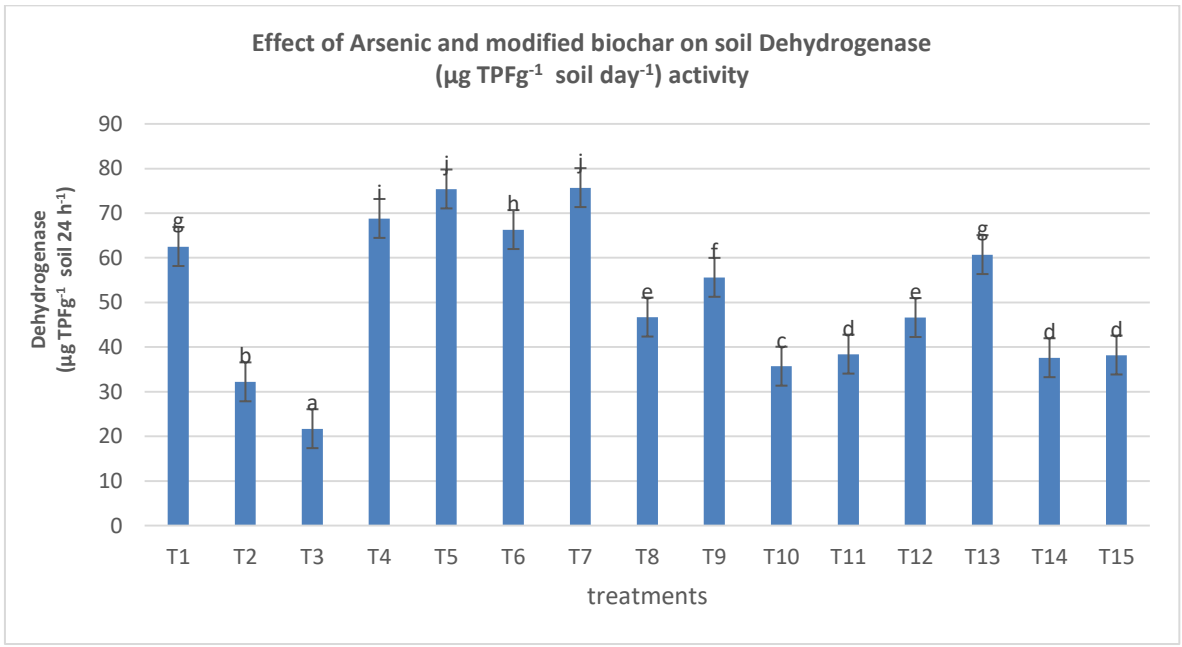

Fig. 4. Modified biochar's impact on the dehydrogenase activity in the soil. Within each column, means that are followed by comparable lowercase letters are not significantly different (p≤ 0.05, Duncan's multiple range tests). Vertical bars show ± standard error of the mean

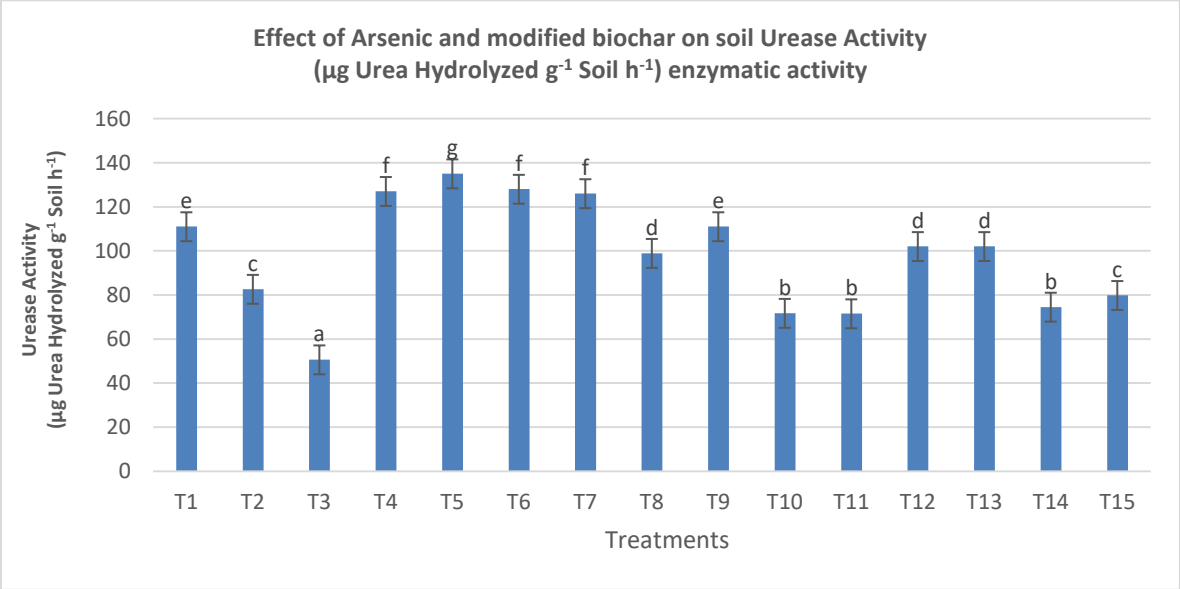

Fig. 5. Effect of modified biochar on urease activity in the soil. Means followed by letter similar lowercase letters within each and every column are not statistically different (p≤ 0.05, Duncan's multiple range tests). Vertical bars indicate ± Standard error of the mean

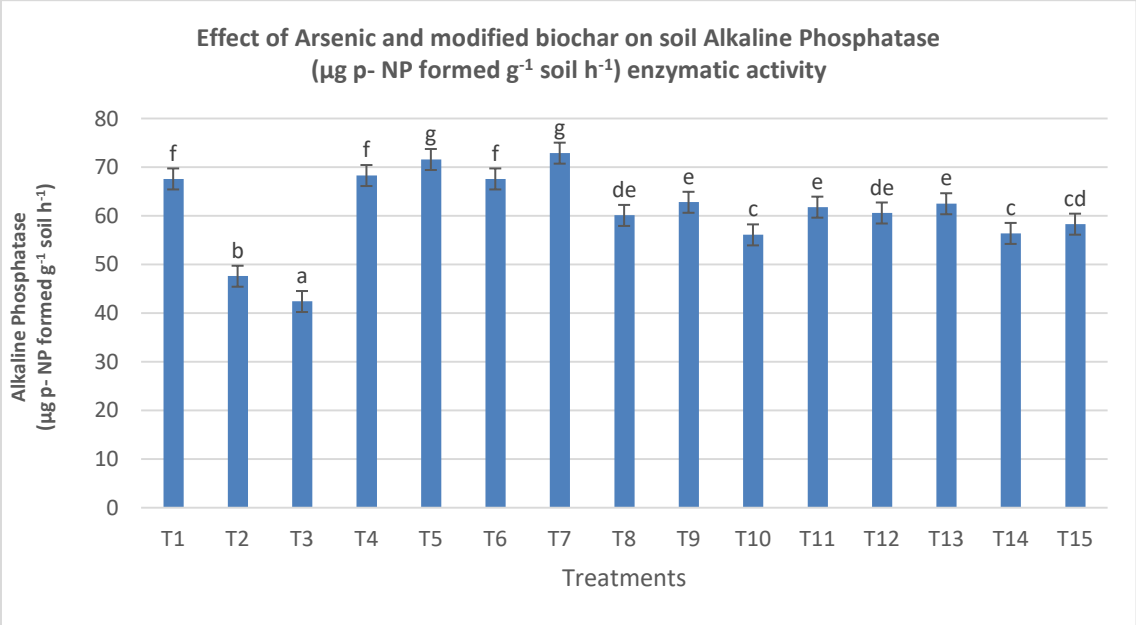

Fig. 6. Effect of modified biochar on alkaline phosphatase activity in soil. Means followed by similar lowercase letters within each column are not statistically different (p≤ 0.05, Duncan's multiple range tests).Vertical bars indicate ± Standard error of mean

The same trends show by APA activity, where, the highest value was recorded with treatment T7 which is statistically at par with treatment T5. The lowest APA activity was found in T3 followed by T2 (Table 1). The combined effect in biochar and As-treated soil was significantly show higher APA activity by T9 which is at par with T13.

4. CONCLUSION

This study reveals that applying simple and Fe-enriched biochar reduces the As toxicity and improves the microbial population and enzyme activity in soil. Microbial population and enzymatic activity was positively responses to

biochar application. These biological properties of the soil shows that how Fe-modified biochar reduces the toxicity of arsenic. Different Biochars application at dose of 10 t ha^{-1} has been found to enhance soil biological properties by mitigating the toxic effects of arsenic.

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CONFLICT OF INTEREST

The authors of this research paper declare that there are no conflicts of interest regarding the publication of this work. We have no financial or personal relationships that could inappropriately influence or bias the findings and interpretations presented in this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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