Effect of different arsenic and biochar levels on soil microbial population and enzymatic activity

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 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.

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 (Pandey et al., 2015). It poses a risk to human health through the food chain as well (Anawar et al., 2018). Currently, the contamination of soil and water with arsenic has become a global problem (Niazi et al., 2018; Shakoor et al., 2019). There is a growing focus on finding ways to remove arsenic from agricultural soils and water or to reduce its availability (Li et al., 2020; Zhai et al., 2020). The structure of bacterial communities, which reflects soil's physical and chemical processes, is considered a crucial indicator of soil function

and fertility (Elzobair et al., 2016). Changes in microbial communities can be associated with a decrease in available cadmium (Cd) after the addition of biochar. Microorganisms can serve as indicators of heavy metal toxicity, such as Cd, 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 (Wang et al., 2015a). 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 (Li et al., 2019). 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 (Dong et al., 2014). 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 (Chen et al., 2017; Chen et al., 2018). Studies have demonstrated that iron has a strong affinity for arsenic and can be utilized for soil remediation (Hu et al., 2014; Wang et al., 2015; Yang et al., 2015). It has also been shown that poorly crystalline Fe-oxyhydroxy sulfate effectively removes arsenic from soils (Yang et al., 2015). 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 (Tatsuyama et al., 1974; Yang and Ge 1984; Maliszewska et al., 1985; Nordgren et al., 1986). 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:

In the Kharif season of 2021 (November to April), a controlled experiment was conducted in a glasshouse at the Department of Soil Science and Agricultural Chemistry,

Institute of Agricultural Sciences, Banaras Hindu University in Varanasi, India. Varanasi is situated at an altitude of 80.2 m above mean sea level, with coordinates between 25°14′ and 25°23′N latitude and 82°56′ and 83°03′E longitude. Prior to transplanting, the field was thoroughly ploughed and flooded for puddling and leveling, with the initial soil conditions showing a pH of 8.02, electrical conductivity of 0.20dS m-1, organic carbon content of 0.47%, and available nitrogen, phosphorus, and potassium levels of 218.02 kg ha-1, 12.8 kg ha-1, and 216.7 kg ha-1 respectively. The dehydrogenase and alkaline phosphatase activity in the soil were measured at 53 µgTPF g-1 soilday-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 Na2HAsO4.7H2O, 2.08 grams of sodium arsenate salt were dissolved in a small amount of water, and the volume was raised to 1000 ml using milliQ 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 (P2O5), and potassium (K2O) was 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 FeCl3 solution. A quantity of 10 g of biochar was mixed with 100 ml of FeCl3 solution (0.75 mol/L) 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. (1964), while alkaline phosphatase and urease activity were determined following the procedures of Tabatabai and Bremner (1969). The population counts of bacteria, fungi, and actinomycetes were determined using the dilution plate technique suggested by Subba Rao (2017) 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. Result and discussion

3.1 Microbial population

Results depicted in table 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 application of simple biochar as well as Fe enriched improve the bacterial population significantly as compare to arsenic contaminated soil. Similar trend was reported by Ghosh *et al*., 2004, Pan *et al*., 2021 and Pathak *et al*., 2022. The fungal population in study show that it was significantly varies with biochar and arsenic treated pot soil. Highest fungal population was observed in the treatment T5 which was statistically at par with T7, T4 and T1. Lowest value of fungal population was recorded in treatment T3, where only As applied with high dose. So it observed that the effect of arsenic toxicity decreases the fungal population.

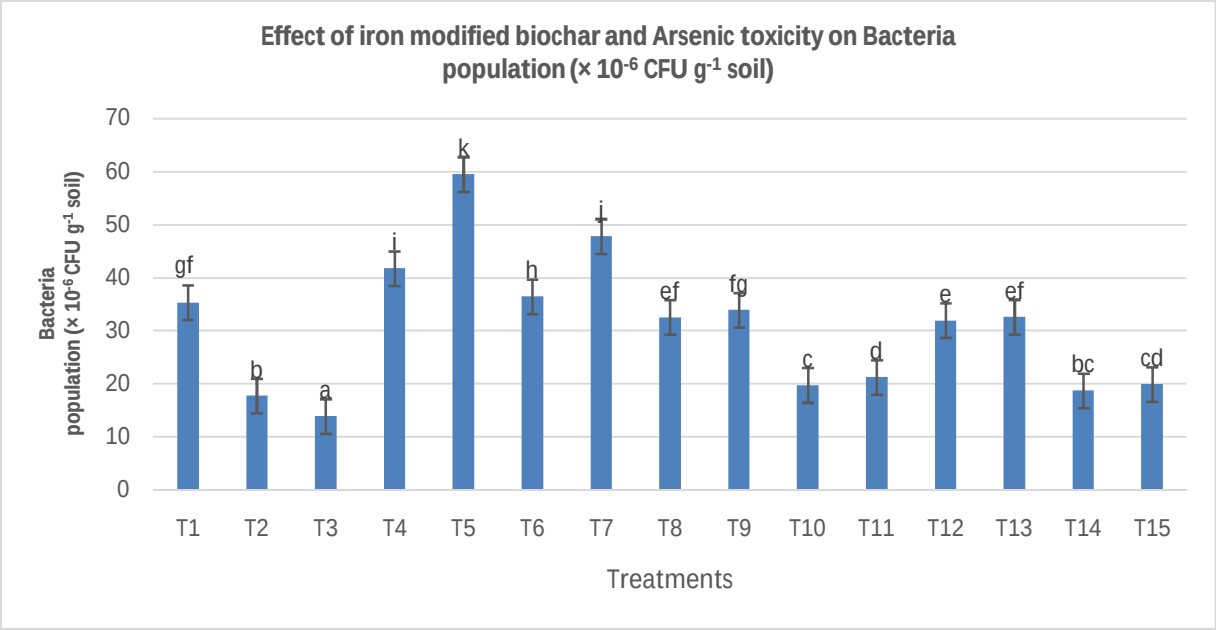

Fig. 1: Impact of modified biochar on soil bacterial population. Within each column, means that are followed by comparable lower case letters are not significantly different ($p \leq 0.05$, Duncan's multiple range tests). Vertical bars show the ± standard error of the mean.

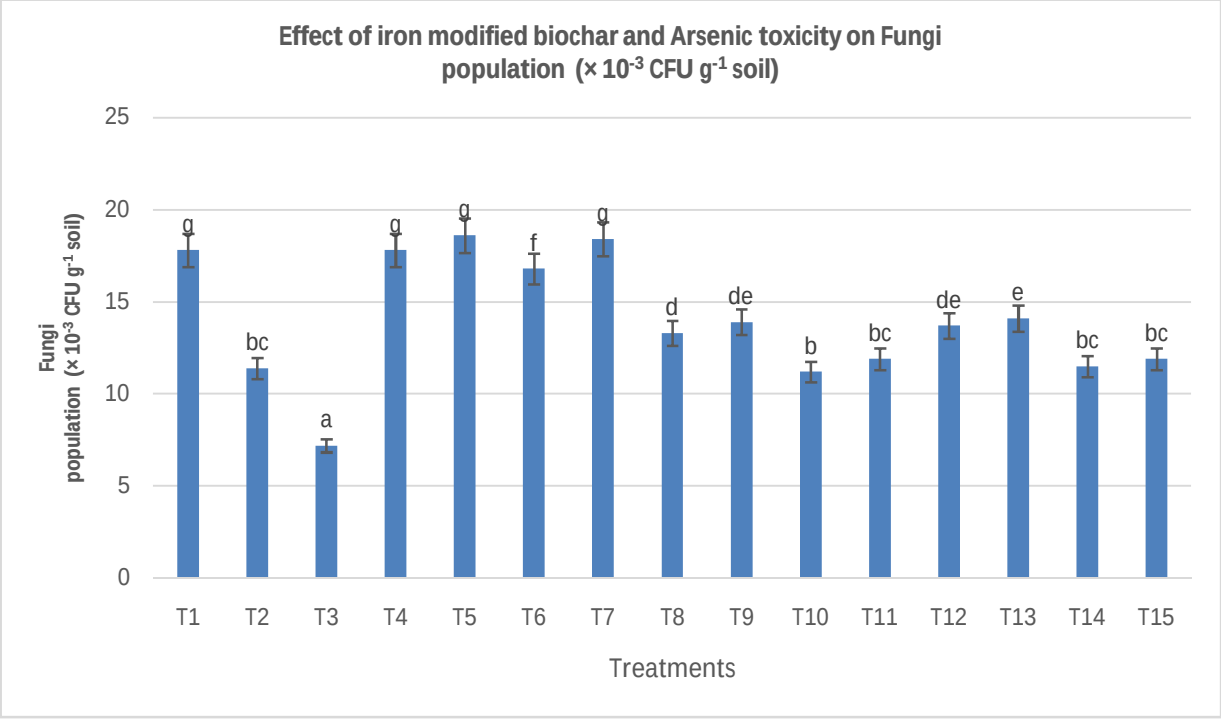

Fig. 2: Effect of modified biochar on soil fungal population. Within each column, means that are followed by comparable lower case letters are not significantly different (p≤ 0.05, Duncan's multiple range tests).Vertical bars show the ± standard error of the mean.

Same result recorded by actinomycetes population show in table 1 and fig. 4. Results revealed that 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 application of biochar (simple and Fe enriched) leads to significant increase in actinomycetes population was found in arsenic contain soil. But 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.

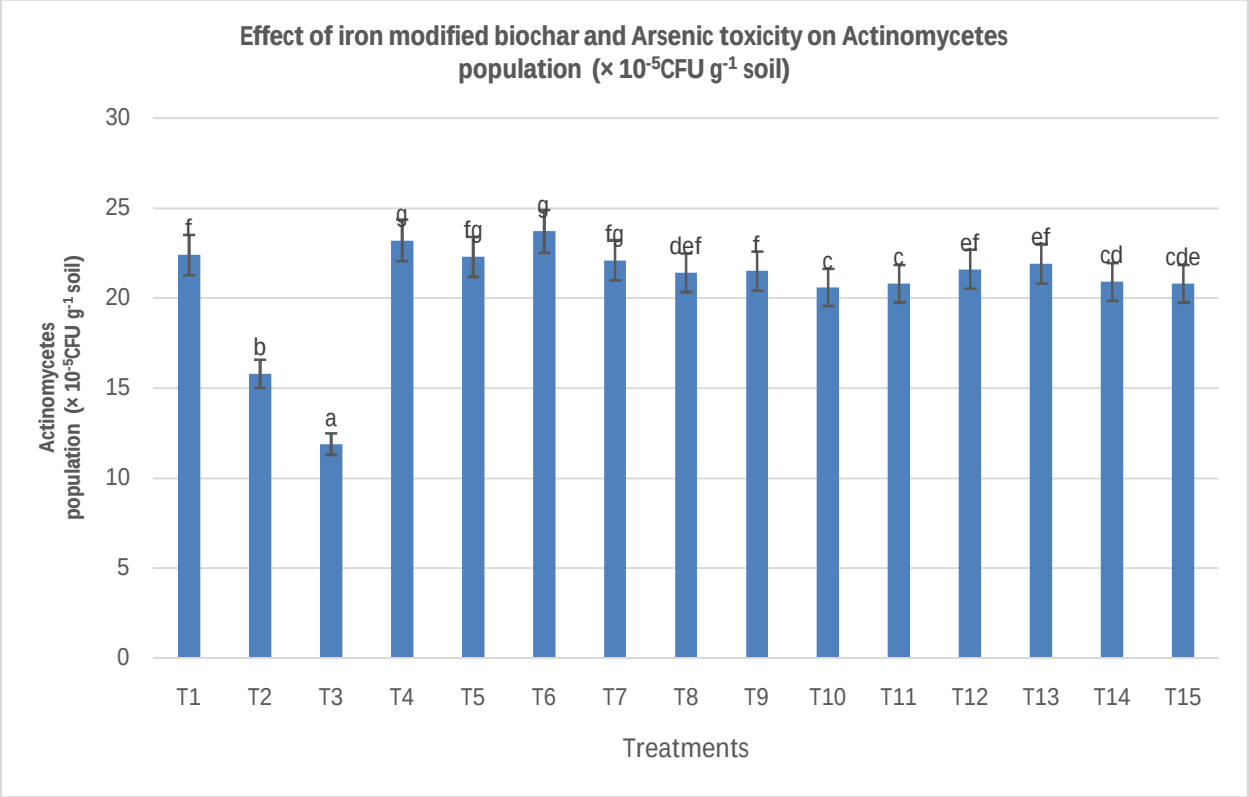

Fig. 3: Effect of modified biochar on the quantity of actinomycetes in soil. Within each column, means that are followed by comparable lower case letters are not significantly different (p≤ 0.05, Duncan's multiple range tests). Vertical bars show the ± standard error of the mean.

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} soil24 h^{-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 a similar lowercase letters within each column are not statistically different (P≤0.05, Duncan's multiple range test

3.2 Enzyme activity

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

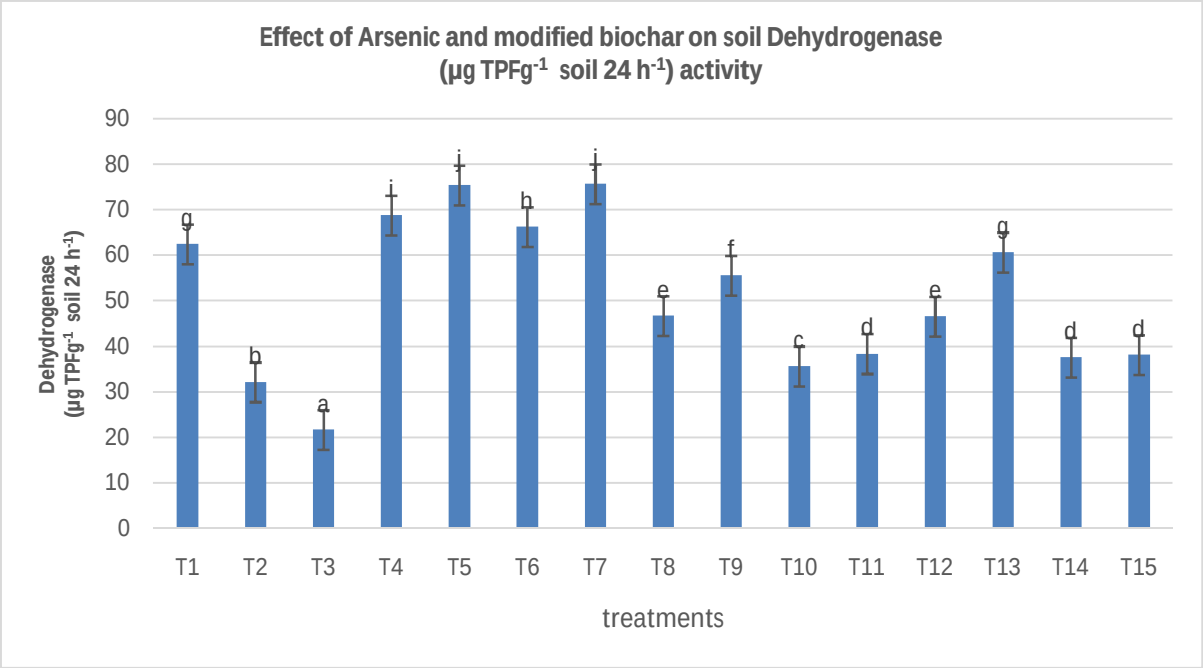

Fig. 4: Modified biochar's impact on the dehydrogenase activity in soil. Within each column, means that are followed by comparable lower case letters are not significantly different ($p \leq 0.05$, Duncan's multiple range tests).Vertical bars show ± standard error of the mean.

Dehydrogenase activity in the soil was observed in pot experiment and shown in table 1 and depicted in fig 4 revealed that highest dehydrogenase activity was found in the treatment T7 which is at par with T5 and 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 show that dehydrogenase activity was reduces in arsenic with biochar applied soil. In 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 high dose. Whereas, the minimum urease activity was found in treatment T3 (only As was applied with high dose). In combine effect of biochar and As applied also show significantly result of higher urease activity were reported with the treatments T9 which is statistically at par with treatments T12 and T13.

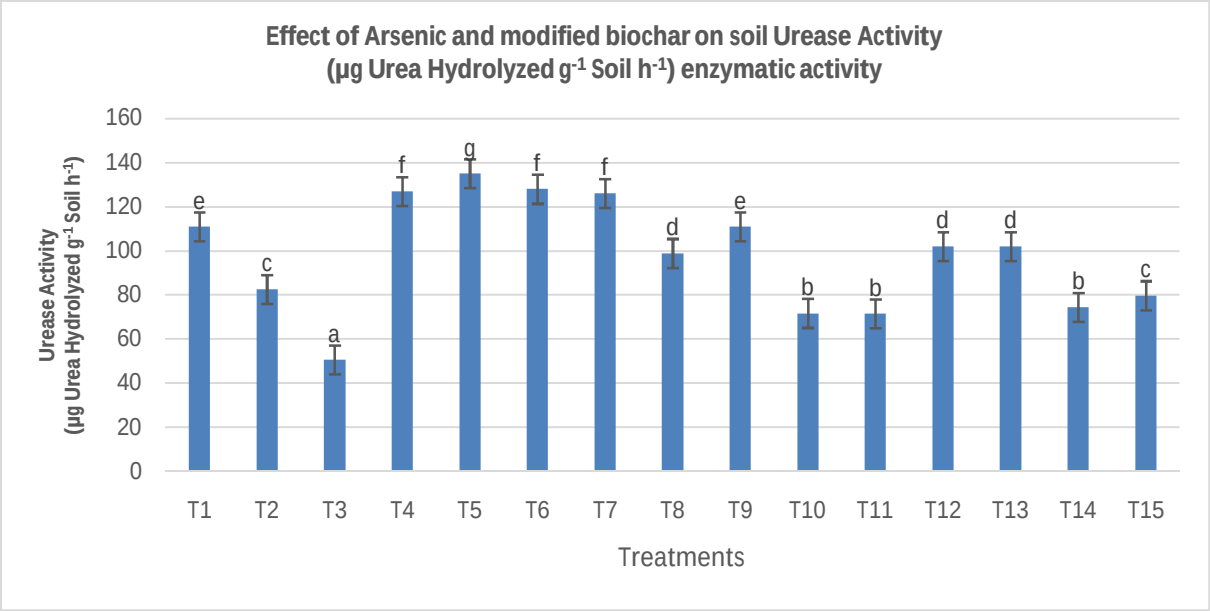

Fig. 5: Effect of modified biochar on urease activity in soil. Means followed by a similar lower case letters within each and every column are not statistically different ($p \leq 0.05$, Duncan's multiple range tests).Vertical bars indicate ± Standard error of mean

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

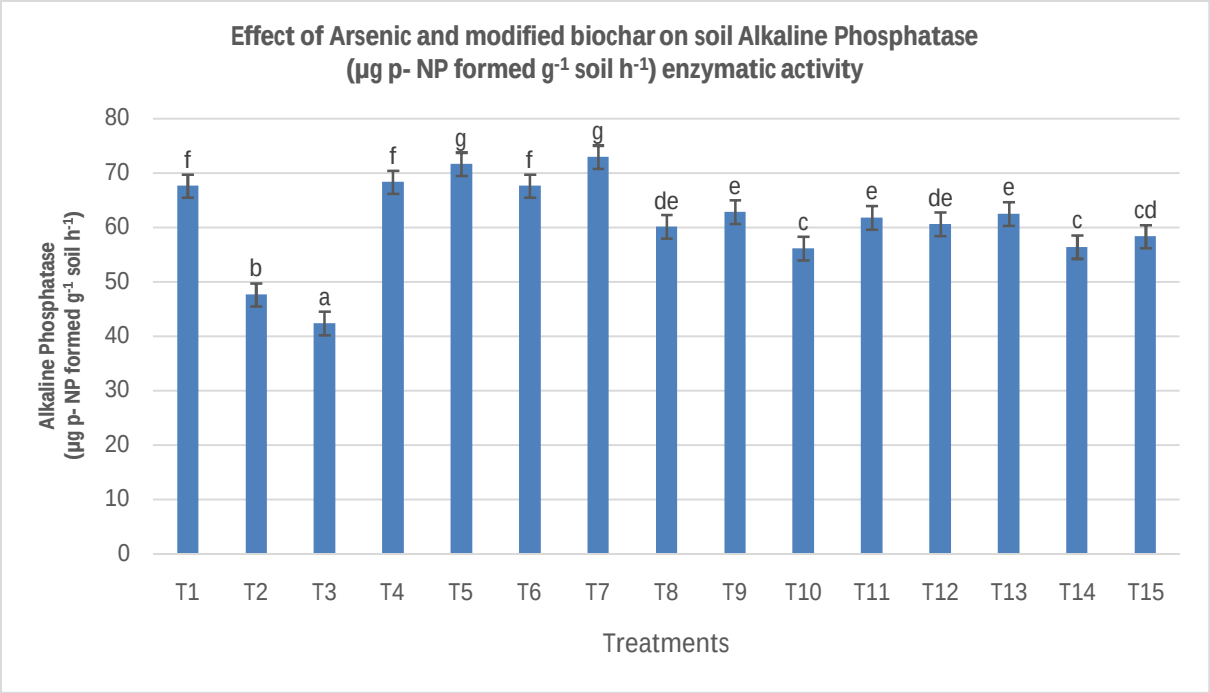

Fig. 6: Effect of modified biochar on alkaline phosphatase activity in soil. Means followed by a similar lower case letters within each column are not statistically different ($p \leq 0.05$, Duncan's multiple range tests).Vertical bars indicate ± Standard error of mean.

Conclusion

This study reveals that application of simple and fe-enriched biochar reduce the As toxicity and improve 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.

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