

Effect of different ~~arsenic~~ Arsenic and ~~B~~ 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~~ 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.

[Keywords missing](#)

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~~levelling, 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⁻¹soil 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 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 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 FeCl₃ solution. A quantity of 10 g of biochar was mixed with 100 ml of

Comment [TTS1]: Not necessary put this information in the introduction

Formatted: Font: Subscript

Formatted: Font: Subscript

Formatted: Font: Subscript

Formatted: Font: Subscript

Formatted: Font: Subscript

Formatted: Font: Subscript

Formatted: Font: Subscript

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.

Formatted: Font: Subscript

Comment [TTS2]: There are many recent references, 1964, 1969, 1986 are too old.

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

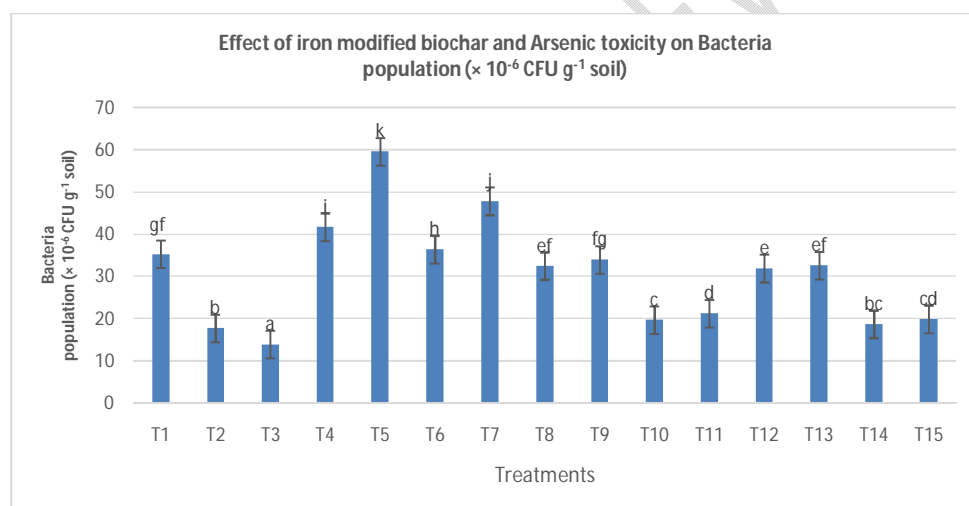


Fig. 1: Impact of modified biochar on soil bacterial population. Within each column, means that are followed by comparable ~~lower ease~~ lowercase letters are not significantly different ($p \leq 0.05$, Duncan's multiple range tests). Vertical bars show the \pm standard error of the mean.

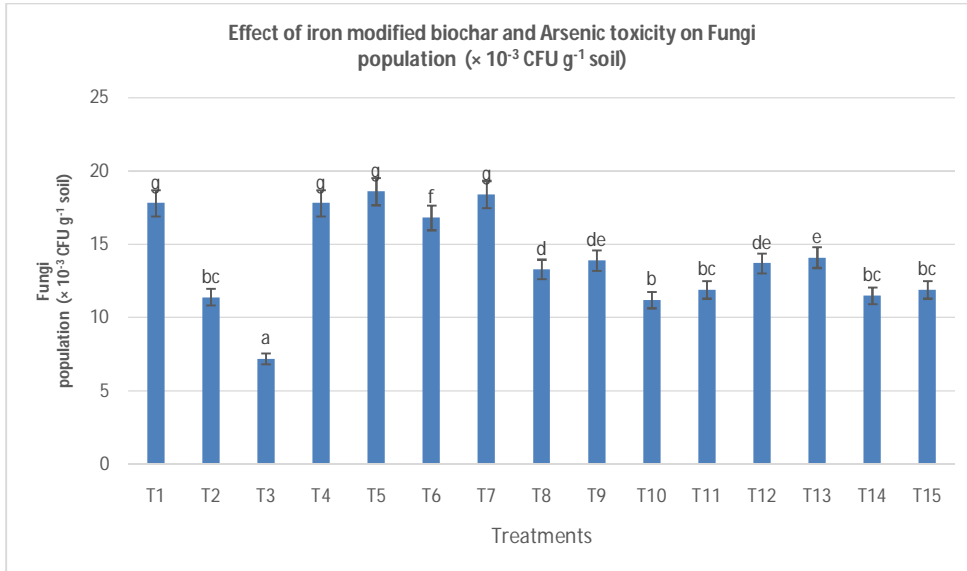


Fig. 2: Effect of modified biochar on soil fungal population. Within each column, means that are followed by comparable ~~lower case~~ letters are not significantly different ($p \leq 0.05$, Duncan's multiple range tests). Vertical bars show the \pm standard error of the mean.

~~Same-The same~~ result recorded by ~~the~~ actinomycetes population ~~show shown~~ in ~~table-Table~~ 1 and ~~fig-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 ~~was~~ 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.

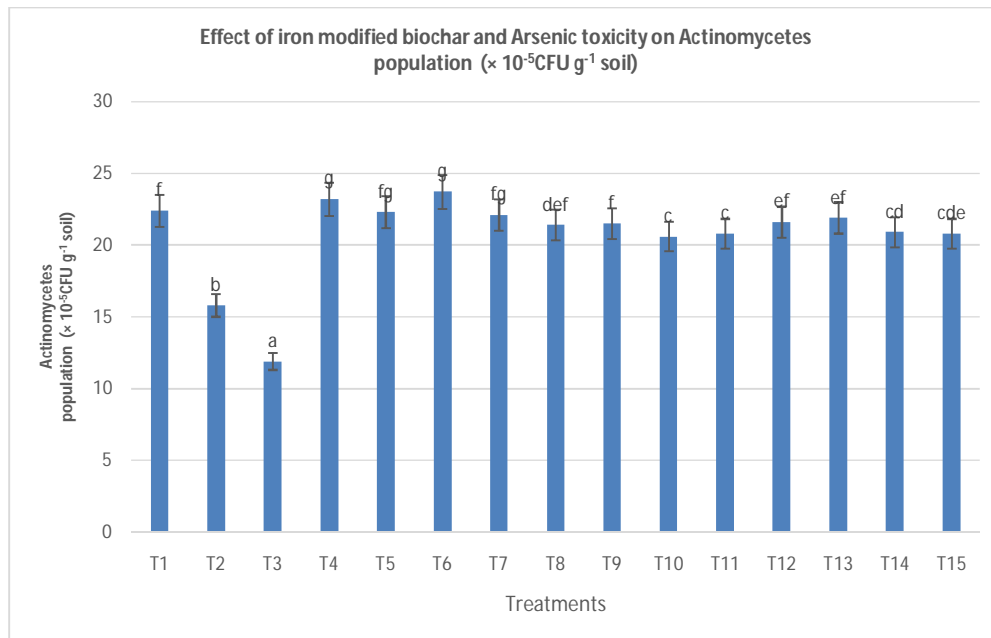


Fig. 3: Effect of modified biochar on the quantity-number of actinomycetes in soil. Within each column, means that are followed by comparable lower case lowercase letters are not significantly different ($p \leq 0.05$, Duncan's multiple range tests). Vertical bars show the \pm standard error of the mean.

Table 1. Biological properties influenced by different treatments

Treatments	Bacteria ($\times 10^6$ CFU g ⁻¹ soil)	Fungi ($\times 10^3$ CFU g ⁻¹ soil)	Actinomycetes ($\times 10^5$ CFU g ⁻¹ soil)	Dehydrogenase ($\mu\text{g TPFg}^{-1}$ soil 24 h ⁻¹)	Urease Activity ($\mu\text{g Urea}$ Hydrolyzed g ⁻¹ Soil h ⁻¹)	Alkaline Phosphatase ($\mu\text{g p-NP}$ 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
<i>SEM</i>	0.50	0.25	0.32	0.67	1.57	1.04
<i>CD 5%</i>	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 ($P \leq 0.05$, Duncan's multiple range test)

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.

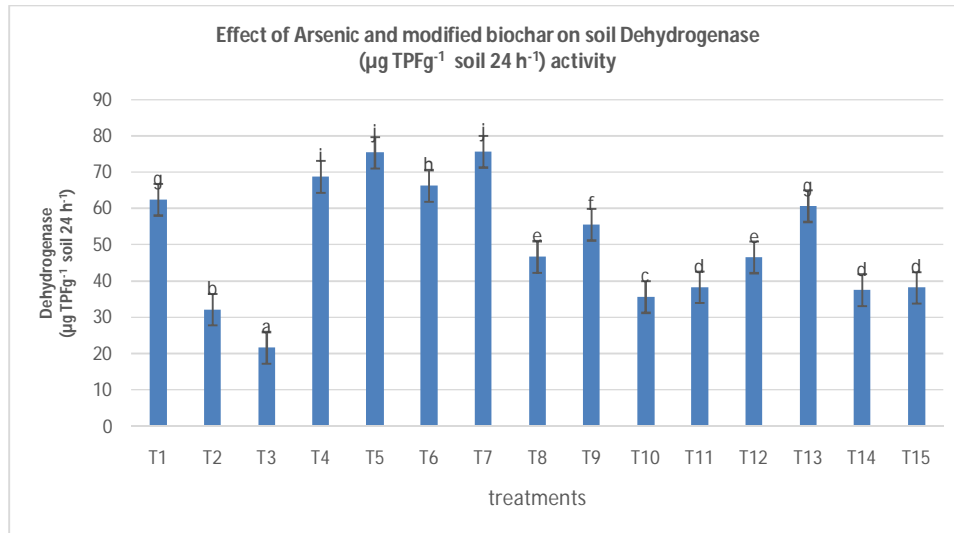


Fig. 4: Modified biochar's impact on the dehydrogenase activity in the soil. Within each column, means that are followed by comparable lower case letters are not significantly different ($p \leq 0.05$, Duncan's multiple range tests). Vertical bars show \pm standard error of the mean.

Dehydrogenase activity in the soil was observed in the pot experiment and shown in table-Table 1 and depicted in fig-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 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). In combine Combined effect-effects of biochar and As applied also show significantly significant result-results of higher

urease activity were reported with the treatments T9 which is statistically at par with treatments T12 and T13.

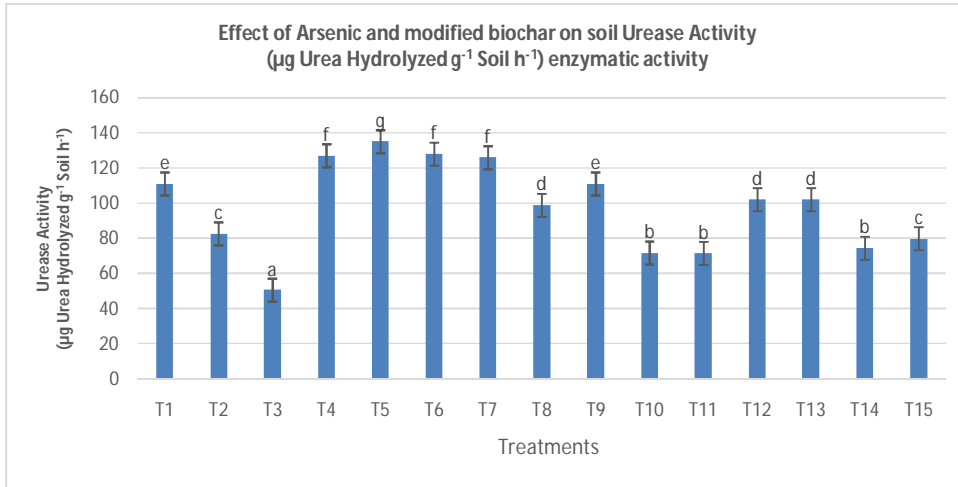


Fig. 5: Effect of modified biochar on urease activity in the soil. Means followed by a similar lower case letters within each and every column are not statistically different ($p \leq 0.05$, Duncan's multiple range tests). Vertical bars indicate \pm Standard error of the mean

Same trends show by APA activity, where, the highest value was recorded with treatment T7 which is statistically at par with treatment T5. Whereas, 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 the higher APA activity by T9 which is at par with T13.

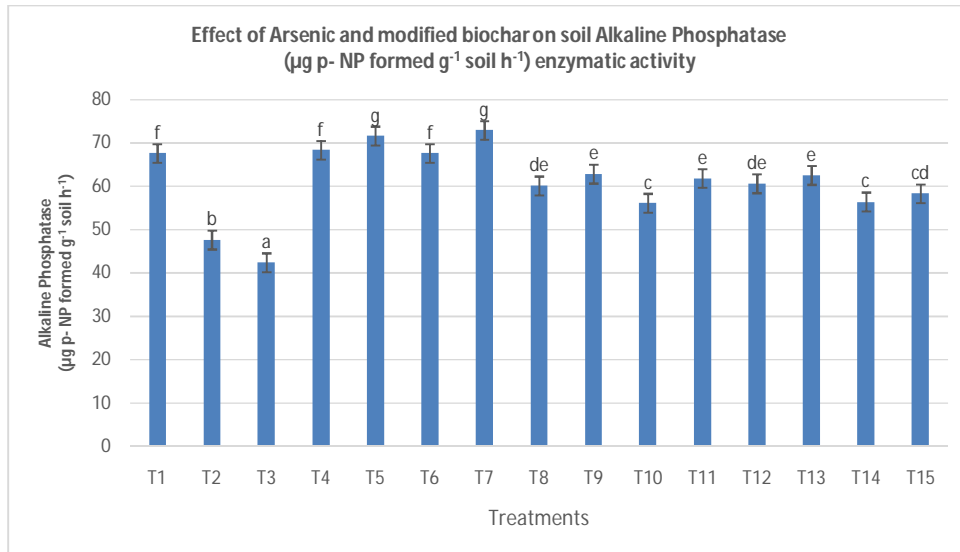


Fig. 6: Effect of modified biochar on alkaline phosphatase activity in soil. Means followed by **a** ~~similar lower case letters~~ **similar lowercase letters** within each column are not statistically different ($p \leq 0.05$, Duncan's multiple range tests). Vertical bars indicate \pm Standard error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

Conclusion

This study reveals that applying simple and Fe-enriched biochar reduces the As toxicity and improves ~~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~~Fe-modified biochar reduces the toxicity of arsenic. Combined this conclusion with a recommendation

References:

- Anawar HM, Rengel Z, Damon P and Tibbett M. Arsenic-phosphorus interactions in the soil-plant-microbe system: Dynamics of uptake, suppression and toxicity to plants. *Environmental Pollution*. 2018; 233, 1003-1012.
- Casida LE, Jr DA Klein and T Santoro. Soil dehydrogenase activity. *Soil Science*.1964; 98:371-376.
- Chen H, Tang Z, Wang P and Zhao FJ. Geographical variations of cadmium and arsenic concentrations and arsenic speciation in Chinese rice. *Environmental Pollution*.2018; 238, 482-490.
- Chen, Y, Han YH, Cao Y, Zhu YG, Rathinasabapathi B and Ma LQ. Arsenic transport in rice and biological solutions to reduce arsenic risk from rice. *Frontiers in plant science*.2017; 8, 268.
- Dong G, Huang Y, Yu Q, Wang Y, Wang H, He N and Li Q. Role of nanoparticles in controlling arsenic mobilization from sediments near a realgar tailing. *Environmental science & technology*.2014; 48(13), 7469-7476.
- Elzobair, KA, Stromberger ME, Ippolito JA and Lentz RD. Contrasting effects of biochar versus manure on soil microbial communities and enzyme activities in an Aridisol. *Chemosphere*.2016; 142, 145-152.
- Ghosh AK, Bhattacharyya P and Pal R. Effect of arsenic contamination on microbial biomass and its activities in arsenic contaminated soils of Gangetic Bengal, India. *Environment International*.2004; 30(4), 491-499.
- Hu J, Weng S, Zheng Z, Pei Z, Huang M and Liu, P. Solvents mediated-synthesis of BiOI photocatalysts with tunable morphologies and their visible-light driven photocatalytic performances in removing of arsenic from water. *Journal of hazardous materials*.2014; 264, 293-302.

- Li C, Zhou K, Qin W, Tian C, Qi M, Yan X and Han W. A review on heavy metals contamination in soil: effects, sources, and remediation techniques. *Soil and Sediment Contamination: An International Journal*.2019; 28(4), 380-394.
- Maliszewska W, Dec S, Wierzbicka H and Woźniakowska A. The influence of various heavy metal compounds on the development and activity of soil micro-organisms. *Environmental Pollution Series A, Ecological and Biological*.1985; 37(3), 195-215.
- Niazi NK, Bibi I, Shahid M, Ok YS, Burton ED, Wang H and Lüttge A. Arsenic removal by perilla leaf biochar in aqueous solutions and groundwater: an integrated spectroscopic and microscopic examination. *Environmental Pollution*.2018; 232, 31-41.
- Nordgren A, Kauri T, Bååth E and Söderström B. Soil microbial activity, mycelial lengths and physiological groups of bacteria in a heavy metal polluted area. *Environmental Pollution Series A, Ecological and Biological*.1986; 41(1), 89-100.
- Pan H, Yang X, Chen H, Sarkar B, Bolan N, Shaheen SM and Wang H. Pristine and iron-engineered animal-and plant-derived biochars enhanced bacterial abundance and immobilized arsenic and lead in a contaminated soil. *Science of The Total Environment*.2021; 763, 144218.
- Pandey VC and Singh N. Aromatic plants versus arsenic hazards in soils. *Journal of Geochemical Exploration*.2015; 157, 77-80.
- Pathak P, Ghosh P, Mukherjee A, Ghosal U, Liang MC, Sikdar PK and Kaushal R. Impact of differential surface water mixing on seasonal arsenic mobilization in shallow aquifers of Nadia district; western Bengal Basin, India. *Journal of Hydrology*.2022; 612, 128270.
- Shakoor MB, Niazi NK, Bibi I, Shahid M, Saqib ZA, Nawaz MF and Rinklebe J. Exploring the arsenic removal potential of various biosorbents from water. *Environment international*.2019; 123, 567-579.
- Subba R. *Soil microbiology*. Oxford and IBH Publishing. 2017
- Tabatabai MA and Bremner JM. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil biology and biochemistry*.1969; 1(4), 301-307.

- Tatsuyama K, Egawa H, Yamamoto H and Inoue K. Influence of metallic compounds on soil microflora in farmland polluted by the chemicals. *Trans. Mycol. Soc. Jpn.;*(Japan).1974; 15(3).
- Tchounwou PB, Yedjou CG, Patlolla AK and Sutton, DJ. Heavy metal toxicity and the environment. *Molecular, clinical and environmental toxicology: volume 3: environmental toxicology.*2012; 133-164.
- Wang S, Gao B, Li Y, Mosa A, Zimmerman AR, Ma L Q., ... and Migliaccio KW. Manganese oxide-modified biochars: preparation, characterization, and sorption of arsenate and lead. *Bioresource technology.*2015; 181, 13-17.
- Yang JR. and Ge JM. Studies on the effects of arsenic on farmland ecosystem. *Acta Ecologica Sinica (China).* 1984
- Yang Z, Liu L, Chai L, Liao Y, Yao W and XiaoR. Arsenic immobilization in the contaminated soil using poorly crystalline Fe-oxyhydroxy sulfate. *Environmental Science and Pollution Research.*2015; 22, 12624-12632.
- Zhai W, Dai Y, Zhao W, Yuan H, Qiu D, Chen J, ... and Xu J. Simultaneous immobilization of the cadmium, lead and arsenic in paddy soils amended with titanium gypsum. *Environmental Pollution.*2020; 258, 113790.