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The Impact of Stubble Burning on Soil Microbial Community: A Comparative Analysis of Colony-forming Units of Rhizospheric Bacteria

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ABSTRACT: India's economy is powered by its agricultural sector. India has the second-largest agro-based economy in the world. A year-round agricultural waste comes from high levels of agricultural production. Even if the costs of collecting, processing, and transportation may be greater, the consequences of burning crop residue are intolerable which has a huge impact on soil ecology as well. An important component of soil ecology is the organisms that are necessary for nutrient cycling, the decomposition of organic matter, the support of plant development, and the production of soil. Therefore, the presence of microbial soil fertility components is essential; unfortunately, they are occasionally ignored or completely discarded. Stubble burning has been a flammable topic concerned with the environment as a whole. Not only has it impacted the air but soil microbial life as well. Viable plate count is a promising method to account for the impact of stubble burning on microorganisms present in the rhizosphere. Soil from wheat (*Triticum aestivum*) fields was collected from different regions of Madhya Pradesh at three intervals and subjected to viable plate count method to assess the effect of stubble burning on the microorganisms present in the soil. Colony forming units (cfu) were calculated in 100 soil samples of stubble burning fields. A significant decrease in the number of viable colony forming cells was observed in the soils collected after the burning of stubble in the wheat fields indicating a harmful effect of this practice in farming. The overall results showed average before burning cfu count as 2.03×10^8 cells/gram of soil. However, after burning average cfu count came as 2.96×10^7 cells/gram of soil. The purpose of this article is to present a concise overview of the existing situation of stubble burning, loss of bacterial biodiversity and potential remedies in order to enhance sustainable agricultural industry. The cfu count proved to be a good measure in assessing microbial flora and the impact of stubble burningon rhizospheric bacteria.

Keywords: cfu count, stubble, Rhizosphere, viable colony, *Triticum aestivum.*

INTRODUCTION

Agriculture is the heart of India which pumps its economy (Deshpande, 2017). In comparison with the world, India has second largest agro-based economy. According to Food and Agriculture Organization (FAO), commonly grown crops make-up around one-third of the world's total land area. (FAO 2018). Nearly 58 percent of the population in India relies solely on agriculture for their livelihood and almost 70 percent of rural households do the same (Balkrishna *et al.*, 2021). With great amount of production comes a year-round agricultural waste, which includes wastes such as stalks, stubbles, leaves, seed pods, and other crop residues as well. However as suggested by United Nations, manure and other wastes from farms, poultry houses, and slaughterhouses; fertilizer run-off from fields; pesticides that enter water, air, or soils; salt and silt drained from fields should also be considered as agricultural waste (Bhuvaneshwari *et al.*, 2019; Prasad *et al.*, 2020).

Focusing specifically on harvest waste, they can be of two types, field residues and process residues. Field residues are left in the agricultural field itself after the crop has been harvested, for example, stalks, seed pods and stubble. Process residues are leftovers after converting a crop in some useable product, such as sugarcane bagasse (Bhuvaneshwari *et al.*, 2019). Farmer's here, in absence of appropriate waste management system at affordable prices, finds it easy to burn crop waste at the cost of the environment. The price of collection, processing and transportation may be higher but the adverse effects of crop residue burning are unbearable (Mohite *et al.*, 2022). Another reason behind this can be least involvement of government in the private lands owned by landowners who manage the waste themselves (Bhuvaneshwari *et al.*, 2019).

Madhya Pradesh lies in the Central Plateau and Hills region (Gangwar and Singh 2011) and half of the state's total geographic area, 14.9 million ha (almost 50%) is devoted to agricultural practices. (Madhya Pradesh, ICAR). In most regions of Madhya Pradesh, the predominant cropping system is wheat-soyabean. To procure maximum economic benefits from a given field, a farmer tries to waste minimum time in stubble management. As soon as crop is harvested farmers rush towards sowing of successive crop and land clearing seems to be a major task here (Kumar *et al.*, 2019).

Soil Ecosystem: The rhizospheric zone. The preservation of healthy, productive soil is crucial for the ecosystem, however, they are sometimes overlooked or entirely ignored (Głodowska and Wozniak 2019). Numerous microorganisms are found in the rhizosphere of plants. They are helpful in promoting plant growth via various mechanisms such as creation of healthy soil, breakdown of organic matter and the cycling of nutrients. Some symbiotic microorganisms increase the bioavailability of nutrients, such as phosphorus (P) (Rawat *et al*., 2011), through arbuscular mycorrhizal fungus (AMF) (Agnolucci *et al*., 2019) or P-solubilizing bacteria, which has a direct beneficial effect on crop yield. Through Nitrogen fixation, i.e. the ability to fix atmospheric nitrogen, soil microorganisms like rhizobium and *Pseudomonas* provides the plants with nitrogen (N) (Wickramasinghe *et al*., 2021).

Hiltner (1904) first used the word "rhizosphere" to describe the region of soil where microorganisms are in close proximity to the root system collectively known as the rhizo-microbiome (Chaparro *et al.*, 2013; Vacheron *et al.*, 2013). The rhizospheric region is the one having contact with plant providing nutrients (Manoharachary and Mukerji 2006). It allows root growth, respiration and abundance of microbial communities which are primarily made up of bacteria and fungus (Qin *et al.*, 2019), in short "hot spot" for microbial colonization. It is a thin zone of 1-2 mm around the roots without a well-defined border. It varies widely depending on the plant, soil, root structure, and most crucially the method used to measure it (Prashar *et al.*, 2013).

According to estimates, the microbial diversity in soil might include hundreds or thousands of different species. Their important role in diversifying soil ecology as well as a supporter to plant growth has come under the light of scientific research recently. They hold a wide range of responsibility as a bioprotectant, biostimulant, biofertilizer and in tackling all sorts of biotic and abiotic stress (Hashem *et al.*, 2019; Latif *et al.*, 2020).

Effect of stubble burning on soil ecology. In majority of studies effect of extreme heat has been researched on shoot and root health (Gonzalez-Garcia *et al.*, 2023), but the impact of extreme heat generated during stubble burning on soil ecosystem is by far not studied in detail. Apart from environmental pollution, crop residue burning has a huge impact on soil ecology. The loss of nutrient content and useful micro-organisms is deleterious for soil's health. The burning of agricultural leftovers from rice, wheat, and sugarcane alone resulted in a loss of around 0.4 MT nitrogen, 0.01 MT phosphorus, and 0.3 MT potassium, according to the main policy-making body in India, the NITI Aayog in 2019 (Prasad *et al.*, 2020). The crop stubbles that are left on fields after mechanical harvesting confront the farmers with considerable issues because of their height, expense of collection and transportation, and lack of suitable on-site residue management solutions. According to farmers, open stubble burning is the quickest and most economical way to dispose of agricultural waste (Chawala and Sandhu 2020). The current study focusses on the harmful effects, stubble burning poses on rhizospheric bacteria and their quantity as a whole.

MATERIALS AND METHODS

Sample collection: Rhizospheric soil samples were collected aseptically from wheat growing fields of different regions of Madhya Pradesh (Narmadapuram22.7519° N, 77.7289° E, Budni 22.7746° N, 77.6826° E, Powarkheda 22.7065° N, 77.7398° E) during March-April, 2022. A total of 50 soil samples were collected from the rhizospheric regions of wheat plant roots, digging the ground approximately 10-20 cm with a clean shovel. Samples were collected at three different time periods. They were categorized as before burning (BB) and after burning (AB). The before burning samples were collected from the wheat fields at 90-100 day crop period. The after burning soil samples were collected immediately within a 10-day period after the stubble was burnt in the crop fields. Sample collection was done manually wearing sanitized gloves in sterilized zipper storage bags and kept at 4ºC up till further use. Combining the BB and AB samples a total of 100 samples were scrutinized.

Serial Dilution: Reducing the abundance of bacteria in given soil sample, technique of serial dilution (Jett *et al*., 1997) was implied. 1g of finely powdered and sieved soil samples were taken and dissolved in 10ml of sterile distilled water and kept on a stirrer for 10 minutes for homogenous mixing of soil sample in distilled water. Also, the continuous stirring helps in detachment of microorganisms from soil particles. After stirring, the solution was allowed to settle down for subsequent serial dilution.1:10 dilutions were made subsequently as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} from the starting culture. 100 µL of 10^{-5}sample was taken and inoculated in Nutrient media Agar (NAM) plates. Samples were taken in triplicates and inoculated by spread plate method. The plates were incubated at 37ºC for 24 hours-48 hours depending upon different types of soil samples taken.

Plate Count Method: Direct viable count is an inexpensive and reliable method to innumerate the viable microorganism count in the soil sample. Colony forming unit (cfu) count was calculated in NAM plates over a 1-3 day incubation period in per gram of soil sample using the following formula:

cfu/ml = (no. of colonies × dilution factor) / inoculum volume

RESULTS AND DISCUSSION

Cfu count is a reliable, quick and easy method to deduce number of viable cells in soil samples (Vieira and Nahas 2005). However, it is a skewed estimate of the cells that can grow in the test conditions (such as the incubation medium, temperature, duration, and oxygen levels) and can form colonies. But still it is largely practiced in analysis of viable cells present in a sample (Sutton, 2011). Plate counting was done with the help of a colony counter. All the 100 plates were calculated for the cfu count. In the 50 samples of before burning (BB) soil, the cfu count ranged from 8.0 × 10^7 cells per gram to 3.58 × 10^8 cells per gram of soil sample (Fig. 1). Soil can be studied as a complex heterogeneous environment comprising of many microhabitats, but the one most valuable is the hotspot of rhizosphere. Such abundance of microorganisms in rhizosphere has been noticed earlier (Kirk *et al*., 2004; Rawat *et al*., 2011). The motive of the study is to elucidate the role of diminishing rhizobacteria during stubble burning, which otherwise can be widely used in replacing chemical fertilizers (Safari *et al*., 2016) due to their capacity in improving nutrient absorption and suppress plant diseases.

Stubble burning showed a drastic effect on rhizospheric soil samples, especially the top soil where heat produced due to stubble burning had a somewhat sterilization kind of effect on the microorganisms found in soil. Population of microorganisms in rhizospheric area is significantly influenced by the levels of disturbance in land management. Hence more cfu count is obtained in forest land as compared to cultivated land (Ram *et al*., 2022).

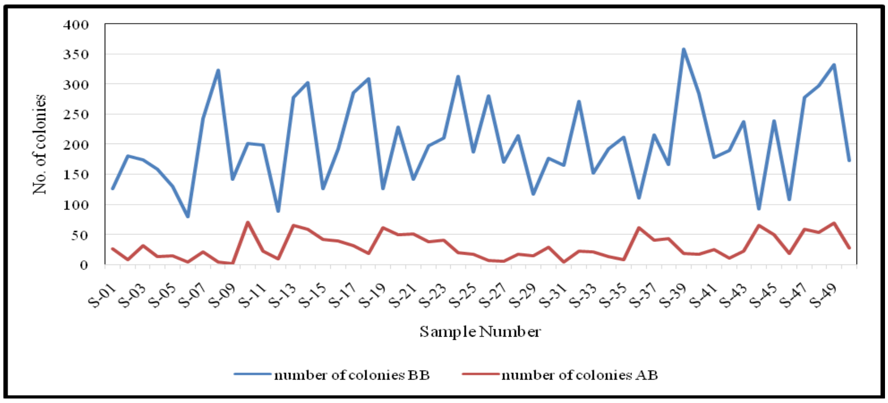

Fig. 1. Comparative Line graph showing the difference in number of colonies in all 100 samples. Blue line shows number of colonies in Before Burning (BB) samples. Orange line shows number of colonies in the After Burning (AF) samples.

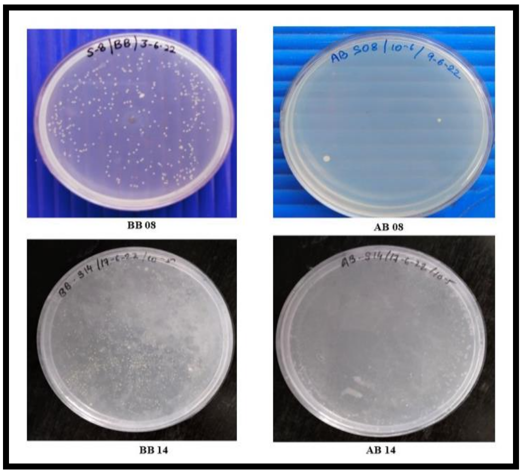

Fig. 2. A comparative picture of two samples of BB and AB showing remarkable difference in microbial growth.

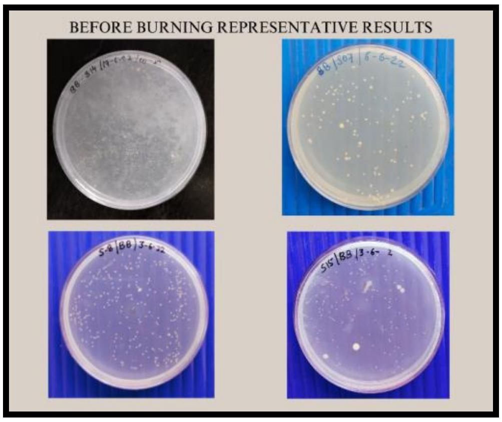

Fig. 3. Representative results on Nutrient media agar plates of Before Burning samples.

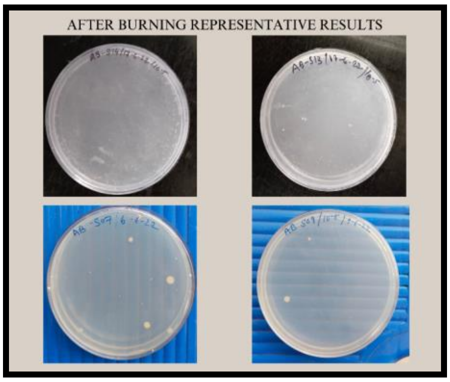

Fig. 4. Representative results on Nutrient media agar plates of after Burning samples.

The microbial count on NAM plates was severely reduced and in some plates the count was as low as <10 colonies. In terms of cfu count such culture plate colonies are considered as Too Few To Count (TFTC) (Fig. 2), but our experiment is based on understanding the impacts of stubble burning on rhizospheric bacteria, hence we have also taken them under consideration. In the 50 samples of after burning soil the cfu counts ranged from 2.0×10^6 cells per gram to 7.0×10^7 cells per gram (Fig. 1). A significant reduction has also been noticed in bacterial cell number (4.97×10^8 cfu/g soil) in stubble burning rice fields as compared to fields where stubble were removed (8.37×10^8 cfu/g soil) (Kumar *et al*., 2019).

All the BB samples showed colony numbers (cfu count) that were way ahead of numbers found in AB samples. The representative images have been shown in Fig. 3 and Fig. 4. The overall results showed average before burning cfu count as 2.03×10^8 cells/gram of soil. However, after burning average cfu count came as 2.96 $\times 10^7$ cells/gram of soil. There have been many studies which reported the importance of stubble removal and stubble retain/return experiments (Liu *et al*., 2014; Wang *et al.*,2015; Huang *et al*., 2021) indicating the significance of organic content of soil. In accordance with this the current experiment also depicted reduction in bacterial population in the plant root adjacent rhizospheric area representing a drop in the probable crop yield and crop quality. This forfeit of soil needs to be replenished by artificial fertilizers later.

The loss of nutrients available on the top layer of the soil, as well as the loss of organic carbon has a negative impact on soil fertility and productivity (Pandey *et al.*, 2023). Government of India has made many policies to curb the practice of stubble burning, but still, it continues in many parts of India. Farming is a big business, and the biggest stakeholders here are farmers. They should feel responsible about all the aspects of farming and not just crop production. Getting rid of the agriculture waste, be it field crop residue or off field crop residue, an easier or cheaper method should not be acceptable. Also, the better means to utilize the stubble should be suggested to farmers, which are under their approach and do not cost them a fortune.

CONCLUSIONS

The impact of stubble burning can be clearly visualized in soil samples collected from wheat fields set on fire. Even the lowest cfu count in the before burning samples were higher than the after burning ones. This issue is serious and a huge lesson has to be taken in terms of soil ecology. Microorganisms are an inherent part of soil and such continuous exposure to stubble burning may lead to some deleterious changes in the microbial biodiversity.

FUTURE SCOPE

The lack of knowledge and infrastructure to clear agricultural lands, especially in rural areas, are the main issues faced by farmers who get involved in stubble burning. India's agricultural development calls for the creation of incredibly innovative ideas to advance this industry. Genetic basis of the biodiversity loss during stubble burning can raise questions about extreme heat acting as a mutagen in soil ecology. In the absence of automation, farming is a physically taxing and back-breaking profession. Agriculture production should definitely be increased but not at a significant ecological cost.

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Conflict of Interest. None.

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