

# MICROFLORA (BACTERIAL) ANALYSIS OF SOIL SAMPLES COLLECTED AROUND VISHNU SUGAR MILL OF GOPALGANJ DISTRICT BIHAR & ROLE OF PRESSMUD IN SOIL FERTILITY.

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## ABSTRACT

Microorganisms originate from the earth's surface and are dispersed in nature through various agencies like air, water, rain and various other media. Soil is a very congenial home for a great variety of microorganisms including nematodes, insects and worms. The contents of resident microorganisms in soil may however depend on physical and physiochemical nature and conditions. Soil samples collected from different areas in and around **Vishnu sugar mill in Gopalganj** district of Bihar were analysed for bacterial composition and population density. Species diversity and population density of soil bacteria were highly reduced at the polluted pressmud dumping sites with the presence of only three species (pseudomonas, micrococcus and Escherichia. coli) and a density of  $11 \times 10^5$  CFU where as in moderately polluted areas around the factory revealed five species (pseudomonas, micrococcus, E. coli, bacillus and staphylococcus.) and a density of  $23 \times 10^6$  CFU. At unpolluted and proper utilization of pressmud area away from the sugar mill seven species were obtained. They are namely – pseudomonas, micrococcus, E.coli, Bacillus, Nitrococcus, Nitrosomonas and agrobacterium with high density of  $32 \times 10^6$  CFU. In general, they succeed in converting insoluble or unavailable materials into forms that can be used by higher plants for better yield.

Key words: - Microorganisms, CFU, Soil bacteria, Pressmud, pseudomonas.

## INTRODUCTION

Soil is the substratum supporting growth of all kinds of terrestrial plants, fungi, slime moulds and several bacteria. All these organisms draw nourishment from soil partly or fully and excrete different metabolic products which are used by soil inhabiting organisms for their growth. Soil is one of the most significant ecological factors, which is an important medium where numerous micro and macro organisms survive. The life on earth depends directly on healthy soil (Daji 1988). The bacterial population of the soil exceeds the population of all other groups of microorganisms in both number and variety. Several billions of bacteria per gram of soil have been reported by direct count method.

Soil ecosystem includes five major groups of microorganisms namely bacteria, actinomycetes, fungi, protozoa and algae. Their number of population are 109,108,106,105 and 124 colony forming unit (CFU)/gram dry soil respectively (Alexendar 1978, Holt 1984). They are heterotrophs, thermophiles, cellulose digesters, sulphur oxidizers, nitrogen fixers and protein digesters. The decomposition of plants and animal remains by microorganisms to humus is an adventitious process in the soil ecosystem (Foth 1978). Unplanned industrialization and haphazard dumping of unprocessed wastes have led to the depletion of these microorganisms of the soil, making soil sterile. Toxic and unprocessed wastes are mainly discharged from textile, pulp mills, chemical industries, oil refineries, tanneries, sugar factories and mineral mining.

Among the various industries, the sugar industry is the second largest industry in India. Sugar Cane and Sugar Beet are the major raw materials for the production of table sugar. The sugar industry produces a large amount of wastes and byproducts namely bagasse, press mud, molasses and distillery spent wash. Bagasse is used as fuel for power generation and in making board and papers. The pressmud contains various organic matters and minerals which is of very great use. But the unprocessed pressmud is dumped on soil in and around the sugar mills. Due to the presence of high content of sugar and strong odour, it creates fly and other insects trapping sanitation problems causing health hazards. If the processed pressmud is used properly then

its water and minerals enriches the soil flora and fauna that enhances soil fertility. In this present study an attempt is made to identify the soil bacteria from the soil samples from different areas in and around sugar factory and processed use of pressmud for enhancing soil fertility.

## **MATERIAL AND METHODS**

**Site Selection :** The soil samples were collected from different areas such as area of pressmud dumping site (Site A), area 500 m away from pressmud dumping site (Site B) and area 2 km away from pressmud dumping site (Site C) of the Vishnu Sugar Mills, Harkua , District Gopalganj, Bihar..

**Collection of Soil Samples :** The soil samples were collected from the various sites using sterile Himedia samples collection containers and enclosed in sterile polythene bags. The samples thus collected were transported quickly to the laboratory and stored in a refrigerator for further microbial analysis. The samples were air dried and saved under laminar air flow.

**Serial dilution method :** One gram of soil samples was taken in a sterilized test tube and dissolved in 10 ml of sterile water and its dilution is  $10^1$ ,  $10^2$ ,  $10^3$ ,  $10^4$ ,  $10^5$ ,  $10^6$ ,  $10^7$ , and so on. From this, serial dilutions were made until a single organism was found in the dilution. From the above diluted samples 1 ml from each was taken and transferred to a separate agar plate and incubated at 30°C for 48 hours. The agar plate with 20 to 200 colonies was taken as pure culture for that organism.

**Staining:** The bacterial culture was taken in an inoculum loop and transferred to a sterilized slide for smear preparation. Crystal Violet and Safranin were used to identify the gram positive and negative bacteria. The cultured colonies were analysed under a light microscope and the species were identified.

## **RESULTS AND DISCUSSION**

Microorganisms constitute a very antique group of living organisms which appeared on the earth surface almost 3000 million years ago. The ultra-structure of bacteria can be examined with the aid of electron microscope and by micro chemical methods that identify the structural and cellular constituents of microorganisms with a high degree of accuracy. Chemical substances from which plants and animals are composed undergo a continuous cycle which is of considerable significance. The transformation of biologically active chemical elements on the earth surface is carried out by the biochemical activity of the microorganisms.

As for as our analysis is concern, The bacterial culture made from the soil samples collected from the selected sites showed three species pseudomonas, Micrococcus and Escherichia coli at site A. Five species namely pseudomonas Micrococcus, E.coli, Bacillus and staphylococcus were obtained from site B. The most amazing thing was the observation of seven species including Nitrosomonas and agrobacterium from site C

**TABLE 1 Bacterial species isolated from soil samples collected in and around a sugar mill.**

S.NO.	SPECIES	SITE 1	SITE 2	SITE 3	SHAPE	GRAM <sup>S</sup> STAIN
1.	Pseudomonas	+	+	+	Rod	-ve
2.	Micrococcus	+	+	+	Coccus	+ve
3.	E. coil	+	+	+	Rod	-ve
4.	Bacillus	-	+	+	Rod	+ve
5.	Staphylococcus	-	+	-	Coccus	+ve
6.	Nitrosomonas	-	-	+	Rod	-ve
7.	Nitrococcus	-	-	+	Coccus	-ve
8.	Agrobacterium	-	-	+	Rod	-ve

Pseudomonas, bacillus, micrococcus and E. coli species were very common in all three selected sites. The maximum bacterial species were obtained from site – C, It is because of the fertile nature and use of processed pressmud that made the soil status very healthy for microorganisms growth.

### Composition of pressmud:-

Constituents	%
Moisture	50–65
Fiber	20–30
Crude wax	7–15
Sugar	5–12
Crude protein	5–10
Nitrogen	2–2.5

Factors which determine the soil environment include soil texture, moisture content and water holding capacity, aeration, temperature, organic matters and nutrient content. Estimations of the number of microorganisms, their composition and variation may indicate the nutritional and agricultural status of the soil. So it is of great importance to analyse the microbial population of a given soil. In the present investigation we find that the members of the genus pseudomonas and E.coli are very common and found in almost all environments. Their activities are very important in the mineralization, nitrogen fixation and decomposition of various wastes. Nitrococcus found in site C is very essential in the process of nitrification of nitrogen fixation. The use of processed pressmud at site C contains more organic matters, minerals and as a result the various species of bacteria were obtained. These bacteria –( pseudomonas, Nitrococcus, bacillus, Nitrosomonas) play very important role in phosphorus and Nitrogen cycles that enriches the soil. The species of agrobacterium – A – tumefaciens and A. rhizogenes inhibits gall diseases in plants such as crown gall disease at the collar region and hairy root disease in the root region respectively (pelczar et al 1977).

Incorporation of pressmud in crop field enhances the soil quality due to the availability of micro and mega nutrients such as iron, calcium, magnesium, silicon and phosphorus. It also supplies carbon to soil microorganism which helps during decomposition and nutrients transformation reactions. (yadav and Solomon 2006). Pressmud can be used as Bio-composting (Chand et al 2011).

### CONCLUSION :-

Soil is one of the most supporting resources that harbours various microorganism. All these microorganism gets nourishment from soil .Their wastes and life process metabolites helps in maintaining different minerals and vital gaseous cycle into biosphere It is clear from very beginning that the soil quality is determined by the moisture content, water holding capacity, Presence of minerals (humus) and quantity of friends microorganism including bacteria. The beneficial effects of soil microbes are drastically altered. From our present analysis it was found that the bacterial population showed great variations with the highest population of  $32 \times 10^6$  CFU at site – c, whereas site A have lowest population density of  $11 \times 10^5$  CFU. The lowest population density at site A is due to the haphazard dumping of sugar factory effluents like pressmud, Molasses and distillery spent wash that inhibits bacterial growth.

Pressmud contains 21% organic carbon along with macro and micronutrients which improves cation exchange and enhances microbe growth. Application of pressmud along with inorganics fertilizers results higher cane yield as reported by Venkatakrishnan and Ravichandran 2013. Patil and shingale (1981) have the opinion that applications of pressmud with bagasse improve the physical condition of soil by reducing bulk density. It enhances macro spore for better growth of roots that ultimately increases the cane yield. Thus it has become clear that processed and proper use of sugar factory by products like pressmud can reduce pollution with the help of microorganism growth and recycling processes. The fertility of soil can also be increased and soil ecology will also be maintained. These favorable conditions will boost crop yield. The use of chemical and toxic fertilizers can be reduced by the use of processed pressmud.

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