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Study Of Soil Micro-Organisms Under Chir Pine Forest In Post Fire Conditions At Purola Range Of Uttarakhand

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Abstract: ***In the whole study we solely tried to understand the post-fire conditions of soil micro-organisms in the forest soils over which Chirpine (Pinus roxburghii) is the dominant species. There was much litter fall in the forest floor of Chirpine (Pinus roxburghii) forests and due to heavy litter fall in some areas of forest, the fire incidences took place so, the population of soil micro-organisms gets affected. Therefore, the post fire (burnt) and pre-fire (unburnt) condition of soil profile has been studied to understand the soil fertility and substrate quality of soil. Soil samples were collected from Purola Forest Range, Uttarakhand. As samples are taken account to observe microbial population so from the surface soil up to 30 cm. depth, the samples were taken from 0 to 15 cm. and 15 to 30 cm. from the forest site. Samples were collected randomly and brought to the laboratory for study of microbial population. After air drying the samples were sieved and weighed and then used as culture inoculums. Various media like Potato Dextrose Agar media, Glycerol Yeast Agar media and Nutrient Agar media was used to study Bacteria, Actinomycetes and Fungi population in the forest soil samples. Both burnt and unburnt samples were collected and compared. The conclusion has also been drawn by comparing the results through discussion that there is impact of forest fire in forest soil microbial population. The population of micro-organism is decreased after fires. Various research finding of different authors were also studied with the study results which has helped in writing of this paper for increase or decrease in soil microbial population in the study area.***

Keywords: ***Soil micro-organism; forest fire; post fire; soil profile; microbial population; soil fertility.***

I. INTRODUCTION

Soils are applied solely to those superficial or nearly superficial horizons of rocks, that have been more or less modified naturally by the interaction of water, air and various kinds of organisms, either living or dead; this being reflected in a certain manner in composition, structure, and color of such formations. Where these conditions are absent, there are no natural soils, but either artificial mixtures or rocks (Dokuchev,1948). Soil generally consists of four major components which are as i) mineral matter, ii) organic matter, iii) water and iv) air, etc. Upon these organic matters and mineral components the whole microbial components are dependent.

Soil microbiology is generally the study of the soil microflora and microfauna. Soil is a good culture media for microorganisms. Microflora generally represents algae but in the case of microfauna they have very dense diversity in the soil as we can say the soil works as a very good culture and growth media for them. They have a vital role in nutrient cycling and promoting plant growth in the soil. There are types of soil microfauna which are as, soil fungi, soil bacteria, soil actinomycetes, and soil protozoa. But other faunal components are there too as small insects which help in the degradation of organic matter.

The earth's life-forms are majorly supported by the atmosphere, hydrosphere, and lithosphere. The all the support mediums in this earth has a genuine population of micro-organisms (e.g. either microflora or microfauna or both). The lithosphere or the soil has a more dense population of these organisms of which many hasn't even been discovered till now. These organisms are least differentiated of all forms of life. They are either unicellular or sometimes a group of few cells arranged distinctively. They are too small to be seen by

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the naked eyes and some cases even much smaller that can be observed under the electron microscope. They vary in different physiological and chemical processes that can be brought under artificial mediums in laboratory conditions and natural soil conditions. Higher life-forms on earth depend upon these micro-organisms because animals and higher plants needs them in most of the physiological processes. As the life has began from unicellular organisms after that the higher life-forms developed from them. But the micro-organisms has not change throughout the time still continue on surviving. The unicellular life-form initiation stated in various theories as,

"Nucleoprotein molecules formed aggregates in the hot soup of primitive sea. These got surrounded by nutrient shells and limiting membrane and formed the first living cell."(Horowitz,1945 and Orgel, 1973).

"The various macromolecules aggregated in the hot dilute soup to form Coacervates. These got isolated from the surroundings by the formation of the polareised membrane of phospholipids. The coacervates with nucleoproteins or in which nucleoproteins were formed, developed into first living cells or protobions" (Oparin AI. 1938 and Haldane JBS. 1929). Uttarakhand has major distinct type of forest features and soil types. Micro-organisms depend on the soil types as well as local and broad climatic factors of the environment. In case of Uttarakhand there are Alpine forests in the extreme North, Tropical deciduous, Sub-tropical temperate forests and thorny forests to the south so, it has distinct soil types under various forest types. Soil types changes from alpine to bhabar and terai which we can broadly say gravels to stiffy clay type. The bhabar area has the highly fertile porous soils. As per rainfall Uttarakhand districts like Dehradun received 1734.1mm, highest to Almora 550.8 mm. actual rainfall during June to September (Report of IMD, 2018). But during the winters-summers due to human activities or extreme dryness the vast forests of Chirpine (*Pinus roxburghii*) are prone to fires and the anxiety of fires came true in the past few years of 2016 and 2018 when large area of Chirpine forests have been burnt down to ashes. As for the primary succession the presence of micro-organisms in the soil found to be have a major role in bringing up the surviving species to grow and adapt. Fire is also of different types which has disturbed the different layers of ecosystem. The surface and ground fires has also effected the soil micro-faunal diversity to some extent. But depending upon their adaptability they have survived and flourished. It also helped the enrichment of soil by assimilating the remaining debris.

However the microscopic forms in the soil are obscured by their environment that they do not get such consideration as they deserve. A survey which has been qualitatively and quantitatively done of the micro-fauna of soil discloses a heterogeneous assortment of forms belonging to the single classified group either by physiological traits or by morphological traits. Even a single organism cannot be visualized under normal microscope they can be seen in a group of organisms i.e. 'Colonies' under microscopes. They can be cultured and studied under laboratory conditions by obtaining and culturing them on natural or artificial substrates/ 'Culture Media' (Robert Koch, 1881).

Forest Fire: Earth crust has been affected by various types of fire especially the natural causes like lightning-caused fire, sparks from rolling stones and causes forest fires, volcanic activity and natural combustion of substances. Prehistoric humans used fire as a tool for hunting and improvement visibility at nights within caves and thickly vegetated habitats. Nowadays forestry and some cases agriculture uses controlled burning as common management purposes and to reduce weeds. For the promotion of the economic growth important plant species and various land-use changes controlled burning is used.

Fire is both a natural and anthropological enhanced factor in ecological systems. Although spontaneous combustion may account for some naturally caused fires, lightning is the primary agent. Climate that consists of hot, dry summer and winter and low humidity level are vulnerable for natural fire occurrence. In chapparal vegetation area characterized by shrubs with leaves that are evergreen, hard, small etc. but after fire dominant, grazing and physical environment produce a static community. The incidence of natural wildfires is a major environmental factor in the development and existence of many vegetation types of the world. Among the ecosystems most influenced by fire are xerophytic communities of Mediterranean ecosystems, African savannas and tropical grassland and alpine/temperate type coniferous species and also the prairie grasslands.

Fire is the key factor in vegetation dynamics of prairies. It is estimated that the fire-prone ecosystems cover about 40% of the land surface (Chapin et al. 2002). In India 80% of fire incidences in forest area, grasslands and agricultural ecosystems are human-induced for grazing, shifting cultivation and collection of minor forest products.

The most frequent disaster in forests is forests fire. Forests fires are as old as the plant life on earth. They pose a threat not only to the forests but also to the entire regime to fauna and flora seriously disturbing the bio-diversity and the ecology and environment of the regime. During summer, when there is no rain for months, the deciduous and semi-evergreen forests become littered with dry senescent leaves and twinges, which could burst into flames ignited by the slightest spark. The Himalayan sub-tropical deciduous forests, particularly, Garhwal Himalayas have been burning regularly during the last few summers, with great loss of vegetation cover of that region.

II. OBJECTIVES

- ✓ To study microbial diversity in reference to fungal, bacterial and actinomycetes population in burnt and unburnt sites.
- ✓ To study future growth of microbial population in burnt site.
- ✓ Correlate the soil fertility and soil biological* properties with unaffected soils to know further succession and establishment of the micro-organisms.

III. REVIEWS OF LITERATURE

The effects of prescribed burning on soil microorganisms in a Minnesota Jack pine (*Pinus banksiana)* forest. They studied selective dilution of plate counts of bacteria, streptomyces and fungi in different media plates. Their study covered 10 acre tract of the jack pine forest which was affected by fire studied before and after two prescribed burns. They were compared with two similar condition of cut and unburnt tract and uncut and unburnt tract. The results showed in a three year span was drastic as the activity and number of several microorganisms reduced drastically after fire but rapidly increased after the first rain in that area. As per their opinion it must has been caused by leachets of ash. Also as per their believe that the intensity of fire and moisture conditions influenced the depth and extent of the effects of fire and rain. The number and activity was still lower in the second season as compared to the normal situation but in the third season there were some changes and growth had been observed. As per the observation streptomyces population was good enough in the third growing season after fire. And towards conclusions it was noted that rainfall was a major variable factor as it caused fluctuation in both the microbial populations of burnt and unburnt sites (Ahlgren and Ahlgren 1965).

It has been studied thatthe effects of surface fire on litter decomposition and occurrence of micro-fungi in a *cymbopogon polyneuros* dominated grassland. (Renbuss et al. ,1973) studied microbiology of an ashbed. Though both studies have different titles they both explains the population of soil microbe dependency upon the intensity of fire. Their study showed the microbial response of soils to the fires from no detectable effect during lower intensity fires to the effect of very hot wildfires where the whole microbial population got wiped out (Joergensen and Hodges 1970);.The magnitudinal studies and persistence of soil NO, N_2O, CH_4 and CO_2 fluxes from burned tropical savannah in Brazil. The major study was about the abundance of the soil microorganisms. This work was not much concerned with the activity of the organisms. Observations in this research made clear that although abundance of microbes were decreased due to the fire but remaining microbes have higher activity than they had in pre-fire conditions (Poth et al. 1995) has studied.

It has been studied forest fire effects on soil microbiology. Fire affects soil microbes directly through heating and indirectly by modifying soil properties (JeorgeMataix-Solera et al. 2009). Microbes also get affected by post-fire environmental factors and re-establishment of vegetation. Severity of fire mostly affects the microbes, top soil temperature the microbes and soil properties related to the post-fire condition. Fungi are more sensitive to heating compared to bacteria and actinomycetes and higher impact under wet soil conditions observed. Negative influence has been seen in the case of mycorrhiza forming arbuscular mycorrhizas. The damaged portions are fungal resistance structures known as 'Sclerotia'. Reduction is also seen due to the change in organic matter. Soluble carbon and nutrient increase cause increase in heterotrophic bacteria population basal respiration is observed. Destruction and creation of new ecological niches and changes in biomass and composition of above ground vegetation species also impact microorganism diversity in soil.

Studies stated that microbial communities in fire impacted soils both underground fire and surface fire conditions (Tobin and Janzen 2008). Surface fires both wildfires and prescribed fires and their impacts are predominantly 'top down' where fire source is above the ground and intense heat sterilize the soil. Fire temperature in that case ranges from 50°C to 1500°C and heat ranges from 2.11 KJ/Kg. of biomass to 2.1 MJ/Kg of biomass has been observed. Its also noted that slow moving fire cause severe impact on top soil and micro-organisms. Microbial impact responses as the availability of nitrogen, sulphur, water, phosphorus and carbon. Nitrogen cycling bacteria when get affected the soil available nitrogen often become a limiting factor in pre-fire environments by combustion of organic molecules released by both surface and underground fires. Sulphur cycling bacterial population also depletes which shows the difference between environments impacted by below ground versus surface fires is the high sulphur that can condense into the soils above surface. As the microorganisms play most important role in bio-geochemical cycles they has major impact in fire prone areas and rhizosphere ecology.

The metagenomic assessment of the potential microbial nitrogen pathways in the rhizo-sphere of Mediterranean forest after wildfire. Soil samples of Lanjaron river based three sites of which two are directly affected by wildfire were studied. Forest that contained holm oak trees (BOF) and burbed bulk soil (BBS) covered by grasses and shrubs and the nearby site in which evergreen undisturbed oak forest (UOF) has been studied. Randomly studied along tracts of 1km. And rhizpsphere of 3 trees/plot had been studied with diameter class of 15 cm. at breast height and separated by 5 cm. each. The whole study was done after 3 years of the fire incident. Rhizospheres of young tree roots were studied. As the microbial dynamics the modification has been done in physical and chemical properties which leads to long-term changes. Even after 3 years of fire soil was alkaline with pH 7.6 whereas unaffected sites were slightly acidic of pH 6.5. The total nitrogen and organic matter content was also found less in the affected soil than the unaffected soil (Josè F. Cobo-Diaz et al. 2015).

Occurrence of fire has been recorded from prehistoric times. The layers of charcoal beneath the surface is the proof. Charcoal layers are indicators of ancient fires recorded in the boundary between Devonian and Carboniferous periods (Komarek 1973, Jones and Rowe 1999). A Wildfire/forest fire is usually lit in the humus layer at the base of lightning struck tree (Granstom, 1993). In present natural dynamics of wildfires/ forest fires is strongly controlled by humans. The invention of deliberate fire and it's control by human has started the anthropogenic modification of biosphere (Pyne and Goldammer, 1997).

In coniferous boreal forest species, they are made by nature, fire prone because of the structure of the forest canopy and the type of litter the tree produces. Generally, conifers burn more easily than deciduous trees (Saari, 1923). The needles of conifers are drier than leaves of evergreen trees and contain larger amount of volatile organic compounds in addition, the litter layer is rich in relatively fine fuels which

are intermingled with a loosely packed moss layer. This promotes ignition (Schimmel and Granström, 1997).

The seasonal weather pattern includes a dry summer when a lighting appears (Chandler et al., 1983). Fire return intervals ranging from 30-250 years have been reported for boreal coniferous forests and the fire frequency has been shown to depend on the climate, quality of fuel, vegetation, tree species, moisture percentage, mean water break distance, topography and degree of human impact. (Zackrisson, 1977; Engelmark, 1984; Masters, 1990; Larsen, 1997; Lehtonen and Huttunen, 1997; Larsen and MacDonald, 1998).

The end products of burning are watervapour, Carbon Dioxide and mineral elements in the ash. Complete oxidation of biomass requires an optimum input of oxygen during the burning process. However under natural conditions this is not the case; Combustion is incomplete producing CO, CH_4, H_2, a wide range of Hydrocarbons and particulates (Cofer et al. 1997). There is a wide variety of microbial response to fire, even in ecosystem. First the response of microbes may be related to the intensity of fire or type of burn. (Vázquez et al. 1993) showed the increasing total numbers of microbes after one month of wildfire in an Atlantic *Pinus pinaster* forest. The effect of interval of burning also depends on the effect of fire (Hossain et al. 1995). In asimilar forest there is no effect detected of prescribed burning on the total number of microbes (Fonturbel et al. 1995).

The microbial response to fire is often studied using plate-count methods, which may vary and give misleading results since only a minor proportion of soil microbes is able to grow in nutrient agar (Zuberer, 1994) and the % of cultivable microbes may vary according to charged soil conditions after fire, e.g.. higher pH (Bååth and Arnebrant, 1994).Thats why different types of media required for studying the interested microbes. However a common observation has been made that burning prefers bacteria over fungi (Dunn et al., 1979; Bissett and Parkinson, 1980; Sharma, 1981; Deka and Mishra, 1983). Humus layer has had its composition altered as a result of heating (Jones et al., 1997). An excess of base (Ca^{2+}, Mg^{2+}, and K^{+}) in the ash leads to increase pH of the soil (Ahlgren and Ahlgren, 1960; Raison, 1979; Wells et al. 1979). The concentration of mineral Nitrogen bond to organic compounds is partly lost during burning (Kivekäs, 1939; Kutiel and Naveh, 1987). The fertilizing effect of burning is also recorded (Austin and Baisinger, 1955). Important feature of boreal Coniferous forests that undoubtedly has an effect on post-fire recovery of is the major role of thick humus layer. Approximately one fourth of the carbon in soil is found in humus layer (Liski and Westman, 1995).

IV. METHODOLOGY

✓ SITE DESCRIPTION AND SAMPLE COLLECTION

The study site was under Sunali Beat and Binai Beat of Purola Forest Range under Tons Forest Division of Uttarakhand. Samples were collected from both burnt and of un-burnt sites which remained unaffected by the fire. Soil depths considered for better observation of microbes were 0-15cm and 15-30cm studied for the post fire effects on soil microbial activity. The samples were collected at an altitude of 1847m and 1321m at msl. Required samples were collected by simple random sampling in line transact method. Collection were done by augurs and forks at different depths of soil under the Chirpine forest. Collected samples were put into polythene bags with marking tags which represents the sample number and location of collection site. Same was also maintained in collection register. The samples were then put into contamination free sealable bags. There is no such proper method by which we can measure total abundance and activities of microbes in natural conditions in soil layers. Only certain groups of organisms present in the soil can be measured and studied under proper condition. Organisms which are counted make up a few part, frequently a very small one, of the total soil organism population and the measured one is few in many. The methods used for counting the microorganisms are based upon the stained preparations of soil or on the behaviour of the microorganisms to sustain on different type of culture media i.e. liquid or solid media which are prepared in lab for cultivation of these microorganisms. Some of the organisms are hard to get in laboratory conditions or lack of suitable media preparations such as actinomycetes and some get destroyed like protozoa in the handling process of handling. Some found in individual clumps or too much scattered to be studied too. Though barely a small proportion of million or trillions of those organisms can be counted but their count can be considered major role in understand what is going inside the soil and how they are surviving the dynamic changes occurring in nature.

V. COLLECTION EQUIPMENTS AND LABORATORY EQUIPMENTS USED FOR CULTIVATION OF THE MICRO-ORGANISMS

✓ COLLECTION EQUIPMENTS

- Augers and Forks (for collecting soil samples),
- Polythene bags (for preserving the collected material from contamination and transporting purpose),
- Marking Tags and collection register (for the general description of sample number, collection site and distinguishing them from one another)

✓ LABORATORY EQUIPMENTS

- Glassware (Measuring cylinder, Beaker, Volumetric flask, Test tubes, Petridishes, Conical flask etc.)
- Autoclave,
- Laminar air-flow,
- Incubator,
- Digital Colony Counter,
- Weighing balance,
- Spatula,
- Micro pipette with micro-tips,
- Sieve (5mm.),
- Paraffin tape, aluminium foil, paper and marker.
- Gloves and lab coat were used for safety measures etc.

✓ MEDIA PREPARED FOR CULTURE

For the isolation of bacteria, fungi and actinomycetes these following media were prepared in lab. They are as, PDA media (Potato Dextrose Agar media); NAM media (Nutrient Agar Media) and Glycerol Yeast Agar media.

✓ COMPOSITION OF POTATO DEXTROSE AGAR

INGREDIENTS	GRAMS./LITRE
1. Agar	20 gm.
2. Potato Infusion	200 gm.
3. Dextrose	20 gm.

✓ IN CASE OF READY-MADE HIMEDIA:

INGREDIENTS	GRAMS/LITRE
1. Potatoes, infusion form	200 gm.
2. Dextrose	20 gm.
3. Agar	15 gm.

pH is adjusted to 5.6± 0.2 at 25^{0}C.

✓ PREPARATION

39 grams of media has been poured in 1000 ml distilled water, thenheated to boiling temperature to dissolve completely. To sterilized by autoclave at 15 lbs pressure at 121^{0}C for 15 minutes. Mixed well before dispensing and then poured into the petriplates.

✓ COMPOSITION OF NUTRIENT AGAR

INGREDIENTS	GRAMS/LITRE
1. Peptone	5 gm.
2. Agar	15 gm.
3. Beef Extract	3 gm.
4. NaCl	5 gm.

pH is adjusted to neutral 7.4 at 25˚C.

✓ PREPARATION

15 grams of agar powder has been poured in 1 litre of distilled water. Then one by one the peptone, beef extract and NaCl is measured and put into the solution. The mixture is heated up to dissolve the ingredients. Then the mixture is put into autoclave at 121^{0}C for 15 minutes at 15 lbs of pressure. The mixture was taken to laminar after autoclaving to avoid contamination. After cooling it was put into petridishes. The petridishes remained open under laminar aseptic conditions for the solidification of media. After the solidification of the media the plates were then closed with the lidand stored.

✓ COMPOSITION OF GLYCEROL YEAST AGAR MEDIA (TOSI, B., DONINI, A., ROMAGNOLI, C., AND BRUNI, A. 1996)

INGREDIENTS	GRAMS OR ML./LITRE
1. Glycerol (anhydrous)	5 ml.
2. Yeast Extract	2 gm.
3. K_2HPO_4	0.10 gm.
4. Peptone	25 gm.
5. Agar	15 gm.

✓ PREPARATION OF MEDIA

At first 5 ml. anhydrous glycerol was put into 1000 ml. of distilled water. Then one by one 2 gram of yeast extract, 0.10 grams of K_2HPO_4 and 25 grams of peptone and 15 grams of agar was pour into the water. The solution was then heated to the boiling point to mix up all the components. Then the whole solution was put into autoclave for 15 minutes under 15 lbs pressure at 121^{0}C temperature. After sterilized in autoclave the whole media was taken into the laminar airflow and then poured into petriplates then left for cooling and solidifying. After solidification the lids were put and all the plates were covered and stored. For the culture the soil sample and the preparation of stock solution most important is the serial dilution technique

✓ PREPARATION OF SOIL SAMPLES

The collected soil samples must have to be prepared for rearing of the microorganisms present in them. The collected soil samples are air-dried under abiotic conditions. But there must be few % of moisture left for the microorganisms to survive. The soils were then sieved by 5mm. sieve and sample replicates were made and number were put into them. That 1 gram replicates made was used to make the stock solution.

VI. SERIAL DILUTION

A SERIAL DILUTION is a series of sequential dilution to reduce the concentration of a solution to be used as a more suitable solution. Each dilution reduces the number of organisms in the next sequential serial. Stock solution is reduced by preparing blanks .And by calculating the total dilution over the entire series it is possible to calculate how many micro-organisms we had started from. This basically helps us to understand that how many micro-organisms as we are saying in this CFU/Colony Forming Units we had taken for this study. In case of micro-organisms the are uncountable in number in soil so, it is a much more needed approach in this aspect. For a countable unit in each and every pertiplate the serial distillation is done.

✓ SERIAL DILUTION FOR BACTERIA INOCULUMS

Diluting the bacteria growing densities are around 109 CFU/ml. the maximum densities vary tremendously depending on the species of bacteria and the media they are growing in. Therefore to get readily countable number of bacteria, we have to make a wide range of dilutions and essay all of them with the goal of having one or two dilutions with countable numbers. We do this method by serial 10-fold dilutions of the bacteria population present in given soil sample that covers the entire probable range of concentrations. Then we transfer 0.1 micro-liter of each dilution to a Nutrient Agar Media plate, which in effect makes another 10-fold dilution, since the final unit is CFU/ml and we only streak 0.1 ml.

✓ MEDIA PLATE INOCULATION PROCESS

- At first the stock solution was made from 1 gm soil sample in 9 ml. of distilled water which made it 10 ml. stock solutions for dilution.
- After that 6 more test tubes with 9 ml. distilled water is filled and prepared for each sample separately.
- From that 10 ml. stock solution with the help of micro-pipette we tool 1 ml. stock solution and poured into the next test tube with 9 mi. of distilled water and the again take 1 ml. solution from that test tube and move on to further dilution upto 5^{th} dilution.
- From the last (5^{th}) dilution we tool 1 ml. and poured into the NAM petriplates under aseptic conditions of the laminar air-flow.
- Then the culture is streaked over the media plate thoroughly just to make sure the similar distribution.
- The 'L'-shaped glass rod has been sterilized after all the needed dilution of a sample with the help of rectified spirit and Bunsen burner flame and cooled before use.

✓ INCUBATION AND COUNTING

All the Petri plates are then incubated at 37 degrees centigrade temperature for next 48 to 72 hours and the colonies formed in the plates are counted over the digital colony counter and noted down. (After counting the plates were sealed in the sealable disposal bags and disposed properly.)

✓ SERIAL DILUTION (FOR FUNGI AND ACTINOMYCETES INOCULUM)

In this inoculation of the sample from the diluted test tubes in the prepared Potato Dextrose Agar plates and Glycerol Yeast Agar Platesby using Pure Plat method or the spread method and then incubated plates in the incubator at 28 degrees centigrade for 48 to 72 hours and for actinomycetes at 28 to 32 degree centigrade for one and a half week. After the specific growth duration of the fungi, the cultural plates are observed as new growth appear in the plates.

✓ MEDIA PLATE INOCULATION PROCEDURE

- 6 test tubes were taken and 9 ml. distilled water for each test tube and then 1 gm. Soil sample for one test tube to prepare the stock solution and each and every test tube were marked.
- 1 gm. Soil sample was taken from 1^{st} tube or the stock solution with the help of micro-pipette and poured into the second test tube.
- 1 ml. from the 2^{nd}test tube is then transferred to the 3^{rd} test tube. As so on for the next dilutions up to the fourth dilution and 5^{th} test tube.
- For pouring the plates media we used spread method.
- We used micro pipette to take 1 ml. from the 4^{th} dilution and dropped over the media and then spreaded with the 'L'-shaped glass rod.

✓ INCUBATION, OBSERVATION AND COUNTING

Fungi plates were observed after 48 hours and actinomycetes plates were observed after one week to the next few days. After that time the colony and growth in the plates were noted down by observing with the help of digital colony counter and the CFU/ml. is calculated. (After observation the plates are disposed of in sealed bags.)

VII. OBSERVATIONS

Sl. No.	Sample Code	Soil Depth (in Cm.)	Soil Status (Burnt/ Unburnt)	Dilution Factor	Bacterial Colony Counted	Bacterial Count in CFU's
1.	252	0-15	Burnt	10^4	NIL	NIL
	253	15-30	Burnt		8	8×10^4
2.	255	0-15	Burnt	10^4	4	4×10^4
	256	15-30	Burnt		3	3×10^4
3.	258	0-15	Unburnt	10^4	6	6×10^4
	259	15-30	Unburnt		12	12×10^4
4.	261	0-15	Unburnt	10^4	8	8×10^4
	262	15-30	Unburnt		NIL	0
5.	268	0-15	Burnt	10^4	11	11×10^4
	269	15-30	Burnt		3	3×10^4
6.	271	0-15	Burnt	10^4	17	17×10^4
	272	15-30	Burnt		NIL	0
7.	274	0-15	Unburnt	10^4	19	19×10^4
	275	15-30	Unburnt		6	6×10^4

Table 1: Calculation of CFU/ml. in Potato Dextrose Agar Plates

Sl. No.	Sample Code	Soil Depth (in Cm.)	Soil Status (Burnt/ Unburnt)	Dilution Factor	Actino. Colony Counted	Actino. Count in CFU's
1.	252	0-15	Burnt	10^4	1	1×10^4
	253	15-30	Burnt		2	2×10^4
2.	255	0-15	Burnt	10^4	NIL	0
	256	15-30	Burnt		NIL	0
3.	258	0-15	Unburnt	10^4	2	2×10^4
	259	15-30	Unburnt		1	1×10^4
4.	261	0-15	Unburnt	10^4	1	1×10^4
	262	15-30	Unburnt		4	4×10^4
5.	268	0-15	Burnt	10^4	NIL	0
	269	15-30	Burnt		3	3×10^4
6.	271	0-15	Burnt	10^4	NIL	0
	272	15-30	Burnt		8	8×10^4
7.	274	0-15	Unburnt	10^4	1	1×10^4
	275	15-30	Unburnt		1	1×10^4

Table 2: Calculation of CFU/ml. in Glycerol Yeast Agar Media Plates

Sl. No.	Sample Code	Soil Depth (in Cm.)	Soil Status (Burnt/ Unburnt)	Dilution Factor	Fungi Colony Counted	Fungi Count in CFU's
1.	252	0-15	Burnt	10^5	24	25×10^5
	253	15-30	Burnt		21	21×10^5
2.	255	0-15	Burnt	10^5	15	15×10^5
	256	15-30	Burnt		18	18×10^5
3.	258	0-15	Unburnt	10^5	18	18×10^5
	259	15-30	Unburnt		4	4×10^5
4.	261	0-15	Unburnt	10^5	17	17×10^5
	262	15-30	Unburnt		27	27×10^5
5.	268	0-15	Burnt	10^5	NIL	0
	269	15-30	Burnt		25	25×10^5
6.	271	0-15	Burnt	10^5	7	7×10^5
	272	15-30	Burnt		4	4×10^5
7.	274	0-15	Unburnt	10^5	NIL	0
	275	15-30	Unburnt		8	8×10^5

Table 3: Calculation of CFU/ml. in Nutrient Agar Media plates

VIII. CALCULATION

Results in this case is observed just to know the population dynamics of microbial population in burnt and unburnt site of certain location. For bacterial study 5thdilution is used as inoculums and for bacteria and actinomycetes4th dilution is used to know their growth and abundance. For each dilution colony forming numbers per plate is counted. Typically numbers between 30 to 800 are considered to be in the range where one's data is statistically accurate. If the number of CFU's on the plate is greater than 100, the record will be TNTC (Too Numerous to Count).Post Fire/ Burnt and Unburnt samples of 0-15 and 15-30 cm. depth for Bacteria, Fungi and Actinomycetes are studied and the lab results are as:

IX. RESULTS

From the CFU units per Milliliter it is clear that the population of fungi is more in case of burnt site and bacterial and actinomycetes population is low in that constraint. But in unburnt site samples the population of bacteria and actinomycets is high. In this population study we can say that after observation of the different biological entities in different media their population is definitely affected by the ground and surface fire under the Chirpine forest. Infavourable conditions the population of fungi is very high it can be because of the burnt litter of the Chirpine forest which stays undecomposed for a long-term because of temperature and other climatic and biotic factors. Plant succession as fungi comes under the kingdom plantae is more in the post fire conditions rather than zoological entities like the bacteria and actinomycetes.

X. DISCUSSION & CONCLUSION

The seven forest sites of Chirpine were studied for post fires conditions of soil microbial populations and research results report that the microbial populations were affected severely. The population of fungi is more in case of burnt site and bacterial and actinomycetes population is low. But in unburnt site samples the population of bacteria and actinomycets is high. In this population study we can say that after observation of the different biological entities in different media their population is definitely affected by the ground and surface fire under the Chirpine forests. Soils take a definite amount of damage which can only be recovered after a certain amount of time in different climatic and natural conditions.

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