RESEARCH PLAN PROPOSAL

PHYSICO-CHEMICAL AND BACTERIAL APPROACH TO ANALYZE THE FERTILITY OF SOIL IN RESTORED AND UNRESTORED AREAS OF CHAKSU RAJASTHAN

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Submitted by

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INTRODUCTION

Restoration of degraded land system can be attained by *in-situ* or *ex-situ* conservation. *Ex-situ* conservation is a technique used to conserve species outside their original habitat in zoos, gardens, aquaria etc. whereas *in–situ* conservation is the conservation in the wild/ original habitat. It is the best strategy for long term protection of biodiversity (Prabhu *et al*, 2009). The Society for Ecological Restoration defines ecological restoration as an "intentional activity that initiates or accelerates the recovery of an ecosystem with respect to its health, integrity and sustainability" (SER, 2004).

Conservation of the environment is the demand of the day and religion can be positively used for the protection of the environment. Sacred groves can also be established to restore certain ecosystems which have been barren in past few years. In India, especially in states like Rajasthan, people are pious, God-fearing and nature loving. Therefore, religion is the best way to instill a sense of belongingness towards nature. Sacred groves can also be considered as a type of *in-situ* conservation because worshipping is done on the site. There has been considerable improvement in soil fertility parameters in areas under sacred grove conservation which is evidently observable in form of higher plant density.

To observe the soil fertility enhancement we plan to undertake this study in the village of Chaksu block which suffered severe floods during 1981 in Dhund River resulting in massive erosion of soil leaving it unproductive.

Communities can play an important role in restoring the degraded ecosystems especially the forest ecosystems. Practices like Joint Forest Management (JFM) and Social Forestry provide a visible role to the local communities in planning, management and protection of forests.

Conservation can be more accurately estimated by studying the soil profile in terms of physico-chemical parameters and analysis of microorganisms responsible for soil fertility. The number and variety of microorganisms present in soil depend on many environmental factors like amount and type of nutrients available, moisture content, degree of aeration, pH, temperature etc.

(Prescott *et al*, 1999). Soil bacteria and fungi play pivotal roles in various biogeochemical cycles (Molin and Molin, 1997; Trevors, 1998; Wall and Virginia, 1999) and are responsible for the cycling of organic compounds. Soil microorganisms also influence above ground ecosystems by contributing to plant nutrition, plant health, soil structure and soil fertility (O'Donnell *et al*, 2001). Remote sensing and GIS are powerful tools, which could be effectively used to study the dynamic behavior of waterlogged areas. Application of remote sensing technology in mapping and monitoring degraded lands, especially salt – affected soils, has shown great promise because of enhanced speed, accuracy and cost effectiveness (Dwivedi, 1998).

We plan to study the restoration of flood affected area of Chaksu block which has been restored by community. The area has been planted with *Acacia tortalis,* 10% fruit and fodder plants of *Cordia myxa, Alianthus excelesa, Emblica officianalis and Zizyphus jujuba.* The idols of the deities were installed in average room-sized temples. The inhabitants are attached sentimentally to their deities and probably fear their wrath so they would not cut any trees for their personal and commercial benefits.

The restoration of the area since 1981 seems to have improved the soil quality which will be analyzed in the present study. We propose to study various soil parameters like pH, electrical conductivity, organic carbon, organic matter, available phosphorous, available potassium, available nitrogen (APHA, 2005) which would provide us with useful information regarding soil fertility.

Moreover soil fertility is also influenced by its microbiota. The fertility of soil is strongly dependent on Nitrogen and Phosphorous which are regularly supplied in soil by soil micro biota. The nitrogen fixing bacteria like *Nitrosomonas, Nitrobacter, Azotobacter, Rhizobium* (Zaharan *et al*, 1999) and Phosphobacteria (Ocampo *et al*, 1975) like *Pseudomonas bacillus* affect the soil fertility. The presence of the phosphobacteria and nitrifying bacteria in the soil and its biodiversity in the restored and unrestored site will provide us with substantial information about the extent of restoration.

Atmospheric nitrogen must be processed, or "fixed" to be used by plants. Some fixation occurs in lightning strikes, but most fixations are done by free-living or symbiotic bacteria. These

bacteria have the nitrogenase enzyme that combines gaseous nitrogen with hydrogen to produce ammonia, which is then further converted by the bacteria to make their own organic compounds (Moir, 2011). When a plant or animal dies, or an animal expels waste, the initial form of nitrogen is organic. Bacteria, or fungi in some cases, convert the organic nitrogen within the remains back into ammonium (NH_4^+), a process called ammonification or mineralization.

The nitrifying bacteria present in soil are responsible for the conversion of ammonia to nitrate. In the primary stage of nitrification, bacteria such as *Nitrosomonas* species is responsible for the oxidation of ammonium (NH_4^+), which converts ammonia to nitrites (NO_2^-), while the oxidation of the nitrite to nitrate (NO_3^-) is performed by the other bacterial species such as *Nitrobacter* (Smil, 2000).
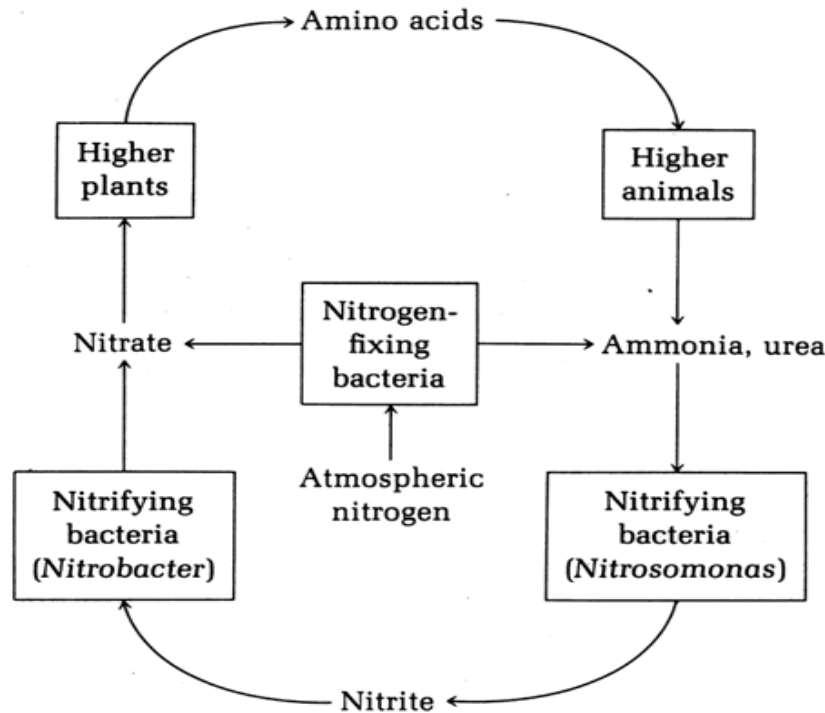
Nitrogen cycle (Bhatnagar, 2007)

Phosphorus (P) is the major plant growth-limiting nutrients despite being abundant in soils. Phosphobacteria have the ability to convert insoluble compounds of phosphorus into available phosphates that enhance nutrient availability to plants (Barea *et al*, 2005; Lugo *et al,* 2008; Rodrıguez and Fraga 1999; Son *et al*, 2006; Souchie *et al,* 2006).

Microorganisms are involved in a range of processes that affect the transformation of soil phosphorous and are thus an integral part of the soil phosphorous cycle. In particular, soil microorganisms are effective in releasing phosphorous from inorganic and organic pools of total soil phosphorous through solubilization and mineralization (Hilda and Fraga, 1999). Strains from the genera *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Micrococcus, Aereobacter, Flavobacterium,* and *Erwinia* are known phosphate solubilizers (Rodrıguez and Fraga 1999).

Thus the research proposed for my doctoral degree involves physico-chemical and microbial analysis of factors responsible for soil fertility of the restored and unrestored soil of Chaksu block, Rajasthan.

REVIEW OF LITERATURE

The Convention on Biological Diversity defined Biodiversity (or biological diversity) as the variability among living organisms from all sources including inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems (Convention on Biological Diversity, 1992).

Biodiversity conservation requires maintaining or re-establishing habitat strips to connect natural forest blocks and protect ecological gradients. Sayer *et al, (*2004) studied the fundamental principles of ecosystem approaches as adopted by the Convention for the Conservation of Biological Diversity and principles for successful common property resource management provide valuable frameworks for forest restoration schemes. Restoration ecology is an emerging field focused on recovering and reinvesting ecological capital presently being quickly spent by humanity, principally in habitat alteration.

According to Choi (1994), ecological restoration is one of the fastest growing fields in applied ecology, providing new ideas and opportunities for biological conservation and natural resource management. He presented a theoretical framework for 'futuristic' restoration, in terms of goals, trajectories, evaluation criteria and monitoring, along with a historical perspective. A 'futuristic'

restoration is: (i) setting realistic and dynamic goals for future environment; (ii) to assume multiple trajectories for unpredictable nature of ecological communities and ecosystems; (iii) to take an ecosystem or landscape approach for both function and structure; (iv) to evaluate the restoration progress with criteria based on quantitative inference; and (v) to maintain long-term monitoring of outcomes after restoration.

The restoration ecologists have to consider two factors before planning the restoration of any area. Firstly, the placement of project in the landscape along with its boundaries and adjoining ecosystem; and receipt and loss of material and energy (Ehrenfeld *et al,* 1997). Secondly the amount of money allocated to restoration efforts, including ecosystem replacement costs, quantifying ecosystem services, contingent valuation, and surrogate market price techniques (Holl *et al*, 2000).The funding for restoration can be collected from private funding by the party responsible for the damage, public funding through taxes, voluntary contributions and various public/private partnerships.

Ecological restoration, including (re)afforestation and rehabilitation of degraded land, is included in the array of potential human responses to climate (Harris *et al*, 2006). According to Benayas *et al*, (2009) ecological restoration is widely used to reverse the environmental degradation caused by human activities. However, the effectiveness of restoration actions in increasing provision of both biodiversity and ecosystem services have not been evaluated systematically. Increases in biodiversity and ecosystem service measures after restoration were positively correlated. In the light of the increasing population pressure, it is of major importance not only to conserve, but also to restore forest ecosystems. According to Aerts *et al,* (2011) ecological restoration has recently started emphasizing the biodiversity-ecosystem functioning (BEF) perspective, which might be the beginning of a paradigm shift in restoration ecology. Stoica (2012) claims that Ecosystem approach proved to be the most efficient strategy for integrated management of soil, water and life which promotes conservation and sustainable use in an equitable application of the ecosystem approach.

Bhagvat *et al,* (2006) believed that communities around the world traditionally protect natural sites that are dedicated to ancestral spirits or deities. Case studies on sacred groves show that

these small forest patches play an important role in biodiversity conservation. They discuss on current threats to sacred groves that need to be addressed through management approaches. Khan *et al,* (2008) studied sacred groves across the globe in general and India in particular, highlighting that the tradition of sacred groves could provide a powerful tool for ensuring biodiversity conservation through community participation. More than half of Ghana's forest cover has been lost to deforestation. Although the Tallensi-Nabdam district has suffered deforestation, portions of the biosphere called sacred groves have survived. Barre *et al,* (2009) aimed to explore the particular reasons why the groves have thrived by articulating precise sacred grove taboos. They also believe that the biodiversity conservation is linked with cultural preservation.

The ecological conservation can be evaluated by forest cover but also by quality or fertility of soil. The physical, chemical and biological properties of soil also called soil indicators are processes and characteristics that influence the capacity of a soil to function. These indicators correlate very well with the overall ecosystem processes giving soil its characteristic properties (Winder, 2003).

International and national calls for management of forestry on a sustainable basis have consistently included maintenance or enhancement of forest soil quality as a criterion of sustainability. Monitoring of function and long-term sustainability of forest ecosystems relies on use of indicators. In the case of soil quality, an indicator is a measurable attribute of a soil that determines how well a soil functions (Burger and Kelting, 1999).

Seasonal variations in biologically driven parameters are somewhat expected and often predictable, but several studies have also demonstrated significant seasonal variations in chemical characteristics that are generally considered more stable (e.g. CEC and exchangeable bases) (Haines and Cleveland, 1981; Peterson and Rolfe, 1982; Johnson *et al,* 1988).

Soil parameters responsible for soil fertility were also reported by many researchers. Paudel *et al,* (2003) analyzed the physiochemical properties of soils like texture, pH, organic matter, humus content, water holding capacity, nitrogen, phosphorous and potassium. Mussa *et al,* (2009)

studied soil samples collected from different places at different depths for the determination of available phosphate, nitrate and sulphate.

The total bacterial and fungal counts of the soil samples were estimated using standard spread plate technique. Ogunmwonyi *et al*, (2008) also suggested that the bacterial and fungal abundance are typical of an environment with high species richness and functional diversity.

Soil texture and depth are soil properties that would change little through time for a given soil, and so they would not be very useful for assessing management effects. Soil bulk density varies among soils of different textures, structures, and organic matter content, but within a given soil type, it can be used to monitor degree of soil compaction and puddling. Changes in soil bulk density affect a host of other properties and processes that influence water and oxygen supply (Taylor *et al*, 1966; Sands *et al*, 1979).

The permeability of soil to water depends on its particle size. Bouwer (1986) determined the soil permeability in the field based by measuring the one-dimensional water flow into the soil per unit time by double-ring infiltrometer at four to six replications. The particle size distribution consisted of coarse sand (0.1–2 mm), very fine sand (0.05–0.1mm), silt (0.002–0.05) and clay (<0.002mm) was determined by the Robinson's pipette method (SSEW, 1982). Gravel (2–8 mm) was determined using the weighting method (Gee and Bauder, 1980). The soil structure was determined based on the size and shape of aggregates according to the Wischmeier and Smith's (1978) procedure.

Soil pH itself provides little direct information but it influences many biological and chemical relationships which in turn critically affects the productive capacity of a soil (Aune and Lal, 1997).

Electrical conductivity as a measure of ion concentration and the potentially negative effect of salinity on the osmotic potential (i.e. water relations) and nutrient imbalances is primarily used in agricultural soils. Its application to forest soils is usually limited to very specific circumstances (e.g. reclamation of mine soils) where highly concentrated soil solutions are known or suspected to inhibit forest growth and productivity (e.g. Burger *et al*, 1994).

Nelson and Sommers, (1982) reported that Soil organic matter (SOM) is commonly recognized as one of the key chemical parameters of soil quality, yet quantitative assessment of its contribution to soil quality is often lacking. It is a critical pool in the carbon cycle and a repository of nutrients, and through its influence on many fundamental biological and chemical processes it plays a pivotal role in nutrient release and availability (Johnson, 1985; Henderson *et al*, 1990; Henderson, 1995; Nambiar, 1997). Organic Carbon is included in the minimum data set (MDS) of soil quality assessment proposed by Larson and Pierce (1994) for agricultural soils, where it is used in pedotransfer functions (Bouma, 1989) to calculate bulk density, water retention capacity, leaching potential, cation exchange capacity (CEC), rooting depth, and soil productivity.

Restoration of the ecosystem eventually improves the geo-environment of any area. This indirectly enhances the soil characteristic especially the microbial diversity. Soil microbiology deals with microorganisms present in the soil, their effect on soil fertility, on plant growth and on the destruction of environmental pollutants (Alexender, 1977). The Tropical Soil Biology and Fertility Programme (TSBF) soil fauna theme states that "soil fauna can be manipulated to improve the physical properties of soil and regulate decomposition processes" (Lavelle *et al*, 1994). Sustainable agriculture practices include agroforestry, intensive fallowing and green manuring, the use of mulch, compost, and the use of natural symbionts (Muller *et al,* 1994). Major factors that constrain tropical soil fertility and sustainable agriculture are low nutrient capital, moisture stress, erosion, high P fixation, high acidity and low soil biodiversity. Most plant species form beneficial associations with arbuscular mycorrhizal (AM) fungi have been reported to enhance physical, chemical, and biological soil quality (Cardoso *et al*, 2006).

The numbers and variety of microorganisms present in soil depend on many environmental factors like amount and type of nutrients available, moisture content, degree of aeration, pH, temperature etc. (Prescott *et al*, 1999). Soil bacteria and fungi play pivotal roles in various biogeochemical cycles (Molin and Molin, 1997; Trevors, 1998; Wall and Virginia, 1999) and are responsible for the cycling of organic compounds. Soil microorganisms also influence above

ground ecosystems by contributing to plant nutrition, plant health, soil structure and soil fertility (O'Donnell *et al*, 2001).

Soil bacteria are also reported to increase or supplement the fertility of soil as biofertilizer. Several microorganisms like *Azolla, Frankia. Rhizobium, Cyanobacteria* and phosphate solubilizing microorganism are considered as potent biofertilizer (Kannaiyan *et al*, 2004).

Nitrogen is important in plant growth and production of food and feed as it is required for cellular synthesis of enzymes, proteins, chlorophyll, DNA and RNA. Biological nitrogen fixation is an important part of the microbial processes (Simon, 2003). Biological nitrogen fixation is carried out only by prokaryotes, which may be symbiotic or free living in nature. The nitrogen fixing activity of free-living, non-photosynthetic aerobic bacteria is strongly dependent on favorable moisture conditions, oxygen concentration and a supply of organic Carbon substrates (Matthew *et al*, 2008).

Nitrogen fixation enables reduction of the atmospheric nitrogen into ammonium ion (NH_4^+) by nitrogenase enzyme. This process introduces nitrogen into the biosphere, which is responsible for its annual fixation by upto 65% of nitrogen while industrial processes represent only 25% (Newton, 1996). Some symbiotic bacteria (most often associated with leguminous plants) and some free-living bacteria are able to fix nitrogen as organic nitrogen. *Rhizobium* is an example of mutualistic nitrogen fixing bacteria, which live in legume root nodules. These species are diazotrophs. An example of the free-living bacteria is *Azotobacter* (Smith, 2004).

These bacteria have the *nitrogenase* enzyme that combines gaseous nitrogen with hydrogen to produce ammonia, which is then further converted by the bacteria to make their own organic compounds (Moir, 2011). When a plant or animal dies, or an animal expels waste, the initial form of nitrogen is organic. Bacteria, or fungi in some cases, convert the organic nitrogen within the remains back into ammonium (NH_4^+), a process called ammonification or mineralization. Nitrifying bacteria such as *Nitrosomonas* and *Nitrobacter* are responsible for the conversion of ammonia to nitrite and nitrate respectively (Smil, 2000).

Nitrogen fixing free living microorganism have frequently been reported as plant growth promoter. Plant Growth Promoting Bacteria (PGPB) was defined as free living soil, rhizosphere, rhizoplane and phylosphere bacteria that under some condition are beneficial for plants. These bacteria are capable of fixing atmospheric nitrogen, solubilize phosphorous and iron and enhance production of plant hormone also capable of promoting plant growth by colonizing the plant root (Kloepper and Schroth, 1978; Kloepper *et al*, 1989; Cleyet-Marcel *et al*, 2001). A variety of symbiotic (Rhizobium sp.) and non-symbiotic bacteria (*Azotobacter, Azospirillum, Bacillus, and Klebsiella sp., etc.*) are now being used worldwide with the aim of enhancing plant productivity (Burd *et al*, 2000; Cocking, 2003). The nitrogen fixing bacteria can be identified by reduction assay but the classification requires standard biochemical and cultural method (Wright *et al*, 1981).

Phosphate is also an essential nutrient required for growth promotion in plants. A greater portion of phosphorous present in the soil is in the form of insoluble phosphate and therefore is not available to the plants (Ranjan *et al*, 2013). Phosphobacteria have the ability to convert insoluble compounds of phosphorus into available phosphates that enhance nutrient availability to plants (Barea *et al*, 2005; Lugo *et al*, 2008; Rodrı´guez and Fraga 1999, Son *et al,* 2006; Souchie *et al,* 2006). The mobility of the phosphorous is very slow in the soil which is not proportionate to the rapid uptake by the plants resulting in phosphate depleted zones around the roots in rhizosphere. This phosphate deficiency leads to formation of plants with small leaves, weak stem and slow developing plants (Ranjan *et al*, 2013).

Phosphate solubilizing microbes are considered as plant growth promoting bacteria (PGPB) which provide plant Phosphate nutrition. PGPB are also giving advantages to sustainable agriculture practice as it protects the soil from negative environmental impact of chemical fertilizers and are also cost effective (Vikram and Hamzehzarghani 2008). The presence of phosphate solubilizing bacteria has been reported to be concentrated in rhizosphere (Vazquez *et al*, 2000).

Secretion of phosphatase enzymes (acid and alkaline phosphatase, phytase, phosphohydrolase) by phosphobacteria is also a common mode of facilitating the conversion of insoluble forms of P to plant-available forms and thus enhance plant Phosphate uptake and growth (Kohler *et al*,

2007). Phosphobacteria not only play a significant role in supplying Phosphate to plants, but also increase plant growth and development through other plant growth promotion activities, like nitrogen fixation, siderophores and phytohormones production (Vassilev *et al*, 2006).

Microorganisms play an important role in transformation of soil phosphorous and thus are an integral component of soil phosphorous. Phosphate-solubilizing soil bacteria could serve as efficient biofertilizer for improving the P-nutrition of crop plants and helps to minimize the P-fertilizer application, reduces environmental pollution, and promotes sustainable agriculture (Mostafa *et al,* 2000). As studied by various workers (Goldstein 1995; Kim *et al,* 1998; Rashid *et al,* 2004; Chen *et al,* 2006; Kohler et al, 2007; Lin *et al*, 2006; Pandey *et al,* 2006; Rodrı´guez and Fraga 1999; Son *et al*, 2006). Phosphate solubilizing bacteria (PSB) release organic acid (low molecular weight) through their carboxyl and hydroxyl group which chelate the cation bound to phosphate resulting in conversion of insoluble phosphate to soluble form (Kpomblekou and Tabatabai 1994). Some Gram negative bacteria have been reported to mobilize insoluble phosphate by producing gluconic acid during the extracellular oxidation of glucose catalyzed by quinoprotein glucose dehydrogenase (Goldstein, 1996).

The discipline of microbiology has gained greatly from the advances especially with respect to detection and identification of micro-organisms. Spratt (2004) suggested a range of appropriate molecular techniques and include aspects of comparative 16S rRNA gene sequencing, polymerase chain reaction detection, strategies for identification of unculturable bacteria, and whole community analysis. Microbial diversity in soil can be measured through biochemical-based techniques and molecular-based techniques. Methods to measure microbial diversity in soil can be categorized into two groups: biochemical-based techniques and molecular-based techniques. Typically, diversity studies include the relative diversities of communities across a gradient of stress, disturbance or other biotic or abiotic difference (Hughes *et al*, 2001).

The isolation and identification of bacteria is an essential diagnostic tool in microbiology. The shape of the bacteria can be determined by microscopy (using gram staining or other staining techniques for acid-fast bacteria), and culturing of the bacteria on various media − selective, differential and certain characteristic (metabolic) media (Willey *et al,* 2008). Selective media

only allow certain bacteria to grow, whilst differential media are used to distinguish bacteria from others, in the presence of some form of dye or indicator (Madigan *et al,* 2009).

Wahba *et al* (1965) studied the diversity of colonial types of *Pseudomonas aeruginosa* which may be encountered is described, together with a series of biochemical tests and the application of serological and pyocine typing which are of use in identifying atypical strains.

Sivakumaran *et al* (1997) reported a hundred strains of non-nodulating, Gram-negative, rod-shaped bacteria were isolated by ribotyping, DNA-DNA hybridization, and partial 16S rRNA sequencing. The strains were identified as *Rhizobium leguminosarum* (6), *Rhizobium loti* (2), *Rhizobium etli* (1), *Rhizobium tropici* (1), and *Sinorhizobium meliloti* (1).

Kumar *et al* (2005) conducted a study in which total of thirty bacteria were isolated and *in vitro* screening was done for different plant growth promotion activities i.e. phosphate solublization, ammonia production, ACC deaminase activity, HCN production and catalase. These isolates were identified as *Acinetobacter sp.*, *Bacillus sp.*, *Enterobacter sp.*, *Micrococcus sp.*, and *Pseudomonas sp.* on the basis of colony morphology, Gram staining and biochemical test.

Thus, recognizing the importance of sacred grove for ecological restoration we aim to undertake a novel study of restored and unrestored area of Chaksu block, Jaipur district for phycico-chemical and microbiological parameters responsible for soil fertility.

OBJECTIVES OF THE STUDY

1. Study of Chaksu block for restored and unrestored area after 1981 flood.

2. Physico-chemical analysis and comparison of restored and unrestored soil parameter related to soil fertility.

3. Analysis of bacterial diversity responsible for soil fertility of the restored and unrestored region of the Chaksu block

METHODOLOGY

Step 1: Selection of study area

The area under the flood of 1981 will be identified by imageries. The restored and unrestored villages in the area will be identified by satellite imageries acquired through IRS-1C satellite system for one time collection of the information. The soil samples will be collected through Global Positioning System (GPS). The most appropriate area will be selected for grid sampling.

Step 2: Collection of soil samples

Soil sample will be collected at various depths, in pre-sterilized polypropylene zip lock bags. The soil sample will be analyzed for the presence of microbes within 2-4 hours of sample collection and stored at 4ºC for physico-chemical characterization.

Step 3: Physico-chemical characterization of soil

The area under this conservation will be analyzed for soil physico-chemical characteristic like pH, electrical conductivity, organic carbon, organic matter, available phosphorous, available sodium and potassium, and available nitrogen. Organic carbon was measured by the procedure given by Walkley and Black (1934), exchangeable potassium and sodium by flame photometry

method, calcium and magnesium of the soil using the versenate method, total nitrogen content by the method of Kjeldahl (AOAC, 1980), phosphorus uptake by Sodium Bicarbonate (Olsen *et al*, 1954) Method, soil pH will be measured by the pH meter using the procedure of Bates (1954). Physical parameters like Water holding capacity, texture and moisture content (oven method) will also be analyzed. These characteristic of soil give an idea about the soil fertility (Woomer *et al*, 1984).

Step 4: Microbial Analysis

1. Colony Forming Unit

Total culturable bacteria will be estimated by making soil dilution and the dilution in which distinct countable colonies are visible will be considered followed multiplying the counted colonies with dilution factor (Reynolds, 2005).

2. Isolation of bacteria responsible for soil fertility:

(i) Nitrogen fixing bacteria

(a) Ammonification:

Colonies will be grown on peptone broth which contains an organic nitrogen substance, is used to demonstrate the ability of some microorganism to degrade proteins, with a resultant formation of ammonia. The presence of ammonia, indicative of ammonification, is detectable by the yellow color with Nessler's reagent (Cappuccino and Sherman 2006).

(b) Nitrification:

Determination of nitrite production

Colonies will be grown on ammonium sulphate broth. Test for the presence of nitrite by use of Trommsdorf's reagent and sulfuric acid (Cappuccino and Sherman 2006).

Determination of nitrate production

Colonies will be grown on nitrite broth and tested for the presence of nitrate by use of diphenylamine reagent and sulfuric acid (Cappuccino and Sherman 2006).

Nitrogen fixation is carried out by bacteria producing the nitrogenase enzyme whose multiple subunits are encoded by the genes nifH, nifD, and nifK (Rubio and Ludden 2002). The nifH gene (encoding the nitrogenase reductase subunit) is the most sequenced and has become the marker gene of choice for researchers studying the phylogeny, diversity, and abundance of nitrogen-fixing microorganisms. The presence of nifH gene community in the soil indicates the presence of N2 fixing population. The presence of nifH in the bacterial isolation showing nitrogen fixation will be performed by PCR amplification

(ii) Phosphate mobilizing bacteria:

Soil is a reservoir of organic and inorganic phosphorous that cannot be utilized by plants. Microorganisms make the bound phosphate available to plants by which insoluble phosphorous compounds are moblilized is by the production of organic acid or inorganic acids.

The enumeration of Phosphobacteria from soil is based on the method of Sperber (1958) using hydroxy apatite medium.

(iv) Characterization of bacteria

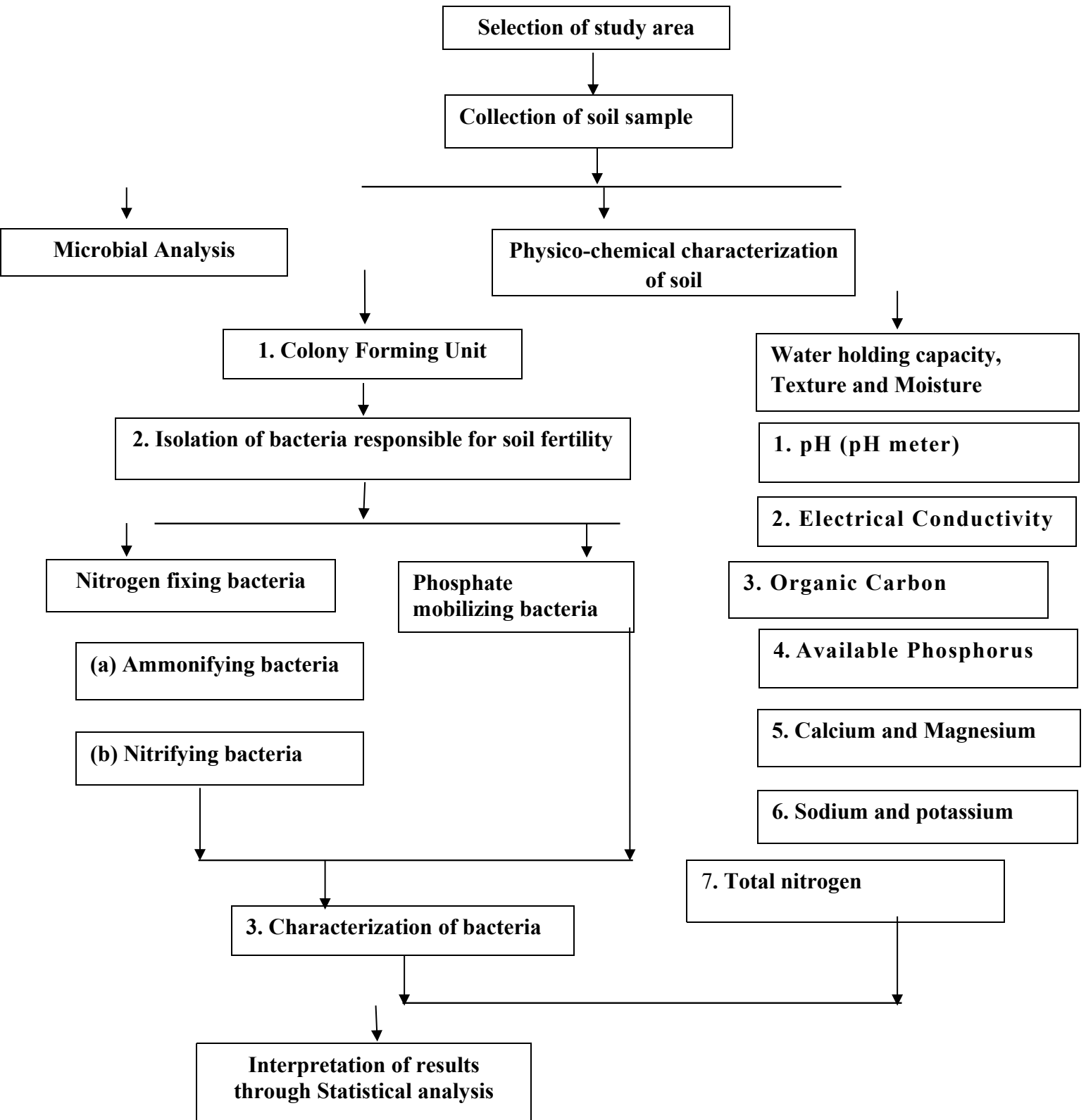Gram staining will be done to study the gram reaction, shape and arrangement. After studying the characteristic responsible for soil fertility the isolated bacteria will be characterized using Bergey's manual of bacteriology.

(v) Statistical Analysis

The quantitative results will be depicted as mean ± S.E. and significance of difference will be computed by two way ANOVA and student t-test.

SCHEMATIC REPRESENTATION OF PLAN OF WORK:

Selection of study area

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Collection of soil sample

Microbial Analysis

Physico-chemical characterization of soil

1. Colony Forming Unit

Water holding capacity, Texture and Moisture

2. Isolation of bacteria responsible for soil fertility

1. pH (pH meter)

2. Electrical Conductivity

Nitrogen fixing bacteria

Phosphate mobilizing bacteria

3. Organic Carbon

(a) Ammonifying bacteria

4. Available Phosphorus

(b) Nitrifying bacteria

5. Calcium and Magnesium

6. Sodium and potassium

7. Total nitrogen

3. Characterization of bacteria

Interpretation of results through Statistical analysis

SIGNIFICANCE OF THE STUDY

The proposed research work would embark on increased soil fertility of the flood affected areas as a consequence of restoration achieved through Joint Forest Management. This research project therefore throws light on various aspects such as soil fertility, ecological restoration, post disaster management, community participation and Joint Forest Management.

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