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Editorial

The global concerns on climate change, emerged more intensively during the past few years, have yielded a clear mandate on emission cuts during COP-21 in Paris, though the same intensified approach could not be maintained in COP-22 due to withdrawal of United States of America (USA) under the New Governance.

Nevertheless, the more vulnerable countries located in subtropical and tropical regions need to prepare themselves to evolve their own adaptation and remediation plans with their own resources, even if some other countries are not supporting. It will help the countries in crisis to secure their sustainability in development with better strength under the new climate crises in their own context.

The Society for Science of Climate Change and Sustainable Environment (SSCE) and its official journal 'Climate Change and Environmental Sustainability' (CCES) have been dedicated to communicate new knowledge on the various aspects of climate variability and climate change, its differential vulnerability to different sectors and different agro-climatic zones. We need to understand the impact of climate change on species, community, ecosystems and biome functioning and develop effective adaption and mitigation strategies to save and sustain their biodiversity, food, feed, livestock, forests, water, mountains and oceans for sustainability of the resources and life.. We need a more humane approach with nature and its biosphere to earn a better future.

The journal has achieved a NAAS rating (C-083) of 4.86 in its fourth year and it has been included in the list of journals accepted by UGC, New Delhi (Journal No. 49284). We expect that from the 5th volume, it will serve the novel purpose of fulfilling the gap in the existing data base and scientific input in the field of climate change and environmental sustainability.

The EC of SSCE has nominated a new editorial board for CCES journal in its 5th Volume with an understanding that the new team will make it more vibrant and more valuable. We are expecting the inputs from New Board Members having expertise and concerns in the different related fields. At the same time, we shall be benefitted with all other colleagues who have contributed in this journal during the last four years as authors, reviewers and editorial team members. We are grateful to all for the valuable input and suggestions.

Rana Pratap Singh
Editor, CCES

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General Article

Commentary

Climate Change and Environmental Sustainability

An official publication of "The Society for Science of Climate Change and Sustainable Environment"

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The society was founded in 2009 to inculcate the multidisciplinary expertise among professionals to address the emerging environmental concerns. The recent realization of the adverse effects of climate change, energy depletion, water crisis, loss of biodiversity and pollution of water, air and soil ecosystems has created a deep concern in people of all walk of life to the environmental issues. The membership of the society is open to researchers, teachers, professionals and anyone else who share its goal and are willing to contribute for the cause. Both annual and life memberships are available subject to a formal approval by the society. The application forms and more details about the foundation are available online at www.ssceindia.org. The executive committee of the society at present is as follows:

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REVIEW ARTICLE

Consequence of Phosphate Solubilising Microbes in Sustainable Agriculture as Efficient Microbial Consortium: A Review

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Abstract Phosphorus is an essential nutrient for plant growth and yield. Currently, phosphorus fixation in soil is a major problem throughout the world as available phosphorus in soil is highly reactive with cationic elements to form complex compounds. Rock phosphate is one of the cheapest fertiliser and most abundant; however, its direct application in soils is not much effective for the availability of phosphorus to plant growth due to its low reactivity. Further, phosphorus is present as a fixed or complex form in soils, which is unavailable for plants. Phosphate solubilising microbes (PSMs) have potential to solubilise the complex form of phosphate in the available form of phosphorus to plants. PSMs have different mechanisms for solubilisation of phosphate by producing various acids and enzymes. Other mechanisms of phosphate solubilisation and mineralisation follow chelation and immobilised cell technology. All such phosphate solubilisation mechanisms by PSMs have been discussed in this article. The current need to develop genetically modified PSM as efficient PSMs strains for sustainable agricultural production is also discussed in the present review. The PSMs is known as effective biofertiliser for enhancing the plant growth, yield and nutrient content in crops as well as improve the soil fertility under sustainable agriculture. The main aim of this review was to elaborate the phosphate solubilising activities and their consequences for sustainable agriculture.

Keywords Rock Phosphate; Phosphate Solubilisation; Genetically Modified Phosphate-Solubilising Microorganism; Sustainable Agriculture; Biofertilizer; Phosphorus; Soil Fertility

1. Introduction

Phosphorus is the second most essential macronutrient after nitrogen that plays important role in plant growth and yield (Scheffer and Schachtschabel, 1992). Lack of phosphorus (P) in soil affects production in agriculture worldwide (Palomo *et al.*, 2006). Farmers are using super phosphate continuously in form of phosphate fertiliser in agricultural land to increase crop production. These phosphates fertiliser get fixed into soils and form an insoluble complex compound (Pundarikakshudu, 1989; Aipova *et al.*, 2010). Super phosphate, an inorganic form of Phosphorus get strongly adsorbed into minerals present in soil such as poorly soluble precipitates of calcium phosphates in alkaline soils and iron and/or aluminium phosphates (Fe-P, Al-P) in acid soils (Richardson, 2001). However, most phosphorus in soil (up to 95–99%) is fixed into the soil as an insoluble complex compound that makes it unavailable to fulfil P for plant nutrition (Corona *et al.*, 1996). As results, only a few poorly soluble phosphorus of soil are considered as highly stable forms with limited availability to plants. P constitute only 5% or even less of the total amount available for plants and others approximate 95% P fixed into soils. In general, tropical and subtropical soils are the mostly acidic type with extremely deficient in phosphorus content. In addition to it, tropical and subtropical soils possess a high phosphate sorption capacity. Factors like strong sorbed capacity or fixed phosphate make the soil P content unavailable for plant uptake. However, a large proportion of applied P fertilisers is easily precipitated into insoluble complexes and becomes unavailable for plants. This, in turn, leads to a need for the excessive dose of soluble P fertilisers, which, in addition to the economic constraint, can also pose a serious threat to

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groundwater [Environmental Protection Agency (EPA), 2013].

Now a days, as substantial inputs of P are always required for promoting plant growth and adequate food yield and fibre production. Soil amendment with phosphate fertiliser, produced by chemical processing of rock phosphate ore (RPO), is, therefore, became an absolute requirement to feed the world's population. The superphosphates are most common and commercially existing phosphatic fertilisers used in most developing countries. As it is not produced locally, therefore, its supplies to poor farmers in the rural area are relatively restricted (Hammond *et al*., 1986). On the other hand, many developing countries have good deposits of natural phosphate rock (PR) mineral which could be used as an alternative [British Sulfur Corporation Limited (BSCL), 1987]. A number of researches have shown that PR can be used directly in agriculture and it is more effective whenever used in acid P-deficient soils of tropical and subtropical countries (Khasawneh and Doll, 1978). Several factors also verify that direct application of PR product is crucial in agronomic effectiveness in the supply of phosphorus to crops. Thus, direct application of PRs is an agronomically as well as economically sound and one of an alternative to the use in place of expensive superphosphates under certain soil and climatic condition of tropical and subtropical countries. Its extensive utility in temperate and tropical agriculture has already been described by several reviewers (Khasawneh and Doll, 1978; Hammond *et al*., 1986). The several factors regarding its utility can be categories into three types namely inherent PR factors, soil factors and plant factors. Consequently, the effectiveness of PR products or materials also differs widely depending on its varying geological origin, mineralogical composition and physical and chemical properties (Hammond *et al*., 1986; Lehr and Mc Clellan, 1972; Binh *et al.*, 1978).

According to the US Geological Survey and the International Association of Fertilizer Producers, the demand for fertilisers over the next 5 years will likely to increase by 2.5–3% annually. At this rate of phosphate consumption, all global phosphate resources would be exhausted within 100–125 years (Gilbert, 2009). In view of the long-term enhance in P and phosphate, production would be maximum within coming 20 years and the importance of partial P recycling continues to grow. Convalescing phosphates from livestock waste is one of such examples of reusing P for agriculture. Other ways to control the wastage of phosphate resources include reducing P run-off into the oceans (Gilbert, 2009; Aipova *et al*., 2010). In future, phosphorus deficiency may cause a crisis of food production because of unavailable phosphorus present in soil as fixed form. Only microbiological processes are well justified alternative methods to solubilise fixed form of phosphate into available form for plant growth. Thus, phosphate solubilising microorganisms (PSMs) play an important role in providing P for plant nutrition and growth promotion, especially when phosphate fertilisers are fixed in soil due to extensive use in the agricultural field. It has been proven that agricultural application of these PSMs boosts crop yields (Khan *et al*., 2007). On the other hand, the activity of PSM more or less depends on physical and chemical properties of the soil. It involves P-solubilisation mechanisms include acid formation, chelating metal ions and exchange reactions. The most active PSMs belong to micromycetes of following genera: *Aspergillus, Penicillium, Curvularia* and phosphate-solubilising yeast. These micromycetes are more active in solubilising phosphates compared with bacteria.

For over 100 years, researchers and scientists have identified the capacity of soil microorganisms in solubilising phosphorus (P) from insoluble (i.e. nutritionally unavailable) organic and mineral phosphates (Whitelaw, 2000). Wide ranges of microbial bio-solubilisation mechanisms exist in nature, so that much of the global cycling of insoluble organic and inorganic soil phosphates are attributed to bacteria and fungi. The genetic and biochemical mechanisms for this solubilisation are as varied as the spectrum of P-containing soil compounds. The limiting level of P in most soils provides the eco-physiological basis for positioning associations between plant roots and mineral phosphate solubilisation (MPS) and/or organic P-solubilising microorganisms. These associations are assumed to play an important role in managing phosphorus nutrition in many natural and agro-ecosystems. As a result, number of researches have been conducted involving isolation and characterisation of MPS and organic P-solubilising microorganisms from a wide range of soils. In general, the goals of these type of research for understanding P cycle and/or to develop P-biofertilisers analogous to biological nitrogen fixation (BNF). With respect to agriculture, bioprocessing of RPO to inorganic phosphate may provide energy efficient, environmentally desirable and sustainable and eco-friendly alternative to improve current technology for industrial development in P fertiliser production. Therefore, present study of this or the aim of the present review is to explore the phosphate solubilisation mechanism and activities of phosphate solubilising microorganism to enhance the available phosphorus in the soil for sustainable agricultural production.

1.2 Significance of Phosphorus

Phosphorus plays a crucial role in photosynthesis, respiration, cell division, cell enlargement and several other processes in plants. An adequate supply of phosphorus promotes plant growth physiological functions at the early root and shoots formation and it is important for laying down the primordial for reproductive parts of plants. It is for seed formation and its content is higher in seeds than in any other part of the plant. It helps the plant to survive in Temperate/

winter region and also contributes to the development of disease resistance in some plants. BNF depends appreciably on the available forms of phosphorus. Phosphorus (P) is an important structural constituent of nucleic acids, phytic and phospholipids.

2. Problems in Availability of Phosphorus by Phosphorus Fixation in Soil

One of the most important problems in tropical agriculture is the low-soil-phosphate availability. Many of the tropical soils are highly weathered and have a high P-fixation capacity that makes their management more difficult (Sanchez, 1976). Sanchez and Logan (1992) estimated that 1018 million ha in the tropics have a high P-fixation capacity. In tropical America, there is 659 million ha affected, 210 in Africa and 199 in Asia. The term 'P-fixation' refers to a series of complex reactions that remove bio-available soil P from the soil solution, where roots directly take up plant nutrients (Barber, 1995). Such reactions consist in the sorption of phosphates on the solid surface of soil colloids and in the precipitation of phosphates with some cations in the soil solution (Havlin *et al*., 1999). The main causes of Phosphate sorption the presence of crystalline or non-crystalline hydrous-oxides of iron and aluminium in highly weathered soils of humid regions and acid savannas (Mattingly, 1975). Allophane (a non-crystalline aluminium-silicate) and humus-Al/Fe complexes are the responsible of the P sorption in soils derived from volcanic parent materials (Schwertmann and Herbillon, 1992; Shonji *et al*., 1993).

The precipitation of P in acidic soils occurs as active forms such as aluminium (Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^+$ and iron (Fe^{2+} and Fe^{3+}). In calcareous soils, P is absorbed on the surface of calcium carbonate (Mattingly, 1975) or precipitated with calcium (Ca^{2+}) (Bohn *et al.*, 1985). The predominance of these mechanisms depends on the extent of weathering and pH of the soil. In past decades, several measures have been formulated to minimise the effect P-fixation effect in soil and to increase the efficiency agricultural field in term of P content. These measures are the use of high rates of P fertilisers, selective use of fertilisers, time and method of application, the combination with amendments and other fertilisers, use of soil tests and others (Engelstad and Terman, 1980).

However, the efficiency of P fertilisers is still not up to the mark, it is low (5–10%) (Havlin *et al*., 1999). Additionally, in the present day, excess use of P fertiliser face to environmental concern regarding high levels of P-fertilisation (Brady and Weil, 1999). Rock phosphates (RPs) (apatite) having lesser P fixing capacity compared with other P fertiliser, therefore, most recommended for soils. RPs are really insoluble, particularly in alkaline soils and a little more reactivity is always desired (Hammond and Leon, 1992; Chien and Hammond, 1978).

2.1 Phosphate Availability in Soils

Phosphorus minerals from rocks and soil deposited during different geological process within different time era constitute the biggest reservoirs of phosphate in this earth. The most principle characteristic of these minerals, which make it important, is their insolubility. In contrary to it, a large portion of inorganic chemical fertilisers are soluble phosphate get rapidly immobilised soon after it applied to agricultural soil and these becomes unavailable to plants as fixed form of phosphate. A second major component of soil P is organic matter, present mostly in the forms of inositol phosphate (soil phytate), accounting for up to 50% of the total organic P. Other organic P in the soil is in the forms of phosphomonoesters, phosphodiesters, including phospholipids and nucleic acids and phosphotriesterase which are converted in to orthophosphate form for plant growth by phosphatase enzyme. In addition to these, large quantities of organic P are xenobiotic phosphonates released into the environment. Despite all these, the concentration of soluble P (that is, bioavailable P) is usually very low in soils due to the phenomenon of chemical fixation of phosphate. Phosphorus (P) is one of the major essential macronutrients needed to plant for their biological growth and development (Ehrlich, 1990). In general, it is present up to 400–1200 mg/kg in soil (Fernándezand Novo, 1988).

The phosphorus cycle in the biosphere is totally called as 'open' as well as sedimentary as there is no any interchange of P from the atmosphere (Begon *et al*., 1990). Microorganisms play an important role in natural phosphorus cycle by acting as mediator in its capacity of conversing P in the useable form for the plant. This cycle involves the cyclic oxidation as well as reduction of phosphorus compounds, where electron transfer reactions between oxidation stages range from phosphine (Glick *et al*., 1998) to phosphate (Broadbent *et al*., 1977). There are about 40 million tons of phosphatic rock deposits in India (Roychoudhury and Kaushik, 1989) and these materials should provide a very good source of phosphate fertiliser for crop production (Halder *et al.*, 1990). Mineral forms of phosphorus are generally represented in soil by its primary minerals, such as apatite, hydroxyapatite and oxy apatite. They are mainly found as part of the stratum rock and are present in their insoluble forms.

2.2 Rock Phosphates

Current concepts in sustainability involve the application of alternative strategies based on the use of less expensive natural sources of plant nutrients like RP. The beneficial effect of RPs has made this material an attractive component for management in agriculture (Rajan *et al.*, 1996). One traditional method of increasing P-availability is the acidulation of RP by adding small amounts of H_2SO_4

or H_3PO_4 to produce partially acidulated RP (Rajan *et al.*, 1996). But this is uneconomical and environmentally nonviable.

The use of commercial P-fertilisers is not cost effective. Among the alternative P sources, the most important are locally available RP resources (Rajan *et al.*, 1996). Not all of the RP resources are readily available for plant and agronomically reactive when applied directly to the soils. Reactivity is defined as the combination of RP properties that determines the rate of dissolution of RP in a given soil under given field conditions. The main factors influencing the agronomic effectiveness of availability of phosphorus by RPs are depending on reactivity/solubility, mineralogy, grain size, surface area and the chemistry of RPs. The availability of phosphorus by RP has been depending on the chemical and physical status of soil, especially pH and P fixing capacity of the soil, type of crops and their nutritional requirements and management practices, including method and time of application.

3. Phosphate Solubilising Microorganisms (PSMs)

PSMs include those groups of microorganisms, which not only assimilate phosphorus from insoluble forms of phosphates, but they also cause a large portion of soluble phosphates to be released in quantities in excess of their requirements. Soil microorganisms play a role in maintaining the ecological balance by active participation in Carbon, Nitrogen, Sulphur and phosphorus cycles in nature. PSMs plays an important role in plant nutrition through an increase in phosphate uptake by plants and used as biofertilisers of agricultural crops (Karpagam and Nagalakshmi, 2014). Phosphate is one of the most vital macronutrients required for the growth and development of plants. Species of *Aspergillus* and *Penicillium* are among fungal isolates identified to have phosphate solubilising abilities. Among the bacterial genera with this capability are *Pseudomonas, Azotobacter, Azospirillum, Bacillus, Rhizobium, Burkholderia, Arthrobacter, Alcaligenes, Serratia, Enterobacter, Acinetobacter, Flavobacterium* and *Erwinia* (Rodriguez *et al.*, 1996; Ahmad *et al.*, 2008). Seed or soil inoculation with PSMs is known to improve solubilisation of fixed soil phosphorus and applied phosphates, resulting in higher crop yields (Jones and Darrah, 1994). Some of the isolated phosphate solubilising organisms are, *Pseudomonas putida*, *Erwinia herbicola*, *Rahnella aquatitis, Enterobacter agglomerans, Pseudomonas cepacia, Pseudomonas aeruginosa, Serratia marcescens, Klebsiella pneumonia, Pseudomonas striata, Burkholderia cepacia, Rhizobium* sp., *Bacillus* sp., *Aspergillus niger, Penicillium* sp. and others. These microorganisms have the genes for MPS and thus exhibiting the MPS phenotype (Asuming-Brempong and Aferi, 2014). PSMs are a low-cost input that enriches the soil giving a thrust to economic development without disturbing ecological balance.

Verma *et al*. (2010a,b) have been reported that the plant growth promoting rhizobacteria, for example *P. aeruginosa* BHUPSB01, *P. putida* BHUPSB04, *Pseudomonas fluorescens* BHUPSB06, *Bacillus megaterium* BHUPSB14, *Paenibacillus polymyxa* BHUPSB16, *B. cepacia* BHUPSB03, *Acinetobacter calcoaceticus* BHUPSB11 and *Klebsiella* sp. BHUPSB08 are strong phosphate solubilising microorganisms, which are isolated from the rhizosphere soil of eastern Uttar Pradesh, India. The phosphate solubilisation in Pikovaskaya agar media showed clear halozone, which is the indicator of strong phosphate solubilisation due to a production of acids (Fig 1) and also tricalcium phosphate solubilisation in broth culture as compared with control (Fig 2). Phosphate solubilisation was

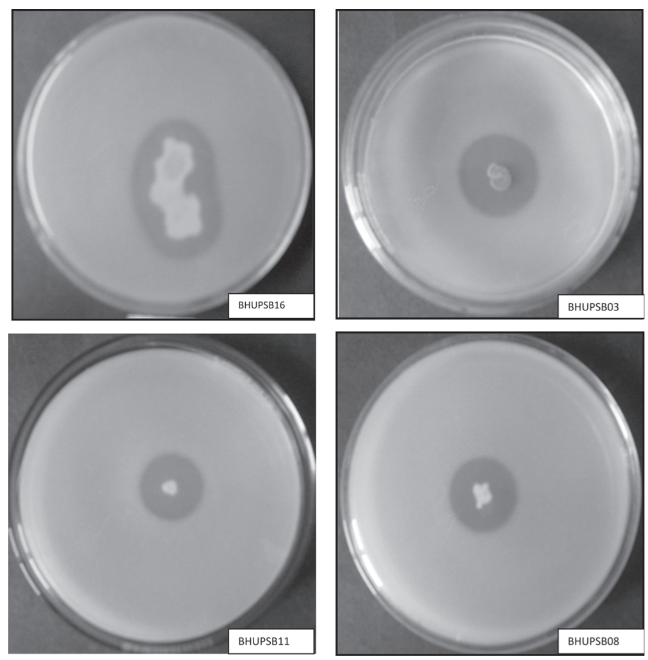

Figure 1. Phosphate solubilising microbes *Paenibacillus polymyxa* strain BHUPSB16, *Burkholderia cepacia* BHUPSB03, *Acinetobacter calcoaceticus* strain BHUPSB11 and *Klebsiella* sp. strain BHUPSB08 showed solubilising zone on Pikovaskaya agar media

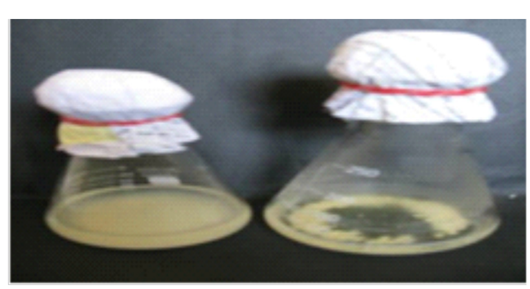

Figure 2. Solubilisation of try calcium phosphate by *Paenibacillus polymyxa* strain BHUPSB16 in Pikovaskaya broth culture after three days incubation at 30^0C as compared with control

most frequently encountered by *P. aeruginosa* followed by *B. megaterium* and least by *Mesorhizobium* sp. and *Azotobacter chroococcum*. *P. aeruginosa* produces largest halos, 20mm (approx.) around their colonies within 5 days of incubation than other isolates (Verma *et al*., 2013).

Filamentous fungi are widely used as producers of organic acids (Mattey, 1992) and in particular, *A. niger* and some *Penicillium* species have been tested in fermentation system or inoculated directly into soil to solubilise RP (Kucey, 1987; Vassilev *et al*., 1996). Reddy *et al*. (2002a,b) found that all the isolates of *Aspergillus tubingensis* and *A. niger* isolated from rhizospheric soils were found to be capable of solubilising all the natural forms of RPs. This is the first report of solubilisation of RPs by *A. tubingensis* and showed that this fungus might serve as an excellent RP solubiliser. Goenadi *et al.* (2000) determined the optimum incubation period and the optimum level of RP for a phosphate solubilising fungus (PSF), *A. niger* BCCF194, isolated from tropical acid soils. They conducted a simple, effective and environmentally sound process to improve P availability of PRs to crops by PSF. Lavakush *et al.*(2014) reported as *P. aeruginosa* BHUJY16, *P. aeruginosa* BHUJY20, *P. putida* BHUJY13, *P. putida* BHUJY23, *P. fluorescens* BHUJY29 are a strong phosphate solubilisers as well as plant growth promoters for rice crop production (Table 1). Thien and Myers (1992) indicated that by increasing soil microbial activities, the bioavailability of P in a bioactive soil was remarkably enhanced.

4. Mechanisms of Phosphate Solubilisation and Mineralisation

The phenomenon of fixation and precipitation of P in soil is highly dependent on soil properties, type and pH. Thus, in acid soils, free oxides and hydroxides of aluminium and iron fix P, whereas in alkaline soils, Ca fixes it. Organic acid and metabolite production of microbes helps in decreasing pH of soil and solubilise the RP. Phosphate solubilisation by microbes is mediated by several different mechanisms, including organic acid production and proton extrusion (Surange *et al*., 1997; Burgstaller and Schinner, 1993; Cunningham and Kuiack, 1992; Dutton and Evans, 1996; Nahas, 1996). Increasing P concentration in the phosphate solubilising fungal containing medium is related to the production of organic-acid-type metabolites, which should correlate with pH of the medium (Illmer and Schinner, 1992; Illmer *et al*., 1995; Narsian *et al*., 1995). It is generally recognised that organic acids solubilise RP through protonation and/or chelation reactions (Kouno *et al*., 1998). In addition to the acidic strength, the type and position of the ligand determine the effectiveness of the organic acid in the solubilisation process (Kpomblekou and Tabatabai, 1994). There is the various mechanism of phosphate solubilisation like an acid production by microbes which directly help in solubilisation and others microbial synthesis of phosphatase enzyme which helps directly for the conversion of organic phosphate to inorganic phosphate as available phosphate for plant growth and yield (Figure 3).

4.1 Role of Chelators on Inorganic Phosphate Solubilisation

The principal underlying mechanism of action of chelators is the formation of unionised association compounds with Ca^{++}, Fe^{++} and Al^{+++} as well as increasing soluble phosphate concentration by scavenging phosphate from mineral phosphates. The ability of low molecular weight organic acids to release P from ores or rocks, related to their ability to form stable metal complexes are well established (Mattey, 1992). Narsian and Patel (2000) reported that *Aspergillus aculeatus* showed phosphate solubilisation by chelator mechanism, which is isolated from rhizosphere soil of gram. This chelator, nitrilotriacetic acid increased RP solubilisation at 2mg/ml, whereas diethylene-triaminepentaacetic acid enhanced phosphate solubilisation only at 6 mg/ml. The ethylenediaminetetraacetic acid, aluminium and oxine also inhibited phosphate solubilisation at all concentrations. They also found that the highest phosphate solubilisation activity, in presence of RP, was recorded up to 50 mg P_2O_5.

4.2 Role of Phosphatase in Organic Phosphate Solubilisation

Phosphorus is the very important nutrient for a structural and functional component of all organisms. Insoluble organic and inorganic compounds of phosphorus are present in soil sediments and it immobilised in the living organism (De *et al*., 2012; Paytan and McLaughlin, 2007), that mostly released by alkaline phosphatase (AP) activity. AP and acidic phosphatase are a key-enzyme which catalyzes the liberation of orthophosphate from organic phosphorus compounds in the alkaline and acidic environment, respectively (Figure 3). Mineralisation of most organic phosphorus compounds that may constitute up to 30–50% of the total phosphorus in most soils is carried out by means of phosphatase enzymes, primarily acid phosphatases. These catalyze dephosphorylating reactions involving the hydrolysis of phosphoester or phosphoanhydride bonds. The main mechanism of organic phosphate solubilisation depends on acid phosphatase activity (Mc Grath *et al*., 1995). Arbuscular mycorrhiza (AM) produces organic phosphate that has the ability to acidify the environment as well as facilitates inorganic P dissolution (Bago *et al*., 1996). Phosphatases are inducible catabolic ectoenzymes of aquatic microorganisms (Boavida, 1990; Vazquez, 2000). Phosphatases play a prominent role in the avoidance of

Table 1. Recent literature for application of phosphate solubilising microbial consortia to provide available phosphorus for crop yields

Phosphate Solubilising Strains	Nature of Study	Finding of Study	Reference
Aspergillus, Penicillium, Klebsiella, Burkholderia, Staphylococcus	To study determined the cell density in the field and the biological activity of culturable phosphate solubilising micro-organisms (PSMs) present in the Elaeis guineensis Jacq. rhizosphere at two locations in a commercial plantation	Palm oil rhizosphere presented a diverse population of PSMs The genera *Aspergillus* and *Penicillium* and three bacteria of the genera *Klebsiella, Staphylococcus* and *Burkholderia* were associated with the solubilisation of calcium phosphate with high soluble P yields. This work may help to enhance oil palm sustainable agriculture by diminishing the demand of inefficient fertilisers	Acevedo *et al.* (2014)
B. cepacia BAM-6	Impact of a phosphate solubilising bacterium and an arbuscular mycorrhizal fungus (*Glomus etunicatum*) on growth, yield andP concentration in wheat plants	Wheat seeds treated with B. cepacia BAM-6 and AMF showed overall better growth and yield Evaluating the crop yield, single inoculation either with PSM or AMF was less effective in comparison to dual inoculation	Saxena and Jha (2014)
Pseudomonas aeruginosa BHUJY16, *P. aeruginosa* BHUJY20, *Pseudomonas putida* BHUJY13, *P. putida* BHUJY23, *Pseudomonas fluorescens* BHUJY29	Evaluation of PGPR and different concentration of phosphorus level on plant growth, yield and nutrient content of rice (*Oryza sativa*)	Combination of PGPR strains of *Pseudomonas* culture with *A. Chroococcum + A. brasilense* + 30 kg/ha P_2O_5 may be used as more effective combination for rice production	Lavakush *et al*. (2014)
Acinetobacter sp. YU-SS-SB-29	Uranium(VI) bioprecipitation mediated by a phosphate solubilising *Acinetobacter* sp. YU-SS-SB-29 isolated from a high natural background radiation site	The bacterium *Acinetobacter* sp. YU-SS-SB-29 isolated from monazite sand exhibited inherent properties of phosphate solubilisation and U tolerance. *Acinetobacter* sp. YU-SS-SB-29 having resistance to U, which could biologically release the bound phosphate and thereby chemically facilitate the formation of U-P for long-term remediation of uranium	Sowmya *et al*. (2014)
Serratia marcescens NBRI1213	To study of solubilisation of tricalcium phosphate by temperature and salt tolerant *Serratia marcescens* NBRI1213 isolated from alkaline soils	*S. marcescens* NBRI1213 can therefore be used as a PGPR agent because its application resulted in better growth even under stressed environment This strain may be helpful in minimising the impact of salt and temperatures stresses which is currently limiting crop production under low input conditions and give rise to a more sustainable agriculture	Lavania and Nautiyal (2013)
Aeromonas RPSB9, *Bacillus* RPSB5, *Enterobacter* RPSB8, *Flavobacterium* RPSB7 and *Pseudomonas* RPSB6	Bacteria showing phosphate solubilising efficiency in river sediment	PSB are widely distributed in different niches and sediment was found to be the most efficient carrier That studies will potentially increase our awareness of the roles of microorganisms in aquatic ecosystems and the process of eutrophication	Paul and Sinha (2013)
B. megaterium	Application of phosphate-solubilising bacteria for enhancing bioavailability and phytoextraction of cadmium (Cd) from polluted soil	This study demonstrated that *B. Megaterium* as phosphatesolubilising bacteria (PSB) has a potential for solubilising phosphorus from soils itself and could promote plant growth by providing soil minerals such as P This bacterialstrain could reduce the plant stress against Cd, especially for *B. juncea*	Jeong *et al.* (2012)
Burkholderia gladioli (MTCC 10216),	Enhanced biomass and steviol glycosides in Stevia rebaudiana	Inoculation with *Burkholderia gladioli* strains 10216 and 10217, *Enterobacteraerogenes* 10208	Gupta *et al*. (2011)

Contd...

Phosphate Solubilising Strains	Nature of Study	Finding of Study	Reference
B. gladioli (MTCC 10217), *Enterobacter aerogenes* (MTCC 10208), *Serratia marcescens* (MTCC 10238)	treated with phosphate-solubilising bacteria and rock phosphate	and *Serratia marcescens* 10238 effectively utilised Mussoorie rock phosphate (MRP) to enhance plant growth and stevioside(ST) and rebaudioside-A (R-A) contents of *Stevia rebaudiana* Solubilisation of MRP by PSB strains varied from 1.4 to 15.2 µg/ml, with the highest solubilisation by *Enterobacter aerogenes* 10208	
Bacillus M-13	Performance of phosphate solubilising bacteria for improving growth and yield of sunflower (*Helianthus annuus* L.) in the presence of phosphorus fertiliser	Application of PSB *Bacillus* M-13strain has beneficial effects on growth, yield and quality of sunflower Highest seed yield of sunflower possible with P fertiliser was achieved with about 50 kg P_2O_5/ha when used in conjunction with PSB, whereas the combined effect of the 100 kg P_2O_5/ha fertiliser level and PSB showed maximum increase in oil yield.	Ekin (2010)
Pantoea sp., *Enterobacter* sp.	Isolation of phosphate solubilising bacteria and their potential for lead immobilisation in soil	PSB increase the solubilisation of insoluble P compounds and the principal PSB-induced P solubilisation mechanism is pH reduction by organic acid production PSB provide plant growth promoting potential through the release of IAA and siderophores PSB enhance the P-induced immobilisation of Pb through the release of P from insoluble P compound	Park *et al*. (2011)
S. marcescens CTM 50650	Characterisation of the mineral phosphate solubilising activity of *Serratia marcescens* CTM 50650 isolated from the phosphatemine of Gafsa	Development of an environmental-friendly process for fertiliser production considering the capacity of *S. marcescens* CTM 50650 to achieve yields of P extraction up to 75% from the Gafsa RP.	Farhat *et al*. (2009)
Pseudomonas, *Aeromonas*, *Klebsiella*, *Enterobacter*	Biodiversity of phosphate solubilising bacteria in rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana	Phosphate solubilisation of these isolates varied from 5.9 to 123.8% and 2.2 to 227.2 µg/ml in solid and liquid Pikovskaya's medium, respectively The highest PSB number and greatest variability were found in the rhizosphere of chickpea, followed by wheat and then mustard	Kundu *et al*. (2009)
Bacillus cereus, Bacillus megaterium, Burkholderia caryophylli, Pseudomonas cichorii, Pseudomonas syringae	Phosphate-solubilising and -mineralising abilities of bacteria isolated from soils	Inorganic P-solubilising bacteria (IPSB) strains exhibited inorganic P-solubilising abilities ranging between 25.4 and 41.7 µg P /ml and organic P-mineralising abilities between 8.2 and 17.8 µg P/ml Organic P-mineralising bacteria (OPMB) strains also exhibited both solubilising and mineralising abilities varying from 4.4 to 26.5 µg P /ml and from 13.8 to 62.8 µg P/ml, respectively	Guang-Can *et al*. (2008)
Aspergillus tubingensis	Phosphate solubilisation by a wild type strain and UV-induced mutants of *Aspergillus tubingensis*	P solubilisation by these isolates is due to lowering of pH of the culture filtrate and also the activity of acid phosphatase and phytase	Achal *et al*. (2007)
Burkholderia cepacia, Burkholderia vietnamiensis, Pantoea ananatis, Pantoea agglomerans, Serratia marcescens	Isolation and characterisation of mineral phosphate-solubilising bacteria naturally colonising a limonitic crust in the south-eastern Venezuelan region	PSB isolates were able to mediate almost complete solubilisation of $Ca_3(PO_4)_2$ in liquid cultures; in contrast, the PSB isolates were less effective when solubilising $FePO_4$ Acidification of culture supernatants seemed to be the main mechanism for P solubilisation	Perez *et al*. (2007)

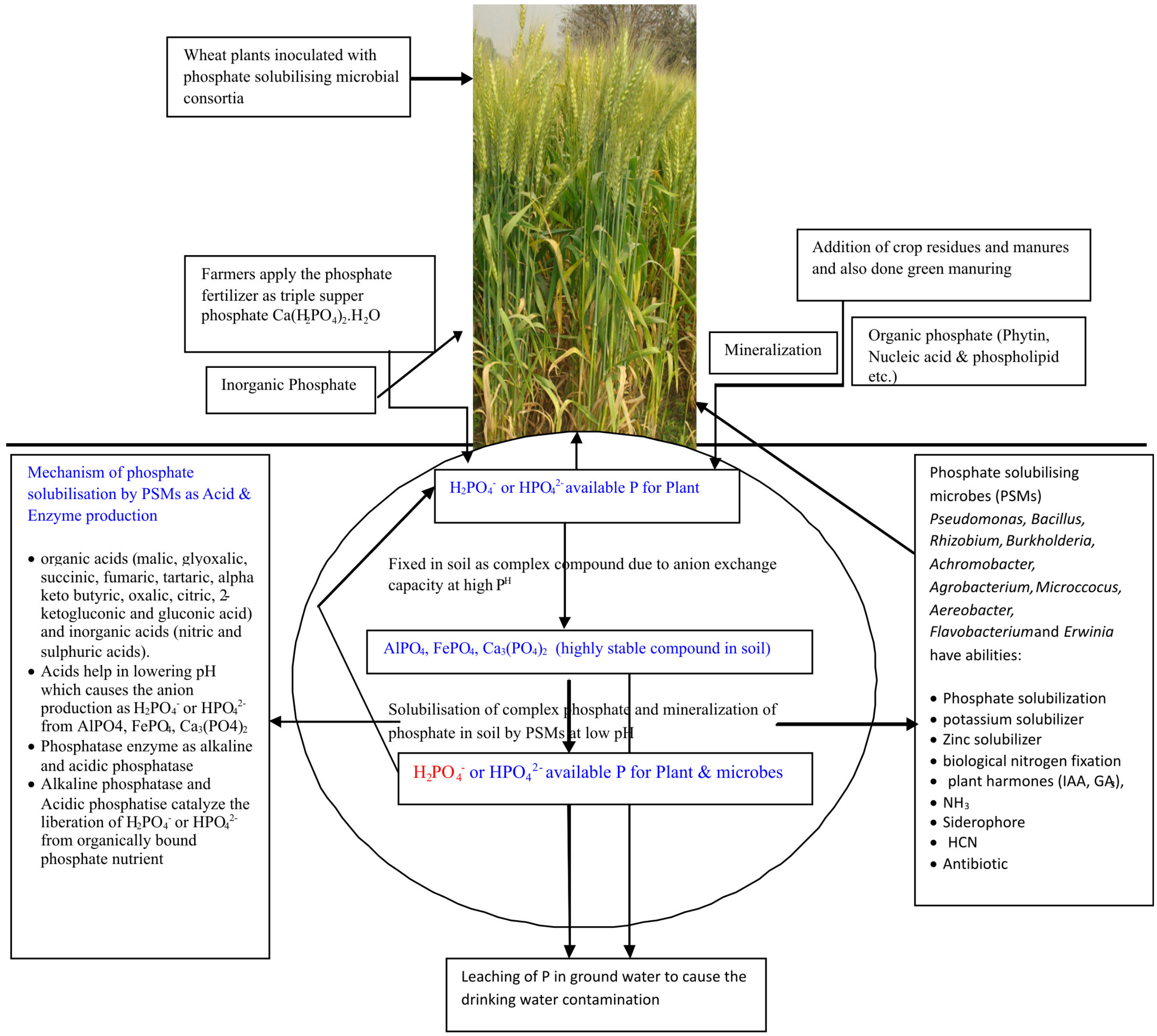

Figure 3. Schematic diagram of phosphate solubilisation process in soil: phosphate solubilising microbes have the ability to solubilise the complex compound of phosphate and organic phosphate into available orthophosphate for uptake of crops

phosphorus limitation in the sea (Sundareshwar *et al*., 2003; Zohary and Robarts, 1998).

4.3 Role of Immobilised Cell Technology in Phosphate Solubilisation

Immobilised microbial P-solubilisers are used for better understanding of the mechanisms of solubilisation of insoluble phosphates. Immobilisation methods are particularly important for filamentous fungi. The mycelial growth of filamentous fungi at high biomass concentrations creates mycelial suspensions having the high viscosity which helps in immobilisation of nutrients. In general, immobilisation methods provide an excellent protection of cells from adverse environmental effects.

Vassileva *et al*. (1998) encapsulated spores of *A. niger* in agar, calcium alginate and k-carrageenan and further applied in citric acid production during six repeated batch cultivations. The highest average citric acid productivity was reached with alginate-bead-encapsulated on RP free culture medium, whereas agar seemed to be the most suitable carrier on RP-supplemented medium (Vassileva *et al*., 2000) found that cell encapsulation favoured the acid-producing activity of *Yarowia lipolytica* that ensured higher average acid productivity and solubilisation levels as compared with treatments with free cells. The reuse efficiency of agar-encapsulated yeast cells for citric acid production was greater than that by freely suspended cells. Alginate and k-carrageenan appeared to be unsuitable carriers when RPs was supplied to the medium solution in place of calcium carbonate. Vassilev *et al*. (1997) immobilised *A. niger* on polyurethane foam. They found that immobilised cells were reused, with higher levels of the acid formation is for longer periods (at least 240 h) than for free cell. Vassilev *et al*. (2001) summarised all available studies that involved immobilised microorganisms related to RP solubilisation and P plant nutrition and pointed out possible future trends in this field of research.

4.4 Role of Microorganisms in Phosphate Solubilisation

By increasing soil microbial activities, the bioavailability of P in a bioactive soil was remarkably enhanced (Thien and Myers, 1992). Looking towards the future, it is reasonable to propose that, using the tools of biotechnology, biophosphorus fertilisation is an achievable goal that lends itself well to the global imperative of sustainable agricultural production. Mineral solubilisation by soil microorganisms is widespread and, with respect to agriculture, this process has been paid considerable attention. Microbial survival following introduction into, particularly natural soils depends on both abiotic and biotic factors (Van Loosdrecht *et al*., 1990). Phosphobacterin is a culture of *B. megaterium* var. *phosphaticum*, phosphate solubilising bacteria (PSB) adsorbed on kaolin. IARI MicroPhos culture (Gaur, 1990) a preparation of carrier-based inoculant of *P. striata*, *Bacillus polymyxa*, *Aspergillus awamori* was used in India. Many free living and symbiotic fungi can solubilise RP and increase phosphorus availability for plants (Kucey, 1987; Illmer and Schinner, 1992; Griffiths *et al.*, 1994).

Several mechanisms of P solubilisation by rhizosphere bacteria have been proposed to explain the P solubilisation by phosphate solubilising rhizosphere bacteria (PSRB); they are associated with the release of organic and inorganic acids and the excretion of protons that accompanies to the NH_4^+ assimilation (Roos and Luckner, 1984; Abd-Alla, 1994; Illmer *et al*., 1995; Asea *et al*., 1988; Whitelaw, 2000). In addition, the release of phosphatase enzymes that mineralise organic P compounds has been also suggested as another mechanism involved (Stevenson, 1986). Azam and Memon (1996) affirm that *Nitrosomonas* and *Thiobacillus* mobilised inorganic phosphates by producing nitric and sulphuric acid. Equally, phosphates may be released from solid compounds by carbonic acid formed as a result of the decomposition of organic residues (Memon, 1996). Many organic acids are effective in solubilising soil phosphates, these acids are produced by rhizosphere microorganisms (Marschner and Dell, 1994). Bolan *et al*. (1994) studied the influence of the addition of organic acids on high P-fixing soils. These acids decreased the P sorption on the clay surfaces which favoured the solubilisation of RP and also increased the dry matter of Rye grass (*Lolium rigidium*) as well as plant P uptake. Hue (1991) found similar results in the availability of P, when added organic acids on tropical soils in Hawaii and concluded that the efficiency of P-fertilisers might be enhanced due to the addition of organic acids with green manures or animal wastes.

Kim *et al*. (1997) point out that the production of organic acid is the major mechanism involved in the solubilisation of hydroxyapatite (RP) by the PSB *E. agglomerans*. Under *in vitro* conditions, the pH of the growth medium has decreased as a result of the release of organic acids commonly, gluconic acid (GA), oxalic acid, citric acid, lactic acid, tartaric acid and aspartic acid (Di Simine *et al*., 1998). These acids are the product of the microbial metabolism, mostly by oxidative respiration or by fermentation of organic carbon sources (e.g. glucose) (Atlas and Bartha, 1997; Prescott *et al*., 1999). Such biological reactions occur in the rhizosphere where carbonaceous compounds are used by PSB and the phosphate released is taken up by the roots or mycorrhiza symbiosis.

PSMs solubilise different forms of inorganic insoluble phosphates. *Pantoea agglomerans* was isolated from an iron-rich ore which has maximum efficient solubility of $Ca_3(PO_4)_2$ as compared with Fe-P ($FePO_4$) and Al-P ($AlPO_4$) (Sulbarán *et al*., 2009; Panhwar *et al*., 2009) (Figure 3). Reddy *et al*. (2002a,b) found that the isolates of *A. tubingensis* and *A. niger* were found to be capable of solubilising all the natural forms of RPs, which is the first report of solubilisation of RPs by *A. tubingensis* and showed that this fungus might serve as an excellent RP solubiliser when inoculated into soils where RP is used as P fertiliser. Goenadi *et al*. (2000) also determined a PSF, *A. niger* BCCF.194 that is isolated from tropical acid soils. *A. niger* strain BHUAS01 was reported as a significant solubilisation of tricalcium phosphate at 1% $CaCl_2$ in saline condition and glucose used as sole source of carbon for more growth of fungus (Yadav *et al*., 2011). Inoculation with PSRB and other soil microorganisms, such as AM fungi (AMF), might enhance even more the benefits of this P solubilisation. The mechanisms involved in the microbial solubilisation of P are the production of organic acids and the release of protons to the soil solution (Kim *et al*., 1997).

5. Application of Phosphate Solubilising Microbes in Agriculture

PSMs have great potential for solubilisation of complex phosphate into available phosphate in the soil for plant growth and yield. Microbiologists have reported that PSM is the best microbial consortium for phosphate solubilisation in the world. Various phosphates solubilisation microbes have been reported to them are given in Table 1. Studied reveal that *Penicillium bilaii* and *Penicillium cf. fuscum* increased total plant phosphorus uptake by 14% and wheat dry matter yield by 16% (Asea *et al*., 1988). The commercial uses microbe like *P. bilaii*spores to increase P % in a wheat field of Canada on has been already started (Cunningham and Kuiack, 1992).

Vassilev *et al*. (1996) observed a higher growth rate and shoot phosphorus content when sugarbeet waste treated with microbes in presence of RP to both mycorrhizal and non-mycorrhizal plants. Also, combine application of both the filamentous and arbuscular fungi show enhancement in plant growth, when degraded organic matter are the supplement or not supplemented with RP was used. The application of RP with Macuna and Lablab as organic matter were found to significant enhancement of grain yield, total N and total P uptake of maize crop in non-acidic soils (Vanlauwe *et al*., 2000). The studied legume–maize rotations supplied with RP during the legume phase and minimal amount of inorganic N during the maize phase are good examples of soil fertility management technologies alleviating N and P deficiencies. Investigations conducted experiments under field level using wheat and maize plants have revealed that PSMs could drastically reduce the usage of chemical or organic fertilisers (Singh and Reddy, 2011). Vessey and Heisinger (2001) reported that the enhancement of plant phosphorus nutrition might be due to stimulation of root growth or elongation of root hairs by microorganisms; thus, there was no direct increase in the availability of soil phosphorus is always expected. PSMs have been isolated from various plants soils such as walnut (Yu *et al*., 2011), rice (Chaiharn and Lumyong, 2009), mustard (Chandra *et al*., 2007), oil palm (Fankem *et al*., 2006), soybean (Son *et al*., 2006), aubergine and chilli (Ponmurugan and Gopi, 2006) and maize (Alam *et al*., 2002). Inoculated with PSB better crop performance was reported to be achieved from several horticultural plants and vegetables (Young *et al*., 2003).

In some cases, the inoculation with known PSB has enhanced the plant growth without affecting plant P uptake. De Freitas *et al*. (1997) found that the inoculation with the PSB's *Bacillus thuringiensis, Bacillus brevis, Bacillus megatherium, Bacillus polymyxa, Bacillus sphaericus* and *Xanthomonas maltophila* increased the growth and yield of canola (*Brassica napus*), but they did not increase the plant P uptake. PSB can also release substances that promote root growth such as hormones, enzymes and antibiotics, enhance availability of other nutrients (e.g. Mn and Fe) and exert biocontrol of plant pathogens (Frankenberger and Arshad, 1995; Bashan and Holguin, 1998). The synergistic effects have been found in sunflower (*Helianthus annuus*) with the triple inoculation of *A. chroococcum, Penicillium glaucum* and *Glomus fasciculatum* (Gururaj and Mallikarjunaiah, 1995); in cotton with the inoculation of *P. striata* and *Azospirillum* sp. (Prathibha *et al*., 1995); in rice, favourable effects were also reported with *P. striata* and *B. polymyxa* (Mohod *et al*., 1991); in chilli (*Capsicum annuum*) with *G. fasciculatum* or *Glomus macrocarpum* and *P. striata* (Sreenivasa and Krishnaraj, 1992); in wheat with *P. putida, P. aeruginosa* and *P. fluorescens* in combination with *Glomus clarum*. The combination of two PSRB, *P. striata* and *Agrobacterium radiobacter*, with *G. fasciculatum* and *Gigaspora margarita* gave higher significant plant growth of wheat when these microbes and fertilisers were added together (Gaur, 1990).

In soybean, the combination of *Bradyrhizobium japonicum*, *P. fluorescens* and *Glomus mosseae* have given equally good results (Shabayev *et al*., 1996). Such results are likely because of to a higher P uptake promoted by the PSRB and AMF, which may satisfy the high P requirements of the symbiotic N_2 fixing process (Azcon and Barea, 1996; Young *et al*., 1990). Toro *et al.* (1996) reported that the combination of AMF (*Glomus* spp.) and eight PSRB found significant increase growth and uptake of P in tropical legume, kudzu (*Pueraria phaseoloides*). These PSRB were isolated from an oxisol and were characterised by their ability to solubilise RP, Fe-P and Al-P. On the other hand, Fe-p solubilisers were more effective alone, whereas Al- and RP-solubilisers performed better when were concurrently inoculated with AMF. Currently, the fungal inoculum *P. bilaii* is commercially available in North America with the name of Provide™, which has been successfully tested to enhance plant P uptake of several plants (Whitelaw, 2000).

PSMs have greater potential for solubilisation of phosphate as well as plant growth promoting properties such as synthesis of phytohormones (IAA, GA3 and Cytokinin), hydrogen cyanide, ammonia, antibiotics, siderophores and various enzymes which help in degradation of cellulose and lignin and also help in nitrogen fixation (Figure 3). Several bacterial and fungal species with varied potentials to solubilise inorganic phosphates, also known as phosphate solubilising microorganisms isolated from rhizosphere soil (Jain *et al*., 2012). There are several previous reports dealing with the application of PSB, either individually or combined, to assess their effects on the growth and biomass production of several crops (Fernandez *et al*., 2007; Mittal *et al*., 2008; Vikram and Hamzehzarghani, 2008; Hariprasad and Niranjana, 2009; Jain *et al*., 2010). Meena *et al*. (2010) reported that the inoculation of the chickpea (*Cicer arietinum*) with the novel symbiotic fungus (*Piriformospora indica*) and a Tn5-lacZ-tagged phosphate-solubilising bacterium (*P. striata*) was found an efficient combination for plant growth and yields.

6. Molecular Approach for Development Genetically Modified Phosphate Solubilising Microorganism (GMPSM)

Various molecular tools and techniques are useful for molecular characterisation as well as gene cloning to develop genetically modified strains for phosphate solubilisation and plant growth promotion (Figure 4). Field demonstration of genetically modified microorganism is controversial, whereas some countries promote it and others proscribe (Rodriguez *et al*., 2006). But this type genetically modified strains should be introduced in field demonstration for higher food production and fertility enhancement through proper government rule and regulations. Chromosomal insertion of the genes is one of the tools to evade horizontal transfer of the introduced genes within the soil rhizosphere. For gene cloning, particular phosphate solubilises gene isolation, identification and their ligation in suitable vector and also an expression of phosphate solubilising activity in appropriate host. After this, the host expressed the function of introducing gene that is called genetically modified strains. In spite of the problems, significant improvement has been made in obtaining genetically engineering microorganisms for agricultural practices (Armarger, 2002).

Genetically modified plant growth promoting microorganisms have many advantages as compared with transgenic plants for enhancing plant growth and yield (Figure 4, Table 2). With current technologies, (1) it is far easier to modify a bacterium than complex higher organisms; (2) several plants growth-promoting character can be combined in a single organism; and (3) as an alternative of engineering crop-by-crop, single, engineered bio-inoculants can be used for multiple crops, especially when using a non-specific genus like *Azospirillum* (Rodriguez *et al*., 2006). Introduction or over-expression of genes involved in soil phosphate solubilisation (both organic and inorganic) in natural rhizosphere bacteria is a very attractive approach for improving the capacity of microorganisms to work as inoculants (Bashan *et al*., 2000). Direct oxidation of glucose is the major pathway for glucose utilisation in gram-negative soil bacteria, in which glucose is directly converted into GA and 2-ketogluconic acid (Goldstein, 1995). These low molecular weight organic acids play a major significant role in MPS ability of soil microbes. The secretion of organic acid has been enhanced by the genetic modification of specially that gene who responsible for MPS. The production of the citric and oxalic acid from soil microbes is due to central carbon metabolism pathway. This is the great approach for converting rhizobacteria into potential PSB. Over expression of citrate synthase gene (gltA) of *E. coli* in *P. fluorescens* ATCC13525 results in secretion of citric acid; however, the secreted citrate was not sufficient to release P (Buch *et al*., 2009).

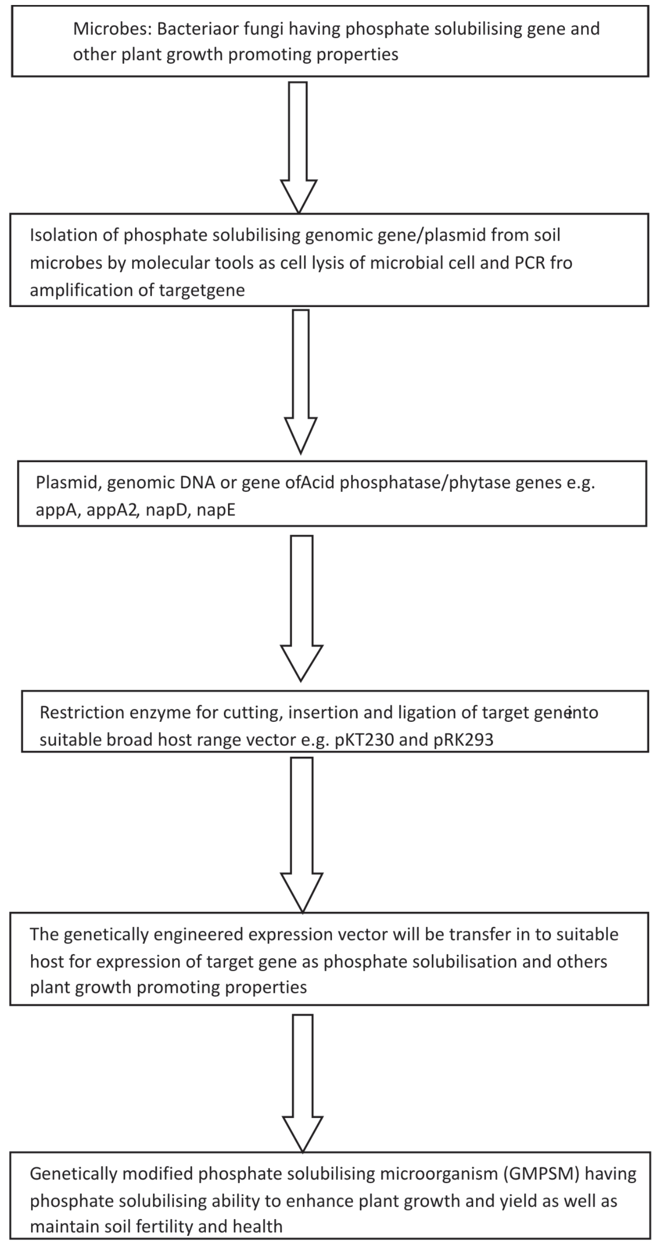

Figure 4. Schematic and general description about molecular gene cloning to develop genetically modified phosphate solubilising microorganism (GMPSM) for enhancing phosphate solubilisation for plant growth and yield

6.1 Molecular Mechanism of Organic Phosphate Solubilisation

Organically bound phosphorus can be released in soil for plant growth by three groups of enzymes: (1) nonspecific phosphatases, which carry out dephosphorylation of phospho-ester or phospho-anhydride bonds in organic matter, (2) phytases, which specifically cause P release from phytic acid and (3) phosphatases and C–P lyases, enzymes that perform C–P cleavage in organophosphates. At present,

Table 2. Microorganism having phosphate solubilising gene and plasmid which is transferred into others microbes to develop genetically modified phosphate solubilisingmicroorganism(GMPSM)

Microorganism which Have PSM Gene	Gene or Plasmid	Host in which gene transferred to develop GMPSM	Mineral Phosphate Solubilise	Acid Production	References
Erwinia herbicola	*Mps, pqq E*	*E. coli* HB101, *Azospirillum* sp.	Tricalcium phosphate	Gluconic acid	Goldstein and Liu (1987), Vikram *et al*. (2007)
Pseudomonas cepacia	*gabY, PQQ Transporter*	*E. coli* HB101, *E. coli* JM109	Tricalcium phosphate	Gluconic acid	Babu-Khan *et al*. (1995)
Enterobacter agglomerans	pKKY	*E. coli* HB101	Tricalcium phosphate	–	Kim *et al*. (1997)
Rahnella aquatilis	*Pqq* ED, pK1M10	*E. coli/E. coli* DH5α	Hydroxy apatite, Tricalcium phosphate	Gluconic acid	Kim *et al*. (2003), Kim *et al*. (1998b)
Serratia marcescens	*pKG3791, PQQ*	*E. coli*	Tricalcium phosphate	Gluconic acid	Krishnaraj and Goldstein (2001)
Enterobacter intermedium	*Pqq ABCDEF*	*E. coli* DH5α	Hydroxy apatite	Gluconic acid	Kim *et al*. (2003)
E. coli	*Ppts-gcd, PgnlA-gcd*	*Azotobacter vinelandii*	Tricalcium phosphate	Gluconic acid	Sashidhar and Podile(2009)
E. coli K12	*g/tA/*citrate synthase	*Pseudomonas fluorescens* ATCC13525	Dicalcium phosphate	Citric acid	Buch *et al*. (2009)
Pseudomonas putida KT2440	*gad/Gluconate dehydrogenase*	*E. asburial* PSI3	Rock phosphate	Gluconic and 2-ketogluconic acid	Kumar *et al.* (2013)
Morganella morganii	Nap A phosphatase, pRK293 vector	*Burkholderia cepacia* IS-16	Organic and Inorganic phosphate	–	Fraga *et al*. (2001)

acid phosphatases and phytases are found most predominantly in soil. The efficiency of the plant and microbial phosphatases on organic P depletion in the rhizosphere and P uptake by plants has been well documented (Tarafdar and Jungk, 1987; Tarafdar and Claassen, 1988). Nonspecific acid phosphatases (NSAPs) are formed by three molecular families, which have been designated as molecular class A, B and C (Thaller *et al*., 1995). These enzymes seem to function as organic phospho-ester scavengers, providing the cell with essential nutrients (Wanner, 1996). The acpA gene is isolated from *Francisella tularensis*, which expresses an acid phosphatase with optimum action at pH 6, with a wide range of substrate specificity (Reilly *et al*., 1996). Also, genes encoding NSAPs class A (PhoC) and class B (napA) isolated from *Morganella morganii* are very promising, as the biophysical and functional properties of the encoded enzymes were extensively studied group of several *Bacillus* having plant-growth promoting activity (Idriss *et al*., 2002). It also showed the highest extracellular phytase activity and diluted culture filtrates of these strains stimulated the growth of maize seedlings under limited phosphate in the presence of phytate. This type study provides strong support that phytase activity can be important for enhancing growth under limited availability of P in soil and supports the potential of using phytase genes to improve or transfer the P-solubilising trait to PGPB strains used as agricultural inoculants. Among soil rhizobacteria, phosphatase activity encoded gene was isolated from *B. cepacia* (Rodríguez *et al.*, 2000). This gene codes present on outer membrane protein, which enhances synthesis of acid phosphatase gene in the absence of soluble phosphates in the medium and could be involved in P transport to the microbial cell. Further, cloning of two nonspecific periplasmic acid phosphatase genes (napD and napE) from *Rhizobium* (*Sinorhizobium*) *meliloti* was accomplished (Deng *et al*., 1998, 2001) (Figure 4, Table 2). The napA phosphatase gene was isolated from *Morganella morganii* and then transferred this gene using the broad-host-range vector pRK293 into *B. cepacia* IS-16, which is an efficient strain used as a biofertiliser (Fraga *et al*., 2001).Therefore, a recombinant strain *B. cepacia* IS-16 was found the significant increase in extracellular phosphatase activity. Insertion of the transferred genes into the bacterial chromosome is advantageous for constancy and ecological protection.

6.2 Molecular Mechanism of Phytases in Phosphate Solubilisation

High molecular weight acid phosphatases belong to phytases (myo-inositol hexakisphosphate phosphohydrolases). Phytate is the primary source of inositol under the basic condition and the major stored form of phosphate in plant seeds and pollen. However, monogastric animals are incompetent of using the P bound in phytate because their gastrointestinal tracts have low levels of phytase activity. Therefore, not quite all the dietary phytate P ingested by monogastric animals is excreted, resulting in phosphorus contamination in areas of intensive animal production and why phytases have emerged as very attractive enzymes for industrial and environmental applications. Genetic studies of phytases began in 1984 and the first commercial phytase, produced by genetically modified microorganisms, appeared on the market in the mid-1990s (Yanming *et al*., 1999).

Phytate is the most important component of organics forms of phosphorus in soil (Richardson, 1994). The capability of plants to acquire phosphorus directly from phytate is very limited. Still, genetically modified Arabidopsis plants with phytase gene (phyA), isolated form of which is *A. niger*, were found as significant enhancement in growth and phosphorus nutrient (Richardson *et al*., 2001). Consequently, development of bioinoculant with high phytase production for sustainable agricultural would be a great interest for improving plant nutrition and reducing P pollution in soil. Although phytase genes have been cloned from fungi, plants and bacteria (Lei and Stahl, 2001). Bacteria are the most realistic for the genetic modification as compared with fungi and plants. Temperature stable phytase genes (phy) from *Bacillus* sp. DS11 (Kim *et al*., 1998a,b) and *B. subtilis* VTT E-68013 (Kerovuo *et al*., 1998) have been cloned. Acid phosphatase/phytase genes (appA and appA2 genes) have been isolated and molecularly characterised from *E. coli* (Golovan *et al*., 1999; Rodriguez *et al*., 1999). Also, neutral phytases have the great possibility for genetic improvement of PGPB. Neutral phytase genes have been currently cloned from *B. subtilis* and *B. licheniformis* (Tye *et al*., 2002). A phyA gene has been cloned from the FZB45 strain of *B. amyloliquefaciens*. It showed the highest extracellular phytase activity, that can be important for stimulating growth under limited P in soil and supports the potential of using phytase genes to enhance or transfer the P-solubilising trait to PGPB strains used as efficient microbial incouclant for sustainable agricultural production.

6.3 Molecular Mechanism of Inorganic Phosphate Solubilisation

Rodríguez and Fraga (1999) reported that the most soil microbes have the ability of MPS due to organic acid production. Goldstein (1994) found it as a major mechanism for MPS in Gram-negative bacteria direct glucose oxidation to GA. Goldstein and Liu (1987) had done the first cloning of MPS gene into *E. coli* HB101 which had been isolated from the Gram-negative bacteria *E. herbicola*. Expression of MPS gene in *E. coli* HB101 allowed the production of GA for hydroxyapatite solubilisation under control condition. *E. coli* can synthesise only GDH, but not PQQ, thus it does not produce GA. The cloned 1.8 kb locus encodes a protein similar to the gene III product of a pqq synthesis gene complex from *A.calcoaceticus* and to pqqE of *Klebsiella neumonie* (Liu *et al*., 1992). PQQ synthase gene found in *E. herbicola* DNA fragment that could be complemented by single open reading frame (ORF) isolated by them also. By accident, a 7.0 kb DNA fragment was isolated from *Rhanella aquatilis* genomic DNA, which has the ability for hydroxyapatite solubilisation in *E. coli* and also showed two complete ORFs and a partial ORF. One of the cloned proteins showed similarity to pqq E of *E. herbicola, K. neumoniae* and *A. calcoaceticus* (Kim *et al*., 1998a,b), whereas the partial ORF is similar to the pqq C of *K. neumoniae*. Kim *et al*. (1998a,b) also report that these genes complement cryptic pqq *E. coli* genes, thus allowing GA production. Another type of gene (gabY) involved in GA production and MPS was cloned from *P. cepacia* (Babu-Khan *et al.*, 1995). In the presence of gabY, GA is produced only if the *E. coli* strain expresses a functional glucose dehydrogenase (gcd) gene (Table 2). A genomic DNA fragment from *E. agglomerans* showed MPS activity in *E. coli* JM109, although the pH of the medium was not altered (Kim *et al*., 1997). For manipulation of a mineral solubilising gene, expression in *E. coli* of the mps genes from *R. aquatilis* supported a much higher GA production and hydroxyapatite dissolution in comparison with the donor strain (Kim *et al*., 1998a,b). The authors suggested that different genetic regulation of the mps genes might occur in both species. Expression of an mps gene in a different host could be influenced by the genetic background of the recipient strain, the copy number of plasmids present and metabolic interactions. Thus, genetic transfer of any isolated gene involved in MPS to induce or improve phosphate-dissolving capacity in PGPB strains, is an interesting approach. An attempt to improve MPS in PGPB strains, using this approach, was carried out (Rodríguez *et al*., 2000) with a PQQ synthetase gene from *E. herbicola*. This gene, isolated by Goldstein and Liu (1987), was subcloned in a broad host range vector (pKT230) (Figure 4). The recombinant plasmid was expressed in *E. coli* and transferred to PGPB strains of *B. cepacia* and *P. aeruginosa*, using tri-parental conjugation. Several of the exconjugants that were recovered in the selection medium showed a larger clearing halo in medium with tricalcium phosphate as the sole P source. This indicates the heterologous expression of this gene in the recombinant strains, which gave rise to improved MPS ability of these PGPBs.

The 16S-RNAgene sequences and microarrays techniques have the ability for detection of functional gene and phylogenetic analysis of soil microbial diversity with next-generation sequencing and soil microbiome analyses, which provide further application of diversity analysis, surrounding particular traits or functional groups of microorganisms (Richardson and Simpson, 2011). Jointly, these molecular tools and techniques provide new opportunity to address key questions in microbial community analysis and to assess the survival and persistence of specific inoculants under changed environmental conditions.

7. Future Perspectives and Challenges

In spite of their diverse ecological niches and numerous functional properties, P-solubilising microorganisms have yet to fulfil their promise as commercial bio-inoculants. Current developments in our considerate of the functional diversity, rhizosphere colonising ability, mode of actions and judicious application are possible to facilitate their application in a consistent way for the management of sustainable agricultural systems. In the last few decades, the significant studies on PSM and their role in sustainable agriculture have been done or also it is going on but the current tool and technique remain less successful. However with a consciousness of the limitations of existing methods, a re-evaluation can be expected, subsequently, the application of PSM as an efficient biofertilisers in different soil environment becomes a reality. Increase in the application of PSM is one of the novel promising options for meeting agricultural challenges imposed by the still-growing demand for food. Therefore, the production of high grain yields is the main challenge for agriculture. In recent years, both producers and consumers have gradually more focused on health and quality of foods, as well as on their nutritional properties due to the higher application of chemical fertiliser and pesticide. For this reason, the biotechnological approach is also likely to ensure conservation of environments. However, PSM can help in phosphate solubilisation, nitrogen fixation, production of phytohormones, ammonia, siderophore, hydrogen cyanide and antibiotics; due to these benefits, scientists must learn more about them and explore ways and means for their better utilisation in agriculture. Future research should focus on management of plant–microbe interactions and their mode of actions and adaptability to extreme environments to get maximum plant growth and yield. In addition, scientists and researchers need to deal with certain issues, like how to enhance the efficiency of phosphate solubilisation ability of microbes or biofertilisers or microbial consortia and what should be an ideal and universal delivery system, how to stabilise these microbes in soil systems and how nutritional and root exudation aspects could be controlled to get maximum benefits from PSM appliance. Biotechnological and molecular tools and techniques could probably develop more thoughtful and alternative about PSM mode of actions that could lead to more successful plant–microbe interaction in soil rhizosphere. Efforts should also be directed towards the use of PSM to reduce pesticide applications. In brief, PSM biotechnology provides an excellent opportunity to develop environment-friendly phosphorus biofertiliser to be used as supplements and/or alternatives to chemical fertilisers. Overall, further studies on this aspect of PSM will provide crucial information in future for the better use of these PSM in varied environmental conditions.

8. Conclusion

Phosphorus is one important essential nutrient for plant growth and yield. It is major use in body building of the plant. Fixed phosphorus in soil is only solubilised by the effective PSM by various mechanisms, such as cell immobilisation and chelating process technology. It is environmentally acceptable and economically sound. The PSM is also known as effective biofertiliser for enhancing the plant growth, yield and nutrient content in crops as well as improve the soil fertility. PSM have saved the chemical fertiliser. In this context, phosphate solubilising microorganism is an important biofertiliser for sustainable agriculture production to enhance environmental and food quality, soil fertility status and soil health. It also provides for basic human food and fibre needs, is economically viable and enhances the quality of life for farmers and society as a whole.

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REVIEW ARTICLE

Assessment of River Ecosystems and Environmental Flows: Role of Flow Regimes and Physical Habitat Variables

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Abstract Globally, rivers are under tremendous anthropogenic stress caused by flow fragmentation, land use changes and regulation. Floodplains had been cut out from rivers by embankments due to urban extension and most of the riparian lands in the river basin are under intensive agriculture. There was also widespread water quality degradation due to the industries and municipal wastewater causing a steep decline in the freshwater biodiversity across the world. It was recognised in the literature that the integrity of flowing water systems depended largely on their natural dynamic character which in turn maintained the habitat. Habitat characteristics and presence and absence of particular species suggests that most fishes of flowing water streams are habitat specialists and physical habitat variables including flow regimes played critical roles in the maintenance of their richness. This paper integrated broad environmental flow concepts and defined a new framework for e-flow assessment and implementation based on a review of supportive understanding of variable flow regimes and their benefits to river ecosystems. The main purpose of the framework was to stimulate the emerging discussion on environmental flows prospects in India and other parts of the world. The paper provided an example of the framework applied to River Ganga in India. We end by examining research needs and gaps in our understanding of the role of variable flow regimes and physical habitat variables and their ecological effects on river ecosystems.

Keywords River systems, River basins, Flow regime, Eco-hydrology, Environmental flows, Water quality, Habitat suitability.

1. Introduction

Human activities such as abstraction of water, disposal of excess waste-water, irrigation and clearing of vegetation can change the natural flow regime. These activities can lead to either an increase or a decrease in quantity of flow as well as changing the timing, duration and seasonal pattern of ecologically important flow events. Climate change is also contributing to changed flow regimes in the rivers such as the reduction in flow seen in various parts of the world (south-west Western Australia, Ganga Alluvial Plain). Many of the major rivers of the world no longer support ecologically and socially valued diversity of native species or sustain healthy ecosystems that provide important ecological goods and services (Dutta *et al.*, 2011, 2015; Dudgeon, 2000; Naiman *et al.*, 1995; Poff *et al.*, 1997a, 1997b). While academicians and researchers have considered rivers as part of living ecosystems whose ecological integrity depends upon their physical, chemical and biological characteristics and interactions within their catchments, river engineers and planners have largely treated rivers as water-delivery networks ignoring ecosystem interactions in designing water allocation schemes and reservoirs.

Restoration and management of riparian and riverine ecosystems are of significant importance in the face of extensive and multiple development pressures (Grygoruk and Acreman, 2015; Arthington, 2012). A thriving river maintains functional integrity under changing social and environmental conditions and performs many functions through its several processes – geo-morphological, ecological, hydrological and socio-cultural. The combined functional integrity gives rivers resilience (Acreman *et al.*, 2015; Poff *et al.*, 1997a, 1997b). Rivers support a multitude of goods and services that benefit mankind and ecology, such as supporting aquatic and riparian biodiversity (flora and fauna), influencing micro-climate, recharging groundwater, diluting pollutants and supporting self-cleaning systems, sustaining livelihoods, transporting silt and enriching the soil. A sustainable river ecosystem not only meets the needs

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of society over the lifetime of the water infrastructure but also maintains key ecological functions that support the provision of ecosystem goods, services and values (Pittock and Lankford, 2010).

A river is not simply water running over the land, but a catchment area – that is, a system linked internally by a hierarchical or cumulative network of river channels. Deteriorating flow regimes and the discharges of pollutants affect a river's health adversely particularly in the light of climate change, population growth and economic development. The focus of water management professionals in developing countries is still largely on maximising water supply for drinking and irrigation, hydropower generation and flood protection. The river ecologists appreciate the ecosystem approach in environmental flow allocations while river engineers are comfortable with integrated water resources management (IWRM) concept with some amount of interests in basin-wide approach. The water and irrigation departments are mainly concerned about human livelihoods and social well-being, while river ecologists and hydrologists wish to define the flow requirements with respect to desirable ecosystem conditions. Acreman *et al*. (2014a) note that 'although hydrological sciences may be at its heart, an environmental flow is truly a cross-disciplinary issue'. Environmental flows are defined as the variable stream-flows necessary to maintain and sustain habitats, including channel morphology and substrate, support spawning and passage of fauna species to previously unpopulated habitats, facilitate the processes upon which succession and biodiversity depend and maintain the desired nutrient structure within lakes, streams, wetlands and riparian areas (MoWR, 2005). Some radical river regulation measures are being taken up in India and other parts of the world under river-restoration projects, but they are largely civil related earth works and channelisation schemes containing the river flow. In such projects, geo-morphological wisdom is seldom considered at design stage with widespread loss of river habitat and floodplain integrity.

2. The Importance of Variable Flow Regime

Flow is generally seen as the master variable influencing the nature of rivers because of its ability to affect all other aspects of the physical and chemical environment in a river (Arthington, 2012). There is no single correct e-flows regime for a river, as every river has a unique ecological system that depends upon complex interactions between physical, biological and other fluvial attributes. In assessing the e-flow for a river, the answer has been largely on what people want from a river. The available knowledge base is very limited and for most of the rivers there is a reference condition of 'natural flow' that existed prior to major river regulation. Very little research has been carried out on understanding implications of environmental-flow releases in managed streams in different climatic regions of the world. There is a lack of evidence, on how managed river ecosystems evolve when flow-regimes are restored. The flow regime of a river system is an important indicator of its health and it represents the variable quantity, duration and seasonal pattern of flows. Many aspects of flow can be analysed but seven key parameters are recognised by river scientists as essential ecological and social attributes of flow. Using these key aspects, hydrological data can be summarised to describe a river's natural, present and possible future flow regimes.

Magnitude**:** quantity of water moving through a given location per unit time

Frequency**:** number of flows of a given magnitude per unit time (also described as the return period)

Predictability**:** certainty of flows of a specific magnitude returning on an annual basis

Timing**:** dates when flows of a certain magnitude begin

Duration**:** length of time of flow of a certain magnitude

Rate of change**:** rate at which the magnitude of flow changes (important for dam releases)

Variability**:** natural daily, seasonal and longer variability of flows.

This variable regime is instrumental in maintaining the distinct ecological richness in the river and influences the flora and fauna compositions. It is observed that some species require permanent inundation of water to a certain depth range while some are naturally adapted to low flow periods. The variable flow regime also influences the lifecycle activities of fauna such as spawning and the survival of larvae and juveniles. The high flow conditions are required to deposit nutrients around the banks, distribute seeds as well as for emigration of certain aquatic species. Any change to a river's flow regime for a longer duration disturbs the composition and richness of the fauna community present, including the fish and macro invertebrates. It can also disturb the river ecosystem by permanently changing aquatic and riparian vegetation, aquatic connectivity, changes in water quality parameters as well as erosion and sedimentation dynamics. Riparian communities are often subjected to multiyear droughts and flood events which help in maintaining the particular habitat (Figure 1).

It is a well-accepted fact that the health of a river and its associated ecosystems deteriorates if the flow falls below a minimum range of values. A poor understanding of river's ecosystem and flow-dependent habitat lies at the heart of the widespread neglect of rivers (Sunding, 2011). As long as the discharge is above a threshold value, the river is able to function satisfactorily. However, researchers have agreed that maintaining the e-flows is not the only thing, but all elements of a flow regime, including high, medium and low flows are important. The fact is, river supports a variety of

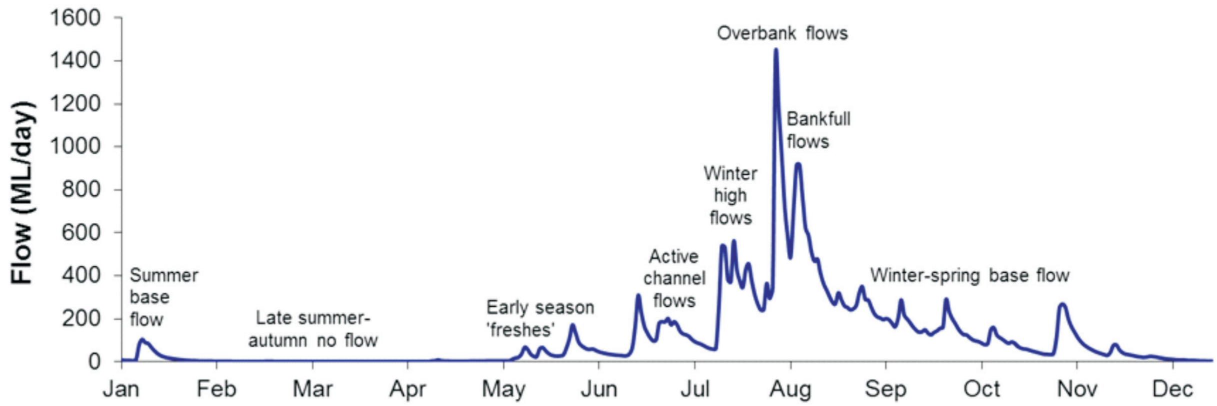

Figure 1. Representative hydrograph showing different components of the variable flow regime in a river (Green *et al.*, 2010)

ecosystem services and the bio-physical and social systems supported by rivers are too intricate to be summarised by a single assessment of minimum flow requirements. No matter how advanced and accurate the environmental-flows assessment is, the output remains on paper if no actual releases are made. There is also a fundamental difference between environmental flow requirements (EFR) and environmental flow allocations/releases (EFA) – while EFR show how different flows would achieve different ecological status, EFR refer to the actual release of water to the environment. Even though the science of eco-hydrology dealing with the flow-ecology relationships is confronting the challenge of uncertainties, the scientists need to show the utility of research outcome rather than stressing upon the challenges of system complexity (Acreman *et al.*, 2014a).

Monthly peak flow, annual water yield and long-term hydrological trends of River Ganga at the downstream of Farakka are shown in Figures 2 and 3. The graph shows how the monthly peak flow and corresponding water yield varies on a long-term basis. There is a change in monthly peak flow after 1985 with more variability than before. In contrast annual water yield seems to show less variability after 1985. During monsoon season flows, the gates are generally left open. They are then gradually lowered as the flood recedes. Although downstream movement of cetaceans through barrages can occur while the gates are open, high-velocity currents within the openings probably prevent, or at least impede to a considerable degree, upstream movement. This characteristic flow pattern has been instrumental in development of a particular habitat type in the river segments. The Padma River that supported dolphins has been greatly affected by the Farakka Barrage which divides the overall population of Gangetic dolphins at approximately the geographical centre of their range. The Kanpur Barrage further fragments dolphins in the Ganges mainstem. A remarkable impact of barrages on migratory fish is that after completion of the Farakka Barrage, landings of hilsa (*Hilsa ilisha*), a commercially important anadromous fish, declined upstream of the barrage by more than 99% (Jhingran, 1982). Also below Farakka, saline encroachment has most probably reduced the amount of habitat available to dolphins thus reducing their population (Figures 2–4).

Reductions in water supplies downstream of barrages have resulted in diminishing of dry season habitat. It is also

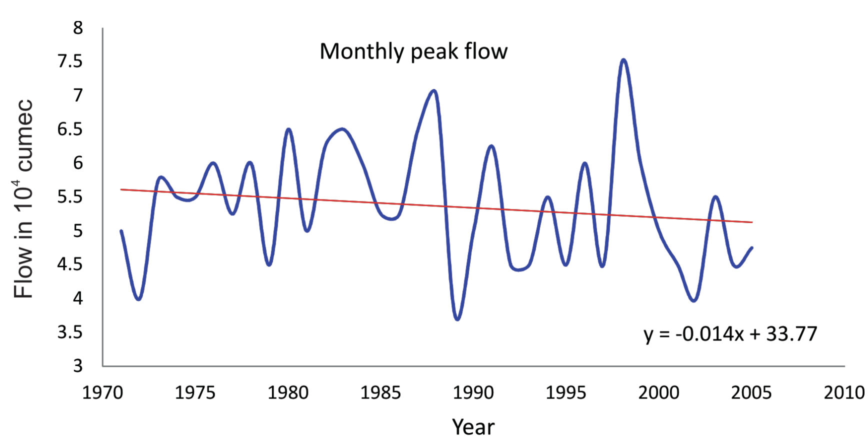

Figure 2. Monthly peak flow and long-term hydrological trends of River Ganga at the downstream of Farakka (flow in 10^4 m^3/s)

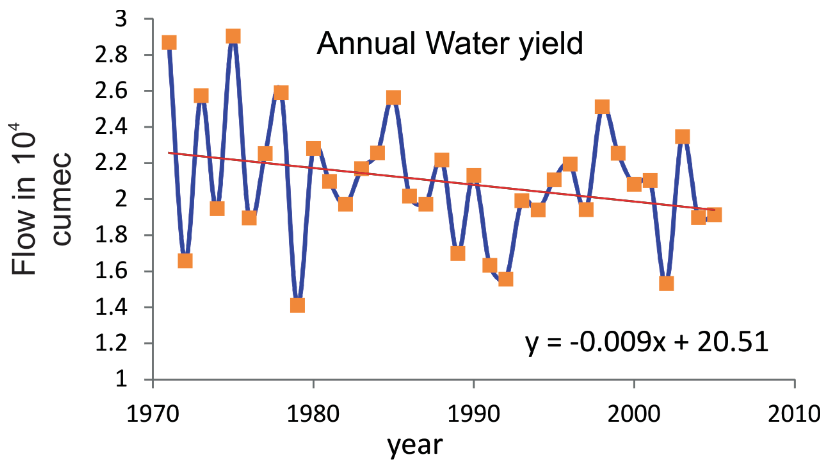

Figure 3. Annual water yield with long-term hydrological trends of River Ganga at the downstream of Farakka (flow in 10^4 m^3/s)

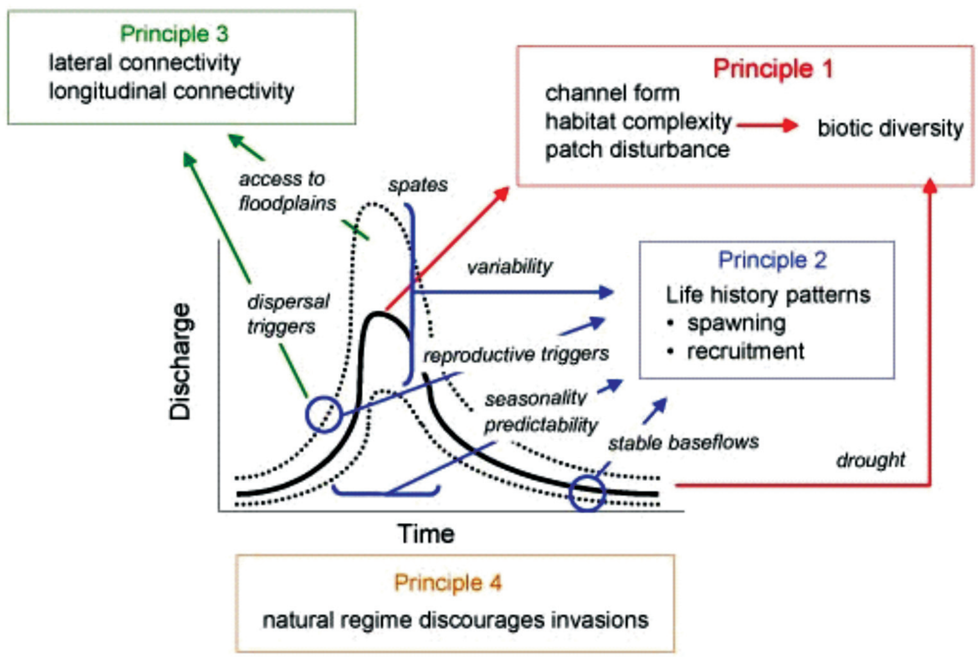

Figure 4. Aquatic biodiversity and natural flow regimes as defined by four principles
Source: Arthington (2012)

noteworthy that magnitude of e-flows varies with local site conditions as well as flow characteristics. Sometimes the flow fluctuates so widely that there is little scope for regulation. If the catchment area is large and flow magnitude is high, the estimates of e-flows are also high and there is a rapid onset of high flows. The computed values of e-flows generally vary according to assessment methods and location. In regulated rivers, e-flows are often defined through vulnerability assessments involving scientists, government agencies and water users to sustain desired ecological processes or conditions downstream of dams (Poff *et al.*, 2010).

Managing river ecosystems sustainably under uncertain climatic and hydrological scenarios poses new and significant challenges (Acreman *et al.*, 2014b). Implementation of e-flow estimates become unpractical in an over-allocated river basin where controversial regional political situations exist, particularly when the river passes through more than two states. In such cases, e-flows are just used as preliminary estimates for initial planning purposes. There would be increasing stress on river basins due to widespread hydrological alterations and climate extremes (Table 4) (Lehner *et al.*, 2011; Vörösmarty *et al.*, 2010). The adaptive management deals with balancing between the acceptable levels of risk that stakeholders are willing to accept and the stakeholder-defined engineering and ecological goals. As river basins across the world become increasingly over-allocated, there is an urgent need for a broader conception of sustainable river management that implements credible e-flows as a necessary ingredient for water ecosystem integrity and the social well-being it supports (Griggs *et al*., 2013; WRI, 2005).

3. Environmental-Flow Management in a Stressed River Basin

The e-flow management in a stressed river basin works better in a continuously dynamic framework which integrates future changes in societal cost functions and shifts in ecological conditions under evolving climate and socio-economic conditions (Poff *et al*., 2015). A critical review of literature published from 2000 to 2015, reveals that new nationwide directives are emerging to develop and manage river ecosystems in more holistic and environmentally sustainable ways that retain social and ecological benefits.

E-flows assessment procedures are both generic and site-specific. While rivers follow the same general pattern irrespective of climatic zone, every river is different – there are no specific type A or B. The best methods employ evidence based principles rooted in geomorphic (landscape) perspectives, catchment specific insights and consideration of restoration strategies using checks and balances. Some of the earlier methods relied upon wetted perimeter (1998) approach, hydrological method (flow-health), holistic approaches. They were mostly used to justify low-flow allocations and assessments were often done in isolation with emphasis on 'getting a number' syndrome. There is also evidence that e-flows will not maintain a pristine river condition.

It is widely observed that water management designs (such as channelisation, river-front development, downstream discharge releases from dams etc.) are often not compatible with socially valued ecological functions (Stratford *et al.*, 2015). Researchers have emphasised that range and value of ecosystem services provided by rivers increase with the degree to which they are allowed to function naturally (Cluer and Thorne, 2014; Palmer *et al.*, 2005). The concept of e-flows was extended and made functional from minimum flow in the early 1980s to strengthen the idea that health and integrity of the entire river ecosystems are fundamental to sustaining human well-being. According to

Table 1. Evolution of environmental-flows assessment in India

Year	Agency/Project	E-flow Criteria	Shortcomings
1999	National Commission for Integrated Water Resources Development Plan (NCIWRDP, 1999)	2% of total natural water requirements for environment and ecology	Not based on scientific reasoning
1999	Supreme Court directed the Govt. to ensure minimum flow of 10 cubic metre per second (m^3/s) in Yamuna in Delhi	Minimum flow of 10 cubic metre per second (m^3/s)	Arbitrary keeping in view the committed utilisations from the river
2003	Water Quality Assessment Authority (WQAA): a separate Working Group made in 2003 to estimate 'minimum flows in rivers to conserve the ecosystems'	EFA used in other parts of the world are unlikely to be applicable in India, advised Tennant method	
2004 and	Amarasinghe *et al.* 2005, Smakhtin *et al.*, 2004	476 km^3 or 25% of total renewable water resources of the country	EWR not EF
2005	NIH-Roorkie, EFA in the Brahmani-Baitarani River Systems	Hydrology-based Range of Variability Approach of Richter et al., 1997 with 7-day minimum and 1-day maximum flows	
2005	Ministry of Water Resources (MoWR) Report of Working Group to advise Water Quality Assessment Authority (WQAA – WG) on minimum flow requirements in Indian Rivers: Tennant Method	For Himalayan Rivers, minimum flow to be not less than 2.5% of 75% of the Dependable Annual Flow (DAF) expressed in m^3/s, one flushing flow during the monsoon with a peak of not less than 250% of the 75% DAF. For other rivers, minimum flow in any 10-day period to be not less than the observed 10-day flow with 99% exceedance (where 10-day flow data is unavailable, this may be taken as 0.5% of 75% of the DAF). One flushing flow during the monsoon with a peak of not less than 600% of 75% of the DAF, expressed in m^3/s	Recommendations of the committee are based on what is practical considering various constraints into the account. It may not lead to desirable outcome for ecological needs

Source: Authors' own elaboration

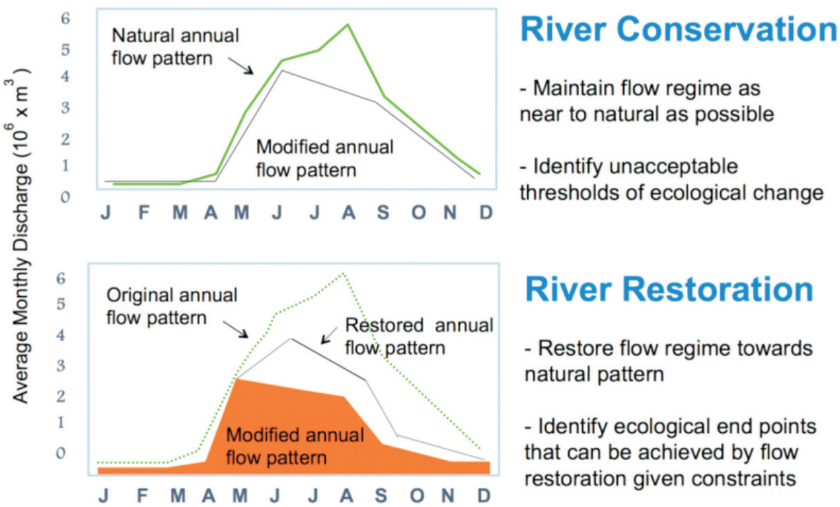

Figure 5. River conservation versus river restoration – designs from 'drawing table' to more proactive approach
Source: Arthington (2016)

Krchnak *et al.* (2009), the term e-flows essentially refers to a 'variable flow regime' that has a definite volume and specific timings that has been designed and implemented – such as through intentional releases of water from a dam into a downstream reach of a river – in an effort to support desired ecological conditions and ecosystem services. Flow in the river is viewed as the master variable because it exerts immense impact on aquatic habitat, river morphology, biodiversity, river connectivity and water quality (Jain, 2012). The early studies carried by Poff *et al.* (1997b) and King and Tharme (1994) led to the understanding that all elements of a flow regime, including high, medium and low flows, are important from the ecosystem point of view (see Table 1). In an earlier study, six environmental management classes (EMC) were defined from A (natural) to F (critically modified) based on expert judgement (DWAF, 1997). The flow duration curve (FDC) for the particular site for natural conditions is drawn and depending upon the desired EMC, the FDC is shifted to the left to obtain the desired e-flow regime. The growing piece of literature has confirmed the idea that e-flows will have to closely follow the natural flow regime, even though in today's context, it is very difficult to maintain the full functional integrity and resilience of river ecosystems by e-flows alone (Arthington, 1998; King *et al.*, 2003; Acreman and Ferguson, 2010). It should be pointed-out here that this contrasts with modern thinking about flow variability, as the FDC loses all temporal sequencing of the flow regime.

The natural flow regime is crucial for sustaining native aquatic biodiversity and maintenance of ecological processes in fluvial ecosystems (Bunn and Arthington, 2002; Jardine *et al.*, 2015) and fragmented riverine ecosystems have altered flow regimes resulting in loss of fluvial habitat. In implementing e-flows in managed rivers, serious questions arise concerning the nature of the pre-disturbance condition to which a given river should be restored. First of all, the likely sequence and habitat impacts of channel adjustments associated with restoration of e-flows are not clear. Second, it may be better to adjust the flow regimes to the prevailing hydrological and sediment regimes to develop a more resilient ecosystem in the long run in the face of climate change (Cluer and Thorne, 2014). The tendency to implement 'static e-flows' rules by water resource practitioners ignore natural system complexity and such mindset will ultimately contribute to further degradation of river ecosystems (Arthington *et al.*, 2006). Similarly, quantification of arbitrary minimum flow is inadequate as it fails to relate patterns of temporal and spatial variation in river flows to the structure and function of a riverine ecosystem (Lytle and Poff, 2004). There is also a difference between river conservation and river restoration, while the conservation approach puts emphasis on maintaining the flow regime as near to natural as possible, identifying unacceptable thresholds of ecological change, the restoration approach stresses on restoring flow regime towards natural pattern (Figure 5).

There are significant engineering and ecological trade-offs, at the same time, unprecedented opportunities for ecosystem restoration (Stratford *et al.*, 2015; Jain, 2012). With initial water allocation from the rivers, economic benefits initially increase sharply and ecological loss is low. However, with further increase in water allocation, the incremental benefits are lesser and ecological costs will be more.

Several EFA methodologies have evolved since early 1980s and they are used globally (Table 2). The first-generation EFA methods are essentially desktop methods which may or may not be ecologically relevant. They ignore

Table 2. Evolution of EFA methodologies and their shortcomings

Timeframe	Approach	Specific Methodology Types	Shortcomings
First generation (1980–1995)	Desktop, rapid assessment, using primarily ecologically relevant hydrological indices or analysis of hydrological time series data, static	Hydrologic methods, Hydraulic Rating Methods	-Used to justify low-flow allocations -Prescriptive and assessments often done in isolation -'Get a number' syndrome for a single flow regime -E-flow will not maintain a pristine river condition
Second generation (1995–2010)	Comprehensive habitat assessment, using primarily methods or habitat modelling	Habitat rating methods, in-stream flow incremental methodology (IFIM), Expert Panel Assessment, DRIFT	-May not be ecologically relevant to advise on suitable flow restoration -Importance of changes in river's physical attributes for the aquatic biota not fully explored
Third generation (2010–2015)	Integration of habitat and ecosystem benefits with human well-being, structured and dynamic with ecological values in a changing climate scenario, synthesis of several EFA techniques	Ecological Limits of Hydrologic Alteration (ELOHA, Poff *et al*., 2010); Stream Evolution Model (Cluer and Thorne, 2014)	-Full tolerance of the flow-ecosystem relationships is still not possible -Adaptive management of flow regimes with respect to changing climate/social preferences being worked out

Source: Authors' own elaboration.

parameters of the flow regime to the response of aquatic species and communities. Hydraulic rating methods (HRM) were based on channel-discharge relationships and considered wetted perimeter to calculate e-flows. Breakpoints were identified in the habitat–discharge response curve where habitat quality degrades with reduction in discharge. They also failed to link channel morphology to habitat supporting aquatic biota. The Building Block Methodology provides an excellent approach to link river objectives to flow requirements (King and Louw, 1998). This method had been applied to Ganga by WWF-India by one of the co-authors. The second generation EFA methods were mostly based on habitat-rating approaches – the most common method of which is the in stream flow incremental methodology (IFIM). IFIM was developed in the USA; it is rarely used in full, but the Physical Habitat Simulation (PHABSIM) model is used. It considers relative contributions to habitat quality and diversity made by different channel forms (King and Tharme, 1994), which was also later advanced by Williams (2010). Stream Evolution Model developed by (Cluer and Thorne, 2014) recognises that river streams may naturally be multi-threaded prior to disturbance and represents stream evolution as a cyclical, rather than linear phenomenon, recognising an evolutionary cycle within which streams advance through the common sequence. The streams skip some evolutionary stages entirely, recover to a previous stage or even repeat parts of the evolutionary cycle.

4. Role of Physical Habitat Variables in Maintaining River Ecosystems

Physical habitat variables play major role in the distribution of fish and other aquatic species. The physical habitat of a river includes sediment size and heterogeneity, channel and floodplain morphology and other geomorphic features (Poff *et al.*, 1997a). Habitat alteration and fragmentation has brought about significant endangerment of freshwater fish fauna in most of the rivers in India. Among various habitat attributes, water depth, dissolved oxygen, flow rate and pH are the most important variables in shaping fish distributions. Fish assemblages are also shaped by the flow rates, faster current has negative impact on the total number of fish species. Higher dissolved oxygen stretches have higher species richness. Similarly, depth less than 1 m has negative impact on fish diversity. Presence of more deep pools with low-to-moderate water velocity supports higher fish diversity. Open river, shallow water and deep pools are the primary habitats contributing to the maximum diversity, therefore, protection of these particular habitats is recommended for conservation and management of the fish biodiversity (Lakra *et al.*, 2010). The literature also suggests that species occurrence and richness are driven more by relationship with abiotic factors (physical habitat variables) than species interaction. Optimum ranges of physical habitat variables are provided in Tables 3 and 4.

Regarding the microhabitat, hydro-morphological parameters (depth and velocity) followed by temperature,

Table 3. Physico-chemical, biological, physiographic and topographic parameters for defining fluvial habitats

Physico-chemical Variable	**Biological Variable**	**Physiographic Variable**	**Topographic Variable**
Turbidity (NTU)	Phytoplankton (cells/L)	River discharge (m^3/s)	Basin area (km^2)
Water temperature (°C)	Phytobenthos (cell/cm^2)	Water velocity (m/s)	Slope (m/km)
Total Dissolved Solids (mg/L)	Macro-invertebrates (individuals/m^2)	Slope (m/km)	Drainage density (km/km^2)
Electrical conductivity (µS/cm)	–	–	–
pH	–	–	–
Dissolved oxygen (mg/L)	–	–	–

Table 4. Fish diversity and habitat relationships – role of physical habitat variables

Physical Habitat Variables	Lower Range	Higher Range	Optimum Range	Remarks
pH	6.1	8.2	7.1–7.5	Both acidic and basic media are not liked by the fresh water species
Temperature (°C)	10.5	25	22–23	A sudden increase or decrease in water temperature may cause fish mortality
Turbidity (NTU)	10.4	46.5	26.5–30.5	While some turbidity may afford greater protection for juvenile fish from predators; excessive high water turbidity showed negative effect on fish egg survival, hatching success, feeding efficiency (mainly on filter feeders) and growth rate and population size
Conductivity (µS/cm)	205	540	300–350	High conductivity areas witness low species richness
TDS (ppm)	135	400	250–300	Higher dissolved solids have adverse effect on abundance of fish diversity
Depth (m)	2.5	9.4	4–5.5	Depth less than 1 m has negative impact on fish diversity in most of the alluvial rivers
Flow (m/s)	0.14	1.5	0.50–0.75	Water discharge is the best predictor of fish species richness patterns in the Himalayan rivers. Faster current has negative impact on total number of fish species. Slow and swift flows are associated with higher fish abundance
Dissolved oxygen (mg/L)	3.63	8.5	4.6–5.8	One of the most important factors for fish abundance and distribution, DO generally effect the survival of fishes especially juvenile

Source: Authors' own elaboration.

turbidity and total dissolve solids are of significance for the structure of the fish community. Conductivity is another important factor that explained the major proportion of the variability affecting fish in their habitat choice. The other local habitat variables like overhanging vegetation and land use were of secondary but significantly important for the assemblage of the fishes. During the flooding season, greater accessibility of aquatic habitat and food resources enhances feeding opportunities and early life-stage survival and storage of fat helps sustain fish during the dry season when resources become limited and most fish populations experience greater competition and predation mortality (Röpke *et al.*, 2017; Lowe-McConnell, 1987) (Figure 6 and Table 5).

4.1 Between Upstream and Downstream Stretches

In flowing water systems, the habitat characteristics are largely shaped by physical processes especially the movement of water and the sediment within the channel and between the channel and floodplains (Poff *et al.*, 1997a). Fish communities in riverine system typically follow a pattern of increasing species richness, diversity and abundance from upstream to downstream. Fish species richness decreases with increasing elevation. However, in some tributaries of Yamuna such as Betwa River, opposite trend has been observed. Species diversity and species richness were both less in the downstream areas compared with the upstream areas (Lakra *et al.*, 2010). It was earlier observed that increasing community and habitat diversity followed stream – order gradients (Gorman and Karr, 1978).

4.2 Land use Pattern of the Catchment Areas

Natural streams support fish communities of high species diversity which are otherwise seasonally more stable than the lower – diversity communities of modified streams (Gorman and Karr, 1978). Land use is a primary factor that cause the declining of aquatic biodiversity in stream ecosystems (Schlosser, 1991; Karr *et al.*, 1985; Salo and

Table 5. Habitat stressors and habitat responses and their associated hydrologic and geomorphic responses

Habitat Stressors	Associated Hydrologic Changes	Geomorphic Responses	Habitat Responses	References
Impoundment by dams and hydroelectric development	Discharge seasonality influences the biota and land–water interactions, results in significant departure from the usual hydrological regimen, reduced discharge downstream of dams, capture sediment moving downstream, reduced magnitude and frequency of high flows, more stabilised flow regimes, change in magnitude of the annual flood pulses	Channel fragmentation, downstream channel erosion, reduced sediment loads, altered frequency of floodplain inundation	Alternating periods of resource scarcity during the dry season and resource surplus during the wet season, emergence of community dominated by few species many of which are tolerant forms, habitat better suited to many non-native biota, during floods some species enter irrigation canals or channels downstream of dams where they die when water levels fall during the dry season	Fausch *et al*. (1990), Rosenberg *et al.* (1997), Sultana and Thompson (1997), Galat (1998) and Ou and Winemiller (2016)
Excessive water extraction for irrigation	Reduced magnitude and frequency of high flows	Reduced baseflows, channel down-cutting, fragmentation of hydrological connectivity between rivers and wetlands	Delayed fish breeding migrations, may not begin until flows have passed a critical threshold; increase in discharge may fail to initiate any population response, juveniles are confined within channels, curtailment of fish migrations	Ferguson *et al.* (2013), Dudgeon (2000), Dudgeon (2010)
Drainage-basin alteration especially deforestation	Retraction of water over the floodplain is hampered, increased run-off peaks, may concentrate pollutants, alter precipitation and evapo-transpiration in the basin, increased sedimentation and increased magnitude and frequency of high flows and flash floods	Bank erosion	Habitat degradation by siltation, altering in stream habitats, food webs and flow conditions during reproductive periods, declines in secondary productivity, declines in reproduction, recruitment and population abundance	Berkman and Rabeni (1987), Dudgeon (1999), Dudgeon (2010), Poff and Zimmerman (2010)
Over harvesting of fishes			Species loss, lower genetic diversity	Pinsky and Palumbi (2014)
Water quality deterioration by pollution	Deoxygenation and acidity, reduced dilution of pollutants	Altered nutrient cycling	Habitat degradation, inland fisheries decline and species loss, native fish and invertebrate populations often are limited by adverse water	Lakra *et al.* (2010) and Sarkar and Dubey (2016)

Source: Authors' own elaboration

Cundy, 1987). The variations in the habitat attributes like pH, turbidity, total dissolved solids and conductivity across different sites are basically attributed to differences in land use pattern, which in turn is responsible for variation of species diversity and distribution (De Silva *et al.*, 2007) and a consequent decline in fish spawn availability in river. To capture the ecological and economic significance in relation to the e-flow requirements for the different reaches of River Ganga a longitudinal river zonation is required, taking into account the main physical, environmental and socio-cultural features and gradients found from the headwaters to the river mouth (Table 6).

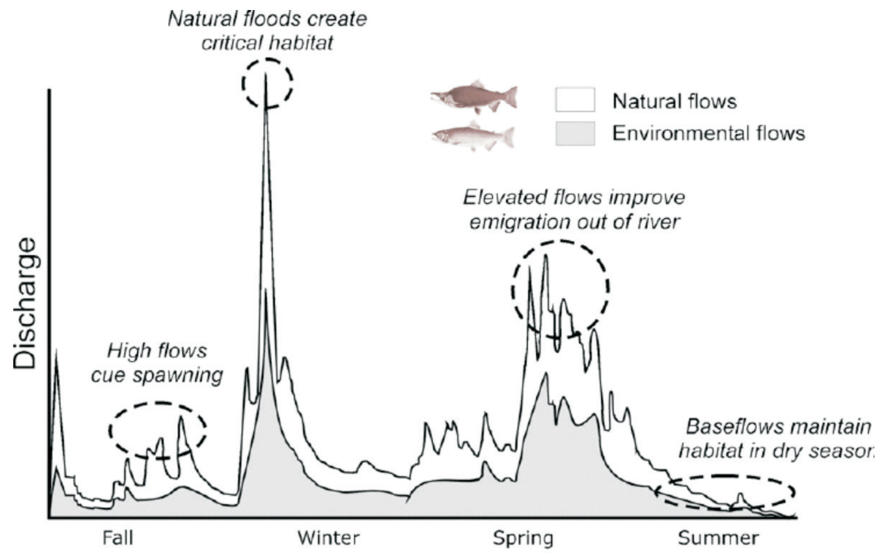

Figure 6. An example of natural and environmental flows for maintaining the salmon habitat for spawning and emigration
Source: Naiman *et al.* (2008)

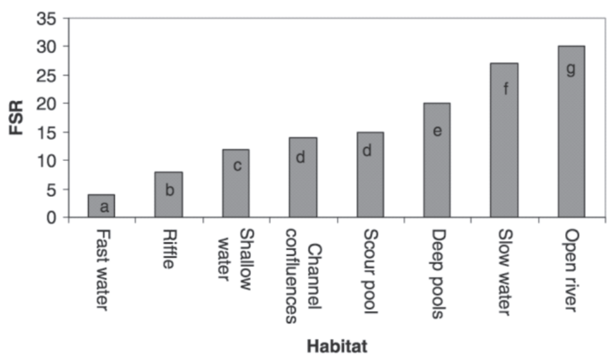

Figure 7. Habitat wise fish species richness (FSR) in River Betwa – tributary of Yamuna. Bars with different superscript letters are significantly different ($P < 0.05$)
Source: Lakra *et al.* (2010)

4.3 Impact of Damming

Damming leads to loss of native species as well as invasion by exotic species (Liermann *et al.*, 2012), because exotic species can establish in modified or degraded freshwaters (Poff *et al.*, 2007). Populations isolated in upstream areas by dams are subject to extirpation when reproductive failure or high mortality rate cannot be counter-balanced by re-colonisation from downstream sources (Winston *et al.*, 1991). Open river habitats are the most preferred habitat for fishes inhabited in the tropical rivers (Sarkar *et al.*, 2010; Lobb and Orth, 1991; Aadland, 1993; Arunachalam, 2000), however, in certain stretch due to damming, there is a minimum depth of water that is maintained throughout the season. There is a positive influence of reservoirs connected with the river as well as due to existence of more 'open river, slow water and pool habitats' along with macrophytes which might have importance in fish assemblage and aggregation (Figure 7). Changes in hydrology especially more reservoir types of situation due to barriers across river seems be responsible for the flourishing of exotoic species *Cyprinus carpio* in Ganga basin. In the middle stretch of the river Ganges (Allahabad), Hilsa (*Tenualosa ilisha*), which used to form a good share in catches below Allahabad, has almost disappeared after inception of Farakka barrage despite fish ladders were installed.

4.4 Altered Habitats as Red Zones

Altered habitat support less biological communities while less disturbed sites are characterised by a diverse fish fauna in a variety of habitats (Shahnawaz *et al.*, 2010). Variables that have negative impact on fish abundance: (red-zones) – are mainly due to low fish richness due to degradation of their breeding grounds. For River Ganga, such altered habitat zones could be:

(a) Stretch having effluent discharge from industries, thermal power plants and sewerage systems – high BOD areas are in Hardwar, Kanpur, Allahabad, Varanasi and Diamond Harbour near Kolkata
(b) High water velocity stretch/due to sudden discharge
(c) High sedimentation rate stretch – Many floodplains have already lost their connection with main channel due to heavy siltation. Floodplains serve as breeding and nursery grounds for several species.
(d) Degraded shoreline habitats
(e) Riverfront development sites which disturb the wetted shorelines/banks
(f) Fishing sites (exploitary zones)
(g) Stretch having exotic species habitats

Table 6. Main criteria for e-flow zoning for River Ganga

Sr. No.	Main Crieteria for E-flow Zoning	Elements to Map
1.	Aquatic ecosystems	River – main channel Streams – tributaries Wetlands Lakes Reservoirs
2.	Distribution of iconic species	Range of Snow Trout (*Schizothorax richardsonii*) Range of Golden Mahseer (*Tor putitora*) Range of Indo-Gangetic Dolphin (*Platanista gangetica*) Range of Ghariyal (*Gavialis gangeticus*) Range of Indian Narrow-headed Softshell Turtle (*Chitra indica*) Range of Northern River Terrapin (*Batagur baska*)
3.	Distribution of protected areas	National Parks Wildlife Sanctuaries Conservation Reserves
4.	Riverine habitats	Open River (Wide Valleys) Confined River (Narrow Valleys) Fast Water (Rapids) Slow Water (Runs) Deep Water (Pools) Shallow Water (Flats) Riffles Confluences Swamps (Marshes) Riparian Forests
5.	Hydrogeomorphological features	Channel Width Sand Banks Sand Bars Islands Floodplains Terraces Braiding Meanders Oxbow Lakes Anastomosing
6.	Hydrological features	Groundwater Groundwater – Surface water connections
7.	Anthropogenic interventions	Dams/Barrages/Weirs Significant Abstractions Punctual Pollution Sites Diffuse Pollution Belts Predominant Landuse of Catchment Cultural Sites

As a result of flow regulation along the River Ganges, major carp fishery virtually disappeared after seasonal inundation of the floodplain was prevented by flood control structures (Natarajan, 1989). The total annual fishing production in River Ganga has declined from 85.21 t during 1959 to 62.48 t during 2004, thus indicating that the fish abundance has decreased during the last 5 decades. The reported catch by the fisher folks have also reduced temporally. Along with Mahseer (*Tor putitora*, *T. tor*) the other migratory species like dwarf goonch (*Bagarius bagarius*), yellowtail catfish (*Pangasius pangasius*), pangas catfish (*Silonia silondia*), hilsa (*T. ilisha*) and long-whiskered catfish (*Sperata aor*) from the middle and upper stretch is under severe threat due to consequences of damming and water diversions projects. Fish production has shown a distinct change in the last two decades in the middle stretch of River Ganga where the contribution of Indian major carps has decreased from 41.4% to 8.3% and that of miscellaneous and catfish species increased (Vass *et al.*, 2008). A total of 143 freshwater fish species have been reported in the all stretches of River Ganga which is about 20% of freshwater fish of the total fishes reported in India. A total of 53 species belonging to 11 families were reported in upper stretch of Ganga up to foothills of Garhwal Himalayas. Out of 143 species, 29 species are listed under threatened category, 133 species were native to River Ganges and its tributaries and remaining 10 species were exotics. High species richness found in orders of *Cypriniformes*, *Siluriformes* and *Perciformes*, accounting for 50.34, 23.07 and 13.99% of the population, respectively. The family Cyprinidae (53.47%), Bagridae (8.46%) and Channidae (1.47%) were found to be the most dominant in the Ganges. Similarly, in Gomti River – a tributary of Ganges, 56 fish species belonging to 20 families and 42 genera were earlier reported from various sampling sites. Of the 56 species, five belong to the 'endangered' (EN) category and 11 belong to the vulnerable (VU) category. Six major categories of habitat were identified and pattern of fish assemblage and dominant genera in each habitat studied. Apart from Indian Major Carps (*Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*), *Chitala chitala*, *Notopterus notopterus*, *Ompok pabda*, *Octopus bimaculatus*, *Labeo bata*, *Labeo calbasu*, *Cirrhinus reba*, *Channa marulius*, *B. bagarius* and *Clupisoma garua* were the important species.

5. Development of a Framework for Guiding Research Questions: Opportunities and Initiatives

The proposed framework as outlined in Figure 8 can be only a small adjustment to the existing water framework. It assesses ecosystem vulnerabilities early in the planning process. It also aids to inform social and economic choice for preferred flow situation. Water allocation trade-offs are identified and addressed accurately during e-flows adjustments. The following questions may be specially investigated during the research studies:

(a) What kind of sensitive ecosystems exist in the river's catchment and adjacent floodplains? Which functions do these ecosystems perform in the maintenance of sustainable water resources and in the provision of other natural resources?
(b) What are the different downstream responses to flow scenarios? How river ecosystems change with flow changes (flow-ecosystem-relationships)? What are the main threats to the maintenance of flow-regimes that maintain integrity of freshwater ecosystems?
(c) What are critical ecological and hydrological thresholds that maintain river functions and guide adaptive management to achieve sustainable outcomes?
(d) How to define threshold values of ecological and hydrological functions as a critical criterion for identification and mitigation of stressors
(e) How can the protection of freshwater ecosystems be integrated into joint riparian management strategies with balancing river ecology and water resource development?

The methodological framework designed for the study comprises of four main components which are further subdivided, as shown in Figure 2. The proposed framework and the stepwise plan are summarised below:

5.1 Background Hydrology

Starting point in the assessment is the understanding of river basin, its condition, present flow regime of the river and how and when that has changed in the past. In the first instance, ecological status of managed rivers compared with their historical, unmanaged counterparts should be characterised. Based on the long-term flow curves, dependable flow and use and non-use allocation should be quantified. The idea is to develop regional e-flow standards vis-à-vis prevailing hydro-climatic and watershed controls.

(a) Characterise ecological status of managed rivers compared with their historical, unmanaged counterparts
(b) Classify aquatic ecosystem of various disciplines like rivers, streams, lakes, wetlands and reservoirs
(c) Develop links between stream evolution and ecosystem services
(d) Develop indices based on time series of river flow data, allocation based on percentage of MAR or values read from flow duration curves (FDC). It should however, be pointed-out here that this contrasts with modern thinking about flow variability, as the FDC loses all temporal sequencing of the flow regime.
(e) Define broader objectives to indicate the type of river desired by stakeholders – achieve specific pre-defined ecological, economic, or social objectives.

5.2 Assessment of Human Welfare (HW) and River-Ecosystem Functions (REF)

Attempts should be made to develop plausible links between stream evolution and ecosystem services. Flow-dependent species should be identified in each of the river basin/segments with attempts to define the reference state. There are available literature and preliminary studies of populations and communities of fish, macro-invertebrates, macrophytes and phytobenthos and phytoplanktons. Simulation of component species is required that would be found in an 'undisturbed' state. Objective-based flow setting as explained in Acreman and Dunbar (2004) would define the broader objectives to indicate the type of river desired by stakeholders – to achieve specific pre-defined ecological, economic, or social objectives. Additionally, critical fish habitats may be identified in the river basin to declare them as fragile areas and conservation reserves. Many fishes might use these protected areas for breeding and spawning grounds. The framework should be able to assess effects of local habitat variables on the structure of fish assemblage. Additionally, there is also a need for integrating religious, cultural and social connection between people and the river.

(a) Characterise and quantify river-ecosystem functions as well as human welfare goods such as water for drinking, hydropower, irrigation, ûoodplain agriculture, fishery yield, desirable geomorphic form and native riverine biodiversity
(b) Estimate dependable flow and quantify use and non-use allocation
(c) Develop regional e-flow standards vis-à-vis prevailing hydro-climatic and watershed controls
(d) Identify major 'fluvial habitat types' based on physical and biological parameters. Within the major habitat types, identify micro-habitats, which could have distinct

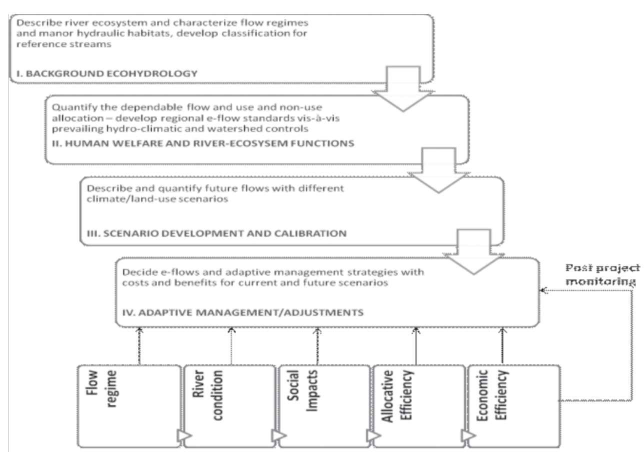

Figure 8. The methodological framework designed for the comparative e-flow adjustments and trade-off assessment
Source: Authors' own elaboration

attributes. Some iconic species should be identified along with their distribution range

5.3 Scenario Development and Calibration

Possible drivers of human well-being, environmental pressures such as climate change and land use change should be assessed and quantified. The dynamics of how river-ecosystem functions interact to affect ecosystem resilience can be simplified through modelling techniques building upon the approach recommended in Arthington *et al.* (2006) and Poff *et al.* (2010). Decision consequences on multiple human welfare (HW) and river-ecosystem functions (REF) can be assessed in a formal analytical framework, including non-market valuation of environmental amenity and social preferences (Martin *et al.*, 2015) in selected stretches. Economically and environmentally acceptable strategies for various river types (based upon evidence gathered from different river basins) can be used to guide development of flow standards for individual rivers and river segments.

(a) Understand how drivers of human well-being, environmental pressures such as climate change and land use change and river-ecosystem functions interact to affect ecosystem resilience – application of simulation models (ecosystem integrity of rivers in its full width of spectrum)
(b) Explore decision consequences on multiple HW and REF indicators in a formal analytical framework, non-market valuation of environmental amenity
(c) Design management decisions that need to balance ecosystem sustainability with desired economic objectives, buffer aquatic and riparian ecosystems against climate change in regulated rivers

5.4 Adaptive Management and Adjustments

The adaptive management toolkit will provide a clear understanding of likely tradeoffs with e-flow implementation that will alter the river-ecosystem function (REF) and their watersheds, including the prevailing legal environmental regulations. Water resources in India are under great pressure. There is no possibility of returning River Ganga flows to anything near natural. Therefore, possible reconciliation of environmental flows and other water uses in Ganga could be arrived at by reducing the water allocation to inefficient irrigation system or by more adaptive management. This could be done by:

(a) Quantify the interacting effects of multiple hydrological and ecological drivers, environmental pressures and intrinsic mechanisms, such as density dependence, on the resilience of flow-dependent species (channel-ecology, flow regimes and riverine ecosystems)
(b) Develop guidance on restoration principles, adaptive management and conflict management principles and test it regionally in a river basin

6. Conclusion

In this paper, some important aspects of the nature of river ecosystems including environmental-flows have been outlined. To attain the goal of freshwater sustainability, the current river management philosophy must make a transition beyond the narrowly defined 'economic criteria' to include socially valued 'ecosystem functions and services'. Satisfying ecological objectives during e-flows management may improve economic performance of water infrastructure systems, thus improving human well-being as well as ecosystem functions. This necessitates identification of critical ecological thresholds that maintain river functions and guide adaptive management to achieve sustainable outcomes. Therefore, the need for scientifically credible flow management guidelines that include stakeholder-defined engineering and ecological goals is very much desired. Therefore, there is a need to design a decision support framework, that explicitly and quantitatively explores trade-offs in implementation of e-flows across a range of possible management actions under unknown future hydrological and climate states, using evidence from river basins across the different parts of the world. Such framework should be able to identify critical ecological thresholds that maintain river functions and guide adaptive management to achieve sustainable outcomes – considering how biological diversity is important in delivering ecosystem services of rivers. The framework should also include metrics of natural capital (such as abundance, diversity or interactions in food web structure) that could help define critical ecological thresholds for e-flows; above which rivers deliver ecosystem services and are resilient, below which services stop and the system degrades. Several approaches can be combined together using grounded theory and bottom-up embedded approaches in operationalising ecologically contextualised sustainable river ecosystem functions. The integrated approach to study variable flow regime in understanding the river ecosystems can help in understanding

(a) River system integrity: Are the proposed environmental flows adequate for maintaining the ecological integrity of the River?

(b) Strategic interventions philosophy: What changes in the Legal and Policy framework are necessary to implement an environmental flow? And

(c) Evidence-based eco-hydrology: What tools can be developed to enhance our understanding of the river systems' integrity and science of eco-hydrological system? Lastly, no matter how advanced and accurate the e-flows assessments are, the ecological integrity of a river basin remains vulnerable if flow regimes are drastically managed and no actual releases are made to restore the flows.

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RESEARCH ARTICLE

Calorific Value and Fuel Wood Consumption Patterns of a Forest Plantation Made by Villagers at Kahinure (Distt Mau), Uttar Pradesh, India

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Abstract In the present study, calorific value of different fuel wood biomass and its consumption patterns of the plantation forest in rural area of Kahinure (Distt Mau) Uttar Pradesh, India had been evaluated. A questionnaire survey of random sampling method was employed for 180 households to understand socio-economic conditions and energy use pattern for cooking purposes in the study area. The study reveals that fuel wood was largely utilised as non-commercial and cheap source of energy, followed by dung cake and agriculture residue. The highest calorific value was estimated by *Prosopis juliflora* (22.56 MJ/kg) followed in *Terminalia arjuna* (21.63 MJ/kg) and *Pithecellobium dulce* (20.67 MJ/kg), whereas the lowest value was found in *Streblus asper* (17.32 MJ/kg). About 65% of household energy consumption was in form of wood biomass fuel derived from the plantation forest. The cow dung was used by 22% households and agriculture residue by 12%. About 1.3% people used kerosene for cooking. Average cooking time was estimated at 4.46 h/day/family, and average value of fuel wood consumption was estimated at 4.5 t/family/year, whereas average market value of annual consumption of fuel wood had been calculated as Rs 8,700.00 per households. Due to poor socio-economic situations in the village, a significant amount of fixed carbon of plantation forest was used for cooking of food which could be saved by providing them renewable source of energy for cooking or cooking gas cylinders.

Keywords Consumption, Calorific value, Fuel wood, Households, Plantation forest, Rural area, Wood biomass

1. Introduction

Globally, wood biomass is considered one of the primary sources of energy for meeting the daily energy need, and it has been estimated that approximately 3 billion people use solid biomass fuel including wood, dung cake, agriculture waste and coal throughout the world (Bond *et al.*, 2004). Wood biomass accounts for about 14% of total energy used globally and is the largest energy source for three-quarters of the world's population living in the developing countries (Sedai *et al.*, 2016). The total average annual production of wood fuel for energy in the developing countries increases nearly 17.6% over the last decade (Simon and Singh, 2015). In India, over 170 million households and almost 800 million people depends on traditional chulha using solid biofuels such as wood, agricultural waste, coal and dried cattle manure (Singh *et al.*, 2014). Rural areas largely depend on locally available resources like forests and agriculture crops to meet their domestic energy needs. Among the various forms of biomass, firewood is the most attractive and occupies a predominant place in the rural energy budget of the country (Jaiswal and Bhattacharya, 2013). Fuel wood is the only source of energy for many people living in the rural areas due to the lack of other available energy sources.

According to 2011 Indian Census, approximately 66% of households relied primarily as solid biomass fuel for energy; this includes 23% urban households and 86% rural households, which adversely affect respiratory health of the individuals. It reduces local forest cover and soil biodiversity dependent on those forests or plant rhizosphere and contributes to carbon release by burning the fuel wood (Anenberg *et al.,* 2013). National Sample Survey resulted that as of 2011–2012, around 80% of Indian households used some form of traditional fuels to satisfy their cooking and heating needs (Jain *et al*., 2015). The adverse health, livelihood, local environment and climate impacts generated by household biomass burning have gained increased attention in the past few years.

The quality of fuel wood is recognised by their calorific value, which is normally governed by the availability, time duration of burning, maximum temperature and the ash content (Lisardo *et al*., 2003). Generally, species burn for

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longer time and emit less smoke is considered more preferred. However, majority of rural people consume fuel wood as whatever fuel wood is available without considering energy value, ecological factor and sustainability. Factors responsible for performance of fuel wood are calorific value, moisture content, bulk density and ash content (Todaro *et al.,* 2015). Higher the moisture content, results decrease in the combustion efficiency of the fuel wood. Important attempts have established the negative effect of moisture content on its calorific value (Kumar *et al*., 2009). The calorific value of fuel wood indicates the amount of heat that develops from the complete combustion of wood sample with a given mass. The variance in calorific value in different species can be attributed to its chemical composition.

This paper is an attempt to document the socio-economic attributes of households in the rural area of Kahinure (District Mau), Uttar Pradesh, India and their dependency on plantation forest in terms of using fuel wood as energy alternatives based on information gathered from the field study and household survey. The study concentrates on the following objectives: (i) calorific value estimation of plantation forest species in study area, (ii) analysing the consumption and collection of fuel wood in the selected households for estimating the fuel wood requirement in the area. This information will be useful in designing and implementing appropriate conservation strategies in the area by understanding the needs of households, livelihood opportunities, forest dependency and their critical consequences in deforestation and forest degradation process under the particular set of ecological, sociological, economic and political conditions.

2. Material and Methods

2.1 Study Area

The study area, a rural area of Mau district, is located in Uttar Pradesh, India with a population of about 3,065. The study area lies between 25°52′274″N and 83°30′578″E. Maximum population of the study area used biomass as fuel wood and cow dung as primary source of energy for daily cooking purposes. Forest covered in the study area consists of plantation forest which is classified under subtropical deciduous forest. Plantation forest was distributed on 118 ha along the studied village. Such plantation forest dominantly has four types of plantation association that is (i) *Tectona grandis* plantation, (ii) *P. juliflora* plantation, (iii) *Eucalyptus* plantation and (iv) mixed plantation forest. Majority of households depend on surrounding plantation forest for their fuel wood.

2.2 Sampling Methods

The current study was conducted during the year 2015–2016. Before the collection of exact data, a reconnaissance survey has been done to understand the number of households, family size or family member, energy use pattern, biomass used for cooking purposes, chulha used for cooking and other important parameters for the study. About 180 households were randomly selected for the present study. The information was collected by questionnaire method which is related to socio-economic condition and energy use pattern of households. Interviews were conducted and recorded using structured questionnaires. The survey questionnaire was translated into local language Hindi, wherever necessary, help of a local person was taken who accompanied the authors during the survey. The questionnaires were supplemented within formal meetings and discussions. One questionnaire was filled by each household. The survey report undertook complete investigation about the selected village depending on surrounding forest for fuel wood. Villager’s dependency on the forest fuel wood, fuel collection per day per household and number of head loads (local units, fuel wood carried on the head for domestic purposes) was counted per day production from the plantation forest and identified which species are maximum in each head loads. Fuel wood consumption on household level was calculated on the basis of family size. On the basis of total number of family members, we have divided the family size in five different family classes (Miah *et al*., 2003): very small (4–8 members), small (9–12 members), medium (13–16 members), large (17–20 members) and very large (21–24 members). The respondents were also asked for their involvement in tree plantation and their support on energy plantation on waste land and degraded land. All the data collected from the respondents were cross-checked with family members and physical verification.

2.3 Calorific Value Determination

Estimation of gross calorific value of wood samples on the basis of per unit biomass was determined by 2 g of oven-dried powdered wood samples in three replicates for each sample. Oxygen bomb calorimeter method was used for calorific value determination (Bhatt and Todaria, 1990).

2.4 Socio-economic Condition of Households

Studied village occupied 490 households with a population of 3,065 individuals, whereas the family size of studied village ranged from 4 to 22 persons per family. Out of 490 households in the selected study area, total 180 respondents between the ages of 22 and 75 years with an average age of 30–50 years were interviewed for the study. The 90% of the respondents were head of the households and responsible for collection of the fuel wood and other sources of energy for cooking daily meal and warming during the winter seasons. According to the respondents, agriculture

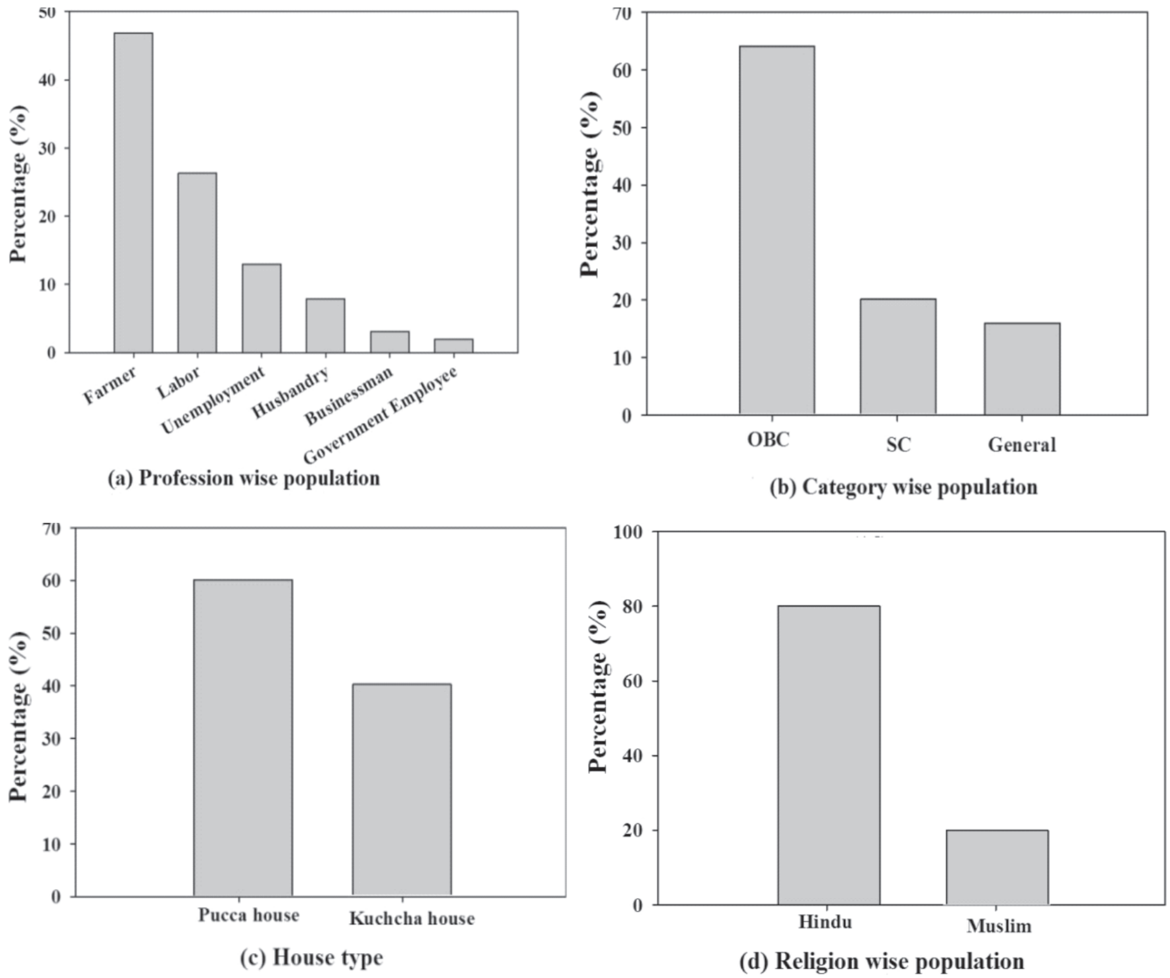

Figure 1. Demography and socio-economic status of the study site: (a) Profession-wise population, (b) category-wise population, (c) house type, (d) religion-wise population

is the main source of livelihood. The forest resources are important for their daily energy need. Demography and socio-economic conditions of the village near the selected study site is presented in Figure 1a–d. Most of the population of study area were farmers (46.83%) and labourers (26.36%) by occupation (Figure 1a). Caste group wise, other backward caste category (64%) was dominated in study area (b). The status of house and population structure of different religions has been presented in Figure 1c and d.

3. Result and Discussion

3.1 Forest Plant Community Composition and Calorific Values of Plant Species

The preferred dominant fuel wood species of study site were *P. juliflora*, *Alangium salviifolium*, *T. arjuna*, *T. grandis* and *Alstonia scholaris.* Details of all species, their vernacular name, calorific values and species ranking on the basis of fuel wood quality are shown in Table 1. Calorific value is an important parameter of tree species that reflects capacity to fix solar radiation during photosynthesis and estimates the amount of heat energy released during combustion of plant tissue. Calorific values of different studied species are given in Table 1. It is an important index for evaluating material cycles and energy conversion in forest ecosystems. Calorific value of species is not only affected by its composition and structure but also other environmental factors as illumination intensity, photoperiod, soil type and nutritional condition (Zeng *et al*., 2014). Highest calorific value occupied by *P. juliflora* (22.56 ± 1.27 MJ/kg), followed by *T. arjuna* (21.63 ± 0.83 MJ/kg) and *P. dulce* (20.67 ± 0.91 MJ/kg), whereas lowest value found in *S. asper* (17.32 ± 0.53 MJ/kg). In general, an ideal fuel wood species should contain high heating value or calorific value with lower value of moisture, and ash content is more desirable. In the present survey report, subtropical tree species were screened and found that these species had a potential as an energy-efficient crops for future energy plantation in this region. Calorific values

Table 1. Species composition of plantation forest and their calorific values and ranking on fuel wood quality

Species	Vernacular Name	Gross Calorific Value (MJ/kg)	Ranking (Fuel Wood Quality)
P. juliflora	Vilayati Babul	22.56 ± 1.27	1
A. salviifolium	Ankol	18.89 ± 1.13	2
T. arjuna	Arjun	21.63 ± 0.83	3
T. grandis	Sagwan	19.92 ± 0.56	4
A. scholaris	Chitvan	19.33 ± 0.63	5
Eucalyptus sp.	Safeda	20.09 ± 1.17	6
S. asper	Sihora	17.32 ± 0.53	7
Madhuca longifolia	Mahva	18.35 ± 0.43	8
Eugenia jambolana	Jamun	18.12 ± 0.31	9
Holoptelea integrifolia	Chilbil	19.35 ± 0.62	10
Ficus benghalensis	Bargad	18.26 ± 1.14	11
P. dulce	Jungle Jalebi	20.67 ± 0.91	12

variation among different tree species and within species has been reported and shows significantly correlative to age too (Bao *et al.,* 2006) however studied plantation forest species having similar age. It has been reported that higher concentrations of extractives and lignin result in greater calorific value (White, 1987; Kataki and Knower, 2001). However, effective calorific value also depends on the moisture content. The higher the moisture content, the less efficient is the wood as a fuel as the net calorific value for heating is reduced (Kumar *et al*., 2011).

3.2 Energy Consumption Pattern of Households

In the selected study area, majority of households used fuel wood for fulfilment of daily energy need. Majority of the households collected fuel wood from plantation forest in vicinity to selected study site. Collection of fuel wood from the plantation forest was considered a sustainable fuel wood production. Composition of energy consumption, their sources and using mode at study area are given in Figure 2. The study resulted that as 65% of households uses fuel wood as primary energy source, followed by cow dung 22%, agriculture residue 12% and 1.3% Kerosene (Figure 2a). Fuel wood using household in study area was higher in per cent value (65%) which was higher than the average value of Uttar Pradesh state, which was reported as 40% (Census of India, 2011). It is may be due to lacking of energy-saving-awareness thoughts and poverty among the population. On the other hand, 71% of all households are using fuel wood in Uttar Pradesh which was higher than our estimated value 65% reported in recent study of Jain *et al.* (2015). It is already a proven fact that traditional cooking stoves in the rural areas are less efficient due to the incomplete combustion of the fuel wood (Miah *et al.,* 2009). This low efficiency is resulting in high consumption of fuel wood which is leading to the more collection of fuel wood from the forests and some health problems also. Approximately 28% households depend on plantation forest for fuel, followed by surrounding (27%) and agriculture (26%), whereas only 1% used government store for their energy needs (Figure 2b). Results show that 74% households used traditional chulha, whereas only 4% households used LPG (Figure 1c). Kitchen types used by households are given in Figure 2d.

In this study, observations support that the energy use pattern in rural India is changing with the uptake of clean energy, but traditional fuels including fuel wood (65%), cow dung (22%) and crop residue (12%) still constitute the major source of household cooking energy because of inadequate and unreliable supply of clean energy options at particular study area, which are almost similar to study which have been done by some previous rural area-based studies (Balakrishnan *et al*., 2004; Das and Srinivasan, 2012). However, due to increasing population and pressure, it is going to become unsustainable due to excessive collection of fuel wood. In addition to this, excessive destruction of natural forests which cause excessive pressure on plantation forest and semi-natural forest make it unsustainable and thereby leading to deforestation (Jashimuddin *et al.,* 2006; Miah *et al*., 2003). Fuel wood collection and consumption from the plantation forest at study site is represented in Table 3. Due to increase in population, the energy demand is increasing tremendously and majority of population living in rural area totally depend on wood biomass as main source of energy. Consumption of fuel wood from the forest ranged between 460 and 470 kg/day. Approximately 45 to 48 head load (average 46.5) were extracted from forest per day, whereas average weight of each head load was 14.5 kg. In study area, the total fuel wood extracted from the plantation forest per day basis was 485 kg/day in summer season and

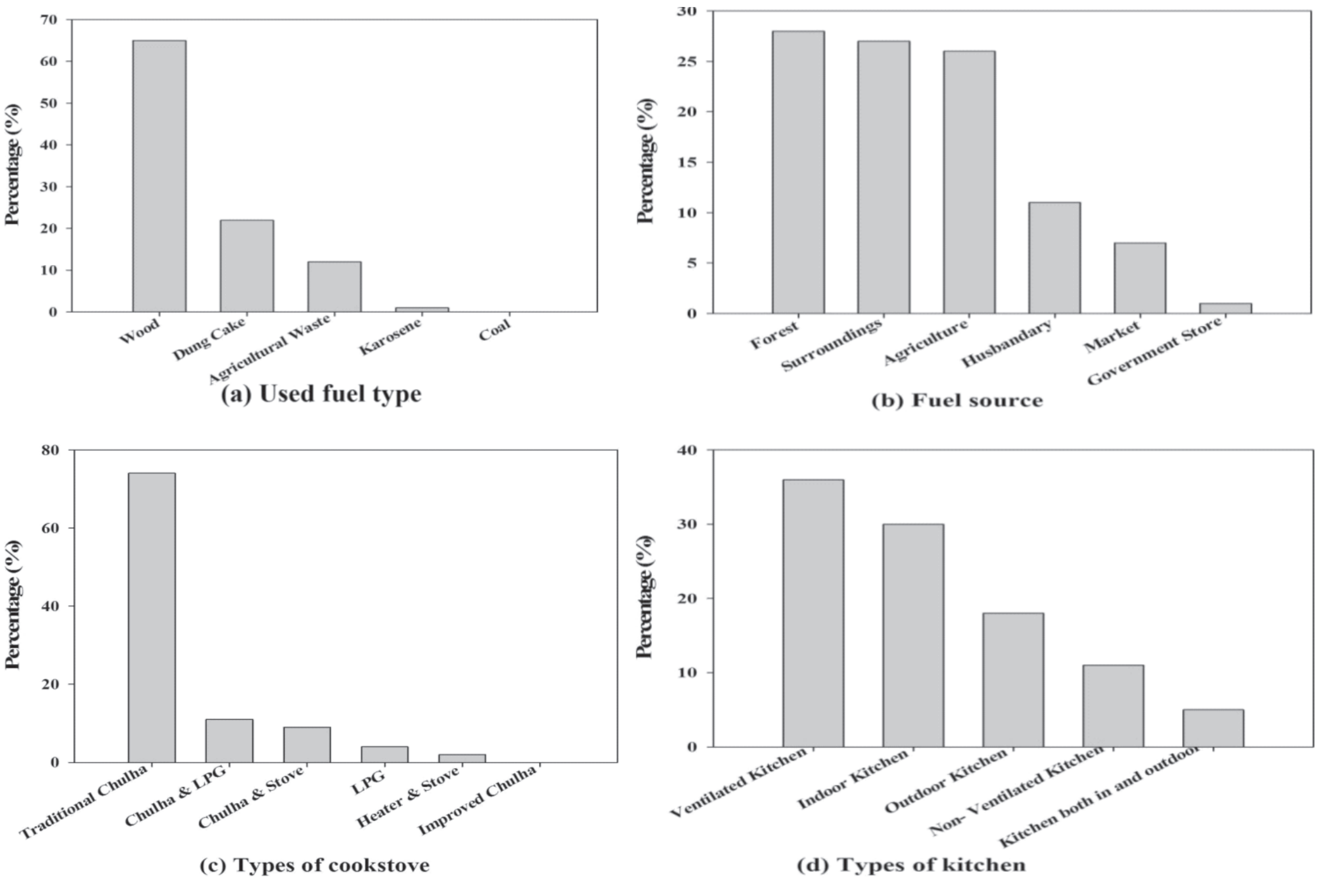

Figure 2. Composition of energy consumption categorised into: (a) used fuel type, (b) fuel source, (c) types of cook stove, (d) type of kitchen

520 kg/day in winter season. The reason of high value of per day forest wood extracted was that majority of households collected fuel wood from the surrounding forests. As per census of 2011, about 49% of households in India use fuel wood for cooking purposes. Fuel wood constitutes the major source of energy for cooking purposes, and about more than 853 million people use fuel wood for daily cooking in India (FSI, 1987, 1993, 1995, 1997, 1999, 2001, 2003, 2005, 2009, 2011). The energy use pattern in rural India is changing with selection of clean energy options, but traditional fuels including fuel wood, crop residue and cow dung are still contributing the main source of household cooking energy due to inadequate and unreliable supply of clean energy.

Excessive usage of fuel wood is associated with rapid degradation of environment and insecurity of energy for rural low-income households (Sedai *et al*., 2016). The average fuel wood collection and fuel wood consumption in two different seasons that is winter season and summer season were studied. There was significant difference in fuel wood collection and fuel wood consumption in different seasons. Fuel wood is a renewable energy resource, and its consumption can only be sustained if the rate of harvesting does not exceed the growth rate (Dhanai *et al*., 2015). Average values of fuel wood collection and consumption were proportionally related to each other and could be clearly differentiated in both seasons as collection range (480–490) was highest in winter with high consuming demand in same season ranges (15–25) compared with summer season (Table 2).

3.3 Households Income, Cooking Time, Consumption of Fuel Wood and Their Market Value

After the collection of data, the households were classified into five family size, that is very small, small, medium, large and very large. Average family income was calculated as maximum income in large family (13,000–25,000 per month) and minimum in very small family (2,000–5,000 per month) (Table 3). Average cooking time of household was 4.46 h/day/family which ranged between lowest (3.2 h/day/family) in very small family to highest (5.9 h/day/family) in very large family. The study showed that average fuel wood consumption from the plantation forest was 4.5 t/family/day with maximum 7.1 family/day

Table 2. Fuel wood collection and consumption from the plantation forest

Parameters	Minimum	Maximum	Average
Consumption of fuel wood from the forest (kg/day)	460	470	465
Number of head load produced from the forest per day	45	48	46.5
Average weight of each head loads (kg)	12	17	14.5
Average fuel wood collected during winter seasons (kg/day)	480	490	485
Average fuel wood collected during summer seasons (kg/day)	250	270	520
Fuel wood consumed by individual households during winter (in per cent kg/day)	15	20	17.5
Fuel wood consumed by individual households during summer (in per cent kg/day)	7	10	8.5

Table 3. Average income of the households, consumption of fuel wood, cooking time and market value of fuel wood in selected study area

Size of Family	Number of Family Members	Monthly Income Level (₹ in Thousands)	Average Monthly Income (₹ in Thousands)	Fuel Wood Consumption Estimates (t/family/year)	Cooking Time (h/day/family)	Market Value of Annual Consumption of Fuel Wood (in ₹)
Very small	28	2–5	2 ± 0.81	2.5	3.2	4,500
Small	40	4–7	5 ± 0.71	3.1	3.9	6,100
Medium	56	8–12	10 ± 1.07	4.2	4.1	8,200
Large	36	6–18	13 ± 3.23	5.6	5.2	10,600
Very large	20	13–25	20 ± 3.35	7.1	5.9	14,100
Average	–	–	–	4.5	4.46	8,700

and minimum 2.5 family/day. Fuel wood constitutes the major source of energy for cooking purposes and about more than 853 million people use fuel wood for daily cooking in India (FSI, 1987, 1993, 1995, 1997, 1999, 2001, 2003, 2005, 2009, 2011). It has been cleared from the present study that large family households consume more fuel wood than small families. It was clear that large families consumed more wood fuel and spent more money than smaller families. Consumption of fuel wood with inefficient chulha consumes higher quantities with maximum time consuming and reduce efficiency. A number of studies reported that fuel wood consumption in traditional chulha is much higher as compared with improved chulha (Alam and Chowdhury, 2010). Most of the chulha user experiences that improved chulha option is time saving during the process of cooking and explained that improved chulha cooks faster than that of traditional mud chulha for the same family members (Kuhnhenn, 2003). High values of fuel wood consumption with high cooking time in study area represent the total lacking of improved chulha and as well as poverty.

The use of fuel wood as a primary source of energy for domestic and commercial use is a cause of severe deforestation in India. Similarly, the present study focuses on forest as one of the important sources of fuel wood and has been meeting the requirement of energy for rural poor households. Due to continue depletion and degradation due to excessive consumption of fuel wood, the sustainability of forest is questioned. This study discusses the production of fuel wood from the Indian forest and visualised technological intervention so that the forest can be sustainably managed. The rural environment is suffering from slow but consistent deforestation and soil erosion, which threatens the entire region's biodiversity. One of the severe impacts of repeated fuel wood harvesting on the structure of the forest is the rapid decline of large, old trees that ultimately results in their complete disappearance. Once these trees are lost, the number of the gaps created by natural tree falls and logging increases (Ruger *et al.,* 2007), which results in forest fragmentation and susceptibility to invasion by ephemerals, that inhibit the regeneration of seedlings of native tree species.

4. Conclusion

Consumption rates are an important variable in defining the potential contribution of a fuel wood source and in assessing the risk of forest over-exploitation. In this regard, study of calorific value of different species in plantation forest of study area will be helpful to recommend their role as a fuel wood species which were investigated here. *P. juliflora* was dominant plant species of studied forest area. It was also a highly ranked species as fuel wood due to their high calorific value. Higher fuel wood consumption in the study area was mainly due to lack of alternative energy sources and poverty. Extensive farming for fuel wood could be one alternative to bridge the gap between supply and demand; consequently, efforts are needed to encourage afforestation of suitable fuel wood species as *P. juliflora, T. arjuna and P. dulce* in the barren areas around the study site, which will help immensely in reducing the degradation of surrounding forests. Information collected in the present study will be helpful in alignment of available resources with future challenges of biomass energy demand and their utilisation in sustainable way and development in rural areas.

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RESEARCH ARTICLE

Long-term Rainfall Trend and Drought Analysis for Bundelkhand Region of India

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Abstract In this study, an effort has been made to characterise the spatial and temporal variability of precipitation in 13 districts of the Bundelkhand region spreading in Uttar Pradesh and Madhya Pradesh of India in the during 1901–2002. The Bundelkhand region receives an average annual rainfall of 1,071.7 mm. The highest annual rainfall of 1,190.6 mm has been recorded in Sagar district, whereas the lowest annual rainfall of 902 mm has been recorded in Jalaun district. On the basis of rainfall records, the Bundelkhand region can be classified into two groups, namely, districts with high rainfall, namely, Panna, Tikamgarh, Lalitpur and Sagar receiving an average annual rainfall of 1,138 mm and low rainfall districts, namely, Jhansi, Jalaun, Hamirpur, Banda, Mahoba, Chitrakut, Datia, Chhatarpur and Damoh receiving an annual average rainfall of 1,011 mm. Standardized Precipitation Index indicates that 5 drought years were observed during 1901–1950 and 12 drought years were noticed during 1951–2000. This indicated a rising trend in the occurrence of drought in the region. Also indicates that the first half of the century was slightly wetter that the second half.

Keywords Bundelkhand, Mann–Kendal test, Rainfall variability, Standardized Precipitation Index, Drought, Time series,

1. Introduction

Rainfall is one of the most important elements that affect the use of land resources for agricultural purposes. Bundelkhand region in central India is recognised as a hard rock plateau region with the rainfall being highly variable both spatially and temporally. The information of inter- and intra-seasonal rainfall variation is reported to be useful for crop planning (Patra *et al*., 2012). In the past, decreasing rainfall trend has been reported from Russia, North-East and North China, Pakistan, Sri Lanka and Australia by Peterson *et al*. (2002), Zhai and Pan (2003), Farooq and Khan (2004), Jayewardene *et al*.(2005) as well as the Lavender and Abbs (2013). Increasing rainfall trend has also been reported from Jinsha river basin of China, Bangladesh and SW Plains of Uttarakhand, India by Wang *et al.* (2013), Farhana and Rahman (2011) and Tripathi *et al*. (2007). Rainfall trend in India has been reported to be highly variable due its geographic conditions by Mooley and Parthasarathy (1984), Patra *et al*. (2012), Rajeevan *et al*. (2008) and Joshi and Pandey (2011).

Drought is a natural and temporary imbalance of water availability, demonstrated by lower-than-average precipitation (Pereira *et al*., 2002). There are four types of droughts, that is meteorological drought (reduced precipitation), agricultural drought (reduced water availability to crop from the soil), hydrological drought (reduced runoff generation) and socio-economic drought (reduced availability for domestic use). The meteorological drought can be very well assessed by calculating Standardized Precipitation Index (SPI) (McKee *et al*., 1993). In India, this technique has been used by Sarkar *et al*. (2010) and Bhuiyan *et al*. (2006).

Bundelkhand is one of the most important drought prone regions of the country. Agriculture is the predominant occupation of a majority of population in the region. Agriculture and allied sectors do provide livelihood to more than 80% of the population in the region. There are limited irrigation facilities and majority of the cultivated area is rain fed. The area comprises rocky outcrops and denuded hills. The rainfall events are generally characterised as small but intense. Therefore, greater part of rainfall goes in to runoff without being absorbed in to the soil. In view of this, the study entitled 'Long term rainfall trend and drought analysis for Bundelkhand region of India' was undertaken with a

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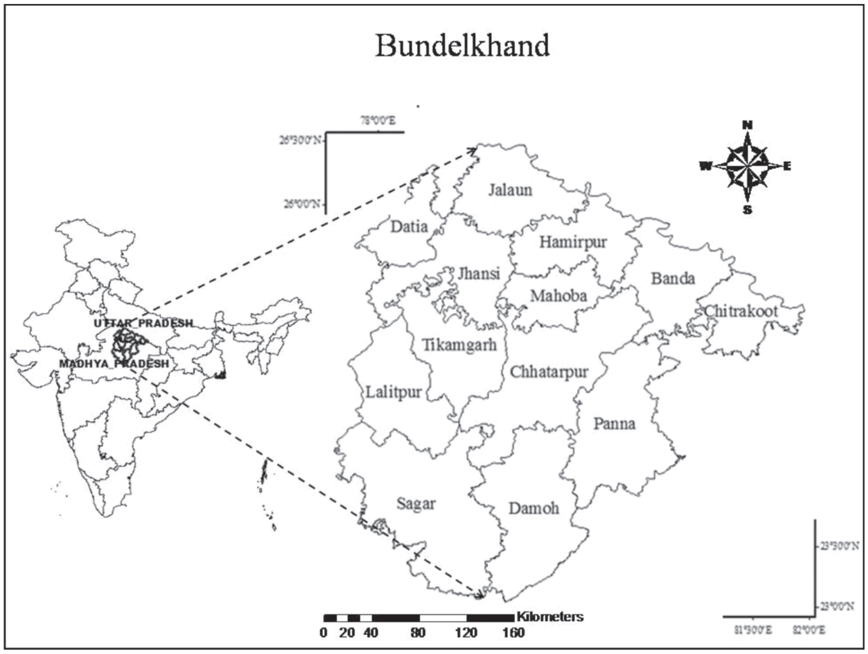


Figure 1: Study area with meteorological stations of Bundelkhand region

reinvestigate to characterise rainfall trend in the region on the basis of its past track record.

2. Materials and Methods

Bundelkhand region comprised 13 districts, 7 in Uttar Pradesh (Jhansi, Jalaun, Lalitpur, Mahoba, Banda, Hamirpur and Chitrakoot) and 6 in Madhya Pradesh (Datia, Chhatarpur, Panna, Sagar and Damoh) covering an area of 294,180 km^2 and lies between 23.20°N to 26.50°N latitude and 78.10°E to 82.20°E longitude (Figure 1).

The monthly precipitation data of all the 13 districts from the periods 1901–2002 was downloaded from India Water Portal (http://www.indiawaterportal.org/metdata). The dataset is frequently used by various researchers of India (Darshana and Pandey, 2013). The data series were plotted to detect the outliers. After visual observation of outliers, the same were corrected using normal ratio method. There was no missing value in the data set; therefore, gap filling was not required. Season-wise data were grouped as defined by IMD, namely, winter (January–February), pre-monsoon (March–May), monsoon (June–September) and post-monsoon (October–December). Annual and seasonal values were calculated. The statistical analysis, including probability, mean, standard deviation and coefficient of variability, was done.

2.1 Standardized Precipitation Index

SPI is a method of defining meteorological drought using the continuous monthly rainfall data. Conceptually, SPI is equivalent to the statistical *Z*-score and is formulated as (McKee *et al*., 1993)

$$\mathrm{SPI}_{ij} \approx \frac{X_{ij} - \mu_{ij}}{\sigma_{ij}} \quad \ldots(1)$$

Where SPI_{ij} is the SPI of *i*th month at *j*th time-scale, X_{ij} is precipitation total for *i*th month at *j*th time-scale, μ_{ij} and σ_{ij} are long-term mean and standard deviation associated with *i*th month at *j*th time-scale.

Computation of the SPI involves fitting a gamma probability density function to a given time series of precipitation, whose probability density function is defined as follows:

$$g(x) = \frac{1}{\beta^{\alpha}\Gamma(\alpha)} x^{\alpha-1} e^{-x/\beta} \quad \ldots(2)$$

where $\alpha > 0$ is a shape parameter, $\beta > 0$ is a scale parameter and $x > 0$ is the amount of precipitation. $\Gamma(\alpha)$ is the gamma function, which is defined as follows:

$$\Gamma(a) = \int_{\alpha}^{\infty} y^{a-1} e^{y} dy \quad \ldots(3)$$

Fitting the distribution to the data requires α and β to be estimated. Using the approximation of Thom (1958), these parameters can be estimated as follows:

$$\alpha = \frac{1}{4A}\left(1 + \sqrt{1 + \frac{4A}{3}}\right), \ \beta = \frac{\mu}{\alpha}, \text{ with } \quad A = \mathrm{In}(\mu) - \frac{\sum \mathrm{In}(\mu)}{n} \quad \ldots(4)$$

where n is the number of observations. Integrating the probability density function with respect to x yields the following expression for the cumulative probability $G(x)$:

$$G(x) = \frac{1}{\Gamma(a)} \int_{\alpha}^{\infty} y^{a-1} e^{y} dy \qquad \ldots(5)$$

It is possible to have several 0values in a sample set. To account for zero value probability, since the gamma distribution is undefined for $x = 0$. Finally, the cumulative probability distribution is transformed into the standard normal distribution to yield the SPI.

2.2 Spatial Distribution

The map showing the boundary of each of the 13 district boundaries was developed. Annual and seasonal rainfall as well as the coefficient of variability was plotted. The Universal Kriging technique is recommended for interpolation spatial variability of rainfall in rolling topographic region.

3. Results and Discussion

3.1 Rainfall in General

The average annual rainfall during the period 1901–2002 was estimated to be 1,071.7 mm with a standard deviation of 218.93 mm and coefficient of variation (CV) of about 20%. The mean annual rainfall at 75% probability was 924 mm with a standard deviation of 210.56 mm. About 91% of the total annual rainfall was received from south-west monsoon. The pre-, post- and north-east monsoon contributed only 1.8%, 3.6% and 3.7%, respectively. The CV during south-west monsoon, pre-monsoon, post-monsoon and north-east monsoon was 22%, 70%, 92% and 62%, respectively. June, July, August and September received 98, 180, 362 and 336 mm, respectively, rainfall with the standard deviation of 86.83, 124.64, 59.65 and 1,116.85 and CV of 48, 37, 61 and 32%, respectively. This shows that rainfall during July and August in Bundelkhand region is not consistent.

3.2 Temporal Variation

The long-term (1901–2002) annual/seasonal rainfall is depicted in Figure 2. In general, the annual rainfall in Bundelkhand region is declining at the rate of 0.24 mm/year. The third, fourth and fifth decades recorded slightly higher rainfall over the annual normal. However, rest of the decades recorded reduced rainfall.

Data presented in Figure 2 showed a remarkable seasonal variation. Pre-monsoon season was highly variable up to 1960, thereafter, became homogeneous. On the contrary, during the south-west monsoon season,

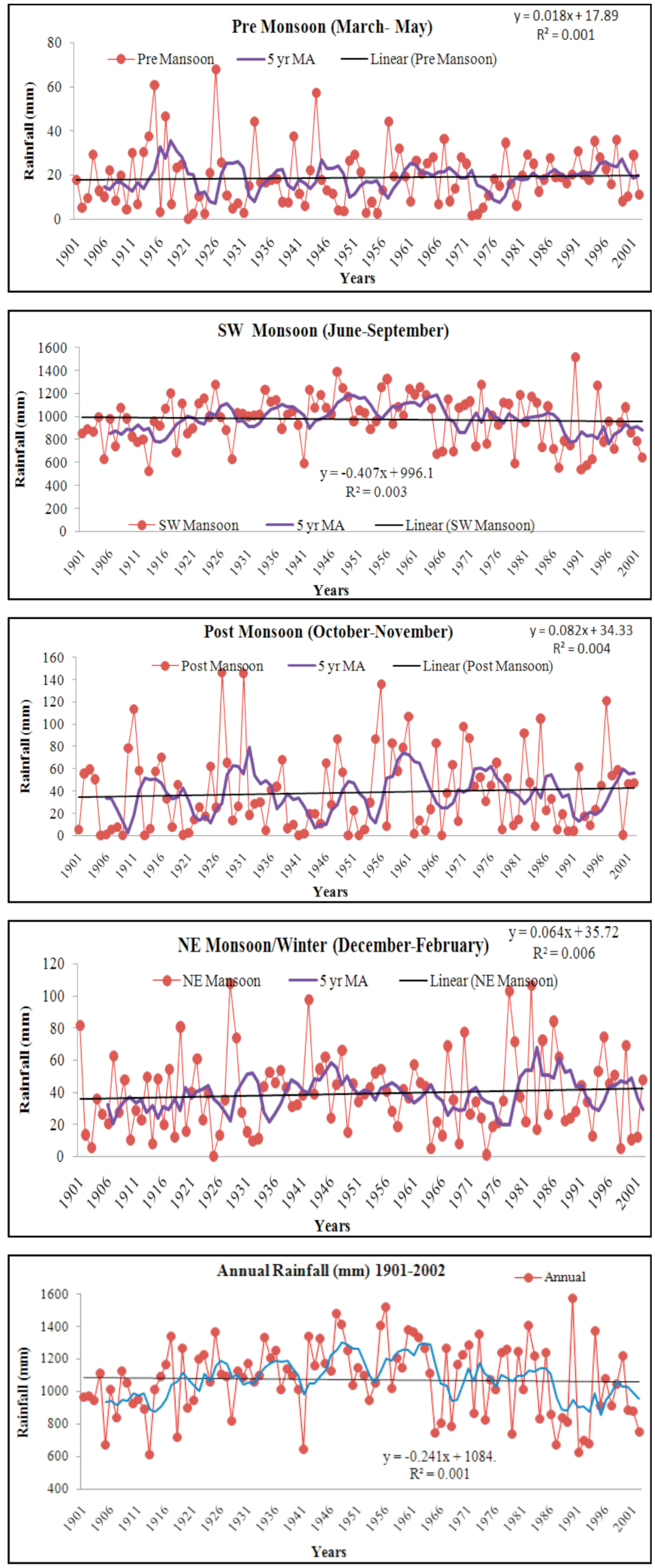


Figure 2: Temporal variation of rainfall in Bundelkhand region (1901–2002)

the rainfall was slightly variable up to 1960 and thereafter, become highly variable. Post-monsoon and north-east monsoon always remained variable with no change in the rainfall pattern. The annual rainfall was slightly variable up to 1960 but became highly variable thereafter. The south-west monsoon (Kharif season) which receives about 90% of the annual rainfall is highly sensitive to agricultural productivity in Bundelkhand region.

3.3 Spatial Variation

The season-wise spatial distribution of annual rainfall (mm) and CV (%) of Bundelkhand region depicted in Figure 3 indicates that the variability is very high in central part as compared to other regions (Banda and Chitrakoot district in northern eastern region, Damoh district in southern region and Jhansi district in northern western region). Thus, it can be concluded that high spatial variability is observed in the districts with higher rainfall.

Figure 3 shows that the northern part of Bundelkhand, that is Datia, Jhansi, Jalaun, Hamirpur, Mahoba and Banda districts experience very low rainfall, whereas southern part like Tikamgarh, Lalitpur, Chhattarpur, Panna and Sagar districts experience high rainfall. The south-west monsoon period (June–Sept.) is main rainy season of Bundelkhand. During this season, rainfall varies from 825 mm in Jhansi to 1,090 mm in Panna. Post-monsoon season rainfall varied from 30 to 58 mm, with maximum being in central and southern region, whereas minimum being in northern region. Tikamgarh, Sagar and Lalitpur districts received maximum rainfall during north-east monsoon season as compared to Jhansi, Chitrakoot and Damoh. The variability in north-east monsoon season rainfall in Panna, Chhatarpur, Tikamgarh, Jalaun, Hamirpur and Panna districts varied from 25 to 44%.

3.4 Standardized Precipitation Index

SPI for each districts of Bundelkhand at 3-, 6- and 12-month interval for the period 1901–2002 was calculated as per the procedure suggested by McKee *et al*.(1993). Classification of drought was done as per the methodology suggested by Hayes (1999).

3.4.1 Three-month SPI

An overview of the SPI calculated for entire Bundelkhand region on tri-monthly time scale is presented in Figure 4. This shows that January–March period experienced several extreme drought events in the first quarter of the century, whereas second

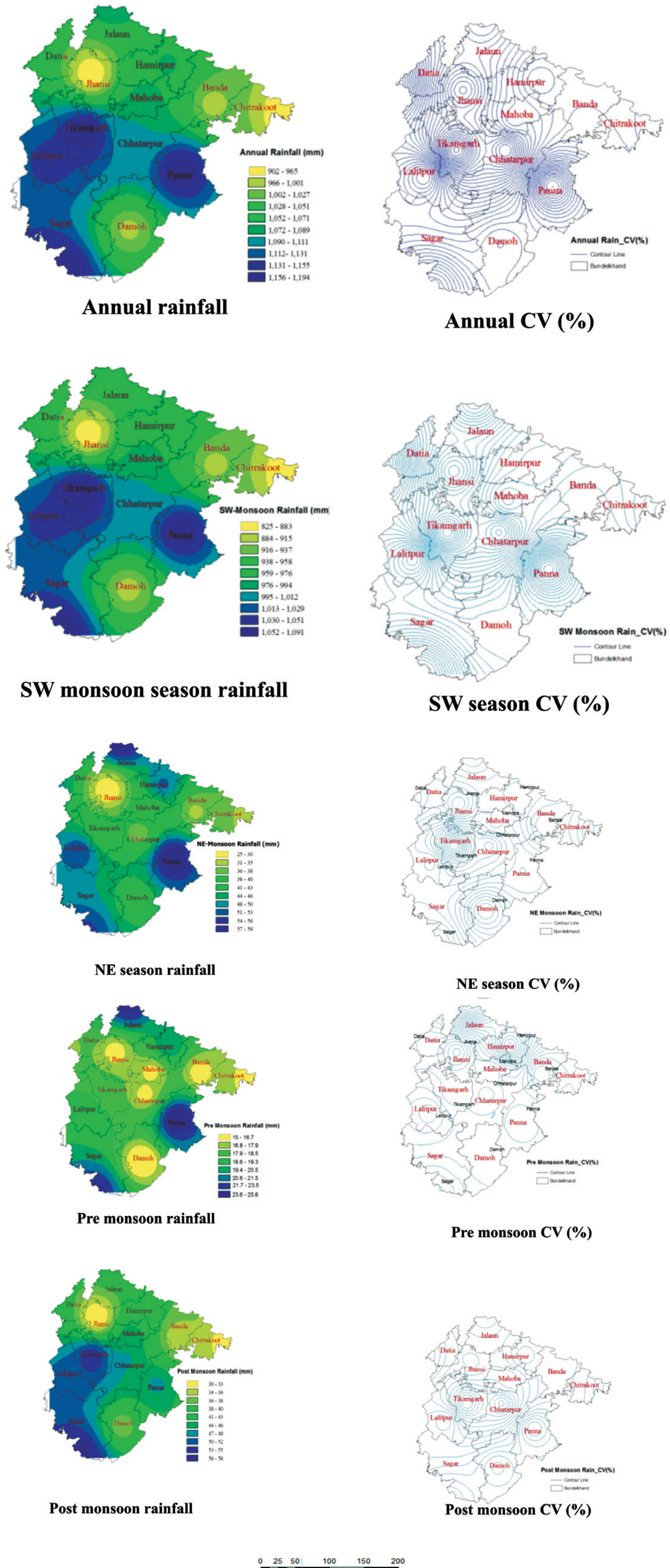
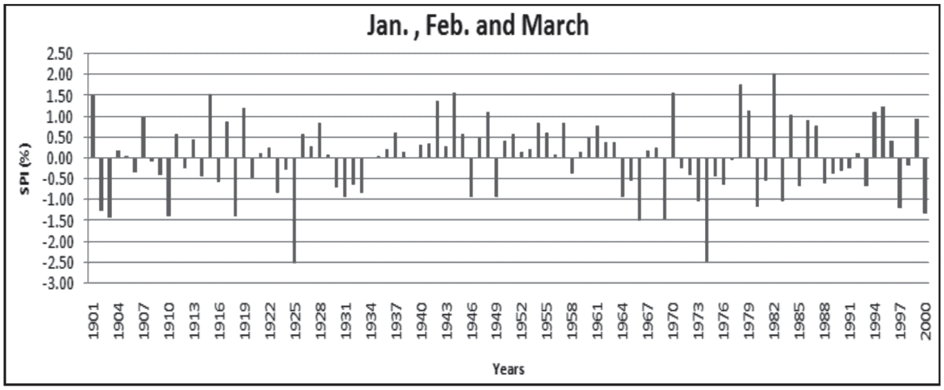
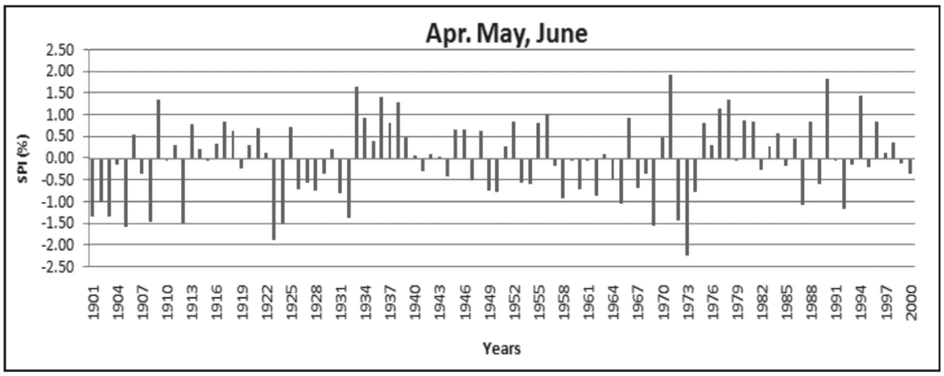
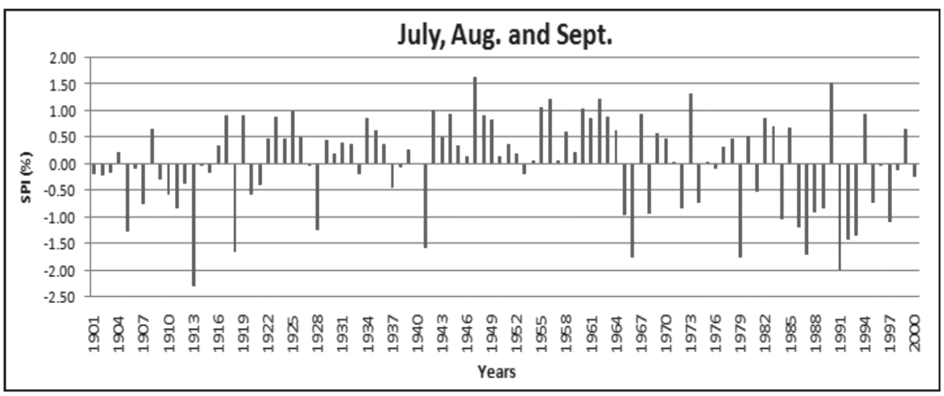
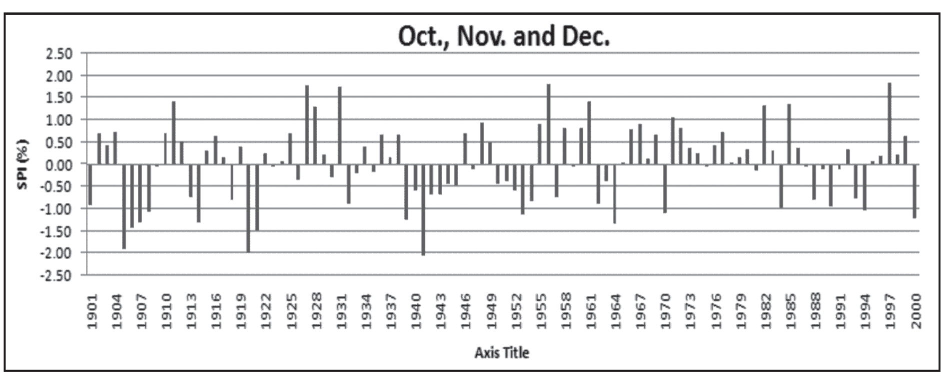


Figure 3: Annual and seasonal spatial distribution of rainfall (mm) and coefficient of variation (%) for Bundelkhand region


Jan. , Feb. and March
SPI (%)
Years


Apr. May, June
SPI (%)
Years


July, Aug. and Sept.
SPI (%)
Years


Oct., Nov. and Dec.
SPI (%)
Axis Title

Contd...

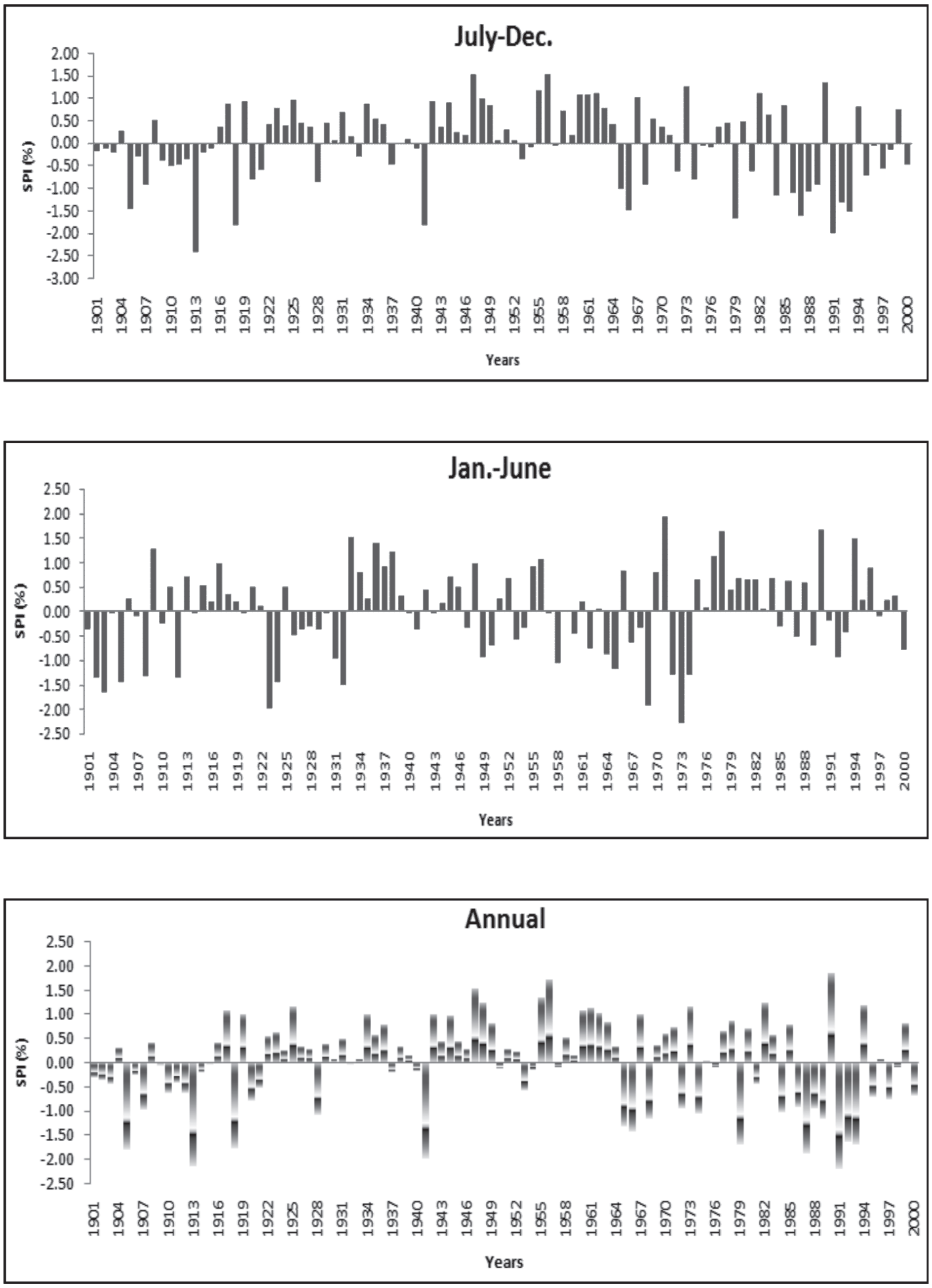


Figure 4: Three-monthly, six-monthly and annual SPI for Bundelkhand region during the period 1901–2002

quarter period from 1925 to 1960 was average wet period. The third quarter period 1960–1980 recorded a mix of dry and wet periods. However, the most severe drought of the century was observed during 1974. The results presented in Table 1 reveals that during the period 1950–2001, a total of 8 moderate-to-severe drought years have been observed, whereas during 1901–1950, only 5 drought years were observed. This indicates that third and fourth quarter of the century was prone to drought during Rabi season.

During April–June, the trend in drought occurrence is almost similar to that during January–March. During July–September, the first half of the century witnessed the extreme drought condition. The second part of the century was somewhat wet. The year 1965 and 1991 were extreme drought years, whereas the 1990 was the wettest. During last tri-month period, that is October–December months, the first decade of the century was one of the driest. During the period 1901–1950, a total of 9 drought years were observed,

Table 1: Drought years in Bundelkhand region based on SPI <-0.99

Time Series	Period						Annual
	Jun–Sep	Jul–Dec	Jan–Mar	Apr–Jun	Oct–Dec	Jan–Jun	
Decadal Distribution							
1901–1910	1	1	3	5	4	4	1
1911–1920	2	2	1	1	2	1	2
1921–1930	1	0	1	2	1	2	1
1931–1940	0	0	0	1	1	1	0
1941–1950	1	1	0	0	1	0	1
1951–1960	0	0	0	0	1	1	0
1961–1970	1	1	2	2	2	2	3
1971–1980	1	1	3	2	0	3	2
1981–1990	3	4	1	1	0	0	3
1991–2000	4	3	2	1	2	0	4

whereas during 1951–2000, only 5 drought years were noticed. Overall result indicates that the drought occurrence during the second half of the century was reduced as compared to the first half of the century.

3.4.2 Six-month SPI

The six-monthly SPI results are presented in Figure 4. During January–June, first half of the century observed frequent droughts of moderate-to-high intensity. On the contrary, during July–December, second half of the century observed frequent droughts of moderate-to-high intensity. Severe drought of the century was observed during 1973, whereas the wettest year of the century was 1971. Overall result indicated the increase in the intensity of drought during monsoon season as compared to non-monsoon season.

3.4.3 Twelve-month SPI

Results presented in Figure 4 indicate that the degree of drought increased in second half of the century. A total of 17 droughts years were observed during the century. Out of which, 5 drought years were observed during 1901–1950 and 12 drought years were during 1951–2000. This indicated a rising trend in the occurrence of drought in the region.

4. Conclusion

The studies showed that the average rainfall of the region during 1901–2000 was 1,050 mm and about 90% of the total annual rainfall occurred during SW monsoon (June–October). Panna, Tikamgarh, Lalitpur and Sagar districts received higher rainfall as compared to other districts. The coefficient of was higher in high-rainfall districts as compared to low-rainfall districts. The SPI observation also revealed that the first half of the century (1900–1950) was less prone to drought as compared to second half of the century. In general, rainfall during July was observed to be decreasing. If a similar trend persists in future then the crop planning should cater to the changing climate.

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RESEARCH ARTICLE

Variation in Juice Quality Traits of Sugarcane Genotypes under Waterlogged Condition in Subtropical India

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Abstract Water-logging is one of the serious environmental constraints for optimum growth, yield and juice quality of sugarcane crop. Twenty three sugarcane genotypes including six commercial varieties were evaluated for water-logging tolerance based on juice quality traits during year 2016–2017 at Kharika block, ICAR-Indian Institute of Sugarcane Research, Lucknow. Significant difference was observed among genotypes for all juice quality traits immediately after waterlogged condition (in the month of November) and at later stage of water-logging (in the month of December and February). Juice quality attributes, namely °Brix, sucrose% juice, juice purity, CCS% juice and S/R ratio were relatively higher in the month of November and lower at later stage of water-logging (December and February) as compared to control plants. While reducing sugar content was slightly higher in waterlogged affected plants in the month of February. Sucrose% juice was ranged from 16.19 (CoLk12202) to 12.48 (LG05020) in November, 16.54 (CoLk12204) to 10.63 (A-46-11) in December and 17.33 (UP9530) to 12.83 (BO91) in February under waterlogged condition. With a general mean value highest increase (14.97% and 6.21% over control) was observed in D-6-13 and highest decrease in CCS% juice, juice purity (12.17%, 13.02% over control) was observed in CoJ 64 due to water-logging. Based on juice quality attributes, CoLk12202, CoLk12204, D-6-13, UP9530, CoLk94184 may be grouped as tolerant genotypes and need to be evaluated at different locations to assess their adaptability under waterlogged condition.

Key words Sugarcane, Genotypes, Waterlogged, Subtropical, Environment, Abiotic stress

1. Introduction

Abiotic stress is the primary cause of crop loss worldwide, reducing average yields by more than 50%. Low temperature, drought, water-logging and high salinity are common stresses that adversely affect plant growth and crop production (Xiong and Zhu, 2002). In subtropical India, water-logging is associated with monsoon rainfall, improper drainage facilities. Higher rate of tiller mortality, reduced growth rate and cane yield are major effects of water-logging. Losses of cane yield and juice quality due to water-logging depends upon genotypes, environmental conditions, stage of development and duration of water-logging (Orchard and Jessop, 1984). In India, usually 5–20% loss in cane yield has been recorded due to water-logging (Dwivedi and Jain, 2014). At active growth stage, water-logging adversely affects stalk weight and plant population (Carter and Floyed, 1974; Carter, 1976), although some varieties are relatively tolerant to high water level and flooding (Roach and Mullins, 1985, Deren *et al*., 1993). Water-logging tolerance for the survival/adaptation is related to many physiological, morphological, anatomical and biochemical changes in plants (Barclay and Crawford, 1982; Gomathi *et al*., 2015; Dwivedi, 2016). The tolerant species are able to form aerenchyma, which helps for functioning of the plant processes under anoxia conditions. To survive and grow under water-logging condition, plant evolves the mechanism of adaptation, avoidance, acclimation or a combination (Gomathi *et al*., 2015). Present study was aimed to investigate the variability among sugarcane genotypes for juice quality attributes under waterlogged condition in subtropical India and identify the genotypes tolerant to water-logging stress based on percent numerical increase/decrease over control in juice quality parameters.

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Table 1: Weather data during experimental period (Feb 2016–March 2017)

Month	Temperature (°C)		Rainfall (mm)
	T Max.	*T* Min.	
Feb'16	27.8	11.6	0.0
Mar	27.7	13.9	23.6
April	46.1	26.2	1.0
May	38.9	25.4	39.6
June	37.9	27.6	92.4
July	33.4	26.3	219.6
Aug	33.6	25.7	243.4
Sept	33.5	25.2	202.4
Oct	33.5	19.7	54.6
Nov	28.7	11.9	0.0
Dec	22.2	8.8	0.0
Jan'17	21.9	7.8	0.0
Feb	26.7	10.4	0.0
Mar	31.8	14.9	6.8

2. Materials and Methods

Field experiment was conducted at Kharika Block (having well established deep plot to conduct experiment on water-logging), Indian Institute of Sugarcane Research, Lucknow, using 23 sugarcane genotypes including six commercial varieties (tolerant/susceptible), CoLk 94184, BO 91, CoS 767, CoJ 64, CoS 97264, UP 9530 and 18 germplasm lines, CoLk 12204, CoLk 12202, CoLk 12206, CoLk 07201, CoLk 04238, LG 06605, LG 04439, LG 05350, LG 05020, LG 03040, A-46-11, B-44-12, A-27-12, D-12-9, D-6-13, S 5085/11, S 5087/11 obtained from different research centres working on varietal screening programme for water-logging. For water-logging treatment, crop was grown in deep plot along with non-waterlogged control treatment. Water-logging condition was imposed for about 90 days to approx. 1 m depth. Planting was performed in the second week of February 2016. Weather data (temperature and rainfall) during the experimental period (Feb 2016–Feb 2017) has been given in Table 1. Sugarcane genotypes were raised in four rows of 10 m length with a

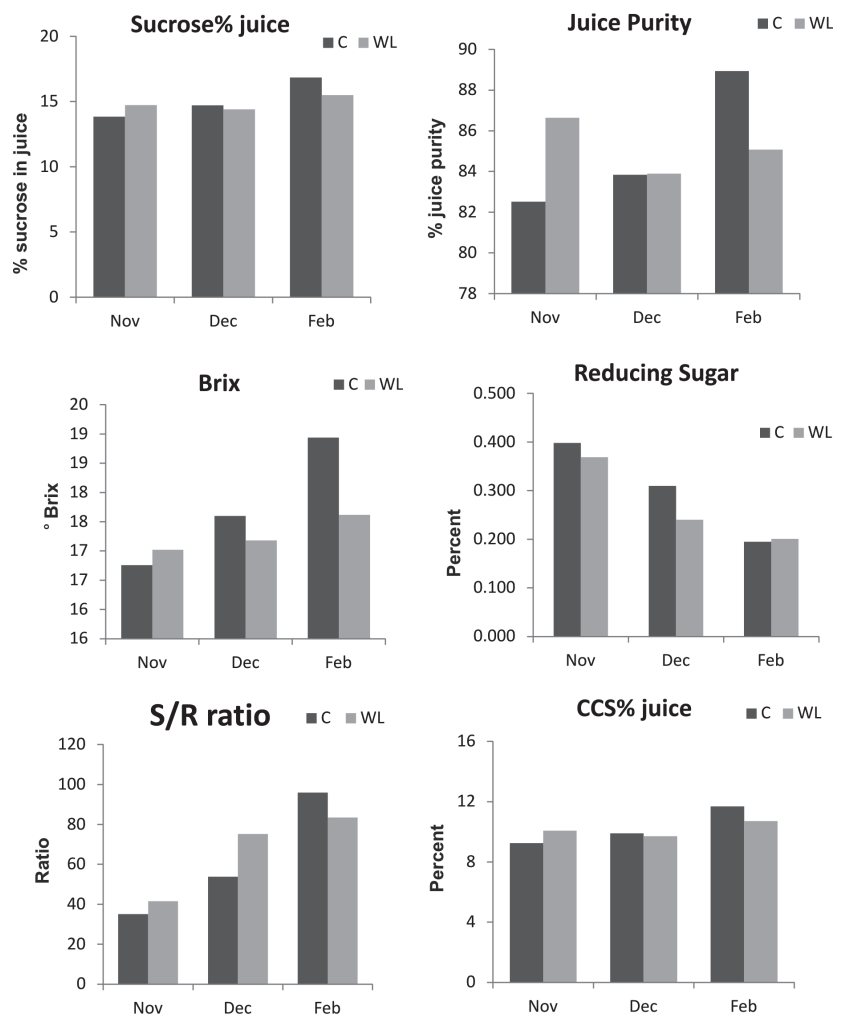

Figure 1: Effect of waterlogging on overall mean of data of juice quality attributes. C – control; WL – waterlogging

row spacing of 75 cm. Three bud setts of each genotype were planted in each row (50 setts/row) in three replications in randomised block design. Full dose of P @ 80 kg P_2O_5/ha, K @ 80 kg K_2O/ha and 1/3rd of N of full dose of 150 kg/ha were applied at the time of planting. Rest 2/3rd of N in the form of urea was top dressed in two equal splits before earthing up in the month of May and June. To study the juice quality attributes, five cane stalks per sample were crushed, immediately after waterlogged condition (in the month of November 2016) and later stage of waterlogged condition (in the month of December 2016 and February 2017) and analysed for juice quality traits; sucrose%, reducing sugars contents, CCS% and sucrose/reducing sugars ratio (S/R), Brix, juice purity in cane juice. Sucrose% juice, Brix and juice purity was determined in clear juice by Automatic Saccharimeter (Rudolph). CCS% juice was determined using formula as reported earlier (Bakshi *et al.*, 2001):

CCS% juice = (1.022 × Sucrose% in juice - 0.292 × °Brix)

The amounts of reducing sugars in cane juice were determined by the method using Nelson's arsenomolybdate reagent (Nelson, 1944).

All the data were determined in three replications and the entire data had been submitted for analysis of SD, SE and coefficient of variance using WASP 2.0 software.

3. Results and Discussion

Based on well established and documented literature on poor juice quality under waterlogged condition, present study was made using breeding material of different research centres along with six commercial varieties (tolerant/susceptible) for identifying sugarcane lines tolerant to water-

Table 2: Changes in °Brix of sugarcane in response to waterlogging

Genotypes	November			December			February		
	C	WL	%Increase/ decrease	C	WL	%Increase/ decrease	C	WL	%Increase/ decrease
CoLK 94184	17.31	17.95	3.67	19.57	18.04	-7.82	19.65	19.21	-2.24
BO 91	16.79	16.22	-3.37	17.99	17.39	-3.34	18.63	14.86	-20.26
CoS 767	17.11	16.76	-2.07	18.74	18.27	-2.51	19.64	15.66	-20.26
CoJ 64	19.26	17.67	-8.23	20.31	18.12	-10.78	20.41	16.87	-17.34
CoS 97264	17.33	16.64	-3.98	19.78	18.83	-4.80	19.24	17.95	-6.73
UP 9530	17.48	17.65	0.97	16.65	18.22	9.43	18.63	19.41	4.16
CoLk 12204	17.75	16.19	-8.76	18.15	19.45	7.16	19.93	15.34	-23.03
CoLk 12202	16.85	18.63	10.60	17.48	19.80	13.27	19.04	18.45	-3.10
CoLk 12206	17.30	17.69	2.28	17.95	17.28	-3.73	20.10	19.12	-4.88
CoLk 07201	16.37	17.69	8.06	18.21	18.23	0.11	20.10	18.92	-5.90
CoLk 04238	16.82	16.64	-1.10	16.96	16.45	-3.01	18.23	17.44	-4.33
LG 06605	17.44	15.78	-9.49	17.94	15.53	-13.43	19.00	14.75	-22.39
LG 04439	15.93	16.63	4.43	17.25	16.97	-1.62	18.98	16.83	-11.35
LG 05350	16.01	17.63	10.12	16.75	18.09	8.00	17.84	19.11	7.12
LG 05020	15.37	15.27	-0.65	16.77	15.74	-6.14	17.46	18.28	4.70
LG 03040	17.67	18.15	2.72	17.93	13.61	-24.09	20.01	19.80	-1.07
A-46-11	15.56	17.67	13.56	16.52	14.72	-10.90	19.18	18.48	-3.65
B-44-12	16.62	18.14	9.15	17.99	17.77	-1.22	19.49	17.65	-9.44
A-27-12	15.03	15.89	5.72	13.45	14.00	4.09	15.75	15.82	0.41
D-12-9	16.70	16.48	-1.32	16.59	15.76	-5.00	18.28	17.88	-2.22
D-6-13	16.24	18.32	12.78	17.58	18.25	3.81	18.35	18.63	1.50
S 5085/11	16.42	15.78	-3.90	15.25	17.63	15.61	17.61	17.11	-2.84
S 5087/11	16.13	15.95	-1.15	18.96	17.00	-10.34	20.09	17.72	-11.82
Mean	16.76	17.02		17.60	17.18		18.94	17.62	
SD	0.920	0.974		1.481	1.633		1.099	1.498	
SE±	0.192	0.203		0.309	0.340		0.229	0.312	
CV (%)	5.5	5.7		8.4	9.5		5.8	8.5	

C – control; WL – waterlogging

logging stress in subtropical India. Juice quality data determined in different month (November, December and February) revealed significant variation among different genotypes planted under control and waterlogged conditions.

3.1 °Brix

Genotypes for °Brix (total soluble solids in juice) in November ranged from 18.63 (CoLk 12202) to 15.78 (S 5085/11 and LG 06605) under waterlogged condition and from 19.26 (CoJ 64) to 15.37 (LG 04439) under control. In December, it varied from 19.80 (CoLk 12202) to 14.00 (A-27-12) in waterlogged and from 20.31 (CoJ 64) to 13.45 (A-27-12) in control. While in February it ranged from 19.80 (LG 03040) to 14.86 (BO 91) in waterlogged and from 20.41 (CoJ 64) to 15.75 (A-27-12) in control condition. °Brix value on mean basis was increased in waterlogged condition in the month of November, decreased in December and February (Table 2) Genotype A-27-12 showed lowest °Brix in December and February under control and LG06605 under waterlogged condition (Figure 1). The present findings are similar to the results reported earlier using a number of sugarcane varieties showing different level of Brix value (Rahman *et al.* 2010; Islam *et al.,* 2007, 2011; Khan *et al.,* 2007; Kabiraj *et al.,* 2007).

3.2 Sucrose% Juice

Sucrose% juice in the month of November was relatively higher in waterlogged condition as compared to control, ranged from 16.19 (CoLk 12202) to 12.48 (LG 05020) and from 16.29 (CoJ 64) to 12.20 (A-27-12), respectively. In

Table 3: Changes in sucrose% juice of sugarcane in response to waterlogging

Genotype	November			December			February		
	C	WL	%Increase/ decrease	C	WL	%Increase/ decrease	C	WL	%Increase/ decrease
CoLK 94184	14.58	15.65	7.30	15.78	15.09	-4.37	17.60	17.31	-1.65
BO 91	14.22	14.13	-0.67	13.48	14.30	6.08	16.66	12.83	-22.99
CoS 767	14.32	14.62	2.06	15.85	15.32	-3.34	17.52	13.33	-23.94
CoJ 64	16.29	15.39	-5.53	16.98	14.82	-12.72	18.43	15.07	-18.23
CoS 97264	14.62	14.64	0.14	16.37	15.04	-8.12	17.10	16.07	-6.02
UP 9530	14.54	15.29	5.16	14.33	15.93	11.17	16.61	17.33	4.30
CoLk 12204	15.46	14.36	-7.12	15.51	16.54	6.64	17.97	13.89	-22.70
CoLk 12202	14.08	16.19	14.99	14.18	16.38	15.51	17.08	16.18	-5.27
CoLk 12206	14.48	15.61	7.80	14.75	13.81	-6.37	18.06	17.09	-5.40
CoLk 07201	13.31	15.26	14.69	15.02	14.67	-2.33	17.99	16.97	-5.67
CoLk 04238	13.52	14.14	4.55	13.91	12.98	-6.69	16.17	15.25	-5.72
LG 06605	14.38	13.78	-4.21	15.60	13.60	-12.82	16.56	13.15	-20.59
LG 04439	13.24	14.37	8.54	14.87	14.83	-0.27	16.86	14.71	-12.75
LG 05350	13.48	15.43	14.51	13.94	15.96	14.49	15.73	17.10	8.71
LG 05020	12.52	12.48	-0.32	14.20	13.55	-4.58	15.10	15.64	3.54
LG 03040	14.88	15.71	5.61	15.87	11.39	-28.23	18.22	17.36	-4.75
A-46-11	12.76	15.24	19.44	14.47	10.63	-26.54	16.93	16.24	-4.08
B-44-12	13.03	15.14	16.20	15.12	15.14	0.13	17.37	15.34	-11.69
A-27-12	12.20	13.81	13.20	10.71	12.63	17.93	13.57	13.56	-0.11
D-12-9	13.24	14.03	5.97	13.79	13.07	-5.22	16.37	15.64	-4.46
D-6-13	12.82	15.98	24.65	14.67	16.63	13.36	16.46	16.00	-2.79
S 5085/11	13.46	13.64	1.37	13.17	15.02	14.05	15.16	15.02	-0.92
S 5087/11	12.98	13.94	7.40	15.98	14.12	-11.64	18.10	15.69	-13.34
Mean	13.84	14.73		14.72	14.41		16.85	15.51	
SD	0.998	0.914		1.310	1.557		1.174	1.411	
SE±	0.208	0.191		0.273	0.325		0.245	0.294	
CV (%)	7.2	6.2		8.9	10.8		7.0	9.1	

C – control; WL – waterlogging

Table 4: Changes in juice purity (%) of sugarcane in response to waterlogging

Genotype	November			December			February		
	C	WL	%Increase/ decrease	C	WL	%Increase/ decrease	C	WL	%Increase/ decrease
CoLK 94184	84.20	87.17	3.53	80.62	83.86	4.02	89.58	90.11	0.59
BO 91	84.70	87.08	2.81	78.56	82.26	4.71	89.44	86.35	-3.45
CoS 767	83.73	87.23	4.17	84.60	83.83	-0.91	89.21	85.22	-4.47
CoJ 64	84.62	87.09	2.92	83.63	81.81	-2.18	90.29	54.36	-39.79
CoS 97264	84.37	87.98	4.28	82.83	81.59	-1.50	88.86	54.58	-38.58
UP 9530	83.18	86.69	4.23	86.11	87.44	1.54	89.18	89.04	-0.16
CoLk 12204	87.09	88.69	1.84	85.49	85.60	0.13	90.16	90.63	0.52
CoLk 12202	83.62	86.90	3.92	81.29	82.76	1.81	89.73	87.62	-2.35
CoLk 12206	83.41	88.24	5.79	82.32	80.02	-2.79	89.86	89.39	-0.52
CoLk 07201	81.24	86.27	6.20	82.51	80.53	-2.40	89.54	89.71	0.19
CoLk 04238	80.36	84.97	5.74	82.04	78.93	-3.79	88.70	87.47	-1.39
LG 06605	82.53	87.32	5.81	86.99	87.65	0.76	87.16	89.28	2.43
LG 04439	83.13	86.42	3.96	86.27	87.39	1.30	88.84	87.32	-1.71
LG 05350	84.20	87.51	3.93	83.20	88.25	6.07	88.26	89.50	1.40
LG 05020	81.43	81.32	-0.14	84.62	86.13	1.78	86.47	85.51	-1.11
LG 03040	84.16	86.45	2.73	88.54	83.59	-5.59	91.05	87.67	-3.71
A-46-11	82.01	86.24	5.16	87.59	72.33	-17.42	88.29	87.86	-0.49
B-44-12	78.39	86.21	9.98	84.05	85.20	1.37	89.14	86.95	-2.46
A-27-12	81.16	86.82	6.97	79.64	88.81	11.51	86.20	85.73	-0.55
D-12-9	79.28	85.10	7.33	83.16	83.07	-0.11	89.59	87.52	-2.31
D-6-13	78.89	87.25	10.60	83.48	91.21	9.26	89.72	88.62	-1.23
S 5085/11	82.01	86.48	5.45	86.39	85.30	-1.26	86.11	87.79	1.95
S 5087/11	80.44	87.35	8.58	84.30	82.09	-2.62	90.14	88.57	-1.74
Mean	82.52	86.64		83.84	83.90		88.94	85.08	
SD	2.142	1.444		2.519	3.986		1.325	9.766	
SE±	0.447	0.301		0.525	0.831		0.276	2.036	
CV (%)	2.6	1.7		3.0	4.8		1.5	11.5	

C – control; WL – waterlogging

December it varied from 16.54 (CoLk 12204) to 10.63 (A-46-11) in waterlogged and from 16.98 (CoJ 64) to 10.71 (A-27-11) under control condition. However, in February it ranged from 17.33 in UP 9530 to 12.83 in BO 91 and from 18.43 in CoJ 64 to 13.57 (A-46-11) in waterlogged and control condition, respectively. On average basis, sucrose% juice increased in November while decreased in December and February due to water-logging stress (Table 3) (Figure 1). The results are similar to the findings of Arefin *et al.,* (2009).

3.3 Juice Purity

Juice purity in November ranged from 88.69 (CoLk 12204) to 81.32 (LG 04439) under waterlogged and from 87.09 (CoLk 12204) to 80.36 (CoLk 04238) in control, while in December it ranged from 91.21 (D-6-13) to 72.33 (A-46-11) in waterlogged and from 88.54 (LG 03040) to 78.56 (BO 91) in control. In the month of February, however it varied from 90.63 (CoLk 12204) to 54.36 (CoJ 64) in waterlogged condition and from 91.05 (A-46-11) to 86.47 (LG 05020) in control condition. Across time periods, on average basis, juice purity varied from 86.64 to 83.90 under waterlogged and from 88.94 to 82.52 under control condition (Figure 1) (Table 4). Islam *et al.,* (2007, 2011) studied juice purity of three commercial varieties/nine clones in response to water-logging and found variability.

3.4 CCS% Juice

Under waterlogged condition, commercial cane sugar (CCS) in November ranged from 11.11 (CoLk 12202) to

Table 5: Changes in CCS% juice of sugarcane in response to waterlogging

Genotype	November			December			February		
	C	WL	%Increase/ decrease	C	WL	%Increase/ decrease	C	WL	%Increase/ decrease
CoLK 94184	9.85	10.75	9.17	10.41	10.15	-2.48	12.25	12.08	-1.37
BO 91	9.63	9.70	0.70	8.52	9.54	11.89	11.59	8.77	-24.27
CoS 767	9.64	10.04	4.20	10.73	10.32	-3.77	12.17	9.05	-25.68
CoJ 64	11.02	10.56	-4.15	11.42	9.86	-13.73	12.88	10.48	-18.64
CoS 97264	9.88	10.10	2.25	10.95	9.87	-9.88	11.86	11.18	-5.69
UP 9530	9.76	10.47	7.35	9.78	10.96	12.03	11.54	12.04	4.37
CoLk 12204	10.61	9.94	-6.31	10.55	11.22	6.38	12.55	9.72	-22.55
CoLk 12202	9.47	11.11	17.27	9.39	10.96	16.73	11.90	11.15	-6.28
CoLk 12206	9.75	10.79	10.66	9.83	9.07	-7.78	12.59	11.88	-5.64
CoLk 07201	8.82	10.43	18.29	10.03	9.67	-3.62	12.52	11.82	-5.56
CoLk 04238	8.91	9.59	7.66	9.26	8.46	-8.65	11.20	10.49	-6.38
LG 06605	9.61	9.47	-1.41	10.70	9.36	-12.52	11.38	9.13	-19.71
LG 04439	8.88	9.83	10.69	10.16	10.20	0.40	11.69	10.12	-13.41
LG 05350	9.10	10.62	16.76	9.36	11.03	17.88	10.87	11.90	9.47
LG 05020	8.30	8.29	-0.14	9.62	9.25	-3.78	10.33	10.64	2.97
LG 03040	10.04	10.76	7.10	10.98	7.67	-30.20	12.78	11.96	-6.43
A-46-11	8.49	10.41	22.59	9.96	6.57	-34.11	11.70	11.20	-4.28
B-44-12	8.46	10.17	20.25	10.20	10.28	0.83	12.06	10.52	-12.75
A-27-12	8.07	9.47	17.27	7.02	8.82	25.67	9.27	9.24	-0.37
D-12-9	8.65	9.53	10.07	9.25	8.76	-5.34	11.39	10.76	-5.51
D-6-13	8.36	10.98	31.38	9.86	11.67	18.33	11.46	10.91	-4.80
S 5085/11	8.96	9.33	4.20	9.01	10.20	13.28	10.35	10.35	0.03
S 5087/11	8.55	9.59	12.11	10.80	9.47	-12.31	12.63	10.86	-14.05
Mean	9.25	10.08		9.90	9.71		11.69	10.71	
SD	0.769	0.665		0.956	1.179		0.882	1.016	
SE±	0.160	0.139		0.199	0.246		0.184	0.212	
CV (%)	8.3	6.6		9.6	12.1		7.5	9.5	

C – control; WL – waterlogging

8.29 (LG 05020) and in control ranged from 11.02 (LG 05020) to 8.07 (A-46-11), while in December it varied from 11.67 (A-27-12) to 8.46 (CoLk 04238) under waterlogged and from 11.42 (CoJ 64) to 9.01 (D-12-9) in control. In February, it varied from 12.08 (CoLk 94184) to 8.77 (CoJ 64) in waterlogged and from 12.88 (CoJ 64) to 9.27 (A-27-12) in control (Table 5). On average basis, CCS% juice was comparatively higher in waterlogged than control in the month of November while it gradually decreased in December and February due to water-logging stress (Figure 1).

3.5 Reducing Sugars Contents

In November, under waterlogged condition, reducing sugars content varied from 0.253% (CoLk 12204) to 0.453% (LG 04439) and under control condition ranged from 0.371% (LG 05350) to 0.416% (LG 05020), while in December, it varied from 0.090% (CoLk 12204) to 0.449% (CoLk 07201) in waterlogged and from 0.133% (LG 05350) to 0.467% (CoLk 07201) in control. However, in February it varied from 0.117% (LG 05350) to 0.332% (CoLk 07201) in waterlogged and from 0.111% (CoS 767) to 0.351% (CoLk 04238) in control. Water-logging stress increased reducing sugars contents in the month of February while in November and December; it was relatively lower in waterlogged affected cane (Table 6, Figure 1). Increase in reducing sugars contents during later stage of water-logging might be due to higher activity of acid invertase enzyme indicating sucrose losses under stress condition. Jabber *et al.* (2005) reported different level of reducing sugar content using sugarcane clones and commercial variety.

Table 6: Changes in Reducing sugars contents (%) of sugarcane in response to waterlogging

Genotype	November			December			February		
	C	WL	%Increase/ decrease	C	WL	%Increase/ decrease	C	WL	%Increase/ decrease
CoLK 94184	0.411	0.397	-3.43	0.265	0.227	-14.23	0.208	0.173	-16.95
BO 91	0.405	0.341	-15.83	0.192	0.160	-16.58	0.127	0.261	105.88
CoS 767	0.391	0.293	-24.97	0.206	0.120	-41.55	0.111	0.200	79.02
CoJ 64	0.385	0.364	-5.43	0.209	0.177	-15.24	0.122	0.285	133.47
CoS 97264	0.401	0.277	-30.88	0.356	0.155	-56.42	0.170	0.157	-7.89
UP 9530	0.421	0.298	-29.36	0.437	0.140	-67.88	0.234	0.161	-31.28
CoLk 12204	0.398	0.253	-36.33	0.214	0.090	-58.14	0.140	0.136	-2.85
CoLk 12202	0.404	0.296	-26.70	0.353	0.144	-59.15	0.257	0.207	-19.38
CoLk 12206	0.383	0.385	0.31	0.449	0.226	-49.67	0.158	0.201	27.04
CoLk 07201	0.401	0.397	-1.19	0.467	0.449	-3.84	0.318	0.332	4.22
CoLk 04238	0.414	0.426	2.98	0.432	0.369	-14.52	0.351	0.294	-16.17
LG 06605	0.412	0.376	-8.72	0.343	0.227	-33.91	0.175	0.260	48.30
LG 04439	0.390	0.453	15.94	0.262	0.216	-17.49	0.166	0.160	-3.59
LG 05350	0.371	0.305	-17.85	0.133	0.111	-16.42	0.149	0.117	-21.40
LG 05020	0.416	0.441	5.97	0.263	0.248	-5.68	0.239	0.250	4.37
LG 03040	0.398	0.436	9.55	0.276	0.433	57.04	0.166	0.168	1.20
A-46-11	0.388	0.388	-0.06	0.258	0.443	71.81	0.151	0.142	-5.92
B-44-12	0.393	0.433	10.24	0.438	0.335	-23.41	0.218	0.247	13.47
A-27-12	0.388	0.416	7.26	0.418	0.324	-22.38	0.238	0.154	-35.15
D-12-9	0.385	0.444	15.54	0.374	0.236	-36.97	0.211	0.151	-28.71
D-6-13	0.402	0.391	-2.65	0.402	0.226	-43.81	0.289	0.178	-38.38
S 5085/11	0.399	0.319	-20.13	0.163	0.170	4.27	0.113	0.231	104.41
S 5087/11	0.406	0.363	-10.65	0.223	0.304	36.61	0.168	0.165	-2.07
Mean	0.398	0.369		0.310	0.240		0.195	0.201	
SD	0.012	0.060		0.103	0.108		0.065	0.058	
SE±	0.003	0.013		0.022	0.022		0.014	0.012	
CV (%)	3.1	16.3		33.4	44.8		33.6	28.8	

C – control; WL – waterlogging

3.6 S/R Ratio

S/R ratio was relatively higher in the month of November and lower in December and February harvested canes of waterlogged affected plants as compared to control on the basis of average (Table 7, Figure 1). Under waterlogged condition, S/R ratio ranged from 56.88 (CoLk 12204) to 28.49 (LG 05020) and in control it ranged from 42.91 (CoJ 64) to 31.73 (A-27-12) in November, while in December it varied from 184.70 (CoLk 12204) to 24.01 (A-46-11) under waterlogged and from 104.55 (LG 05350) to 25.63 (A-27-12) in control. In February, it varied from 146.26 (LG 05350) to 49.12 (BO 91) in waterlogged and from 157.21 (CoS 767) to 46.10 (CoLk 04238) in control. Decrease in S/R ratio after water-logging condition might be due to higher reducing sugar contents causing sucrose deterioration and drying of canes.

In present study, juice quality attributes, namely °Brix, sucrose% juice, juice purity, CCS% juice and S/R ratio, were relatively higher at early stage of cane ripening (in the month of November) under water-logging condition and decrease at later stage of waterlogged condition (in the month of December and February). Reports are available on sugar inversion and higher non protein N, reducing sugars and gums and decreased sucrose content in juice of flood affected sugarcane plants (Gomathi *et al.,* 2015, Dwivedi, 2016). Such imbalance resulted in poor juice quality and decreased sugar recovery. Cane deteriorates and increase in non sugars contents occurs due to longer duration of water logging. It has also been observed that canes of waterlogged areas start drying at a faster rate after receding of water in comparison to normally grown canes (Gomathi *et al.,* 2015). It is, therefore, revealed that sugarcane matures earlier in

Table 7: Changes in S/R ratio of sugarcane in response to waterlogging

Genotype	November			December			February		
	C	WL	%Increase/ decrease	C	WL	%Increase/ decrease	C	WL	%Increase/ decrease
CoLK 94184	35.75	39.62	10.81	59.62	66.52	11.57	84.43	99.98	18.42
BO 91	35.77	41.59	16.26	70.19	89.26	27.17	131.32	49.12	-62.59
CoS 767	37.27	49.88	33.83	76.95	127.25	65.35	157.21	66.79	-57.51
CoJ 64	42.91	43.85	2.21	81.26	83.68	2.97	151.20	52.96	-64.98
CoS 97264	36.61	52.83	44.32	45.96	96.89	110.84	100.50	102.54	2.03
UP 9530	34.92	51.52	47.54	32.81	113.54	246.11	71.03	107.81	51.77
CoLk 12204	39.02	56.88	45.77	72.50	184.70	154.75	128.54	102.27	-20.44
CoLk 12202	35.15	55.55	58.04	40.14	113.53	182.81	66.53	78.18	17.50
CoLk 12206	38.74	40.68	5.00	32.87	61.14	86.02	114.15	85.00	-25.54
CoLk 07201	33.41	38.52	15.32	32.19	32.69	1.57	56.50	51.14	-9.49
CoLk 04238	32.71	33.51	2.45	32.21	35.16	9.16	46.10	51.85	12.47
LG 06605	35.27	37.11	5.22	45.44	59.95	31.92	94.56	50.64	-46.45
LG 04439	34.00	32.15	-5.44	56.82	68.68	20.87	101.46	91.82	-9.50
LG 05350	36.49	50.85	39.37	104.55	143.21	36.98	105.74	146.26	38.32
LG 05020	30.22	28.49	-5.73	54.06	54.69	1.17	63.10	62.60	-0.79
LG 03040	37.63	36.25	-3.65	57.58	26.31	-54.30	109.98	103.51	-5.88
A-46-11	32.87	39.21	19.29	56.15	24.01	-57.24	111.94	114.13	1.96
B-44-12	33.26	35.05	5.37	34.54	45.15	30.74	79.71	62.04	-22.17
A-27-12	31.73	33.70	6.19	25.63	38.94	51.93	57.06	87.89	54.02
D-12-9	34.86	31.51	-9.63	36.86	55.42	50.37	77.42	103.75	34.01
D-6-13	31.90	41.14	28.98	36.49	73.63	101.75	56.94	89.83	57.76
S 5085/11	34.02	42.96	26.30	80.71	88.28	9.38	134.24	65.07	-51.53
S 5087/11	32.28	41.86	29.69	71.70	46.37	-35.32	107.64	95.25	-11.51
Mean	35.08	41.51		53.79	75.17		95.97	83.50	
SD	2.849	8.025		20.75	40.46		31.65	25.52	
SE±	0.594	1.673		4.327	8.436		6.599	5.320	
CV (%)	8.1	19.3		38.6	53.8		33.0	30.6	

C – control; WL – waterlogging

waterlogged areas (Gomathi and Chandran 2013), so cane should be harvested early after water recession so that maximum amount of sucrose can be obtained.

Findings obtained indicated wide variation among different genotypes for juice quality attributes. Highest decrease in CCS (12.17%), purity (13.02%) was observed in CoJ 64 and highest increase was observed in D-6-13 (14.97% and 6.21%) waterlogged affected plants over control. LG 05350 showed highest increase in sucrose (12.57%), Brix (8.41%) value while highest decrease was observed in LG 06605 (12.54 and 15.11%) under water-logging condition. Genotypes CoLk12202, CoLk12204, D-6-13, UP9530, CoLk94184 may be classified as water-logging tolerant genotypes based on juice quality attributes.

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RESEARCH ARTICLE

Struggle from Subsistence to Sustainability and Threat to Local Biodiversity under Changing Climate: A Case Study on Ladakh Folk Agriculture

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Abstract The traditional agroforestry system in Trans-Himalayan region is unique with very old history and the land-based economy in cold and arid tract, commonly known as Ladakh essentially comprised crop-tree/shrubs-livestock-man continuum. Families rely essentially on subsistence agriculture on the basis of vegetables and principal crops like naked barley, wheat and potato. Although subsistence-oriented production remains the economic mainstay, livelihood strategies have diversified in the light of growing geostrategic relevance and significant socio-economic changes. The present study was carried out 15 to find out the crop production systems and their constraints under the changing climate and energy budget of different cropsin Saboo village of Leh, having 623.7 ha land cover with 20.6% of cultivated land with artificially created soils in agroforestry systems and being maintained with repeated manure application. This practice compensate with loss of organic matter due to frequent erosion and nutrient uptake by plants as the region faces fast winds blowing at 40–60 km/h mainly in the afternoon and experiences the combined condition of both arctic and desert climate. Agroforestry as a sustainable alternative is suitable to traditional subsistence farming practices and provide flexible and affordable model which combines tree and crop species using a number of techniques. Wheat and barley although assumed very low energy input (29.2 and 25.4×10^5 kcal/ha/year, respectively) but from total energy point of view, staple crop production was found to be energy efficient. Small scale interventions by research institutes draw the attention of farming community to adopt for sustainable hike in productivity by saving-oriented energy.

Keywords Subsistence, Ladakh, Sustainability, *Cirsium arvense*, Energy budget, Biodiversity, Productivity

1. Introduction

The traditional farming systems of Tethys (Zanskar Valley) and Trans-Himalayan (Leh and beyond) are unique with more than 400 year old history. Agriculture and livestock husbandry sectors both together comprise land-based economy. Ladakh is a poor region with many diverse and often isolated rural groups, living across remote vastly different climatic and topographic locations. The Himalayan mountain range had a significant bearing on the climate of India and the current recession in available resources, sustainability recedes from subsistence farming situation. Since last many decades, Ladakh region has been dependent mainly upon subsistence agriculture and pastoralism. With the modernisation and doubling of its population from 70,000 in 1981 to 145,000 in 2011, farming sector of Ladakh region has not been properly investigated but significantly affected under the shadow of various livelihood options/avenues like tourism, government sectors and so on, leaving Leh with no more self-sufficient in food-grain production. Due to heavy snowfall around Ladakh valleys and declining trend in glacier recharge, that is by 21% (with glaciers less than 1 m^2 retreating faster) keep the region cut-off for almost seven months. But with the opening of Srinagar–Leh highway after

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1962, the process of modernisation became faster and new lifestyles, practices and social mores have pervaded the Ladakhi community against a backdrop of secular indigenous traditions and culture (Norberg-Hodge, 1991). On the other hand, families mostly rely essentially on subsistence agriculture based on principal crops like wheat, barley, peas and potato. Due to delayed and declined snow, recharge in glacierised water has shifted the date of sowing (delaying by 15–25 days) and stresses being posed by biotic and abiotic factors is an another challenge in the valley for attaining the sustainability. The art of crop production, which is as old as civilisation itself and its essential features have remained largely unchanged over the ages. Reckoning the 'needs' would assess the situation by developing policies in regard to the sustainable food security for the region. Altitude is the empirical factor dictating climatic and environmental conditions, allowing certain species to be grown using specific techniques. Energy projection for farmyard manure in traditional crop cultivation has been found to be 80–90%of the total energy cost; thus, traditional crop cultivation has been cited as more efficient in energy and economics (Nautiyal *et al*., 2007).

2. Methods and Materials

Bio-geographical survey was carried out by Regional Research Station, ICAR-Central Arid Zone Research Institute (CAZRI), Leh during 2013–2014 and 2014–2015 to find out the crop production systems and their constraints under the changing climate and energy budget of different crops in Saboovillage of Leh Valley of under Trans-Himalayan region. A survey was carried out for 40% households (112) selected randomly using questionnaire regarding food, fodder and vegetables grown and input–out thereon. The data collected from the village have been used to work out the energy budget of the crops grown and input and out were converted to caloric equivalents as reported by Mitchell (1979). Energy budget calculated separately for each crop and fodder. Being a cold and arid region, the temperature ranges between -40°C in winter and 35°C in summer. In general, the region has short, mild summer and long, cold winter. Mean precipitation ranged between 80 and 300 mm with potential evapo-transpiration to the order of 700–800 mm/year. The growing period varies from location to location, ranging from 80 to 150 days. The soil of Saboo village is gravelly and sandy to sandy-loam in texture and medium to medium-high in organic matter and pH 7.85, electrical conductivity(µS/cm) 141.7 and total dissolved solids (ppm) 66.9, Sodium Adsorption Ration 1.47, soluble sodium percentage 35.1 and residual sodium carbonate (mel^{-1}) 0.37 and poor water holding capacity. More than 90% soils are low in phosphorous and high in potassium.

3. Results and Discussion

3.1. Rationale

Now this region is in transition characterised by population increase, general economic growth, consistent human development, raised in life expectancy, literacy rate and GDP per capita. With the import of large quantities of food grains in addition to sugar and kerosene from the plains every year in Leh and distributed at subsidies prices/ at free to the local population by public distribution system (PDS) and resulted in lack of interest in agrarian economy as also the traditional subsistence agriculture systems have been progressively and indirectly influenced with the socio-economic dynamics with new off-farm income opportunities with more and more employment avenues open to the local people in the form of booming tourism industry, government jobs, as wage labourers or soldiers. The economic limitations of the region serve to demonstrate the innate interconnectivity between sustainable agriculture and sustainable development. According to Thomson and Metz (1998), the concepts of food self-sufficiency and food security differ, whereas food self-sufficiency is linked to an overall perspective on self-reliance development, that is an auto-centric approach, food security taking into account commercial imports, international specialisation, comparative advantage and food aid as possible sources of commodity supply. Subsistence food-grain production in Ladakh is more likely to be used for family consumption than cash income. Modifications over the past three decades of the land use system in Leh District have also reflected the changing in household strategies and local dietary habits. Moreover, subsidised rice supplied through the PDS is increasingly replacing locally grown barley as the main staple of the summer diet (Dame and Nüsser, 2011).

3.2. Agriculture in Ladakh: A Unique Farming System Approach and Constraints

People, through the ages, have developed need-based and location-specific indigenous technologies for enhancing productivity and the concept of quality seeds is well known, but the agronomic practices have not so far been standardised for improved seeds being introduced. Small-scale agriculture is still the predominant characteristic of the Ladakh. It has still great role in the economy of the region. Total number of villages in Ladakh is 244 and 80% land holding in the region are 2 ha or less. The agricultural economy is based on production of barley, wheat and peas and rearing livestock especially, yak, cows, dzos (yak – cow cross breeds), sheep and goat. Thus, essentially the farming systems are mixed livestock – crop based. Such farming systems are of very small scale and adapted to a unique environment. The land is irrigated by a system of channels which funnel water from the ice and snow of the mountains. At lower elevation temperate, fruits are grown in pockets, whereas the high altitudes are the preserve of nomadic herders. Apricots and *Pashmina* are important export items. Currently, largest commercial agricultural products are vegetables-sold in large amounts to defence establishment and as well as in local market. Production remains mainly in the hands of small land owners, who work on their own lands, often with help of migrants. Farming system in Ladakh region feature interaction among the following five components:

- crop fields,
- a private land support system (fruit and multipurpose trees in an around crop fields),
- community woodlands and pastures,
- livestock and
- man in uniquely specific socio-, economy-cultural

Researches show that pre-chemical input agricultural yields in Ladakh were very good by international standards. The traditional system attained these yields without the use of artificial fertilisers, by a careful system of recycling essential crop nutrients through human and animal consumers (i.e. use of manure and night soil), supplemented by rotational cropping with peas to provide additional nitrogen. Livestock plays an essential part in the agricultural cycle, especially for the production of dung, for ploughing and threshing. Families also tended small vegetable gardens with crop fields and sufficient number of apple or apricot trees as per the land size. As a result of these efforts, Ladakh produces enough food for the entire year per family for their livelihood only. Surplus grain was being traded previously for salt, tea or jewellery and made into gallons of *chhang*, the local beer. By almost any measure, its traditional culture was extremely successful. It was ecologically sustainable and just as important, people were supported by strong family and local self-governance (community bonds) that provided a deep sense of psychological security. Still entire agriculture and new plantation are irrigated either by summer snow melt from high mountains or channels of many kilometres long driven through river belt, ferry the water to the villages via small channels and an equally finely tuned social system of determining the distribution to every resource poor farmer of small terraced fields locally known as *Chhures*. Between the fields, mini meadows of a rich assortment of grasses and herbs add their colour (mauve of lavender, yellow of a local clover) and winter fodder to the equation. Wood is produced with coppices of willow and poplar in their silvi-pastoral system along with some fruit and nut trees at lower altitudes (Figure 1). Diversified and integrated systems are perhaps the most relevant one for this investigation. Here at Ladakh region, diversified systems consist of components such as crops and livestock that co-exist independently from each other as quite independent unit. In this case, the mixing of crops and livestock primarily serves to minimise risk and to recycle resources efficiently. Grazing of livestock under trees such as salix, poplar is a form of crop–livestock integration that is often found in Ladakh. Animals graze under trees or on stubble of harvested fields, they provide draught and manure for improving soil fertility (Figure 2). On the other hand, climate change is rendering native species more vulnerable to weeds either directly or indirectly (Figure 3).

Figure 1: Silvi-pastoral system as integrated part of integrated farming

Figure 2: Wheat field applied with manure mixed with night soil

Figure 3: Weeding in cereals is not followed and crop infested with weeds is additional source of surplus fodder for livestock for winter period

Import of fodder and crops seeds from one place to another helps in stabilising new species that are violent in nature due to invasiveness. Even the residents of much higher altitude (>4,500 m) who are traditionally nomads and solely dependent on livestock rearing for livelihood have started washing their hands off from this sector. Elevated CO_2 commonly stimulate the growth of roots and rhizomes and weeds like *Cirsium arvense* and *Phragmites australis*, having strong enough move with allelopathic potential, have established to new areas and affect native vegetation on frost heaves (Figure 4, 5, 6).

3.3. Initiatives to sustain Agricultural Productivity

Agricultural development in Ladakh is driven by the Govt. of Jammu and Kashmir, which in turn directed by Ladakh Autonomous Hill Development Council (LAHDC) of Leh and Kargil. In fact, LAHDC in both the districts has the final say in all plans relating to agriculture and other developmental sectors. Agricultural developmental projects undertaken by district Department of Agriculture, Leh include the introduction and subsidising of high yielding variety seeds, chemical fertilisers, mechanisation (such as threshing machine, mechanical tillers), irrigation and various export-oriented produce such as floriculture, vegetable production and mushroom production. The aim of these programmes is both to increase the volume of crops in the region and create livelihood options through substantial income for Ladakhi farmers. No doubt, these programmes increased the agricultural productivity in the region but still-small scale scientific interventions are needed to be incorporated in packages of practices for easier adoption to improve the sustainability under changing climate. For example, in Leh district between 1981–1982 and 1995–1996, the crop productivity increased from 16,000 to 22,000 t. However, this high input agriculture has many negative impacts on environmental and social scale. Institutes like CAZRI, RRS, Leh and DIHAR, SKUAST (K) carry out exhaustive research to sustain the productivity.

Figure 4: Group of frost heaves with desired flora of pasture

Figure 5: Frost heaves infested with *Cirsium arvense*

Figure 6: Disrupted frost heaves are deteriorating pasture

CAZRI, Leh has been successful in making farmers aware about the weed menace in staple and vegetable crops and increasing productivity by 10–15% as indicated in onion in which amongst the varieties, Brown spinach performed significantly well in terms of bulb yield (13.41 t/ha) as compared to Red coral (12.39 t/ha) and Liberty (11.88 t/ha). Although amongst the herbicides, hand weeding has yielded significantly higher bulb yield of 15.87 t/ha which was at par to pendimethalin (38%) (14.99 t/ha), pendimathalin (30%) (14.43 t/ha) and oxyfluorfen (14.51 t/ha) as compared to weedy check (2.87 t/ha). Although in case of potato, maximum tuber yield was recorded under black polythene mulch (21.65 t/ha) followed by hand hoeing 30 days after sowing (DAS) (19.13 t/ha), Metribuzin (18.81 t/ha) and earthing-up at 30 DAS (18.89 t/ha) as compared to weedy check (5.70 t/ha). It was recorded that more yields from potato can be expected if weeds are suppressed using black polythehene mulch between the rows of potato followed by hand hoeing and earthing-up during the critical period of crop–weed competition. These efforts could reduce the gap indicated in Table 1 by Mishra *et al* (2010).

For the last three decades, Ladakh has been increasingly exposed to modern influences brought on largely by tourism and economic 'development'. The results have been mixed

Table 1: Total requirement and local production of vegetables in Ladakh (Mishra *et al.*, 2010)

Vegetables	Requirement (mt/year)	Production (mt/year)	Gap
Vegetables	17,000	8,160 (>30 TYPES)	8,840
Potato	13,000	8,450	4,550
Onion	5,400	1,080	4,320

Table 2: Land use pattern in village Saboo, Leh

Land use	Area (ha)
Cultivated land	128.5 (20.6%)
Orchards*	0.7 (0.1%)
Woodlot (CPR)**	177.2 (28.4%)
Permanent woodland	21.9 (3.5%)
Land put on non-agricultural use	23.0 (3.7%)
Land not available for cultivation (including permanent fellow)	272.4 (43.7%)
Total	623.7

* Orchards of Local apricot and apples
** Common property resources (CPR) also includes Monastery land for livestock use

at best. In the capital, Leh, a range of consumer goods is now available in most households have a television and most men and children wear Western clothes. For many Ladakh is, life is becoming less physically demanding. However, modernisation is undermining the very foundations of the traditional culture and giving rise to a familiar pattern of devastating environmental and social problems. Subsidised food trucked into Ladakh over the Himalayas is often cheaper in the bazaar than food grown five minutes' walk away. Local agriculture now seems 'uneconomic' and many Ladakh is are abandoning their farms in pursuit of paid jobs in Leh or outside Ladakh. Children who once learned from relatives and neighbours how to grow barley at 14,000 ft (4,300 m) and how to build a house, to tend animals and recognise useful plants and herbs, are gradually stop thinking about these values due to ever increasing Western culture. Villagers are now flocking to Leh in search of jobs available in rapidly expanding tourism sector and large presence of armed forces, leading to a population explosion in the city. In the 'modern' economy being created by global economic forces, women are increasingly marginalised. Traditionally, women in Ladakh enjoyed remarkably high position, being at the centre of every household and involved in all decisions. The paid-jobs available now are generally filled by men, while the women are left behind to do the agricultural work that was once shared by both.

Due to limited period of cropping season in the region, rotation has been confined to cash crops rather promoting legumes for improving fertility in a majority of the situations in small land holdings. Though cash crop production is not energy efficient; however, perhaps in terms of economics, cash crops provide good revenue to the farmers. In Saboo village, the total area is 623.7 ha, of which cultivated land only 20.6%. CPR of the village is accounted to be 28.4% of the total village area (Table 2). In all the surveyed villages, majority of area (75%) is under vegetable crops as compared to cereals like wheat/barley (25%) (Tables 3 and 4 and Figure 7). It was noted that the efforts/energy (unit in each case= value × 105 kcal/ha/year) being put to grow vegetables are significantly more than the total output and the situation is just reverse in case of cereals. Survey also revealed that even though realising the need of weed management, critical periods of crop–weed competition is always overlooked due to higher labour cost and that has resulted in direct yield loss (40% approx.)by crop lodging, reduced crop quality and create direct access to various insects and diseases on crops/fruit orchards under the pressure of different weed canopies.

The land use pattern of the study village Saboo is as Tables 2–4.

It is apparent from Table 4 that in all the crops of village, manure was a major input which accounted 8.2×10^5kcal/ha/year for barley and 217.5×10^5kcal/ha/year for potato. Total energy input was highest for potato followed by pea. Although minimum energy input was recorded for staple crops like barley and wheat. It was very interesting that farmers sow wheat and barley and do not carry out any intercultural operations like weeding and weeds are treated as additional fodder as shown in Figure 4. The energy input of animal and human was also very low in these crops. But interestingly, it was although noted that these crops required

Table 3: Crop productivity of village Saboo

Crop	Seed Input (kg/ha)	Yield Output (kg/ha)		Manure Used (kg/ha)
		Grain	Straw	
Barley	200.0	528.0	1,320.0	200.4
Wheat	283.6	819.5	2,104.8	242.0
Pea	260.0	1,350.0	450.0	3,960.0
Tuber (mainly potato)	1,720.0	12,900	2,150.0	5,340
Other vegetables*	2.6	612.2	895.5	3,900.0
Onion	2.6	342.5	–	3,512
Alfalfa (fresh)	26.4	9,784.0	–	3,320.8

*Other vegetables includes spinach, cabbage, cauliflower, tomato, carrot, radish and chilli

Table 4: Energy budget of different crops in Saboo

Particular	Barley	Wheat	Pea	Potato	Onion	Other vegetables	Alfalfa
Input							
Human labour	2.4	3.0	6.1	0.4	1.5	2.2	1.8
Dzo labour	3.1	4.3	6.9	2.2	2.4	1.6	0.2
Seed	7.7	11	10.6	16.2	0.1	0.1	1.0
Manure	8.2	9.9	161.3	217.5	143.1	158.9	135.3
Fertiliser	4.0	1.0	–	3.0	3.0	2.0	–
Total	25.4	29.2	184.9	239.3	150.1	164.8	138.3
Output							
Arable crop/commodity yield	20.4	31.6	54.9	121.5	36.8	24.9	92.2
Crop straw/vegetable residue	43.9	70	4.2	20.2	3.3	8.4	-
Total	64.3	101.6	59.1	141.7	40.1	33.3	92.2
Output: input ration	2.5	3.5	0.3	0.6	0.3	0.2	0.7

very low energy input but from total energy point of view, staple crop production was found to be energy efficient. However, the energy input of pea, potato, onion, other vegetables and alfalfa was much higher than the output. Similar findings were also reported by Tewari *et. al.*(2003). Percentage of total cultivated area

On the other hand, interaction of climate change with the processes like land management and introduction of new cultivars, there is likelihood to turn the native/non-native into invasive species that may enable the sleeper weeds becoming more active and weedy. Under the pressure of abiotic stresses, challenges are ahead in the form of biotic stresses (problems like pests, increase pollinators, breeding cycles, infiltration of invasive weeds) and wake up for sleeper local biodiversity of Ladakh region. On the other hand, climate change is rendering native species more vulnerable to weeds either directly or indirectly. Import of fodder and crops seeds from one place to another help in stabilising new species that are violent in nature due to invasiveness. Even the residents of much higher altitude (>4,500 m) who are traditionally nomads and solely dependent on livestock rearing for livelihood have started washing their hands off

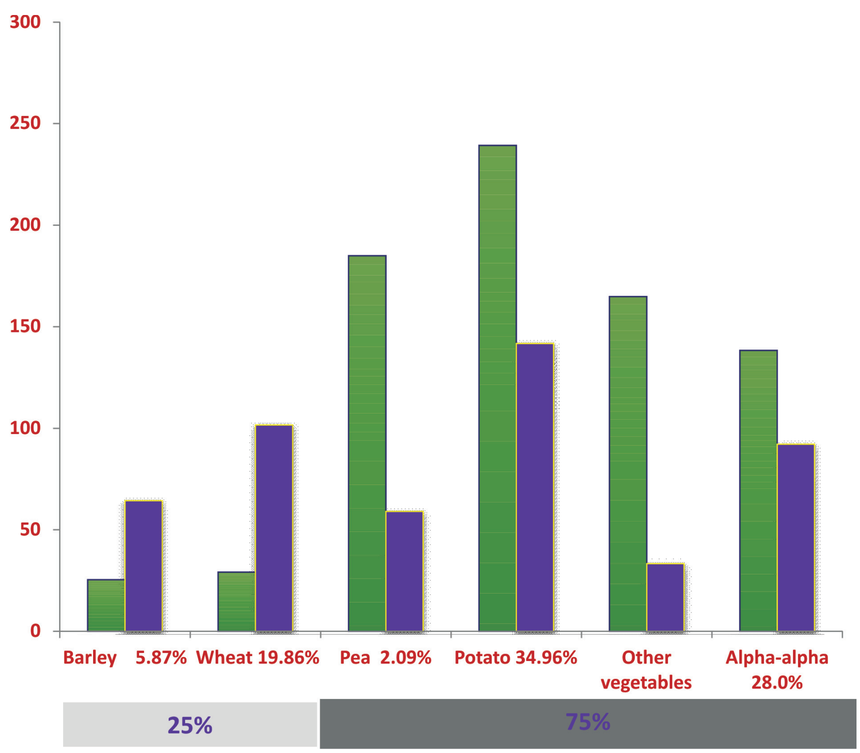

Figure 7: Energy budget of different crops in village Saboo (unit in each case= value×10^5 kcal/ha/year)

from this sector. While in this region, elevated CO_2 commonly stimulate the growth of roots and rhizomes and weeds like *C. arvense* and *P. australis* having strong enough move with allelopathic potential have established to new areas and affect native vegetation. During the survey made to understand the grassland ecology of Tsangse part of Changthang Valley revealed tiny rosette, sedges and other cohesive group of vegetation such as *Kobresia* spp., *Carex* sp., *Leontopodium pusillum, Astragalus* spp., *Triglochin* spp., *Puccinellia* spp. and *Glaux maritima*. Although these pastures are dominated by one of the smallest *Cyperaceae* endemic, growing not taller than 2 to 3 cm, covering more than 90% and consist of only 8 to 10 mostly tiny rosette species (e.g. *Thalictrum alpinum*, *Potentilla saundersiana*, *Aster flaccidus*, *Primula walshii*, *Pedicularis* spp., *Cortiella caespitosa*). The open humic soil is colonised by rosettes (e.g. *Lancea tibetica*, *Lagotis brachystachya*, *Potentilla bifurca*, *Przewalskia tangutica*, *Persicaria glacialis*, *Lasiocaryum densiflorum*). Invasion of *C. arvense* in pastoral system posed allelopathic influence on these rosette (Raghuvanshi *et al.*, 2017). It was noted that *Cirsium* stand was recorded where these native species (on which flocks depend) were absent. Fast depletion of natural resources which include the major concern of these days, that is receding of glaciers (21% decrease glacierized area Indian Himalayan Region) purportedly due to effect of climate change. Impact of alarming situation of global warming in cold and arid region likely to reduce the amount of snowfall, reducing the water flow in snow-fed rivers during the summer months, thereby affecting agriculture production, rangeland coverage, reduced forest cover and livelihoods of cold desert communities. All four dimensions of food security are predicted to be affected by changing climate: food availability, food accessibility, food utilisation and food system stability. Not everyone has same capacity to adapt to changing climate. Looking to the livestock, animal husbandry is in general state of decline in whole Ladakh region. Now with the entrance of new research interventions, the process of struggle from subsistence to sustainability has gradually originated.

4. Recommendations

For sustainable agriculture and food security in Ladakh, it is the need of hour to intensify the production of main staple crops barley, wheat, newly introduced maize as cereals and alfalfa as fodder with the introduction of new cultivars and fodder maize as they are source of very good income for Ladakhi farmers and need for sustainable agriculture in the Himalaya, suggesting agroforestry as a potentially successful and apt method for alleviating poor productivity, land stability and water supply. Small-scale interventions by research institutes draw the attention of farming community to adopt for sustainable hike in productivity by energy-oriented saving. Further, protected cultivation is one of the most viable options for the region. Already polyhouses have become very popular in the cold and arid parts of Ladakh region, which holds almost 70% of the total area under polyhouses in India. Considering the productivity potential of Ladakh region and impacts of climate change on the Himalayan ecosystem, concerted efforts are to be taken for cross-sectoral planning improving the agricultural lands in mountain landscapes; promoting local action, need to harmonise with national policy/programmes/imperatives; distinction of forest and non-forest areas; reduced emissions, black carbon and other non-CO_2 gases as mitigation strategy; investment and capacitation for community innovation for adaptation and better understanding local experiences/strategies; linking community to science; learn from 'failures'; database to enable informed agriculture; ecological forecasting to minimise losses and/or damages; and augment traditional knowledge on climate variability and coping-up strategies to help formulating strategies to achieve climate resilience in agriculture, particularly in the biodiversity rich, ecologically fragile, Indian Himalayan region.

In Ladakh region, the supply of required food grain will be probably assured with a mix of local grain produced and lessening imported food grain, but the ratio between the two quantities will be reduced only when the small scale interventions are integrated with production packages of resource poor farming practices.

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RESEARCH ARTICLE

Soil Properties in Response to Different Plant Community Structures in Tropical Moist Deciduous Forest from Northern India

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Abstract We selected Katerniaghat Wildlife Sanctuary in which five plant community structures (PCSs) were identified namely dry miscellaneous forest, sal mixed forest, teak plantation, lowland miscellaneous forest and savanna whose response in physical, chemical and microbial soil properties were assessed. Response of PCSs on soil pH, electrical conductivity and soil organic carbon (SOC) were although insignificant, but soil texture, bulk density, soil temperature, water-holding capacity, total nitrogen (N_t), available phosphorus (P_{av}), potassium (K), microbial biomass carbon (MBC) were significant. Principal component analysis revealed that in dry miscellaneous forest and sal mixed forest, SOC, MBC and K were dominant soil properties and N_t was principal component of lowland miscellaneous forest; while, soil pH and P_{av} were identified as principal components of savanna ecosystem. The study concluded that different PCSs developed on a large tract of the same landscape changed their soil properties according to species association and niche formation.

Key words Plant community structures, Protected area, Principal component analysis, Seasonal variation, Soil properties, Species association, Tropical Moist Deciduous Forest

1. Introduction

Protected Areas (PAs) have been established throughout the world to protect natural habitats and to conserve the biodiversity within these habitats (Chape *et al.*, 2005). PAs play important roles to maintain existing populations of tree species and to reduce extinction rates of endangered, threatened wildlife species and indigenous communities (Villamor, 2006). The PAs are repositories of biodiversity in a biogeographic unit (Dhar *et al.*, 1997). PAs are the cornerstone of conservation efforts (Cantu-Salazar and Gaston, 2010), but land-use changes can trigger habitat loss and fragmentation outside PAs and may affect ecosystem processes within them (Hansen and DeFries, 2007). Analysing land use changes in and around PAs is therefore critical for assessing the effectiveness of this common biodiversity conservation strategy.

Soil is the substrate of all living organisms in the terrestrial ecosystems, which contains nutrient reserves and supports many biological processes in vegetation development. Forest biodiversity directly depends on abiotic factors like forest soil and microclimate variables, which have vital roles to sustain forest biodiversity (Pandey *et al.*, 2002). Soil quality may be affected by plant association because this may cause alterations in soil physical and chemical properties and even in soil biotic community (Caravaca and Masciandaro, 2002) which, in turn, affects land productivity (Sanchez-Maranon *et al.*, 2002; Singh *et al.*, 2012). The importance of species composition and key factors which broadly control structure of tropical forest communities remains enigma in community ecology (Bell, 2005); whereas, in case of forest structure and soil properties there is ample evidence that species distributions affect the soils and habitat factors at landscape level. Hence, under natural conditions it is very difficult to estimate reliably the influence of vegetation on soil properties. However, the

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magnitude of this influence can be evaluated. There are some studies in which vegetation and soil properties were collated (Yadav, 1968; Islam and Weil, 2000; Tripathi and Singh, 2005; Zornoza *et al*., 2008; Mishra *et al*., 2013b) but these are not sufficient to describe the integrated effect of microclimate variations with plant functional types on the soil properties. The evaluation of soil properties under the diverse tree cover is important to understand the role of different tree species on soil differentiation (Sharma and Sharma, 2004).

The high local diversity of tropical forest communities poses a unique challenge for testing plant community structures (PCSs) working theories based on soil resource availability. We have already studied the effect of abiotic factors in under story vegetation composition (Mishra *et al*., 2013a); role of soil properties in different forest in northern India (Mishra *et al*., 2013b) and seasonal variation in microclimate in different forest communities at Katerniaghat Wildlife Sanctuary (KWLS) (Behera *et al*., 2012). Now let us examine the soil factors such as texture and chemical and biological factors, which are affected by the change in PCSs. We have identified soil principal component at five different PCSs in KWLS. The objectives of the present study were: (i) to assess the soil physical, chemical and biological properties in different PCSs and (ii) to characterise the response of PCSs and soil principal components along the species composition.

2. Materials And Methods

2.1 Description of the Study Area

Study site KWLS constitutes a moist deciduous forest in the Upper Gangetic Plains in Bahraich district of Uttar Pradesh, India. It is situated between 27°410–27°560N and 81°480–81°560E with elevation ranges from 116 to 165 m along the southern border of Nepal. The forests spread in 40 km length and 10 km width with an area of 440 km^2. KWLS is one of the PAs, which was established in year 1975. In this sanctuary, we have identified five different PCSs such as dry miscellaneous forest, sal mixed, teak plantation, low land miscellaneous and savanna (Behera *et al*., 2012). The whole area is subjected to the typical climatic variations of the plains of northern India with their extremes of heat and cold. The winter nights are very cold, foggy and heavy dew falls regularly. Frosts occur generally in January. The nights remain cool and dew falls until late in the spring, the hot weather commences from April and extends until the rains break towards the end of June. Monsoon season is followed onwards until September. An average annual rain fall of about 1,300 mm was recorded during last five years (2008–2013). The different forest types constitute different microclimates (Behera *et al*., 2012) which indirectly influence many soil properties. In the study area dry miscellaneous forest and sal mixed forest were old natural forests having high density and diversity in each forest strata whereas lowland miscellaneous forest was dense and highly diverse forest type. Teak plantation was established before it was declared a PA sanctuary, dominated by 90% teak plants. Savanna ecosystem distributed in edge of forest near Geruva river.

2.2 Forest Composition

Five PCSs of the sanctuary area were surveyed using a stratified random sampling technique. The size and number of quadrates were determined using the species area-curve (Misra, 1968). Phytosociological attributes of each species were studied by randomly laying of 160 quadrats of 20 × 20 m size, for trees (≤5 cm diameter at breast height) and 220 quadrats of 5 × 5 m for shrubs and 220 quadrats of 1 × 1 m for herbs in the each selected forests. Frequency, density and basal area were calculated following Misra (1968). Relative frequency, relative density, relative dominance and importance value index (IVI) for individual tree, shrub and herb species in each forest community were calculated according to Cottam and Curtis (1956). Based on IVI dominant plant species of five PCSs were segregated among all individuals. Plant Voucher specimens collected from different forest communities were identified from herbarium of NBRI-CSIR and regional flora. Detail study of structure and tree species composition of five PCSs are available in our earlier publication (Behera *et al*., 2012) and given in Table 1.

2.3 Soil Sampling and Analysis

Soil samples were collected seasonally (in February for late-winter season; LW and in September for post-monsoon season; PM) from two soil depths 0–15 cm and 15–30 cm in 2011. All soil samples were collected randomly from three permanent plots of 5 m × 5 m size in each identified plant communities. Three composite samples were drawn from each plot by excavate soil core monoliths (10 × 10 × 15 cm). Soil samples were gently sieved through a 2 mm mesh to remove stones, roots and large organic residues, sealed in plastic bags and stored at room temperature for analysing soil properties. Soil texture was analysed using Hydrometer method (Bouyoucos, 1962). Water-holding capacity (WHC) was measured gravimetrically by weighing water-saturated and oven-dried soil samples (24 h at 105°C) with the help of Keen's box method (circular brass boxes with perforated base) (Black, 1965). Bulk density (B_d) was determined by measuring the weight of dry soil per unit volume with the help of pycnometer (specific gravity) bottles. Soil pH and electrical conductivity (EC) were determined in 1:2.5 soil: water suspension by the electrodes of Thermo Orion ion metre (Thermo Fisher Scientific Inc, Waltham, MA USA). Soil organic carbon (SOC) was determined by dichromate oxidation and titration with ferrous ammonium sulphate (Walkley and Black, 1934). Soil total nitrogen (N_t) was analysed by micro-Kjeldahl technique (APHA, 1985), available phosphorus (P_{av}) was estimated by phosphormolybdic blue colorimetric method and potassium (K) was measured by flame photometry (Jackson, 1967). Microbial biomass carbon (MBC) was estimated by chloroform fumigation direct extraction method (Vance *et al*., 1987).

2.4 Statistical Analyses

Data were subjected to two-way analysis of variance using the software package 'SYSTAT-9.0' version (Systat Software, Inc., San Jose California USA) to compare the effects of PCSs, on physical, chemical and biological properties of soil. Values of soil properties that differed at $P \leq 0.05$ and $P \leq 0.01$ were considered significant trends. Annual means of each parameter were used for multivariate analyses, that is five PCS_S and 13 soil parameters were used for Cluster Analysis and Principal Component Analysis (PCA). Cluster Analysis and PCA were assayed by software PAST 3.5, Natural History Museum University of Oslo (Hammer *et al*., 2001) and correlation done by SPSS 16 (Chicago, SPSS Inc.).

3. RESULTS

3.1 Floristic Compositions

Structure and composition of different PCSs of KWLS are presented in Table 1. *Mallotus philippensis, Syzygium cumini, Shorea robusta, Lagerstroemia parviflora, Ehretia laevis* were dominant species of dry miscellaneous forest. *Shorea robusta, Mallotus philippensis, Terminalia elliptica* and *Syzygium cumini* were predominant tree species of sal mixed forest. Teak plantation was homogeneous, mainly dominated by *Tectona grandis*. Lowland miscellaneous forest was wet land PCS dominated by low land species *Mallotus philippensis, Ficus hispida, Mallotus nudiflorus* and *Acacia catechu*. *Bombax ceiba, Sterculia villosa, Lannea coromandelica* were the dominant species in savanna.

3.2 Characteristics of Soil Properties in Response of Plant Community Structures

Soil physical properties of different PCSs were significantly different in two seasons (Figure 1 and Table 2). The soil textural class of all the PCSs was sandy-loam in which of dry miscellaneous forest, teak plantation and savanna had higher sand % (53.2 ± 1.6; 71.4 ± 6.3 and 63.5 ± 1.1) whereas (48.1 ± 3.2) silt particles were relatively high in sal mixed forest community and clay particles in lowland

Table 1. Description of the selected PCSs successional structure in Katerniaghat Wildlife Sanctuary

PCSs	Age	Alt	Lat	Long	T	S	H	C	T	D	B	Dominant Plant Vegetation
DMF	80 year	147 m	28°15.621″	81°12.259″	58	46	62	6	172	813	62.32	*Mallotus phillipensis, Shorea robusta, Syzygium cumini, Lagerstroemia parviflora, Ehretia laevis*
SMF	80 year	176 m	28°13.885″	81°16.998″	35	39	41	4	119	793	55.34	*Shorea robusta, Terminalia allata, Mallotus phillipensis, Syzygium cumini*
TP	50 year	168 m	28°13.416″	81°12.992″	25	42	46	2	115	765	77	*Tectona grandis*
LMF	80 year	96 m	28°13.020′	81°12.383′	51	42	62	9	164	740	50.3	*Mallotus phillipensis, Ficus hispida, Ficus glomerata, Trewia nudiflora, Acacia catechu*
SAV	100 year	125 m	28°19.332′	81°08.102′	15	19	46	0	80	350	51.7	*Bombax ceiba, Sterculia villosa, Dalbergia sisso, Lannea coromandelica, Acacia catechu*

PCSs = plat community structures, DMF =dry miscellaneous forest, SMF = sal mixed forest, TP = teak plantation, LMF = low land miscellaneous forest, SAV = savanna ecosystem, D = density and B = basal cover, TSR = total species richness.

Table 2. Results of two-way analysis of variance in physical properties of the soils under five PCSs, two seasons and two soil depths. Soil physical properties (annual means with standard deviations) in five different PCSs. *F* and *P* values from two-way ANOVA applied to each parameter against season and depth are reported in the last line

PCSs	Texture Class	Sand	Silt	Clay	WHC	B_d (g/cc)	ST (°C)
DMF	Sandy silt loom	53.17 ± 1.45	12.1 ± 1.19	34.4 ± 5.9	46 ± 17.2	1.32 ± 0.54	17.2 ± 4.0
SMF	Silty loom	19.33 ± 1.37	48.1 ± 3.2	32.57 ± 1.16	46 ± 7.15	1.29 ± 0.3	21.1 ± 5.2
TP	Sandy soil	71.43 ± 6.26	6.17 ± 1.08	22.4 ± 2.25	48 ± 11.1	1.4 ± 0.64	21.3 ± 4.9
LMF	Clay loom	41.56 ± 2.24	12.07 ± 1.88	46.37 ± 2.21	46 ± 10.2	1.21 ± 0.18	19.4 ± 6.2
SAV	Sandy soil	63.53 ± 1.13	8.07 ± 1.11	28.4 ± 5.25	40 ± 7.3	1.39 ± 0.82	23.1 ± 6.9
P	–	0.001	0.001	0.001	0.004	0.001	0.001

PCSs = plant community structures, DMF = dry miscellaneous forest, SMF = sal mixed forest, TP = teak plantation, LMF = low land miscellaneous forest, SAV = savanna ecosystem, WHC = water holding capacity, B_d = bulk density and ST = soil temperature.

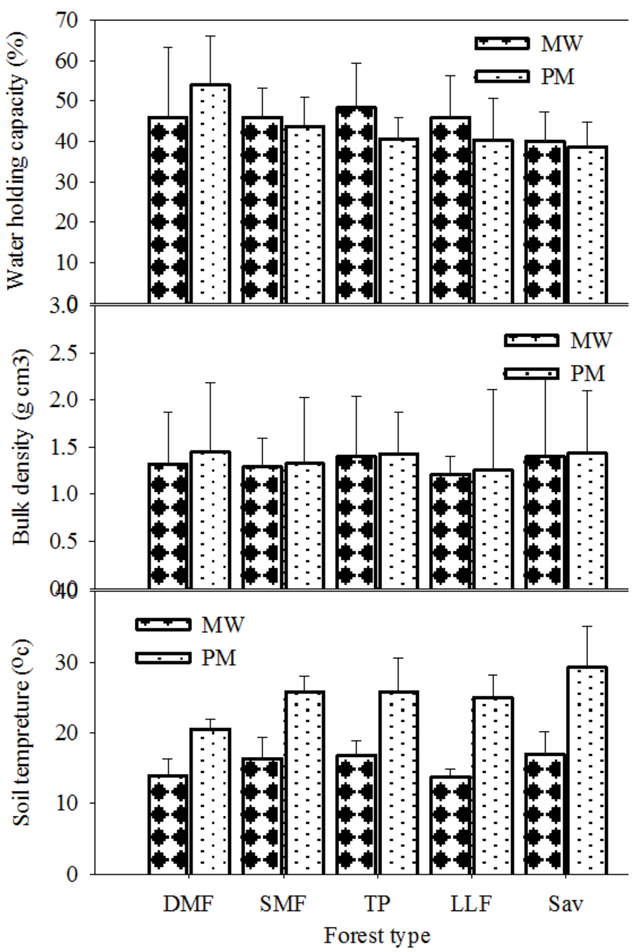

Figure 1. Mean and standard deviations of physical properties of the soils at five different plant community structures (PCSs) in Ketarniaghat wildlife sanctuary in two seasons. DMF = dry miscellaneous forest, SMF = sal mixed forest, TP = teak plantation, LMF = low land miscellaneous forest, SAV = savanna ecosystem, LW = Late-winter season and PM = Post-monsoon season

miscellaneous forest (46.4 ± 2.2). Soil WHC ranged between 40 and 48.33 % with highest values in teak plantation and lowest values in lowland miscellaneous forest. The effect of season was significant on WHC which decreased significantly in PM in comparison to LW. Soil B_d and soil temperature (ST) both were significantly different among the PCSs, whereas ST varied significantly in different seasons.

The interaction of PCSs and season on soil chemical properties (EC, SOC, N_t, P_{av}, K) and soil MBC was significant (see Figure 1 and Table 3). Soil pH and EC were influenced significantly by PCSs. dry miscellaneous forest consisted of relatively high SOC and sal mixed forest, lowland miscellaneous forest, savanna were more or less similar whereas, teak plantation was lowest in SOC. The C:N ratios did not vary among the forest types. N_t content was high under lowland miscellaneous forest compared to levels in the other forest soils. Soil K and P_{av} were significantly higher in dry miscellaneous forest and sal mixed forest forest soils, whereas lowest in plantation forest teak plantation (Table 3), except for soils from savanna showing higher values P_{av} content. The values of MBC changed significantly with the seasons in all the plant communities.

Soil of lowland miscellaneous forest represents its wetland nature whereas soil properties of teak plantation and savanna have lower nutrient status compared to other PCSs. In cluster analysis, soil properties of savanna were exceedingly different from others (Figure 2). Three principal clusters were generated on the basis of soil properties: first dry miscellaneous forest and sal mixed forest, second lowland miscellaneous forest and teak plantation making a similar group and third one was savanna which shows highest distance than either two groups. PCA analysis was done with 13 soil properties in five PCSs. PCA biplot shows similar results as cluster analysis (Figure 3). The variance of the first three PCA axes accounted for 40.4%, 28.2 % and 18.5 %. The first two axes covered 70% variance so here we used first two axes for data representation. PCA results revealed that dry miscellaneous forest and sal mixed forest are similar in association and this is principally governed by SOC, MBC

Table 3. Results of two-way analysis of variance in chemical and biological properties of the soils under five PCSs, two seasons and two soil depths. Values of soil chemical and biological properties (annual means with standard deviations) in five different forest types. *F* and *P* values from two-way ANOVA applied to each parameter against season and depth are reported in the last line

PCSs	pH	EC	SOC (%)	N_t (%)	C:N	P_{av} (mg/kg)	K (ppm)	MBC (µg/g)
DMF	6.7 ± 0.1	163.2 ± 78.8	1.5 ± 0.3	0.11 ± 0.1	13.6	499.1 ± 206.8	4,501.1 ± 812.9	506.8 ± 346.4
SMF	6.5 ± 0.2	154.1 ± 79.5	1.3 ± 0.3	0.09 ± 0.01	14.4	436.4 ± 178.6	4,170.5 ± 912.7	421.3 ± 258.6
TP	6.8 ± 0.1	137.3 ± 78.6	1.1 ± 0.3	0.07 ± 0.01	15.7	372.6 ± 155.3	3,580.4 ± 1,159.3	294.8 ± 179
LMF	6.1 ± 0.1	165.9 ± 15.4	1.2 ± 0.3	0.92 ± 0.6	1.3	427.1 ± 156.6	4,145.1 ± 685.1	278.8 ± 150.3
SAV	7.9 ± 0.5	44.9 ± 10	1.3 ± 0.3	0.12 ± 0.01	10.8	622.2 ± 226.6	3,954.5 ± 922.1	367.1 ± 266.9
P	ns	Ns	Ns	0.001	0.001	0.001	0.001	0.001

PCSs = plant community structures, DMF = dry miscellaneous forest, SMF = sal mixed forest, TP = teak plantation, LMF = low land miscellaneous forest, SAV = savanna ecosystem, pH = soil pH, EC = electrical conductivity, SOC = soil organic carbon, N_t = total nitrogen C:N = soil organic carbon and total nitrogen ratio P_{av} = available phosphorus K = potassium MBC = microbial biomass carbon.

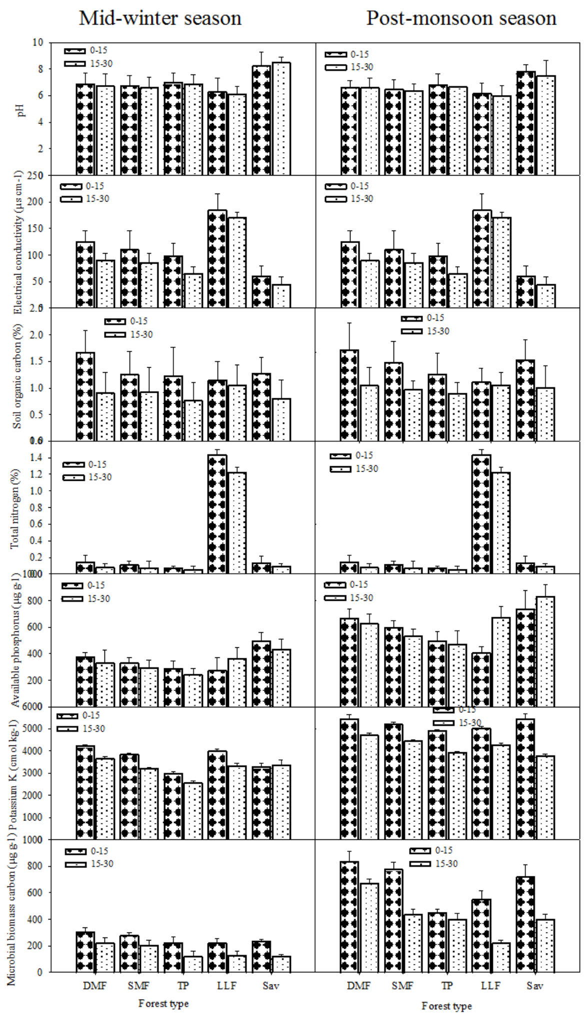

Figure 2. Mean and standard deviations of chemical and biological properties of the soils at five different plat community structures (PCSs) in Katerniaghat Wildlife Sanctuary in two depths and seasons. DMF = dry miscellaneous forest, SMF = sal mixed forest, TP = teak plantation, LMF = low land miscellaneous forest, SAV = savanna ecosystem, LW = Late-winter season and PM = Post-monsoon season

and soil K. Correlation between PCA axis 1 and 2 with all 13 forest soil parameters of five PCSs are presented in Table 4. PCA axis 1 shows that many soil properties were negatively correlated with soil pH and positively with EC, whereas PCA axis 2 shows that soil properties were positively correlated with SOC, MBC and soil K. Use of PCA analysis and their results to assess the role of different forest composition in soil properties is reviewed in Table 5.

4. Discussion

Our results showed significant difference in physical, chemical and microbial properties in different PCSs. Most of the soils under study were fine textured and exhibited textural variations ranging from sandy loam to clay loam, which are suitable for good sal regeneration and high quality trees (Gupta, 1951). Soil texture under teak plantation was sandy and showed lowest amount of silt as observed by Yadav and Prakesh (1969) at Chakrata forest. These results show that during the PM season most of the physical, chemical and biological properties increased compared to the LW season due to low moisture content and higher temperature, supported with litter and decaying fine roots, which release nutrients in soil, although uptake of nutrients increased. But in old forest generally growth became saturated. Soil properties decreased with the depth as major supply comes through surface litter. The WHC was greater in forest soils compared to savanna which is in agreement with previous studies conducted in tropical dry forest (Singh *et al.,* 2009). So both, the increase in WHC and decrease in B_d in forest soils compared to teak plantation and savanna soils, favours the soil porous structures and the hydrothermal conditions improved as vegetation cover increased. Forest soils are mostly acidic in nature. Flood and water logging was the main reason of higher acidic nature in lowland miscellaneous forest soil, whereas in sal mixed forest, sal regenerating capacity makes its pH acidic in nature

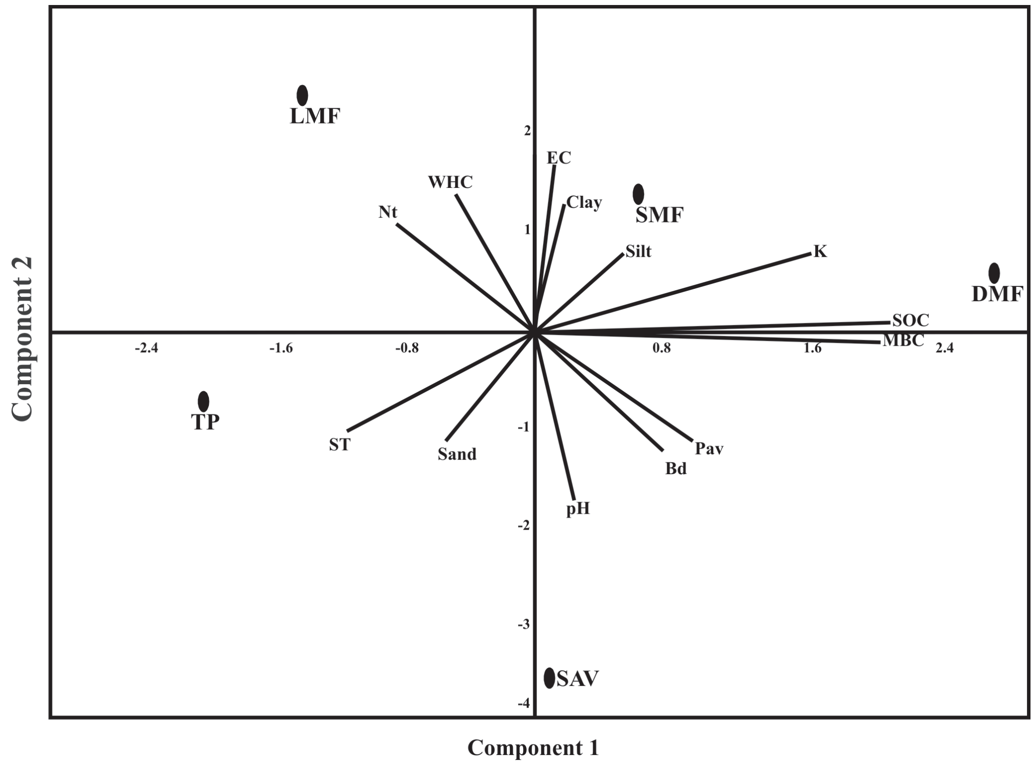

Figure 3. Results of Principal Component Analysis (PCA): the five plant community structures (PCSs) following 13 soil physical chemical and biological properties. DMF = dry miscellaneous forest, SMF = sal mixed forest, TP = teak plantation, LMF = low land miscellaneous forest, SAV = savanna ecosystem, WHC = water holding capacity, B_d = bulk density and TS = soil temperature, pH= soil pH, EC = electrical conductivity, SOC = soil organic carbon, N_t = total nitrogen, P_{av} = available phosphorus, K = potassium and MBC = microbial biomass carbon

Table 4. Correlation coefficients between PCA two axes scores and soil physical, chemical and biological parameters of five PCSs in Katerniaghat Wildlife Sanctuary

–	Axis 1	Axis 2	Clay	Silt	Sand	WHC	B_d	ST	PH	EC	SOC	MBC	N_t	P_{av}	K
Axis 2	0	1	–	–	–	–	–	–	–	–	–	–	–	–	–
Clay	0.69	0.09	1	–	–	–	–	–	–	–	–	–	–	–	–
Silt	0.43	0.26	0.113	1	–	–	–	–	–	–	–	–	–	–	–
Sand	-0.67	-0.27	-0.532	-.902*	1	–	–	–	–	–	–	–	–	–	–
WHC	0.68	-0.23	0.02	0.136	-0.126	1	–	–	–	–	–	–	–	–	–
B_d	-0.73	0.396	-0.753	-0.418	0.68	-0.217	1	–	–	–	–	–	–	–	–
ST	-0.58	-0.558	-0.307	0.003	0.137	-0.582	-0.096	1	–	–	–	–	–	–	–
PH	-.98**	0.106	-0.591	-0.317	0.528	-0.805	0.663	0.604	1	–	–	–	–	–	–
EC	.93*	0.088	0.465	0.308	-0.468	0.856	-0.45	-0.773	-.963**	1	–	–	–	–	–
SOC	-0.01	.983**	0.139	0.092	-0.145	-0.218	0.429	-0.622	0.098	0.098	1	–	–	–	–
MBC	-0.02	.978**	-0.071	0.338	-0.264	-0.124	0.464	-0.553	0.105	0.113	.938*	1	–	–	–
N_t	0.57	-0.384	0.873	-0.167	-0.235	0.09	-0.799	-0.047	-0.54	0.342	-0.303	-0.536	1	–	–
P_{av}	-0.69	0.462	-0.055	-0.193	0.187	-.955*	0.41	0.343	0.804	-0.78	0.473	0.364	-0.213	1	–
K	0.42	0.841	0.61	0.297	-0.524	-0.107	-0.107	-0.646	-0.286	0.377	0.851	0.746	0.161	0.28	1

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

WHC = water-holding capacity, B_d = bulk density and ST = soil temperature, pH = soil pH, EC = electrical conductivity, SOC = soil organic carbon, Nt = total nitrogen P_{av} = available phosphorus K = potassium MBC = microbial biomass carbon.

Table 5. Comparative study of principal component analysis results to assess role of soil properties in different forest structures and plant biodiversity

SN	Forest Type and Location	Maltivariate Technique	Software	Result	Reference
1	Moist tropical forest, Bolivia	PCA	SPSS 17	Soil nutrient specially P and soil texture have independent effects on forest structure and diversity	Pena-claros *et al*. (2012)
2	Dry tropical forest, Bolivia	PCA	SPSS 17	Ca, organic matter, Nitrogen, Mg and cation exchange capacity strongly related with vegetation structure	Pena-claros *et al*. (2012)
3	Coniferous mixed forest, Italy	PCA	PCA, Sintax 2000	Several physical, chemical and biological parameters, in soil affected by different land use types	Marzaioli *et al*. (2010)
4	Alpine forest, Poland	PCA	Win ISI II-1.02	Tree species had a significant effect on the chemical and microbial properties of soil	Chodak and Niklinska (2010)
5	Dry tropical forest, Vindhyan range	PCA	PC-ORD	Soil Nitrogen with disturbance intensity determining the constitution and distribution of the dry tropical forest communities	Sagar *et al*. (2003)
6	Alpine forest, Gadhwal Himalaya	PCA	PAST 1.92	Among habitat characters, Soil bulk density also have a great role in soil lichens species richness distribution	Rai *et al*. (2011)
7	Alpine forest, Kumaon Himalaya	PCA and DCA	DECORANA	Forest canopy cover, trees diversity and grasses abundance majorly represents forest community structure	Hussain *et al*. (2008)
8	Tropical moist deciduous forest, Northern India	CA and PCA	PAST 2.00	Species association greatly affects soil health but teak plantation does not contribute in soil quality improvement and rather deteriorates the soil properties	Present study

(Bhatnagar, 1965). Forest trees contributes a lot of organic matter to the soil in the form of leaves, twigs, stems, flowers, fruits and fine roots which after decomposition form the organic carbon resource and release nutrients. Plant litter serves as a resource for decomposer organisms (Boeken and Orenstein, 2001) and a nutrient source for plants (Boeken and Orenstein, 2001), as well as enhancing the availability of soil resources such as water (Segoli *et al*., 2012). So among all forests SOC value ranged from 1.1 to 1.5 % and MBC value ranged from 278.8 to 506.8 μg/g, which was a good indication, may be due to PA. Therefore, soil physical and chemical properties can be improved significantly in the vegetation systems having higher organic matter content.

Correlation result exhibited that the SOC and MBC were positively correlated with each other. This could be because of increased microbial activity at the higher ST and moisture conditions, because the microbial activity is sensitive to water potential and the increase in soil water potential in the rainy season in the natural forest showed higher value of SOC and MBC than teak plantation and savanna soils. Mineralisation among forest type regulated by combination of inter specific differences in litter, its production and decomposition rate, whereas quality of litter is one of the important factor which control nutrient cycling so we find teak plantation and savanna have lower soil nutrients compared to other forests. N_t content, higher in lowland miscellaneous forest than other forests probably due to higher dominance of *Acacia catechu* in upper canopy and *Acceciya suniyata* in ground flora. P_{av} and K have same trends in all the forest types; these were not affected much by vegetation composition. Over all, monoculture plantations might disrupt the nutrient cycling (Brown *et al.*, 1994) and often increases the incidence of surface runoff (Tang *et al*., 2006, as a consequence low nutrient content was found in teak plantation.

In our results, the natural forests dry miscellaneous forest and sal mixed forest had positive effects on the soil organic matter and nutrients than teak plantation and savanna which might be due to the presence of maximum deciduous plant species at dry miscellaneous forest and sal mixed forest, compared to teak plantation where vegetation was homogenous (Tripathi and Singh, 2009) whereas, savanna was moderate in diversity and density. Several studies have demonstrated that species composition modify the soil environment of tropical forest in India (Bhatnagar, 1965; Banerjee *et al*., 1986; Paudel and Sah, 2003) and our cluster analysis and PCA grouping against 13 soil physical, chemical and microbial properties clearly demonstrate that different PCSs have significant difference in their below ground ecosystem resources. PCA is a fact-finding tool that reduces measurement problems, such as bias and reduces the complexity of correlated data, as it extracts only variables that have significant contributions among a set of variables or principal components, which account for most of the variance in the observed variables (Johnson and Wichern, 2002). However, the PCA result for this study showed that the reciprocal effects of soil and vegetation were influenced by five PCSs of soil-vegetation variables. PCA identified SOC, MBC and K as principal components in dry miscellaneous forest and sal mixed forest, as most of the organic matter produced by vegetation is returned to the soil. In Savanna ecosystem, animal consumption, forest fire and grasses used as fodder and house construction were the threats by which relatively marginal amount is returned back to the soil. pH was principal component for savanna, may be due to high ash accumulation from biomass burning (control fire), which would have returned enough base-forming cations to increase pH of the surface soil, at least temporarily.

The spatial distributions of tree species at particular tropical forest community show strong associations to soil nutrient distributions (Pena-claros *et al*., 2012). It should be mentioned; however soil not only affects vegetation, but that vegetation may drive some of the soil variability that we measured. Neutral dispersal assembly of any forest community strongly governed by dominant plant and its soil associations or the observed community association controls soil properties. Our results indicate that belowground resource availability depends on forest vegetation type in tropical forest at local scales and provide the basis for future investigations on the mechanism of resource competition among tropical tree species in a forest community.

5. Conclusion

The study showed that, as compared to natural forest, teak plantation and savanna forest had a negative response on soil properties. We find significant differences in soil properties in each PCSs as well as seasons and soil depths have significant effect on soil properties. The heterogeneous behaviour of tropical moist forest indicates that belowground resource availability depends on forest vegetation types that sustain the vegetations reciprocally. PCA results showed that species association greatly affects soil health but teak does not contribute in soil quality improvement and rather deteriorates the soil properties.

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RESEARCH ARTICLE

Temporal Variation and Trace Metal Characterisation of Particulate Matter in Ambient Air of Rural and Urban Areas of Lucknow, India

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Abstract Temporal variation in the concentration of particulate matter (PM) was monitored at an institutional and rural area of Lucknow. The concentration of coarse (PM_{10}) and fine particulates ($PM_{2.5}$) was found to be in the range of 134.14–387.64 (225.79±76.34), 108.47–233.38 (147.15±36.47) and 90.68–220.8 (136.19±37.08), 33.13–113.67 (83.47±24.73) $\mu g/m^3$ at institutional and rural area, respectively. Average PM_{10} and $PM_{2.5}$ concentration at both the locations were found to be beyond the permissible limits, that is 100, 60 $\mu g/m^3$(National Ambient Air Quality Standards) and 150, 35 $\mu g/m^3$ of (United State Environmental Protection Agency) for institutional and rural areas, respectively. Average $PM_{2.5}$-to-PM_{10} ratio was found to be .63±.11; .57±.15 at institutional and rural area, respectively, which indicates the higher contribution of fine particulates in PM_{10}. Further, the PM_{10} was subjected for trace metal characterisation in terms of Fe, Pb, Zn, Cd and Cu. Trace metal concentration (ng/m^3) associated with PM_{10} at both the locations was found to be in the order of Fe (823.87) > Pb (198.67) > Zn (93.19) > Cd (88.10) > Cu (37.60). The Pearson correlation of trace metals associated with PM_{10} and meteorology showed significant correlation at $P<.05$ and $P<.01$ levels; which inferred that the PM and trace metals were originated from similar sources.

Key words Correlation, Meteorology, NAAQS, PM_{10}, $PM_{2.5}$, Trace metal, Windrose

1. Introduction

Rapid urbanisation, uncontrolled traffic growth, reduced forest cover, sudden expansion in the number of vehicles and so on have rapidly deteriorated the air quality of developing and developed countries (Kim *et al*., 2015; Von Schneidemesser *et al*., 2015; Terrouche *et al*., 2016). Urban metropolitan areas are uncompromisingly exposed to considerable amount of particulate matter (PM), commonly accredited to direct emissions from vehicle exhaust, wood burning and industrial activities (Chen *et al*., 2011). PM can be defined as small solid or liquid droplets suspended in ambient air. PM were broadly classified as PM_{10} and $PM_{2.5}$ based on their aerodynamic diameter; PM_{10} (diameter ≤ 10 µm) and $PM_{2.5}$ (diameter ≤ 2.5 µm). PM_{10} arise predominantly from mechanical processes including brake lining abrasion, tire wear, windblown soil and dust, sea salt and bio-aerosols such as pollen and fungal spores, whereas $PM_{2.5}$ are known to primarily originate from combustion and gas-to-particle conversion processes in the atmosphere (Almeida *et al*., 2005; Edgerton *et al*., 2009). PM concentrations in the atmosphere is regulated by micrometeorological condition, local sources, dispersion and long rate transport pattern, land-use pattern, topography and fossil fuel combustion (Maenhaut *et al*., 2016; Von Schneidemesser *et al*., 2015). Distribution and transport of PM is conspicuously coupled with meteorological parameters such as wind speed and direction, relative humidity (RH), rainfall and temperature (Pakbin *et al*., 2010). Further, temperature and humidity influence secondary gas particle formation and particle hygroscopic growth, whereas wind speed can alter the dispersion, while wind direction can influence the path of pollutants (Luvsan *et al*., 2012; Tie *et al*., 2015). Chemically PM is a multifarious mixture of organic and inorganic compounds and their toxicity is primarily governed by composition, shape and size of particles and presence of other associated pollutants

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(Clements *et al*., 2014; Rashki *et al*., 2013). PM, especially ultrafine particles (aerodynamic diameters < 100 nm) are of high health risk as particles of small diameter penetrate deep into the lungs, contribute to reduced lung function and can be transported to the vital organs via the bloodstream (Rückerl *et al*., 2011; Wu *et al*., 2013). Since last several decades' air pollution is mainly accredited to urban and industrial areas, but now it is frightening to the rural areas also (Abu-Allaban *et al*., 2007). Urban population is particularly exposed to elevated levels of PM including trace metals (V, Fe, Pb, Zn, Cd, Mn, Ba, Sr, Al, U, Th, Zr, Cs, Rb, Sb, Sn and Cu) due to vehicular exhaust, whereas combustion of fossil fuels produces V, As, Cu, Co, Mo, Ni, Sb, Cr, Fe, Mn and Sn (Moreno *et al*., 2006; Sharma *et al*., 2006). Most of the trace metals have toxic effects on living organism if their concentration is exceeded to desired limit (Shinggu *et al*., 2010). Toxicity and bioavailability of any metal depends on their chemistry in atmosphere; however, chemistry of every element varies due to different physical and chemical properties (Magalhaes *et al*., 2015). Fe and Cu are essential elements but Fe is less toxic than Cu because it is generally present as Cu^{+2} and forms strong complexes with nitrogenous bases (ATSDR, 1993). It has been reported that the level of Pb can induce severe neurological and haematological effect, especially in infants, whereas an elevated level of Cd and Ni will produce carcinogenic effects in human (Benoff *et al*., 2000). Several studies have reported the trace metal concentration in PM across Indo-Gangetic planes and their adverse effect on human health (Murari *et al*., 2015; Kumar *et al*., 2015; Barman *et al*., 2008; Khillare *et al*., 2004; Tiwari *et al*., 2014a). Vehicular traffic is the main source of particulate air pollution in Lucknow city (Kisku *et al*., 2003; Sharma *et al*., 2006). The total vehicular population of the Lucknow is 1,709,662 as on 31.03.2015 which is 10.08% higher over the last year (Regional Transport Office, Lucknow, 2015). The daily introduction of newer vehicles and continuation of old and fuel efficient vehicle, aggravate the 'canyon effect' among urban population (Kisku *et al*., 2013). Keeping in view the ever-growing vehicles population in Lucknow, this study has been conducted to monitor temporal variation in the concentration of the PM_{10} and $PM_{2.5}$ at an institutional and rural area. Further, the sampled PMs have been characterised for the presence of trace metals, namely Fe, Pb, Zn, Cd and Cu.

2. Material and Methods

Lucknow is the administration capital of Uttar Pradesh, India, located in Northern India. The population of Lucknow is 45 lakh (2011 census) and lies between 26°52′ latitude and 80°56′ longitude at 128 masl and covers an area of 310 km^2 (Figure 1). Lucknow is having subtropical climate which consists of cool dry winter and temperature vary from 45°C during summer to 3°C in winters. Average rainfall is about 100 cm. Babasaheb Bhimrao Ambedkar University is India's premier educational institute situated at 80°30′ E longitude and 26°30′ N latitude. Mohanlalganj is a rural area situated in southeast region with coordinates 26°41′ N and 80°58′E.

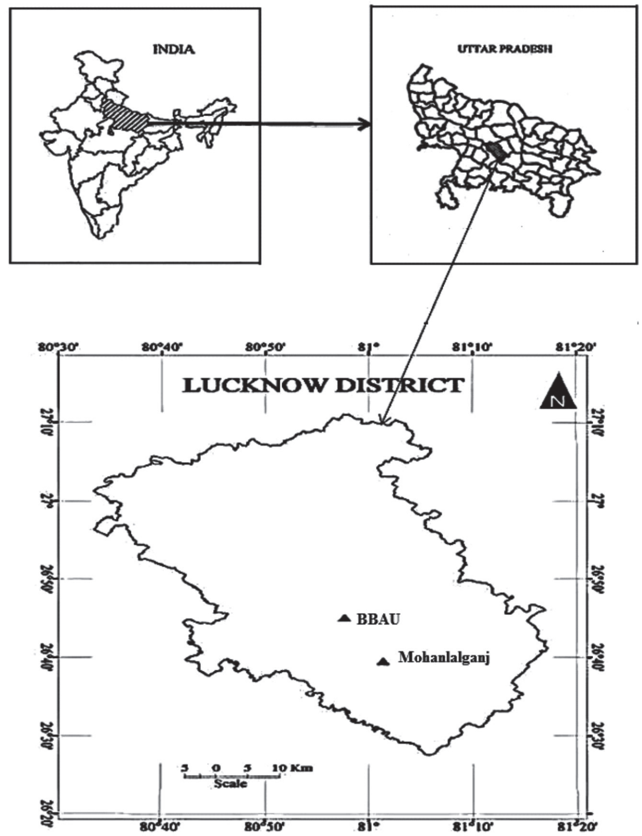

Figure 1. Location of sampling sites

2.1 Air Monitoring

PM_{10} were collected onto glass fibre filters (GF/C, Whatman Cole-Parmer India Pvt. Ltd. Mumbai, Maharastra, India,) using respirable dust sampler (Envirotech, Envirotech Instruments Pvt. Ltd. New Delhi, India, APM 460) at flow rate of 1.3 m^3/min, whereas $PM_{2.5}$ was collected using fine particle sampler (Envirotech, Envirotech Instruments Pvt. Ltd. New Delhi, India, FPM 550) at flow rate of 13.7 l/min. Glass fibre filter paper of 8 ×10 in and glass microfibre filter paper of 47 mm diameter were used for sampling of PM_{10} and $PM_{2.5}$, respectively. Continuous monitoring was carried out for 24 h with a frequency of once a week, to have about 48 samples in a year. The $PM_{10\ \mathrm{and}\ 2.5}$ concentrations in ambient air was determined by following formula:

$$\text{Particulate matter}\left(\mu g/m^3\right) = \frac{\left(W_f - W_i\right)\times 10^6}{V}$$

Where W_f is the final weight of sampled filter paper (g), W_i is the initial weight of blank filter paper (g), V is the total air volume (m^3) and $10^6$is the conversion of g into μg.

2.2 Trace Metal in PM_{10} and Quality Control

Filter paper of 23×13 mm were cut and digested using US EPA Method IO-3.2 (US-EPA, 1999), in an extracting solution of nitric acid and perchloric acid (3:1, v/v). Digested samples were filtered through no. 42, Whatman filter paper and final volume was made up to 25 ml by Milli-*Q* water. The filtrate was examined for concentration of trace metal by atomic absorption spectrophotometer (AA 240 FS; Varian, Agilent Technologies Pvt Ltd. Australia). AAS was calibrated thrice for each metal using known certified reference material (Qualigens, Qualigen Fine Chemical Pvt. Ltd , USA) before analysis. Blank filters were also subjected to the similar procedure for extraction and analysis to quantify the background trace metal concentration. Background contamination of trace metals was determined by subtracting the field blank values from sample concentrations. To check the recovery efficiency spiking of known amount of trace metal onto the unused filter paper was done before extraction and recovery efficiency was found to be approximately 94%.

3. Result and Discussion

3.1 Temporal Variation in Particulate Matter Concentration

Continuous monitoring of PM (PM_{10} and $PM_{2.5}$) was performed during January 2015 to December 2015 (*n*=48) and its variability was depicted in Figure 2. Average concentration of coarse and fine particles was found to be 225.79 ± 76.34, 147.15 ± 36.47 and 136.19 ± 37.08, 83.47 ± 24.73 µg/m^3 at institutional and rural area, respectively. PM was exceeded many times of 24 h standards of 100, 60 µg/m^3 (NAAQS, 2009) and 150, 35 µg/m^3 (USEPA, 2012). Similar results were also reported with some variability at Indo-Gangetic planes and the primary sources were reported to be vehicular exhaust, waste incineration and biomass combustion (Kisku *et al*., 2013; Tiwari *et al*., 2014b; Pipal *et al*., 2014). Maximum concentration of particulates (PM_{10} and $PM_{2.5}$) was observed during winter season (Figure 2); probably due to thermal inversion (Tiwari *et al*., 2012; Kumar *et al*., 2015). During summers concentration of PM was also found to be higher, possibly due to enhanced re-suspension of road and crustal dust favoured by higher temperature and wind speed, coupled with low rainfall and RH (Pakbin *et al*., 2010; Shah and Shaheen, 2010). However, during monsoon season concentration of PM was found to be lowest reason being high RH reduces re-suspension of road and crustal dust (Celo and Dabek-Zlotorzynska, 2010; Shah *et al*., 2012). Correlation analysis between PM_{10} and $PM_{2.5}$ suggested that local meteorology have significant role in controlling the particulate pollution at both sites. Typically the ratio of $PM_{2.5}$ to PM_{10} at both location was very high

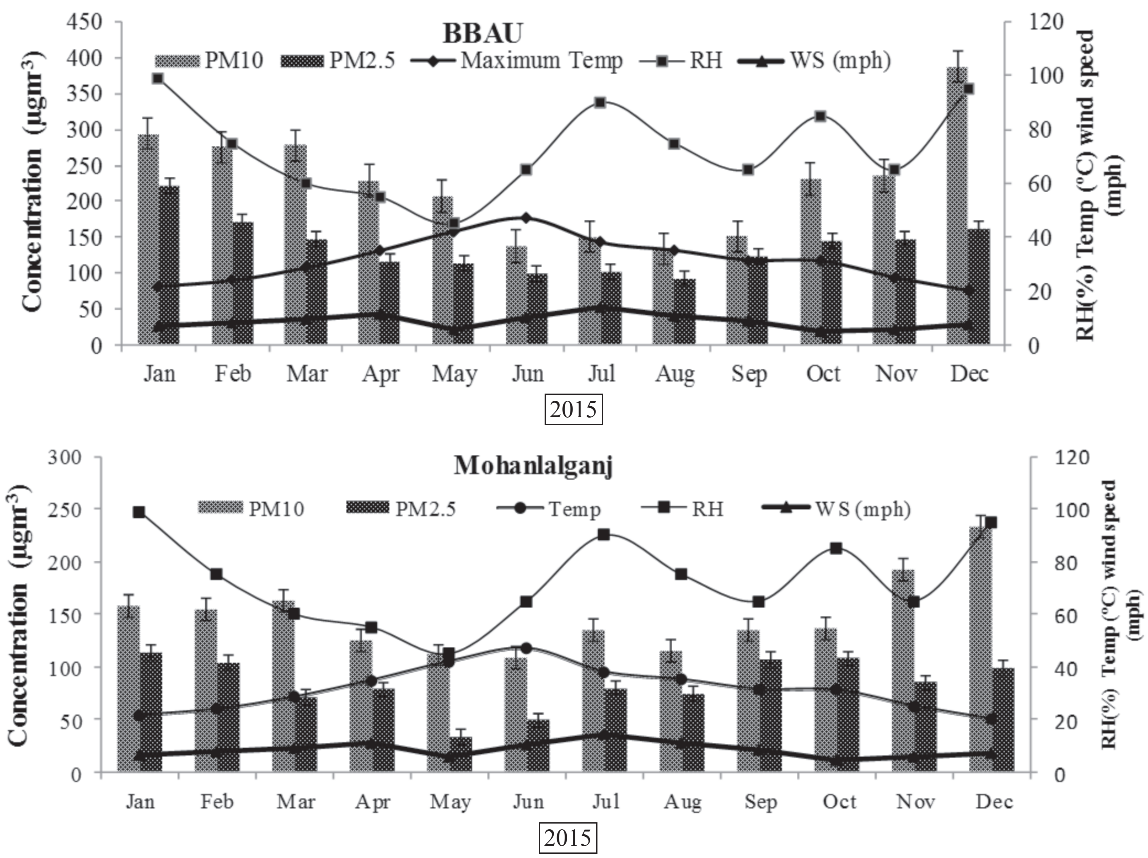

Figure 2. Temporal variations of PM_{10} and $PM_{2.5}$ with prevailing meteorological conditions at institutional area (A) and rural area (B)

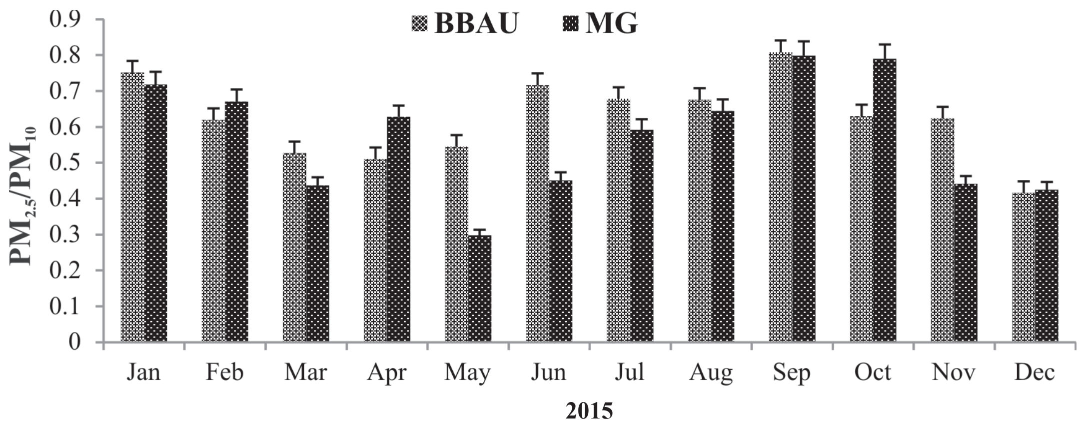

Figure 3. $PM_{2.5}$ to PM_{10}ratio at institutional and rural area of Lucknow

indicating the high fraction of finer particle in suspended PM (Figure 3). The ratio of $PM_{2.5}$ to PM_{10} was recorded to be very high in rural area of Lucknow; indicated the considerable contribution of secondary aerosol to $PM_{2.5}$ concentration.

3.2 Influence of Meteorology on Particulate Matter

It is important from the view of health aspect to know not only the chemical composition and emission sources, but also the meteorological influences upon the particle matter concentration, particle number concentration, different particle size fractions and particle chemical composition (Schafer *et al.*, 2016). These factors strongly influence the exchange processes of air pollutants and particle size distributions (Alföldy *et al*., 2007; Schäfer *et al*., 2011; Barmpadimos *et al*., 2012). The average maximum RH varied among 60–99%, 25–80% and 57–100% during winter, summer and monsoon, respectively (Figure 2). Long residence time, low wind speed, low mixing height and temperature inversion favour the high level of PM in the atmosphere during winter season (Krar *et al.*, 2006). During winter season as the RH increases, simultaneously hydrophilicity of aerosol increases and radius of particle will be doubled by coating with water vapour on the surface which ultimately enhanced the atmospheric PM(Liu *et al.*, 2011). Atmospheric temperature near the earth surface was recorded maximum during summer and enhanced vertical mixing height, which ultimately lower the concentration of PM (Jayamurugan *et al.*, 2013). During monsoon humidity is on higher side enhances the rate of absorption of PM, whereas rain brings down the concentration of PM in atmosphere by acting as natural scrubber during monsoon (Bhaskar and Mehta, 2010). Average wind speed varied from minimum of 5.75 in winter and maximum of 14 mph during entire monsoon. Annual variation of wind vector has been illustrated in Figure 4. Wind direction was found to be westerly during summer and winter season whereas easterly during monsoon.

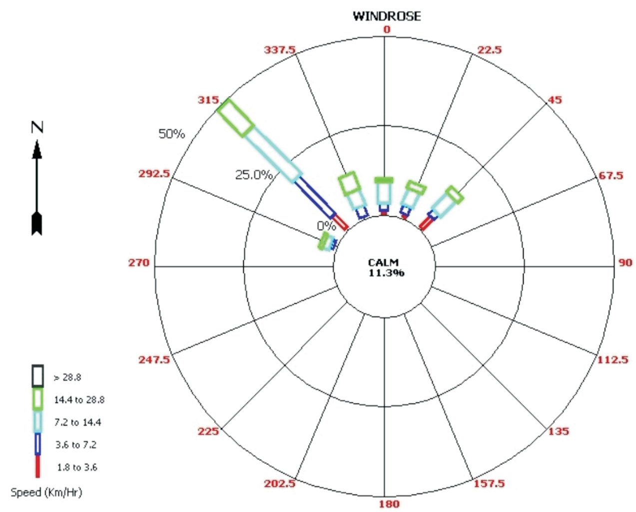

Figure 4. The annual variation of wind vector for the entire monitoring period Lucknow

3.3 Trace Metal Characterization of Particulate Matter

Average concentration of Fe, Pb, Zn, Cd and Cu present in PM and their seasonal variation at both the sites is presented in Table 1 and Figure 5, respectively. The Fe concentration varied from 267.78–823.87 (610±145.65) to 193.36–518.56 (397.97±105.96) ng/m^3 at institutional and rural areas, respectively. At both sites higher Fe concentration in PM_{10} implies their crustal origin and from re-suspension of dust (Kwangsam *et al.*, 2004; Lee *et al.*, 2005). However, airborne Pb concentrations was found to be ranged from 70.10–134.46 (99.52±22.18)to 102.54–198.67 (136.38±33.97) ng/m^3. The elevated levels of Pb are emitted by vehicular exhaust and it constitute over 20% of total mass of fine particles and 25% coarse fraction of PM (Tripathi, 1994; Lili and Gao, 2011). Cadmium concentration was found to be ranged from 22.60–58.00 (40.95±11.26) and 44.10–88.10 (60.60±11.46) ng/m^3 at both location. Airborne Cd is mostly originating from combustion of accumulators

Table 1. Trace metal concentrations (ng/m^3) in the PM_{10} collected from institutional and rural area of Lucknow

Months	Cd		Cu		Fe		Zn		Pb	
(2015)	MG	BBAU	MG	BBAU	MG	BBAU	MG	BBAU	MG	BBAU
Jan	58.00	55.50	11.60	21.30	518.56	635.55	56.73	62.43	90.20	108.36
Feb	37.00	75.20	18.70	25.50	378.23	789.23	54.87	74.07	77.60	169.67
Mar	37.50	59.40	19.20	25.60	511.19	823.87	57.16	78.56	70.10	108.78
Apr	22.60	59.60	29.80	3.60	475.74	608.23	66.32	93.19	119.10	129.58
May	30.60	52.30	19.80	17.60	193.36	267.78	25.07	35.52	83.78	104.78
Jun	33.30	54.20	7.90	15.60	291.36	582.87	25.79	30.93	125.67	154.98
Jul	40.30	53.40	8.60	19.70	287.37	356..87	26.45	35.84	134.46	198.67
Aug	32.10	44.10	6.50	16.70	372.15	565.56	34.78	42.23	90.56	104.34
Sep	51.10	61.80	7.90	17.80	482.87	673.45	33.75	37.73	109.46	174.67
Oct	51.60	63.80	8.70	16.50	285.75	664.45	33.36	40.11	124.78	163.56
Nov	39.70	59.80	25.80	26.50	441.13	572.36	70.05	83.19	93.20	116.68
Dec	57.60	88.10	14.40	18.20	321.89	536.45	58.70	67.33	75.30	102.54
Maximum	58.00	88.10	29.80	37.60	518.56	823.87	70.05	93.19	134.46	198.67
Minimum	22.60	44.10	6.50	15.60	193.36	267.78	25.07	30.93	70.10	102.54
Average	40.95	60.60	14.91	21.55	379.97	610.89	45.25	56.76	99.52	136.38
SD	±11.26	±11.46	±7.72	±6.35	±105.96	±145.65	±16.86	±22.06	±22.18	±33.97

Table 2. Pearson correlation of trace metal associated with PM_{10} and meteorology at rural area

	Cd	Cu	Fe	Zn	Pb	Temp	RH	WS	PM_{10}
Cd	1								
Cu	-.457	1							
Fe	.144	.271	1						
Zn	.068	.716**	.669*	1					
Pb	-.122	-.305	-.237	-.463	1				
Temp	-.623*	-.213	-.528	-.738**	.572	1			
RH	.752**	-.510	.041	.068	.072	-.523	1		
WS	-.421	-.173	.070	-.200	.422	.410	.030	1	
PM10	.580*	.248	.245	.667*	-.504	-.821**	.457	-.330	1

*Correlation is significant at the .05 level (two-tailed).
**Correlation is significant at the .01 level (two-tailed).

and carburetors of motor vehicles (Divrikli *et al.*, 2006). It is a major industrial pollutant which is used in battery and smelting of zinc (Hassan *et al.*, 2009). Cd is highly toxic metal, because it get dispersed in the air and becomes available to physiological activities; once inhaled can cause respiratory illness, hypertension, heart enlargement and premature death (ATSDR, 1993). The observed concentration of Cu and Zn in PM_{10} were found to be 15.60–37.60 & 6.50–29.8 and 30.93–93.19 & 25.07–70.05ng/m^3 at institutional and rural area, respectively. Airborne Cu and Ni mostly originate from combustion of fossil fuel and incineration of waste (IPCS, 1991; Sub-committee on Nickel, 1975). Measured levels of trace metals were well below the prescribed limits NAAQS, except for zinc, cadmium and iron at both locations. To study the inter-elemental relationships among PM and associated trace metals, Pearson's correlation matrices were computed (Tables 2 and 3). Inter-correlation was found to be as follows: PM_{10} with Cd (r = .58, P<.05), Zn (r = .667, P<.05), Zn with Cu (r = .716, P<.01), Zn with Fe (r = .669, P<.05) and PM_{10} with Cd (r = .913, P<.01), Zn (r = .616, P<.05), Zn with Pb (r = .620, P<.05), Zn with Cd (r = .664, P<.05), Zn with Cu (r = .869, P <.01), Pb with Cd (r = .727, P<.01), Pb with Zn (r = .924, P<.05) at institutional and rural area, respectively. Positive correlation among PM with heavy metal indicates that, as the concentration of PM increases in atmosphere

Table 3. Pearson correlation of trace metal associated with PM_{10} and meteorology at rural area

	Cd	Cu	Fe	Zn	Pb	Temp	RH	WS	PM_{10}
Cd	1								
Cu	.324	1							
Fe	.286	.3	1						
Zn	.664*	.869**	.47	1					
Pb	.727**	.762**	.522	.924**	1				
Temp	-.281	.394	.133	.166	-.008	1			
RH	-.128	-.478	-.598*	-.552	-.013	-.421	1		
WS	.062	.109	-.303	-.071	.047	.258	.539	1	
PM10	.913**	.259	.283	.616*	.620*	-.768**	.181	-.330	1

*Correlation is significant at the .05 level (two-tailed).
**Correlation is significant at the .01 level (two-tailed).

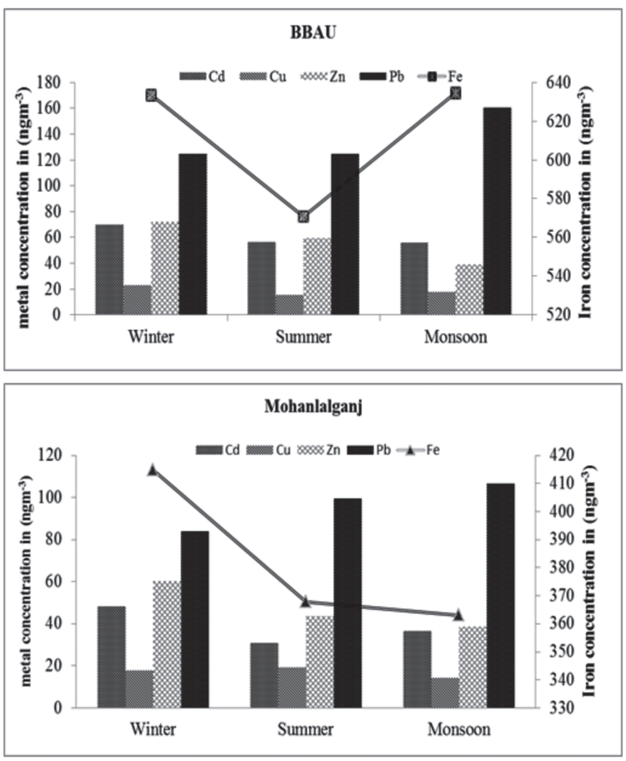

Figure 5. Seasonal variation of trace metal concentration (ng/m^3) associated with PM_{10} at both location

metal concentration simultaneously increases (Devi *et al.*, 2015; Barman *et al.*, 2008). Positive inter correlation between metals also points that the source of these metals may be same or they may be originated from a similar sources.

4. Conclusion

Study revealed that the PM concentration at studied locations exceeded the ambient air quality standard (NAAQS, 2009). The meteorological factors, namely temperature, RH and wind speed influence the atmospheric pollutants. Temperature and concentrations of PM had shown negative correlation, whereas RH has positive correlation with finer fraction of PM. Both PM_{10} as well as $PM_{2.5}$ were found to be laden with Fe, Pb, Cd, Zn and Cu. Trace metal concentration associated with PM has shown significant positive inter-correlation within metals which confirms their similar origin. Finding of present study emphasise the need of regular ambient particles monitoring both in terms of concentration and trace metal.

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RESEARCH ARTICLE

Climatic Variability Analysis at Ballowal Saunkhri in Submontane Punjab (India)

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Abstract The variabilities in maximum and minimum temperature, rainfall, relative humidity, wind speed, evaporation and sunshine hours were analysed from historical 30 years of meteorological data (1984–2013) recorded at Regional Research Station (Punjab Agricultural University), Ballowal Saunkhri, district SBS Nagar (Punjab). Two distinct crop growth seasons of *kharif* (1st May to 30th September) and *rabi* (1st October to 30th April) were characterised for seasonal trends. The analysis of data revealed that the climate had changed slightly over the past three decades. The annual maximum and minimum temperature in last three decades ranged from 28.5 to 31.0°C and 15.2 to 17.4°C. The annual average maximum temperature had shown increasing trends over the years and increase was non-significant in annual minimum temperature. The rainfall of this region ranges from 617 to 2,041 mm with average value of 1,067 mm, out of which about 80% was received during the monsoon period. The declining trend in average annual and *kharif* rainfall was observed over the years. The average annual relative humidity showed significant increase over the years and ranged from 58 to 71% with mean value of 64%. The significant decrease in wind speed was recorded from 1988 to 2000. The sunshine hours also showed significant decreasing trends over years.

Key words Climate change, Rainfall, Relative humidity, Sunshine hours, Temperature, Wind, Seasons, Shiwalik.

1. Introduction

The agriculture sector in India is highly susceptible to the frequent and erratic climatic irregularities. The projected changes in the climate due to intensive anthropogenic activities may cause worst conditions. India has been witnessing too many climatic shifts and natural calamities in the last one decade. Climatic disturbances have adversely affected public health, food security, water resources and biodiversity in the country. In India, Satyanarayana and Srinivas (2008) studied the variation in amount and duration of Indian monsoon conditions and observed high risk for extreme rainfall events in Indian subcontinent with the change in climate. The average frequency of extreme rainfall events along with the contribution of extreme rainfall events to the seasonal rainfall showed increasing trend during monsoon season and also during June and July months. The increasing trend of contribution from extreme rainfall events is balanced by a decreasing trend in low rainfall events (Pattanaik and Rajeevan, 2010). The Intergovernmental Panel on Climate Change (IPCC, 2007) has projected that increase in temperature is expected to be in the range of 1.8 to 4.0°C by the end of 21st century. For South Asia, the IPCC has projected that rise in temperature will be 0.5 to 1.2°C by 2020, 0.88 to 3.16°C by 2050 and 1.56 to 5.44°C by 2080, depending on the future human activities. The new findings indicate that warming is more pronounced than expected and impact would be severe in tropical areas, which mainly consist of developing countries, including India (Sathaye *et al.,* 2006). Goswami *et al.* (2006) observed the increase in heavy rainfall events and decrease in low and medium rainfall events in India.

In Punjab, the maximum and minimum temperatures have increased by 0.5–1.0°C and by 0.5 to 1.5°C, respectively, in 2010 with respect to the base line 1971–2000 (IMD, 2011). The projection indicates that the mean annual precipitation is likely to increase by 13 to 22% in the mid century. Though the precipitation is highly variable spatially, but the monsoon precipitation shows a definite increase by 11 to 21% across the entire state (Jerath *et al.,* 2014). The minor variations in maximum temperatures, significant increase in minimum temperature at the rate of

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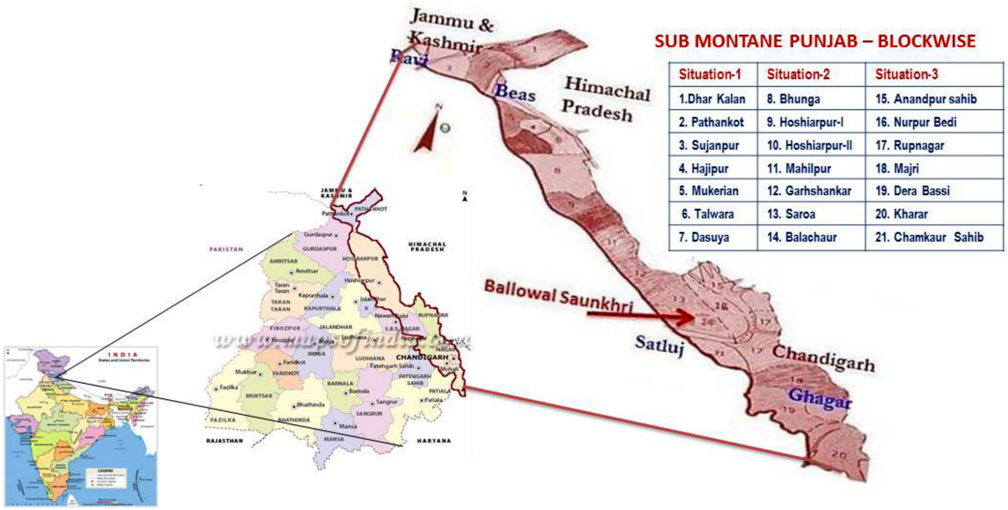

Figure 1. Block-wise representation of submontane region of Punjab

0.07°C/year and significant increase in the annual rainfall (6.5 mm/year) in past three decades at Ludhiana were reported by Kaur *et al.* (2006).India has to face the challenge of sustaining its rapid economic growth in the era of rapidly changing global climate and needs a strong national strategy to adapt to climate change and also to enhance the ecological sustainability. Climate change and its research have gained lot of momentum in recent years and globally it has been scaling down to regional or local concerns. In fact it makes an impetus to our planning and policies on decadal time frame (Li and Calum, 2014). Thus study of regional climatic variability in decadal time scales do possess an immense potential for planning of socio-economic activities. An effort has been made to study and analyse the change in different weather parameters on the submontane region on the foothills of Shiwalik ranges in Punjab.

2. Materials and Methods

Submontane region of Punjab covers nearly 3.93 lakh ha which is about 7.8% of the total area of the state (Figure 1). The area lies in the region of 10–20 km wide strip lying immediately next to Shiwalik hills locally known as "*Kandi*". This zone is located between 30°44′ and 32°32′ N latitude and 75°52′ and 76°43′ E longitude at an elevation of 300–500 m above mean sea level. The climate of the region varies between semi-arid to sub-humid. This region comprises of 21 blocks of 5 districts, namely Pathankot, Hoshiarpur, SBS Nagar, Rupnagarand Ajitgarh. Based upon the annual rainfall and vegetative flora, mainly classified into three situations that is Situation-I (Block – DharKalan, Pathankot, Sujanpur, Hajipur, Mukerian, Talwara and Dasuya), Situation-II (Block–Bhunga, Hoshiarpur-I, Hoshiarpur-II, Mahilpur, Garhshankar, Saroya and Balachaur) Situation-III (Block – Anandpur Sahib, NurpurBedi, Ropar, Majri, Kharar, Derabassiand Chamkaur Sahib).

Regional Research Station (Punjab Agricultural University), Ballowal Saunkhri dist SBS Nagar represents the situation 2 of submontane Punjab and different weather parameters were recorded at this station. The weather parameters like maximum and minimum temperature, rainfall, relative humidity, sunshine hours, wind speed and so on recorded from 1984 to 2013 that is for 30 years have been used to study the climatic variability over the years in this region.

3. Results and Discussion

3.1 Temperature

The annual maximum and minimum temperature in last three decades ranged from 28.5 to 31.0°C and 15.2 to 17.4°C with average values of 30.0°C and 16.2°C, respectively (Table 1). The annual average maximum temperature has shown increasing trends over the years. The annual minimum temperature has shown significant decreasing trends from 1984 to 1997; thereafter significant increase in annual minimum temperature has been recorded (Figure 2). Highest annual maximum temperature was recorded in the year 2002, whereas annual value of minimum temperature was lowest in 1993 and1997.

Among seasons, *rabi* season (October–April) showed the significant increase in the maximum and minimum temperatures over the years; however, no consistent trend of increase and decrease has been observed during *kharif* season (Figure 3). In extreme events, maximum temperature (46.8°C) was observed in the month of June 1995, while extreme minimum temperature(-3.0°C) was recorded during January 2003 followed by -1.0°C during December 1996 (Table 2). The gradual increase in the maximum and minimum temperature was also reported by Hundal and Kaur (2002). Kaur *et al.* (2006) reported in Ludhiana (Punjab)

Table 1. Range and average of maximum and minimum temperature at Ballowal Saunkhri in submontane regions of Punjab (1984–2013)

	Max.Temp. (°C)			Min.Temp. (°C)		
	Annual	***Kharif***	***Rabi***	**Annual**	***Kharif***	***Rabi***
Range	28.5–31.0	34.2–36.0	24.4–27.8	15.2–17.4	22.6–24.8	9.2–12.2
Average	30.0	34.9	26.4	16.2	23.7	10.9
Max.temp.year	2002	2002	1999	1988	2012	2006
Min.temp.year	1997	1990, 1997	1997	1993, 1997	1997	1993
SD	0.63	0.58	0.91	0.58	0.46	0.76
CV	2.04	1.70	3.55	3.55	1.92	7.05

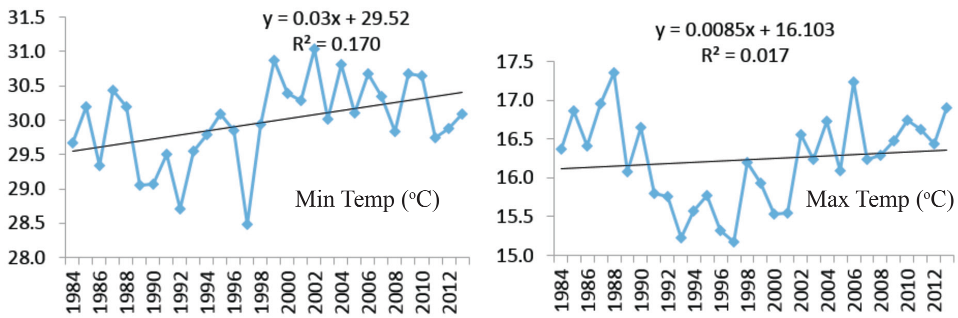

Figure 2. Variation in average annual maximum and minimum temperature (1984–2013)

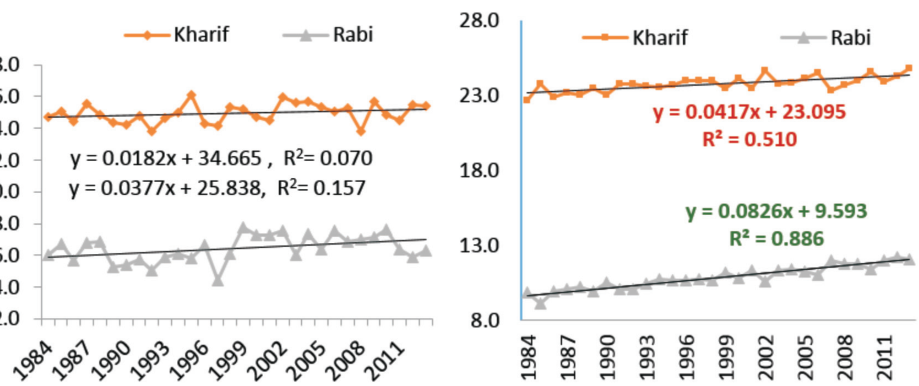

Figure 3. Variation in average seasonal maximum and minimum temperature (1984–2013)

Table 2. Monthly extreme events of maximum and minimum temperature at Ballowal Saunkhri in submontane regions of Punjab (1984–2013)

Month	Extreme Average Max. Temp. (°C)	Year of Extreme Max. Temp.	Extreme Average Min. Temp. (°C)	Year of Extreme Average Min. Temp.
Jan	30.5	2007	-3.0	2003
Feb	32.0	2006	0.0	2001
March	38.2	2010	4.5	2003
Apr	44.0	1999	7.0	1996
May	46.6	1995	13.6	2004
June	46.8	1995	17.6	2011
July	42.3	1987	19.5	2005
Aug	38.4	1987	19.6	2005
Sept	36.2	1987, 2004	13.2	1994
Oct	36.2	2000	8.0	1993
Nov	33.6	2001	1.5	2000
Dec	28.8	1996	-1.0	1996

that maximum temperature has remained near normal over the past three decades as the annual and *kharif* season maximum temperature revealed a slight decreasing trend while the *rabi* season maximum temperature revealed a slight increasing trend. On the other hand, the annual *kharif* and *rabi* minimum temperature have increased significantly at the rate of 0.07°C/year.

Schewe and Levermann (2012) predicted the increasing temperature in the late 21st century and early 22nd century will cause frequent changes and shifts to the monsoon precipitation up to 70% below normal levels. Not only will this affect the Indian summer monsoon, but the onset of monsoon over Southeast Asia may also be delayed up to 15 days in the future as indicated by Ashfaq *et al.* (2009). Hundal and Kaur (2006) examined the climate change impact on productivity of wheat, rice, maize and groundnut crop in Punjab. If all other climate variables were to remain constant, temperature increase of 1, 2 and 3°C from present day condition, would reduce the grain yield of wheat by 8.1, 18.7 and 25.7%, rice by 5.4, 7.4 and 25.1%, maize by 10.4, 14.6 and 21.4% and seed yield in groundnut by 8.7, 23.2 and 36.2%, respectively.

3.2 Rainfall

Total precipitation in the form of rainfall plays an important and significant role in different agricultural practices. The variations in the rainfall are plotted in Figure 4. The declining trend in average annual and *kharif* rainfall has been observed from 1984 to 2013, but *rabi* season did not show any significant change over years. Pal and Al-Tabbaa (2010) and Gill *et al.* (2010) found decreasing trends in the spring and monsoon rainfall and increasing trends in the autumn and winter rainfall in India during 1954–2003. The rainfall of this region ranges from 617 to 2,041 mm with average value of 1,067 mm, out of which about 80% is received during the monsoon period coinciding with *kharif* season (Table 3). The highest average rainfall was received during the month of July (281.6 mm) and August (303.1 mm) and lowest during the months of November (6.7 mm) (Table 4). The highest rainfall (2,041 mm) was received

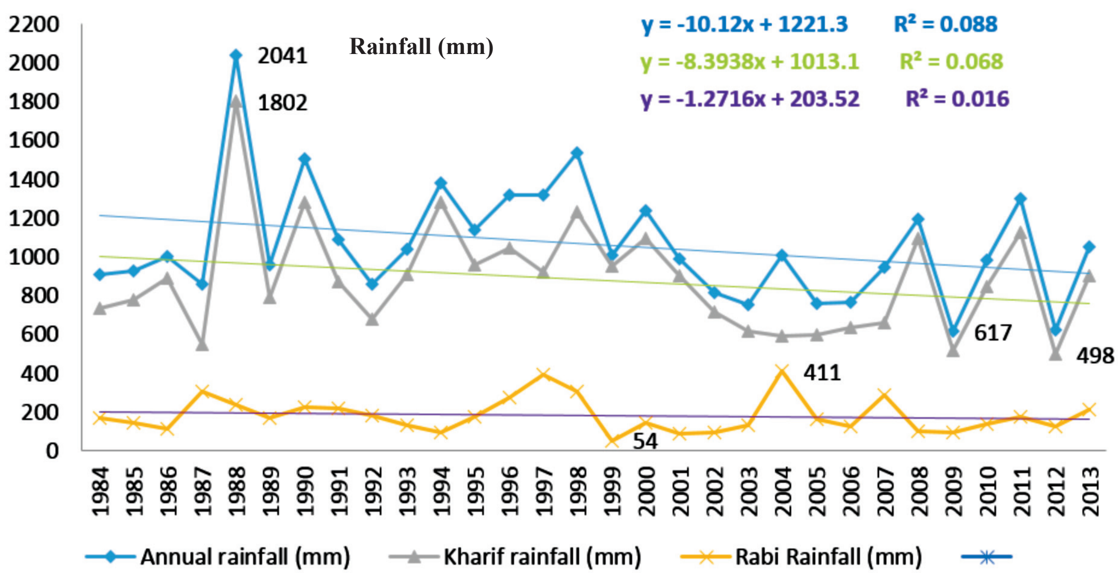

Figure 4. Rainfall pattern at in submontane regions of Punjab (1984–2013)

Table 3. Range and average of rainfall (mm) and rainy days at Ballowal Saunkhri in submontane regions of Punjab (1984–2013)

	Rainfall (mm)			Rainy Days		
	Annual	*Kharif*	*Rabi*	Annual	*Kharif*	*Rabi*
Range	617–2,041	498–1,802	54–411	39–69	25–50	6–24
Average	1067	883	184	50	37	13
Year with minimum value	2009	2012	1999	1984	1987	2002
Year with maximum value	1988	1988	2004	1997	1988	1997
SD	295.7	278.1	87.3	7.9	6.9	4.4
CV	37.7	31.5	47.5	15.7	18.5	31.1

Table 4. Range and average of month-wise rainfall (mm) at Ballowal Saunkhri in submontane regions of Punjab (1984–2013)

	Range	Mean	SD	CV	Minimum Rainfall Year	Maximum Rainfall Year
Jan	0.0–117.5	32.9	28.9	87.9	1986	2004
Feb	0.0–112.8	42.3	34.8	82.3	2006	2007
Mar	0.0–105.6	28.1	23.3	83.1	2004, 2008	2007
Apr	0.0–78.1	19.8	17.6	89.2	2010	1997
May	0.0–114.8	32.5	29.3	90.1	1995	1999
Jun	9.9–435.0	109.9	91.6	83.4	1998	2008
Jul	66.1–769.6	281.6	158.2	56.2	2002	1988
Aug	45.9–549.9	303.1	115.3	38.0	1993	1994
Sep	10.6–594.7	156.0	122.6	78.6	2004	1988
Oct	0.0–229.0	27.8	48.8	175.7	–	2004
Nov	0.0–59.6	6.7	12.5	186.7	–	1997
Dec	0.0–180.9	26.3	41.3	156.7	–	1997
Annual	616.6–2041.1	1066.8	295.7	27.7	2009	1988

during 1988, whereas it was lowest (617 mm) during 2009. The average rainfall during *kharif* (maximum in 2012 and minimum in 1988) and *rabi* (maximum during 2004 and minimum in 1999) season was different from the average annual rainfall. Rainfall ranged from 498 to 1,802 mm with average value of 883 mm during the *kharif* season; however, it was 54–411 mm with average value of 184 mm during the *rabi* season. The seasonal monsoon rainfall for Punjab during the period 1901–2011 was lowest and second lowest during 1911 (-51.0%) and in 1987 (-67.6%), respectively, and highest and second highest in year 1950 (+91.2%) and in 1988 (+119.1%) (IMD, 2012). Krishan *et al.* (2015) analysed the rainfall trends for 17 districts of Punjab and reported that rainfall has indicated an increasing trends in annual, monsoon, pre-monsoon and post-monsoon seasons; however, winter rainfall is found decreasing in 11 districts. The variability of rainfall is likely to increase in Punjab in future.

The number of rainy days along with total rain also plays an important role in agriculture. The number of rainy days in a year varied from 39 to 69 with an average value of 50. During *kharif* season it varied from 25 to 50 with average value of 37 and *rabi* season 6–24 with average value of 13. It has been observed from the data that there is significant decrease in the rainfall quantity over the year, but non-significant difference has been observed in the number of rainy days (Figure 5). It also shows the occurrence of extreme events of rainfall, because maximum rainfall has been observed during the year 1988 but the numbers of rainy days were maximum in 1997. Guhathakurta *et al.* (2011) reported that frequency of heavy rainfall events are decreasing in major parts of central and north India and increasing in

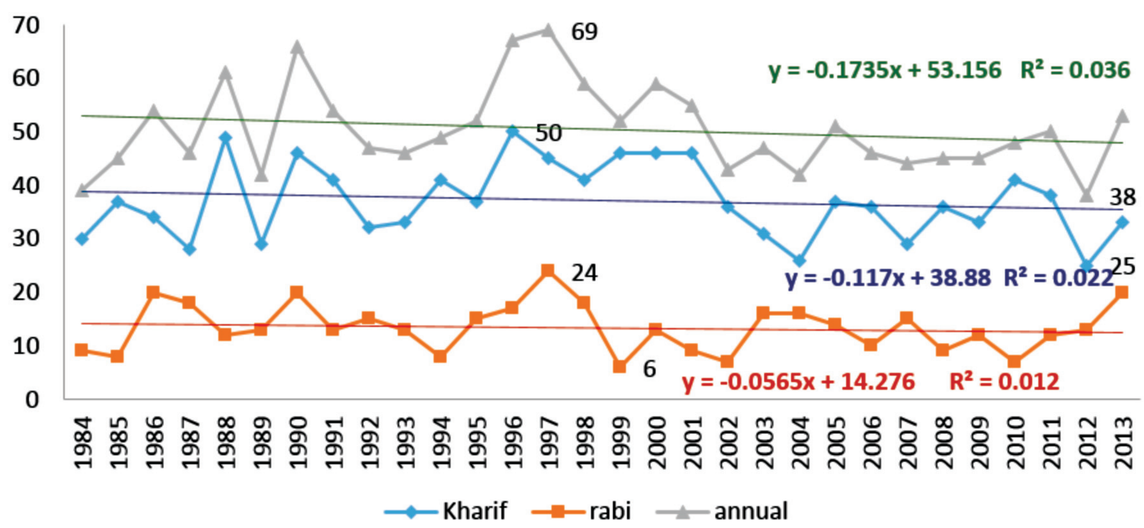

Figure 5. Number of rainy days at in submontane regions of Punjab (1984–2013)

peninsular, east and north east India. However, the extreme rainfall and flood risk are increasing significantly in the country except some parts of central India.

3.3 Relative Humidity

The average annual, *kharif* and *rabi* season relative humidity in submontane region of Punjab showed significant increase over the years and it ranged from 58 to 71, 60 to 74 and 53 to 70 with mean values of 64, 66 and 62%, respectively (Table 5). The annual variations in relative humidity are depicted in Figure 7. The mean average relative humidity (%) during 1990s increased significantly and consistently over the 1980s, whereas it declined after 2002 and again started increasing but inconsistently. The average relative humidity was minimum in the month of April (41%) and May (39%); however was maximum in the month of August (83%) followed by July (78%) and September (77%) due to rainy season (Figure 6).

3.4 Wind Speed

The perusal of the data revealed significant decrease in the wind speed over years (Figure 8). In last 30 years, the average wind speed varied from 2.0 to 5.5 (annually), 2.0 to 5.8 (*kharif* season) and 1.7 to 5.3 km/h (*rabi* season) with

Table 5. Range and average of annual season and month-wise relative humidity (%)at Ballowal Saunkhri in submontane regions of Punjab (1984–2013)

Month	Range	Mean	SD	CV	Year with Minimum RH	Year with Maximum RH
Jan	60–86	74	6	8.2	1984	1999
Feb	53–77	66	6	9	1985	2013
Mar	42–68	57	6	10.5	1985	2013
Apr	29–56	41	6	15.2	2010	1997
May	27–54	39	7	17	1988	1987
Jun	38–74	53	10	19.3	2012	2008
Jul	56–86	78	6	8	1987	2013
Aug	76–89	83	3	3.7	1987	1995–1996
Sep	70–83	77	4	4.7	1987	1998
Oct	54–80	65	6	9.8	1984	2013
Nov	52–79	64	5	8.4	2005	1997
Dec	58–88	70	6	8.7	1984	1997
Annual	58–71	64	3.3	5.2	1984	2013
Kharif season	60–74	66	3.8	5.7	1987	2008
Rabi season	53–70	62	3.7	6.0	1984	1997

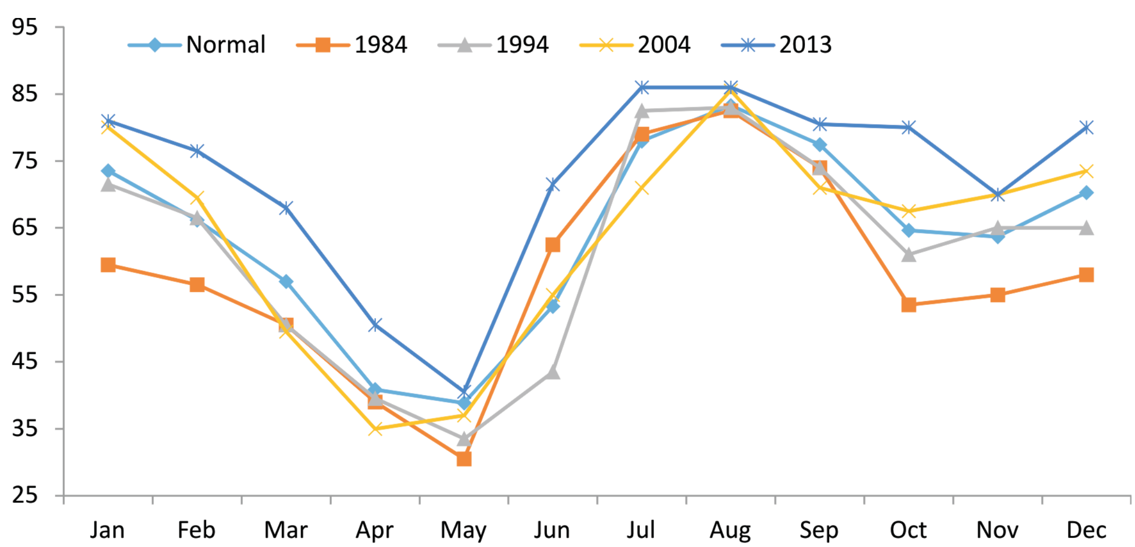

Figure 6. Decade-wise and month-wise relative humidity (%) pattern at in submontane regions of Punjab (1984–2013)

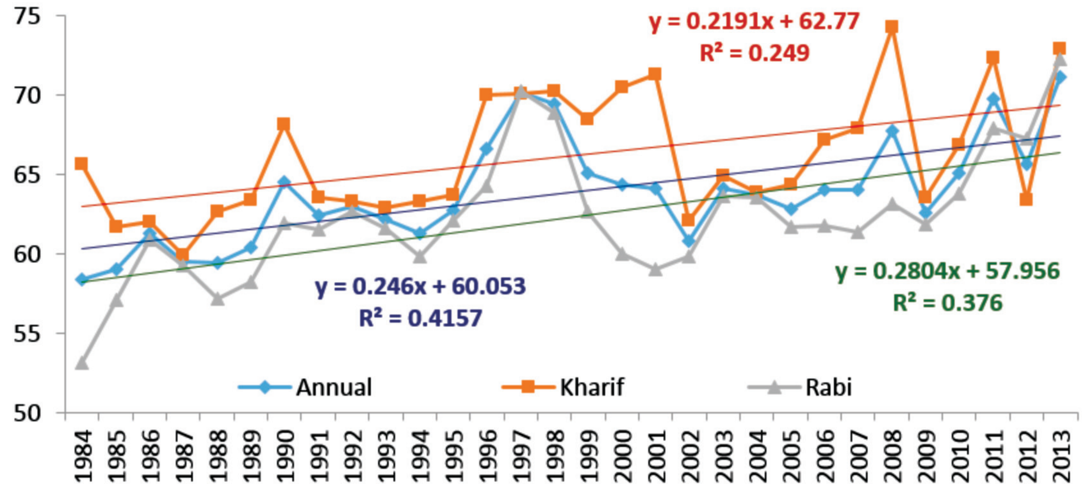

Figure 7. Relative humidity (%) pattern at in submontane regions of Punjab (1984–2013)

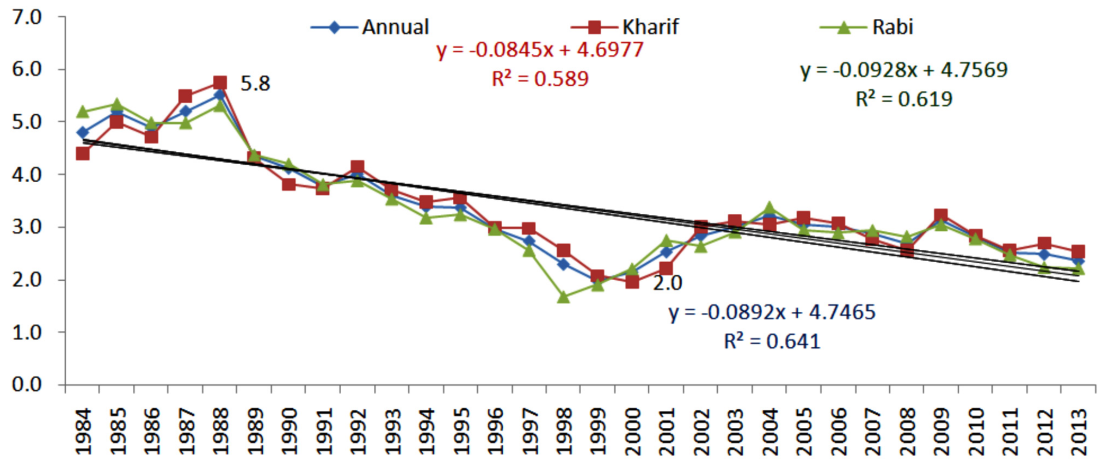

Figure 8. Wind speed (km/h) pattern at in submontane regions of Punjab (1984–2013)

mean values of 3.4, 3.4 and 3.3 km/h, respectively (Table 6). The decrease in wind speed was more severe from 1988 to 2000 and did not show significant difference thereafter. The average wind speed was maximum during the year 1988 (5.5 km/h) and minimum during the year 2000 (2.0 km/h). Wind speed was highest in May (5.0 km/h) and minimum during monsoon season that is September (2.2 km/h) and August (2.3 km/h). Kulkarnia *et al.* (2015) studied the effect of climate change on wind persistence among three offshore regions along the eastern, southern and western parts of the coastline, namely Rameshwaram, Kanyakumari and Jakhau, recorded decrease in wind persistence for future of the order of 3% and 8% at Rameshwaram and Jakhau, respectively, and an increase by around 3% at Kanyakumari.

3.5 Sunshine Hours

The study of the sunshine hours over the years revealed that average annual sunshine hours varied from 7.0 to 8.7 with mean value of 7.9 hours. The sunshine hours showed significant decrease in the sunshine hours over years (Figure 9). The maximum sunshine hours (8.7) was recorded in the year 1987 and minimum (7.0) in the year 2011. The sunshine hours were highest from April (9.5), May (10.0) and June (8.8) mainly due to clear sky and longer day lengths and minimum during July (6.1), August (6.2) and January (6.2) due to cloudy weather (Table 7) in the monsoon months and dense fog in the month of January as this region is situated in the foothills of Shiwalik ranges and more prone to fog. Soni *et al.* (2012) observed that the decline in global solar radiation (solar dimming) continues over India and the decrease in sunshine duration was concomitant with the decrease in global solar irradiance. Changes in aerosol loading and cloud cover, as well as cloud properties, are the most probable causes for the reduction in surface solar radiation.

Table 6. Range and average of annual season and month-wise wind speed at Ballowal Saunkhri in submontane regions of Punjab (1984–2013)

	Range	Average	SD	CV	Min. Average Wind Speed	Max. Average Wind Speed Year
Annual	2.0–5.5	3.4	0.9	28.0	1999	1988
Kharif	2.0–5.8	3.4	1.0	28.0	2000	1988
Rabi	1.7–5.3	3.3	1.0	29.4	1998	1985, 1988
Jan	0.4–4.7	2.7	1.0	36.7	1998	1988
Feb	1.2–6.2	3.6	1.1	30.8	1998	1988
March	2.5–7.4	4.3	1.3	29.9	1998	1985
Apr	2.9–6.9	4.8	1.1	23.6	2013	1988, 1984
May	3.2–7.9	5.0	1.2	23.2	2012	1988
June	2.6–7.7	4.5	1.2	26.7	1998	1988
July	1.5–6.5	3.0	1.1	37.6	2000	1987
Aug	1.2–5.2	2.3	0.8	36.4	1999, 2001	1987
Sept	0.6–4.1	2.2	0.8	37.2	2000	1988
Oct	0.6–5.3	2.6	1.0	37.4	1999	1987
Nov	1.1–4.9	2.6	0.9	34.6	2000	1987
Dec	0.8–4.9	2.5	0.9	36.6	1999	1985

Table 7. Range and average of annual season and month-wise sunshine hours at Ballowal Saunkhri in submontane regions of Punjab (1984–2013)

	Range	Mean	SD	CV	Minimum Average Sunshine Hours	Maximum Average Sunshine Hours
Annual	7.0–8.7	7.9	0.4	5.1	2011	1987
Kharif	6.8–9.0	7.8	0.6	7.7	2011	1987
Rabi	7.0–8.8	8.0	0.5	6.3	2013	1988
Jan	4.0–8.1	6.2	1.1	17.4	2003	2007
Feb	5.3–8.9	7.3	1	14.0	2011	2004
Mar	6.5–9.7	8.2	0.7	8.5	1992	2004
Apr	7.8–10.7	9.5	0.7	7.4	2013	2007
May	8.1–11.2	10	0.7	7.2	2009	1988
June	5.9–11.4	8.8	1.3	14.9	2008	1987
July	4.1–9.5	6.1	1.3	20.8	2000	1987
Aug	3.6–8.3	6.2	1.1	18.2	2012	1986
Sep	6.0–9.5	7.9	0.8	10.3	1998	2001
Oct	6.4–10.4	9	0.9	10.3	2013	1988
Nov	6.9–9.8	8.4	0.7	8.1	2011	1987
Dec	3.5–8.5	6.8	1.1	16.0	1997	1990

4. Conclusions

The analysis of 30 years data of Ballowal Saunkhri in submontane Punjab revealed that annual average maximum temperature has shown increasing trends over the years and increase was non-significant in annual minimum temperature. The declining trend in average annual and *kharif* rainfall was observed over the years. The average annual relative humidity showed significant increase over the years and ranged from 58 to 71% with mean value of 64%. The wind speed and sunshine hours also showed significant decreasing trends over years.

The present study has great applicability for the submontane region of Punjab, especially for selection of crops for the region, crop diversifications, implementation of improved agronomic practices and incidences of diseases and pests.

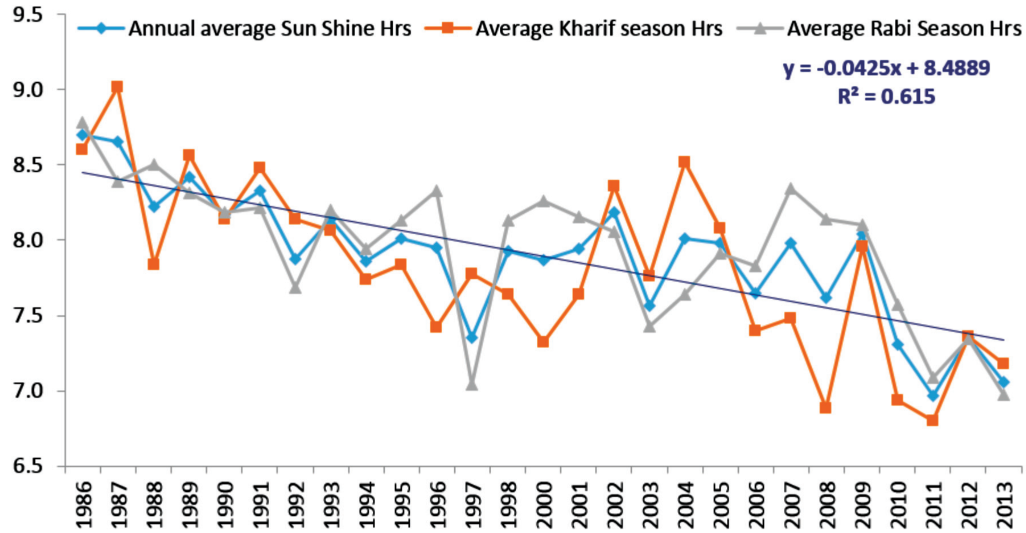

Figure 9. Average of annual season and month-wise sunshine hours in submontane regions of Punjab (1986–2013)

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GENERAL ARTICLE

Caterpillar Fungus Gold Rush: Growing Dependence on a Lucrative Trade with Disputes among Communities in the Himalaya

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Abstract Caterpillar fungus (*Ophiocordyceps sinensis*) is a flagship species of the Himalaya and is one of the world's most expensive natural medicinal resources. In the Nanda Devi Biosphere Reserve and Dharchula–Munsiyari landscape of the western Himalaya, it inhabits ioslated patches of alpine grasslands (elevation from 3,000 to 4,500 masl) of Mana, Niti, Rishi, Pindar, Gori, Dhramganga and Kali Valleys. Although there was a steady increase both in price and demand of the resource, overall harvest at the local level was decreasing and the number of harvesters were increasing every year. With the gradual increases in the market value of *O. sinensis*, the dependency of local communities was becoming more prominent on the income generated through its collection, whose livelihoods were earlier based on pastoral and agricultural activities. Caterpillar fungus played a significant role in the economy of communities who were living in the region. Thus, the caterpillar fungus harvest-boom was facilitating the integration of rural upper Himalayan households into regional, national and international economic cycles by providing the necessary product and cash in exchange for sharing in this commodity trade. There was a drastic growth in the economy empowerment of villagers and in contrast the growing dependence of the local community on this remarkable gold rush had promoted violent confrontation among harvesters.

Key words NTFPs, Caterpillar fungus, Biosphere reserve, Alpine meadows, Himalaya

1. Introduction

In India, natural resources and people are inextricably linked since millions of people live adjacent to or within protected areas and harvest forest products (Davidar *et al*., 2010). However, often the human pressure on natural resources is not sustainable and can result in habitat loss and degradation (Sagar and Singh, 2004; Arjunan *et al*., 2005), and together with intensive livestock grazing can reduce carrying capacity, that is the net primary productivity available for herbivores in a year (Madhusudan, 2005). Improving the sustainability of relationship of humans with the natural resource is firmly established as a societal goal for the twenty-first century. Over the last two decades, devolution of resource management and access rights from the state to local communities and user groups has become an important policy tool in developing countries (Shackleton and Pandey, 2014). The sustainability of non-timber forest products (NTFPs) extraction for the long-term ecological integrity of forests depends on a variety of considerations, including its importance to the local economy, possibility of alternative sources of income to the people, ecological impacts of NTFP extractions and legal status of the forests (Mutenje *et al*., 2011).

During the last two decades significant progress has been made in understanding the role of biological resources in the lives of poor people, particularly their contribution to the household economy and their role in alleviating poverty (Vira and Kontoleon, 2013). Trade and collection of NTFPs including medicinal plants have a long history in the

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Himalayas (Olsen, 2005), but harvesting of Caterpillar fungus has become extremely popular in recent years, surpassing all other species in terms of revenue. The caterpillar fungus (*Ophiocordyceps sinensis*), locally known as Kira Jari (in India), Yartsa Gunbu (in Tibet) and Yarsa gumba (in Nepal), is among the most valuable NTFP products in the world almost like gold and plays a major role for the local economies in its distribution area on the Tibetan Plateau and adjacent regions. Large proportion of its habitat in the Indian Himalaya has been designated under the protected area network.

2. Caterpillar Fungus (*Ophiocordyceps Sinensis*)

The caterpillar fungus is a parasitic species of fungus and is endemic to the Himalaya and the Tibetan Plateau including the adjoining high altitude areas (between 3,000 and 4,500 msl). In the western Himalaya, it has been documented in alpine meadows of protected area like Nanda Devi Biosphere Reserve and Askot Wildlife Sanctuary (Figure 1). *O. sinensis*, belonging to the family Ophiocordycipitaceae, is a parasitic fungus on Lepidopteran larvae. It infects the caterpillars of ghost moths (family Hepialidae) and produces a fruiting body (stroma) which emerges from the head of the larvae and eventually kills it by paralysis and mummification. As the insect is the sole source of food for the fungus, the size of its stroma is dependent on the size of host caterpillar (Negi *et al.,* 2014). Caterpillars prone to infection by *O. sinensis* generally live 5–7 in underground and thrive in subalpine and alpine grasslands or meadows as well as open dwarf scrublands around the potential timberline.

For centuries, caterpillar fungus has presumably been used in traditional Tibetan and Chinese medicine as a tonic, aphrodisiac and as relief medicine for lung, liver and kidney problems (Holliday and Cleaver, 2008; Zhou *et al*., 2009). Nevertheless, it is widely traded as an aphrodisiac and a powerful tonic in the name of 'Himalayan Viagra' (Winkler, 2009; Shrestha and Bawa, 2013). Global trade of caterpillar fungus rapidly expanded after the 1993 World Athletic Championships in Stuttgart, Germany, when Chinese athletes reportedly training on dietary supplements of *O. sinensis* and turtle blood set multiple records in distance running (Winkler, 2010). Now-a-days, it is the world's highest-priced biological lucrative trade, more expensive by weight than gold (The Economist, 2015). Best quality fungus in China fetched up to US\$100,000 kg^{-1} in March 2012, and in Singapore it reached US\$ 130,000 kg^{-1} at a time when the price of gold was about US\$68,000 kg^{-1} (Shrestha and Bawa, 2013).

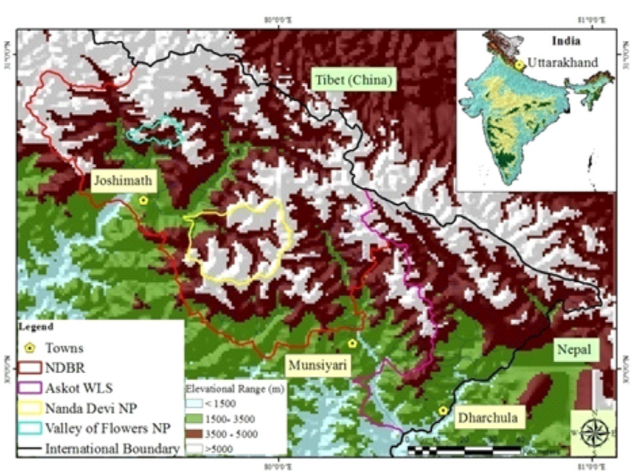

Figure 1. Location of caterpillar fungus' inhabited protected areas in Uttarakhand

3. Harvesting Techniques and Collection Period

The collection season starts in the beginning of May and lasts till the end of June. The collection period depends on many factors like the local weather, condition of snow in the pasture and elevation of the collection site. The harvesters recline on the ground over the high-altitude expanses, attentively scanning the terrain. It is a difficult task requiring attention and tolerance for harvesting. Indeed, the height and thickness of caterpillar fungus are so small almost like stalk of the apple that it cannot be easily seen. During spring the ground is covered with short vegetation stumps as brownish as the small caterpillar fungus. But mountain people work hard since it is considered particularly strenuous, the enterprise is highly profitable. Caterpillar is first dug out of the ground it is covered in dirt (Figure 2A) and the best way to remove this layer is with a toothbrush. During cleaning process careful consideration must be taken not to damage or break the caterpillar fungus. After drying in shade, species is ready to trade (Figure 2B) and people store it on dray place to save from moisture.

A B

Figure 2. Caterpillar fungus: uprooted from the ground (A) and cleaned and dried which is ready to sell (B)

4. Medicinal Value of Caterpillar Fungus

The caterpillar fungus is one of the most highly priced natural resource used in traditional oriental medicine. It was discovered about 1,500 years ago by Tibetan herdsmen who observed their livestock become energetic after eating certain mushroom. Consequently, the King's physicians in Ming Empire explored to develop powerful and potent medicines.

It is usually consumed by cooking with aged duck to treat patients suffering from cancer and asthenia, or cooked with hen's meat to treat hypo-sexuality and male impotence, especially emission (Jiang, 1994). Moreover, it is also cooked with pork, sparrow and turtle to treat fatigue (Miller, 2009). In some parts of Nepal, *O. sinensis* is powdered and combined with the rhizome of *Dactylorhiza hatagirea* for consumption (Devkota, 2006). A combination is made with *D. hatagirea,* honey and cow's milk for tonic and aphrodisiac (Lama *et al*., 2001). It has also been reported to possess a range of more specific therapeutic properties, including action against asthma and bronchial inflammation (Kuo *et al*., 2001), cure of renal complaints (Guo and Yang, 1999), stimulation of the immune system (Kuo *et al*., 2005), potent cytotoxic effect on various human cancer cells, including human lung carcinoma cells (Park *et al*., 2009) irregular menstruation (Zhu *et al*., 1998; Francia *et al*., 1999) and anti-inflammatory (Qian *et al*., 2015).

5. Harvesting and Trade of the Caterpillar Fungus

The collection session starts at the beginning of May till the end of June. Although, collection period depends on many factors like local weather, condition of snow in the pasture and elevation of the collection site. Most of the harvesting areas are located on the north-facing slopes of the mountains. The gatherers recline on the ground over the high-altitude expanses, attentively scanning the terrain. It has been observed that when the caterpillar is first dug out of the ground it is covered in dirt and the best way to remove this layer is with a toothbrush. Careful consideration must be taken in order to avoid damage to the caterpillar during the harvesting and cleaning process. It is a challenging task, requiring careful attention and patience for harvesting. Indeed, the height and thickness of the fungus is so small that it cannot be easily seen. During spring, the ground is covered with short vegetation stumps, which appear as brown as the small caterpillar fungus. Despite being a strenuous process the collectors put in immense efforts as the enterprise is highly profitable.

Due to resource scarcity and high publicity, both demand and price of caterpillar fungus have very high causing high competition among harvesters and traders. Over the last decade Himalayan villagers have become astute to the commercial potential of caterpillar fungus. After harvesting it, the produce is sold to the traders. These traders feed the growing demands in Asia's fast growing urban centres, as well as that of the western countries. In India, a single fungus sells for about US$4.00 to US$7.00, depending on the health and size of the fungus while traders sell the product to wholesalers or exporters for US$12,365 to US $18,307 kg^{-1} (Yadav, 2016). About 5–6 years ago, people could collect around 55 to 60 individuals, but due to engagements of more people in the harvesting, now villagers can scavenge as many as 15 to 20 of these per day, making it a new gold rush for the Himalaya. Owing to the upsurge in consumer demand for this ingredient in the last decade, local people have been gathering more quantity of caterpillar fungus over the high-altitude expanses in upper Himalayan regions and this activity has become one of their most prosperous sources of income in the landscape.

6. Opportunity and Challenges for the Community

The villagers who harvest caterpillar fungus in the Nanda Devi Biosphere Reserve and Dharchula–Munsiyari landscape belong to the marginal community, historically shepherds, porters and traders. Woolen handlooms and beverage production are the traditional cottage industries. Investigation for socio-economic contribution of caterpillar fungus among mountain dwellers illustrates that stream of cash income to harvester from this gold rush has caused a far-reaching revolution in social and economic conditions in the last 12–15 years. During our filed visits in the study area harvesters informed us that as early as 2 years, after initiating harvesting of caterpillar fungus, the households' income from its trading during the month of June, July and August had increased tremendously. Harvesters spend this income on child education, family healthcare and subsistence needs for whole year. Furthermore, they do not have to rely completely on agriculture which again is subjected to rainfall and wildlife depredation. Thus, the income derived through the collection and trade of this precious fungus has led to an enhanced empowerment of marginal communities, often living in extremely remote locations, who used to secure their survival only through pastoral and agricultural activities. Furthermore, the cash influx has led to a commoditization of local production and services. Thus, the caterpillar fungus boom is facilitating the integration of rural upper Himalayan households into regional, national and international economic cycles by providing the necessary product and cash in exchange. Although a proper scientific investigation is required to establish these assumptions.

But there is dark side also to the harvesting of caterpillar fungus. In addition to having to brave harsh climates to find caterpillar fungus, its rarity means that there are no guarantees that a collector will find anything at all. Some villagers return with nothing to show, for their weeks of hardship in high altitude snow fields and many falls ill. People often return to the village with snow-blindness, painful joints and problems of breathing. In the past, community disputes mostly occurred over grazing rights, now they are mostly fought over access to caterpillar fungus resources, and some of these turn violent. Thousands of villagers go for mass-collection of the species each year, along with their tents, food, other consumables and domestic

Figure 3. Poor logistics is representing challenges for the harvesters in the alpine meadows

Figure 4. Harvesters' camps and cattle in alpine pasture during harvesting season

animals. These huge aggregations in the remote pastures are bound to destroy the pristine nature of the ecosystems and the threatened species that inhabit them (Figures 3 and 4). Local people and ecologists alike have been complaining about the sharp decline in the abundance of the caterpillar fungus as well as the destruction of the habitats in the concerned areas within a span of a few years. Ultimately, increasing trade-induced over-harvesting seems almost certainly responsible for declining populations of the caterpillar fungus, which needs to be assessed more scientifically.

7. Discussion and Conclusion

Hundreds of villagers go for the mass-collection of the species each year, carrying with them tents, food, other consumables and domestic animals. These huge aggregations in the remote pastures are bound to destroy the pristine nature of the ecosystems and the threatened species that inhabit them. Despite increase in price and demand of caterpillar fungus, results show that the harvest at local level is decreasing and on other hand number of harvesters has increased. Ultimately, increasing trade induced over-harvesting seems almost certainly responsible for declining populations. Over harvesting and decreasing population of the caterpillar fungus is leading the species towards extinction from natural habitat of its occurrences. Other studies Shrestha and Bawa (2013, 2014), Winkler (2009) and Stone (2008) also show that over-harvesting is one of the primary causes of population decline of the species. Studies conducted by Negi *et al*. (2014) and Sharma (2004) in the Dharchula–Munsiyari region conclude that since it was discovered in those areas by the villagers, massive exploitation has occurred, leading to a drastic decrease in populations. The regulation of rampant exploitation and implementation of scientific sustainable harvesting is thus the need of the hour for the sustainability of the species.

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COMMENTARY

Cyanobacterial Biotechnology: An Opportunity for Sustainable Industrial Production

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Abstract In this communication, a comprehensive commentary on contribution of cyanobacteria and micro-algae in well being of humans and environment has been postulated. In the recent years cyanobacteria and micro-algae have gained much more attention because of their valuable biotechnological applications. These valuable bio-agents are potential source of various compounds such as exopolysaccharides, lipids, proteins, vitamins, sterols, enzymes, pharmaceuticals and other valuable life supporting chemicals. Secondary metabolites derived from cyanobacteria and micro-algae have been reported to be beneficial as antibacterial, antifungal, antiviral, anticancer, anti-malarial and immunosuppressive properties and can open new doors to develop more effective drugs in agriculture, industrial and human welfare. Some cyanobacteria and micro-algae can accumulate polyhydroxyalkanoates intracellularly; having similar properties to polyethylene and polypropylene. These biodegradable plastics can be alternative to oil-derived thermoplastics incoming future. An inclusive knowledge on the diversity, physiology and inherent genome organisation of cyanobacteria and their beneficial genetic manipulations may be helpful to provide better opportunity for sustainable industrial products and environmental development. This article remarks meaningful information about the possible uses of cyanobacteria and micro-algae in industry sector and also expresses an outlook on the challenges and future prospects of cyanobacteria and micro-algal biotechnology.

Keywords Biotechnology, Cyanobacteria, Colorants, Micro-algae, Nutrition, Pharmaceuticals, PHA,

1. Introduction

Cyanobacteria (Blue-Green Algae, BGA) comprise a highly diverse group of prokaryotic microorganisms throughout the globe performing oxygenic photosynthesis (Garcia-Pichel and Pringault, 2001; Kulasooriya, 2011). Oxygen generated by cyanobacteria helped the ancient reducing atmosphere of the earth to an oxidising one (Olson, 2006) and facilitates the evolution of biodiversity on the planet Earth. It is also suggested that origin of chloroplasts of eukaryotic algae and higher plants have developed from endosymbiotic relationships by cyanobacteria. This event in the early evolution of life has stimulated the advent of oxygen tolerant flora and fauna capable of aerobic respiration, resulted in the predominance of oxygenic and aerobic species diversity globally (Kulasooriya, 2011). Cyanobacteria have been reported to flourish in different morphological forms ranging from unicellular to filamentous (Castenholz and Phylum, 2001). While unicellular cyanobacteria exist as single cells, suspended or benthic, or aggregate while filamentous structures either thin or thick, single trichome or bundles with or without a sheath (Abed *et al.*, 2009).

Micro-algae are evolutionarily diverse, unicellular, eukaryotic organisms, use sunlight to produce biomass and oxygen from carbon dioxide (CO_2), water and simple mineral nutrients (Kulasooriya, 2011). In most of the terrestrial (paddy soils), aquatic ecosystems (marine waters, hypersaline, brackish waters, soda lakes, freshwater etc.) and extreme environments (hot springs and Polar Regions), cyanobacteria and micro-algae are the primary producers at the base of the food web in an ecosystem. Cyanobacteria have been reported to exists as symbiont in association with a variety of organisms, that is the marine diatom *Rhizosolenia*, leaves of *Azolla* and the roots of *Cycas* (Thajuddin and Subramanian, 2005; Abed *et al.*, 2009). Cyanobacteria and micro-algae, an interesting natural source of diverse bioactive compounds, can be used as functional ingredients of several medicines, cosmetics and new

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generation food resources (Singh *et al*., 2011a–c; Singh and Singh, 2013; Singh, 2014; Kumar and Singh, 2016). The high biomass producing attributes within a short duration make cyanobacteria and micro-algae as promising biological agents for production of high-value industrial products for human welfare [Prasanna *et al*., 2010; Priyadarshani and Rath, 2012).

However, recent screening programmes for the discovery of bioactive compounds from cyanobacteria and micro-algae show unique similarities with chemical compounds derived from plants and animal products. At present, the attention of scientific communities is on identifying the antiviral, antibacterial, anti-mitotic, antifungal, anti-coagulating, hem-agglutinating anti-helminthic and toxic metabolites (Beccari *et al*., 2009; Bui *et al*., 2014). Cyanobacterial and micro-algal metabolites are also now explored as important sources of pharmacologically active compounds useful in diagnostics or pigments as fluorescent probes and as nutraceuticals and food/feed supplements (Singh, 2015a–c; Singh *et al*., 2016a,b). Allelopathic interactions between cyanobacteria and micro-algae have been reported for the tremendous implications for interactive biology (Vimal *et al*., 2017). The chemicals produced by these unique bio-agents may also make these as a competitive advantage and permit proliferation in particular environments, especially eutrophic lakes or undisturbed marine habitats. The algal water blooms, a widespread phenomenon reported from different geographical area of the world, presents a considerable threat to the flora and fauna, risk to human health, with specific socioeconomic impact. These algal blooms are also found to be a rich source of secondary metabolites having new and significantly improved chemicals and molecular structures (Mundt *et al*., 2001; Prasanna *et al*., 2010; De Morais *et al*., 2015). Many of these have pharmaceutical value frequently used as life saving drugs in critical problems such as hepatotoxins (liver damaging), neurotoxin (nerve damaging), cytotoxins (cell damaging) and toxins responsible for allergic reactions. Over the past decade, cyanobacteria have also been recognised as a primary source of novel classes of pharmacologically active natural products with potential therapeutic applications in the treatment of cancer and HIV-related diseases (Prasanna *et al*., 2010). Though the information related to cyanobacteria and micro-algae in soil fertility enhancement, bio-fuel and bio-energy is available in abundance but, literature on cyanobacterial tools in production of industrial products is very limited. So, this communication discusses the sustainable industrial products generation in benefits of agriculture and environment from cyanobacteria and micro-algae.

2. Secondary Metabolites

The cyanobacteria and micro-algae have a significant attraction as a natural source of bioactive molecules with the wider range of activities, such as antibiotics, anti-virals, anti-tumoural, antioxidant and anti-inflammatory (Figure 1) (Patra *et al*., 2004). Micro-algae are one of the largest

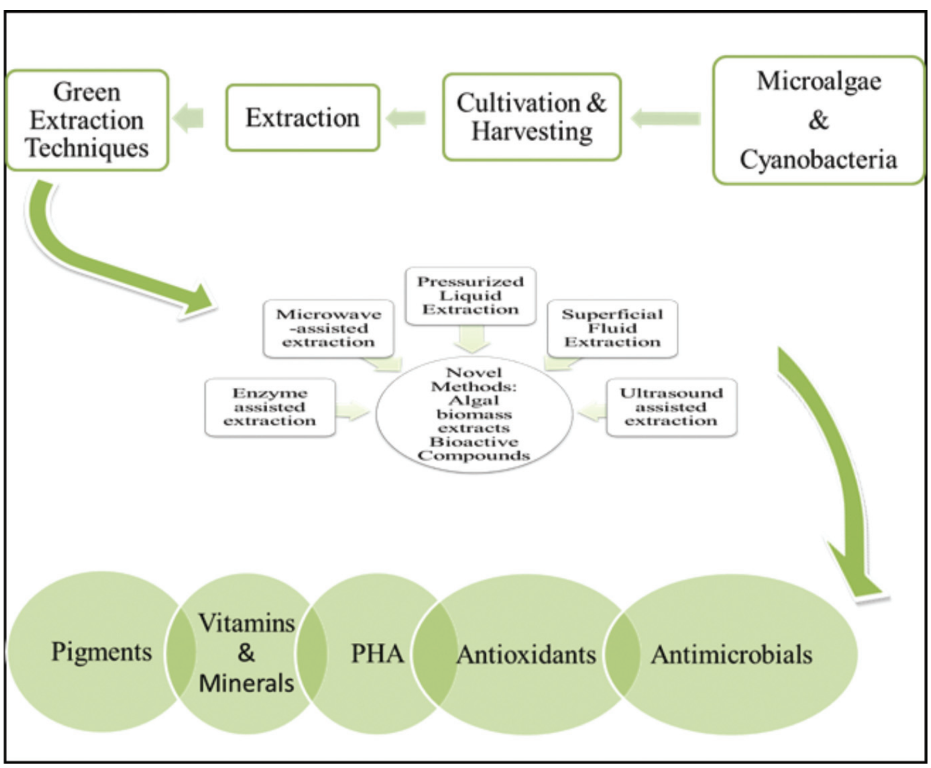

Figure 1. Novel extraction methods for production of bioactive compounds from bio-agents

Table 1. Cyanobacterial species as a source of various vitamins

Vitamins	Cyanobacteria
B_1 (Thiamine)	*Nostoc*
Pentothenate	*Oscillatoria*
Nicotinic acid	*Nostoc*
B_{12} (Cobalamine)	*Anabaena*, *Chroococcus*, *Oscillatoria*, *Nostoc*, *Spirulina*
Biotin	*Nostoc*
Inositol	*Spirulina*
Vitamin E	*Spirulina*

Source: Prasanna *et al*. (2010).

producers of biomass across different ecosystems and produce a large number of chemically active metabolites in their surroundings, potentially protect themselves against the other invading organisms. These active metabolites called as biogenic compounds, such as halogenated compounds, alcohols, aldehydes, terpenoids which are produced by several species of micro-algae and have antibacterial, anti-algal and antifungal properties, useful in the prevention of bio-fouling and have other uses like in therapeutics (Bhadury and Wright, 2004). More recently, research has turned to the creation of novel structures (micro/nanospheres, polymer beads and capsules) with safe edible materials, wherein bioactive compounds (e.g. antioxidants, vitamins, probiotics or prebiotics) are encapsulated. Encapsulation techniques are designed to protect the bioactive substances and to promote their controlled release.

2.1 Vitamins and Minerals

Cyanobacteria and micro-algae synthesises vitamins such as tocopherols, ascorbic acid, B_1, B_2, B_6, B_{12}, nicotinic acid, biotin and so on as well as macrominerals (Na, K, Ca and Mg) and micro-minerals (Fe, Zn, Mn and Cu) (Table 1). *Spirulina*, *Anabaena* and *Nostoc* are used as human nutrition in many countries including Chile, Mexico, Peru and Philippines. *Arthrospira platensis* (misidentified as *Spirulina platensis*) is commercially harvested in either outdoor ponds or sophisticated bioreactors and sold in the markets in the form of powder, flakes, tablets and capsules. It is a very nutritive and easily digestive food supplement; possess more than 60% proteins of total dry weight and also rich in beta-carotene, thiamine and riboflavin. It is also considered to be one of the richest sources of vitamin B_{12} (Abed *et al*., 2009).

It is reported that 1 g *Spirulina* contains about 0.5–2.0 mg of vitamin B_{12}, a peculiar herbal product, which shows a close phylogenetic link between these algae and bacteria that produce vitamin B_{12} (Kelman *et al*., 2012). Thus, *Spirulina* may be used as rich source of vitamin B_{12} and may be included in the diets of vegetarians. Extraction of vitamins from cyanobacteria and micro-algae has received increasing attention due to the high antioxidant activity associated with this family of compounds. Besides its well-known antioxidant activity, recent studies have demonstrated that certain synthetic vitamins are less effective than natural vitamins (Kelman *et al*., 2012). At present, they are also cultivated in wide open raceway ponds with paddle wheel stirring under high pH and high temperature to minimise contamination by human pathogenic microorganisms. Such commercial production facilities are today found in the United States, Thailand, India, Taiwan, China, Pakistan, Myanmar and Chile (Thajuddin and Subramanian, 2005). However, some of the claims of the high nutritive value of these cyanobacterial products have been questioned. Kulasooriya (2011) reported that *Spirulina* contain a pseudo-Vitamin B_{12} stated that it cannot replace the proper Vitamin B_{12} requirement, particularly incomplete vegetarian diet? The consensus appears to be that cyanobacterial-based food supplements could provide a high level of nutrition particularly to poor sectors of the population who are malnourished, but they should not be considered as a replacement diet for animal protein sources such as fish, eggs, meat and milk.

2.2 Plant Growth Promoting Substances and Allelopathic Chemicals

Some cyanobacteria and micro-algae are reported to produce naturally occurring phytohormones. A variety of microbes is known to synthesise indole-3-acetic acid (IAA), including soil, epiphytic and tissue colonising bacteria. In fact, it has been suggested up to 80% of bacteria isolated from rhizosphere (the area around the roots of plants) can produce IAA. *Nostoc*, *Chlorogloeopsis*, *Calothrix*, *Plectonema*, *Gloeothece*, *Anabaena*, *Cylindrospermum* and *Anabaenopsis* are capable of IAA biosynthesis. The independent tryptophan pathway, more common in plants, is also found in microorganisms like *Azospirillum* and cyanobacteria. Cyanobacteria can benefit plants by producing growth promoting regulators/hormones, that is gibberellin, cytokinin and auxin-like compounds or abscisic acids (Table 2) (Prasanna *et al*., 2010; Singh *et al*., 2016a,b; Vimal *et al*., 2017).

Table 2. Phytohormone produced by some cyanobacteria and micro-algae

Phytohormones	Cyanobacteria
Auxins	*Anabaena*, *Anabaenopsis*, *Calothrix*, *Chlorogloeopsis*, *Cylindrospermum*, *Glactothece*, *Nostoc*, *Plectonema*, *Synechocystis*
Gibberellins	*Anabaenopsis*, *Cylindromum*
Cytokinins	*Anabaena*, *Chlorogloeopsis*, *Calothrix*

Source: Prasanna *et al*. (2010)

Some heterocystous and non-heterocystous cyanobacteria can fix atmospheric nitrogen to plant usable inorganic-N (Singh and Seneviratne, 2017a,b). It is also reported that the nitrogen-fixing cyanobacteria have enriched the fertility of many tropical rice field soils and it is estimated that cyanobacteria added about 18 kg N/ha/year to the soils. Therefore, it is assumed that a more advanced technology related to cyanobacterial inoculants could be beneficial to increase the fertility of soils the through the inoculation of cyanobacteria inoculants in agriculture. Garcia-Pichel and Pringault (2001) obtained that the addition of *Azolla* supports the growth of soil microorganisms including heterotrophic N_2 fixers. It is also found that nitrogen-fixing cyanobacteria successfully dominate the desert crusts worldwide (Garcia-Pichel and Pringault, 2001) and contribute significantly to the fertility of desert soils and may eventually facilitate vegetation of deserts (Abed *et al*., 2009). Based on the importance of cyanobacteria in enriching the soil fertility and degraded land restoration, it is commented that these bio-agents can be cultivated on commercial scale in the various laboratory and industries and can be used to get rid of from lethal chemicals in agriculture industry.

Cyanobacteria and eukaryotic algae are known to excrete bioactive compounds into the environment, which are important determinants of allelopathic activity in water and soil. Allelochemicals are secondary metabolites or non-nutritional primary metabolites that affect growth, reproduction or behaviour of individuals other than the ones producing them or influence the structure and dynamics of populations or communities of either plants or animals or microbes (Mundt *et al*., 2001). Allelopathic chemicals play a role in the interactions between the emitter organisms and their direct competitors or predators; they are categorised according to their toxic stimulatory effect on several organisms, including some that may not be present in their immediate environment (Prasanna *et al*., 2010). In recent years, the prospects of exploiting allelopathy as an alternative strategy for controlling weeds, insects and diseases gaining much attention.

2.3 Antioxidants

Due to very fast life and changed modern life style, we are getting experience with a lot of stresses and mental problems. Recent decades have witnessed a growing interest in compounds with antioxidant properties because such compounds can play favourable roles in get rid of stresses due to fast and restless working style. This expands the role of antioxidants from classically functioning as a food preservative to that of a therapeutic, which can be either ingested in native foods or as a part of formulated functional foods. Carotenoids are potent biological antioxidants that can scavenge the singlet oxygen radicals. The radical scavenging behaviour of antioxidants protects the DNA, proteins and membrane lipids from damage. Therefore, production of these compounds in cyanobacteria at larger scale after bioprocess through photoautotrophic biotechnology optimisation urgently required (Muller-Feuga *et al*., 2003). Cyanobacteria and micro-algae promised to be an alternative source of antioxidants (Table 3). Several cyanobacteria and micro-algae such as *Botryococcus*, *Chlorella*, *Dunaliella*, *Nostoc*, *Phaeodactylum*, *Spirulina*, *Haematococcus* and *Chaetoceros* evaluated for antioxidant properties (Goh *et al*., 2010). Micro-algae are already commercially produced as a source of carotenoid antioxidants (e.g. *Haematococcus* for astaxanthin, *Dunaliella* for β-carotene) for use as additives in food and feed applications, as well as for use in cosmetics and as dietary supplements (Pulz and Gross, 2004).

Some protease inhibitors obtained from cyanobacteria like microginins, aeruginosins and cyanopeptolins can apply for the treatment of high blood pressure, asthma and viral infections (Takaichi, 2011). Apart from this, Hayashi *et al*. (1994) reported that *Spirulina* can enhance phagocytic activity and antigen production in mice. Similar results that is increased phagocyticactivity, increased antigen production and increased natural killer cell-mediated anti-tumour activity in *Spirulina* (Qureshi and Ali, 1996). In a preliminary small clinical study, an increase in 13.6-fold in interferon and 3-fold in interleukin (IL)-1b and -4 was observed in human blood cells incubated with *Spirulina* extracts. Khan *et al*. (2005) reported that different products obtained from *Spirulina* might be helpful to increase the phagocytic activity of macrophages, stimulate antibody and cytokine production, increase accumulation of natural killer cells into tissues and activate T and B cells which effectively modulate the immune systems.

2.4 Antimicrobial Compounds

Cyanobacteria and micro-algae has been well considered as an alternate source of antimicrobial, antibacterial, antifungal and antiviral compounds (Table 4) (Rodrıguez-Meizoso *et al*., 2010). Screening of cyanobacteria and micro-algae for antibiotics has opened a new horizon for formulating innovative and more effective drugs (Abed *et al*., 2009; Singh *et al*., 2011; Pradhan *et al*., 2014). A large number of algal extracts have been found to have antimicrobial activity (Mao and Guo, 2010). A major group of antimicrobial agents found in freshwater micro-algae are fatty acids, lipids, pigments, polyphenols, carbohydrates, hydrocarbons and some other derivatives.

Much attention has been focused on the micro-algae and cyanobacteria as sources of the novel biologically active compounds such as phycobilins, phenols, phenolicglycosides, saponins and phytoalexins terpenoids,

Table 3. Antioxidant compounds derived from cyanobacteria/micro-algae

Cyanobacteria/Micro-algae Species	Bioactive Compounds	References
Spirulina swartzii	Sulphated polysaccharide	Vijayabaskar *et al*. (2012)
Padina gymnospora	Sulphated polysaccharides: iota, kappa and lambda carrageenans, fucoidan, fucans	De Souza *et al*. (2007)
Bifurcaria bifurcata	Phenols	Zubia and Payri (2009)
Padina pavonica, *Spirulina vulgare*	Phenols	Khaled *et al*. (2009)
S. platensis	Antioxidants – carotenoids	Jaime *et al*. (2005)
Chlorella vulgaris	Polyphenol, flavonoid	Wang *et al*. (2010)
Haematococcus pluvialis	Nutraceutical astaxanthin	Minhas and Peter (2016)
S. platensis	Phycocyanin	Minhas and Peter (2016)
Chlorococcum humicola	Bioactive compounds	Sanmukh *et al*. (2014)
Chlorella vulgaris	Biomass, Ascorbic acid	Pulz and Gross (2004), Priyadarshani and Rath (2012)
Dunaliella salina	Carotenoid, β-carotene	Pulz and Gross (2004), Priyadarshani and Rath (2012)
Haematococcus pluvialis	Carotenoids, astaxanthin	Pulz and Gross (2004), Priyadarshani and Rath (2012)
D. salina, *Chlorella sorokiniana*, *Chlorella prothecoides*	Lutein	Elena *et al*. (2015)
Synechocystis, *Chlorella*, *Saccharophila*	Zeaxanthin	Elena *et al*. (2015), Singh *et al*. (2013)
S. platensis	Phycobiliproteins	Elena *et al*. (2015)
Chlorella pyrenoidosa	Peptides	Elena *et al*. (2015)
Spirulina maxima, *Chlorella ellipsoidea*	Phenolic compounds	Elena *et al*. (2015), Abd El-Baky *et al*. (2010)

steroids and polysaccharide. The important compounds also identified as antimicrobial are fatty acids, acrylic acid, halogenated aliphatic compounds, terpenes, sulphur containing hetero-cyclic compounds, carbohydrates and phenols (Kannan *et al*., 2010). Nowadays multi-drug-resistant bacteria causing nosocomial infections like methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococci* and AmpC *b*-lactamase producing Enterobacteriaceae have posed therapeutic challenges and are of great concern worldwide (Reinert *et al*., 2007). Therefore, searches for new antibiotics to treat bacterial infections are urgently needed. To develop new antibiotics, it is urgently required to screen and identify the cyanobacterial and micro-algae extract for their antibacterial activity (Biondi *et al*., 2008) which may more potentially effective against various bacterial diseases.

At present, tropical diseases caused by *Plasmodium*, *Trypanosoma*, *Leishmania*, *Schistosoma* and others are the big concern for the world community (Simmons *et al*., 2008). Besides this, current drugs are ineffective, especially in cases of malaria (Lanzer and Rohrbach, 2007) and leishmaniasis (Priotto *et al*., 2007) due to the development of resistance by these protozoa. On the other hand, progress in the advancement of drug discovery programmes against these diseases is very slow (Sheifert *et al*., 2007). To provide the efficient and affordable treatment, Panamanian International Co-operative Biodiversity Group, reported the isolation of five classes of antiprotozoal compounds from *Cyanobacteria*. Barbaraus *et al*. (2008) indicated that an alkaloid isolated from *Nostoc* sp. 78-12 A, nostocarboline effectively control the *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania donovani* and *Plasmodium falciparum* with IC_{50} values ranging from 0.5 to 0.194 mM. Portmann *et al.* (2008) isolated Aerucyclamide C from *Microcystis aeruginosa* PCC 7806 which found to be active against *T. brucei* and the already known aerucyclamide B against *P. falciparum* with submicromolar IC_{50} values. Clark *et al*. (2008) isolated six new acyl proline derivatives, tumonoic acids D-I, from the marine cyanobacterium *Blennothrix cantharidosmum* among which tumonoic acid I displayed moderate activity in an antimalarial assay (IC_{50} 2 mM).

2.5 Anticancer Compounds

At present, available drugs, like vinca alkaloids and taxanes, which is thought to be one of the leading causes of failure in the chemotherapeutic treatment of cancers because

Table 4. Antibacterial and antifungal compounds reported from cyanobacteria and micro-algae

Cyanobacteria/ Micro-algae	Compound	Type	Effective Against	References
Fischerella	Ambiguine Isonitrils	Alkaloids	*C. albicans*	Raveh *et al.* (2010), Mo *et al.* (2009)
	Fischerellin A	Other	*U. appendiculatus* *E. graminis*	Hagmann *et al.* (1996)
	Ambigol A–C	Polychlorinated Polyaromatic Phenols	*B. megaterium*	Wright *et al*. (2005)
Anabaena	Balticidins	Glycosylated Lipopeptide	*C. maltose*	Bui *et al*. (2014)
	Laxaphycins	Cyclic peptides	*A. oryzae*	Bonnard *et al*. (2007)
	Bromoanaindolone	Alkaloid	*B. cereus*	Volk *et al*. (2009)
Nostoc	Nostofungicidine Noscomin	Diterpene	*A. candidus* *B. cereus* *S. epidermidis*	
	Muscoride A	Peptide alkaloid	*E. coli* *B. subtilis*	
	Nostocyclyne A	Polyketide	*S. aureus* *B. subtilis*	Ploutno *et al.* (2000)
Microcystis	Aeruginazole A	Cyclic peptide	*B. subtilis*	Raveh *et al.* (2010)
	Kawaguchipeptins	Cyclic peptides	*S. aureus*	Bruno *et al.* (2011)
Lyngbya	Lobocyclamide A–D	Cyclic lipopeptides	*C. albicans*	MacMillan *et al.* (2002)
	Lyngbyabellin B	Cyclic depsipeptide	*C. albicans*	Milligan *et al.* (2000)
	Majusculamide C	Cyclic depsipeptide	*R. solani* *P. aphanidermatum*	Pettit *et al*. (2008) Zainuddin *et al.* (2009)
	Lyngbyazothrins	Cyclic peptides	*B. subtilis* *E. coli*	Zi *et al*.(2012) Leão *et al*. (2010)
	Malyngamides	Fatty acid amides	*S. aureusB. subtilis*	Gunasekera *et al*. (2011)
Scytonema	Scytoscalarol	Sesterterpene	*C. albicans*	Mo *et al*. (2009)
Tolypothrix	Tjipanazoles	Indolocarbazoles	–	
	Tolybyssidins A/B	Cyclic peptide	*C. albicans*	Jaki *et al*. (2001)

of resistance of tumour cells to these drugs. There are some examples of promising anticancer cyanobacterial metabolites with established mechanisms of action (Table 5). Cryptophycins are potent anticancer agents produced by the cyanobacteria and micro-algae. Cryptophycin 1 was isolated from *Nostoc* sp. GSV224 as an anticancer agent. Scytonemin isolated from *Stigonema* sp. is a compound which has anti-proliferative and anti-inflammatory activities. Cell extracts of *Calothrix* isolates are found helpful in inhibiting the growth of human HeLa cancer cells in a dose-dependent manner. Calothrixin A (I) and B (II), pentacyclic metabolites are obtained from micro-algae, which have growth inhibitory effects (Foster *et al*., 1999). Curacin-A obtained from the organic extracts of Curacao collections of *Lyngbya majuscule* is found to be an unusually potent anti-proliferative agent. It shows an inhibitory effect against colon, breast and renal cancer cells. Largazole is another compound having anti-proliferative activity was obtained from *Symploca* sp. and Apratoxins, derived from micro-algae and cyanobacteria, have the ability to inhibit a variety of cancer cells. Cyanobacteria and micro-algae notably *Dunaliella* and *Spirulina* are also a rich source of natural beta carotene (precursor of vitamin A) and have been extensively tested for anticancer effects that are well documented. Besides beta-carotene, a BGA pigment, cryptophycin demonstrates a powerful anticancer property, especially useful in the chemotherapy of drug-resistant tumours (Mozzachiodi *et al*., 2001). It has been shown that of the two freshwater green

Table 5. Anticancer compounds obtained from cyanobacteria and micro-algae

Cyanobacteria/ Micro-algae	Bioactive Compounds	Function	References
Nostoc	Borophycin	Cytotoxicity against human epidermoid carcinoma (LoVo) and human colorectal adenocarcinoma activity	
	Cryptophycin-1	Cytotoxicity against human tumour cell lines and human solid tumours	Mozzachiodi *et al*. (2001)
		Leukaemia U937, CCRF-CEM and HL-60, colon carcinoma HT-29	
		GC3 and Caco-2, mammary carcinoma MCF-7 and MDA-MB-231 and cervical carcinoma HeLa	
	Cryptophycin-8	Greater therapeutic efficiency and lower toxicity than cryptophycin-14 in vivo	
Lyngbya	Apratoxin A	Cancer, U2OS osteosarcoma, HT29 colon adenocarcinoma and HeLa cervical carcinoma	Leusch *et al*. (2001)
	Apratoxins B–C	KB oral epidermoid cancer and LoVo colon cancer	Leusch *et al*. (2001)
		H-460 lung cancer	
	Apratoxin D	U2OS osteosarcoma, HT29 colon adenocarcinoma and HeLa	
	Apratoxin E	Epithelial carcinoma	Leusch *et al*. (2001)
		H-460 lung cancer	
	Apratoxins F and G	HCT-116 colorectal cancer cells	
	Aurilide B	H-460 lung tumour	Leusch *et al*. (2001)
Calothrix	Calothrixin A	HeLa epithelial carcinoma	Foster *et al*. (1999)
	Calothrixin B	HeLa epithelial carcinoma	Foster *et al*. (1999)
Oscillatoria	Ethyl tumonoate A	H-460 lung cancer	Tidgewell *et al*. (2010)
Microcystis	MicroviridinToxin BE-4, siatoxin	Antibiotic, anticancer	Soria-Mercado *et al*. (2009)
Phormidium	Caylobolide B	HT29 colorectal adenocarcinoma and HeLa cervical carcinoma	Andrianasolo *et al*. (2005)
Leptolyngbya	Coibamide A	Cytotoxicity against NCIeH460 lung and mouse neuro-2a cells	
Symploca	Symplostatin 3	Epidermoid carcinoma cell line	McPhail *et al*. (2007)
	Tasiamide	KB oral epidermoid cancer and LoVo colon cancer	Kwan *et al*. (2009)
	TZT-1027 analogue of dolastatin 10	Effective against MX-1 breast carcinoma and LX-1 lung carcinoma in both p53 normal and mutant cells	McPhail *et al*. (2007)

algae studied, that is *Cladophora glomerata* and *Microspora floccosa*, the former is a potential source of biologically active compounds that may be useful as therapeutic agents including an anticancer.

2.6 PHB (Poly-Hydroxy-Butyrate)

Poly-Hydroxy-Butyrate (PHB) is an ideal biodegradable material that can be completely mineralised into water and carbon dioxide by the action of naturally occurring micro-organisms (Abed *et al.*, 2009). It could be useful in the biomedical and biopharmaceutical fields (Sudesh, 2004; Abed *et al.*, 2009). PHB is a widespread intracellular storage compound typically in prokaryotic organisms (Liu *et al.*, 2010). The properties of pure PHB including thermoplastic, total resistance to water and complete biodegradability suggest that PHB could be attractive to conventional plastics and would fit well with new waste management strategies (Chen *et al*., 2008). Its high production cost limits the use of PHB produced by bacterial fermentation as a commodity polymer compared with some widely used petroleum-derived plastics. The number, as well as the types and potential qualities, have significantly increased the production of superior materials such as epoxides and polysulphones and have become one of the most widely used products all over the globe (Tian *et al*., 2010; Balaji *et al*., 2013). Cyanobacteria and micro-algae can be considered as an

Table 6. Types and characteristics of pigments in cyanobacteria and micro-algae

Chemical Nature and Colour	Photosynthetic Pigments	Predominant Pigment
Tetrapyrole (Porphyrin derivatives-green bluish green)	Chlorophyll Phycobilin	Chlorophyll *a* Phycoerythrin, phycocyanin and allophycocyanin
Tetra-terpenoids (Carotenoids-yellow to red or orange)	Carotene Xanthophyll	ε-Carotene, lycopene, γ-carotene, β-carotene Astaxanthin, canthaxanthin, β-cryptoxanthin, echinenone, myxoanthophyll, oscillaxanthin

alternative host system due to their minimal nutrient requirements and photoautotrophic nature for the production of PHB (Jyotsana *et al*., 2010). *Chlorogloea fritschii* is first reported cyanobacterial species for the presence of PHB in the year 1966. Today several cyanobacteria such as *Spirulina* sp., *Aphanothece* sp., *Gloeothece* sp. and *Synechococcus* sp. have been reported for occurrence of PHB (Fernandez-Nava *et al*., 2008). The *Synechocystis* sp. possesses genes corresponding to PHB synthase and conducted research on its characteristics (Quillaguaman *et al*., 2010). The detection of PHB was done electron microscopically respectively in *Gleocapsa* sp. and *Nostoc* sp. under photoautotrophic conditions (Shrivastav *et al*., 2010). The presence of PHB in *Oscillatoria limosa* and *Gloeothece* sp. is detected by Gas–liquid chromatography. Cyanobacteria and micro-algae can be easily harvested in wastewaters due to their ability to use inorganic nitrogen, phosphorous and wastewaters such as effluents of farmyards, fish farms and rubber industries and sewage treatment plants (Andreessen *et al*., 2010). The primary advantage of cyanobacterial – and other bacterial PHB is relatively low weight due to their small size and mass. The advantage of the environment friendly and highly processes synthesis of biodegradable and in many cases biocompatible polymers will become increasingly attractive for the industry.

Cyanobacterial and micro-algal bioplastics manufactured using biopolymers derived from two ways like biopolymers from living organism and polymerisable molecules (Beccari *et al*., 2009). Biopolymers from living organisms are typically made from cellulose, starch and soy protein. Polymerisable molecules are usually made from lactic acid and triglycerides. Micro-algae and cyanobacterial (BGA)-based plastics have been a recent trend in the era of bioplastics compared with traditional methods (Castilho *et al*., 2009). Algae-based plastics are in their infancy; once, they are into commercialisation, they are likely to find applications in a wide range of industries. Cyanobacteria and micro-algae serve as an excellent feedstock for plastic production owing to its many advantages such as high yield and the ability to row in a range of environments (Seiichi and Doi, 2004). The use of cyanobacterial and micro-algal plastics opens up the possibility of utilising carbon, neutralising greenhouse gas emissions from factories and power plants (Wang *et al*., 2008; Lee *et al*., 2008). This provides the double advantages like conservation of fossil resources and reduction in carbon dioxide emissions, which makes a significant innovation of sustainable development.

2.7 Pigments

At present, synthetic colorants are used in food, cosmetic, nutraceutical and pharmaceutical industries. However, due to problems associated with the harmful effects of artificial colorants, exploitation of micro-algal pigments as a source of natural colours becomes an attractive option (Begum *et al*., 2016). Cyanobacterial and micro-algal pigments (Tables 6 and 7) comprise the most colourful and attractive components in these micro-organisms. Screening programmes all over the world have further confirmed the diversity and rich repertoire of pigments, which can revolutionise the industrial uses of 'colours' with their nutraceutical and pharmaceutical value (Prasanna *et al*., 2010). The extensively utilised pigments in bio-industry are the phycobiliproteins comprising phycocyanin (PC), for about 20% of total dry weight (Prasanna *et al*., 2010) of many cyanobacteria. The phycobiliproteins represent the primary photosynthetic accessory pigments in cyanobacteria, along with chlorophyll *a*. Phycobiliproteins are a family of highly soluble and reasonably stable fluorescent proteins derived from cyanobacteria. There are three basic types of biliproteins – phycoerythrin (PE, λ_{max} 560 nm), (PC, λ_{max} 615 nm, blue pigment) and allophycocyanin (APC, λ_{max} 652 nm, bluish green pigment).

Cyanobacteria have all three types of phycobilins: APC and PC are always present and PE is found in some organisms and not in others, but forms the most spectroscopically variable class of phycobiliproteins. Carotenoids are the most common, naturally occurring terpenoid pigments. They are of keen interest in many scientific disciplines because of their wide distribution, diverse nature and unusual properties. The colour of these carotenoids ranges from yellow, orange to red with variations of brown and purple and several types of carotenoids are accumulated as part of their response to various stresses. They carry out important functions in photosynthesis, nutrition and protection against oxidative damage. Most common carotenoids in micro-algae and cyanobacteria are β-carotene, zeaxanthin, ketocarotenoid,

Table 7. Pigments extracted from cyanobacteria and micro-algae

Cyanobacteria/Micro-algae	Pigments	References
S. platensis	Phycocyanin, C-phycocyanin	De Morais *et al*. (2015), Ibanez and Cifuentes (2013)
Haematococcus pluvialis	Astaxanthin, lutein, zeaxanthin, canthaxanthin, lutein, β-carotene	De Morais *et al*. (2015), Markou and Nerantzis (2013)
Chlorella vulgaris	Canthaxanthin, astaxanthin	Priyadarshani and Rath (2012)
Chlorella ellipsoidea	Zeaxanthin, violaxanthin	De Morais *et al*. (2015)
Chlorella zofingiensis	Astaxanthin	De Morais *et al*. (2015), Markou and Nerantzis (2013)
Chlorella protothecoides	Lutein, zeaxanthin, canthaxanthin	De Morais *et al*. (2015), Markou and Nerantzis (2013)
Chlorella pyrenoidosa	Lutein	De Morais *et al*. (2015)
D. salina	β-Carotene	De Morais *et al*. (2015), Markou and Nerantzis (2013)

Table 8. Some of UV absorbing pigments reported in cyanobacteria

UV Absorbing Pigments	Cyanobacteria	References
Porphyra 334, Shinorine	*Nodularia*	–
Mycosporine-alanine	*Oscillatoria*	–
Mycosporine-glutaminol	*Leptolyngbya*	–
Mycosporin-2-glycine	*Euhalothece*	–
Euhalothece-362	*Euhalothece*	Volkmann and Gorbushina (2006)
Shinorine	*Anabaena*	Sinha *et al*. (2001)
Mycosporine-glutaminol-glucoside	*Leptolyngbya*	Volkmann and Gorbushina (2006)

echinenone and myxoxanthophyll. The carotenoids are grouped into two groups: carotenes – that do not contain oxygen atom and xanthophylls – the oxygenated derivatives of carotene (Britton *et al*., 2004).

Micro-algae and cyanobacteria are the major photosynthesisers on earth and produce valuable pigments that include chlorophyll *a*, *b* and *c*, β-carotene, astaxanthin, xanthophylls and phycobiliproteins. The primary pigments include chlorophyll *a*, *b* and *c*, *b*-carotene, PC, xanthophylls and PE. All these pigments are of great potential for applications in food, pharmaceuticals and cosmetics. There is an increasing demand for natural colours which are of use in food, pharmaceuticals, cosmetics, textiles and as printing dyes (Dufosse, 2005).

2.8 Mycosporine-like Amino Acids

Mycosporine-like amino acids (MAAs) are a family of secondary metabolites known to protect organisms exposed to solar UV radiation. Micro-algae as well as c*yanobacteria* biosynthesise MAAs while other marine algae acquire MAAs by diet transfer, symbiotic or bacterial associations (Table 8).

MAAs are a type of pigments, having a role in protection from absorbing harmful UV radiation in micro-algae and cyanobacteria. They are water soluble substances characterised by acyclohexenone or cyclo-hexen-imine chromophore conjugated with the nitrogen substituent of an amino acid or its imino alcohol, having absorption maxima ranging from 310 to 360 nm (Volkmann and Gorbushina, 2006). MAAs has been identified in some taxonomically diverse organisms. MAAs such as asterina 330, mycosporine Gly, porphyra 334 and shinorine are common in various types of organisms. Most of the c*yanobacteria* reported to contain MAAs have been identified from terrestrial habitats (Prasanna *et al*., 2010).

2.9 Toxins

About 300 species of micro-algae and cyanobacteria are reported at times to form so-called algal blooms. Nearly one-fourth of these species are known to produce toxins (Table 9) (Hallegraeff *et al*., 2003). The extreme toxicity of algal toxins and the potential misuse in bioterrorist activities have led to stringent restrictions on sale and transport of toxins standards, which could eventually limit the capacity to detect and monitor these toxins. Cyanobacteria, as well as marine and freshwater micro-algal toxins, are a varied group of compounds that can occur on the coast and offshore, in lakes and water reservoirs, especially in eutrophicated areas (Bittencourt-Oliveira *et al*., 2005). Both marine and freshwater toxins can bio accumulate in the food chain to

Table 9. Cyanobacterial toxins, their source, chemical nature and effects

Toxins	Source	Chemical Nature	Effects	LD50
Hepatotoxins				
Microcystins	*Anabaena*, *Anabaenopsis*, *Hapalosiphon*, *Microcystis*, *Nostoc*, *Oscillatoria*, *Planktothrix*	Cyclic heptapeptide	Inhibition of protein phosphatases (PP1 and PP2A)	50–160 µg/kg
Nodularin	*Nodularia*	Cyclic heptapeptide	Inhibition of protein phosphatases (PP1 and PP2A)	50 µg/kg
Neurotoxins				
Anatoxin-a	*Anabaena*, *Aphanizomenon*, *Cylindrospermum*, *Oscillatoria*, *Phormidium*, *Rhaphidiopsis*	Alkaloid	Binds irreversibly to the nicotinic acetylcholine receptors	375 µg/kg
Anatoxin-a(s)	*Anabaena*	Guanidine methyl phosphate ester	Inhibits acetylcholinesterase	200–250 µg/kg
Saxitoxins	*Aphanizomenon*, *Anabaena*, *Cylindrospermopsis*, *Lyngbya*, *Planktothrix*	Carbamate alkaloid	Binds and blocks the sodium channels in neural cells	10 µg/kg
Cytotoxins				
Cylindrospermopsins	*Anabaena*, *Aphanizomenon,* *Cylindrospermopsis*, *Raphidiopsis*, *Umezakia*	Guanidine alkaloid	Inhibitor of protein biosynthesis and genotoxic	2.1 mg/kg
Dermatotoxins				
Lyngbyatoxin-a	*Lyngbya*, *Oscillatoria*, *Schizothrix*	Alkaloid	Cause erythema (dermatitis), blisters and necrosis in mammals; potent tumour promoters	–
Aplysiatoxins	*Lyngbya*, *Oscillatoria*, *Schizothrix*	Alkaloid	Inflammatory agents, protein kinase C activators	0.3 mg/kg
Endotoxins				
Lipopolysaccharide	All cyanobacteria	Lipopolysaccharide irritants	Inflammatory agent, gastrointestinal	45–190 mg/kg

Source: Yadav *et al*. (2011).

very high concentration in seafood, molluscs, fish and other aquatic organisms (Cazenave *et al*., 2005). For these reasons, these compounds pose a health hazard for humans, domestic animals and wildlife with toxicological effects including neurotoxicity, hepatotoxicity, cytotoxicity and dermatotoxicity (Table 9) (Carmichael, 2001; Kujbida *et al*., 2006). The microcystins, cylindrospermopsin, homo- and anatoxin-a, anatoxin-a(s) and saxitoxins are the most common freshwater algae toxins and are associated with *Microcystis*, *Anabaena*, *Oscillatoria* and *Nostoc* sp. (Codd *et al*., 2005). Prokaryotic and eukaryotic micro-algae produce a vast array of compounds with biological activities.

These include antibiotics, algicides, toxins, pharmaceutically active compounds and plant growth regulators. Toxic micro-algae thus are common only among the cyanobacteria and dinoflagellates. The micro-algal toxins is either important as material for useful drugs.

3. Extraction of Bioactive Compounds from Cyanobacteria and Micro-Algae

Micro-algal and cyanobacterial functional ingredients obtained from new matrices and to fulfil this there is a need to combine appropriate, selective, economical and green extraction techniques with the help of food-grade solvents and procedures. Commonly used extraction methods such as soxhlet, solid–liquid extraction or liquid–liquid extraction have some negative points like high volumes of solvents and long extraction times. These techniques often produce small extraction yields of bioactive and present little selectivity. Besides this, these methods are usually not automated procedures and their reproducibility can, therefore, green extraction techniques (Figure 2), such as supercritical fluid extraction, pressurised liquid extraction, accelerated solvent extraction pressurised hot water extraction, ultrasound-assisted extraction and microwave

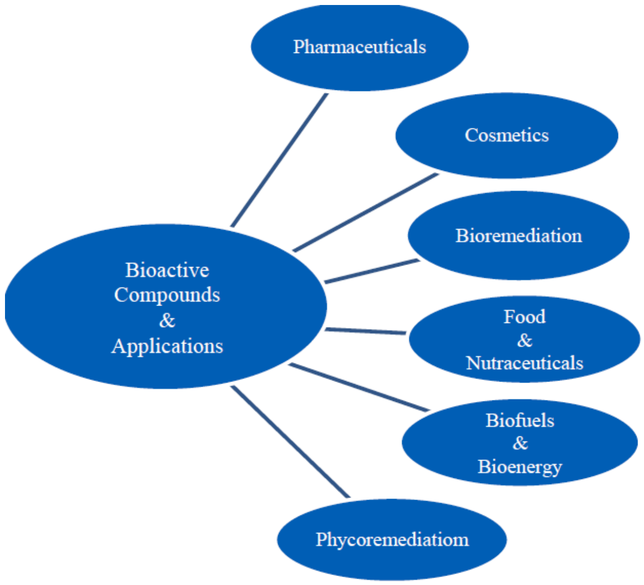

Figure 2. Bioactive compounds from cyanobacteria and micro-algae and their applications

assisted extraction techniques, among others, provide an efficient alternative to the problems encountered with the use of traditional extraction procedures.

4. Conclusions and Recommendations

Based on the above discussions and available literatures it is proposed that cyanobacteria and micro-algae are versatile bio-agents and their biotechnological applications will contribute significantly in future industrial applications. They are potent sources of bioactive compounds, biofertilisers, bioplastics, energy, food and have currently been used in drugs formula, medical diagnostics and bioremediation. The consensus appears to be that cyanobacterial and micro-algae-based food supplements could provide a high level of nutrition particularly to poor and marginal society peoples who are malnourished, but they should not be considered as a replacement diet for animal protein sources such as fish, eggs, meat and milk. The biotechnological potentials of cyanobacteria and micro-algae as a source MAAs and phycobiliproteins have gained much attention. MAAs and phycobiliproteins show the potential applicability of food, cosmetics and biomedical research as well as in the design of very specific and potent new pharmaceuticals against a variety of diseases. The availability of bioactive compounds derived from cyanobacteria and micro-algae show advantages in the production of commercially valuable products can be optimised to produce sustainable yields on an industrial scale. Continued technical improvement and market demand will result in further significant advances and an expansion of the commercially available cyanobacterial, micro-algae species and their products.

Genetic engineering can be used for cyanobacterial and micro-algae strain improvement. Further, multidisciplinary and supportive efforts will expedite the more sensitive and fast methods in the analysis of the cyanobacterial bioactive compounds. Several patents dealing with some applications of PBPs have been licensed. Investigations on cyanobacteria and micro-algae are still needed to be much more improved with reference to their basic biology and culture techniques. Hence, many more biological and ecological investigations are needed to optimise the biotechnological potential of cyanobacteria and micro-algae for industrial production at sustainable level. More attention is required to explore the cyanobacteria as well as micro-algae and their metabolites that may to lead the discovery of useful compounds for a range of agriculture and environmental applications.

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