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Editorial

I am happy to lead a team that brought to fruition the third issue of Spectrum: Science and Technology. The current issue has articles from information technology, geology and the biological sciences.

Radical changes in cellular connectivity is the felt need across India. One of the articles dwells on that theme. The other article on information technology explains the concept of machine translation. Application of the concept in India will greatly benefit communication between citizens belonging to different linguistic groups.

Water is an important element of life on earth. An article deals with the water resources of Shillong, city from the geologist's point of view. In another article the authors present their findings on the potability of water of four major rivers of East Khasi Hills district of Meghalaya. Herbicides, although important inputs in agriculture, can inhibit growth of beneficial microorganisms residing in the soil when used indiscriminately. Authors of one article present their findings on the effect of one such herbicide on soil bacteria.

Diabetes is a major disease in the country. An article explains the innovative concept of artificial pancreas, which, if successfully implemented will bring succour to thousands of patients. Our immune system provides natural defence against infectious agents; the article on regulation of immune system throws light on a less known regulatory mechanism. The authors also present their own perspective on the topic. The pathogenicity of human viruses is a subject of great interest. The present issue has an original research article on this subject.

Plant-microbe interactions and microorganisms of potential applications in biotechnology industry are thrust areas of many research groups around the world. Three articles focus on those aspects. The biotechnology industry is also interested in the discovery of food species that can serve as both sources of nutrition and of income generation. An article on minor fruits of Meghalaya and another on eel are relevant to those areas.

This science and technology journal of St. Anthony's College, Shillong has been providing authors a platform to express opinion in their respective domain areas and the present issue has followed that trend. I thank the reviewers for their contribution in improving the quality of manuscripts. I am also grateful to members of the editorial board for their valuable inputs. A special thanks to Prof. Thy Answer Challam, Prof. Jeremy N. Syiem. and Prof. Stevenson Thabah for devoting their valuable time and labour in the publication stage of this third issue of Spectrum: Science and Technology.

I, on behalf of the editorial board of Spectrum: Science and Technology and on my behalf thank Rev. Br. Albert L. Dkhar, Principal; Rev. Fr. Joby Joseph, Vice-Principal and Rev. Fr. Saji Stephen, Vice-Principal for their support and encouragement at all stages of the publication of the current issue of the journal.

Dr. M.A. Laskar
Chief-Editor

Contents

7. **Effect of pretilachlor on rice field bacteria of Sung Valley of Meghalaya**

   - Thyanswer Challam and Stevenson Thabah

8. **Morphometric analysis of the mud eel *Monopterus Cuchia* From Meghalaya, India**

   - Barisha Mary Kurbah and Rabindra Nath Bhuyan

9. **Artificial pancreas project-a review**

   - Violina Kalita and Linu John

10. **Isolation of α- amylase producing bacterial strain from soil**

    - Jahangir Alom and Dr. Manabendra Mandal

11. **Challenges in future 5G networks and some potential solutions: A survey**

    - Probidita Roychoudhury

12. **Market potential of some minor fruits of East Khasi Hills District, Meghalaya**

    - D. Bokolial W.Majaw, M. W. Sanglyne, R. Lytan, J. Kurbah and W.Lakiang

13. **Scenario of water resources in Shillong City, Meghalaya.**

    - V Jennifer Joan Wallang

1. Notch signal and its emerging role in T cell biology

Dimpu Gogoi[1,2], Dipankar Biswas[1], Biswajyoti Borkakoty[1] and Jagadish Mahanta[1]

Abstract

Notch signaling pathway facilitates short range communication between cells. The initiation of Notch signal requires a cell to cell contact wherein the ligand binds to the Notch receptor. The Notch acts both as a receptor and a transcription factor. The role of Notch signal has been implicated in αβ versus γδ T cell lineage commitment, and is essentially dissimilar in mice and human. Notch signal is also necessary for regulation of T cell response and influences T cell function. In this review, we explain Notch signaling pathway, and underscore the function of Notch signal in the development and activation of T cells.

Keywords: *Notch signal, αβ T cells, γδ T cells and effector function*

Introduction

Several signaling pathways contribute to cellular diversification and guide them to execute its function. The Notch signaling pathway is one among the many signals that facilitate short range communication between cells. Notch gene was discovered by John S. Dexter in 1914, who observed a mutant in

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Drosophila, which generated serrations on the wing margin that lends the name Notch to the gene. Over the past 35 years, Notch field has grown exponentially and it is now clear that Notch plays an essential role in both embryonic and adult development. Its function can be found in neuronal development, stem cell biology, wound healing, angiogenesis, immune system, etc. Notch signaling activity is necessary for the T cell maturation in the thymus as well as in regulating effector functions of the matured T cells. Here, we review our current understanding of Notch signal and its role in the development and effector functions of T cell.

The structure of Notch receptor and its ligands

The Notch is ~300 kilodalton transmembrane receptor, which requires ligand to trigger the signaling event in the cell. The ligand for Notch is also transmembrane protein that binds to the receptor (Lissemore and Starmer, 1999). There are four isoforms of Notch receptor (Notch 1-4) and five Notch ligands, three Delta-like (Dll1, 3 and 4) and two Jagged (Jag1 and 2) (Fiuza and Arias, 2007). Initially, the Notch receptor is formed as a single big protein in the endoplasmic reticulum. Then, in golgi, the Notch receptor undergoes first cleavage by furin like protease to generate non-covalent linked heterodimeric Notch that then migrates to the cell membrane (Logeat *et al.*, 1998). The Notch receptor spans the cell membrane with part of it inside and part outside (Figure 1). The heterodimeric Notch extracellular domain (NECD) is composed of arrays of up to 36 epidermal growth factor (EGF) repeats, involved in ligand interaction. The membrane-tethered Notch intracellular domain (NICD) includes sets of ankyrin repeats (ANK) flanked by nuclear localizing signals (NLS), RBP-jκ associated module (RAM), a

proline, glutamine, serine, threonine-rich (PEST) domain and a transactivation domain (TAD) (Fleming, 1998; Lubman *et al.*, 2004).

Notch receptor isoforms differ from each other in their extracellular and intracellular regions. Notch1 & 2 have 36 extracellular epidermal growth factor repeats, whereas, Notch3 has 34 and Notch4 has 29 repeats. Notch1 and Notch2 are differentiated by the fact that Notch1 has a strong TAD region, while, Notch2 has a weak TAD region, and Notch3 as well as Notch4 lack TAD region (Chillakuri *et al.*, 2012; Thurston *et al.*, 2007). The Notch ligands are also transmembrane proteins with an extracellular region, a transmembrane region and an intracellular region (Figure 1). The main structural difference between the Dll and Jag ligands is that the Jag contain a larger number of EGF repeats in the extracellular region and also insertions within the EGF repeats (Fiuza and Arias, 2007). In addition, the Jag contain a cysteine-rich region that is entirely absent from the Dll (Leimeister *et al.*, 2000).

The Notch signaling pathway

The initiation of Notch signal requires binding of a ligand to the 29-36 tandem EGF repeats of NECD (Figure 2). The repeats 11-12 of NECD are required for productive interaction with the ligand presented by neighboring cells (trans-interactions). While, the repeats 24-29 of NECD are involved in cis-interaction with the ligand expressed in the same cell (Kopan and Ilagan, 2009). Many EGF repeats bind to calcium ions, which play a vital task in determining the structure and affinity of Notch in ligand binding (Cordle *et al.*, 2008). These EGF-like repeats can be modified by O-linked glycans at specific sites. GDP-fucose protein, O-fucosyltransferase1 (POFUT1) adds O-

fucose which is absolutely required for Notch function (Shao *et al.*, 2002). The other part of the Notch, i.e., NICD is essentially a membrane-tethered ranscription factor whose release is regulated by the ligand binding (Greenwald and Kovall, 2013).

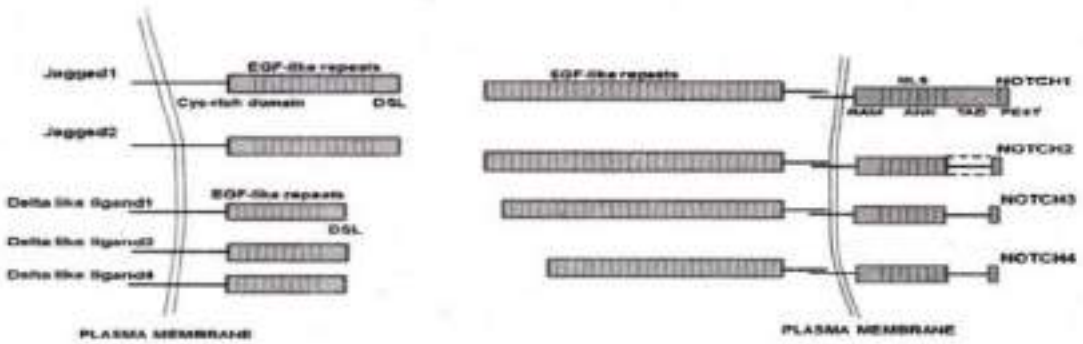

Figure 1: Structure of Notch receptor isoforms and its ligands. The four Notch receptors-Notch1, Notch2, Notch3 and Notch4, share homology with a difference in epidermal growth factor (EGF)-like repeats (29-36) present in the Notch extracellular domain (NECD). Notch intracellular domain (NICD) includes sets of ankyrin repeats (ANK), nuclear localizing signals (NLS), RBP-jK associated module (RAM), a proline, glutamine, serine, threonine-rich (PEST) domain and a transactivation domain (TAD). The five membrane ligands, Delta like ligand (Dll)-1, 3, 4, Jagged (Jag)-1 and 2, also share structural homology, including Delta Serrate and Lag2 (DSL) domain, followed by a number of EGF-like repeats (6-18), a cysteine rich region in Jag1 and Jag2 and then a membrane-tethered domain.

The interaction between NECD and any of its ligands result in the shedding of the ectodomain and exposure of an extracellular metalloprotease site. The exposed site, thus, becomes susceptible to cleavage by transmembrane proteases of the ADAM/TACE (a disintegrin and metallopeptidase/tumor

necrosis factor-α converting enzyme) family. This cleavage generates a membrane-tethered intermediate called Notch extracellular truncation (NEXT) that is a substrate for γ-secretase, a multicomponent member of a growing family of intramembrane cleaving proteases (I-CLiPs) (Kopan *et al.*, 1996; Struhl and Adachi, 1998; Wolfe, 2006). Consequently, γ-secretase mediates an additional cleavage at the transmembrane position to release NICD. The NICD of Notch is released and translocates into the nucleus where it forms a complex with CSL (CBF1, Suppressor of Hairless, Lag-1) that activates the transcription of target genes (Pursglove and Mackay, 2005; Struhl and Adachi, 1998). To date, many Notch target genes have been identified and one of the best characterized is the *hairy enhancer of split* (*HES*) family of transcription factors (Davis and Turner, 2001). Other genes include *Hes-related repressor protein* (*Herp*) transcription factor family (Iso *et al.*, 2003), the cell cycle regulator *Cdkn1a* (Rangarajan *et al.*, 2001), the gene for *Notch-regulated ankyrin repeat protein* (*NRARP*) (Krebs *et al.*, 2001), *Deltex* (*DTX*) (Deftos *et al.*, 2000).

This well-documented Notch signaling pathway is evolutionary conserved across species. Recently, non-canonical Notch signaling has also been demonstrated that occur independent of CSL (Minter and Osborne, 2012). The evidence for non-canonical Notch signaling was also first observed in Drosophila where it was shown to be required for axon guidance (Giniger, 1998). Subsequently, non-canonical Notch activity was implicated in dorsal closure during embryonic development which occurs independent of Su(H) (Drosophila CSL) (Zecchini *et al.*, 1999). Similarly, it was found that that Notch signal in epithelial transformation does not require CSL and therefore occurs through non-canonical Notch signaling (Liu *et al.*, 2009). In human peripheral T cells, it was observed that Notch regulates transcription

independent of CSL by associating with NF-κB proteins, p50, or c-Rel (Cho *et al.*, 2009; Shin *et al.*, 2006). The non-canonical non-nuclear Notch signaling pathway has also been reported that supports survival of the peripheral T cells and occurs in the cytosol (Perumalsamy *et al.*, 2009; Perumalsamy *et al.*, 2010).

Notch signaling in T cell development and functions

Notch signaling is required for the commitment of T cell lineage from the hematopoietic progenitor cells. The Notch has been implicated in the differentiation of T versus B lymphocytes from a common lymphocyte precursor and favors differentiation of T cell lineage (Pui *et al.*, 1999). Notch signaling is also intimately involved in γδ versus αβ lineage decision (Tanigaki *et al.*, 2004). It is interesting to point out that the molecular event triggering T cell development are essentially different in mice and human (Figure 3). Several reports in mice suggest that Notch favors αβ T cell lineage over γδ T cell (Nelson *et al.*, 2011; Pui *et al.*, 1999; Tanigaki *et al.*, 2004; Washburn *et al.*, 1997). The development of γδ T cell from T cell progenitor cell requires the absence of Notch ligand interaction (Ciofani *et al.*, 2006). In contrast, there is an opposing functions of Notch signal in αβ versus γδ lineage decision of human. The induction of γδ-lineage precursor cell to split off from the progenitor cell requires the presence of Notch signal in human (Garcia-Peydro *et al.*, 2003). The high level of Notch activation generates γδ T cell and to inhibit differentiation towards αβ lineage (Van de Walle *et al.*, 2009). Hence, these differences in thymic T cell development between mice and human must be considered when using mouse models as a basis for understanding aberrations associated with human T cell development.

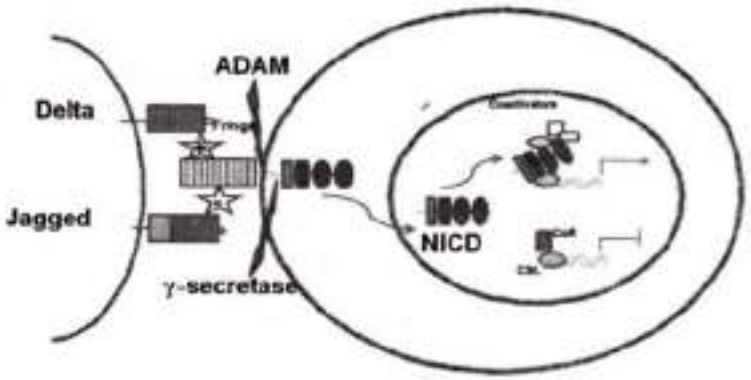

Figure 2: Canonical Notch signaling Pathway. Ligand binding to the Notch extracellular domain (NECD) brings conformational changes in the bound Notch receptor. This conformational change exposes ametalloprotease site. The exposed site is then cleaved by disintegrin and metallopeptidase/tumor necrosis factor-α converting enzyme (ADAM/TACE) family. Notch cleavage by ADAM generates the membrane-anchored Notch extracellular truncation (NEXT) fragment, a substrate for γ-secretase. Thus, γ-secretase cleaves the Notch transmembrane domain to release Notch Intracellular domain (NICD). NICD then migrates into the nucleus where it associates with the DNA-binding proteinCBF1, Suppressor of Hairless, Lag-1 (CSL) and act as a transcription factor after recruiting other co-activators. In the absence of NICD, CSL associate with ubiquitous co-repressor (Co-R) proteins to repress transcription of target genes.

The T cells, thus generated in the thymus, then join the pool of matured T cells wherein their functions are regulated by Notch signal. The αβ T cells consisting of $CD4^+$ and $CD8^+$ T cells are activated by recognizing antigen presented to them by antigen presenting cells (APCs) through major histocompatibility complex-(MHC) class-II and MHC class-I respectively in the presence of co-stimulation. Naive $CD4^+$ and $CD8^+$ T cells are known to express Notch1 and Notch2 receptors on their surface (Amsen *et al.*, 2004; Hoyne *et al.*, 2000). The detection of NICD and its Notch target gene-*HES1* in stimulated T cells indicate the participation of Notch signaling pathway

during activation of T cells (Adler *et al.*, 2003; Palaga *et al.*, 2003). It was also observed that TCR-mediated signaling in both $CD4^+$ and $CD8^+$ T cells induces the activation of Notch signal (Cho *et al.*, 2009; Palaga *et al.*, 2003). The activation of Notch signaling pathway has been shown to be important for proliferation and IFN-γ secretion by αβ T cells (Adler *et al.*, 2003). Recent data showed that Notch signaling potentiates downstream PI3K-dependent signaling

event that allows $CD4^+$ T cell to respond to the antigen (Laky *et al.*, 2015).

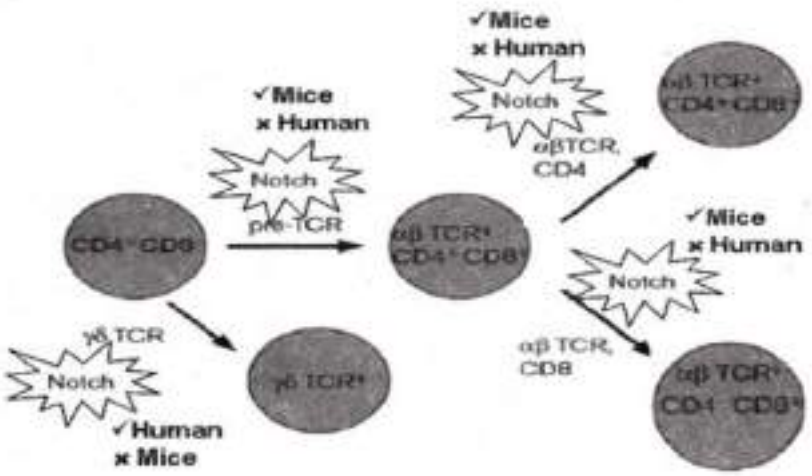

Figure 3: Notch signaling in human T cell development differs than that in mice. Notch signaling play a role in T cell lineage decision at double negative T cell stage, which is negative for CD4 and CD8 co-receptors. In human, increased Notch signal results in increased γδ T cell development besides a decreased in Notch activation makes αβ T cells. However, in mice, αβ T cell lineage commitment into $CD4^+CD8^+$T cell and then subsequently into either $CD4^+$T cell or $CD8^+$T cell requires a higher level of Notch signal and reducing or absence of Notch signal flavored γδ T cell development

Besides, Notch signaling is involved in the expression of effector molecules-perforin and granzyme B to mediate cytolytic effector function of $CD8^+$ T cells (Cho *et al.*, 2009). It was also shown that Notch2 is required for

mediating potent anti-tumor immunity by $CD8^+$ T cells (Sugimoto *et al.*, 2010). Furthermore, the formation of short-lived effector memory $CD8^+$ T cells is dependent upon the Notch signaling pathway as demonstrated in *Listeria* infection and DC vaccination (Mathieu *et al.*, 2015). Notch signaling is also essential for Th17 cells that are known to play a role in host defense against extracellular pathogens and are involved in mediating inflammation. Notch1 was shown to be involved in the regulation of IL-17 as well as RORγt promoters of Th17 cell (Keerthivasan *et al.*, 2011). It was shown that DLL4 enhances differentiation of Th17 cells in a STAT3 dependent manner, whereas, Jag1 inhibits Th17 differentiation from $CD4^+$ T cells by attenuating RORγt (Meng *et al.*, 2015; Wang *et al.*, 2015). The Notch signaling pathway is also required for the antigen-specific proliferation of γδ T cells and their ability to kill tumor cells (Gogoi *et al.*, 2014). Thus, Notch plays an important role in the regulation of different subsets of peripheral T cell effector functions. In a recent report, T cell was engineered with the synthetic Notch receptor and chimeric antigen receptor (CAR) that can efficiently kill target cancer cells, without destroying bystander normal cells (Roybal *et al.*, 2016). In effect, synthetic Notch receptor can direct the engineered T cells to perform duty such as sense cells with one tumor antigen, but activate the killing program of T cells only when the second antigen is also present.

Conclusion

In this review, we have described our current understanding of Notch signal and its role in regulating T cell fate. Notch signaling is involved in the regulation of T cell development in the thymus as well as in the activation of peripheral T cells. The αβ versus γδ lineage commitment is controlled by

Notch signal and is essentially dissimilar in human and mice. In mice, Notch signal is necessary for αβ T cell lineage commitment and inhibits development towards γδ T cell lineage, whereas, the opposite is true for human. The Notch signaling pathway is also important in regulating the effector functions of different subsets of peripheral T cells. Notch signal is essential in the activation of T cell response to act against the invading pathogens and cancer. Thus, Notch is playing a vital role in supporting both the thymic and peripheral life of T cells. The significance of Notch in T cell functions could have an important implications in clinical situations where new strategies for the manipulation of T cells for cancer immunotherapy are being investigated.

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Author Disclosure Statement

The authors have no financial conflicts of interest.

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2. Plant/Microbial Association - Reviewing the beneficial aspects of interaction

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Abstract

Plants in the environment do not exist in an isolated standalone system. Microorganisms and plants share evolutionary coexistence that shaped complex interaction systems. These microorganisms can be classified on the basis of their historical succession in endosphere and ectosphere regions in plants which forms different communities. This association of plant and microorganisms is influenced by many biotic and abiotic factors which exert significant effect on the physiology and functioning of physiological processes of the host. The physiological effects on host are a result of metabolic regulation induced by its interacting partner. It has been reported that these microbial communities have a role to play in controlling disease, regulating nutrient acquisition, and also inducing stress tolerance by the plants. This review highlights some of the factors that are essential for microbial communities to form plant-microbial associations at different levels. The impact of microbes in plant development and performance has also been reviewed which offers significant scopes for sustainable development in agriculture.

Keywords: *rhizosphere, microbiome, endophytes, symbiosis, mycorrhizal, host*

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Introduction

The growth and development of plants is dependent on the supply of nutrients and water present in soil. Vascular plants previously considered of independent existence have now widely accepted and evidently reported to associate with several microorganisms which greatly influenced their metabolic growth and development. Many research groups have revealed important roles performed by associated microorganisms in the formation or modification of soil (Pate *et al.* 2001; Pate and Verboom, 2009; Taylor *et al.* 2009). de Bary (1887) introduced the term 'symbiosis' to define interaction between plant and microbe which includes both beneficial and non-beneficial interactions. Although the presence of microbes in plants was initially related to the occurrence of diseases, it is now recognized that the vast majority of microbial organisms are not causal agents of damage in plants (Mendes *et al.* 2013). Most microbes inhabiting plant-related niches have neutral or beneficial roles in plant health and development (Beattie and Lindow, 1995; Hallmann *et al.* 1997; Mendes *et al.* 2013; Philippot *et al.* 2013).

Boon *et al.* (2014) proposed that the best definition of 'microbiome' would relate to the set of microorganisms along with their genomes encountered in association with the host or a defined environment, thus diminishing the importance of the link between taxonomy and functionality of the microbial community members. Although the discovery and identification of the fixation of nitrogen during rhizobia-legume interaction was observed in the 19th century (Hellriegel and Wilfarth, 1888) but the majority of the plant microbiome, and its contribution to the extended phenotype of the host, is not yet well defined. Analysis of the plant microbiome involves linking

microbial ecology and the plant host's biology and functioning, and viewing microorganisms as a reservoir of additional genes and functions for their host (Vandenkoornhuyse *et al.* 2015). Most of the research on this topic has focused on the functional roles of single microbial groups (e.g., specific species or organisms from the same genera) associated with plants, mostly because of methodological limitations to assess non-culturable microbial groups (Amann *et al.* 1995; Andreote *et al.* 2009).

Microorganisms can affect agricultural productivity by regulating nutrient availability/uptake and promoting stress tolerance (Doornbos *et al.* 2011; Ferrara *et al.* 2012; Kavamura *et al.* 2013). Undertaking to manipulate plant microbiome for reducing incidence of plant diseases, increasing agricultural production, reducing chemical inputs and reducing emissions of greenhouse gases is vital for sustaining the world's growing population.

Types of plant-microbial interactions

The spectrum of plant-microbe interactions is highly complex which comprises of diverse microbial species with potential for forming consortia (Hirsch, 2004). In this review, emphasis has been given to discuss rhizosphere (plant root-soil interface), phyllosphere (plant aerial surfaces) and endosphere (internal tissues). Microbes in these niches can establish symbiotic, neutral or pathogenic associations of varying intimacy with their host plants.

Rhizosphere

Rhizosphere is a region of rich, largely soil-derived, microbial diversity, influenced by deposition of plant mucilage and root exudates. Root exudates

contain a variety of compounds, predominately organic acids and sugars, but also amino acids, fatty acids, vitamins, growth factors, hormones and antimicrobial compounds (Bertin *et al.* 2003). Root exudates are key determinants of rhizosphere microbiome structure (Shi *et al.* 2011). Walker (2003) demonstrated that root tip produces antimicrobial products which provide a defensive zone around the meristematic and elongating root cells. Abundance and composition of microbes in rhizosphere therefore influence oxygen availability (Philippot *et al.* 2013), organic content, pH and activity of microbial enzymes (Shi *et al.* 2011). Rhizosphere-colonizing microbes are plant-growth-promoting rhizobacteria (PGPR), which act through a variety of mechanisms (Bloemberg *et al.* 2001). Free-living nitrogen-fixing bacteria (*Azotobacter* spp.) as well as symbiotic (*Rhizobium* spp.) fix atmospheric nitrogen for plant, and many bacteria can solubilize phosphorous-containing minerals, increasing its bioavailability (Turner *et al.* 2013). Many PGPR act antagonistically towards plant pathogens by producing antimicrobials or by interfering with virulence factors *via* effectors delivered by type 3 secretion systems (T3SSs) (Rezzonico *et al.* 2005). Turner *et al.* (2013) reported that fungal antagonist, *Pseudomonas fluorescens* produced antifungal compound diacetylphloroglucinol (DAPG) which modulates transcription in another plant-growth-promoting rhizobacterium, *Azospirillum brasilense and* increasing expression of genes involved in wheat root colonization and plant-growth promotion (Combes-Meynet *et al.* 2011).

Phyllosphere

By contrast, the phyllosphere is relatively nutrient poor and subject to extremes temperature, radiation and moisture. Organisms colonizing the

external area of the aerial plant tissue make up the phyllosphere. Although this term can be used for any external surface of plants, it is commonly referred to the leaf surface (Vorholt, 2012). The phyllosphere community is composed of fungi (filamentous and yeasts), bacteria, algae, and, at lower frequencies, protozoa and nematodes (Lindow and Brandl, 2003). Bacterial communities are the most abundant group in the phyllosphere whose numbers ranging between 105 and 107 cells per cm^2 (Beattie *et al.* 1995; Andrews and Harris, 2000). Phyllosphere-living organisms are the most tolerant as they can thrive harsh environmental conditions with limited nutrients and variable conditions of wind, humidity, UV radiation, pH and temperature (Andrews and Harris, 2000; Lindow and Brandl, 2003). The microbial communities found in the phyllosphere have essential roles in processes related to plant development, for instance, nitrogen fixation, protecting plants against invading pathogens and biosynthesizing phytohormones (Jones, 1970; Freiberg, 1998; Brandl *et al.* 2001; Kishore *et al.* 2005). They can be utilized in global carbon sequestration processes (Bulgarelli *et al.* 2013), and they can be used as potential sources for the development of sustainable agricultural practices.

Endosphere

Large and diverse populations of microbes colonize plant tissues without causing signs of disease and are broadly termed endophytes. Bacterial endophytes reside either inside the host cells or in the intracellular fluids, and have been isolated from all plant tissues (Rosenblueth and Martinez-Romero, 2006). They can be considered to sit at the benign end of the spectrum between mutualists and pathogens (Hirsch, 2004). The presence of non-

pathogenic organisms inside plants was first described by de Bary (1866) in microscopically analyzed plant tissues. This observation remained unexplored until the definition of endophytes which was provided by Petrini (1991) as "organisms that at some part of their life cycle colonize internal plant tissues without causing apparent harm to the host". Endophytes are distinct from rhizospheric bacteria in that they change their metabolism and become adapted to their internal environment (Monteiro *et al.* 2012). It has been suggested that microorganisms enter host plants at lateral root junctions, most likely at naturally occurring cracks (Figure 1A, B) (Monteiro *et al.* 2012), or more passively through natural breaks in roots or root tips and/or by vegetative propagation (James *et al.* 1998).

The presence of endophytes has also been described in plants maintained in vitro, where they seem to be intimately associated with plants rather than with the culture medium (Almeida *et al.* 2009; Abreu-Tarazzi *et al.* 2010).

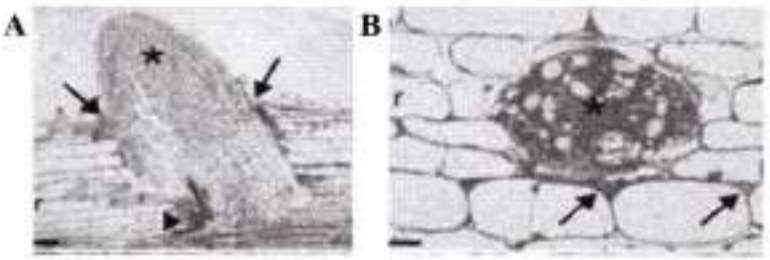

Figure 1. Routes of *Herbaspirillum seropedicae* invasion and colonization in rice. A, penetration point through the lateral root junction (longitudinal section). **B,** colonization and establishment in the intercellular spaces surrounding the emerging region of lateral root (transverse section).

Most of the knowledge about endophytic bacteria interactions has come from well-studied 'model' organisms, such as *Azoarcus*, *Burkholderia*,

Gluconacetobacter, *Herbaspirillum* and *Klebsiella* spp., which were all isolated from non-legumes, particularly grasses (Suarez *et al.* 2012). High-throughput sequencing of 16S rRNA has recently been used to define the core endophytic bacterial microbiome of *A. thaliana* (Lundberg *et al.* 2012).

Factors affecting microbial diversity

The factors that influence the microbial composition include biotic and abiotic factors, specially the genotype, the phenological stage of the plant, and some agricultural practices that influence the physicochemical characteristics of the soil. Soil factors such as soil type, nutrient availability and pH can affect indirectly by affecting the nutrient and root exudate composition.

- Plant genotype

The plant genotype influences the composition of microbial communities associated with plants through specific metabolic processes (Garbeva *et al.* 2008; Philippot *et al.* 2013). The two halophilic plants used as forage in North America, *Atriplex canescens* and *A. torreyi*, showed diversity in the prevalence of diazotrophic bacteria (Lucero *et al.* 2011). This is due to metabolic differences observed between the two plant genotypes.

- Soil

In model plant *Arabidopsis thaliana*, the diversity in the rhizospheric microbial community is mostly determined by the soil composition rather than the plant genotype (Lundberg *et al.* 2012) whereas in the phyllosphere which is less subjected to the high density of microbial inoculum from the soil, the plant genotype seems to have a stronger influence (Dias *et al.* 2012;

Rigonato *et al.* 2012). Some hypotheses also suggest that clay soil have stronger influence on rhizosphere community (Buyer *et al.* 1999). However the effect of soil may be indirect through plant growth and nutrients.

- Developmental stage

It has been observed that developmental stages of the host plants influence the microbial communities which was caused by plant metabolism during the life cycle (van Overbeek and Elsas 2008; Chaparro *et al.* 2014). For instance, the rhizosphere in maize plants is mostly determined by the developmental stage of the maize plant rather than its soil type or the genotype. Andreote *et al.* (2010) reported that the composition of bacterial communities (both rhizosphere and endosphere) in potato was driven by the plant genotype and the plant developmental stage.

- Physical and chemical changes in matrix

Agricultural practices have a direct impact on the structure of microbial communities associated with plants. This is due to the change in the physical and chemical propertiesin the soil matrix, ultimately influencing the way plants exert selection criteria for microbiome. In sugarcane (*Saccharum officinarum*), soil chemical fertilization alters the composition of bacterial communities in cultivated fields (Wallis *et al.* 2010).

- Microbial interactions

Pathogenic microorganisms can alter the composition of microbial communities in the rhizosphere (Mendes *et al.* 2011) and in the endosphere (Araújo *et al.* 2002). On the other hand there are reports demonstrating the ability of beneficial microorganisms to confer resistance of *A. thaliana* to

pathogenic microorganisms (Nongbri, 2012; van der Ent *et al.* 2008). Comprehensive studies on these topics can uncover essential components of plant microbiomes or specific stimuli for beneficial groups that are able to suppress the development of pathogens in plants.

- Root secretions

Plant roots release photosynthetic products in soluble form known as root exudates which consists of free oxygen, ions, enzymes, mucilage, secondary metabolites (Uren, 2000). The composition of root exudate varies from plant to plant (Somers *et al.* 2004). Microorganisms capable of metabolizing these compounds for use as carbon source establish themselves around the roots or in the rhizosphere. The symbiotically associated bacteria confer many benefits to host plants and these bacteria are called plant growth promoting bacteria (PGPBs). *Azospirillum,* in particular, has been studied extensively both as a PGPR and as an endophyte and is used as a commercial inoculant to improve yields and/or reduce expensive fertilizer use (Bashan 1998; Hungria *et al.* 2010; Okon and Itzigsohn, 1995).

Effects on host metabolism and physiology

Phytohormone signaling

PGPRs inoculation leads to alteration in host architecture such as increased root hair and reduced root length. This is due to phytohormone action which acts as an interface between plants and microorganism signaling (Spaepen *et al.* 2007; Yuan *et al.* 2008).

- Auxin

Khan and Doty (2009) had shown the rapid generation of roots in sweet potato inoculated with indole acetic acid (IAA) producing strains. IAA can be generated in bacteria through different biosynthetic pathways. It was shown that there was a direct positive effect of IAA produced by *Pseudomonas putida* on the root development (Patten and Glick, 2002).

- Cytokinin

Arkhipova and colleagues (2005) studied the production of different cytokinins by *Bacillus subtilis* and showed improved growth for lettuce plants (*Lactuca sativa* L.) after inoculation with cytokinin-producing bacteria.

- Ethylene

Bacteria such as *Pseudomonas spp., Burkholderia caryophylli, Achromobacter piechaudii* were shown to lower the endogenous ethylene level in planta by producing a degradative enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Mayak *et al.* 2004a, b; Shaharoona *et al.* 2007).

Nutrient acquisition

Many studies on 'endophytic diazotrophs' (nitrogen-fixing bacteria that live in plants, particularly in grasses) have provided evidences that they express *nif* genes and proteins which fix significant amounts of nitrogen (Reinhold *et al.* 2011). It has been shown that Brazilian sugarcane plants harbouring N-fixing endophytes grow well in conditions with low fertilizer inputs showing no symptoms of N deficiencies (Boddey *et al.* 2003). Other non-leguminous

plant species were also shown to benefit from association with diazotrophic endophytes, such as wheat (*Triticum aestivum*; Iniguez *et al.* 2004) and rice (*Oryza sativa*; James *et al.* 2002).

It was hypothesized that nutrient uptake by plants increases with increased root surface area triggered by PGPRs. PGPRs such as *Pseudomonas, Bacillus, and Rhizobium* have been demonstrated to stimulate ion transport and dissolution of insoluble forms of phosphate (Richardson *et al.* 2009; Saleh *et al.* 2004). Solubilization processes occur through: acidification of the external medium through the release of low molecular weight organic acids (such as gluconic acid) that chelate the cations bound to phosphate, and production of phosphatases/phytases that hydrolyse organic forms of phosphate compounds (Miller *et al.* 2009). Phosphate acquisition from soils with low concentration of phosphorus can also be enhanced by mycorrhizal symbioses (Bolan, 1991; Richardson *et al.* 2009). Nitrogen acquisition can be upregulated greatly by symbiotic nitrogen fixation which is common in legumes forming root nodules with rhizobia (Franche *et al.* 2009). Actinorhizal species (e.g., *Alnus, Casuarina, Myrica*) form symbiotic N_2-fixing nodules (rhizothamnia) with Actinobacteria, and cycads (e.g., *Ceratozamia, Macrozamia*) form N_2-fixing structures (coralloid roots) with cyanobacteria (Vessey *et al.* 2005; Franche *et al.* 2009).

Abiotic stress tolerance

The association of plants with microbes has shown to increase plants resistance to abiotic stresses. Rhizospheric microorganisms affect plant cells by induction of osmoprotectors and heat shock proteins. Yang *et al.* (2008) introduced the term 'induced systemic tolerance' (IST) that is caused by

PGPRs. According to this concept, IST causes physical and chemical changes in plants, which result in plant tolerance to abiotic stresses.

- Drought

Grover *et al.* (2010) proposed that certain microbes may lessen the impact of soil drought through formation of exopolysaccharides, induction of resistant genes, increased circulation of water in the plants, synthesis of ACC-deaminase, indole-acetic acid and proline. PGPRs reduce soil drought through the following processes:

- Cytokinin production induces abscisic acid accumulation in leaves which in turn causes closing of stomata (Figueiredo *et al.* 2008; cit. Yang *et al.* 2008)
- Production of antioxidants to degrade active form of oxygen (Yang *et al.* 2008)
- Bacteria expresses ACC deaminase enzyme which degrades the ethylene precursor 1-amino- cyclopropane-1-carboxylate (ACC) (Yang *et al.* 2008).

- Temperature

The bacterium *Burkholderia phytofirmans* PSJN colonizes grapevine residues and confers temperature tolerance against heat and frost through increases in the levels of starch, proline and phenols (Miloševic, 2008).

- Salinity

Different strategies have been used by microorganisms to confer host tolerance against salinity (Grover *et al.* 2010). Wheat seedlings inoculated with bacteria produce exopolysaccharides (EPS) which restrict sodium

uptake and stimulate plant growth under high salinity (cit. Grover *et al.* 2010). AM fungi play essential role in osmoregulation and proline production which enhance salinity tolerance in Corn, beans and clover (Feng *et al.* 2002, cit. Grover *et al.* 2010).

Phytoremediation

Phytoremediation is the reclamation of polluted land by plants through a series of processes. Plant-microbe interaction processes can increase phytoremediation efficiency, reclaiming organic and inorganic metals, triggering the physiology of the plant and influencing water and metal uptake (Farrar *et al.* 2014). *Burkholderia cepacia has been shown to increase the efficiency of remediation* (Weyens *et al.* 2009). Plant growth promoting bacteria (PGPB) synthesize siderophores which can sequestrate iron from the soil to the plant cells. They also solubilize minerals such as phosphorus making it more accessible to plants for their uptake (Amora-Lazkano *et al.* 2014). The genes involving in polychlorinated biphenyls-degradation have been isolated in bacteria (Brazil *et al.* 1995). Idris *et al.* (2004) investigated the involvement of endophytes and rhizobacteriain metal uptake by *Thlaspi goesingense*, a hyper accumulator of Nickel. Fungi have shown to have symbiotic relationship with the plants which can be used in the phytoremediation technology (Khan, 2006). Arbuscular mycorrhizal fungi have been shown to increase uptake of metals (Liao *et al.* 2003) and arsenic (Liu *et al.* 2005) in plants. Fungi influence the pollutant bioavailability in three ways:

- Competing with roots and microorganism for metal uptake

- Protecting roots from direct exposure to pollutant by forming ectomycorrhizal sheath
- Pollutant transport through increased soil hydrophobicity

Modulation of host resistance and disease protection

Interactions with bacteria can induce two types of plant defense responses: systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Wu *et al.* 2009). A major distinction between the two is the involvement of salicylic acid pathway in SAR and salicylic acid-independent pathway in ISR. Mendes *et al.* (2007) have shown the ability of endophytic *Burkholderia* spp. to control the growth of the pathogen *Fusarium moniliforme*. Other example of plant disease control is the use of *Pseudomonas* strains to induce resistance in apple (*Malus domestica*) against the pathogenic fungus *Venturia inaequalis*, which causes apple scab (Gau *et al.* 2002). Some pathogenic bacteria rely on signalling molecules for the development of infection. However, other microbes may degrade these signalling molecules. Pretreatment of potato slices with *Bacillus thuringiensis* resulted in reduced maceration from *Erwinia carotovora* virulence (Dong *et al.* 2004).Interestingly, the inoculation of sugarcane and rice with defined strains of endophytic and/or rhizospheric bacteria, particularly *Azospirillum*, *Burkholderia*, *Gluconacetobacter* and *Herbaspirillum*s pp. showed induction of plant defense-related genes, such as resistance (R) genes and leucine-rich repeat (LRR)-containing-receptor-like kinases (Brusamarello-Santos *et al.* 2012). Apart from bacteria there are some fungi that contribute to disease protection in plants. Waller *et al.* (2005) reported the protective role of *Piriformospora indica* on barley plants.

When infected by *P. indica* these plants become resistant to root infection caused by *Fusarium culmorum*. Also they induce a systemic resistance in leaves against biotrophic fungus *Blumeria gramanis* which causes powdery mildew in barley.

Future perspectives

The important scope to study plant-microbial interaction is to uncover promising alternative for the improvement in agricultural sectors. Better understanding of the processes and mechanisms of interaction with microorganisms will immensely contribute to the stability and sustainability of the ecological environment and landscape. Selecting specific type of plant-microbe interaction system to suit a particular agricultural or phytoremediation application is difficult but would help to explain the sometimes confounding results from field applications that may result from varying environment conditions or species specificity. Some microbial species and strains could be used as model systems for understanding the molecular mechanisms of plant tolerance and adaptability to stressful conditions. Scientific endeavors on available techniques and continual improvisation should be taken in order to address the complexity of this network.

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3. High prevalence of Torque Teno Virus in Arunachal Pradesh of North-Eastern India

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Abstract

Torque teno virus (TTV), a circular DNA virus is found in the plasma of over 80% of people worldwide, however its prevalence varies from place to place. At present, the epidemiology of TTV in India is preliminary, with no documented studies from northeastern region. Understanding the epidemiology of TTV is important for clarifying the endemicity and pathogenic potential of the virus which is mostly neglected. The present study was aimed to get baseline information on the prevalence of TTV in the general community as well as in patients with hepatitis B virus (HBV). The study group comprised of patients infected with HBV and healthy individuals without any underlying disease. All the subjects belonged to Arunachal Pradesh, a state of northeast India, dominated by indigenous tribal population. TTV DNA was detected in sera of collected specimens by semi-nested PCR utilizing primers targeting 5'untranslated region of TTV. TTV was detected in 88.6% (93/105) of HBV infected individuals and 83.5%

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(106/127) in healthy individuals. TTV prevalence in pediatric age group (<15 years) was found to be 80% in healthy subjects. The study for the first time documents the high prevalence of TTV in a tribal community of India which seems to be acquired early in childhood. Although, the study detected very high endemicity of TTV in Arunachal Pradesh, it appears to be a silent bystander than a potential pathogen.

Keywords: *TTV, Torque teno virus, HBV, Northeast India*

Introduction

Torque teno virus (TTV), earlier known as transfusion transmitted virus, is a non- enveloped and circular DNA virus (3.6-3.9kb) of negative polarity (Mushahwar et. al., 1999). The genome is divided into coding region (2.8kb) and an untranstranslated region of approximately 1.2kb. It was first isolated from a Japanese patient with post transfusion hepatitis of unknown etiology (Nishizawa et al., 1997).

Worldwide, information regarding epidemiology, endemicity and pathogenic potential of TTV is limiting including the Indian sub- continent. Arunachal Pradesh, one of the seven northeastern states of India, is mostly inhibited by tribal populations of diverse ethnic origins. Earlier studies have reported that hepatitis B is endemic to Arunachal Pradesh with prevalence ranging from 8.6% to 21% (Prasad, 1983; Biswas, 2007). Since TTV also shares common mode of transmission with HBV, it is hypothesized that the former may also be present in the community of Arunachal Pradesh along with HBV; however, there is no documented study to clarify the same. In absence of adequate information on the epidemiology and the pathogenic potential of

TTV, the prime objective of the present study was to get baseline data regarding the prevalence of TTV in the general community as well as in high risk population such as patients with HBV in the present setting.

Materials and methods

Patients and samples: Patients with hepatitis B virus infection were recruited by conducting hepatitis B mass screening at selected places in Arunachal Pradesh during 2012-2014. Patients hailing from Arunachal Pradesh, visiting or admitted to major hospital in Dibrugarh district of Assam were also enrolled. Confirmation of HBV infection was based on the sero-positivity of hepatitis B surface antigen (HBsAg) and antibodies to hepatitis B core antigen (anti-HBc).The subjects (from Arunachal Pradesh) found negative for HBV and other hepatotropic viruses such as Hepatitis C (HCV) and without clinical history of any chronic diseases served as apparently healthy control. About 5 ml of venous blood samples were collected in sterile K_2EDTA vials (BD, Sparks, USA) by trained phlebotomist from enrolled subjects with prior informed consent.

Laboratory assays: The plasma specimens were processed for serological investigations such as markers of HBV, HCV, HIV using enzyme immune assay following kits and methods from DRG international USA. Hepatitis B DNA viral load was estimated from 500µL of collected plasma specimens in Cobas TaqMan 48 analyzer (Roche, Pleasanton, CA, USA) using viral nucleic acid extraction kit (Roche). Viral DNA was extracted from 100-200 µL of plasma samples using magnetic bead method in an automated nucleic acid extraction system (Magnapure LC 2.0. Roche;Rotkreuz, Switzerland). TTV DNA was detected by semi-nested PCR targeting 5'untranslated region

as described by Kenar et al (Kenar Koohi, 2012). For further confirmation, few TTV isolates were subjected to dideoxy sequencing using inner nested primers for 5' UTR region used in PCR. Nucleotide sequences were edited manually in BioEdit softtware V.7.0 (Hall, 1999) and Molecular phylogenetic analysis conducted in MEGA software v 6.0 (Tamura, 2013) using Neighbor joining method (Saitou & Nei, 1987) and employing Kimura 2- parameter (Kimura, 1980).

Results and Discussion

The study group consisted of 105 HBV patients and 127 healthy individuals. The distinguish features of such study subjects are given in Table1.TTV was detected by nested PCR (Figure 1) in 88.5% (93/105) and 83.5% (98/119) among patients with HBV and healthy control respectively. The detection was also supported by the sequence analysis (GenBank Accession No. **KM596844-48**). Among the HBV cases, co- infected with TTV, about 2.15% (2/93) had acute HBV marked by anti-HBclgM positivity. Thus majority of cases were suspected to have chronic hepatitis B infection. TTV DNA was detected in patients with HBV irrespective of viral load, Alanine aminotransferase (ALT) level, and HBeAg and HBeAb status as shown in Table 2. No statistical difference with regard to age and sex were observed in both the groups of subjects processed for TTV (Table1). Among healthy individuals tested for TTV, the overall anti HBc prevalence was 48.8% (62/127), where TTV was detected in 90.0% (56/62) of individuals with anti-HBc Positivity. TTV was also found in about 76.9% (50/65) subjects without any prior exposure to HBV (P value=0.31). The phylogenetic analysis

revealed that the 4 of 5 TTV isolates belonged to TTV genomic group 3 while one belonged to genomic group 2 (Figure 2).

It is believed that TTV is present in the plasma of over 80% of world's population; however its prevalence varies in the Indian subcontinent. In north India, Irshad et al (Irshad, 2008) have reported a prevalence of TTV ranging from 22%-35% in patients with various liver diseases and 27% in healthy control. In yet another study from north India, Chattopadhya et al (Chattopadhyay, 2005) have found TTV prevalence to as 26% (6/23) in chronic Hepatitis B and 12% (12/100) in healthy control. In western India, a low prevalence of TTV has been reported in patients with chronic hepatitis (6.7%, 5/75) and among blood donors (7.4%, 4/54) (Arankalle, 2000). In the present study, high prevalence of TTV has been found in both healthy individuals (83.5%) and in patients with Hepatitis B (88.5%), which is higher than studies reported so far in other parts of India. The reported prevalence, as done using UTR primers, is analogous to studies from Egypt (85%), Japan (70-93%), Nepal (82%) and Bolivia (82%) (Bendinelli et al., 2001). TTV prevalence in pediatric age group (<15 years) was found to be 80% in healthy control which may possibly explain that TTV is acquired early in childhood and persist throughout life

in the target population. However, further studies are required to firmly confirm the same.

In India, the epidemiology of TTV is mostly known from limited studies conducted in northern and western part with no documented studies from eastern and northeastern region. The study for the first time provides evidence for the existence of TTV in greater proportion among the inhabitants of Arunachal Pradesh. The high rate of positivity among healthy

individuals suggests that TTV is widely prevalent in the general population of Arunachal Pradesh, causing no significant disease manifestation. The precise cause of such high prevalence of TTV in the study population is not known although ethnic differences may partly account for the same.

Table 1: Demographic differentiation of hepatitis B patients and healthy individuals recruited in the study

	HBeAg[1]		Anti HBeAg[2]		ALT		HBV Viral load	
	Positive (%)	Negative (%)	Positive (%)	Negative (%)	>40 (%)	<40 (%)	>log4 (%)	<log4 (%)
TTV Positivity	36/42 (85.7)	56/62 (90.3)	47/52 (90.4)	44/51 (86.3)	14/15 (93.3)	27/29 (93.1)	61/66 (92.4)	32/39 (82.1)
Odds ratio	0.64		1.5		1.03		2.2	
95% CI	0.19-2.1		0.44-5.0		0.08-12.4		0.69-7.2	
P value (based on chi square test)	**0.76**		**0.76**		**0.99**		**0.45**	

1: Hepatitis B e antigen; 2: Antibody to hepatitis B e antigen; ALT: Alanine amino transferase; HBV: Hepatitis B virus

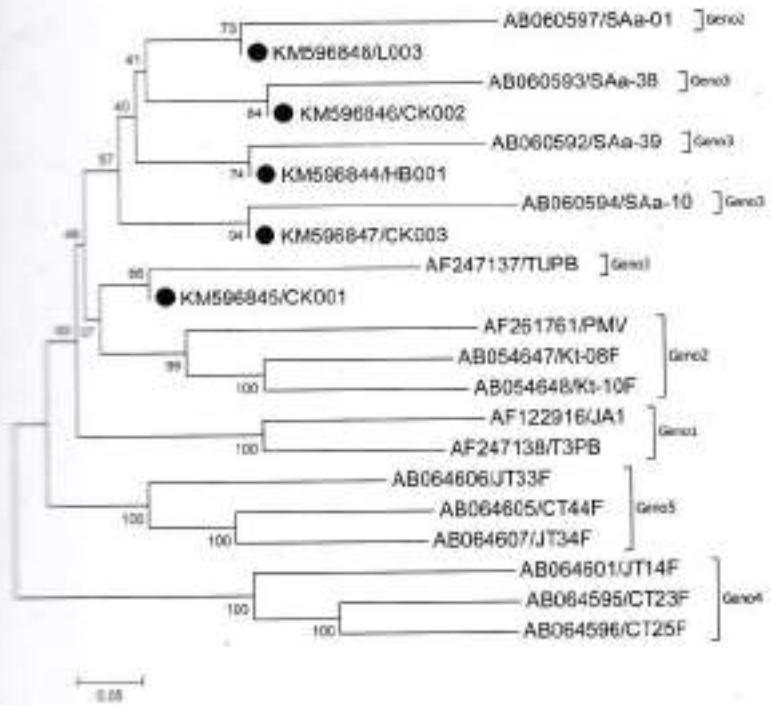
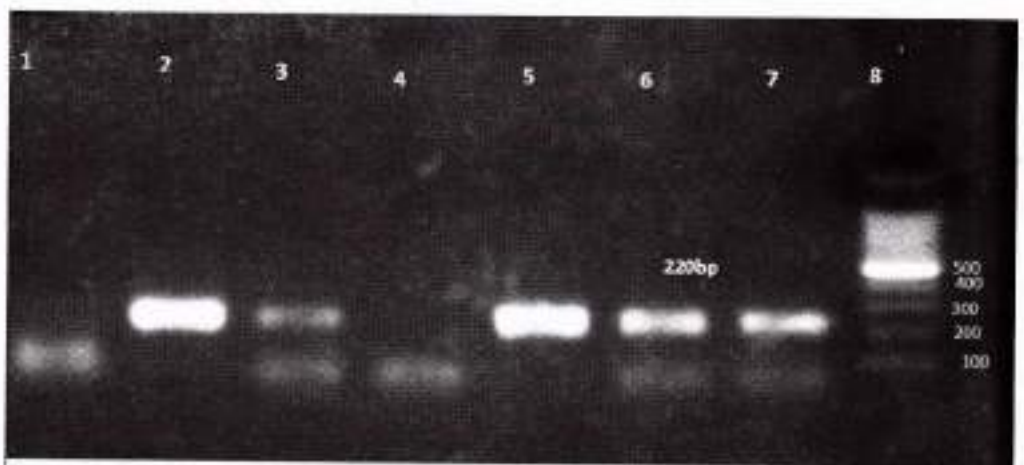


Figure 2: Dendrodrogram showing clustering of five TTV isolates from Arunachal Pradesh to TTV genomic group 2 and 3. TTV reference sequences belonging to all the five major genomic groups were downloaded from NCBI-GenBank and aligned by multiple alignments using CLUSTAL W in MEGA software v 6.0. The individual isolates are shown with their name and GenBank accession numbers. The northeastern TTV isolates are shown with solid circles. Phylogenetic tree was constructed by Neighbor-Joining method using the Kimura 2-parameter method. All ambiguous positions were removed for each sequence pair. Robustness of tree was inferred by bootstrap of 500 replicates. Evolutionary analyses were conducted in MEGA6.

In absence of any significant differences (Table1), the presence of TTV does not seem to aggravate hepatitis B virus replication or cause profound pathogeneicity. Thus based on this observation along with high prevalence in healthy subjects led us to conclude that TTV appears to be silent bystander than a potential pathogen in the present setting.

About past exposure to HBV, TTV was detected in healthy subjects with high number of anti HBc IgG antibody, although not significant. Thus it may be possible that TTV was co transmitted along with HBV in the study population, however, possibility of other risk factors cannot be ruled out, since it was also detected in subjects with no previous contact to HBV.

Although TTV is a DNA virus, it shows unusual high degree of genetic variability (Okamoto, 1998; Tanaka et al., 1998). Worldwide there is a dominance of geno groups 1, 2 and 3. In Asia there is predominance of genogroup1 (Gallian et al., 2000) while in India, geno group 1 is mostly prevalent (Abraham, 2005) besides genogroup3 and genogroup 5 (AbuOdeh et al., 2015). In the present study, majority of the isolates belonged to genogroup 3 while one isolate of genogroup 2 was also detected. This is a distinctive feature noted in this part of India which demands further studies to be carried out in the northeastern region of India to document genetic diversity of TTV and its clinical relevance, if any.



Lane 1: Negative template control, Lane 2: Positive control, Lane 3-7: clinical specimens, Lane 8: 100 bp DNA ladder

Figure1: Gel photograph showing 220bp DNA product obtained using primers targeting 5'UTR region of TTV genome.

The choice of primers in the PCR has been found to have profound effect on the rate of TTV detection. Whereas primers targeting 5'UTR, a well conserved region has been shown to detect most of TTV genotypes, the detection rate is limited when using ORF1 primers (Biagini et al., 2006; Kato et al., 1999). In the present study, UTR-5 primers were employed to aid in enhanced detection limit.

Table 2: Intra-group comparison of various serological markers of hepatitis B infection with respect to TTV positivity.

Category	No. of cases	TTV positive (%)	Age (In years)		TTV positivity in individuals aged (in yrs)			Proportion of male and female found TTV positive	
			Range	Mean± SD	<15 (%)	16-45	>45	Male Proportion	Female proportion
HBV case	105	93 (88.6)	3-73	24± 14	21/24 (87.5)	65/74 (87.8)	7/7 (100)	57/64 (89)	36/41 (87.8)
Healthy control	127	106 (83.5)	5-65	29.5± 14	20/25 (80)	73/86 (84.8)	13/16 (81.3)	56/70 (82.8)	48/57 (84.2)
P value (case & control) based on chi square test		**0.36**		**0.41**	**0.64**	**0.82**	**0.16**	**0.93**	**0.19**

Conclusion

In conclusion, high prevalence of TTV has been noted in parts of Arunachal Pradesh in northeast India. Large scale studies including various diseased conditions and in other places of northeastern states would be required to firmly understand the epidemiology, endemicity and clinical relevance of TTV genotypes in the region.

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4. Morphological Analyzer and Generator for Indian languages processing: A brief survey

Alusha Vellintihun Hujon[8]

Abstract

This paper is a survey of Morphological Analyzer and Generator for Indian Languages. The first part of this paper is an introduction to the use of Morphological Analyzer, it also points out some of the various Morphological approaches that can be used to build Morphological Analyzer and Generator (MAG). The second part of this paper presents a brief explanation about different morphological analyzer and generator developments for some Indian languages.

Keywords: *Natural language processing, Machine translation, Morphological analyzer and generator, MAG, MAG approaches, MT for Indian languages*

Introduction

Machine Translation (MT) is the automatic translation from one natural language to another natural language using computers. It is the main key application in the field of Natural Language Processing (NLP). To understand and analyze a particular natural language, automated tools are required. Morphological analyzer and generator are such tools and they have been developed for many natural languages which aid in the translation of one natural language to another. Morphological analyzer is used to analyze a

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given word and Morphological generator is used to generate word given the stem and its features. In order to develop a full-fledged morphological analyzer and generator (MAG) tools is a challenging task.

The morphological structure of any language is different and capturing its complexity in a form that a machine can analyze and generate is a challenging job. Analyzing the internal structure of a particular word is an important intermediate stage in many natural language processing applications especially in bilingual and multilingual MT system. To build a MAG for a language one has to take care of the morphological peculiarities of that language, specifically in case of machine translation. Some peculiarities of language such as, the usage of classifiers, excessive presence of vowel harmony, make it morphologically complex and thus, a challenge in natural language generation (NLG).

Morphological Analyzer and Generator Approaches

In general there are several approaches that have been attempted for developing Morphological analyzer. Among these approaches that are used for developing morphological analyzer and generator, some are language dependent and some are language independent. These approaches are briefly discussed below:

1. Corpus Based Approach:

In case of corpus based approach, a large sized well generated corpus is required for training. Any machine learning algorithm is used to train the corpus and collects the statistical information and other necessary features from the corpus. The collected information is used as a MAG model. The performance of the system will depends on the feature and size of the corpus.

The disadvantage is that corpus creation is a time consuming process. This approach is suitable for languages having well organized corpus.

2. **Paradigm Based Approach:**

For a particular language, each word category like nouns, verbs, adjectives, adverbs and postpositions will be classified into certain types of paradigms. Based on their morphophonemic behavior, a paradigm based morphological compiler program is used to develop MAG model. In the paradigm approach a linguist or the language expert is asked to provide different tables of word forms covering the words in a language. Based on this information and the feature structure with every word form a MAG can be build. The paradigm based approach is also well suited for highly agglutinative language nature and this or the variant of this scheme has been used widely in NLP. Literature shows that morphological analyzers are developed for almost all Indian languages using paradigm based approach.

3. **Finite State Automata (FSA) Based Approach:**

Finite state machine or finite state automation (FSA) (or finite automation) uses regular expressions and is used to accept or reject a string in a given language (Saranya, 2008). In general, an FSA is used to study the behavior of a system composing of state, transitions and actions. When FSA start working, it will be in the initial stage and if the automation is in any one of final state it accept its input and stops working.

4. **Finite State Transducers (FST) Based Approach:**

FST is a modified version of FSA by accepting the principles of a two level morphology. A finite state transducer essentially is a finite state automaton

that works on two (or more) tapes. The most common way to think about transducers is as a kind of "translating machine" which works by reading from one tape and writing onto the other. FST's can be used for both analysis and generation (they are bidirectional) and it act as two level morphology. By combining the lexicon, orthographic rules and spelling variations in the FST, we can build a morphological analyzer and generator at once (Jurafsky & Martin, 2008).

5. Stemmer Based Approach:

Stemmer uses a set of rules containing list of stems and replacement rules to stripping of affixes. It is a program oriented approach where developer has to specify all possible affixes with replacement rules. Potter algorithm is one of the most widely used stemmer algorithm and it is freely available. The advantage of stemmer algorithm is that it is very suitable to highly agglutinative languages like Dravidian languages for creating MAG.

6. Suffix Stripping Based Approach:

For highly agglutinative languages such as Dravidian languages, a MAG can be more successfully build using suffix stripping approach. The advantage of the Dravidian language is that no prefixes and circumfixes exist for words. Words are usually formed by adding suffixes to the root word serially. This property can be well suited for suffix stripping based MAG. Once the suffix is identified, the stem of the whole word can be obtained by removing that suffix and applying proper orthographic rules. A set of dictionaries like stem dictionary, suffix dictionary and also using morphotactics and sandhi rules, a suffix stripping algorithm can successfully implements MAG.

7. Rule Based Approach:

In the rule-based approach, human experts specify a set of rules to describe the translation process. This approach applies to large collections of linguistic rules and it requires a good linguistic knowledge to write the rules. It is often used for Machine translations.

MAG for Indian Languages

An extensive work has been done already for developing MAG in various Indian languages from the last ten to fifteen years. Some of these works are as follow:

1. MAG for Tamil Language:

One of the MAG for Tamil language is the AMRITA Morph Analyzer and Generator for Tamil. This MAG uses a Rule Based Approach. Menon *et al.*, (2010) developed a rule based Morphological Analyzer and generator for Tamil using finite state transducer called AMAG

The performance of the system is based on lexicon and orthographic rules from a two level morphological system. The system consists of list of 50000 nouns, around 3000 verbs and a relatively smaller list of adjectives. The proposed AMAG is compared with the existing Tamil morph analyzer and generator called ATCHARAM and proved better performance.

2. MAG for Kannada Language:

Kannada Morphological Analyzer and Generator Using Trie. This MAG is also Rule based with Paradigm approach, proposed by Sambhavi *et al.*, (2011) which is a morphological analyzer and generator for Kannada language. They used *Trie* as a data structure for the storage of suffixes and

root words. The disadvantage of *Trie* is that it consumes more memory as each node can have at most 'y' children, where 'y' is the alphabet count of the language. As a result it can handle up to maximum 3700 root words and around 88K inflected words.

3. MAG for Malayalam Language:

One of the MAG system for Malayalam language is the Morphological analyzer for Malayalam verbs, which was developed by Saranya (2008) as a prototype morphological analyzer for Malayalam language based on hybrid approach of Paradigm and Suffix Stripping Method .

4. MAG for Bengali Language:

Development of a morphological analyzer for Bengali is also present. It is an open-source morphological analyzer for Bengali Language. It uses finite state technology. This Analyzer was developed by Zaher *et al.*, (2009).

5. MAG for Assamese, Bengali, Bodo and Oriya Languages:

Morphological analyzer using rule based affix stripping approach (2011). The design and development of morphological analyzers for four Indian languages-Assamese, Bengali, Bodo and Oriya was proposed by Parakh & Rajesha (2011). At present it is an ongoing work based on dictionary based and suffix stripping approach and the performance of the system directly related to the size of the dictionary. The developed prototype model currently can handles inflectional suffixes and work is going to handle derivation as well as prefixation.

6. MAG for Manipuri:

A Manipuri Morphological analyzer has been developed by Singh and Bandopadhyay (2005). It uses a Manipuri – English dictionary that stores the Manipuri root words and their associated information. It uses the string pattern stripping and matching technique. This Morphological analyzer can handle five types of words. A word without any affix, word with a prefix, word with one or more suffix, compound word, semantic reduplicative words. There is also another Morphological analyzer which has been developed by Choudhury *et al.*, (2008).

Conclusion

There are many researches in the field of Machine translation and Natural language processing and many systems and tools have been developed. This paper has presented a study on different developments of morphological analyzer and generator for a few Indian languages. Additionally it also gives a brief idea about the existing approaches that have been used to develop morphological analyzer and generator for Indian languages. There are still other analyzers and generators for many other languages that were not possible to present in this paper. The Table below summarizes the Analyzers discussed and the approaches used to develop them.

Table 1: Existing Morphological analyzers

Languages	Approach used	Analyzer/Generator
Tamil	Rule Based Approach	AMRITA (Menon *et al.*, 2010)
Kannada	Trie	Kannada Morphological Analyser and Generator Using Trie (Shambhavi *et al.*, 2011).
Malayalam	hybrid approach of Paradigm and Suffix Stripping Method	Morphological analyzer for Malayalam verbs (Saranya, 2008).
Bengali	finite state technology	Morphological analyzer for Bengali (Saranya, 2008).
Assamese, Bengali, Bodo and Oriya Languages	using rule based affix stripping approach	Morphological analyzer using rule based affix stripping approach (Shambhavi *et al.*, 2011).
Manipuri	It uses the string pattern stripping and matching technique	Morphological Analyzer for Manipuri. (Singh & Bandyopadhyay, 2005)

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5. Phytoremediation Technologies: An Overview of the Different Mechanisms Currently in Use and their Associated Advantages and Limitations

Joanica Delicia Jyrwa[9] and Aldenna M. Susngi[9,10]

Abstract

Phytoremediation, a process that uses plants and their associated microorganisms for cleaning up the environment, is an emerging technology for reducing the risk posed to humans, other organisms and the ecosystem as a whole by toxins, heavy metals, radioactive substances in contaminated sites. Being cost-effective and eco-friendly, phytoremediation techniques are gaining worldwide recognition. Phytoremediation is a general term which includes several techniques: phytoextratction, rhizofiltration, phytodegradation, phytostimulation, phytovolatilisation, phytostabilisation and phytohydraulics. The objective of this overview is to discuss in brief the basic mechanism of the different phytoremediation technologies currently in use, and their associated advantages and drawbacks.

Keywords: *Phytoremediation, green technology, phytoremediator, contaminants, heavy metals, hyperaccumulators.*

1. Introduction

The build-up of toxic pollutants in land, surface as well as underground waters from domestic, industrial, military and agricultural activities causes

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imbalance in ecosystem and affects natural resources. Remediation of such places, therefore, is not only important but necessary to prevent any further contamination. Plants with their efficient metabolic and absorption capabilities can take up nutrients or non-essential contaminants as deep as their roots grow, making them useful agents for cleaning up the environment. Metals are generally absorbed by specific transporters (channel proteins) or hydrogen ions carrier proteins present on the roots (Grapsson, 2011).

Phytoremediation is a "green" technology that uses plants to degrade, extract, contain, or immobilize contaminants from soil and water which serves as an innovative and a cost-effective alternative to the established treatment methods used at hazardous waste sites (Flathman & Lanza, 1998; Salt *et al.*, 1998; USEPA, 2000). The term "Phytoremediation" comes from the Greek *phyto* meaning "plant" and Latin *remedium* meaning "able to cure" or "restore". It was coined by Dr. Ilya Raskin of Rutgers University's Biotechnology department, USA, in 1991 to describe the process of using plants to clean up pollution in the environment (Laghlim *et al.*, 2015; Vamerali *et al.*, 2010). This technology can be used to immobilise and/or clear up both organic and inorganic contaminants.

The idea behind phytoremediation was conceived when scientists tried to find out if plants could be used to remove radioactive toxic pollutants, like iodine, strontium, cesium-137 and plutonium, from soil or underground water after the nuclear explosion which took place on the 26th April 1986 at Chernobyl Nuclear Plant Reactor 4 in Ukraine (Kipp, 2000). The green plants that are involved in cleaning up the environment are termed "Phytoremediators" (USEPA, 2001).

Phytoremediation works best at sites with low to medium amounts of pollution and in low permeability soils (USEPA, 2001). Depending on the chemical nature and properties of the contaminant and the plant characteristic, contaminants and pollutants may be cleaned up by the plants using different phytoremediation techniques (**Figure 1**). These techniques include: (i) **Phytoextraction** - toxins and pollutants can be absorbed by the roots and the plants are allowed to grow, after which, they will be harvested, destroyed or recycled; (ii) **Rhizofiltration** - contaminants that are in sofution surrounding the root zone are adsorped or precipitated onto plant roots; (iii) **Phytodegradation** - usually in roots or shoots, toxins can be converted into less toxic chemicals by the plant's metabolic activities; (iv) **Phytostimulation** - contaminants are broken down in the roots by microbial activity; (v) **Phytovolatilisation** - pollutants may also be absorbed by the roots from the soil and given out through the process of transpiration; (vi) **Phytostabilisation** - immobilisation of a contaminants through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone of plants; (vii) **Phytohydraulics** - the use of plants to rapidly uptake large volumes of water to contain or control the migration of subsurface water (Baker & Brooks, 1989; Dushenkov *et al.*, 1995, 1997, Salt *et al.*, 1995; USEPA, 2000). Examples of some phytoremediators and related pollutants for different phytoremediation techniques are highlighted in **Table 1.**

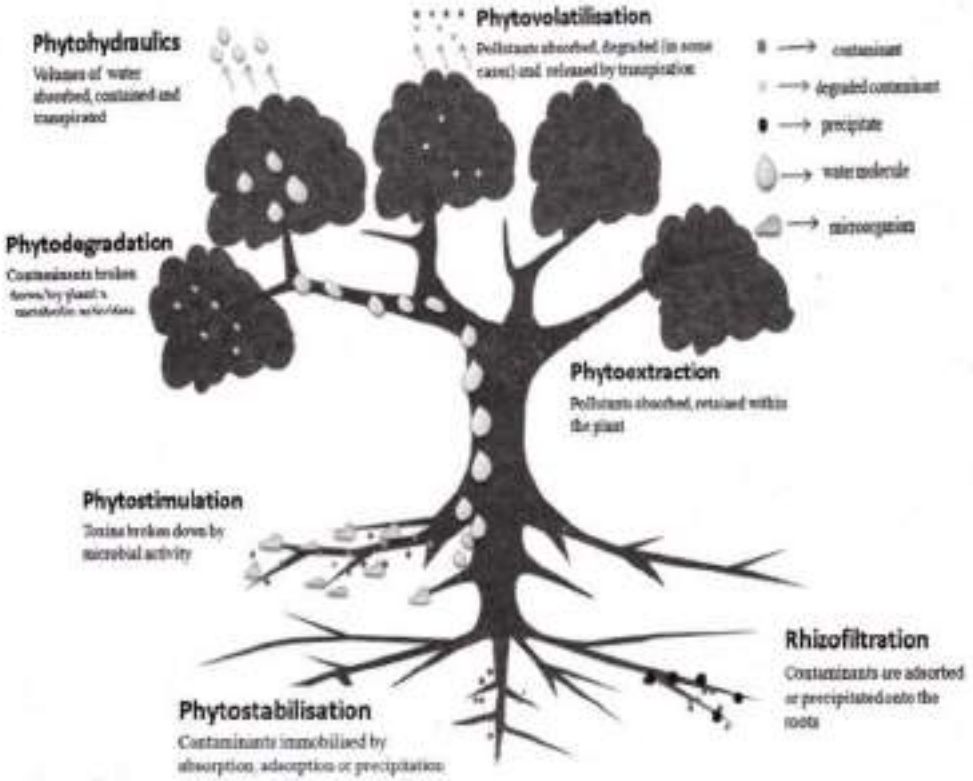

Figure 1: Overview of the basic mechanism of action of the different phytoremediation technologies.

2. Phytoextraction

2.1.What is Phytoextraction?

Phytoextraction involves the uptake of contaminants, usually metals, from the soil by the plant roots, their storage in the roots or their translocation and subsequent accumulation in the above-ground regions of the plants (Garbisu & Alkorta, 2001; McGrath *et al*., 2001).After the plants

have grown sufficiently, they are harvested or completely , and safely processed to

Table 1. Examples of phytoremediators and related pollutants for different phytoremediation techniques.

Phytoremediation technique	Contaminant Removed and/or Immobilised	Plant(s) involved	Reference
Phytoextraction	Zinc	*Thlaspi caerulescens* and *Arabidopsis halleri*	Dhankhar *et al.*, 2012
	Cadmium	*Brassica napus*	Cojocaru *et al.*, 2016
	Endosulfan	*Helianthus annuus*	Mitton *et al.*, 2016
Rhizofiltration	Uranium	*Phaseolus vulgaris*	Mater, 2010
	Iron, zinc, cadmium, copper, boron and chromium	*Eicchornia crassipes*	Elias *et al.*, 2014
Phytodegradation	Pyrene	*Festuca arundinacea*	Chen *et al.*, 2003
	2,4-dichlorophenol	*Brassica napus*	Agostini *et al.*, 2003
	Polychlorinated biphenyls (PCBs)	*Brassica nigra*	Singer *et al.*, 2003
Phytostimulation	Cadmium	*Astragalus sinicus* (microbe: *Mesorhizobium huakuii*)	Sriprang *et al.*, 2002
	Polychlorinated biphenyls (PCBs)	*Beta vulgaris* (microbe:*Pseudomonas fluorescens*)	Brazil *et al.*, 1995
	3-Methyl benzoate	*Zea mays* (microbe: *Pseudomonas putida*)	Ronchel *et al.*, 2001

Phytovolatilisatio	Carbon tetrachloride	Hybrid poplars	Newman *et al.*, 1997
	Selenium	*Medicago sativa*	Terry *et al.*, 1992
	Mercury	*Salix spp*	Wang, 2004
Phytostabilisation	Lead, zinc and copper	*Agrostis tenuis*	Salt *et al.*, 1995
	Calcareous lead and zinc	*Festuca rubra*	Salt *et al.*, 1995
Phytohydraulics	Gasoline and diesel	*Populus spp.*	Nelson, 1996
	Water-soluble organics and inorganics	*Populus spp., Salix spp.*	ITRC, 2009

facilitate permanent removal of the contaminant. Processing can be achieved by drying, ashing or composting the harvested plant parts (Garbisu & Alkorta, 2001). Occasionally, the accumulated metals can be extracted from the ash enabling them to be recycled (Comis, 1996).

The process of phytoextraction, in some cases can be enhanced by the addition of chemical amendments or chelators such as ethylenediamine tetraacetate (EDTA) and nitrilotriacetate to the soil (Vamerali *et al.*, 2010). The contaminants must be dissolved in a solution and come in contact with the roots in order for them to be absorbed. The amount of contaminant immobilised or cleared from the soil depends on the root zone of the plants being used. Depending on the plant species, the soil depth can range from a few inches to several meters (Schnoor *et al.*, 1995).

Some plant species have a natural ability to absorb metal 50–100 times more than ordinary plants (Chaney *et al.*, 1997; Baker *et al.*, 1998) and sometimes

up to 500 times more (Chibuike & Obiora, 2014). Such species are termed as "hyperaccumulators". Hyperaccumulators are found in metals-rich regions of the world. Generally, for a plant to be classified as a hyperaccumulator, it must be able to accumulate at least 1,000 mg/kg (dry weight) of a specific metal or metalloid. For some metals or metalloids, however, the concentration must be 10,000 mg/kg (Baker *et al.*, 1998). Examples of some hyperaccumulators include, *Thlaspi rotundifolium grown* in a lead-zinc mine area contained 8,200 g/g lead (0.82%) and 17,300 g/g zinc (1.73%), and *Armeria maritima* var. *halleri* contained 1,300 g/g lead, dry weight basis (Reeves & Brooks, 1983).

Four processes are assumed to be essential for hyperaccumulation: root uptake of metal contaminant, transport from roots to the shoots, formation of complexes with chelating molecules and compartmentalisation into the vacuole (McGrath & Zhao, 2003).

2.2.Advantages and Limitations

Phytoextraction has several advantages over conventional techniques, including the fact that the contaminants can be removed *in situ*, the use of green plants which lends added benefits, and the low cost involved in using this technique (Garbisu & Alkorta, 2001). However, most metal hyperaccumulators are slow-growing plants with a small biomass and shallow root systems. Therefore, it takes time to completely remove a particular metal contaminant from a contaminated site and the amount of contaminants accumulated is also less. Additionally, the metals may also have a phytotoxic effect (Kumar *et al.*, 1995) on the plants employed for extracting the metals.

Another problem associated with phytoextraction is that the plant biomass must be harvested and removed, followed by metal reclamation or proper disposal of the biomass (Kumar *et al.*, 1995). In some cases, however, the plant biomass containing the extracted contaminant need not be disposed of; it can be a useful resource. For instance, biomass that contains selenium (Se) has been transported to areas that are deficient in Se and used as animal feed (Bañuelos, 1997).

3. Phytodegradation

3.1. What is Phytodegradation?

Phytodegradation is the phenomenon in which plants breakdown compounds either through metabolic processes within the plant or by the action of enzymes produced. It is also known as phytotransformation (USEPA, 2000).

About 88 species of plants have been reported to successfully take up and degrade 70 organic chemicals (Paterson *et al.*, 1990). The uptake of contaminants mainly depends on hydrophobicity, solubility, polarity of the compounds, type of plant, age of contaminant, and many other physical and chemical characteristics of the soil. Moderately hydrophobic and non-polar molecules will be efficiently absorbed by the roots (Schnoor *et al.*, 1995; Cunningham *et al.*, 1997; Bell, 1992).

Plant enzymes belonging to the classes oxygenase, nitroreductase and dehalogenase are capable of degrading hydrocarbons, explosives and halogen subgroups and many of these enzymes metabolise the chemicals

completely into carbon dioxide and water (Dhankher *et al.*, 1999; Schnoor, 1997).

The technique is used in treating soil, sediments, sludge, groundwater as well as surface water and it also reduces harmful contaminants into stable safer molecules like water (USEPA, 2000; Schnoor, 1997).

3.2. Advantages and Limitations

Plants can be grown in sterile soils where microorganisms are killed by the high contaminants levels proving to be more advanced than biodegradation (USEPA, 2000; Schnoor, 1997). Another advantage is the metabolism of diverse groups of organic compounds including herbicides, insecticides, dichlorodiphenyltrichloroethane (DDT) and fungicide (Burken & Schnoor, 1997; Newman *et al.*, 1997; Thompson *et al.*, 1998; Komossa *et al.*, 1995).

The major limitation of this process is that toxic intermediates or degradation products may form. In a study unrelated to phytoremediation research, pentachlorophenol (PCP) was metabolised to the potential mutagen tetrachlorocatechol in wheat plants and cell cultures (Komossa *et al.*, 1995). It is also quite difficult to confirm contamination destruction since ways of detecting and identifying the metabolites have not been developed (USEPA, 2000).

4. Rhizofiltration

4.1. What is Rhizofiltration?

In rhizofiltration, plants are used to filter contaminants from water onto root systems. It has been used to concentrate chemicals like lead (Pb), cadmium

(Cd), copper (Cu), nickel (Ni), zinc (Zn) and chromium (Cr), which are initially retained primarily within the roots, after which they are accumulated or immobilised within the plant or on the plant surface. The plants are gradually acclimatised to the contaminated water which requires to be treated. They are then planted on the site, where the pollutants are taken up. Once the plants have become saturated with contaminants, they are harvested and the collected biomass might be subject to some form of final treatment (Evans & Furlong, 2003).

Extracted groundwater, surface water, and wastewater with low contaminant concentrations are remediated by this technique (USEPA, 2000).

4.2. Advantages and Limitations

Rhizofiltration has the ability to use both terrestrial and aquatic plants for either *in situ* or *ex situ* applications. An advantage of rhizofiltration over some of the other phytoremediation technologies is that contaminants do not have to be translocated to the shoots. Thus, species other than hyperaccumulators may be used. Terrestrial plants are preferred because they have a fibrous and much longer root system, increasing the amount of root area (Raskin & Ensley, 2000).

Limitations of this technique include the possible need to grow the plants in a greenhouse or nursery, the periodic harvesting and plant disposal and a good understanding of the chemical interactions. Additionally, the tank design must be well engineered and the pH needs to be adjusted constantly. The cost of remediation by rhizofiltration is also expensive and has been estimated to be $2-$6 per 1000 gallons of water (USEPA, 2000).

5. Phytostimulation

5.1. What is Phytostimulation

Phytostimulation, also known as rhizodegradation, involves the breakdown of contaminants in soil through microbial activity that is enhanced by the presence of the root zone. This technique is also known by names like plant-assisted degradation, plant-assisted bioremediation, plant-aided in situ biodegradation, and enhanced rhizosphere biodegradation (USEPA, 2000).

The microbial populations and activity in the root zone are facilitated by the presence of certain compounds produced by plants. These compounds are known as root exudates and include sugars, amino acids, organic acids, fatty acids, sterols, growth factors, nucleotides, flavanones (Shimp *et al.*, 1993; Schnoor *et al.*, 1995). Active microbial degradation of contaminants in the rhizosphere leads to an increase in surface area of the rhizosphere. However, phytostimulation can also occur in the absence of root exudates. Microbial activity is mainly influenced by aeration and moisture, which is favourable for their growth (USEPA, 2000). The association of exudates or microbial activity may also change the pH of the soil.

5.2.Advantages and Limitations

An advantage of using this technique is that contaminant destruction occurs *in situ*. Furthermore, translocation of the compound to the plant or atmosphere is less likely than with other phytoremediation technologies since degradation occurs at the source of the contamination, and the contaminants may undergo mineralisations which is stable. Installation and

maintenance cost is also much lower as compared to other remedial options (USEPA, 2000).

Development of an extensive root zone likely to require substantial time is one of the main drawbacks of using this technique. Root depth can be limited due to the physical structure or moisture conditions of the soil. Another disadvantage is that rhizosphere might affect an increase in the initial rate of degradation compared to a non-rhizosphere soil (Molina *et al.*, 1995). Besides, laboratory and field studies need to account for other loss and phytoremediation mechanisms that might complicate the interpretation of rhizodegradation. For example, if plant uptake occurs, phytodegradation or phytovolatilisation could occur in addition to rhizodegradation.

The phytoremediators in most cases require additional fertilization because of microbial competition for nutrients. The added exudates might stimulate microorganisms that are not degraders, at the expense of degraders. Moreover, the organic matter from the plants may be used as a carbon source instead of the contaminant, which could decrease the amount of contaminant biodegradation (Molina *et al.*, 1995).

6. Phytovolatilisation

6.1. What is Phytovolatilisation?

In phytovolatilisation, contaminants are taken up by plants and released through the leaves into the atmosphere: normally in a modified form. The ability of contaminants and pollutants to volatilise depends on properties like Henry's constant and vapour pressure. Many contaminants

react with hydroxyl radicals, formed during the photochemical cycle in the atmosphere (USEPA, 2000).

Compounds volatilised can also be products simplified from rhizodegradation and/or phytodegradation, for example, *trichloroethylene (TCE)* in poplars, of the parent contaminant along the transpiration pathway (Chappell, 1998). Tobacco plants are another example of phytoremediators that makes use of phytovolatilisaton to clean up the highly toxic methyl-mercury. These plants degrade, and phytovolatilise elemental mercury into the atmosphere in relatively safe levels (Heaton *et al*., 1998).

The technique is used to treat groundwater, soils, sediments, sludge and generally relies on the transpiration pull of fast-growing trees, which accelerates the uptake of the pollutants (USEPA, 2000).

6.2.Advantages and Limitations

The advantage of using phytovolatilisation is that the contaminants could be altered to less-toxic forms, such as elemental mercury and dimethyl selenite gas. Furthermore, contaminants released to the atmosphere might be subject to more effective or rapid natural degradation processes such as photodegradation (USEPA, 2000).

One obvious limitation of phytovolatilisation is that there is no control on the destination of the volatilised compounds. The hazardous contaminants (such as vinyl chloride formed from TCE) maybe be released into the atmosphere. In addition, the harmful contaminants may accumulate in the plants and consequently be passed on in the products such as fruit or lumber (Newman *et al.*, 1997).

7. Phytostabilisation

7.1. What is Phytostabilisation?

Phytostabilization, also known as phytorestoration, treats soils, sediments and sludge by reducing the mobility of the contaminants and prevents their migration to the surface water, groundwater or air (Cunningham *et al.*, 1995; Flathman & Lanza, 1998). The use of certain phytoremediators to immobilize contaminants in the soil or ground water through absorption and accumulation by roots, adsorption onto roots, or precipitation within the root zone is termed phytostabilisation. Translocation of metals to the above ground portion must be avoided because it may disturb the main purpose of the technique (Tangahu, *et al.*, 2011; USEPA, 2000).

To prevent an increase in metal solubility and leaching the root zone, root exudates, and soil amendments may be required. For example, various phosphate compounds have been found to be effective in the immobilisation of lead (Pb) in soils (McGowen *et al.*, 2001). Root exudations, such as organic acids, from some plant species may acidify the rhizosphere by up to 2 pH units (Salisbury & Ross, 1978). These plants can be used to increase the solubility of non-essential metal cations such as Cd^{2+} (Naidu *et al.*, 1994).

At sites of high metal concentrations, phytostabilisation can be used to re-establish a vegetative cover. A vegetative cover is a technique of growing plants in and/or over materials that pose environmental risk (USEPA, 1999; USEPA, 2000).

7.2 Advantages and Limitations

One of the benefits is that soil removal is unnecessary, since the contaminants will stay in one place and there is no threat of contamination spreading. Entry of contaminants into food chains and is also considerably reduced. Phytostabilisation is less disruptive than other more-vigorous soil remedial technologies (Berti & Cunningham, 2000; Schnoor, 2000) and less expensive than other remediation technologies such as capping and soil removal (Miller & Miller, 2007). Besides, restoration of ecosystem by re-vegetation and disposal of hazardous materials or biomass is not required (USEPA, 2000).

Phytostabilisation has a few disadvantages associated with it. Since the contaminants remain in place, the vegetation and soil may require long-term maintenance to prevent re-release of the contaminants and future leaching. Vegetative covers are long term and self sustaining; which requires minimal cost. But on the other hand, the periodic application of soil amendments to enhance immobilization increases the overall maintenance cost (Keller *et al.*, 2005; USEPA, 2000).

8. Phytohydraulics

8.1. What is Phytohydraulics?

In order to limit the spread of comtaminants or pollutants by groundwater, a phytoremediation technique called phytohydraulics can be used. Removal of groundwater is facilitated through uptake, consumption and transpiration by plants (Clothier *et al.*, 2008; ITRC, 2009; USEPA,

2000). Phytohydraulics control is also known as hydraulic control or hydraulic plume control (USEPA, 2000).

For an effective site water balance, it is essential that appropriate phytoremediators are chosen and the growing conditions optimized. The phreatophytes, a class of water-loving trees, are deep-rooted, high-transpiring plants that send their roots into regions of high moisture and can survive in conditions of temporary saturation. Phreatophytes, for example poplar trees, are widely used in phytohydraulics (Gatliff, 1994).

8.2 Advantages and Limitations

One of the benefits of phytohydraulics over conventional methods, such as the employment of a pumping well, is that an engineered pump-and-treat system does not need to be installed. Hence, costs will be lower. Furthermore, roots will penetrate into and be in contact with a greater volume of soil than if a pumping well is used (USEPA, 2000).

Water uptake by most plants is affected by climatic and seasonal conditions. The rate of water uptake will, therefore, not be constant. For instance, water uptake by deciduous trees will slow considerably during winter (USEPA, 2000). In high-rainfall regions, plant transpiration may also not sufficiently reduce drainage from the site. Another drawback is that groundwater removal is limited by the root depth of the vegetation.

9. Conclusion

The success of applying a phytoremediation technology at a given contaminated site cannot always be attributed to just one of the mechanisms discussed above because a combination of processes may be at work. The

major challenge in using the different phytoremediation technologies efficiently is that a thorough knowledge is required about the various plant-contaminant interactions, the physiological stress and damage on the plants, and the long term effects on the ecosystem.

This technology could also pose a number of environmental concerns. Unscientific disposal of plants after remediation is the main cascade factor. Leaching of metals into water supply, or ingestion of contaminants present in phytoremediators by animals and humans could affect the food chain and their overall health. Additional research and field trials are, therefore, required to make the widespread implementation of phytoremediation to reclaim contaminated land, a reality in the near future.

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6. Assessment of the potability of water obtained from Umkhrah, Umshyrpi, Umshing and Mylliem rivers of East Khasi Hills District Meghalaya.

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Abstract

The dwindling number of clean rives in and around the East Khasi Hills District of Meghalaya from which potable water can be obtained for domestic use and for drinking has led to the scarcity of water sources especially in the dry winter months. In this study, water samples from four main rivers of the afore mentioned district namely the Umkhrah river, Umshyrpi river, Umshing river and Mylliem river has been analysed for their potability with regards to the presence and number of coliforms. The findings of this study indicated the presence of coliforms in the water samples which further indicated fecal contamination being present in these rivers. This can be attributed to the dumping and discharge of domestic wastes especially from toilets directly into the rivers. These findings can thus help us to understand the gravity at which humans are polluting the environment and thereby creating a huge risk to human life itself from water borne infections.

Keywords: *Potable water, rivers, coliforms, Most Probable Number, fecal contamination, water borne infections.*

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Introduction

Freshwater rivers and streams are some of the main sources of potable water for people around the world. They also provide a means of livelihood for the people living around them through fishing and other aquatic products. Meghalaya which is one of the North Eastern States of India and having eleven districts is blessed with many natural freshwater rivers and streams which have now been polluted like other major rivers elsewhere in mainland India. This pollution of the rivers can be attributed to the ever growing population and the encroachment of houses near the river banks which discharge their domestic wastes directly into the rivers or indirectly through drains which all converge and empty into the rivers. One of the major risks to the health of the populace is water borne infections be it bacterial, fungal, parasitic (helminthes), protozoan or

viral. The most common type of water borne infection that affects most of the people is considered to be bacterial. Of all the bacterial types and species that are involved with water borne infections like gastroenteritis and diarrhea are enteric pathogens including *E. coli*, *Klebsiella sp.*, *Citrobacter sp.* etc. (Ibe & Okplenye, 2005). These bacteria are usually found in the intestine of warm blooded animals and belong to the family Enterobacteriaceae. They are also one of the members of the group Coliform which includes bacteria from other families (Rompré et al., 2002). Enteric coliforms enter water bodies through the faeces of animals including humans and are therefore considered as indicator organisms of fecal contamination (Girdoniya, 2011). Among the fecal coliforms, *E. coli* is an important member and its presence alone may indicate the presence of fecal contaminants as well as the presence

of other pathogenic bacteria in water (Borah, Dutta & Misra, 2010; Kambhaskar, Dandia & Palav, 2015).

Many methods have been adopted for the isolation of coliforms especially *E. coli* from water samples including the "Wurtz method" which dates back to 1893, where water samples were directly inoculated by spread plating onto Litmus lactose agar and the bacterial colonies growing thereafter were counted (Ashbolt, Grabow & Snozzi, 2001). However, the method of preference till date is the Most Probable Number (MPN) method (Sami & Rahman, 1985; Sutton, 2010; Ahmed et al., 2013; Dadwal et al., 2014; Gogoi et al., 2016)

In the present study, the potability of water from the Umkhrah river, Umshyrpi river, Umshing river and Mylliem river present in the East Khasi Hills District of Meghalaya was tested for the presence of coliforms using the MPN method. This study was carried out with the objective of determining the extent of fecal contamination of these once pristine rivers which may pose a health hazard for the people who still depend on them for many domestic activities.

Materials and methods

Collection of water samples

100 ml of water samples from the Umkhrah, Umshyrpi, Umshing and Mylliem rivers were collected in sterile sample containers.

The MPN test

In the Presumptive test, which is the first part of the MPN test, 3 tubes containing sterile double strength lactose broth were inoculated with 10 ml

of the water samples each. Another set of 3 tubes containing sterile single strength lactose broth were inoculated with 1 ml of the water samples each and finally a last set of 3 tubes containing sterile single strength lactose broth were inoculated with 0.1 ml of water samples each. Each of the tubes also contained a Durham's tube to check for gas production after incubation for 24 to 48 hours at 35°C. Using the MPN index table (Table 1), the number of tubes containing gas after 48 hours were checked to get an estimate of the number of coliforms present per 100 ml of water sample.

The second part of the MPN test is called the Confirmed test. In this test, inoculum from a lactose broth tube from each set (10 ml, 1 ml and 0.1ml) showing the maximum gas production was inoculated with the help of an inoculation loop into a sterile brilliant-green lactose bile broth which again, also contained a Durham's tube. The tubes were incubated for 48 hours at 35°C.

The last part of the MPN test called the Confirmed test involved transfer of inoculum by the streak plate method from the brilliant-green lactose bile broth tubes showing gas production onto sterile Eosin Methylene Blue (EMB) agar plates. Any colonies growing after incubation for 24 hours at 35°C and showing a dark center or a metallic green sheen were then transferred into fresh sterile brilliant-green lactose bile broth tubes containing Durham's tubes to check for gas production. Gram staining of the bacteria growing in the tubes and on the plates EMB plates was then carried out.

Results and Discussion

The presumptive test yielded positive results for all the three sets of 3 tubes (10 ml, 1 ml and 0.1 ml) with the presence of gas in the Durham's tubes. This result gave a most probable number of $\geq$ 2400 bacteria being present per 100 ml of water sample as per the MPN index (Table 1 (("Environmental science methodology," 2012)) which is above the recommended limit of 50 or less number of bacteria per 100 ml for potable water (CPCB, 2008). The confirmed test also showed the presence of gas in all the brilliant-green lactose bile broth tubes. The completed test showed colonies having the characteristics of coliforms growing on the EMB agar plates including some with a metallic green sheen which is a characteristic of *E. coli* colonies growing on EMB agar (Figs. 1 – 4). This indicated the presence of fecal contamination being present in the rivers. Gram staining of the bacteria showed all to be gram negative bacilli, another characteristic of coliforms. The presence of coliforms in such huge numbers in all the four rivers studied showed that fecal contamination is very high. This is largely because of the lack of proper hygienic disposal of fecal matter from domestic households which empty their toilets directly into the water bodies rather than into septic tanks. Also, the Umshing and Mylliem rivers flow mostly through sub urban and rural areas in which the awareness for sanitation and hygiene are lacking. At the same time, in these areas, domestic animals like cattle, goats and pigs are sometimes allowed to roam freely in the open thus leading to a greater probability of fecal contamination of the rivers from these animals.

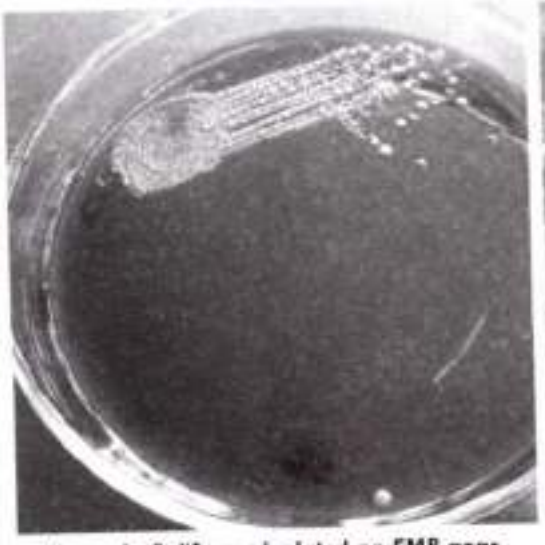

Figure 1: Coliforms isolated on EMB agar from Umkhrah river

Figure 2: Coliforms isolated on EMB agar from Umshyrpi river

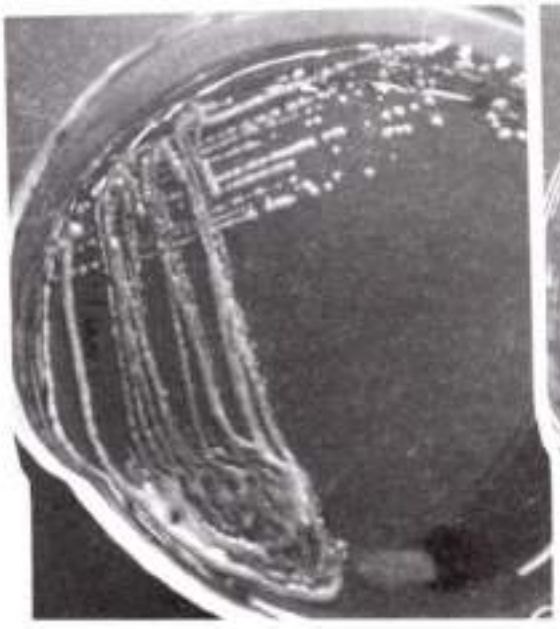

Figure 3: Coliforms isolated on EMB agar from Umshing river

Figure 4: Coliforms isolated on EMB agar from Mylliem river

Table 1: Most Probable Number Index when 3 tubes of 10 ml, 3 tubes of 1 ml and 3 tubes of 0.1 ml are used.			
No. of tubes giving positive result out of			MPN index per 100 ml
3 of 10 ml each	3 of 1 ml each	3 of 0.1 ml each	
0	0	0	<1
0	0	1	3
0	1	0	3
1	0	0	4
1	0	1	7
1	1	0	7
1	1	1	11
1	1	0	11
2	0	0	9
2	0	1	14
2	1	0	15
2	1	1	20
2	2	0	21
2	2	1	28
3	0	0	23
3	0	1	39
3	0	2	64
3	1	0	43
3	1	1	75
3	1	2	120
3	2	0	93
3	2	1	150
3	2	2	210
3	3	0	240
3	3	1	460
3	3	2	1100
3	3	3>	>2400

Source: ("Environmental science methodology," 2012)

Conclusion

This study revealed the level of fecal contamination of four main rivers flowing through the East Khasi Hills District of Meghalaya. The presence of coliforms in all the rivers under study indicates the possible presence of enteric and non-enteric pathogens which may cause life threatening diseases in humans (Girdoniya, 2011). It is to be noted that to determine the potability of water from a river with regards to the presence of coliforms, it is recommended to be test the water at least three times in a year ("Good agricultural practices and water testing guidelines," 2013). The lack of awareness regarding hygiene and the standards of drinking water should be addressed so that the local people using the contaminated river waters especially those in rural areas will know of the dangers of water borne diseases which is one of the major causes of death worldwide (Fripp, Woodyard, & Hanna, 2015).

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7. Effect of pretilachlor on rice field bacteria of Sung valley of Meghalaya

Thyanswer Challam[13, 14] and Stevenson Thabah[13]

Abstract

Pretilachlor is a very popular herbicide that is used in rice cultivation especially in Meghalaya, India. The impact of herbicides on the soil microflora and the surrounding environment is assumed to be negative. Although some studies on the impact of herbicides on soil microflora seem to confirm this assumption, no study has been done on the development of resistance to herbicides, specifically to pretilachlor. In this study pretilachlor was applied at recommended field rate (RFR), twice recommend field rate (2RFR), 5RFR and 10RFR and the effect on soil bacteria was assessed every 5 days for 30 days. It was seen that at RFR and 2RFR, pretilachlor actually has a population enhancing activity on the soil bacteria. At higher concentrations (5RFR and 10RFR) small reductions in bacterial population were observed. To this end, development of herbicide resistance to pretilachlor was also investigated. The effect of the herbicide on isolated phosphate solubilizing bacteria was also studied.

Keywords: Herbicide, rice cultivation, bacteria, pretilachlor, phosphate-solubilising, herbicide-resistance

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Introduction

The Sung Valley of West-Jaintia Hills District of Meghalaya is one of the most fertile regions in Meghalaya and has traditionally been associated with the cultivation of the red variety of rice (*Oryza sativa* Linn). Due to its productivity, the area is sometimes referred to as the rice bowl of Meghalaya. The fertile soil of the valley also supports the growth of weeds of the grass, sedge and broad-leaf variety. Weeds such as the barnyard grass (*Echinochloa colona*), cockspur grass (*Echinochloa crus-galli*) (Neogi, 1989), finger-grass (*Digitaria setigera*), hoorah grass (*Fimbristylis miliacea*), false daisy (*Eclipta prostrate*), small flower umbrella grass (*Cyperus difformis)*, common sedge (*Schoenoplectus juncoides*), gooseweed (*Sphenoclea zeylanica*, Umbrella sedge (*Cyperus iria*) and Indian goosegrass (*Eleusine indica*) are especially prevalent and can be controlled by hand weeding or chemically using pre-emergence herbicide application.

Over the past few decades the growing demand for sustainable production of rice and the inflating cost of manual labour has caused farmers to increasingly turn to herbicides. The most popular herbicides for transplanted rice as well as wet seeded rice in the Sung Valley continue to be butachlor and pretilachlor. Pretilachlor was selected because of its popularity among the farmers since it was among the cheaper herbicides available. Pretilachlor (2-chloro-N-(2,6-diethylphenyl)-N-(2-propoxyethyl) acetamide) ($C_{17}H_{26}ClNO_2$) belongs to the chloroactamide class of herbicides, which inhibits plant growth and reduces cell division. It offers reliable pre-emergence to early post-emergence control of annual grasses, some sedges and broad-leaf weeds for high yield in transplanted and dry-sown rice

cropping systems. It is especially designed for early season weed control in wet-sown rice and rice nursery beds.

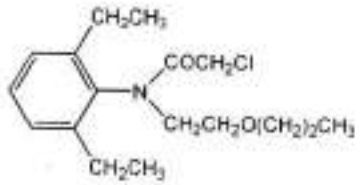

Figure 1: Structure of Pretilachlor

Although there is a lot of literature on the effect of various agrochemicals, including pretilachlor, on soil microflora, the corresponding processes are still poorly understood (Imfeld& Vuilleumier,2012).A non-apparent impact of applied herbicides or pesticides is on non-target species, particularly soil bacteria. These are essential to not only plant health but also to nutrient cycling. Among the most important microflora in the rice field are the phosphate-solubilizing microorganisms (PSMs). Grennan (2008) pointed out that phosphate is the most limiting mineral in cultivation. Generally, phosphate fixation limits the amount of phosphate available to plants. This affects plantgrowth to the extent that plants suffering phosphate starvation show stunted growth as well as poorly developed root and shoot systems (Hewitt *et al*., 2005). In the light of this fact, the role of PSMs in plant growth becomes abundantly clear. Among the PSMs, bacteria contribute majorly to the phosphate solubilizing activity in the plant rhizosphere since they make up to 50% of the total soil microbial population (Alam, 2002). These phosphate solubilizing bacteria belong to the genera *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Serratia*, *Enterobacter*, *Rhodococcus* and

Arthrobacter. Among the phosphate-solubilising bacteria, Bacillus sp. show remarkable tolerance to herbicides. The aim of this work is to not only show the effect of the herbicide pretilachlor on PSMs but also show the development of resistance to the same.

Materials and Methods

Pretilachlor was purchased from a local store supplier of agricultural products as Pretila® containing 50% EC w/w pretilachlor.

All other chemicals and dehydrated media used were from HiMedia™ Laboratories.

Sample Collection

The samples were collected from 3 designated areas within the Sung Valley of West Jaintia Hills District of Meghalaya, (25.553494 N, 92.112995 E). The soil of the rhizosphere of rice plants were collected by uprooting the rice plant and collecting the root along with the soil in a sterile plastic bag. The same was then transported to the laboratory for further study. In all a total of 30 rhizosphere soil samples were collected in individual sterile bags.

Soil pH determination

Soil that had been air-dried for 4 days was used in a 1:1 suspension in Millipore water. The soil suspension was then stirred over a hot plate magnetic stirrer for 10 minutes. The temperature was maintained at 25°C for all samples to maintain uniformity. A pre-calibrated pH meter equipped with a glass electrode was then used to measure the pH.

Determination of soil organic content

The determination of soil organic carbon is based on the Walkley-Black chromic acid wet oxidation method. Oxidisable matter in the soil is oxidised by 1 N $K_2Cr_2O_7$ solution. The reaction is assisted by the heat generated when two volumes of H_2SO_4 are mixed with one volume of the dichromate. The remaining dichromate is titrated with ferrous sulphate. Organic content of soil was determined by the method described by Sebiomo (2011) with slight modifications. Soil samples (RFR, 2RFR, 5RFR, 10 RFR and Control) were taken every 10 days for a period of 30 days. These were sieved through a 0.5mm sieve and 1g was suspended in 10ml of 1M $K_2Cr_2O_7$ contained in a 250ml conical flask and swirled to distribute the soil uniformly. To this 20ml of concentrated H_2SO_4 was added and the contents were vigourously mixed. The flask was placed in an ice bath for 30 minutes after which 100ml of water was added. 0.5ml of ferroin indicator (1.485 g *o*-phenanthroline monohydrate and 0.695 g ferrous sulphate in 80 ml millipore water, diluted to 100 mL) was added to the flask. The flask contents were then titrated with 0.5 N $FeSO_4$ solutions till the end point was reached with the colour changing from green to dark maroon. The calculation shown below was used for determining the percentage organic content:

$$Organic\ Carbon\ (\%) = \frac{0.003\ g\ x\ N\ x\ 10\ mL\ x\ (1\ T/S)\ x100}{ODW}$$

Where:

N = Normality of K2Cr2O7 solution

T = Volume of FeSO4 used in sample titration (mL)

S = Volume of FeSO4 used in blank titration (mL)

ODW = Oven-dry sample weight (g)

Basal Soil Respiration (BSR)

The basal respiration of soil samples was studied on rhizosphere soils treated by spraying with pretilachlor i.e., RFR (5.5 mg/sq ft. soil), 2RFR, 5RFR and 10RFR) and untreated rhizosphere soil as control. The methods described by Cheng et al., (2013) with modifications adapted from Benslama & Boulahrouf (2013) were used. 50 g samples were removed from each of the treated samples and incubated for 24 hours at 25^{0}C in 500ml conical flasks with an absorption vial containing 2 ml of 0.1M NaOH suspended at the *mouth of the flask. The flask was then sealed to be airtight. This is done to* capture the CO_2 produced by the soil respiration. Control flasks without soil were also set up in the same way with 2ml vials of 0.1M NaOH suspended at the mouths of the flasks. This was followed by a 24 hour incubation period at 25^{0}C after which time the NaOH solution samples were each poured into a flask containing 4ml of 0.05M $BaCl_2$ solution. The precipitated carbon was in the form of $BaCO_3$. Phenolphthalein indicator was added into the flasks followed by titration with 0.1 M HCl. The difference of consumed volume of HCl between the treatment and the control in titration was used to calculate the quantity of CO_2 evolution from soil microbes, 1 ml 0.1 M consumed NaOH was equivalent to 2.2 mg CO_2. All soil samples were studied every five days for 30 days.

Enumeration of Bacteria from the rhizosphere soil before and after pretilachlor treatment

Soil from the rhizosphere was collected by gently shaking the roots of the rice plants to dislodge the soil aggregates around primary, secondary and tertiary roots of the plants. Adhering soil was gently scraped with a sterile

spatula. Serial dilution was then performed to isolate the bacteria from the rhizosphere soil. 100µl aliquots were plated onto LB agar. The plates were then incubated at 30°C for 24-48 hours after which colony counts were conducted. Bacterial and isolates were characterized based on cultural characteristics and biochemical tests. Identification was thereafter made with reference to Bergey's Manual of Systemic Bacteriology (1994). The bacterial colonies were isolated individually on nutrient agar plates and maintained on agar slants for further study. Rice plants removed from Sung Valley were replanted in pots and flooded. While maintaining a control with no pretilachlor added, plants were treated with Recommended Field Rate (RFR) of 0.5 and 1.5 kg a.i. ha^{-1} (Bhowmick *et al.*, 2014), twice Recommended Field Rate (2RFR), five times Recommended Field Rate (5RFR) and ten times Recommended Field Rate (10RFR). Soil samples were removed every 5 days for 30 days and bacterial colonies were isolated using serial dilution plating on LB agar and enumerated.

Isolation of Phosphate Solubilising Bacteria from the rhizosphere soil

All the isolates were screened for their phosphate-solubilizing ability on Pikovskaya agar (Pikovskaya, 1948). The bacterial isolates were streaked on Pikovskaya agar and incubated for 72 hours at 30°C. Pilovskaya Agar has the composition (Surange et al., 1997): glucose, 10 g/L; $Ca_3(PO_4)_2$, 5 g/L; $(NH_4)_2SO_4$, 0.5 g/L; NaCl, 0.2 gL; $MgSO_4 \cdot 7H_2O$, 0.1 g/L; KCl, 0.2 g/L; yeast extract, 0.5 g/L; $MnSO_4 \cdot H_2O$, 0.002 g/L; and $FeSO_4 \cdot 7H_2O$, 0.002 g/L. The presence of a zone of clearance around the bacterial colony indicates that it is positive for phosphatase activity.

Demonstration of susceptibility to pretilachlor

The isolated phosphate solubilizing bacteria were plated in a series of petri dishes containing Mineral salt medium (MSM) agar and pretilachlor amounts ranging between 10ul/ml – 50 ul/ml pretilachlor. The MSM agar has the composition : KH_2PO_4, 4.6 g/L; K_2HPO_4, 1.3 g/L; NH_4NO_3, 1.0 g/L; $MgSO_4.7H_2O$. 0.2 g/L; $Ca(NO_3)_2.4H_2O$, 0.04 g/L; and $Fe(SO_4)_3$, 0.001 g/L. The pH was adjusted to 7.0 ± 0.2 with H_3PO_4 or above 10ul/ml. Plates were then

Results and Discussion

Soil pH

The soil samples collected were of the clay loamy type of top soil. The soil samples were generally dark in colour, almost black with slight differences in colour between the samples. The soil was of the silty loamy variety. The soil pH was found to range from slightly acid (6.2) to neutral (7.2). **Table 1.** describes the values of pH seen in the 30 soil samples. Bhaskar *et al.*, (2004) that generally soils of the Meghalaya plateau range in pH from strongly acidic to moderately acidic. As evident from the table, our findings of the pH of the Sung Valley are more in line with Syiem *et al.*, (2010).

Determination of soil organic content (SOC) or organic carbon %

The soil organic content did not vary too much between the untreated samples and those which were treated with the recommended rate (RFR) even twice the recommended field rate (2RFR). However it became increasingly apparent that soil organic carbon % decreased drastically upon

adding 5RFR and even more so with 10RFR. From **Table 2**, it can be noted that from day 20 onwards the organic carbon percentage begins to recover in 5RFR and 10RFR treated samples, indicating that the toxic effect of the herbicide becomes more pronounced at higher field rates. At lower RFRs, there was only a slight repression of soil organic content and the SOC began to increase more than the control at 2RFR indicating that at lower concentration pretilachlor promotes growth of soil microorganisms and a proportional increase in organic content. This is probably due to the ability of the soil microbiota to degrade and use pretilachlor as a nutrient source. The same effect could not be observed at higher concentrations of pretilachlor.

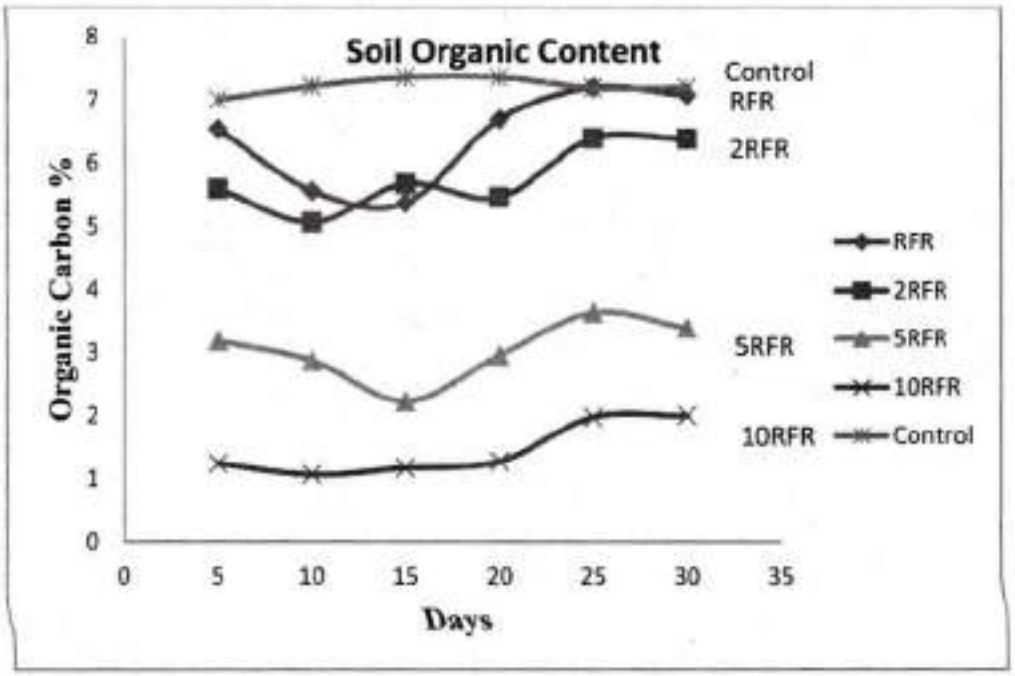

Figure 2: Change in Soil Organic Content over 30 days

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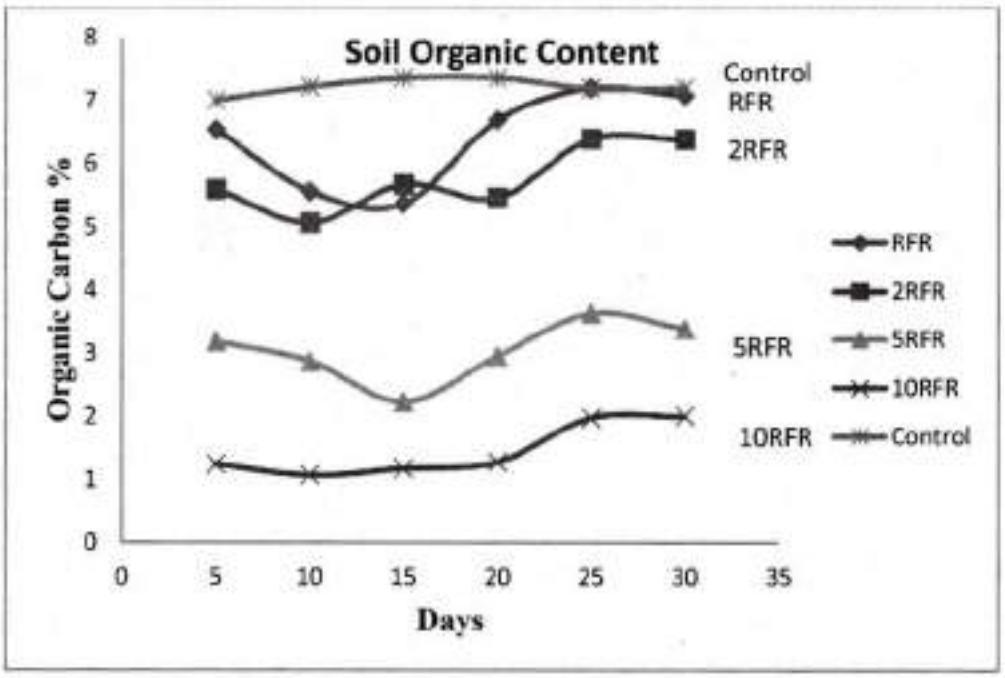

Figure 2: Change in Soil Organic Content over 30 days

Table 1. pH Values			
Plot	pH Values		
		With $CaCO_3$	
1	7.02	7.24	7.35
2	6.35	7.34	6.58
3	6.74	7.32	7.14
4	7.32	6.90	6.72
5	7.32	7.31	6.97
6	6.29	6.60	6.27
7	7.34	6.21	6.40
8	7.21	7.06	6.82
9	6.49	6.43	6.86
10	6.85	6.77	6.73
11	7.06	7.18	6.42
12	6.37	6.58	6.44
13	6.30	6.97	7.13
14	7.23	6.23	6.88
15	6.97	6.25	7.17
16	7.08	6.66	6.74
17	6.33	7.04	7.39
18	6.67	7.02	6.90
19	6.41	7.23	6.53
20	6.77	6.75	6.67
21	6.65	6.38	6.52
22	6.37	6.96	6.53
23	6.56	7.00	6.65
24	7.03	7.20	7.36
25	7.32	6.57	7.24
26	6.55	6.59	6.63
27	6.25	6.64	6.62
28	6.61	6.46	6.92
29	6.32	6.99	7.00
30	6.53	6.90	6.97

Table 2: Soil Organic Content.					
Days	RFR	2RFR	5RFR	10RFR	Control
5	6.54	5.59	3.19	1.25	7.00
10	5.56	5.07	2.87	1.07	7.21
15	5.37	5.67	2.23	1.18	7.36
20	6.69	5.46	2.96	1.28	7.36
25	7.19	6.39	3.63	1.98	7.18
30	7.07	6.38	3.39	2.00	7.19

Basal Soil Respiration (BSR)

There was little difference in BSR between the RFR and 2RFR treated samples. However, in both cases BSR is seen to increase more than the control samples. This may be indicative that similar to the findings of Radosevich *et al.*, (1995), low doses of herbicides actually promotes BSR. At higher concentrations of pretilachlor, the BSR is severely repressed, reflective of the toxic effect of the herbicide. This is evident at 5RFR and 10RFR.

Enumeration of Bacteria from the rhizosphere soil before and after pretilachlor treatment

At low dosage of pretilachlor, RFR and 2RFR, the no of CFUs were similar to untreated control soil samples (**Table 3** and **Figure 3**). At higher concentration 5RFR and 10RFR, the number of CFUs was seen to decrease, albeit by a very small amount showing that the presumed toxic effect is has a minimal impact on rhizosphere soil bacteria. However, even at day 30, the recovery of 5RFR and 10RFR back to untreated CFU levels has not begun. The difference in the number of CFUs is however, not very large.

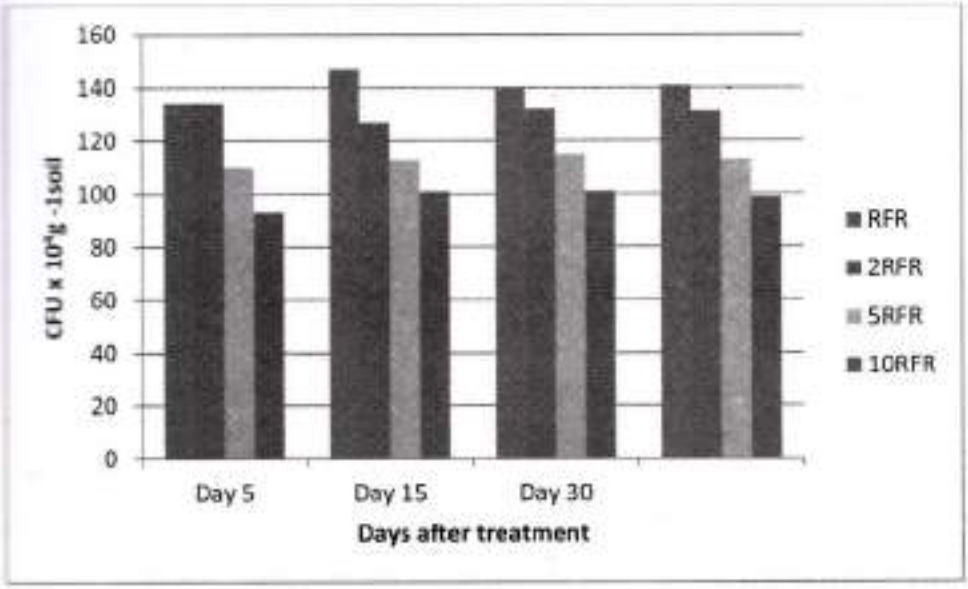

Figure 3: Population count of heterotrophic bacteria after application of pretilachlor

Table 3. Total Population of total heterotrophic bacteria after pretilachlor application (CFU x $10^4 g^{-1}$soil)

	Day 5	Day 15	Day 30	Mean
Control	113	114	116	115
RFR	112	113	115	114
2RFR	111	112	114	113
5RFR	109	111	113	111
10 RFR	104	107	111	108

Isolation of Phosphate Solubilising Bacteria from the rhizosphere soil

Phosphate solubilizing bacteria were isolated from the rhizosphere soil. 8 colonies were isolated with three being moderately active phosphate solubilizers, with a 5-6mm zone of clearance, and the remaining had only very little phosphate solubilizing activities 1-2 mm zone of clearance.

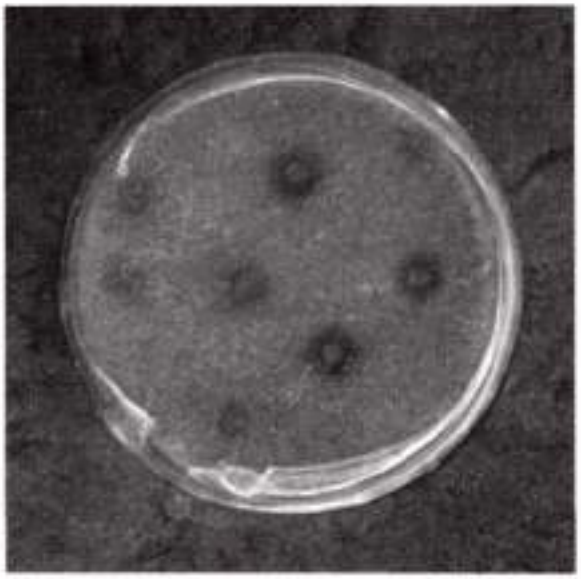

Figure 4: Colonies of phosphate solubilizing bacteria

Demonstration of pretilachlor susceptibility and isolation of pretilachlor-resistant phosphate solubilizing bacteria

The phosphate solubilizing bacteria isolated from the rhizosphere soil showed susceptibility to pretilachlor at concentrations above 20ul/ml when plated on Mineral salt medium (MSM) agar enriched with phosphates.

Conclusion

This study observes that herbicide concentration does affect the microbial count. Pretilachlor at RFR was not observed to affect the soil microbial communities (Murato *et al.*, 2004). At low doses, bacterial counts are not affected but basal soil respiration and organic carbon percentage do show observable reductions with increase in the herbicide concentration. It has been suggested (Radosevich *et al.*, 1995) that at low concentrations herbicides such as glyphosate can be nutrients for bacterial growth. This study has also demonstrated the phosphate solubilizing bacteria are negative affected by high concentrations of pretilachlor. It would make for an interesting study to show how other parameters of growth, such as protease and dehydrogenase activities are affected.

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8. Morphometric Analysis of the Mud Eel *Monopterus Cuchia* From Meghalaya, India

Barisha Mary Kurbah[15] and Rabindra Nath Bhuyan[16,17]

Abstract

Monopterus cuchia (Mud eel) belong to the family Synbranchidae is fresh water air breathing. In Meghalaya, the species locally known as *Khabseiñ* and is considered as a nutritious and oriental medicinal value. The population of this species has been declined to a great extend due to various environmental and anthropogenic factors. This study is based on the examination of specimens from two different areas of Meghalaya using 12 different morphometric parameters. It was found out that there is a positive increase in the morphometric characters with increase in the length of the fish. Variation between the morphological characters was also noticed between two different habitats of the state. The correlation coefficient (r) values of different morphometric characters on total length were > 0.8 indicating a high degree of positive correlation. However, the fishes from the two different habitats viz. Khasi Hills and Garo Hills belong to the same species.

Keywords: *Monopterus cuchia*, morphometric parameters, positive correlation

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Introduction

Mud eel (*Monopterus cuchia*) is fresh water air breathing; swamp dwelling fish belong to the family Synbranchidae of the order Synbranchiformes. Locally known as *Khabseiñ* in Khasi language in Meghalaya and considered as a nutritious and tasty fish as well as a valued remedy in oriental medicine. The species is commonly available in the fresh water of Bangladesh, Pakistan, Northern and North-East India and Nepal (Jingran and Talwar, 1991). The habitat of this species is fresh and salty water as they are mainly found in shallow well vegetated water and mud. They often spend their daytime hiding under stones and mud as they have a burrowing habit (Nasar, 1997). The population of this species has been declined to a great extend due to various environmental and anthropogenic factors. According to (Das and De, 2002), the status of *Monopterus cuchia* in India is endangered.

Several workers have reported on the biological studies of various fish species at different level. However, a study on *Monopterus cuchia* in Meghalaya is scanty. Morphometric analysis is important for identifying fish species and their habitat as well as its ecological criteria. It is common to use morphometric measurements to identify and classify fishes (Bagenal and Tesch, 1978). Therefore, the morphometric analysis of this species from Meghalaya was undertaken to find out the variations, if any, and growth pattern from two climatically different habitats viz. West Khasi Hills District and Garo Hills Districts of Meghalaya.

Materials and Methods

For accomplishing the objective of the study, specimens of *Monopterus cuchia* were collected during the period of December, 2015-March, 2016. This study is based on the examination of five specimens from two different areas of Meghalaya i.e. Balat, West Khasi Hills and Garo Hills. The specimens collected were brought to the Hatchery Complex, Department of Fishery Science, St. Anthony's College, Shillong and reared in specially designed cemented tank (Plate-1). Overall, 12 morphometric parameters have been taken up for study according to the methods described by Lowe-McConnel (1971). Divider and measuring board, having graduations in centimeter have been used for various measurements and were made to the nearest millimeter. The regression equation used for the morphometric characters against the total length was obtained by using the given formula:

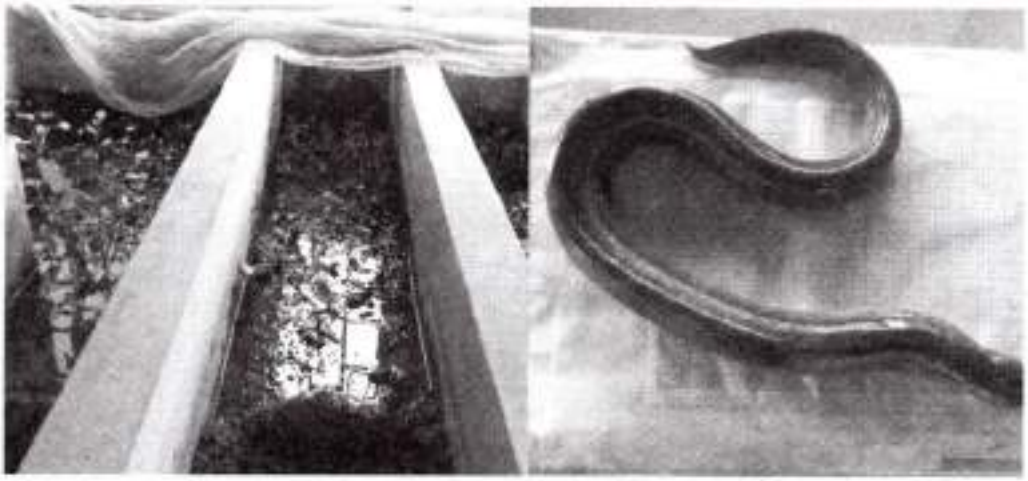
$$\mathbf{Y = a + b\,X}$$

Where, 'Y' is the variable character such as head length, body depth etc., 'a' is the constant value, 'b' is the regression co-efficient and 'x' is the total length. The correlation coefficient 'r' of these regressions was computed using standard statistical formula.

Results and Discussion

The detail findings of the fish in terms of morphometric measurements are listed in Table 1 and 2, respectively. The present study showed a positive increase in the morphometric characters with increase in the length of the fish from both habitats. Similar result was also reported by on *M. cuchia* from West Bengal (Jana and Dasgupta, 2007).

Plate 1. *Monopterus cuchia*



Cemented tank where cuchia is reared ***Monopterus cuchia***

The regression equation as well as correlation coefficient (r) of different morphometric characters (Y) on total length (X) of *M. cuchia* from Garo Hills and West Khasi Hills was presented in Table 3 and 4, respectively. The correlation coefficient (r) values of different morphometric characters on total length were > 0.8 indicating a high degree of positive correlation. Moreover, if $r > 0.8$, it shows a highly significant relationship between the two variables (Jyrwa *et al.*, 2015).This shows that the growth of the fish is good from the two areas studied.

It was found out that, there was variation of morphological characters among the species from two different localities. However, this variation may be due to sex, nutrition and different environmental factors. This variation is found to be similar with the earlier report (Gould, 1966). The morphometric indices exhibited a great variability in their behavior also (Mekkawy and Ashraf 2011).

Table 1: Morphometric analysis of *Monopterus cuchia* from Garo Hills Meghalaya

Sl. No.	Parameters	Mean (mm)	Range (mm)
1	Total Length (TL)	678	640-730
2	Length of Caudal Peduncle (LCP)	163.6	153-180
3	Body Depth (BD)	47.6	45-50
4	Head Width (HW)	79.4	72-90
5	Head Depth (HD)	34	30-40
6	Head Length (HL)	46.2	42-55
7	Pre-orbital Length (PL)	9.4	9-10
8	Post-orbital Length (POL)	37	35-40
9	Upper Jaw Length (UJL)	31	30-32
10	Lower Jaw Length (LJL)	34.8	34-36
11	Gape of Mouth (GOM)	10	9-12
12	Inter-orbital Length (IOL)	9.4	9-10

Table 2: Morphometric analysis of *Monopterus cuchia* from Balat, West Khasi Hills, Meghalaya

Sl. No.	Parameters	Mean (mm)	Range (mm)
1	Total Length (TL)	730.6	620-800
2	Length of Caudal Peduncle (LCP)	184	150-210
3	Body Depth (BD)	52.6	50-55
4	Head Width (HW)	75.8	62-81
5	Head Depth (HD)	37.8	31-40
6	Head Length (HL)	49.2	45-54
7	Pre-orbital Length (PL)	12.6	10-15

8	Post-orbital Length (POL)	42.8	35-47
9	Upper Jaw Length (UJL)	24	23-25
10	Lower Jaw Length (LJL)	30.8	30-31
11	Gape of Mouth (GOM)	10.4	9-12
12	Inter-orbital Length (IOL)	9.6	9-10

Table 3: Regression equations of morphometric parameters of *Monopterus cuchia* from Garo Hills, Meghalaya

Parameters	Regression equation (Y = a + b X)	Correlation coefficient (r)
Length of Caudal Peduncle (Y) on Total Length (X)	Y = -39.8 + 0.30 X	0.9973
Body Depth (Y) on Total Length (X)	Y = 12.34 + 0.05 X	0.9315
Head Width (Y) on Total Length (X)	Y = -74.51 + 0.23 X	0.9525
Head Depth (Y) on Total Length (X)	Y = -47.66 + 0.12 X	0.9614
Head Length (Y) on Total Length (X)	Y = -49.31 + 0.14 X	0.9568
Pre-orbital Length (Y) on Total Length (X)	Y = -14.60 + 0.04 X	0.912
Post-orbital Length (Y) on Total Length (X)	Y = 1.13 + 0.05 X	0.9793
Upper Jaw Length (Y) on Total Length (X)	Y = 13.67 + 0.03 X	0.9456
Lower Jaw Length (Y) on Total Length (X)	Y= 20.22 + 0.02 X	0.9526
Gape of Mouth (Y) on Total Length (X)	Y = -11.03 + 0.03 X	0.9375
Inter-orbital Length (Y) on Total Length (X)	Y = 0.24 + 0.01 X	0.9125

Table 4: Regression equations of morphometric parameters of *Monopterus cuchia* from

Balat, West Khasi Hills, Meghalaya		
Parameters	**Regression equation (Y = a + b X)**	**Correlation coefficient (r)**
Length of Caudal Peduncle (Y) on Total Length (X)	Y = -35.18 + 0.30 X	0.9527
Body Depth (Y) on Total Length (X)	Y = 35.10 + 0.02 X	0.9536
Head Width (Y) on Total Length (X)	Y = -0.39 + 0.10 X	0.9489
Head Depth (Y) on Total Length (X)	Y = 0.91 + 0.05 X	0.9367
Head Length (Y) on Total Length (X)	Y = 14.20 + 0.05 X	0.9360
Pre-orbital Length (Y) on Total Length (X)	Y = -9.31 + 0.03 X	0.910
Post-orbital Length (Y) on Total Length (X)	Y = -8.34 + 0.07 X	0.9830
Upper Jaw Length (Y) on Total Length (X)	Y = 18.15 + 0.01 X	0.8798
Lower Jaw Length (Y) on Total Length (X)	Y= 27.14 + 0.01 X	0.856
Gape of Mouth (Y) on Total Length (X)	Y = -0.03 + 0.01 X	0.9057
Inter-orbital Length (Y) on Total Length (X)	Y = 4.67 + 0.01 X	0.8910

Morphometric measurements are widely used to identify differences between fish populations. The growth rate of the fish might be associated with the size of fingerlings stocked, quality of feed supplied, culture period and season (Miah *et al*, 2015).'

Conclusion

The study of morphometric characters *M. cuchia* from Garo Hills District and West Khasi Hills District reveals that the growth pattern of the species is in positive correlation though both the region differs climatically. The study showed a positive growth pattern with increasing length of the fish and a high degree of positive correlation with reference to the total length.

The present analysis also confirms that though there is slight variation in morphological characters, but the species is same as that are found in other parts of the country.

However, as population of the species is declining over the years, its induced breeding is the need of the hour to conserve and increase the natural population.

The analysis of *Monopterus cuchia* from Garo Hills and Balat, West Khasi Hills of Meghalaya showed a positive growth of the morphometric characters with increasing length of the fish and a high degree of positive correlation with reference to total length. Variations between the various morphometric characters with respect to the total length were also reported.

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9. Artificial Pancreas: A Review

Violina Kalita[18] and Linu John[18,19]

Abstract

An artificial pancreas is a closed-loop system containing only synthetic materials which substitutes for an endocrine pancreas. It will consist of functionally integrated components like a sensor that will continuously sense glucose levels, determine appropriate insulin dosages through algorithm device and deliver the insulin. Closed loop control utilizes models of glucose homeostasis which account for the influences of factors such as feeding, stress, insulin, exercise, on blood glucose levels. Models are necessary for understanding the relationship between blood glucose levels and insulin dosing; to develop algorithms controlling insulin dosage; and customizing each user's system based on individual responses to such factors that influence glycemia. Components of an artificial pancreas are now being developed which includes continuous glucose sensors; insulin pumps for parental delivery; and control software, all linked through wireless communication systems which are greatly benefitted to patients with diabetes.

Keywords: Diabetes, Artificial pancreas, Bioengineering approach, Gene Therapy Approach, Continuous blood glucose monitor, Insulin pump, Control Algorithm Device.

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Introduction

Diabetes is a long term condition that causes high blood sugar level in a body. After the consumption of food, body breaks down the sugars present in the blood and turns it into glucose which travels through blood stream and provides energy. To accomplish this, the pancreas needs to produce a hormone called insulin. In a person with diabetes also termed as Diabetes Mellitus, the pancreas either produces too little insulin or none at all, or the insulin can't be used effectively. This allows blood glucose levels to rise while the rest of the cells are deprived of much needed energy. Commonly used terms in diabetes are-

Hypoglycemia – It is a term used when blood glucose is low.

Hyperglycemia – It is a term used when blood glucose is too high.

In 2013 it was estimated that over 382 million people throughout the word have diabetes (Today, 2004).

Different types of Diabetes are-

Type 1 Diabetes-

Figure1: Type 1 Diabetes.

(source:
https://www.google.co.in/search?q=type+1and+type+2+diabetes+photos&espv=2&biw=1366&bih=677&tbm=isch&tbo=u&source=univ&sa=X&ved=0ahUKEwiAyZDZ8MLLAhWWCI4KHVJHBKUQsAQIHw#imgrc=vXNrrXsJmxc3wM%3A)

In this type of diabetes the body doesn't produce insulin. Approximately 10% of all the diabetes cases are Type1 (Campus, 2016).Type 1, also known as juvenile diabetes or insulin-dependent diabetes, is an immune system disorder where, the patient's own immune system attacks the insulin-producing cells in the pancreas, destroying the ability to manufacture insulin. People with type 1 diabetes are dependent on insulin intake (fig 1). Most people with Type 1 diabetes are diagnosed as children or young adults (Foundation, 2016).

In Type2 diabetes body doesn't produce enough insulin for proper function. Approximately 90% of all the cases of diabetes worldwide are of this type (Foundation, 2015). The major challenges of type 2 diabetes is the presence of insulin resistance, where the cells that need energy don't respond normally to the usual amounts of insulin. The pancreas has to produce much higher levels of the insulin in order to manage blood glucose levels. Over time, the insulin-producing cells in the pancreas can burn themselves out due to this overproduction which is the point at which a person with Type 2 diabetes begins to require insulin medication (fig. 2).

Type 2 Diabetes-

However, in earlier phases of this more common type of diabetes, the illness can be effectively managed with diet, exercise, and careful monitoring of blood sugars. People with Type 2 diabetes may require a variety of oral medications and eventually insulin (Foundation, 2015).

Figure 2: Type 2 Diabetes

(source-
https://www.google.co.in/search?q=type+1and+type+2+diabetes+photos&espv=2&biw=1366&bih=677&tbm=isch&tbo=u&source=univ&sa=X&ved=0ahUKEwiAyZDZ8MLLAhWWCI4KHVJHBKUQsAQIHw#imgrc=vXNrrXsJmxc3wM%3A)

Full cure for diabetes has not yet been discovered but different approaches like The Bioengineering approach, The Gene Theory approach and The Medical Equipment approach have been taken up by different medical institutions. The review is based mainly on the medical equipment strategies. The Juvenile Diabetes Research Foundation (JDRF) is orchestrating a major initiative that promises to give the world its first fully automated artificial pancreas by using Continuous Glucose Monitor (CGM) of transforming Type 1 Diabetes to Type None.

The Medical Equipment Approach

It is the equipment with a close loop control using real time data from a continuous blood glucose sensor along with a control algorithm device and an insulin pump.

Continuous Blood Glucose Monitoring

Technology for blood glucose monitoring supports the mission of the Artificial Pancreas by:

1. Providing a blood glucose reading without finger sticks from the user automatically after every few minutes,

2. It helps in the prediction of blood glucose levels by monitoring trends pertaining to rising and falling of blood sugars which is helpful in the immediate future,

3. Prompting the user for a correction bolus from an insulin pump that is needed immediately by comparing the sugar levels and predictions which begins at high blood sugar and at low blood sugar threshold ("Glucose control therapy research," 2015).

Basal Control

It is the first step in controlling an insulin pump based on a continuous blood glucose data which automatically controls the basal rate of the insulin pump. When a bolus has not been recently performed, the pump manages the blood glucose level by adjusting the basal rate as needed:

1. When the blood sugar is increasing, a small correction bolus can be automatically delivered and a higher basal rate can be set.

2. When the blood sugar is decreasing, the basal rate can be halted to deny the quantity of insulin needed to bring the blood glucose level back up until the basal rate can be continued at a new lower rate.

3. The pump has adaptive filtering techniques, which 'learn' the unique basal rates for the person as a function of the time of bay (Gerace, Martiniello-Wilks, O'Brien, & Simpson, 2014).

History

Artificial Pancreas (AP) development can be traced back to 50 years from the year 2015, the possibility for external blood glucose regulation was established by studies in individuals with type1 diabetes using intravenous glucose measurement infusion of insulin and glucose ("Glucose control therapy research," 2015).

After pioneering work by Kadish in 1964, expectations for effectively closing the loop were inspired by the nearly simultaneous work of five teams reporting closed-loop control results between 1974 and 1978 ("Glucose control therapy research," 2015). In 1977, one of these realizations resulted in the first commercial device – the biostator, followed by another impatient system, the Nikkiso STG – 22 Blood Glucose Controller, now in use in Japan ("Glucose control therapy research," 2015).

Juvenile Diabetes Research Foundation

Juvenile Diabetes Research Foundation (JDRF) has spearheaded great progress in forming the ARTIFICIAL PANCREAS PROJECT (APP) in 2005 ("Artificial pancreas," 2016), which bring together the world's leading experts to speed the development of automated closed-loop system.

JDRF launched the Artificial Pancreas Project to accelerate the development of a commercially viable artificial pancreas as a system to ultimately mimic the biological function of the pancreas for patients with the Type1 diabetes.

More recently, studies outside of the hospital are beginning to show that the people with the Type1 diabetes can successfully use these systems. Additionally, advancing the artificial pancreas could prevent costly complications that are burdening our economy – a recent study by leading health economics experts showed that an artificial pancreas could save Medicare nearly $1billionover the next 25 years (Gilles, 2016).

An Artificial Pancreas will consist of a continuous glucose monitor (CGM) and an insulin pump programmed with a computer algorithm that "closes the loop," calculating insulin doses from blood glucose readings and telling the pump to deliver the medication. In early versions of the artificial pancreas, the CGM and insulin pump will be similar to the Biostators, if not identical. The main difference will be that one of these devices will house the algorithm, a set of mathematical steps that takes into account various factors to determine the proper amount of insulin to dispense CGMs transmit. The Artificial Pancreas is a transformational technology and one that is urgently needed by the patients. The Artificial Pancreas combines a continuous glucose monitor, smart phone, two insulin pumps with sophisticated computer software to provide just the right amount of insulin and glucagon at just the right time (fig. 5).

The technology will be especially helpful overnight, when 50 to 70 percent of hypoglycemic emergencies (dangerous low blood sugar) occur (Rawlings & Director, n.d.). Even with diligent monitoring, the majority of the people

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with Type1 diabetes still spend many hours a day outside healthy blood sugar levels.

Artificial Pancreas Project (APP) uses will reduce dangerous high and low blood sugars, providing a better quality of life now for those suffering from the decease, and helping lower the risk of developing complications.

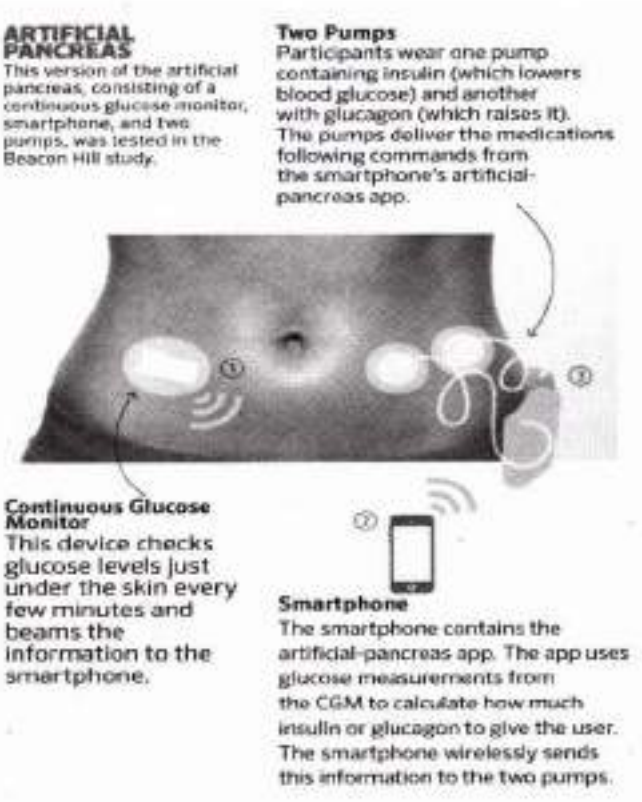

Figure 5: Artificial Pancreas System Using Two Pumps

(source-http://www.diabetesforecast.org/2014/mar/the-artificial-pancreas-aces.html?referrer=https://www.google.co.in/)

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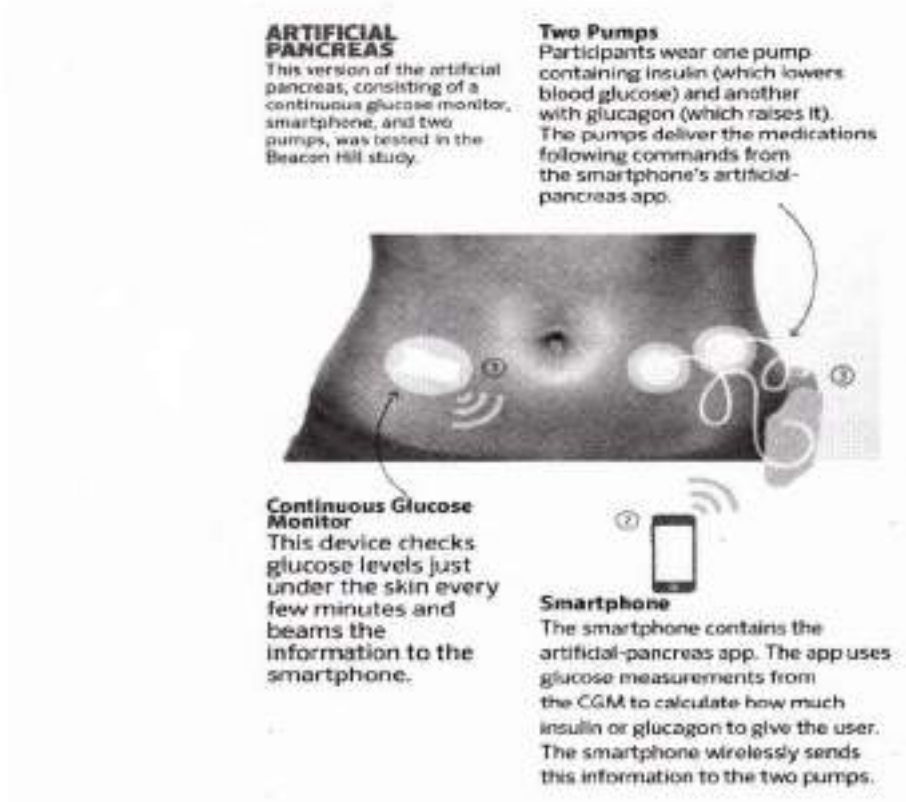

Figure 5: Artificial Pancreas System Using Two Pumps

(source-http://www.diabetesforecast.org/2014/mar/the-artificial-pancreas-

aces.html?referrer=https://www.google.co.in/)

Mechanism Of Artificial Pancreas Project

An artificial pancreas consist of a continuous glucose monitor (CGM) and an insulin pump programmed with a computer algorithm that "closes the loop," ascertaining insulin measurements from blood glucose readings and advising the pump to convey the medication steps. CGMs transmit glucose readings each one to five minutes from under the skin sensor to a handheld receiver, which can be coordinated into a pump. This is a huge measure of data, which is effectively handled by an algorithm. Three CGMs are currently available, each having received Food and Drug Administration (FDA) approval within the past five years (Gilles, 2016; Berg, 2014). Unlike blood glucose meters, CGMs measure glucose levels in the interstitial fluid. Interstitial glucose readings commonly linger behind genuine blood glucose levels by 8 to 10 minutes, which is most significant during periods of rapid change, such as after a meal. That's a key consideration when designing a closed loop device, since insulin doses will rely on those estimations (Rawlings & Director, n.d.). "CGMs are only approved for tracking and trending" blood glucose, not for determining an insulin dose," says Chip Zimliki, PhD, the chair of the artificial pancreas working groups, the units of the FDA that review research proposals related to the development of the device (Rawlings & Director, n.d.).

The following segment to CGM is an insulin pump, is a beeper sized device that is frequently tethered to the body through an adaptable tube embedded into the tissue simply under the skin. It conveys insulin as coordinated by the user. Yet not at all like insulin that originates from the pancreas, insulin conveyed into tissue requires some investment to get into the circulation

system, where it can bring down blood glucose. Individuals with diabetes regularly take insulin 15 minutes or so before a meal, giving it time to start working. It might appear as an obstacle however programmed premeal and postmeal boluses input will make it work easier.

The two model based algorithms furthest along in clinical studies are those of Kovatchev and Roman Hovorka, of the University of Cambridge in England. Both algorithms compute insulin measurements once more as new glucose readings stream in. The primary contrast is that Hovorka alters itself, as well, while the Kovatchev algorithm, after initialization, doesn't change (Rawlings & Director, n.d.). While the artificial pancreas is intended to totally assume insulin dosing and keep blood glucose in the objective reach, there are as of now some incomplete "half loop" solutions becoming accessible. A Medtronic device available in Europe give an algorithm a chance to have some control over an insulin pump, however it's not a genuine artificial pancreas. The device, the Paradigm Veo, combines a CGM, a pump, and an algorithm, which can shut off insulin for two hours if blood glucose dips below a threshold. Medtronic sells a similar system, the Paradigm Revel, in the United States—minus the shutoff feature.

University of Virginia's DiAs artificial pancreas system is on clinical trial testing and named as DiAs system. This research platform consists of an Android Smartphone running a control algorithm, a Roche insulin pump, and a Dexcom G4 Platinum CGM (Foundation, 2016) with a special Bluetooth box (fig.6).

Components of DiAs system?

1. The Dexcom CGM value is received after every five minutes wirelessly from an Android Smartphone with a control algorithm.
2. The amount of insulin to be delivered is calculated by the algorithm running on a phone based on the current and predicted blood glucose and insulin on board. If glucose is predicted to go too high or too low then additional insulin is given or suspended respectively. The glucose target varies by time of day which is more aggressive at night and conservative during the day.
3. A wireless command is sent to the pump to give the calculated amount of insulin.

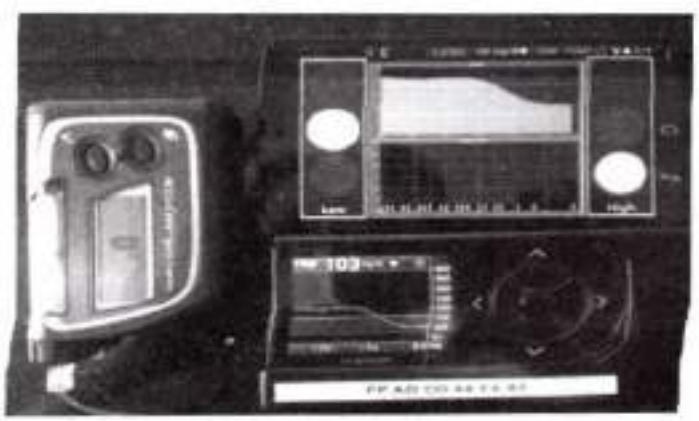

Figure 6: The DiAs Artificial Pancreas System

(source-http://diatribe.org/takingartificialpancreashome24hoursday)

4. The same is repeated every five minutes during the day or at night while the closed-loop is running. The meal information have to be entered into the system (number of carbs) during the day, which makes this a "hybrid closed-loop" ("treat-to-range") system (Foundation, 2016).

Development Pathways of Artificial Pancreas Project

The 6-stage Artificial Pancreas Project (APP) advancement pathway demonstrated as follows (fig.7) serves as the APP's key subsidizing arrange and characterizes the needs of item innovative work. Every progressions in the arrangement speaks to incremental advances in automation beginning with devices that shut off insulin conveyance to avoid scenes of low glucose and advancing eventually progressing to a completely robotized "closed loop" system that maintains blood glucose at an objective level without the need to bolus for meals or adjust for exercise. The APP pathway is depicted in three generations- the first, second and the third generations respectively which are described in detail ("A pathway to an artificial pancreas: An interview with JDRF's Aaron Kowalski," 2014).

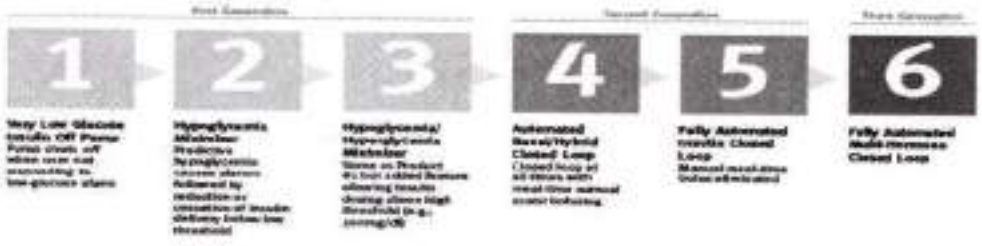

Figure 7: The 6 step APP pathway combined together in three generations.

(source-
https://myglu.org/articlesApathwaytoanartificialpancreasaninterviewwithjdrfsaaronkowalski)

First Generation

1. The first generation items insteps 1-3 concentrates on anticipating perilous high and low glucose levels and means to maintain the level approximately between 70 and 180mg/dL ("Artificial pancreas," 2016).

2. Preventing episodes of low glucose is the main focus of step 1 and step 2 and thus aim to constrain the time a person spends below 70mg/dL. While a Step1 product suspends insulin conveyance in react to low glucose levels,Step2 product enhances the capacity to foresee approaching episodes of low blood sugar and consequently shuts off a reduce insulin delivery if the person is lethargic to notice alerts. Such a framework may not prevent all episodes, but rather will altogether diminish this events contrasted with current treatment alternatives.

3. Step3 in the arrangement includes a component that anticipates risky high glucose levels, not withstanding low glucose levels, consequently the name 'hypoglycemia/hyperglycemia minimizer'. This product represents First Generation, semi –automated, artificial pancreas product concept since the user will still need to set baseline insulin delivery, as well as bolus for meals.

Second Generation

1. The Second-Generation product concept is represented by Step 4 and Step 5. Step 4 is referred to as a "Hybrid Closed-Loop" product as it targets a specific blood sugar level instead of a range. Bolusing for meals will still be necessary in this plan of step 4.

2. The next advance step is shown in Step5 that eliminates manual premeal boluses to result in a fully automated, closed-loop artificial pancreas product. This product concept will require research and development of advanced insulin and improved glucose sensing technologies ("Artificial pancreas," 2016).

Third Generation

The third generation product concept is shown in Step6. This final concept adds the ability to dose potentially more drugs to closely mimic the way the body maintains blood sugar levels and thus improves blood sugar control. Pumps are required in this concept that can deliver two or more solutions (insulin pump and glycogen pump), as well as the development and approval of additional hormonal drugs ("Artificial pancreas," 2016).

Global Study of Artificial Pancreas Project

Western Australia's wellbeing division specialists at Princess Margaret Hospital for Children (PMH) added to an insulin pump which acts like artificial pancreas to treat Xavier Hames Type1 diabetes. Xavier Hames, a four year old kid in Australia who has turn into the primary individual on the planet to be fitted with a manufactured pancreas in a historic innovation to treat diabetes. This new insulin pump framework has been created by expert group at PMH and also a system of healing facilities crosswise over Australia financed by Juvenile Diabetes Research Foundation (JDRF), a non-benefit organization (Press, 2015). Hames was found to have diabetes when he was only 22 months old. He has been getting general treatment at PMH since his diagnosis and is the first child, outside of the clinical trials, to use the new gadget (fig. 8).

Francis J. Doyle and colleagues from the University of California

Santa Barbara uncovers how the gadget can persistently quantify an individual's blood glucose levels and consequently convey insulin when required. They distribute the subtle elements of their creation in the journal

Fig. 8-Xavier Hames after the implementation of artificial pancreas.

(source-
https://www.google.co.in/search?q=Xavier+Hames&tbm=isch&imgil=mXd78ZHm30utKM%253A%253BmNTtEQCSwom23M%253Bhttp%25253A%25252F%25252Fpresstv.com%25252FDetail%25252F2015%25252F01%25252F23%25252F394374%25252FAussie-boy-gets-1st-artificial
pancreas&source=iu&pf=m&fir=mXd78ZHm30utKM%253A%252CmNTtEQCSwom23M%252C_&usg=__oKH1NIX4Wo7HxofvRB2LG55z4Bw%3D&biw=1366&bih=677&ved=0ahUKEwiKvML6yMXLAhUCwY4KHfhzCfMQyjcIJw&ei=OYLpVsq7GYKCuwT456WYDw)

Industrial & Engineering Chemistry Research. As per the American Diabetes Association, around 1.25 million grown-ups and kids in the US have type1 diabetes, of which around 2 lakh populations are less than the age of 20.Doyle and colleagues say their new device of artificial pancreas could make overseeing type1 diabetes much less demanding, nullifying the requirement for finger pricking and various infusions (Whiteman, 2015).

In their study, the specialists detail the making of a completely implantable artificial pancreas that uses an algorithm to screen patients' blood glucose levels and ascertain the insulin measurement required, which is then

consequently conveyed to the body. On leading PC testing of the device including recreation of an ascent and fall in glucose levels that happens after meals for the duration of the day, the team found that it was kept up the ideal blood glucose scope of 80140mg/dL, 78% of the time, "with no time spent in hypoglycemia". Speaking about the device at a press conference when it was initially divulged back in January, Doyle says she trusts it can possibly change type 1 diabetes administration, adding:

"The closed circuit system provides much tighter control at an unprecedented level to minimize complications and to improve the quality of life. It will have immediate benefits, as it will lower health care costs in the country and it will reduce the amount of decisions people with diabetes need to make on a constant basis" (Whiteman, 2015).

European Association for the Study of Diabetes

The European Association for the Study of Diabetes reported a trial study in Annual Meeting in Stockholm. The volunteers for the study were fitted with both a glucose sensor and an insulin pump. Now and again they settled on their own choices about when to receive insulin. Amid the remaining time, the computer program naturally ascertained a suitable measure of insulin and like clockwork, remotely advised the insulin pump the amount to discharge into the body wirelessly after every 12 minutes. In the investigation of 33 adults, glucose stayed in the objective scope of 70 to 180 milligrams for each deciliter, 68 percent of the time when the software controlled the discharged, versus 57 percent of the time when the volunteers controlled their insulin discharged. The change was most processed during the evening, when levels were in the ordinary extent 59 percent of the time versus 29 percent without

utilizing the software. The adult study members lived in the U.K., Germany and Austria. The 25 youngsters and youths in the companion study all in the U.K. just utilized the trial framework during the evening meal, turning it off before breakfast. Glucose stayed in the target range of 70 to 145 mg/dl 60 percent of the time with the system and 34 percent of the time when the insulin infusions weren't automatic. The benefits of tighter night time control seemed to carry over to the day as well, said the research team, led by Dr. Hood Thabit of the University of Cambridge (Pierson & Clarke, 2016).

Cambridge University and De Montfort University

The artificial pancreas being created by Cambridge University is comprised of a remotely worn insulin pump which imparts by remotely to a continuous glucose monitor worn as a patch on the skin.

Another solid contender is an implantable insulin conveyance device including a gel that reacts to changes in blood glucose levels. At higher blood glucose levels, the gel permits a more prominent rate of insulin to be discharged and at lower sugar levels; the gel diminishes the measure of insulin it discharges. The implantable artificial pancreas is being developed by researchers from De Montfort University.

Cambridge University's closed loop artificial pancreas has been created and has additionally been tried on people under controlled and home conditions. Examines demonstrated that the artificial pancreas framework could expand the measure of time study members spent in the right blood glucose level extent by 22%.

Dr Roman Hovorka at the University of Cambridge is chipping away at a five and a half year undertaking to produce a counterfeit pancreas model ("Artificial pancreas," 2016) and assess its capacity to enhance blood glucose control at home and decrease the danger of overnight mesmerizing adults with Type 1 diabetes. Also at the University of Cambridge, Dr Helen Murphy is driving on a five year venture to adjust the artificial pancreas ("Artificial pancreas," 2016) to control blood glucose levels amid pregnancy. This examination could definitely diminish instances of stillbirth and mortality rates among pregnant women with Type 1 diabetes.

Australian Artificial Pancreas Program

Set up in 1987, the Diabetes Australia Research Program bolsters and builds up the field of Diabetes exploration in Australia. The system stores research into counteractive action, administration and a cure for diabetes. AAPP scientists as of late started introductory clinical trials of their calculation. Presented underneath is their first trial member, Brock Mueller, with some of their group in the clinical trials office at Hunter Medical Research Institute (fig.9). Information from Brock's trial gave check of the AAPP approach and is currently being investigated to propel the calculation. The trial proceeded with their second member who went along with them in August and three more members in 2015. Australia Artificial Pancreas Research Program gave $1.1 million in stipends to 17 scientists in NSW. This incorporated the prestigious Millennium Award for Type 1 Diabetes Research, granted to AAPP's Clinical Director Dr Bruce King for his exploration into the Australian Artificial Pancreas calculation for reported and unannounced meals (Program, 2015).

Figure9- Pictured (L to R): Dr Adrian Medioli, Brock Mueller, A. Prof Bruce King, and Prof Graham Goodwin.

(source-http://www.artificialpancreas.com.au/news)

Europe Diabetes Research Centre

The GlucoSitter artificial pancreas calculation is endorsed in the EU which got a CE Mark in Europe, making it the principal ever artificial pancreas algorithm to get administrative endorsement on the planet. The endorsement is an essential stride for the whole artificial pancreas field. The GlucoSitter programming depends on the MD-Logic closed loop algorithm (Foundation, 2015) and is the result of the Diabetes Wireless Artificial Pancreas Consortium (fig. 10). The gathering – made out of diabetes focuses in Germany, Israel, and Slovenia – has been trying GlucoSitter in a huge number of trials in the course of recent years. Utilization of the MD-Logic calculation has altogether diminished patients' opportunity spent in hypoglycemia and expanded their time spent in-extent in overnight and 24-hours/day use. In the group's study distributed in the *New England Journal of*

Medicine (Foundation, 2015), diabetes campers on the framework had 68% less hypoglycemia scenes and invested about twofold the energy in the scope of 70-140 mg/dl overnight. In studies to date, the GlucoSitter programming has keep running on a tablet or laptop that corresponds with a Medtronic pump (Foundation, 2015) and Medtronic Enlite CGM (Foundation, 2015).

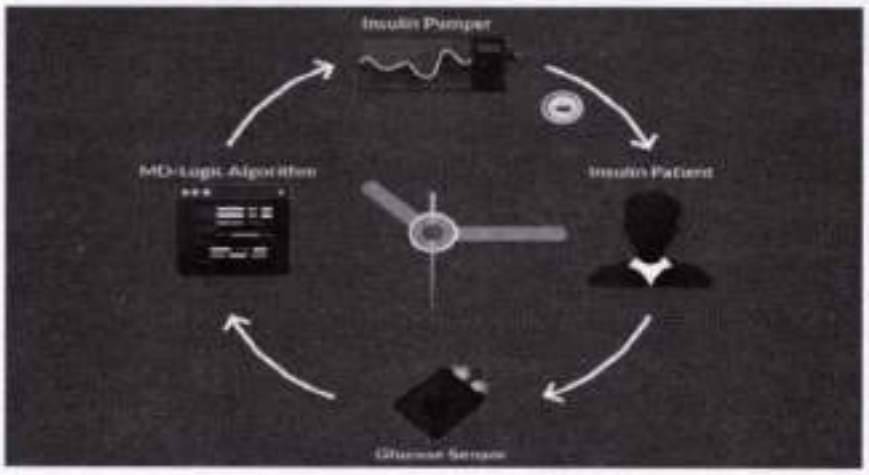

Figure10-The GlucoSitter Artificial Pancreas Algorithm.

(source-http://diatribe.org/firsteverartificialpancreassoftwarereceiveseuropeanapproval)

Israeli medical tech firm DreaMed Diabetes

Israeli therapeutic tech firm DreaMed Diabetes has hit an arrangement with Medtronic, the world's greatest medicinal gadget organization, to utilize its MDLogic Artificial Pancreas calculation in Medtronic's insulin pumps created at an Israeli healing center to help patients in 120 nations. DreaMed will hold responsibility for calculation, in order to have the capacity to utilize

it to create different items. The calculation is as of now being used in a gadget the organization itself has created, called the GlucoSitter, a completely mechanized, simulated pancreas framework for controlling glucose levels. The framework connects the glucose sensor with the insulin pump through electronic control algorithm. It utilizes information of glucose levels from a persistent glucose sensor, investigates them and guides the insulin pump to convey the right measurement of insulin to be discharged for the body to keep up adjusted blood glucose. In actuality, the product constantly screens glucose levels, and characterizes correctly when and how to change insulin levels (Shamah, 2015).

The deal falls right in with Medtronic's long standing efforts to develop an artificial pancreas. Over the past several years, the company has come out with several insulin pump and Continuous Glucose monitoring systems.

Boston University and Massachusetts General Hospital

The community oriented gathering from Boston University and Massachusetts General Hospital cooperating to make robotized blood glucose controls a reality. Engineers from Boston University have added to a bionic pancreas framework that uses persistent glucose checking alongside subcutaneous conveyance of both fast acting insulin (to lower blood glucose) and glucagon (to raise blood glucose) as coordinated by a PC calculation. The bionic pancreas consequently settles on once again choice about insulin and glucagon dosing each five minutes; That's 288 choices for each day, 7days every week, and 365 days for every year.

Artificial Project in India

On December 17, 2013 Narayana Multispecialty Hospital started offering treatment based on the artificial pancreas system for diabetes patient in Hyderabad.

Sudhaker Jadhav, Facility Director of Narayana Hospital, Hyderabad told news person that they were offering the Artificial Pancreas System for the first time in India in Hyderabad and will also expand this to other hospitals located in Bangalore and other places(fig 11).

Doctor Ford Gilbert, who holds a patent on the system that has been approved by the US Food and Drug Administration, said the artificial pancreas treatment will help diabetics by improving their metabolism.

The treatment procedure will need at least 10 sessions to find some improvement, according to Jadhav (Bureau, 2013).

Conclusion

When JDRF started The Artificial Pancreas Project, little was happening in the field. But through a strategic approach of direct funding and collaborative ventures, dramatic advances using integrated smart technology to automate insulin management have already occurred – with more in development and being applied to real – world solution. Today, the Artificial Pancreas (AP) systems are drawing closer to the market for the ultimate goal of Type None. Outpatient trials of first generation system are already underway. Immense benefits can be drawn from artificial pancreas project but along with it there could be some cons/challenges associated with the device.

Fig. 11: (from left) Dr. Ford Gilbert of Trina Health explaining the Artificial Pancreas System in Hyderabad on Tuesday. Beside him are G. J. D. Rao, Senior Physician and Diabetologist of Narayana Multispecialty Hospital and Dan Fisher of Trina .Photo. V. Sivakumar.

(source-
http://www.thehindubusinessline.com/news/national/narayanahospitaloffersartificialpancreas/article5470472.ece).

It has been argued that a machine may not be able to ever replace the human mind when it comes to administering a correct dose of insulin, because everyone's body is different from each other, it may not be a flawless system. And in some cases people may become too dependent on piece of equipment, any malfunction of the device can be huge risk for their lives as all devices can have malfunctions, and this piece of equipment will be holding people's lives in its hands.

Its benefits although could not be neglected. The initiatives that has been taken is bringing us closer to making an artificial pancreas that fully automates insulin dosage and achieves the ultimate goal of turning Type One into Type None.

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10. Market Potential of some minor Fruits of East Khasi Hills District, Meghalaya

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ABSTRACT

The article describes about the commercial potential of some minor fruits available in the markets of East Khasi Hills district, Meghalaya. Indigenous people of Meghalaya use a number of wild or semi wild fruits which lack wide scale cultivation and trade. These minor fruits are essential for maintaining a balanced nutritional profile and also provide some extra income tothe rural people. Due to urbanization and change in life style, these fruits are slowly being forgotten. Because of this, there is a need to create awareness among people about the importance of minor fruits. In this investigation efforts were made to identify the minor fruits with better commercial potential. From the market surveys, 25 fruit species were recorded, out of which *Elaeagnus latifolia, Myrica esculenta, M. nagi, Prunus nepalensis, Passiflora edulis, Docynia indica* and *Averrhoa carambola* were identified as potential species for commercial exploitation, which can be used for large scale cultivation and trade.

Keywords: rhizosphere, microbiome, endophytes, symbiosis, mycorrhizal, host

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Introduction

Minor fruits are those which lack wide scale cultivation and trade. They grow wild or in homestead gardens without much care. Some of these fruits are as delicious and highly nutritious as the commonly cultivated (major) fruits. Minor fruits play an important role in day to day life of rural people. They are important sources of essential nutrients like vitamins, minerals etc. These fruits are important sources of food and medicine, thus playing a vital role in providing nutritional and economic security to the poor masses in the rural areas (Sankaran et al., 2006). These minor fruits, which are mostly wild are collected and consumed by people of all age groups in rural areas. However, with rapid urbanization and consequent changes in life style and food habit of people, the minor fruits, which otherwise was an important part of diet, is being neglected and slowly displaced by major commercial fruits. Also, due to habitat destruction and rapid urbanization, these wild fruits are becoming rare in their natural habitats (Kharshandi, Lyngdoh & Bokolial, 2015). Thus studies related to different aspects of lesser known fruits are necessary to bring them back to the limelight.

The northeastern state of Meghalaya (25°47'-26°10' N latitude and 89°45'-92°47' E longitude) is a part of the Indo-Burma biodiversity hot spot of the world (Myers, et al., 2000).The East Khasi hills district is one of the eleven districts of Meghalaya and located between 25°07" - 25°41" N and 91°21" - 92°09" E and covers an area of 2752 sq km. Average annual rainfall of the district is around 1200 cm and temperature varies from 3.8°C to 28° C (www.eastkhasihills.gov.in). The climate of the district varies from temperate to sub-tropical and tropical in different stretches. The inhabitants

are mainly Khasis and their sub-tribes. These indigenous people of the state, through their experience, have developed a rich traditional knowledge of a variety of plant uses. The present investigation was carried out to record the market potential of minor fruits used by local people of East Khasi Hill district of Meghalaya.

Materials and Methods

The study was carried out in the year 2014-2015.Local markets from different locations of the East Khasi Hills district were visited and fruit sellers were interviewed regarding price, availability and demand of the minor fruits available in markets. Markets were visited on a monthly basis keeping in mind the seasonal variations in fruit markets. Specimens were collected and identified with the help of standard literature (Kanjilal et al., 1934-40; Haridashan & Rao, 1985-1987;Hajra, Nair & Daniel, 1997). For highlighting the importance of the fruits, data about their nutritive values are collected from published literature and incorporated in the paper. Collected specimens were preserved as bottled specimen in the Department of Botany, St. Anthony's college, Shillong for future reference.

Results and Discussion

Table -1 contains names of minor fruit species found in markets of East Khasi Hills district together with their local names, mode of utilization and reported nutritional values. A total of 25 numbers of species of minor fruits belonging to 16 families of angiosperms were recorded during the investigation. Rutaceae and Moraceae has the highest number of species recorded (3 species each) followed by Rosaceae, Flacourtiaceae, Ericaceae, Arecaceae, and Myricaceae (2 species each). Some of the fruits recorded are

available in most of the markets studied, whereas availability of someothers is confined to local markets only. Fruits like *Artocarpus heterophyllus, Baccurea ramiflora, Castanopsis indica, Elaeagnus latifolia, Flacourtia jangomas, Morus australis, Myrica esculenta, Passiflora edulis, Prunus nepalensis* etc. were available in most of the markets studied, while *Aphananthe cuspidata, Artocarpus lacucha, Citrus latipes, Docynia indica, Entada rheedii, Gynocardia odorata, Rhuschinensis, Viburnum foetidum*etc were recorded mainly from the local markets among the minor fruits *Entadarheedii, Gynocardia odorata* and *Viburnum foetidum* are marketed in a small scale and found less frequently in markets. *Agapetes variegata, Citrus latipes* and *Rhus chinensis* were rarely sold in the markets. Our survey also revealed that most of the minor fruits recorded from markets are collected from the wild. Fruits like *Entada rheedii, Gynocardia odorata, Rhuschinensis, Viburnum foetidum* etc are harvested from forests and sold in the market. Species like *Baccurea ramiflora, Elaeagnus latifolia, Flacourtia jangomas, Myrica esculenta, Phyllanthus emblica, Passiflora edulis, Prunus nepalensis* etc. are semi wild and found growing in the forest edges, near human settlement or in home gardens. It was also observed that all the species of fruits available in the markets did not have equal demands. Some of these minor fruits are more popular;fruits like *Averrhoa carambola, Elaeagnus latifolia, Myrica esculenta, Morus australis, Passiflor aedulis, Prunus nepalensis* etc. are preferred by all, while consumption of *Aphananthe cuspidata, Docynia indica, Gynocardia odorata, Pinanga gracilis, Viburnum foetidum* etc. is mostly confined to local tribal population. Also, the prices of the minor fruits were found to vary widely depending on location of the markets. However, the fruits having more demand are sold in

more or less constant rates in all the markets surveyed. Nutritional values of some of these minor fruits suggest that they are easily comparable to the common major fruits in terms of nutrition. Data available about nutritional qualities of minor fruits shows that they are rich in micronutrients like vitamins and minerals and high in antioxidants(Table-1). This data can be quite significant, as malnutrition and vitamin deficiency is of common occurrence in the state of Meghalaya (ICDS, 2010).

Table-1

Sl No	Botanical name	Family	Local name	Mode of utilization	Market price	Reported nutritional values
1	*Aphananthe cuspidata* (Blume) Planch.	Cannabaceae	Sohbrai	Ripe fruits are edible	Rs 50 per kg	Not found
2	*Agapetes variegata* (Roxb.) D. Don ex G. Don	Ericaceae	Soh-jalamut	Fruits are eaten raw, also used for making pickles.	Rs 20 per kg	Not found
3	*Artocarpus Heterophyllus* Lam.	Moraceae	Sohphan	Ripe fruits edible, Seeds eaten roasted or boiled , unripe fruits are used to	Rs 40-100 per piece depending on size	Seeds good source of starch and dietaryfibre (Hettiarachchi, Eikanayak

				make pickles.		e and Welihinda 2011).
4	*Artocarpus lacucha* Buch.-Ham.	Moraceae	Soh ram	Ripe fruits edible, Seeds roasted and eaten.	Rs.20 per packet	Contain high amount of carotenoi ds, Znc, Cu and K (Shajib, Kawser, Miah, Begum, Bhattacha rjee, Hossain, Fomsgaar d and Islam 2012).
5	*Averrhoa carambola* L.	Oxalidac eae	Soh-pyrshon g	Ripe fruits edible, also made into pickles.	Rs.10 per 3 piece or Rs.80-120	Good source of Ca, Fe, P and K. Also contain

					per kg	vitamins and antioxidants (Chang, Chen, Fang and Huang 2002).
6	*Baccurea ramiflora* Lour.	Euphorbiaceae	Soh ram-dieng, sohmyn dong	Ripe fruits edible	Rs 60-80 per kg	A significant source of vitamin C, Fe, Cu,Zn and other micro and macro minerals (Shajib et al. 2012).
7	*Calamus erectus* Roxb.	Arecaceae	Soh-thri	Fruits eaten raw	60-80 per kg	Not found
8	*Castanopsis indica* (Roxb. ex Lindl.) A.DC.	Fagaceae	Sohot	Nuts eaten raw	Rs60-120 per kg	Nuts rich in Ca (Agrahar-Murugkar and Subbulaks

						hmi 2005). Contain high concentration of essential micro and macro-minerals, carbohydrates and other nutrients. Contain high amount of phenolics, exhibit strong antioxidant activity (Seal 2011a)
9	*Citrus maxima* (Burm.)Osbeck	Rutaceae	Soh-myngor, soh bah	Mature fruits edible	Rs 20-30 per piece	Contain high concentration of Vitamin C,

						rich in K, P and Mg, also contain dietary fibres and other essential micronutrients (USDA National Nutrient Database for Standard Reference)
10	*Citrus latipes* (Swingle) Yu.Tanaka	Rutaceae	Soh Kynphur, soh syiem, soh dkhar	Ripe fruits edible	Rs 50per kg or Rs10 per - piece	Not found
11	*Citrus macroptera* Montr.	Rutaceae	Sohkwit	Mature fruits edible	Rs10-20 per piece	Rich sources of fiber, total phenols,

						and natural antioxidants with high amount of ascorbic acids, contain high amount of beta carotene, Ca, P, Mg and other micronutrients(Islam, Hoque, Asif-Ul-Alamand Monalisa 2015)
12	*Docynia indica(Wall.)* Decne.	*Rosaceae*	Soh-phoh khasi	Fruits eaten raw, also made into pickles	Rs 10 per 4 pieces	Contain high amount of raw proteins,Ca,P,K, proline and

						aspartate (Wenquan, Chengyao ,Luxiang and Jinyu2002)
13	*Elaeagnus latifolia* L.	Elaeagnaceae	Sohshang	Ripe fruits edible, unripe fruits used for making pickles.	Rs 80 per kg	Antioxidant activity (Seal 2011b). Fruit a richsource of vitamins and minerals and other Bioactive compounds(Dandge, Kasabe and Patil 2011)
14	*Entadar heedii* Spreng.	Fabaceae	Mei nup, Ka-nup, Soh-nup	Seed kernel (cotyledons) after removing	Rs .10 per 3-4spoons of sliced	High amount of K and P, alsohigh in Ca, Na

				the seed coat isroastedan dboiled and sliced for eating.	seed.	and Fe (Okba, Soliman, El Deeband Yousif 2013)
15	*Flacourtia jangomas* (Lour.) Raeusch.	Flacourtiaceae	Sohmluh	Ripe fruits edible	Rs. 80 per kg	Fruits a good source of protein, vitamin, Ca, K, P, Fe, Mg. (Khare 2007)
16	*Gynocardia odorata*R. Br.	Flacourtiaceae	Sohling, sohliang	Seeds boiled for 3-4 hours and cut into thin pieces, washed thoroughly and eaten. Other method is keeping the sliced seeds	Rs.20 per cup	Seeds have high nutritive value, contain almost all the essential minerals (Seal 2011d)

				under running water for 3-4days to remove toxic substances and eaten.		
17	*Morus australis* Poir.	Moraceae	Soh-lyngd khur	Ripe fruits edible	Rs 10 per packet	Fruit contains invert sugar, pectin, fruit acids (including malic and citric acid),ascorbic acid, and flavonoids (Khare 2007)
18	*Myrica esculenta* Buch.-Ham. ex D. Don	Myricaceae	Sohphie	Ripe fruits eaten raw, also used for making pickles.	Rs 40-80 per kg per kg	Antioxidant activity(Seal 2011b).High in

						nutrients (Seal 2011c)
19	*Myricanagi* Thunb.	Myricaceae	Soh-phie-rit	Ripe fruits eaten raw, used for preparation of pickles and refreshing drink	Rs 80 - 100 per kg	Antioxidant activity(Seal 2011b). Rich in minerals and essential nutrients(Seal 2011c).
20	*Passiflora edulis* Sims.	Passifloraceae	Sohbrap	Ripe fruits edible, used for preparing refreshing drinks	Rs 5 per piece	Fruit juice good source of ascorbic acid, carotene and sugars(Khare 2007).
21	*Pinanga gracilis* Blume	Arecaceae	Sohkwai-laper	Ripe fruits are taken as masticatory	Rs 60-120 per kg	Not found
2	*Prunus nepalensis*	Rosaceae	Sohiong	Ripe fruits	Rs120	Rich in β

2	Ser.			edible; used in preparation of squash, wine and jam.	per kg	carotene and vitamin C (Agrahar-Murugkar and Subbulakshmi2005). Contain high amount of Na, Fe, Ca, and crude protein and available carbohydrates (Seal 2011c).
23	*Rhuschinensis* Mill.	Anacardiaceae	Sohma	Ripe fruits are eaten raw.	Rs 60 per kg	Fruits have high nutritive value, contain high amount of carbohydrate and

						fats, also contain essential minerals and fibres (Seal and Choudhury 2014).
24	*Viburnum foetidum* Wall.	Caprifoliaceae	Sohlang	Ripe fruits edible, also used as fishing bait.	Rs 10-20 per bundle	Not found
25	*Vaccinium griffithianum*Wight.	Ericaceae	Soh-ryng-kham	Mature fruits edible	Rs 80 per kg	Not found

Some of the minor fruits have found use in large scale production of food items. *Averrhoa carambola, Artocarpus heterophyllus, Elaeagnus latifolia, Myrica esculenta, Myricanagi, Prunus nepalensis* and *Passiflora edulis* have been used by Department of Agriculture, Government of Meghalaya for making jam, squash and pickles (http://megagriculture.gov.in /public/sales_corner/meg_products.aspx.).Pickles made from *Docynia indica* are also available in the market. Considering all these aspects, we identified *Docynia indica, Elaeagnus latifolia, Myrica esculenta, M. nagi, Prunus nepalensis, Passiflora edulis* as species having potential for large scale production and commercial exploitation. These wild or semi wild species can

be introduced into an organized agriculture system for maximizing production and commercial exploitation. Integration of these fruits into agricultural systems will also serve the purpose of conserving these precious genetic resources.

Minor fruits, once an integral part of a day to day life in rural areas are gradually being forgotten and replaced by commercial fruits. These minor fruits are not only important for maintaining a balanced and healthy diet, but also for providing financial benefits to the poor, especially in rural areas. The need of the hour is to make people aware of the health benefits of minor fruits, identification and mass production of potential species and framing proper strategies for their commercial utilization.

Fig-1: *Aphananthe cuspidata* (sohbrai)

Fig-2: *Baccurea ramiflora* (soh ram dieng)

Fig-3: *Entada rheedii* (soh nup)

Fig-4: *Gynocardia odorata* (soh liang)

Fig-5: *Passiflora edulis* (soh brap)

Fig-6:*Prunus nepalensis* (sohiong)

Fig-7: *Pinanga gracilis* (soh kwai laper)

Fig-8: *Vaccinium griffithianum* (soh-ryng-kham)

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11. Challenges in future 5G networks and some potential solutions: A Survey

Probidita Roychoudhury[22]

Abstract

The mobile communication scenario is expected to witness an unprecedented growth in terms of capacity and traffic volume in the next few years. The current Fourth Generation (4G) mobile networks also known as Long Term Evolution – Advanced (LTE-A) will not be able to guarantee customer satisfaction as perceived in the future connected society. Hence, it is anticipated that by the year 2020 the Fifth Generation (5G) mobile services will be rolled out. The 5G mobile networks promises to provide a fast, efficient, ubiquitous and energy efficient mobile platform for users by providing connectivity "everywhere", at "anytime" and between "anything". However, the path to achieving this is not free of challenges. In this paper, we delve into some possible challenges in implementation of 5G services and also explore a few potential solutions in order to overcome the challenges.

Introduction

The market for cellular data is growing at a tremendous pace (Schiller, 2009). Starting from the humble First Generation (1G) Advanced Mobile Phone Service (AMPS) analog mobile communication systems of 1983, to

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be followed by 2G - Global System for Mobile Communication (GSM) with digital voice only services in 1990, 2.5G – General Packet Radio Service(GPRS), Enhanced Data Rates for GSM Evolution (EDGE) with digital voice and limited data services in 1995, 3G-Universal Mobile Telecommunication System (UMTS) with both voice and enhanced data services in 1999 to the current 4G- Long Term Evolution Advanced (LTE-A), WiMax with high speed data service, cellular systems have seen the birth of a new generation at every decade. This has be largely prompted by the explosive growth in mobile traffic. The growth in mobile traffic worldwide in 2015 alone was 74% and this figure is expected only to grow in the future. It has reached 3.7 Exabytes per month at the end of 2015, up from 2.1 Exabytes per month at the end of 2014. Mobile data traffic has grown 4,000-fold over the past 10 years and almost 400-million-fold over the past 15 years. Global mobile devices and connections in 2015 grew to 7.9 billion, up from 7.3 billion in 2014 and 36% of these were smart devices. These smart devices generate 14 times more traffic as compared to non-smart devices ("Cisco Visual Networking Index," 2016). Given the above statistics, it is evident that even the current 4G cellular networks will not be able to meet the growing demand for higher data rates and increased capacity. Hence, we have the next generation 5G wireless networks which promise ubiquitous connectivity, ultra-high transfer rates, near zero latency and enhanced capacity of base stations in order to bring about a significant improvement in user satisfaction.

Taking into consideration the rapid pace at which newer technologies and applications in the field of mobile wireless communication are emerging, it can be rightly predicted that the society of the future will be an "always

connected information society". Innovative and challenging applications and services in the areas of infotainment, transportation, industrial and professional applications etc. provide impetus for the development of 5G networks. We now have intelligent transportation systems for efficient traffic management, smart grids for delivery and management of utility services like gas, electricity, water etc, smart connected homes, smart security and surveillance services for providing security to personal and industrial sites, e-heathcare for improved quality of healthcare, virtual reality, industrial automation and many more. While some applications require enormous volume of data transfer like huge video files in virtual reality applications, there may be others with periodic low data volume requirements like smart grids. Certain applications require real time data transfer and others may be delay tolerant. These diversities in the requirements of application make the task of designing an efficient 5G network all the more difficult (Osseiran et al., 2015).

The rest of the paper is organized as follows – section II explores some of the challenges that can come in the way of implementation of 5G networks and enlists potential solutions that can be applied to address each of the challenges mentioned and finally Section III concludes the paper.

Major challenges

The flagship program of the EU on 5G, Mobile and wireless communication Enablers for Twenty-twenty Information Society (METIS) (Droste, 2015), have attempted to prepare the groundwork regarding 5G network before any standardization activities are carried out. This project has studied diverse use

cases and forecasted the requirements and challenges of 5G in order to support –

- 1000 times higher mobile data volume per area
- 10 to 100 times higher number of connected devices
- 10 to 100 times higher user data rate
- 10 times longer battery life for low-power massive machine communication (MMC)
- 5 times reduced end-to-end latency

Based on this analysis, the requirements for 5G can be enumerated as follows –

1. Massive number of heterogeneous devices within a single cell – in order to sustain applications like smart grid, industrial automation etc, including devices like sensors, actuators, regular user equipment, vehicles etc,
2. Ultra-high data rates – to provide for applications like virtual office
3. Spectrum efficiency – to optimize the available spectrum to support large number of users in crowded places
4. Zero latency - to support real time applications like traffic efficiency and safety
5. Energy Efficiency – applied to all applications

The aforementioned requirements pose multiple challenges to the design of 5G cellular networks. We look at some of the most notable challenges for each requirement and their potential solutions in the following sub section.

Massive number of heterogeneous devices within a single cell

With the ever growing number of connected devices in the future 5G networks, it can be expected that the network capacity of the existing base stations in macro cells will increase hundredfold. Typical solutions like increasing the cell size, using higher frequency band and improving the spectral efficiency will not suffice. Increasing the size of cells will not be energy efficient just as increasing the number of base stations will not be practical due to the prohibitive costs involved. Moreover, the scarcity of the currently uses ultra-high frequency band makes it all the more difficult to increase the network capacity. Spectral efficiency has already reaching the Shannon's capacity limit and the use of high frequency bands comes with their own set of issues.

Potential Solutions

One possible solution to handle the projected capacity, is to create small cells powered by low power base stations like Micro cells and Pico cells or even cells with distributed antenna arrays which relays the data to the macro base station (Vahid et al., 2015). These cells can co-exist with existing macro cells and give rise to a heterogeneous network of small cells. This assists in not only offloading the traffic to the small cells but is also energy efficient as the stations as well as the devices need not transmit at full power since the network becomes closer to the users.

Ultra-high data rates

With the increasing number of powerful devices like smartphones that use the cellular network to access data, the demand for higher data rates has also grown by leaps and bounds. Applications such as virtual office, streaming video, online gaming etc requires very high data rates which is expected to

be 10 -100 times more than the current figures. In order to support these high data rates, both the radio access technology as well as the backhaul to the core network needs to be revamped.

Potential Solutions

Given that the current radio frequency spectrum is scarce and also expensive, in order to meet the requirements of high data rates, we need to explore alternate radio access technologies. Millimetre Wave (mmWave) Communication technology appears to be the first choice in this regard (Wang, 2014). The use of mmWave spectrum (3 – 300 GHz range) as the carrier frequency coupled with opportunistic traffic offloading to unlicensed spectrum can help in achieving the targeted data rates. Additional speed gains can be achieved by use of advanced antenna technologies like massive MIMO and beamforming. Furthermore, the latency can be further reduced by disassociating the radio resource management from the base stations and moving them to the cloud

.

Spectrum Efficiency

Increased network capacity and high data rates are closely linked to spectral efficiency. Thus, increasing the spectral efficiency can assist in providing fast data access to people even in crowded locations like shopping malls, railway stations etc. The existing 4G network use Orthogonal Frequency Division Multiplexing (OFDM) and Orthogonal Frequency Division Multiple Access (OFDMA) as the modulation and multiple access technique. However, whether the same techniques will be suitable for the proposed technologies like mmWave is still under study.

Potential Solutions

Studies have shown that alternate modulation techniques such as Filter Bank Multi Carrier (FBMC), Universal Filtered Multi Carrier (UFMC), Bi-orthogonal Frequency Division Multiplexing (BFDM) and Generalized Frequency Division Multiplexing (GFDM) provides better spectral efficiency as compared to OFDM (Gohil, 2013). 5th Generation Non-Orthogonal Waveforms for Asynchronous Signaling (5GNOW) is a research group currently exploring these modulation techniques (5GNOW Deliverable 2.2, 2015).

Zero Latency

For time-critical applications like security, healthcare, industrial automation etc, the amount of delay that can be tolerated is almost zero. Huge mishaps can occur if an unwarranted amount of delay is present. Thus, the 5G network should be capable of supporting these types of applications. This delay can be caused by several factors like number of elements in the architecture, the speed of the backhaul link, the number of users in the cell and so on.

Potential Solutions

The architecture in 5G networks need to be less complex with reduced number of elements between the user and the core network but at the same time maintaining the security of the system. There has to be a paradigm shift from base station centric design to a device centric design (Agiwal, 2016). Restructuring the responsibilities of the core network and the base stations can also assist in reducing the latency. For example, if the handoff process is handled entirely by the base stations instead of the core network nodes, the

latency can be reduced to a large extent. The use of efficient backhauling technologies like mm-Wave coupled with distributed antenna systems can also assist in achieving zero latency.

Energy Efficiency

The greater the complexity of the wireless communication system, the higher is the energy consumption of the different network elements. It has been found that 70% of the electricity bills of the network operators come from the energy consumed by the base stations (Wang, 2014).

Potential Solutions

Designing an energy efficient system cannot be looked in isolation. Every aspect of the network has to be taken into consideration starting from the architecture, the network deployment, radio transmission technologies used and backhauling solutions employed. The network architecture should incorporate suitable procedures for handling signalling for idle mode devices (Olsson, 2013). Flexibility in the architecture like separation of control, data and management planes makes it possible to scale up or scale down operations in an energy efficient manner. A flexible network deployment with small cells together with indoor-outdoor, day-night traffic makes the network more energy efficient as compared to a static rigid deployment (Agiwal, 2016). Use of energy efficient transmission systems like massive-MIMO and backhauling technologies will also go a long way in achieving the aforementioned requirement.

Conclusion

In this paper, we have tried to look at a number of challenges to the future 5G networks and also explored some potential solutions for the same. As discussed, keeping in consideration the requirements of 5G networks and the challenge mentioned in the previous section, the design of the 5G cellular network has to be viewed from both an evolutionary as well as a revolutionary perspective. While optimizing the current cellular system to suit the new and emerging scenarios is important, at the same time, the need to explore alternative technologies is also essential. Further research is required in the fields of small cell HetNets, radio access technologies like mm-Wave and energy efficient technologies in order for 5G networks to provide a satisfying user experience.

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12. Isolation of α- Amylase Producing Bacterial Strain From Soil

Jahangir Alom[23] and Dr. Manabendra Mandal[24]

Abstract

This study was aimed at isolating the α-amylase producing bacteria, and colony morphology-characterization of α- amylase produced by the selected strains at different pH. A total of 48 bacterial strains were isolated from soil containing vegetable waste ,decaying materials. Amylases are among the most important enzymes and are employed in the starch processing industries for the hydrolysis of polysaccharides such as starch into simple sugar constituents. With the advent of new frontiers in biotechnology, the spectrum of amylase applications has expanded into many new fields such as clinical, medicinal, industrial processes and analytical chemistry .Amylases can be obtained from several sources, such as plants, animals and microorganisms. α-Amylase has been in increasing demand due to its crucial role of starch hydrolysis and the applications of this hydrolytic action. α-Amylases (E.C.3.2.1.1) are enzymes that catalyses the hydrolysis of internal α-1,4-glycosidic linkages in starch result into low molecular weight products, such as glucose, maltose and maltotriose units.

Keywords: Saccharification of starch, polysaccharides, α-amylase

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Introduction

The soil is considered as the land surface of the earth which provides the substratum for plant and animal life. The soil represents a favorable habitat for microorganisms and is inhabited by a wide range of microorganisms, including bacteria, fungi, algae, viruses and protozoa. There are billions to hundreds of billions of soil microorganisms in a mere handful of a typical, garden soil. That single handful might well contain thousands of different species of bacteria, hundreds of different species of fungi and protozoa, dozens of different species of nematodes plus a goodly assortment of various mites and other micro arthropods. Almost all of these countless soil organisms are not only beneficial, but essential to the life giving properties of soil.

Enzymes are biological catalysts which are an indispensable component of biological reactions. The use of chemical catalysts has been followed for a very long time. Chemical catalysis though widely used was very cumbersome. The disadvantages that this method poses include need for high temperature and pressure for catalysis and the moderate specificity. These limitations were overcome by the use of enzymes. Enzymes work at milder conditions when compared to that required by chemical catalysts for operation. Also enzymes are highly specific and catalyse reactions faster than chemical catalysts . Enzymes are now being used in various sectors of industry. They are used in detergents, paper industry, textile industry, food industry and many others industrial applications. Enzymes have been in use since ancient times[8] and they have been used in saccharification of starch, production of beverages like beer, treatment of digestive disorders and

production of cheese from milk. Among the many enzymes that are widely used, α-Amylase has been in increasing demand due to its crucial role of starch hydrolysis and the applications of this hydrolytic action. α-Amylases (E.C.3.2.1.1) are enzymes that catalyses the hydrolysis of internal α-1,4-glycosidic linkages in starch result into low molecular weight products, such as glucose, maltose and maltotriose units .Amylases are among the most important enzymes and are of great significance for biotechnology, constituting a class of industrial enzymes having approximately 25% of the world enzyme market . They can be obtained from several sources, such as plants, animals and microorganisms. Today a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry. The amylases of microorganisms have a broad spectrum of industrial applications as they are more stable than when prepared with plant and animal α-amylases. The major advantage of using microorganisms for the production of amylases is the economical bulk production capacity and the fact that microbes are easy to manipulate to obtain enzymes of desired characteristics. α-Amylase has been derived from several fungi, yeasts and bacteria. However, enzymes from fungal and bacterial sources have dominated applications in industrial sectors. .α-Amylases have potential application in a wide number of industrial processes such as food, fermentation, textile, paper, detergent, and pharmaceutical industries. Fungal and bacterial amylases could be potentially useful in the pharmaceutical and fine-chemical industries. However, with the advances in biotechnology, the amylase application has expanded in many fields such as clinical, medicinal and

analytical chemistry, as well as their widespread application in starch saccharification and in the textile, food, brewing and distilling industries .

Materials and Methods

Sample collection

Soil sample was collected from the vegetable waste dumping area of the local market of Napaam, Tezpur, Assam. The sampling was done from the upper surface and from 10-15 cm below the surface in small pre-labelled sterile plastic containers, which were tightly sealed and transported to the laboratory.

Preparation of culture media for Isolation of bacterial strains

Preparation of Nutrient Agar Plates with different P^H:

Nutrient agar media plates with different pH range was prepared for isolation of bacterial strains. For 100 ml of the Nutrient agar media, 1.3grams of nutrient broth was dissolved in 50ml distilled water. Then the pH of media was adjusted to 5, 6, 7, 8 and 9 using pH meter, after that the final volume was makeup up to 100ml by adding distilled water. Then 1.6gm of agar powder was added into the flask and the flasks were capped with cotton plugged. The flasks were then allowed for sterilization by autoclaving. After sterilization the media was allowed to cool and were the poured onto the sterile Petri plates.

Preparation of 0.85% saline solution

For preparation of saline solution 0.85 gm of the NaCl was dissolved in 100ml of distilled water and sterilized for further use.

Serial dilution of the soil sample

Serial dilution of the soil sample was done in 0.85% normal saline solution. For this,7 test tubes were taken and were fill with 9ml saline solution and were labelled as 10^{-1}, 10^{-2} ..... 10^{-7}. 1g soil sample was then measured and added to the test tube labeled as 10^{-1} and mixed thoroughly by vortexing. Using a pipette, 1ml of the solution then transferred to the next test tube and the process was continued till the last test tube.

Spreading of diluted soil sample on agar plates

100ul of the diluted soil sample from the dilution series prepared was then spread on the agar plated by spread plate technique. The plates were then incubated at 37 ^{0} C for 24 hours. The different colonies observed then were note down.

Pure culture preparation

Pure culture of the bacterial strains was prepared by streak plate method. Single colonies were picked up by sterile inoculating loop followed by repeated streaking on the nutrient agar media plates. The plates were then incubated at 37 ^{0} C for 24 hours and then the cultures were observed.

Screening for amylase production

Preparation of Starch Agar Plates

Starch agar media plates with pH-7 were prepared for screening of amylase production of different bacterial strains. For 200 ml of the Starch agar media, 0.8 grams of yeast extract,0.2 grams of K_2HPO_4 and 0.3 grams of $MgSo_4.7H_2O$ was dissolved in 100ml distilled water, the P^H of media was adjusted to 7 using P^H meter. After that the final volume was makeup up to 200ml by adding distilled water, then 3.2grams of agar powder and 3.6 grams soluble starch was added into the flask and the flask were capped with cotton plug. The flasks were then allowed for sterilization by autoclaving. After sterilization the media was allowed to cool and were then poured onto the sterile Petri plates. Then with a sterile loop a single colony was picked up from the pure culture plate and streak onto the starch plate in the form of a circle. The plates were then incubated at 37 °C for 24 hours. After incubation period the plates were flooded with Gram Iodine solution.

Interpretation of the test

Positive Test - A clear and colourless zone around the colonies

Negative Test - No clear and colourless zone around the colonies; a blue colour remains.

Results and Discussion

Isolation of the bacterial strain based on colony morphology

Different types of bacteria will produce different-looking colonies, some colonies may be coloured, some colonies are circular in shape, and others are

irregular. A specific terminology is used to describe common colony types. These are:

- Form - What is the basic shape of the colony? For example, circular, filamentous, etc.
- Size – The diameter of the colony. Tiny colonies are referred to as punctiform.
- Elevation - This describes the side view of a colony. Turn the Petri dish on end.
- Margin/border – The edge of a colony. What is the magnified shape of the edge of the colony.
- Surface - How does the surface of the colony appear? For example, smooth, glistening, rough, wrinkled or dull.
- Opacity - For example, transparent (clear), opaque, translucent (like looking through frosted glass), etc.
- Colour - (pigmentation) - For example, white, yellow, buff, red, purple, etc.

A total of 48 different bacterial strains were isolated based on their colony morphology (Table 1). At Different media pH the morphology of the strains were observed different. At pH 5 most of the strains were white in color and small in size. At pH 6, most of the isolates were medium in size. Maximum number of isolates was observed at neutral media pH. At pH 8 the colonies were mainly large. At pH 9 the most of the colonies were small in size.

Table No. 1: Isolated strains at different pH and their colony morphology			
SL. No	**Strain No.**	**pH of Media**	**Colony Morphology**
1	DEH-35	5.0	Large, white, irregular, flat.
2	DEH-36	5.0	Small, white, circular, convex.
3	DEH-37	5.0	Small, white, circular, raised
4	DEH-38	5.0	Punctiform, white, circular, convex.
5	DEH-39	5.0	Circular, small, white, irregular, flat.
6	DEH-40	6.0	Large, white, smooth, flat
7	DEH-41	6.0	Large, white, irregular, flat
8	DEH-42	6.0	Medium, circular, raised
9	DEH-43	6.0	Small, white, circular, raised.
10	DEH-44	6.0	Punctiform, white, circular, convex
11	DEH-45	6.0	Punctiform, light yellow, circular, raised
12	DEH-46	6.0	Medium ,white, irregular, flat.
13	DEH-47	6.0	Punctiform, white, circular, convex
14	DEH-48	**7.0**	Large ,white, smooth, flat
15	DEH-49	7.0	Small ,light yellow, circular, raised
16	DEH-50	7.0	Punctiform, white, circular, convex
17	DEH-51	7.0	Punctiform, white, circular, Raised
18	DEH-52	7.0	Small white irregular, flat
19	DEH-53	7.0	Small white, irregular, flat
20	DEH-55	7.0	Small white, circular, raised
21	DEH-56	7.0	Medium, white, circular, flat.
22	DEH-57	7.0	Small, white, smooth, flat
23	DEH-58	7.0	Small, white, irregular, raised
24	DEH-59	7.0	Medium, white, circular, convex
25	DEH-60	7.0	Small, white, circular, flat
26	DEH-61	7.0	Small, yellow, circular, convex

SL. No	Strain No.	pH of Media	Colony Morphology
27	DEH-62	7.0	Large, white, irregular, flat
28	DEH-63	7.0	Small, white, circular, raised.
29	DEH-64	8.0	Large, white, filamentous, irregular
30	DEH-65	8.0	Large, white, smooth, flat
31	DEH-66	8.0	Small, light yellow, circular, raised
32	DEH-67	8.0	Medium, white, circular, flat
33	DEH-68	8.0	Punctiform, yellow, circular, raised
34	DEH-69	8.0	Small, white, circular, flat
36	DEH-70	8.0	Small ,yellow ,circular, flat
37	DEH-71	8.0	Small, white, irregular, flat
38	DEH-72	8.0	Punctiform, yellow, circular, convex
39	DEH-73	8.0	Small ,white, irregular, raised
40	DEH-74	8.0	Small ,white ,irregular, rraised
41	DEH-75	8.0	Small ,white, irregular
42	DEH-76	9.0	Small, white, circular, flat
43	DEH-77	9.0	Small, white, smooth, raised
44	DEH-78	9.0	Small, yellow, circular ,raised
45	DEH-79	9.0	Punctiform, white, circular, flat
46	DEH-80	9.0	Punctiform, white, circular, convex
47	DEH-81	9.0	Punctiform, white ,circular, flat
48	DEH-82	9.0	Small ,white ,smooth, flat

Screening of bacterial isolates for α-amylase production

All the isolated strains were screened for extracellular α-amylase production. Among the isolates, 24 of them produced little or more α-amylase. Table-2 showing the results of enzyme production. Among the 24 amylase positive

isolates, 5 of them were found potent strain showing higher enzyme activities than others. They include, isolates DEH-40, DEH-46, DEH-56,DEH-63,DEH-68.

Table No. 2: Screening of bacterial strain producing extracellularα- Amylase

Serial	Strain	α- Amylase activity	Serial	Strain	α- Amylase activity
1	DEH-35	++	21	DEH-60	+
2	DEH-36	+	22	DEH-61	++
3	DEH-38	+	23	DEH-62	-
4	DEH-39	-	24	DEH-63	+++
5	DEH-40	+++	25	DEH-64	+
6	DEH-41	+	26	DEH-65	++
7	DEH-42	+	27	DEH-66	-
8	DEH-43	+	28	DEH-67	+
9	DEH-44	+	29	DEH-68	+++
10	DEH-45	+	30	DEH-69	+
11	DEH-46	+++	31	DEH-70	+
12	DEH-47	-	32	DEH-72	+
13	DEH-50	-	33	DEH-73	-
14	DEH-51	-	34	DEH-76	-
15	DEH-53	+	35	DEH-77	-
16	DEH-55	-	36	DEH-79	-
17	DEH-56	+++	37	DEH-80	+
18	DEH-57	-	38	DEH-81	-
19	DEH-58	-	39	DEH-82	-
20	DEH-59	++	40	DEH-49	-

**** [(+++)more than 20mm; (++), more than 15mm and(+), below 10mm clear zone, (-) no zone of hydrolysis)**

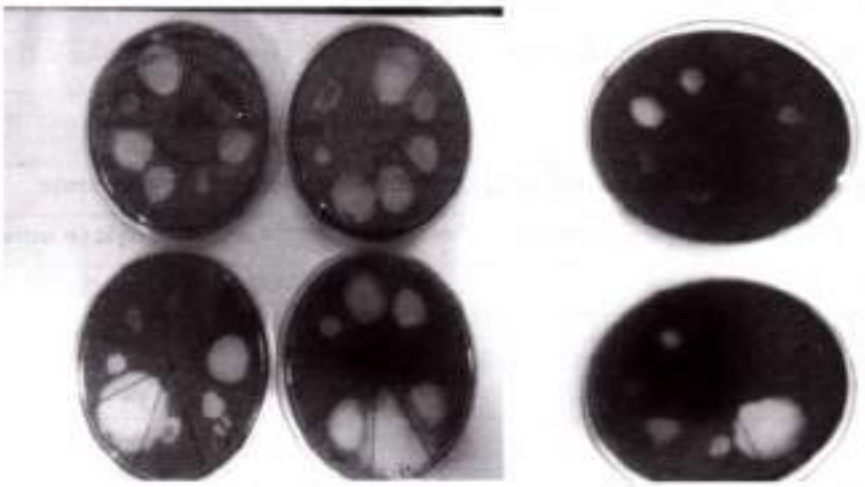

Fig. 1: Plates showing zone of hydrolysis after iodine flooding

Conclusion

A total of 24 bacterial strains which produced clear halos in the starch-nutrient agar medium were isolated and purified. Among the 24 bacterial strains, five strains were selected as best α- amylase producer. The use of α-amylase in starch based industries has been prevalent for many decades and a number of microbial sources exist for the efficient production of this enzyme, but only a few selected strains of fungi and bacteria meet the criteria for commercial production. The search for new microorganisms that can be used for amylase production is a continuous process. Amylases are among the most important enzymes used for industrial purposes, and now in the light of biotechnology they are considered useful for biopharmaceutical applications. They are useful tools in medicinal and clinical chemistry. It is hoped that amylases will continue to provide new opportunities in

biotechnology as biocatalysts and that new applications will emerge in the biopharmaceutical sector. The increasing importance of sustainable development has inspired man to use enzymes for various reactions as they are biodegradable and can be produced using biological sources. α-Amylase can be produced using microbes from soil and waste products of other processes. The enzyme has crucial applications including the production of fructose syrup, environmentally safe detergents, and baked products. Living in an era of depleting fossil fuels with a desperate need to produce alternate forms of energy, this enzyme is a ray of hope. It is used for biofuel production with starch as a raw material. As the production yields an industrially important enzyme and helps keep the environment clean, more researches are focusing on the microbial production of the enzyme.

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13. Scenario of Water Resources in Shillong City, Meghalaya.

V Jennifer Joan Wallang[25]

Abstract

Shillong the capital city of Meghalaya is a rapidly growing urban centre. Water scarcity is common across the urban complex even though it receives a very high rainfall of the order of 2400mm/ year. Shillong city has been exclusively relying on surface water sources viz., rivers and springs, but during recent years it has started using groundwater to meet the requirements of growing population. The present study is an attempt to account the quantity of water supply from ground water and surface water for domestic consumption within Shillong city and prospective groundwater zones in the study area. The study reveals that Shillong Municipal Board and Public Health Engineering Department water supply services in the city accounts to 8.96 million liter per day with 31.44 million liter per day water is supplied by Greater Shillong Water Supply Scheme. The total water production from Shillong city is around 17.10 % from surface water and 05.07% is pumped from groundwater and remaining 77.82 % is imported from Umiew river. Besides the supply from Umiew river a deficit of 17.25 million liter per day is experienced. Geological and geomorphological studies indicate that occurrence and distribution of surface water and ground water of Shillong city is not uniform due to inhomogeneity of lithology and degree of weathering as well as soil profile development. Geomorphologically the ground water potential is limited to intermontane valleys. Hydrological data

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indicates that groundwater occur both under artesian as well as water table condition with moderate to high yield. In general depth of water level is shallower in topographic depression than in the upland areas or slopes.

Keywords: *Water supply, water quantity, account, Shillong city, Umiew river.*

Introduction

Water is essential for sustenance of life on earth. Its sustained supply is needed for socio-economic development and for maintaining healthy ecosystems. Water resources are extremely limited but renewable, exhibiting diversity in their quality and quantity (Rokade et.al. 2004).The demand for water has increased over the years making the assessment of the quantity and the quality of water resources and its optimal utilization most critical (Gebre et.al. 2015). Many parts of India experienced acute shortage of water for different purposes and the problem of water crises is likely to become more severe and serious which will continue well into the 21st century (Biswas, 1991). The quantity of water consumed in most of the Indian cities is not determined by the demand but the supply. People attempt to adjust to the quantity as well as quality of water supplied (Kaur et.al. 2016). As population increases, development calls for an increased allocation of groundwater and surface water for the domestic, agriculture and industrial sectors. It will be a great challenge to meet increased demand for water due to increasing population, economic growth and technological changes (Kaur et.al. 2016). In the long term, given the ever-increasing urbanization and

population growth, the urban water problems are expected to escalate rather than attenuate unless serious planning and management are carried out (Sim and Balamurugan, 1991). There is an urgent need for the evaluation of water resources as water plays a primary role in the sustainability of livelihoods and regional economy (Gebre et.al. 2015).To maintain the availability of water all needful efforts are being made in the field of research and development to meet the demand for domestic, agricultural and industrial uses (Bhaisare and Goel, 1992).

This paper attempts to account the quantity of water supply from ground water and surface water for domestic purposes based on limited available secondary data. An attempt is made to develop a ground water prospect zone map through the convergence of evidence method by integrating collateral data and field inputs.

Study area

The study area (Figure 1) encompasses the urban growth center in and around Shillong, the capital of Meghalaya, covering about 208 sq km as per the Shillong Master Plan 1991-2011. It is defined by the boundary coordinates 25°30'29" to 25°42'10" North and 91°46'50" to 92°00'32" East. It has a population of 3,54,759 as per census 2011. The Shillong Master Plan Area (SMPA) is an agglomeration of twelve urban centres viz., Shillong Municipality, Shillong Cantonment, townships such as Nongthymmai, Madanriting, Mawlai, Pynthorumkhrah, Nongmynsong, Mawpat, Umpling, Umlynka, Nongkseh , Lawsohtun and 35 fringe villages.

Database and Methodology

Available data on hydrological aspects particularly on status of ground water in the Shillong Master Plan is very scanty. Considering limited scope for primary data collection, the present study for water resources was made based on the secondary data available with agencies like Public Health Engineering Department (PheD) and Shillong Municipal Board (SMB), Government of Meghalaya, Shillong. Data on quantity of discharge from PheD sources and ground water level was collected Public Health Engineering Department, and quantity of discharge from SMB sources was collected from Shillong Municipal Board, Government of Meghalaya.

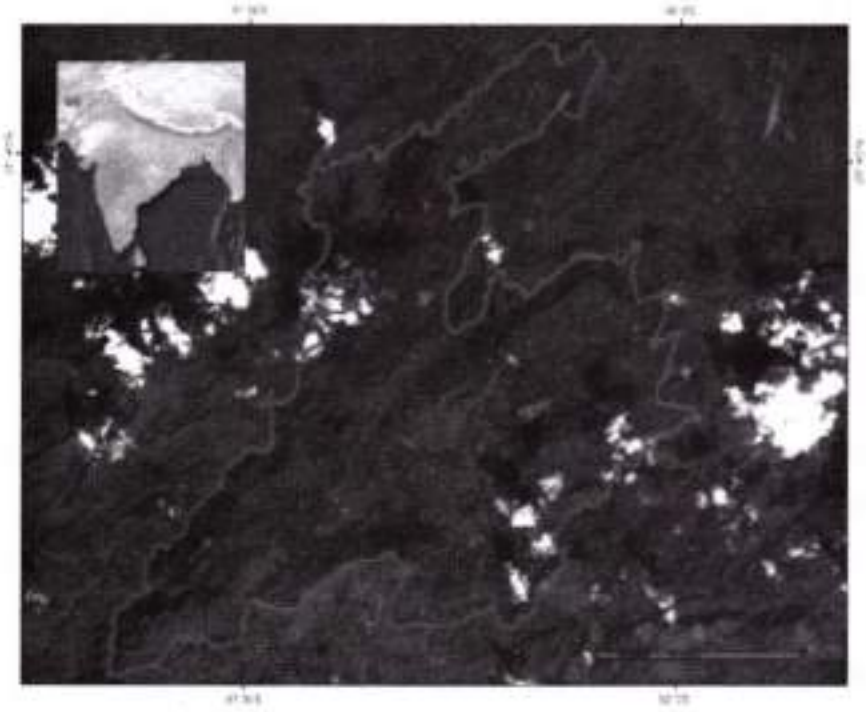

Figure 1: Location map of the study area shown as overlay on the IRS LISS III FCC image

The analysis for water supply and production was confined to Shillong city due to non-availability of data for the whole study area. Thirty six sample points were obtained for hydrogeological data of wells during dry seasons distributed over the study area. The location of these sources was brought to GIS format using ARC GIS software. The study area boundary was prepared on 1:50,000 scale with the help of data collected from Urban Affairs Department of Government of Meghalaya.

Results and Discussion

Shillong City is a rapidly growing urban centre. Water scarcity is common across the urban complex even though it receives a very high rain fall of the order of 2400mm/ year. Shillong City has been exclusively relying on surface water, but during recent years it has started using groundwater to meet the requirements of growing population. The total potable water supply quantum is obtained from numerous springs and a few low discharge streams, formed due to seepage of inland water. Besides private bore wells and hand pumps are also being used extensively for water supply in the area. These water sources are controlled and taken care of by several institutions such as the Shillong Municipal Board (SMB), Public Health Engineering Department (PheD), Shillong Cantonment Board (SCB) and local Dorbars, who are responsible for providing the water supply service. The SMB and PheD provide the bulk of the water supply services. In order to meet long-term projection of water demand, a dam was constructed under Greater Shillong Water Supply Scheme (GSWSS) in 1978 to divert water from Umiew River located 25 km from the main city.

The Shillong Municipal Board (SMB) obtains water supply from several surface water sources such as springs and streams which were identified long back probably when the town was established. These surface water sources include Wahrisa, Wahjalynoh, Umjasai, Crinoline, Madan Laban, Patta Khanna and Wahdienglieng. For example the water supply scheme from Umjasai stream was undertaken in the year 1958 and since then water is being distributed in the municipal area without any treatment. It was observed that these sources are utilized for water supply either by construction of a pumping station and thereby supplying water through the pipes, or by inserting the pipe directly into the spring source. The former method is adopted where there is conspicuous discharge of the spring. The quantity of water produced from these sources for pre-moonsoon and monsoon period is given in Table 1.

The average quantity of water produced per day is 3.27 mld (million liter per day) from these sources and it is distributed within Shillong Municipality area. Sources of water supply which were implemented and maintained by PheD or implemented by PheD but maintained by the local committee are given in Table 2. Average production from these sources is 5.69 mld out of which 3.64 mld is contributed by surface sources and 2.05 mld from underground sources.

In areas beyond the SMB and PheD networks water supply service is being provided by the local dorbar by implementing small water schemes supplying through stand posts and small local springs. Households without access to institutional services depend upon supply made by water tankers or

purchase water from private vendors/ operators or draw water from private bore wells or hand pumps.

Table 1: Quantity of discharge from SMB sources

Sl. No.	Name of the Source	Discharge	
		Premonsoon (mld)	Monsoon (mld)
1	Wah Risa	0.45	0.64
2	Wah Jalynoh	0.45	0.64
3	Umjasai	0.91	1.14
4	Crinoline	0.36	0.5
5	Madan Laban	0.23	0.36
6	Patta Khanna	0.11	0.23
7	Wahdienglieng	0.23	0.29
	Total	2.74	3.8
	Average	3.27 mld	

Source: Shillong Municipal Board

Accounts of water

The revised guidelines/norms on water supply by the Govt. of India, prescribes supply of 135 lpcd(liters per capita daily) and 40 lpcd for the urban and rural population (Bureau of Indian Standards, IS: 1172- 1993). According to this guideline 57.65 mld is required for a projected population of 427076 (PheD, 2006). SMB and PheD water supply accounts for 8.96

mld; thus the town has a net deficit of 48.69 mld. This deficit is compensated by balancing through imports from Umiew river through Greater Shillong Water Supply (GWSS). The average production capacity of the dam is 51.30 mld out of which 31.44 mld is supplied to Shillong City (PheD, 2014). At present a total of 40.40 mld (Table 3) is being supplied to the consumers which is hardly sufficient to meet the growing demands of water. Therefore the total water production available for Shillong City is around 17.10 % from surface water and 05.07% is pumped from groundwater and remaining 77.82 % is imported from Umiew river (Figure 2). Shillong City's reliance on Umiew river water source is evident. Without this external source, the city's rapid development would have been impossible, since consumption has exceeded total local water supply. Besides the supply from Umiew river a deficit of 17.25 mld is experienced or in other words, 70 lpcd at an average is available to the consumers which is very much below the required norms. With the current trend of reduction of discharge of the water sources as reported by local residents during interviews, the small spring sources are likely to be dried up thus reducing the yield considerably in the coming decades. In such a situation there will be almost total dependence on Umiew river.

Groundwater prospective zones in the study area

Geological and geomorphological studies indicate that occurrence and distribution of surface water and ground water of Shillong City is not uniform due to inhomogeneity of lithology and degree of weathering as well as soil profile development.The Shillong group of rocks is characterized by quartzite with some metabasic intrusive of low interstitial porosity but high

fracture porosity. The rocks are widely weathered and the degree of weathering is found to be more in the topographic depression than in other areas. The metabasic rocks are more prone to weathering than the quartzitic rocks.

Table 2: Quantity of water supplied by PheD under various water supply schemes

Sl. No.	Name of the Scheme	Quantity (mld)
1	Mawlai Umsohlang WSS	1.95
2	Pynthor Umkhrah DTW WSS	0.65
3	Pynthorbah Lum Shiap DTW WSS	0.75
4	Umkhen WSS	0.99
5	Lawjynrew,Lumiawblot,Pohkseh,Demthring WSS	0.50
6	St Edmunds(Lumawrie) DTW WSS	0.04
7	Madanrting WSS	0.20
8	Wahdingdoh(Jingthangbriew) DTW WSS	0.08
9	Jaiaw Laitdom DTW WSS	0.16
10	Nongmynsong DTW WSS	0.24
11	U Tirot Singh Nagar-Lumshngain DTW WSS	0.13
	Total	5.69

Source: Public Health Engineering Department

Table 3: Water availability in Greater Shillong Planning Area			
Sl. No	**Source**	**Discharge (mld)**	%
1	Surface water from sources within city	6.91	17.10
2	Ground water from sources within city	2.05	05.07
3	Umiew River	31.44	77.82
	Total	40.40	

Figure 2: Total water availability for Shillong City

The thickness of weathered zones varies from a thin veneer to more than 10-15 m at places. The NE-SW direction of lineaments coincides with the strike direction of the rocks and has well developed joint sets, and therefore it will be more conducive for ground water storage and circulation. However,

hydrologically, the intersection point of various lineaments in the area are very important, as they indicate the places where there will be several source of ground water circulation and the ground water withdrawal, therefore bore well/tube well located at such places will have a good supply of water. Geomorphologically, landform varies from highly dissected hills and hills slope with small and narrow intermontane valleys. The high relief areas of the southern and south-eastern corner occupied by Laitkor range and Shillong Peak with steep topographic slope, and characteristic geological set-up offer high run-off and little scope for rain water infiltration. The discharge at this level is meager to very little in several of the streams, which are mostly of low order. The ground water potential therefore in these terrains is limited to intermontane valleys.

The hydrogeological data indicate that groundwater occur both under artesian as well as in water table condition (Table 4). Hydrogeological data shows that depth of water level is shallower in topographic depression than in the upland areas or slopes. It varies from 4 m to 8 m in topographic depressions whereas in upland areas it is about 55 meters.

Table 4: Hydrogeological data of wells in Shillong City.

Sl. No	Village	Borewell Diameter (mm)	Borewell Depth (m)	Casing Depth (m)	Yield (l/m)	Static Water Level (m)	Drawdown (m)
1	Auxillium	150	130		18	21.8	NA

2	12 mile	100	60	18	153.9	7.5	NA
3	Umpling	56	18.75	4	NA	27	NA
4	Umpling	62	12.5	6.25	NA	10	NA
5	Umpling	68.75	10	Artesian	NA	15.4	NA
6	Rynjah	62.5	15	3	NA	27	NA
7	Rynjah	87.5	15	Artesian	NA	27	NA
8	Rynjah	87.5	NA	NA	NA	NA	NA
9	Rynjah	66.25	22	10	NA	55	NA
10	Rynjah	68.75	18.75	28	NA	55	NA
11	Rynjah	66.25	20	26	NA	55	NA
12	Umtyngar	100	45.45	20	10	4.5	NA
13	Mawtawar	150	107	31	27	NA	NA
14	Umshing,Mawkynroh	150	100	8	30	8.4	NA
15	Mawlai Umshing	150	100	15	27.5	15	NA
16	Mawlai Umshing	150	100	23	27.5	18	NA
17	Mawlong	150	101	36.5	45	27	NA

18	Tynring-111	150	134	12	153	31.53	NA
19	Tynring-1	150	129	10	120	33	NA
20	Mawlynghat	150	72	30	40.36	19	NA
21	Umphyrnai-111	150	81	16.15	97.65	9	NA
22	Umphyrnai-11	150	60	12	40	17.5	NA
23	Umphyrnai-1	150	60	12	55.8	12	NA
24	Kynton-U-Mon	125	81	33	97.65	3.6	NA
25	Nongmynsong DTW (w/s/s)- 1	150	100	6.25	262.5	Artesian	18
26	Nongmynsong DTW (w/s/s) -2	200	100	30	371	8	60
27	Nongmynsong DTW (w/s/s)- 3	200	100	30	614	4	50
28	Mawpat DTW (w/s/s)	150	152	26	315	Artesian	25
29	Pynthorbah DTW (w/s/s) -1	150	67	6.25	265	15	24
30	Pynthorbah DTW (w/s/s)- 2	150	60	6.9	155	27	20
3	Mawlai-Mawroh DTW	200	109.5	30.85	132	21	60

1	(w/s/s) -1						
32	Mawlai-Mawroh DTW (w/s/s) -2	200	100.5	6.2	483	15	50
33	Mawlai-Umsaw Umkhiew - 1	110	61.64	22	NA	24.72	30
34	Mawlai-Umsaw Umkhiew -2	110	60	32.12	NA	30.44	38
35	Mawlai-Umsaw Umkhiew -3	110	60	32	NA	30	3.8
36	Permanent Campus, NEHU	250	89.67	30	6	20.2	50

Source: Public Health Engennering Department, Government of Meghalaya

The water level towards the SW is shallow (within 10 meters) while it is deeper towards NW side (Figure 3). It is also observed that the depth of water level is comparable in different litho- units. The tube wells constructed between the depth range of 100 m to 152 m yield 132 to 614 liters per minute for a drawdown of about 3.8 m to 60 m. The overall yield capacity of the tube wells indicate moderate to high yield potentiality. Some deep tube wells for example, Nongmynsong DTW indicates high yield of about 614 liter/minute. In spite of this, Shillong city depends mostly on the surface water such as rivers, streams and few springs for water supply. Ground water extraction for public use is insignificant.

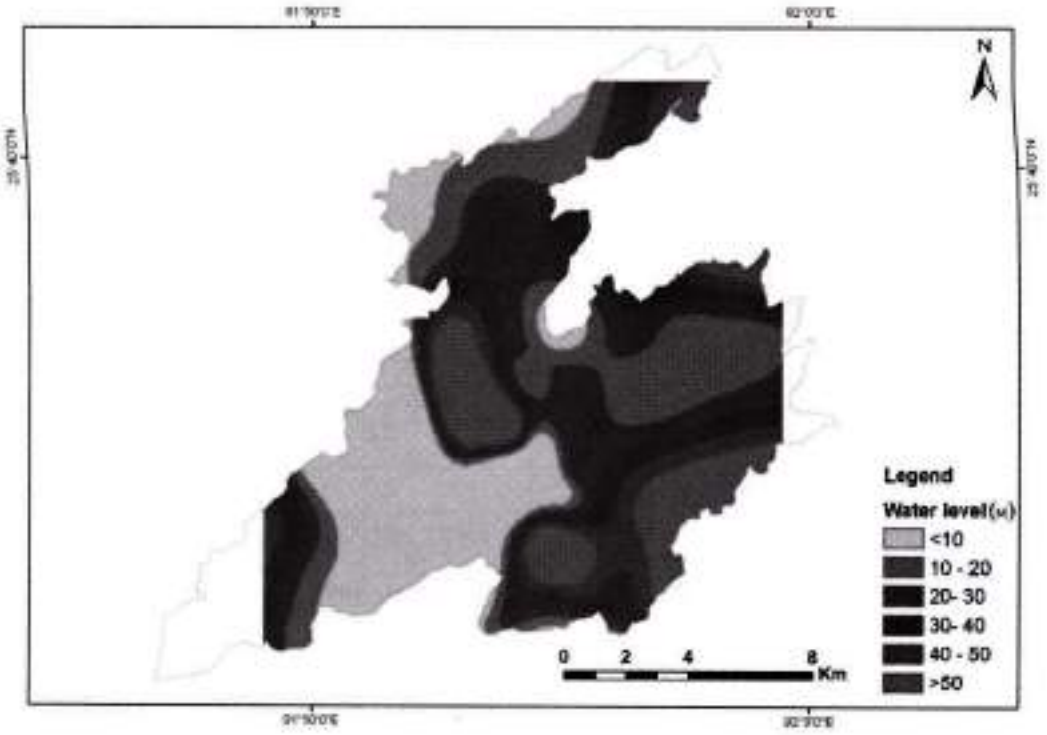

Figure 3: Water level map of Shillong City

Conclusion

SMB and PHeD provide the bulk of the water supply services to Shillong city. In order to meet long-term projection of water demand, a dam was constructed under Greater Shillong Water Supply Scheme to divert water from Umiew River located 25 km from the main city. In areas beyond the SMB and PheD networks water supply service is being provided by the local dorbar by supplying water through stand posts. Out of the total amount of water supply from various sources to Shillong city a deficit of 17.25 mld is experienced. Thus only 70 lpcd water is available to the consumer, which is very much below the Government of India norms (130 lpcd for urban area).

Geological and geomorphological studies indicate that occurrence and distribution of surface water and ground water of Shillong City. Geomorphologically the ground water potential is limited to intermontane valleys. Hydrological data indicates that groundwater occur both under artesian as well as water table condition with moderate to high yield. In general depth of water level is shallower in topographic depression than in the upland areas or slopes.

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St. Anthony's College, Shillong, established in 1934, became the first institute of higher education of the Don Bosco Society world-wide. The college was accredited by NAAC with the rank of Five Stars in 2000. It was also awarded the status of College with Potential for excelence by the UGC in 2006, 2011 and again in 2015. The college was reaccredited with Grade 'A' (3.14/4.00 CGPA) by NAAC in 2014. With more than 75 years of experience and expertise, St. Anthony's College, Shillong excels as one of the top colleges in the country.

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