# **Annual Report**

Integrating Bio-treated Wastewater Reuse with Enhanced Water Use Efficiency to Support the Green Economy in EU and India (India side)





Submitted to

Department of Biotechnology (DBT) Ministry of Science and Technology New Delhi - 110 003, India

Submitted by



International Crops Research Institute for the Semi-Arid Tropics **Annual Report** 

# **Integrating Bio-treated Wastewater Reuse with Enhanced Water Use Efficiency to Support the** Green Economy in EU and India (India side)

Submitted to

Department of Biotechnology (DBT) Ministry of Science and Technology New Delhi - 110 003, India

Submitted by



CRISAT International Crops Research Institute for the Semi-Arid Tropics

## Contents

Sι	mmary	1
Ba	ickground	2
O	pjectives of the project	3
St	rategy	3
1.	Work package: Agro-food industry wastewater valorization and reuse	6
	Objectives	6
	Task 1.1: Complete characterization of selected wastewaters	6
	Subtask 1.1.1 Selection of sites and collection of wastewater samples	6
	Subtask 1.1.2 Wastewater characterization	6
	Task 1.2: Biological wastewater treatment and its impacts (NEERI, TERI, ICRISAT)	16
	Subtask 1.2.1 Screening and isolation of microorganisms from soils utilizing contaminated wastewater for growth	16
	Subtask 1.2.1a Decolourization of wastewater using microorganism (MSSRE)	17
	Task 1.3: Waste based media for recovery of selected compounds (TERL SABM, UGSG)	
	Subtask 1.3.1. Preparation and characterization of separation media	
	Subtask 1.3.2 Performance evaluation for phenolics/pigments recovery	40
	Task 1.4: Assessment of treated water quality and its suitability for irrigation (ICRISAT, JISL)	40
	Subtask 1.4.1: Assess effect of untreated and treated wastewater on soil properties (biological	
	and chemical) <i>in-vitro</i> studies	40
	Subtask 1.4.2 Study effect of untreated and treated wastewaters on plant growth in pot	
	culture	40
	Subtask 1.4.3 Understand changes in soils irrigated with treated and untreated wastewater on	
	biological and nutrient dynamics	43
	Deliverables (brief description and month of delivery)	43
2.	Work package: Bio-treatment of municipal wastewater for reuse and bioremediation of degraded la	ands
	Objectives:	43
	Task 2.1a Site selection and wastewater characterization	43
	Pandherkawada, Nagpur (NEERI)	43
	ICRISAT, Patancheru, India (ICRISAT)	43
	Musi river area, Hyderabad, India (ICRISAT)	44
	Muduvatti, Kolar, India (ICRISAT)	45
	UAS, Dharwad (UASD)	47
	Task 2.1b Biological wastewater treatment (NEERI, TERI, ICRISAT, MSSRF, UASD)	47
	Subtask 2.1.1 Screening and isolation of microorganisms utilizing contaminated wastewater for	
	growth	47
	Subtask 2.1.2 Formation and optimization of microbial consortium for removal of contaminants .	48
	Subtask 2.1.3 Design and demonstration of CWs and HRTS systems	48
	Task 2.2: Bio-remedial regeneration of degraded land irrigated with city wastewater for long term	
	(ICRISAT, NEERI, TERI, MSSRF)	50
	Subtask 2.2.1: Site selection and characterization	50
	Subtask 2.2.2 Baseline data generation with respect to wastewater used for irrigation, soil	
	(Physical, chemical and Biological properties), groundwater quality, cropping pattern	51
	Task 2.3: Impact assessment of treated wastewater use in agriculture (ICRISAT, NEERI, TERI,	
	MSSRF)	56
	Subtask 2.3.1 Assess effect of untreated and treated wastewater on soil properties in-vitro	
	Studies	56
	Subtask 2.3.2 Study effect of untreated and treated wastewaters on plant growth in pot culture .	56

	Subtask 2.3.3 Understand changes in soils irrigated with treated and untreated wastewater on biological and nutrient dynamics	57
	Deliverables (brief description and month of delivery)	58
З	Work package: Agricultural water management	58
5.	Ohiertives	58
	Task 3.1: Identification of henchmark sites baseline data collection and report preparation	58
	Subtask 3.1.1: Benchmark sites will be identified based on the representative abundance and	
	severity of industrial and municipal wastewater	58
	ICRISAT Patancheru India (ICRISAT)	
	Subtask 3.1.2: Inventory of wastewater including guality assessment and status of present	
	usage (ICRISAT, MSSRE, UASD, JISL, SABM)	60
	Subtask 3.1.3: Soil analysis for physical, chemical and biological properties (ICRISAT, UASD)	60
	Subtask 3.1.4: Data analysis and report preparation (ICRISAT, UASD, MSSRF, JISL)	60
	Task 3.2: Design and implementation of irrigation systems based on crop water requirements	62
	Subtask 3.2.1: Selection of efficient irrigation systems (JISL)	62
	Subtask 3.2.2: Selection of irrigation strategies (ICRISAT, JISL, UASD)	64
	Task 3.3: Identify improved agronomic practices for enhanced water use efficiency (ICRISAT,	
	UASD, SABM and JISL)	64
	SAB Miller Sangareddy	64
	ICRISAT, Patancheru	65
	Task 3.4: Impact modelling through simulation	65
	Task 3.5: Design and implementation of integrated agro-aqua farming systems using industrial	
	treated water	65
	Subtask 3.5.1: Exploring the quantum of availability of treated water from industries and saline	
	affected land surrounding the industries along Tamil Nadu and Andhra Pradesh coast	65
	Deliverables (brief description and month of delivery)	69
	3.1 Benchmark sites characterized (month 12)	69
4.	Work package: Development of water efficient crop varieties	69
	Objectives	69
	Task 4.1a: Analyze comparative abilities of maize, sorghum and millet association panel genotypes	
	for biomass production and water use efficiency (ICRISAT)	69
	Task 4.2a: Characterization and response of maize, energy-dedicated sweet sorghum and pearl	
	millet isogenic lines to water deficits (ICRISAT)	71
	Task 4.2b: Mapping of genomic regions controlling traits related to drought tolerance/WUE in	
	tomato (UASB)	72
	4.2b.1 Evaluation of tomato germplasm for drought tolerance	73
	4.2b.2 Studies on assessment of variability among root traits for identification of water use	
	efficient genotypes	79
	4.2b.3 Evaluation of wild and cultivated genotypes to identify water use efficient lines	83
	4.2b.4 Characterization or standardization of root characters among different species of	
	tomato	83
	Task 4.3: Deep sequencing of mRNA and smRNA transcriptome of sorghum and pearl millet for	
	identification of genes and smRNAs functioning in abiotic stress tolerance, with a focus on	
	drought and salinity (MSSRF)	84
	Task 4.4: Improving drought adaptation in chickpea through marker-assisted breeding and trait	00
	(A) Improving drought adaptation in chickness through reaction conists discussion	89
	(A) Improving drought adaptation in chickpea through marker-assisted breeding	89
	(b) Interventing arought adaptation in chickped through trait based selection	90
	rask 4.5. Capacity building of NARS in research on drought adaptation of crops and integrated broading for drought adaptation (ICPISAT)	റാ
E	Work package: Epabling Green Growth using water treatment and rouse innovations	9Z
J.	איטוא אמנאמפר. בוומטוווא טו כבוו טוטאנוו נואוא אמנכו נופמנוופווג מונו ופנאפ ווווטימנוטוא	

Objectives	93
Task 5.1: Stakeholder mapping (EIRC, TERI)	93
Task 5.2: Future trends and boundary conditions (all partners)	93
Task 5.3: Co-creation process of identifying innovation potentials to enable green economy (all	
partners)	94
Task 5.4: Evaluation of shortlisted business opportunities (all partners)	94
Task 5.5: Synthesis of results and initiation of an implementation process (EIRC)	94
Deliverables	94
6. Work package: Dissemination and technology exchange	95
Objectives	95
Task 6.1: Exchange of experiences and results within the innovation platforms (IP's) (EIRC)	95
Task 6.2: Organization of special entrepreneur and SME knowledge brokerage event	
(establishment of the Science Practice interface (EIRC)	95
Task 6.3: Providing Mass Media dissemination (EIRC)	96
Task6.4: Dialogue with EU delegations on Green Economy (EIRC, TERI)	98
Task 6. 5: Capacity building (all partners)	98
Deliverables	98
7.Work package: Coordination and Management	99
Objectives	99
Task 7.1: Communication and coordination (EIRC, ICRISAT)	99
Task 7.2 Administrative management of the project (EIRC)	101
Task 7.3 Management of knowledge generated by the project (EIRC, ICRISAT)	101
Deliverables	101
Appendix A	102
Appendix B	104
Appendix C	105
Appendix D	106
Waste Water Details:	106
Appendix E	107
Untreated Waste Water From Fruit (Banana) 2013 :	107
Treated Waste Water From Fruit (Banana) 2013:	108
Appendix F	109
Table B1: Guidelines for interpretations of water quality for irrigation	109
Appendix G	112
Filter	113
Appendix H	113
Protocol for Clogging Test Using Food Processing Water	114
Appendix I	118
Snapshot of Project Directory	122

# Integrating Bio-treated Wastewater Reuse with Enhanced Water Use Efficiency to Support the Green Economy in EU and India (India side)

### **Executive Summary**

Water, food and energy securities are emerging as increasingly important and vital issues for India and the world. Most of the river basins in India and elsewhere are closing or closed and experiencing moderate to severe water shortages, brought on by the simultaneous effects of agricultural growth, industrialization and urbanization. Current and future fresh water demand could be met by enhancing water use efficiency and demand management. Thus, wastewater/low quality water is emerging as potential source for demand management after essential treatment. In order to address the issues of water scarcity for food production as well as to dispose of domestic and industrial wastewater safely, Water4Crops project through India-EU collaboration under "FP7-KBBE-2012-6-Singlestage" has been approved. The Indian consortium consists of 15 research partners including private companies and the research institutions. The main objective of the project is to enhance the safe use of treated wastewater in agriculture through valorization and improved water use efficiency through genetic enhancement as well as management practices including irrigation practices.

In all, there are seven work packages with different work packages coordinators are identified and all the partner institutions have implemented the project after the approval and launching workshop held in Rome 2012 and detailed planning workshop at ICRISAT, Patancheru during January 2013. All the partners have employed approved project staff as well as procured the equipments needed for undertaking the research. As per the milestones, the activities on complete characterization of selected wastewater samples from SAB Miller India at Sangareddy, fruit processing plant, Jain Irrigation System Ltd, Jalgaon, Maharashtra; Ugar Sugar Works, Belgaum, Karnataka; and K.C.P. Sugar and Industries Corporation Ltd, Chennai, Tamilnadu along with domestic wastewater samples from different areas.

In order to treat the industrial wastewater as well as domestic wastewater, wet-lands have been constructed at ICRISAT, Patancheru, SAB Miller India, Sangareddy; UAS, Dharwad and NEERI, Nagpur have been completed to understand the effect of continued application of untreated wastewater from the industries, samples from Ugar Sugar mill in Belgaum district soils have been collected and analyzed along with the plant samples for isolating microflora from sugarcane. Using the standard protocol, the soil samples have been analyzed and it has been observed that the major problems in this degraded soils is of salinity build-up and appropriate management technologies are needed. An experiment on different land degradation and reclamation along with soil fertility management, treatments like green manuring, use of inoculation with microorganisms have been evaluated with soybean as a test crop. The domestic wastewater samples from Musi river in Hyderabad, BHEL housing using wastewater at ICRISAT and Pandherkawada village, near Nagpur; Muduvatti, Kolar Watershed and UAS, Dharwad and Mavanur, Katnur and Gabbur in Dharwad have been collected and characterised. The wetland construction is in progress.

For the Work Package 3, agricultural water management, benchmark sites have been identified and the work on characterisation of the soils as well as designing and implementation of irrigated systems have been undertaken. Baseline survey in Vuyyuru and Lakshmipuram is conducted and initiated work on community mobilization and organize them in groups as well as capacity building have been initiated.

In Work Package 4, different genotypes of sorghum, millet, maize and tomato have been evaluated for water use efficiency along with phenotyping and genotyping have been completed and wild germplasm accessions particularly for drought tolerance has been undertaken. The detailed results are presented in the main body of the report and the root growth pattern and their relationship with drought tolerance of targeted crops are detailed using the SSR markers linked to a QTL. Root traits in chickpea have studied at ICRISAT for the selected germplams accessions and cultivars. The innova platform has been formed in India and the technologies from partner institutions have been identified for sharing with the industries and other partners. The co-creation process and identification of agri-business opportunities using the bio-treatment have been initiated and development of website for the project has been completed <u>(www.water4crops.org/)</u> which is combined site for India-EU projects.

The "Project Document Store" or the "Intranet" has been developed for exchange of information amongst the partners, for dissemination brochure for the project was disseminated, prepared and released during the kick off meeting in Hyderabad (India), from 28-30 January 2013. Subsequently common brochure for India-EU project is prepared. Project Directory of all the research consortium partners for India-EU consortium is prepared and uploaded on the website. The Project logo has been developed and small videos highlighting the kick-off meeting as well as related videos are uploaded to the water4crops website and videos are also disseminated through social media networks using youtube (<u>http://www.youtube.com/watch?v=tOCC7z2fUdQ</u>). Number of press releases related with the projects are there during the year. Recently, the first joint EU-India review meeting was held during 3-5 December 2013 at Bari, Italy and at the same location on 5<sup>th</sup> December, the first joint innovative platform meeting was also held.

In brief, the consortium project Water4Crops is very much on the tract and have achieved very good progress during the first year in spite of large number of partners working in the consortium. The consortium team is working as one team and substantial progress has been achieved and plans for strengthening and expanding the work during the second year are already in place.

### Background

Ensuring global food security for the ever growing population that will cross nine billion by 2050 and reducing poverty is a challenging task. The increased food production has to come from the available and limited water and land resources which are finite. Neither the quantity of available water nor land has increased since 1950, but the availability of water and land per capita has declined significantly due to increase in global human population. For example, in India per capita water availability has decreased from 5177 m<sup>3</sup> in 1951 to 1820 m<sup>3</sup> in 2001 due to increase in population from 361 million in 1951 to 1.02 billion in 2001 which is expected to rise to 1.39 billion by 2025 and 1.64 billion by 2050 with associated decrease in per capita water availability of 1341 m<sup>3</sup> in 2025 and 1140 m<sup>3</sup> by 2050 respectively. There is an urgent need to manage water resource efficiently through enhancing water use efficiency and demand management. Water availability for food production is not only restricted to fresh water but wastewater re-use is also emerging as an integral part of demand management (Al-Jayyousi 2003; Al-Hamaiedeh and Bino 2010).

With rapid expansion of cities and domestic water supply, quantity of gray/wastewater is increasing in the same proportion. Almost 90% of total water supplied for domestic use was generated as wastewater which would be diverted for agriculture purpose. Grey water use in agriculture contributes significantly to the supply of fresh fruits and vegetables to urban markets. However, there is higher risk associated with human health and the environment on the use of wastewater especially in developing countries, where rarely the wastewater is treated and large volumes of untreated wastewater are being used in agriculture (Buechler and Scott 2006). In the above context, the water4crops project explores the possible opportunities of wastewater use in agriculture both in Indian and in European context. Since, the project is involving both research and industries as consortium partners, this will help in identifying efficient treatment methodologies. This co-creation process will boost the business development in the field of bio-treatment, wastewater re-use, and agricultural innovations to reduce the water footprint. This process would integrate the role of co-learning, links between traditional and industrial agri-production systems, better utilization of market opportunities. This project would facilitate researchers and project partners to conduct science based research on wastewater treatment and its management would open-up various avenues for up-scaling process. This project aims at twinning leading examples from cases in Europe with cases in India for exploiting agricultural water use in better ways.

### **Objectives of the Project**

- 1. Develop and demonstrate integrated treatment processes for agro-food industry effluents targeted at recovery of economically useful components and recycling of water suitable for irrigation
- 2. Selection and optimization of microbial consortium to reclaim degraded lands and biotreatment of municipal wastewater for re-use in agriculture
- 3. Enhancing water use efficiency through improved irrigation systems, agronomic practices and using validated simulation models
- 4. Assess impacts of treated wastewater on soil, crop produce and groundwater quality
- 5. Increasing saline wastewater use efficiency through Integrated Mangrove-Fishery Farming System
- 6. Mapping and characterization of quantitative trait loci (QTL) for drought tolerance related traits in maize, sorghum, pearl millet, chickpea and tomato
- 7. Improving drought adaptation using marker-assisted breeding and trait-based selection approaches in maize, sorghum, pearl millet, chickpea and tomato
- 8. Evaluate and optimize the proposed combinations of bio-treatment and wastewater reuse from a perspective of supporting green growth and to boost interaction between knowledge organizations and industries of the European and Indian parties.

## Strategy

The Water4Crops consortium partners have a common mandate to find solutions for emerging water and related problems for achieving sustainable development in Europe and India. The consortium is designated to satisfy all the project objectives, permitting to treat and reuse wastewaters for non-potable uses. The consortium is a conglomeration of public research institutes, private non-government research institutes, universities, private industries both large and small, and consulting firms from Europe and India thus forming a perfect example for international public private partnership. The list of consortium member is given in Table 1. This include premier research institute from the countries in the field of environmental and agricultural sciences. ICRISAT is lead institute for Indian consortium and IRSA-CNR is lead institute for EU consortium.

National research institutes like The Energy and Resources Institute (TERI) and National Environmental Engineering Research Institute (NEERI), who are the pioneer institutes of industrial wastewater research, will be engaged in finding solutions for reusing wastewater in different sectors. On field research institutes, along with the strategic research on water use efficiency, International Crops research Institute for the Semi-Arid Tropics (ICRISAT), University of Agricultural Sciences Dharwad (UASD), and Bangalore (UASB) are involved for conducting the research on water and crop management aspects. For dissemination, coordination and management, Euro-India Research Center (EIRC) and ICRISAT have vast experience. Industry partners of Water4Crops India consortium include – SAB Miller, Ugar Sugar, ION Exchange, Larsen & Toubro who will work towards

developing and demonstrating integrated treatment processes for bio-refinery effluents. Another industry JISL will be involved in agricultural and water management activities including bioremediation of degraded wasteland (due to untreated wastewater irrigation) and bio-treatment of municipal wastewater for reuse in agriculture. MSSRF will develop water efficient crop variety for selected crops and on integrated mangrove-fishery farming system to optimise use of saline wastewater.

Indian consortium	EU consortium
<ul> <li>Indian consortium</li> <li>International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)</li> <li>The Energy and Resources Institute (TERI)</li> <li>University of Agricultural Sciences Dharwad (UASD)</li> <li>MS Swaminathan Research Foundation (MSSRF)</li> <li>National Environmental Engineering Research Institute (NEERI)</li> <li>Jain Irrigation Systems Limited (JISL)</li> <li>Euro India Research Centre (EIRC)</li> <li>SABMiller (SABM)</li> <li>University of Agricultural Sciences Bangalore (UASB)</li> <li>JalSri, Jalgaon</li> <li>PRAJ Matrix (PRAJM)</li> <li>Ugar Sugar (UGSG)</li> <li>Larsen &amp; Toubro (L&amp;T)</li> <li>ION Exchange</li> <li>KCP Sugar Industries</li> </ul>	<ul> <li>EU consortium</li> <li>Istituto di Ricerca Sulle Acque - Consiglio Nazionale delle Ricerche (IRSA-CNR), Dept. of Bari, Italy</li> <li>Natural Environment Research Council, NERC - Centre for Ecology and Hydrology, (NERC-CEH), Wallingford, UK</li> <li>University of Applied Sciences Northwestern Switzerland (FHNW), Muttenz, CH</li> <li>Alma Mater Studiorum – University of Bologna (UNIBO), Bologna, Italy</li> <li>VITO - Flemish Institute for Technological Research, Brussels, Belgium</li> <li>Technical University of Crete (TUC), Crete, Greece</li> <li>Helmholtz Centre for Environmental Research (UFZ), Germany</li> <li>University of Catania (UNICT) – Department of Agri-food and Environmental Systems Management [GESA], Catania, Italy</li> <li>Unité Mixte de Recherche Gestion Eau Acteurs Usages (GEAU-Cemagref), France</li> <li>Institut National de la Recherche Agronomique (INRA), France</li> <li>Stichting Dienst Landbouwkundig Onderzoek (ALTERRA), Wageningen, NL</li> <li>Consorzio di Bonifica di Secondo Grado per il Canale Emiliano Romagnolo (CER), Bologna, Italy</li> <li>Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ), Eschborn, Germany</li> <li>INOFEA GmbH, Basel, Switzerland.</li> <li>SIMA-tec GmbH, Germany</li> <li>BionActis International Group SA (Bionactis), Valais, Switzerland</li> </ul>
	<ul> <li>Consorzio di Bonifica di Secondo Grado per il Canale Emiliano Romagnolo (CER), Bologna, Italy</li> <li>Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ), Eschborn, Germany</li> <li>INOFEA GmbH, Basel, Switzerland.</li> </ul>
	<ul> <li>SIMA-tec GmbH, Germany</li> <li>BionActis International Group SA (Bionactis), Valais, Switzerland</li> <li>PHYTOREM S.A., Miramas, France</li> <li>BioPlanta GmbH, Leipzig, Germany</li> </ul>
	<ul> <li>Environmental Nutritional and Health Services S.A.(Envinhealth), Greece</li> <li>Horta srl (HORTA), Piacenza, Italy</li> <li>S.T.E.P. Consulting GmbH (STEP), Germany</li> </ul>

Table 1 List of	consortium	members from	India and FLL
TADIE I. LISUUI	CONSOLUUII	IIIeIIIbels IIOIII	

Besides consortium approach other important part of strategy are mirror case approach, innovative modular biotechnological approach, co-learning, co-creation of new products leading to be business opportunities. The miror cases are at the Emilia Romagna region (Italy) and at Hyderabad (Andhra Pradesh State, INDIA). Both regions offer potential for excellent application of technology development research in increasing/diversifying agricultural production. Water4Crops is aimed at providing for the first time an innovative combination of individual technical improvements to bridge bio-treatment of wastewater and increased water efficiency with a trans-disciplinary identification of agri-business opportunities and the related requirements for tailoring technological innovations. Water4Crops is based on three Pillars: P1: Biotechnological wastewater treatment, P2: Improved water use efficiency, P3: Enabling Green Economy. Each of them is structured into Work Packages (Table 2) (P1-WP1: Valorization, treatment and reuse of agrofood industry wastewater; P1-WP2: Innovative municipal wastewater bio-treatment for agricultural reuse; P2-WP3: Agricultural water management; P2-WP4: Improving water use efficiency and drought tolerance via genomic approaches and modelling; P3-WP5: Methodology for trans-disciplinary approach; P3-WP6: Dissemination and technology transfer. WP7: Coordination and Management covers the whole project.

WP No	Work package title	Co-ordinator
1	Agro-food industry wastewater valorization and reuse	Dr. Malini Balkrishnan
2	Bio-treatment of municipal wastewater for reuse and bioremediation of degraded lands	Dr. Asha Juwarkar
3	Agricultural water management	Dr. Suhas Wani
4	Development of water efficient crop varieties	Dr. Pooran Gaur
5	Enabling green growth using water treatment and reuse innovations	TERI
6	Dissemination and technology exchange	Ms. Surbhi Sharma
7	Coordination and management	Dr. Suhas Wani Ms. Surbhi Sharma

Table 2. List of work packages and work package leader.

#### Table 3.List of deliverable during first year.

No	Deliverable name	Package	Lead	Institute
1 1	Detailed characterization of selected	WP1	TERI	ICRISAT, UASD, JISL,
1.1	wastewaters			NEERI, MSSRF
21	Report on microbial consortium formed	WP2	NEERI	ICRISAT, UASD, TERI,
2.1	using available strains			MSSRF
21	Benchmark sites characterized	WP3	ICRISAT	UASD, MSSRF, TERI,
5.1				JISL, NEERI
5.1	Database of stakeholders	WP5	ERIC	ERIC
	Internal report on customer /	WP6	ERIC	ERIC
6.1	entrepreneur demands and technological			
	offer			
6.2	Webpage and Public Dissemination	WP6	ERIC	ERIC
0.2	material			
63	Report on training course including	WP6	ERIC	ERIC
0.5	online curricula			
	Workshop to workout common protocols	WP7	ERIC/	ERIC/ICRISAT
7.1	to be adopted by the partners in the		ICRISAT	
	project			
72	First year appual report to DBT	WP7	ERIC/	ERIC/ICRISAT
1.2			ICRISAT	

Mileston e number	Milestone name	Package	Lead	Institute
1.1	Sites selected and wastewater samples collected	WP1	TERI	ICRISAT, UASD, JISL,
1.2	Detailed wastewater characterization done			NEERI, MSSRF
2.1	Strains for microbial consortium selected	WP2	NEERI	ICRISAT, UASD, TERI, MSSRF
3.1	Baseline data collected, analysed and reported	WP3	ICRISAT	UASD, MSSRF, TERI, JISL, NEERI
3.9	Suitable areas for development and demonstration of integrated agro-aqua farming system selected			MSSRF
3.10	Mobilising and organising community and other stakeholders and build their capacities in integrated agro-aqua farming system			MSSRF
5.1	Innovation platform established	WP5	ERIC	ERIC
6.1	Establishment of dissemination plan with EBTC	WP6	ERIC	ERIC
7.1	Kick-off Meeting	WP7	ERIC/ICRISAT	ERIC/ICRISAT
7.2	Project Review Meeting			
7.3	Project Review and Planning Meeting			

Table 4. List of milestones during first year.

#### 1. Work Package: Agro-food Industry Wastewater Valorization and Reuse

#### Objectives

To develop and demonstrate integrated treatment processes for agro-food industry (biorefinery) effluents targeted at (a) recovery (direct or after conversion) of economically useful components from agro-food industry/biorefinery wastewater and (b) production of treated water suitable for irrigation purposes.

#### Task 1.1: Complete characterization of selected wastewaters

#### Subtask 1.1.1 Selection of sites and collection of wastewater samples

Following site are identified for wastewater sources

- SAB Miller, Sangareddy, Andhra Pradesh
- Jain Irrigation System Ltd, Jalgaon, Maharashtra
- Ugar Sugar Works, Belgaum, Karnataka
- K.C.P. Sugar and Industries Corporation Ltd, Chennai, Tamilnadu

# Subtask 1.1.2 Wastewater characterization

#### SAB Miller, Sangareddy, India (NEERI, ICRISAT)

SAB Miller factory at Sangareddy has Effluent Treatment Plant (ETP) of capacity 1000 cubic meter per day. Beside this ETP, SABM also has Reverse Osmosis (RO) plant. Water treated in RO plant is

reused in factory for cleaning and washing purpose. Reject from RO also had high TDS. This high TDS water is being treated through solar evaporation ponds. The wastewater samples were collected from six different unit operations viz. equalization tank, UASB (Up flow Anaerobic Sludge Bed) reactor, aeration tank, secondary clarifier, tertiary clarifier and final treated wastewater. During August, 2013, CSIR-NEERI has made a second visit to SABMiller, Hyderabad, during which CSIR-NEERI was requested to tackle the problem with disposal of Reverse Osmosis (RO) rejected wastewater from SABMiller ETP. Apart from that, CSIR-NEERI has collected wastewater (sludge) samples from Aeration tank 1 and 2, for developing the microbial consortia, which would be applied in constructed wetlands for enhanced removal efficiency. Samples were analysed as per the standard methods for the examination of water and wastewater (APHA, 2012) for various physico-chemical parameters. The secondary data for characteristics of effluents at ETP collected from SAB Miller laboratory (Table 1.1). Effluent Treatment Plant (ETP) reduces COD, BOD, and TSS by almost 99%, but reduction in TDS is only 37%. Total dissolve solids in ETP effluent is still high (2149 mg L<sup>-1</sup>).

The characteristics of the wastewater collected from various unit operations of ETP are presented in Table 1.2 and Table 1.3. The results indicated that the pH of the wastewaters varied in the range of 4.82 to 8.44 with an electrical conductivity (EC) ranging from 145 to 16256  $\mu$ S/cm respectively. The TDS contents in the wastewater samples varied in the range of 101 to 8168 mg L<sup>-1</sup>. The total alkalinity and suspended solids in the wastewater samples varied in the range of 2 to 15 mg L<sup>-1</sup> and 28 to 1360 mg L<sup>-1</sup> respectively. The total hardness in the wastewater samples varied from 8 to 64 mg L<sup>-1</sup> and 2 to 38.9 mg L<sup>-1</sup> respectively.

The concentrations of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) varied from 38 to 5200 mg L<sup>-1</sup> and 18 to 3200 mg L<sup>-1</sup> respectively. The concentration of ammoniacal nitrogen and nitrate varied from 5.4 to 12.8 mg L<sup>-1</sup> and 0.88 to 32.78 mg L<sup>-1</sup> respectively. The levels of cations with respect to sodium and potassium in the wastewater samples varied from 26 to 83 mg L<sup>-1</sup> and 18 to 47 mg L<sup>-1</sup> respectively. The levels of anions with respect to chloride varied from 20 to 400 mg L<sup>-1</sup> whereas sulphate varied from 0.2 to 20.1 mg L<sup>-1</sup> in the wastewater samples respectively. The level of chloride and sulphate in the wastewater samples was within the permissible limit for discharge of treated wastewater onto the land for irrigation as per Indian standards. The concentration of different heavy metals such as cadmium, lead, copper, nickel, zinc, iron, manganese, arsenic, boron, chromium presented in Table 1.2 showed that their concentrations were within the toxicity limit and do not pose any toxicity to plants species.

The focus is on treatment of RO reject as well, along with the UASB effluent. It is observed that the concentration of TDS and ions are found to be inordinately high, which cannot be treated economically with the ECWs. Therefore, CSIR-NEERI would suggest the disposal of high TDS RO reject wastewater with the High Rate Transpiration System (HRTS). HRTS has already been endorsed by CPCB as a method to dispose the high TDS containing wastewater.

The results presented in Table 1.2 and Table 1.3 indicated that the concentration of BOD, COD and TSS of the wastewater has considerably decreased after being treated in UASB reactor. The status of wastewater quality after UASB with respect to salinity falls under the group "severe restriction on use" for irrigation. Whereas, the combined effect of EC and SAR when considered to ascertain its effect on in soil infiltration rate, the wastewater belongs to the group "moderate restriction on use". Hence, it was decided that to reduce the cost of the treatment processes, the wastewater after treatment in UASB reactor will be taken up for treatment in a specially designed Engineered Constructed Wetland system (ECWs) and utilised for crop irrigation.

Dete	Raw Effluent					UASBR effluent			ETP effluent						
Date	рΗ	COD	BOD	TSS	TDS	рΗ	COD	BOD	TSS	рΗ	COD	BOD	TSS	TDS	DO
		mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>		mg L <sup>-1</sup>	mg L <sup>-1</sup>	mg L <sup>-1</sup>		mg L <sup>-1</sup>				
6-Jul-2012	5.0	10240	-	2800	3700	7.0	704	-	420	8.0	112	-	30	1900	3
6-Aug-2012	5.0	7280	-	3500	3400	7.5	840	-	340	8.0	98	-	40	2100	2.9
6-Sep-2012	5.5	10880	-	2800	3400	7.5	768	-	360	8.0	80	-	30	2200	3
6-Oct-2012	6.0	10440	3100	3700	4300	7.0	864	140	380	8.0	72	20	30	1888	2.9
6-Nov-2012	5.5	-	2900	2950	3700	7.0	-	160	550	8.0	-	25	40	2000	2.8
6-Dec-2012	5.5	-	2900	3200	3400	7.5	-	150	470	8.0	-	25	40	1900	
6-Jan-2013	5.5	9100	-	3300	4400	7.0	1024	-	630	8.0	128	-	70	2600	2.0
6-Feb-2013	5.5	9600	2700	3650	4200	7.5	896	150	550	8.0	128	30	30	2100	2.4
6-Mar-2013	5.5	9600	2600	2700	3300	7.5	896	145	530	8.0	96	30	30	2200	2.9
6-Apr-2013	5.5	9920	2400	3450	4000	7.5	704	190	600	8.0	128	40	70	2100	2.2
6-May-2013	5.5	9920	2500	3500	3800	7.5	960	280	720	8.0	149	50	90	2800	2.1
6-Jun-2013	5.9	8680	2400	3100	3550	7.5	750	210	410	8.2	93	30	50	2180	1.4
6-Jul-2013	5.0	11600	-	3500	2920	7.5	800	-	620	8.0	80	30	30	1798	1.4
6-Aug-2013	5.5	10000	-	3100	2170	7.7	880	-	460	8.5	133	-	30	1490	2.8
6-Sep-2013	5.8	13600	-	3400	1926	7.6	880	-	390	8.4	120	-	30	2140	1.9
6-Oct-2013	7.0	8000	2450	2900	2820	7.5	560	140	310	8.2	100	30	20	2980	2.3
6-Nov-2013	10.9	8400	-	3000	-	7.9	260	-	150	8.6	106	-	30	-	2.3
Average	6	9817	2661	3209	3437	7	786	174	464	8	108	31	41	2149	2

**Table 1.1** Physico-chemical characteristics of effluents from different unit operations of ETP at M/s Charminar Breweries of SAB Miller India at Sangareddy,

 Andhra Pradesh, India [Source: SAB Miller]

SI. No.	Parameters	Equalization Tank	UASB Reactor	Aeration Tank	Secondary Clarifier	Tertiary Clarifier	Treated Water	Standards for disposal of Environmental Pollutants (Land Irrigation)
1.	рН	4.82	6.85	8.29	8.22	8.44	6.91	5.5 to 9.0
2.	EC, μS/cm	1786	3946	3613	2722	2835	145	
3.	TDS, mg L <sup>-1</sup>	940	1954	1843	1307	1464	101	2100
4.	Total Alkalinity, mg L <sup>-1</sup>	10	15	10	8	5	2	200
5.	TSS, mg $L^{-1}$	1360	350	220	210	50	28	
6.	Total Hardness, mg L <sup>-1</sup>	120	320	160	220	220	20	
7.	Calcium, mg L <sup>-1</sup>	20	64	24	40	40	8	
8.	Magnesium, mg L <sup>-1</sup>	17	38.8	24.3	29.1	29.1	2	
9.	COD, mg L <sup>-1</sup>	5200	410	215	108	85	38	
10.	BOD, mg L <sup>-1</sup>	3200	165	85	48	30	18	100
11.	Ammonical Nitrogen, mg L <sup>-1</sup>	8.2	12.8	5.4	7.3	7.9	5.9	
12.	Nitrate, mg L <sup>-1</sup>	0.88	1.32	26.13	31.45	32.78	2.65	
13.	Sodium, mg L <sup>-1</sup>	50	83	74	63	66	26	
14.	Potassium, mg L <sup>-1</sup>	22	40	42	45	47	18	
15.	Chloride, mg L <sup>-1</sup>	120	400	300	240	200	20	600
16.	Sulphate, mg L <sup>-1</sup>	9.2	20.1	0.5	0.2	0.3	0.2	1000
17.	SAR	1.99	2.03	2.57	1.86	1.95	2.21	
Heavy I	Metals and Oxyanion, mg L <sup>-1</sup>							
18.	Zinc	0.227	0.076	0.06	0.071	0.104	0.104	
19.	Lead	BDL	BDL	BDL	0.003	BDL	BDL	
20.	Cadmium	BDL	BDL	BDL	BDL	BDL	BDL	
21.	Nickel	0.021	0.013	0.008	0.004	0.028	0.028	
22.	Manganese	0.155	0.203	0.187	0.185	0.194	0.194	2.0
23.	Iron	1.596	1.847	1.795	1.198	0.704	0.704	3.0
24.	Chromium	BDL	BDL	BDL	BDL	BDL	BDL	
25.	Copper	0.02	0.057	0.002	0.011	0.005	0.005	
26.	Boron	2.890	1.458	BDL	BDL	BDL	1.372	
27.	Arsenic	BDL	BDL	BDL	BDL	BDL	BDL	0.2

**Table 1.2**. Physico-chemical characteristics of wastewater collected from different unit operations of ETP at M/s Charminar Breweries of SAB Miller India at Sangareddy, Andhra Pradesh, India (March 2013)

SI. No.	Parameters	Aeration Tank -1	Aeration Tank- 2	R.O rejected Waste water
1	рН	8.04	8.06	7.78
2	E.C. μS/cm	3233	3261	16256
3	TDS mg L <sup>-1</sup>	1616	1635	8168
4	Alkalinity as CaCO3, mg L <sup>-1</sup>	1500	1200	4400
5	Total Hardness as CaCO <sub>3</sub> , mg $L^{-1}$	220	245	980
6	Total suspended solids, mg L <sup>-1</sup>	9360	6180	1540
7	COD, mg L <sup>-1</sup>	2120	1480	310
8	BOD, mg L <sup>-1</sup>	142.5	140.0	57.5
9	Calcium, mg L <sup>-1</sup>	68.0	68.8	192.0
10	Magnesium, mg $L^{-1}$	12.2	17.5	121.8
11	Sodium, mg L <sup>-1</sup>	731	691	2620
12	Potassium, mg L <sup>-1</sup>	39	39	162
13	Chloride, mg L <sup>-1</sup>	274.9	579.8	1674.5
14	Sulphate, mg L <sup>-1</sup>	5.0	4.8	21
15	Nitrate, mg L <sup>-1</sup>	3.01	21.93	23.22
16	Phosphate, mg L <sup>-1</sup>	38	40	58
17	Ammonia, mg L <sup>-1</sup>	0.58	0.58	1.18
18	Cadmium, mg L <sup>-1</sup>	BDL	0.01	7.62
19	Lead, mg L <sup>-1</sup>	0.002	0.10	0.002
20	Copper, mg L <sup>-1</sup>	1.27	0.41	0.08
21	Nickel, mg L <sup>-1</sup>	0.05	0.04	0.07
22	Zinc, mg $L^{-1}$	0.8	0.6	0.36
23	Iron, mg L <sup>-1</sup>	15.61	36.38	1.34
24	Manganese, mg L <sup>-1</sup>	0.3	2.7	0.06
25	Arsenic, mg L <sup>-1</sup>	BDL	BDL	BDL
26	Chromium as $Cr^{+6}$ , mg L <sup>-1</sup>	0.16	0.10	0.02
27	Boron, mg L <sup>-1</sup>	7.1	16.9	44.5
28	Cobalt, mg L <sup>-1</sup>	0.01	0.02	0.03

Table 1.3. Physico-chemical characteristics of wastewater collected from different unit operations of ETP at M/s Charminar Breweries of SAB Miller India at Sangareddy, Andhra Pradesh, India (Aug 2013)

#### JISL, Jalgaon, India (JISL)

Land for crop cultivation has been identified and soil characterization is going on. Irrigation facilities are being installed for this land, which will be connected to wastewater from fruit processing and onion dehydration plant. Historical data of effluent water quality before treatment and after treatment is collected (Table 1.4). More data on wastewater quality is given in Appendix D.

Sr.No	Parameters	Limits	Units	Sample		
				Before	After	
				Treatment	Treatment	
1	P <sup>H</sup>	6.5-9.0	-	7.33	7.52	
2	Total Dissolved Solids	<2100	mg/lit	1896	1424	
3	Total Suspended Solids	<100	mg/lit	322	50	
4	B.O.D(27 <sup>0</sup> C, 3 days)	<100	mg/lit as O <sub>2</sub>	910	28	
5	C.O.D	<250	mg/lit as O <sub>2</sub>	1865	87	
6	Chloride	<600	mg/lit as cl	750	70	
7	OIL & Grease	< 10	mg/lit	24	< 0.5	
8	Sulphates	< 100	Mg/lit as SO4	91.2	76.3	
9	Detergents	< 1.0	mg/lit as	11.6	0.4	
			MBAS			
10	Residual free chlorine	< 0.20	mg/lit	< 0.05	< 0.05	
11	Total Ammonical	< 50	Mg/lit	12.5	0.56	
	Nitrogen					

Table 1.4. Quality parameters assessed at JISL Effluent Treatment Plant

#### K.C.P. Sugar and Industries Corporation Ltd (MSSRF)

K.C.P. Sugar and Industries Corporation Ltd in Vyuru and Laxmipuram was approached and discussed about the Water4Crops project and ascertained the availability of wastewater and land. Major concern is about the colour and odour of the effluent discharged from the distillery.

#### Vuyyuru (L1)

The spent wash is generated from distillery at the rate of 9-11 liters for every 1 liter of alcohol produced with continuous fermentation. The generated spent wash is stored in raw spent wash pond at a temperature of 40°C. The raw spent wash is pumped to two digesters at the rate of 24 m<sup>3</sup>/hr. For better reactions in the digesters diammonium phosphate and urea are used in required quantities. As a result of anaerobic digestion, methane gas and bio-methanated spent wash from the digesters are generated. The methane gas obtained from the digesters is being used as a fuel for boilers. Thus huge amount of the bagasse is saved.

Parameters	Before Digester	After Digester
рН	3.5 - 6.0	7.0 – 8.5
Temperature °C	70 – 90	Ambient
COD, mg/L	40,000 - 1,76,000	60,000 - 70,000
BOD 5 day, 20 °C, mg/L	30,000 - 40,000	4,000 – 7,000
Total solids, mg/L	28,000 - 1,45,000	6 – 8
Suspended solids,	350 - 8,000	1-2

Table 1.5 Wastewater characteristics after anaerobic treatment

About 500-600  $M^3$  /day of spent wash is generated from distillery and 300  $m^3$  of wash per day is fed to RO plant. Treated water from RO plant stored in water tank for reuse. Nearly 45-50 % of permeate which is arising from RO is used for gardening. The reject from RO is taken for processing of compost.

#### Lakshmipuram Sugar Unit (L2)

The treated effluent is treated anaerobically in anaerobic pond by overflow arrangement. The neutralized effluent is mixed with cow dung and urea daily to reduce BOD and COD. As a result of anaerobic treatment, carbon dioxide ( $CO_2$ ), methane ( $CH_4$ ), & ammonia ( $NH_3$ ) gasses are formed.

Here the water pH will be 5.50 to 7.00. The treated effluent is passed to Contact Filters Pond by underflow arrangement. This treated effluent is filtered through contact filters, which will result in removal of Suspended particles. Here the water pH will be 7.00 to 7.50. After this the treated effluent enters in to Aerobic Pond by overflow arrangement. The treated effluent will undergo Aeration by floating aerators, which results in reduction of the BOD and COD values. The purpose of Aeration is mainly to increase the dissolved oxygen content of the effluent and to convert the Total Dissolved Solids to Total Suspended Solids. Here the water pH will be 7.0 to 8.0.

#### Wastewater characterization

The wastewater samples were collected from the ETP storage ponds of the Distillery unit at Vuyyuru (L1) and sugar unit at Lakshmipuram (L2) of KSICL industry. The wastewater samples were collected manually in plastic containers which were sterilized using alcohol to ensure that the containers were free of contaminants. The wastewater samples were collected from four storage ponds *viz*. Molasses Spent Wash (MSW), Anaerobically treated Molasses Spent Wash (AnTMSW), sugar wastewater anaerobically treated (SWWAP) and sugar wastewater aerobically treated (SWWAP) storage tanks. Wastewater samples were collected from the middle level depth at five different points from the individual tanks and the individual samples were pooled and stored in the containers which were tightly sealed, labeled and shifted to the lab and stored at 4°C till use. The details of the sample collected are represented in Table 1.6 and Figure 1.1.

Information	L1		L2		
Sample ID	MSW AnTMSW		SWWAnP	SWWAP	
Volume of water sample collected	2.5 L	10L	2.5L	10L	
Location GPS points	16.21° 820′ N, C	).51°898′ E	16.07°390' N, 80.	57° 627′ E	
Location address	Vuyyuru		Lakshmipuram		
Date and time	28 June 2013, 10.00 am		29 June 2013, 10.30 am		
Sample type (Grab or composite)	Composite sample		Composite samples		
Photograph	Available		Available		
Water temperature	Ambient		ambient		
Water level	3 feet		3 feet		
Weather while collecting	Sunny day		Sunny day		

Table 1.6 Details of the wastewater collected



Collection of waste water sample from distillery



Sample collection from Sugar industry waste water

Figure 1.1. Collection of wastewater from the storage tanks of L1 and L2

The details of various physicochemical properties of the AnTMSW and SWWAP are compared with the ICRISAT guidelines and listed in Table 1.7. All these parameters analysed were very high in the distillery wastewater when compared to ICRISAT standard. The salinity was 100 folds high in the AnTMSW. But in the SWWAP parameters such as salinity COD, BOD and the TSS were moderately high whereas the other parameters were comparatively low.

Baramotors	MSSRF data		ICRISAT	
Farameters	AnTMSW	SWWAP	Guidelines	
рН	8.05	8.15	6-8.05	
EC (ms/cm)	38	1.85	0.3	
BOD (mg/L)	13750	33	30	
COD (mg/L)	46080	129.54	90	
Total suspended solids (mg/L)	15180	134.5	50 - 100	
Total Dissolved solids (mg/L)	69084	1204	0 - 2000	
Sulphate (me/L)	86.0	2.08	0 - 20	
Chloride (me/L)	473.1	6.624	0-30	
Nitrate (me/L)	129.2	1.1	0-10	
Potassium (me/L)	7610	72.9	0 -2	
Calcium(me/L)	157.8	2.104	0-20	
Magnesium (me/L)	262.7	2.733	0-5	
Sodium (me/L)	262.7	7.287	0-40	
Carbonate as CaCO <sub>3</sub> (mg/L)	133.33	3.33	0-1	
Bicarbonate as HCO <sub>3</sub> (mg/L)	163.93	6.961	0-10	
Sodium Adsorption Rate (mg/L)	3.18	6.20	0 - 15	
Phosphorous (mg/L)	1619.6	732.9	0 – 2	
Total Iron (mg/L)	35.39	2.53	0-5.0	
Copper (mg/L)	0.72	BDL	0.05	
Lead (mg/L)	0.230	BDL	0.5	
Zinc (mg/L)	0.32	BDL	0.2	
Total Nitrogen (mg/L)	2263	10.17	XXX	
Volatile acids (mg/L)	39492	140	50 - 100	

Table 1.7 Physicochemical properties of the wastewater sample collected from L1 and L2

#### Microbial parameters

The microbial load in the wastewater in MSW and AnTMSW was detected up to Log 7 and it was around Log 6.in the SWWAnP (Figure 1.2). No fungal growth was observed in PDA medium from all the wastewater samples. The bacterial colonies isolated from different wastewater samples in Luria Bertani (LB) agar is represented in (Figure 1.3). The morphology of the colonies of individual sample was similar but varied within the samples.



*Figure 1.2. Microbial load of wastewater from L1 and L2* 



Figure 1.3. Determination of bacterial and fungal population in wastewater and soil

The pathogenic population in the wastewater was determined using selective medium as represented in Table 1.8. An important aspect of wastewater microbiology is the presence of disease causing pathogenic microorganisms. Hence the wastewater samples were screened for the presence of the most common waterborne pathogenic microorganisms using selective medium and were identified based on the characteristic growth in the respective medium. *Yersinia enterolitica, Staphylococcus aureus, Shigella* sp. *Legionella* sp. and *Salmonella* sp. were observed in AnTMSW, whereas in the MSW only *Y. enterolitica* and *Salmonella* sp could not be detected whereas all the other three organisms were present. (Figure 8). In the SWWAP the presence of *Enteric bacilli, Staphylococcus aureus, Vibrio cholera* and *Shigella* sp. and *Legionella* sp. were observed. Whereas in SWWPAnTP *Y. enterolitica Staphylococcus* sp., *Shigella* sp. and *Legionella* sp were detected but *V. cholera* was found in addition (Figure 1.4).

S. No	Pathogen	Medium	Identification characters
1	Yersinia enterolitica	Yersinia Isolation Agar	Good-luxuriant growth on selective medium
2	Enteric bacilli	EMB agar	Purple with black centre & green metallic sheen
3	Staphylococcus	Staphylococcus Agar No.110	Positive based on the selective supplement
4	Vibrio cholera TCBS Agar		Yellow colour colony
5	Shigella sp.	Shigella broth base	Positive based on the selective supplement
6	<i>Legionella</i> sp.	Modified Buffered Charcoal Agar base	Light blue-grey, white grey to blue grey
7	Salmonella typhi	Xylose lysine deoxycholate agar	Red with black centers colony
8	<i>Klebsiella</i> sp.	Hichrome Klebsiella selective agar	Purple-magenta (Mucoid)
9	Campylobacter	Campylobacter agar base	Positive based on the selective supplement
10	Clostridium perfringens	Perfringens agar base	Positive based on the selective supplement

	Table 1.8 List of media used for the determination of the	pathogenic population in the wastewater
--	---	---



Figure 1.4. Determination of pathogenic population wastewater

The total DNA was Isolated from the wastewater sample. The protocol of Tabatabaei *et al.*, 2010 followed by purification of DNA using soil DNA isolation kit was found to yield good Quality DNA. Hence this method was adopted for the isolation of DNA from the wastewater samples and the Isolated DNA was quantified in the Nano drop and the quality was checked in 1% agarose gel.



Figure 1.5. Amplification of total DNA

The DGGE of the PCR amplified 16S rRNA partial gene fragments were performed in a denaturing gradient of 20–80% at 150 V for 14 h. The gel was then stained for 45 min using Silver nitrate. DNA band patterns were digitized, photographed, and analysed using the GelDoc system and Quantity One software (Bio-Rad Laboratories). The dominant bands were eluted by incubating overnight in 30  $\mu$ L sterile water at 4°C. The presence of the different microbial population in the water sample is represented in DGGE gel (Figure 1.6).The bands represented in the gel needs to be eluted, reamplified and sequenced. The sequence data will be analyzed using the BLAST search and the population would be identified. Each band in the gel represents the individual taxa. Lane1. SWWANP, 2-SWWAP, 3-MSW, 4-AnTMSW



Figure 1.6. DGGE profiling of isolated DNA

#### Task 1.2: Biological Wastewater Treatment and its impacts (NEERI, TERI, ICRISAT)

Subtask 1.2.1 Screening and isolation of microorganisms from soils utilizing contaminated wastewater for growth

#### SAB Miller (NEERI)

The microbial analysis of the wastewater samples collected from different unit operation of SAB Miller is presented in Table 1.9. The initial microbial plate count was done using standard serial dilution method. The number of different microbial groups viz. bacteria, fungi, actinomycetes, *Azotobacter* and *Rhizobium* were counted using spreading specific dilutions on their selective media. The results presented in Table 1.8 suggested that appreciable amount of nitrogen fixing bacteria i.e. *Azotobacter* and *Rhizobium* were found in the wastewater samples. The variation in the counts of different microbial groups may be attributed due to the physico-chemical and nutrient status of the wastewater samples.

Sample		Microbial Count (CFU/ml)					
Details	Bacteria	Fungi	Actinomycetes	Azotobacter	Rhizobium		
Equalization Tank	26×10 <sup>5</sup>	31×10 <sup>2</sup>	-	48×10 <sup>3</sup>	20×10 <sup>3</sup>		
UASB Reactor         22×10 <sup>6</sup> 21×10 <sup>2</sup> 50×10 <sup>3</sup>		UASB Reactor 22×10 <sup>6</sup> 21×10 <sup>2</sup> 50×10 <sup>3</sup>		41×10 <sup>4</sup>	20×10 <sup>4</sup>		
Aeration Tank	20×10 <sup>5</sup>	-	22×10 <sup>3</sup>	-	23×10 <sup>3</sup>		
Secondary Clarifier	36×10 <sup>4</sup>	20×10 <sup>2</sup>	103×10 <sup>3</sup>	46×10 <sup>4</sup>	30×10 <sup>3</sup>		
Tertiary Clarifier	34×10 <sup>4</sup>	-	20×10 <sup>3</sup>	76×10 <sup>4</sup>	22×10 <sup>3</sup>		
Treated Water	30×10 <sup>4</sup>	-	22×10 <sup>3</sup>	57×10 <sup>4</sup>	20×10 <sup>3</sup>		

Table 1.9. Microbiological characteristics of wastewater collected from different unit operations of ETP at SAB Miller India at Sangareddy, Andhra Pradesh, India

CFU – Colony Forming Unit

#### KCP Sugar and Industries (MSSRF)

Soil and wastewater samples from the L1 and L2 were used for isolating bacteria with decolourising potential of the AnTMSW. For the isolation of the degrading microorganisms two different media viz., Basal medium and LB agar amended with 20% of AnTMSW was used.

Growth of Bacterial colony with diverse morphology was observed in the wastewater amended minimal medium when soil was used as the inoculum source compared to the wastewater used as inoculum. Bacterial strains with decolourization potential were isolated from AnTMSW and from soil

sample from L2 sites (Figure 1.7). Whereas the growth in LB amended medium colonies with similar morphology was observed (Figure 1.8).



Figure 1.7. Isolation of bacteria strains with decolourization potential from soil samples in minimal medium amended with 20% wastewater.



Figure 1.8. Isolation of bacteria strains with decolourization potential from soil samples in LB amended with 20% wastewater.

#### Subtask 1.2.1a Decolourization of wastewater using microorganism (MSSRF)

Lab tests were done to estimate the decolouring potential of *S. potatorum* seeds and biochar and the results are presented in this section. The bacterial culture with decolourization potential was inoculated in 100ml of minimal medium containing 20% of AnTMSW (Table 1.10). One ml of overnight grown culture was inoculated into the medium in sterile condition and incubated in a shaker at 150 rpm for 10 days. The reduction in the colour was measured in UV-Spectrophotometer at 475nm. Based on the OD value at 475nm Percentage decolourization was calculated as follows: Percentage decolourization = Final OD-initial OD/Initial ODX100.

Around 44 strains with decolouration efficiency were isolated from the soil samples of L1and L2 and from the wastewater and their decolouration potential was determined by spectrophotometric analysis. The decolourization of the AnTMSW in agar plates is represented in Figure 1.9. Among the 44 strains around 14 strains were chosen based on their decolourization efficiency and the percent colour reduction compared to the control was determined (Figure 1.10). Strain MSSRFW14 and MSSRFW20 isolated from the AnTMSW exhibited 6% decolouration potential compared to control followed by MSSRFW28 and MSSRF 46 and *Peudomonas putida* strain which showed ~ 4.0% decolourization.

Bacterial strain	Source	OD at 475nm
Control		0.685
MSSRFW11	L2-soil	0.669
MSSRFW12	L2 soil	0.886
MSSRFW13	AnTMSW	0.681
MSSRFW14	AnTMSW	0.642
MSSRFW15	AnTMSW	0.790
MSSRFW16	AnTMSW	0.878
MSSRFW17	AnTMSW	0.857
MSSRFW18	L2-soil	0.897
MSSRFW19	L2-soil	0.883
MSSRFW20	AnTMSW	0.640
MSSRFW21	AnTMSW	0.671
MSSRFW22	AnTMSW	0.690
MSSRFW23	AnTMSW	0.669
MSSRFW24	AnTMSW	0.689
MSSRFW25	AnTMSW	0.803
MSSRFW26	AnTMSW	0.873
MSSRFW27	AnTMSW	0.829
MSSRFW28	AnTMSW	0.652
MSSRFW29	AnTMSW	0.742
MSSRFW30	AnTMSW	0.663
MSSRFW31	AnTMSW	0.677
MSSRFW32	AnTMSW	0.701
MSSRFW33	AnTMSW	0.770
MSSRFW34	AnTMSW	0.812
MSSRFW35	Millet rhizo	0.657
MSSRFW36	Millet rhizo	0.659
MSSRFW37	L2-soil	0.732
MSSRFW38	L2-soil	0.800
MSSRFW39	L2-soil	0.917
MSSRFW40	L1-soil	0.728
MSSRFW41	L1-soil	0.943
MSSRFW42	L2-soil	0.784
MSSRFW43	AnTMSW	0
MSSRFW44	AnTMSW	0.754
MSSRFW45	AnTMSW	0.679
MSSRFW46	AnTMSW	0.654
MSSRFW47	AnTMSW	0.788
MSSRFW48	AnTMSW	0.936
MSSRFW49	AnTMSW	0.853
MSSRFW50	L2-soil	0.752
MSSRFW51	L2-soil	0.662
MSSRFW52	L2-soil	0.791
MSSRFW53	L2-soil	0.892
MSSRFW54	L2-soil	1.168

Table 1.10 Quantification of the decolourization potential of the bacterial strains



AnTMSW L1 soil L2- soil Figure 1.9. Potential decolourizing strains isolated from wastewater and soil



Figure 1.10. Degradation of colour using efficient bacterial strains

#### Treatment using Strychnos potatorum seeds

The wastewater was diluted up to 5% with distilled water and 100 ml was taken in 250ml conical flask. Then diluted wastewater was treated with powdered seed 2%, 4% and 6% and 4%, 8% and 16 % of bio char and incubated under shaken condition for 7 days. After which the treated wastewater samples were centrifuged to remove any debris and the reduction in color was analyzed at 475 nm in the UV-Spectrophotometer. Percentage of colour reduction was calculated with the above formula.

The seed material reported to have coagulating properties were used as powdered and whole seeds. The powdered seeds coagulated the water and also reduced the colour at 6% concentration similarly the whole seed at 6% conc. was found to reduce the colour by 6% but no coagulation was observed. The reduction in colour and coagulation is represented in Figure 1.11.



Figure 1.11. Decolourization using Stychnos potatorum seeds in a powdered form



Figure 1.12. Decolourization using Stychnos potatorum seeds in a powdered form

The whole seeds were used to in 5% diluted AnTMSW at different percent namely 2, 4 and 6 %.of the whole seed of *Strychnos potatorum*. The absorption was read by spectrophotometric analysis after dilution Reduction in the colour of the distillery was observed with increasing seed percent as represented in Figure 1.12. The percent colour reduction using the different concentration of the seeds is presented in Table 1.13. 2% and 4% seeds enhanced the colour though it coagulated the suspended solid material in the waste water, whereas 6% seed treatment resulted in color reduction of around 6%.

Seed on 5% wastewater	OD @ 475	Color reduction in %
2%	0.258	-
4%	0.263	-
6%	0.187	6.03
С	0.199	-

Table 1.11 Decolourisation using whole seed material

#### **Treatment using Biochar**

Biochar were used for the decolourization of AnTMSW. In 250ml conical flask 5%AnTMSW were taken and different percentage of biochar was used as given in the Table 1.12 and Figure 1.13. The flasks were incubated at room temperature for 15 days. Then wastewater was centrifuged to settle down the aggregated matter and the absorbance was measured at 475 nm. Reduction in OD value was observed with 4% Biochar which increased with increasing concentration of the Biochar. Biochar used for decolourisation showed the reduction of the OD value compared to control. Nearly 20% reduction in the colour was observed.



Figure 1.13. Decolourization using biochar

Biochar on 5% wastewater	OD @ 475	Color reduction in %
4%	0.165	17.1
8%	0.163	18.1
16%	0.158	20.6
С	0.199	-

Table 1.12 Decolourisation using biochar

Subtask	1.2.2	Design	and	demonstration	of	Constructed	wetlands	and	High	Rate	Transpiration
Systems.											

#### SAB Miller, Sangareddy, India

The design of Engineered Constructed Wetlands (ECWs) for treatment of wastewater generated at SAB Miller after UASB reactor is developed and submitted to SAB Miller India, Hyderabad (Figure 1.14). The studies on substrate (filter media consisting of gravel and coarse sand in ECWs) performance especially for treatment of brewery wastewater is under progress. The field scale implementation of ECWs at brewery's premises has been done in consultation with officials of SAB Miller India.

#### K.C.P. Sugar and Industries Corporation Ltd (MSSRF)

Architecture for bio-treating the industrial wastewater is developed and presented in Figure 1.15. Three levels of treatment are proposed for this project as microbial, constructed wetlands, phytoremediation using duckweed. Since decolouration of the distillery wastewater is a major concern of the industry, a separate treatment is being proposed using microbes. For sugar units this may not be included. At second level, constructed wetland is the medium for treating all the parameters. Based on the water quality that flows out of constructed wetlands option for tertiary level treatment will be decided. Finally the treated water will flow into aqua farm which has a dual purpose as to culture fish as well as a treatment process. From aqua farm the water flows to agro farm. Before and after each levels water quality monitoring as well as analysis will be done to ensure treatment process and reuse efficiency.

An innovative approach is proposed in the treatment process by using *Strychonos potatorum* seeds. This seed is traditionally used by people in Ramanathapuram district of Tamil Nadu to purify the turbid water collected from the pond (Oorani) and used for drinking and cooking purposes for centuries. This project aims to give scientific blend to the traditional knowledge and practice through the lab experiments which shows better results as discoloring material. There is an issue of coagulation which will be sorted out with technical support from industry and consortium partners of this project. Also biochar which has proven record as bio-treatment material will also be used as discoloring agent. This would be one of the innovations of this project.

**Microbial treatment:** As a first step of bio-treatment of partially treated distillery wastewater will be done using suitable microbes mainly for decolourization. The design comprises of two aeration tanks each with the capacity of 10000 cu m since the process requires incubation time f 7 to 10 days. To maintain the aerobic condition for the microorganisms' air will be supplied through agitator or air blowers. Assuming air requirement of 0.7 cum/hr per volume of tank, 1 HP agitator will be used in each tank.



Figure 1.14. Design of ECW fir treatment of industrial wastewater generated at M/s Charminar Breweries of SAB Miller India, A.P., India



#### All dimensions are in m



Figure 1.16. Filter media of S. potatorum and biochar

From step 1 the microbial treated wastewater flows to step 2 through a filter media that comprises of *S. potatorum* and Biochar packed in columns for decolourisation, removal of heavy metals, TDS and TSS. *S. potatorum* acts as a coagulant agent and might clog and affect water flow which will be addressed in the pilot testing. The packet columns are designed for a detention time of 2 hrs with a surface area of 0.07 sq m. The width of each layer is assumed to be 0.15 m.

**Equalization tank:** From step 2 where wastewater is treated using *S. potatorum* seeds and biochar the treated water flows to the equalization tank. Equalization tanks are designed with size  $0.7 \times 0.7 \times 1$  m for storing treated wastewater for 8 hr of retention time. The equalization tank is mainly to ensure continuous flow of water to step 3 that is constructed wetlands. From equalization tank the treated water will flow into constructed wetland. The design and specification of the constructed wetlands is described the next section.

**Subsurface flow constructed wetlands:** The technology of wastewater treatment by means of constructed wetlands with horizontal sub-surface flow (HSSF) was started in Germany. The aerobic zones occur around roots and rhizomes that leak oxygen into the substrate (Brix, 1987; Cooper et al., 1996). Major design parameters, removal mechanisms and treatment performance have been reviewed by Kadlec and Knight (1996), Cooper *et al.* (1996). Billore et al. (2001) reported on the use of an horizontal flow constructed wetland to treat the secondary treated distillery effluent from a private distillery, Associated Alcohols and Breweries, Ltd. at Khodigram village in the outskirts of Baraha town in Central India. The study indicated that constructed wetlands may be a suitable treatment option.

The constructed wetland cell is of 9.2 m<sup>2</sup>surface area and aspect ratio of 2:1. The surface area is calculated using method of Crites & Tchobanoglous. The depth of the bed should not exceed the potential root penetration depth for the plant species to be used. This will ensure availability of some oxygen throughout the bed profile. The root depths shown in table 10 are considered to be near the maximum practical limit to be expected (US EPA manual, 1993).

Horizontal subsurface flow constructed wetland will be planted with common reeds (*Typha* sp. and *Phragmites australis*). The wetland will comprise of five compartments of which the middle portion will be a plant bed area. Two compartments each on either side of the plant bed will be filled with gravels of size 20-40 mm and 40-60 mm. The hydraulic conductivity will be determined to find the velocity of flow to be maintained inside the wetland. Flexible IV tube (used for intravenous therapy) is proposed as inlet piping. To maintain a constant flow throughout the wetland, the inlet pipe provided with perforations will be laid parallel along the width. The wastewater will be let inside through inlet tube and collected through outlet perforated pipe to the collection tank.

#### Subtask 1.2.3\*: Bioremediation of degraded land at Ugar Sugar

#### Ugar Sugar, Belgaum (TERI, Delhi)

After detailed discussion by TERI with industry partner (Ugar Sugar, Miraj, Belgaum), sampling was carried out from different agricultural fields from Maharashtra having different history of biomethanated spent wash application (Table 1.13). During sampling, plant (sugarcane) roots along with rhizosphere soil were collected with the aim of isolating native bacteria from plant rhizosphere. Photos from specific sites are shown in Figure 1.17.





Figure 1.17. Photos of different sites

Table 1.13 Details of sampling sites

S. No.		Sample site descript	tion
5. NO	Field Plot	Duration of biomethanated spent wash	Farmer's Name
1.	А	5-10	Dhanapal Amble
2.	А	5-10	Balu Veerbhadra Katagire
3.	А	5-10	Iragonda Katagire
4	В	10-15	Suresh Korabu
5.	В	10-15	Chandrkant Shinde
6.	С	Above 15	Ugar Sugar Pvt.ltd, Miraj, Belgaum,
7.	С	Above 15	Ugar Sugar Pvt.ltd, Miraj, Belgaum,
8.	С	Above 15	Ugar Sugar Pvt.ltd, Miraj, Belgaum,

#### Soil analysis

Soil samples were processed according to standard protocol prior to analysis for pH, EC, OC, and available N (Table 1.14).

Davamatava	Donth	Duration of application				
Parameters	Depth	5-10 years	10-15 years	15 years & above		
	0-30 cm	8.01±0.03a	7.61±0.03b	7.80 ±0.08a		
рН	30-60 cm	8.11±0.02a	8.02±0.05a	7.98±0.11a		
	>90 cm	8.00±0.10a	8.02±0.02a	8.06±0.05a		
	0-30 cm	0.63±0.04a	0.70±0.02a	2.06±0.78a		
EC (dSm <sup>-1</sup> )	30-60 cm	0.55±0.04a	0.61±0.02b	1.43±0.40a		
	>90 cm	0.71±0.12a	0.54±0.03b	1.32±0.18a		
	0-30 cm	0.81±0.09a	1.02±0.11a	0.70±0.06a		
OC (%)	30-60 cm	0.49±0.06b	0.45±0.06b	0.44±0.08b		
	>90 cm	0.58±0.04b	0.43±0.11b	0.39±0.04b		
	0-30 cm	0.0085±0.0006a	0.0070±0.001a	0.0080±0.0003a		
Av-N (%)	30-60 cm	0.0078±0.0005a	0.0052±0.001a	0.0069±0.0003b		
	>90 cm	0.0092±0.0007a	0.0074±0.002a	0.0073±0.0002ab		

Table 1.14 General physico-chemical properties of rhizosphere soil from sugarcane

\*Different letters in same column indicate significant difference at p < 0.05 according to Duncan multiple range test.

Irrespective of depths, rhizosphere soil from all fields found alkaline. Maximum salinity (EC > 1.0  $dSm^{-1}$ ) was found in field treated with bio-methanated spent wash more than 15 years. As expected, organic carbon was found decreasing with depth. There was no significant difference with respect to nitrogen content in different depths of a given site.

#### Isolation of bacteria

Bacteria associated with sugarcane crop grown in alkaline soil conditions, were isolated from the following (Table 1.15 and Figure 1.18):

- 1. Soil samples of Site A and C
- 2. Rhizosphere of Site A and C
- 3. Rhizoplane of Site A and C

(Bashan Y, Holguin G, and Lifshitz R, 1993 *Isolation and Characterization of Plant Growth-Promoting Rhizobacteria.* Methods in Plant Molecular Biology and Biotechnology 331-344) The bacteria were isolated at pH 7 to capture the diversity and pH 8.5 to select out the isolates surviving at this alkalinity. Medium with 5% (w/v) salt solution was also used to isolate microorganisms.

S.No	Culture Code	Morphology-from slides
1	AMY1.2	3 types - small individual rod shaped; small rod shaped in chains; and
2	AMW1.2	Single colony - small rod shaped
3	CMT8.5e	
4	AM1.1	
5	CMT8.5a	
6	AMT+S1.1 (duplicate slide of no. 4 culture)	
7	СВТа	Rod Shaped

Table 1.15 Preliminary data based on Gram staining for identification of pure colonies

8	CMT8.5a (duplicate slide of	
9	CMT8.5c	
10	AMY1.2 (duplicate slide of	Small rod shaped organisms observed, plus longer rod shaped
11	CRT8.5e	Cocci shaped organisms present with both violet and red stains
12	CRT8.5c	
13	CRT8.5a	Cocci shaped
14	ART8.5b	Cocci shaped
15	CRT8.5d	Cocci shaped
16	ART8.5a	Cocci shaped
17	ART8.5e	Cocci shaped
18	ART8.5d	
19	ART8.5c	Cocci shaped
20	CRT8.5b	
21		Rod snaped, some present in clusters
22	CM18.5d CBTd	Rod shaped
24	АВТа	Rod shaped
25	ABT+Sb	Rod shaped
26	ABTb	Rod shaped
27	ABTc	Rod shaped
28	CBTb	Rod shaped
29	ABTd	Rod shaped
30	ABT+Sa	Rod shaped - long rods or in long chains
31	AMT8.5h	Rod shape - 2 different types
32	AMT8.5c	Соссі
33	AMT8.5e	Cocci
34	CBT+Sc	Small rod
35	CBT+Sd	Small rod
36	AMT8.5f	Rod shape
37	AMT8.5a	Rod shape
38	AMT8.5g	Small rod shape
39	AMT8.5b	Rod shape
40	AMT8.5d	Rod shape
41	CSUT+Sa	Соссі
42	ASUT8.5a	Cocci
43	CBT+S1 (b1)	Multiple cultures - long rod; rod; and cocci
44	CSWTa	Multiple cultures - long rod; rod; ring shape; rods in chains; and cocci
45	CSWT+Sb	Rod shape
46	ASUT+Sa	Rod shape
47	ASUT8.5b	Mixed culture - long rod; cocci (ring shaped); rods; cocci in chains;
48	CBT+Sb	Long rod shape; and cocci
49	CBT+Sa	Rod shape
50	CSUT+Sb	Rod shape



Figure 1.18. Images of different isolates

The microorganisms produce organic acids during their metabolic processes which are able to reduce rhizospheric alkalinity. Some of the isolates were qualitatively analyzed for acid production using the pH indicator Bromothymol blue (Table 1.16). Estimation of other chemical properties of soil and biochemical characterization of isolates are ongoing.

Culture Name	Acid Production
C.M. T8.5 BTBa	++
C.M. T8.5 BTBb	+
C.M. T8.5 BTBc	+++
C.M. T8.5 BTBd	++
C.M. T8.5 BTBe	+
A.M. T8.5 BTBa	+
A.M. T8.5 BTBb	+
A.M. T8.5 BTBc	+++
A.M. T8.5 BTBd	+
A.M. T8.5 BTBe	+++
A.M. T8.5 BTBf	++
A.M. T8.5 BTBg	++
A.M. T8.5 BTBh	++
A.R. T8.5 BTBa	++
A.R. T8.5 BTBb	++
A.R. T8.5 BTBc	++
A.R. T8.5 BTBd	+
A.R. T8.5 BTBe	+
C.R. T8.5 BTBa	++
C.R. T8.5 BTBb	++
C.R. T8.5 BTBc	++
C.R. T8.5 BTBd	+
C.R. T8.5 BTBe	

Table 1.16 Qualitative comparison of acid producing isolates

#### Bioremediation of degraded soil using microbial consortia at Ugar Sugar, Belgaum(UASD)

The suitable bioremedial measures have been implemented at Ugar, Khurd to improve soil and crop productivity. The details of microbial consortia being used is as follows:

A consortia comprising of four salt-tolerant bacterial strains (*Enterobacter aerogenes* S63(1)R, *Azospirillum irakense* S173E, *Enterobacter cloacae* S125R and *Pseudomonas* sp. S4 (1) S) was developed at Department of Agricultural Microbiology, UAS, Dharwad and evaluated under various salinity levels ranging from 4.9 to 14.3 dS/m in wheat, Sorghum and Sunflower at Agricultural Research Station, Gangavati, UAS, Raichur from 2010 to 2013. The details of each strain are given below.

- Enterobacter aerogenes S63(1)R, a free-living  $N_2$  fixing bacteria tolerating 15 per cent NaCl concentration.
- Azospirillum irakense S173E, an associative N<sub>2</sub> fixing bacteria tolerating 10 per cent NaCl concentration.
- *Enterobacter cloacae* S125R, a phosphate solubilizing bacteria tolerating 15 per cent NaCl concentration.

*Pseudomonas* sp. S4(1)S, a fluorescent pseudomonas bacteria tolerating 17.5 per cent NaCl concentration.

In the view of efficiency of above bacterial strains, the technical programme was planned and implemented at Ugar Khurd. The productivity of Ugar Sugars "R" and "D" fields declined due to continuous application of spent wash and resulted in build-up of soil salinity. Thus, reclamation of affected lands with integrated measures, bio-remedial measures and land grading has been initiated to improve soil fertility and productivity with the following treatments.

#### Main plot: (Land grading and reclamation)

M1: Land levelling and gradingM2 : Land levelling and grading with bunding (compartment)M3 : Use of amendment (Bunds)M4 : Control

#### Sub-plot : (Soil fertility build up)

- S1 : Green manuring in situ (Dhaincha)
- S2 : Use of organics (Press mud)
- S3 : Microbial inoculation
- S4 : Green manuring *insitu* (Dhaincha) + Microbial inoculation
- S5 : Use of organics (press mud) + Microbial inoculation
- S6 : Control

**Plant height (cm):** Plant height at 30 days after sowing (DAS) differed significantly with land management practices (Table 1.17). Significantly higher plant height was recorded with use of amendments (27 cm) as compared to land leveling, grading and compartment (25 cm), land leveling and grading (21 cm) and control (20 cm). However, the plant height did not differ significantly at 60 DAS.

Soil fertility management practices were also found to have significant effect at 30 and 60 DAS. At 30 DAS use of pressmud with microbial culture recorded significantly higher plant height (27 cm). Whereas at 60 DAS use of pressmud recorded significantly higher plant height (36 cm) as compared to the rest of the treatments.

The interaction effect due to land management practices and soil fertility practices was significant. Combined use of pressmud with microbial culture recorded significantly higher plant height (31 and 42 cm, at 30 and 60 DAS, respectively) over control (12 and 31 cm at 30 and 60 DAS, respectively).

**Number of branches/plant:** Land management practices (Table 1.18) influenced significantly the number of branches per plant at 30 and 60 DAS. Significantly higher number of branches were recorded in use of amendments and land leveling, grading and compartment as compared to land leveling and grading (0.9) and control (1.2).

Similarly, fertility management practices also significantly affected the number of branches per plant at 30 and 60 DAS. Use of pressmud recorded significantly higher number of branches per plant (1.6) as compared to control (1.1). However, it was on par with microbial culture and use of pressmud with microbial culture.

The interaction effect due to land management practices and soil fertility management practices did not differ significantly at 30 DAS. However, at 60 DAS the combined effect of land leveling, grading and compartment and use of press mud with microbial culture recorded significantly higher number of branches per plant (9.1) as compared to the rest of the treatment combinations.

**Number of pods/plant:** Among the land management practices, use of amendments recorded significantly higher number of pods per plant (12) at 60 DAS as compared to control (8). Soil fertility management practices were found non-significant with respect to number of pods per plant. Similarly, interaction effect due to land management practices and soil fertility management practices on number of pods per plant was non-significant (Table 1.19).

,				Diant haisht (and) at CO DAC						
Land	PI	AS	Plant height (cm) at 60 DAS							
management	Soil fe	ictices	Soil fertility management practices							
practices	S <sub>2</sub>	S <sub>3</sub>	$S_5$	$S_6$	Mean	S <sub>2</sub>	S <sub>3</sub>	$S_5$	$S_6$	Mean
M <sub>1</sub>	21	22	24	15	21	39	42	34	28	36
M <sub>2</sub>	26	27	29	17	25	34	31	32	29	31
M <sub>3</sub>	29	28	31	17	26	32	33	42	31	34
$M_4$	21	21	25	12	20	40	29	29	31	32
Mean	24	25	27	15		36	33	35	30	
		CD				S.Em	CD			
	S.Em±	(0.05)				±	(0.05)			
Main (M)	0.82	2.62				3.16	10.07			
Sub (S)	0.71	1.46				2.69	5.56			
MXS	0.82	2.38				1.80	3.71			

Table 1.17. Effect of land management and soil fertility management practices on plant height of soybean

Land	Numb	er of brai	nches/pl	lant at 30	) DAS	Number of branches/plant at 60 DAS				
managem	Soil fertility management practices					Soil fertility management practices				
ent practices	S <sub>2</sub>	S <sub>3</sub>	S <sub>5</sub>	S <sub>6</sub>	Mean	S <sub>2</sub>	S <sub>3</sub>	<b>S</b> <sub>5</sub>	S <sub>6</sub>	Mean
M <sub>1</sub>	0.6	0.9	1.1	0.7	0.8	7.8	6.7	7.1	6.5	7.0
M <sub>2</sub>	2.2	2.1	1.6	1.3	1.8	5.9	6.7	9.1	4.7	6.6
M <sub>3</sub>	2.1	1.8	2.2	1.1	1.8	6.1	4.9	4.9	4.3	5.1
M <sub>4</sub>	1.7	1.1	0.6	1.3	1.2	4.4	5.2	5.4	4.8	5.0
Mean	1.6	1.5	1.4	1.1		6.0	5.9	6.6	5.1	
	S.Em±	CD				S.Em±	CD			
		(0.05)					(0.05)			
Main (M)	0.16	0.46				0.31	1.42			
Sub (S)	0.16	0.48				0.28	0.82			
MXS	0.96	NS				0.79	1.63			

Table 1.18. Effect of land management and soil fertility management practices on number of branches per plant of soybean

Table 1.19. Effect of management practices and microbial culture inoculation on number of pods per plant of soybean

	Number of pods/plant at 60 DAS						
Land management practices	Soil fertility management practices						
	S <sub>2</sub>	S <sub>3</sub>	$S_5$	S <sub>6</sub>	Mean		
M <sub>1</sub>	11	13	12	9	11		
M <sub>2</sub>	12	10	16	9	12		
M <sub>3</sub>	12	13	13	11	12		
$M_4$	10	7	7	9	8		
Mean	11	11	12	9			
	S.Em±	CD (0.05)					
Main (M)	0.44	1.99					
Sub (S)	0.92	NS					
MXS	1.26	NS					



Figure 1.19 Treating soybean seeds with biocultures


Figure 1.20. General view of the experiment initiated at the Ugar Khurd



Figure 1.21. Growth of soybean at 50 days after sowing



Figure 1.22. Growth of Dhaincha at 50 days after sowing

**Microbial load:** The rhizosphere soil was collected for determination of bacteria, fungi and actinomycetes in the soil. It is evident from the data presented in the Table 1.20, Table 1.21 and Table 1.22 that both land management practices and soil fertility management practices significantly influenced the population of soil bacteria, fungi and actinomycetes. However, the extent varied with the combination of nutrient management and land management practices. It was interesting to note that the bacteria and actinomycetes showed significant variation in their population due to either nutrient or land management practices. The variations were significant at 30 DAS for bacteria and actinomycetes.

However, at 60 DAS, the population of bacteria and fungi varied significantly due to land management practices which clearly suggest the importance of these practices in promoting beneficial micro-organisams.

Further, it is interesting to know that the population variations as influenced by the combination of management practices were significant both at 30 and 60 DAS. From the two way interaction it is observed that the changes in population of both bacteria and fungi showed an increase from 30 to 60 DAS irrespective of the management practices. This trend was seen only with the selective land management practices in case of actinomycetes. The application of pressmud ( $S_2$ ) appears to result in gradual increase in the population of all three groups of micro organisams suggesting their role in amelioration. This trend is also reflected in all the treatments receiving soil management practices except for  $S_3$  which appears to have reduced the actinomycetes population.

					0		,	0						
					Ро	pulation	of Bacteri	a (CFU X 1	0 <sup>6</sup> /g of soil)					
			30	DAS				60 DAS						
Land Management		Soil Fertility management practices							Soil Fertility management practices					
practices (M)	<b>S</b> <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	<b>S</b> <sub>4</sub>	S <sub>5</sub>	<b>S</b> <sub>6</sub>	Mean	<b>S</b> <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	<b>S</b> <sub>4</sub>	S <sub>5</sub>	<b>S</b> <sub>6</sub>	Mean
M <sub>1</sub>	1.10	1.30	1.80	20	2.30	1.65	1.69	3.75	2.95	2.50	4.00	3.70	3.25	3.36
M <sub>2</sub>	1.90	2.25	2.45	2.45	1.95	1.65	2.11	4.40	4.55	4.05	4.05	2.35	2.50	3.65
M <sub>3</sub>	2.00	2.55	2.90	2.80	2.60	1.85	2.45	2.05	2.25	1.85	3.25	2.75	3.20	2.56
M <sub>4</sub>	2.40	2.95	2.55	2.45	3.05	1.95	2.56	3.50	3.00	3.10	3.10	2.45	2.55	2.95
Mean	1.85	2.26	2.43	2.43	2.48	1.78		3.43	3.19	2.88	3.6	2.81	2.88	
	S.Em±	CD (0.05)							S.Em±	CD (0.05)				
Main	0.14	0.33						Main	0.22	0.55				
Sub	0.25	NS						Sub	0.45	NS				
MXS	0.29	0.61						MXS	0.52	1.1				

Table 1.20. Bacterial population in soil under different land management and soil fertility management practices

Main plot (Land management practices)

M<sub>1</sub> – Land leveling and grading

M<sub>2</sub> – Land leveling, grading and compartment

M<sub>3</sub> – Use of amendments (Big compartment bunds)

M<sub>4</sub> – Control

Sub plot (soil fertility management practices)

 $S_1$  – Green manuring in-situ (Dhaincha – wheat)

S<sub>2</sub> – Use of press mud (Soybean- wheat)

S<sub>3</sub> – Microbial culture (Soybean-wheat

 $S_4 - S_1$  + microbial culture (Dhaincha-wheat)

 $S_5 - S_2$ + microbial culture (soybean – wheat)

S<sub>6</sub>-Control

						Populatio	on of Fungi (	CFU X 10⁵/	g of soil)					
Land				30 DAS				60 DAS						
Management		Soil Fe	ertility m	management practices				Soi	il Fertility mar	nagemer	nt practic	es		
practices (M)	<b>S</b> <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	<b>S</b> <sub>4</sub>	S₅	S <sub>6</sub>	Mean	<b>S</b> <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	<b>S</b> <sub>4</sub>	<b>S</b> <sub>5</sub>	S <sub>6</sub>	Mean
M1	3.45	3.55	3.05	3.45	3.40	3.15	3.34	3.25	3.70	3.55	4.25	3.25	5.5	3.92
M <sub>2</sub>	3.30	2.75	2.75	2.85	3.10	3.85	3.10	5.90	7.95	7.85	7.15	4.90	5.35	6.52
M <sub>3</sub>	3.65	3.25	3.10	3.35	2.80	3.05	3.20	3.90	5.65	6.60	4.00	4.30	4.10	4.76
M <sub>4</sub>	3.00	3.00	3.10	3.65	3.15	3.70	3.27	2.90	2.90	3.65	4.35	3.85	3.20	3.48
Mean	3.35	3.14	3.00	3.33	3.11	3.44		3.99	5.05	5.41	4.94	4.08	4.54	
	S.Em±	CD (0.05)							S.Em±	CD (0.05)				
Main	0.09	NS						Main	0.48	1.18				
Sub	0.21	NS						Sub	0.54	1.61				
MXS	0.24	0.52						MXS	0.62	1.31				

Table 1.21. Fungi population in soil under different land management and soil fertility management practices

Main plot (Land management practices)

 $M_1$  – Land leveling and grading

M<sub>2</sub> – Land leveling, grading and compartment

M<sub>3</sub> – Use of amendments (Big compartment bunds)

M<sub>4</sub> – Control

Sub plot (soil fertility management practices)

 $S_1$  – Green manuring in-situ (Dhaincha – wheat)

S<sub>2</sub> – Use of press mud (Soybean- wheat)

S<sub>3</sub> – Microbial culture (Soybean-wheat

 $S_4 - S_1$  + microbial culture (Dhaincha-wheat)

 $S_5 - S_2$ + microbial culture (soybean – wheat)

S<sub>6</sub>-Control

					Popula	ation of A	ctinomyce	tes (CFU X	10 <sup>4</sup> /g of so	il)				
Land			30 E	DAS				60 DAS						
Management		Soil Ferti	lity mana	nanagement practices					Soi	l Fertility mai	nageme	ent practi	ces	
practices (M)	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	<b>S</b> <sub>4</sub>	S₅	S <sub>6</sub>	Mean	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	<b>S</b> <sub>4</sub>	S <sub>5</sub>	S <sub>6</sub>	Mean
M <sub>1</sub>	2.00	1.60	1.75	1.60	1.25	1.30	1.58	2.25	2.40	1.20	2.25	3.25	2.95	2.38
M <sub>2</sub>	1.75	2.20	1.95	1.90	2.15	2.35	2.05	2.95	3.45	1.50	3.20	1.85	1.80	2.46
M <sub>3</sub>	2.25	2.60	2.85	3.10	2.75	2.60	2.69	2.75	2.25	2.50	2.10	1.80	2.25	2.28
M <sub>4</sub>	2.95	2.75	2.25	2.35	3.25	1.90	2.58	2.95	1.60	1.55	2.00	2.25	2.30	2.11
Mean	2.24	2.29	2.2	2.24	2.35	2.04		2.73	2.43	1.69	2.39	2.29	2.33	
	S.Em±	CD (0.05)							S.Em±	CD (0.05)				
Main	0.16	0.38						Main	0.37	NS				
Sub	0.18	NS						Sub	0.39	NS				
MXS	0.21	0.44						MXS	0.46	0.97				

Table 1.22. Actinomycetes population in soil under different land management and soil fertility management practices

Main plot (Land management practices)

M<sub>1</sub> – Land leveling and grading

M<sub>2</sub> – Land leveling, grading and compartment

M<sub>3</sub> – Use of amendments (Big compartment bunds)

M<sub>4</sub> – Control

Sub plot (soil fertility management practices)

 $S_1$  – Green manuring in-situ (Dhaincha – wheat)

S<sub>2</sub> – Use of press mud (Soybean- wheat)

S<sub>3</sub> – Microbial culture (Soybean-wheat

 $S_4 - S_1$  + microbial culture (Dhaincha-wheat)

 $S_5 - S_2$ + microbial culture (soybean – wheat)

S<sub>6</sub>-Control

## Task 1.3: Waste based media for recovery of selected compounds (TERI, SABM, UGSG)

### Subtask 1.3.1. Preparation and characterization of separation media

### Carbon from bagasse ash at Ugar Sugar, Belgaum (TERI)

The sourced bagasse ash was sieved and subjected to water floatation to recover the unburnt carbon fraction. The separated carbon so obtained was oven dried at 50°C before use. The unburnt carbon was modified by (a) deashing (treatment with 15% HCl followed by treatment with 25% HF; the samples were washed thoroughly with RO water and dried before use) (b) steam activation at 800°C at different carbon to water ratios; this was done for samples with and without prior deashing. The procedures are described in our earlier work (Batra et al., 2011). The carbon samples tested in this work are presented in Table 1.23.

Name	Description
UC	Unburnt carbon
UC-ASH	Deashed Carbon
3AC UC	Steam activation; Unburnt carbon-water ratio 1:3
5AC UC	Steam activation; Unburnt carbon-water ratio 1:5
7AC UC	Steam activation; Unburnt carbon-water ratio 1:7
3AC UC-ASH	Steam activation; Deashed carbon-water ratio 1:3
5AC UC-ASH	Steam activation; Deashed carbon-water ratio 1:5
7AC UC-ASH	Steam activation; Deashed carbon-water ratio 1:7

Table 1.23. Carbons tested in this work

The unburnt carbon was analyzed for moisture, ash, volatile matter, and surface area. Moisture content was determined using ASTM D 2867-04. Ash content was determined using ASTM D 2866-94. Volatile matter was measured using ASTM D 5832-98. All samples were analyzed in duplicate. pH was determined by the method of Ahmedna (1997). Particle size was determined by sieving through a series of standard sieves and weighing the amount retained on each sieve. Particle size reduction was achieved by ball milling. BET surface area was determined in Smart Sorb 93(Mumbai, India) Surface Area Analyzer. The properties of the prepared carbons are summarized in Table 1.24

Name	Moisture (%)	Ash (%)	Volatile matter (%)	Surface Area (m <sup>2</sup> /g)
UC	8.2 ± 0.1	33.3 ± 2.1	27.7 ± 0.29	29
UC-ASH	8.0 ± 0.1	2.1 ± 0.1	29.0 ± 1.03	130
3 UC	$1.7 \pm 0.1$	31.6 ± 0.1	14.7 ± 1.22	335
5 UC	$1.6 \pm 0.1$	33.9 ± 0.4	13.5 ± 0.09	365
7 UC	3.1 ± 0.1	31.1 ± 2.7	13.3 ± 2.36	346
3 UC-ASH	$1.0 \pm 0.3$	4.9 ± 0.7	15.8 ± 3.81	553
5 UC-ASH	1.2 ± 0.3	5.04 ± 0.7	12.3 ± 1.39	604
7 UC-ASH	2.3 ± 0.1	4.9 ± 0.1	25.7 ± 1.41	591

Table 1.24 . Properties of different carbons

Commercial activated carbons have moisture content in range of 3% to 10%. In the present study, moisture content of as-is UC was highest at 8.2% and that of 3 UC-Ash was lowest (1%). After steam activation moisture content of the carbon samples was in the range of 1 to 3%, which is within the accepted limits for commercial carbons.

Ash content indicates the amount of inorganic constituents associated with the carbon. Ash content of as-is UC was very high (33.3%). Deashing is therefore essential. The HF used during deashing dissolves the silica in the unburnt carbon and thus reduces the ash content. Ash content of steam activated UC is marginally less than that of the as-is UC. However, steam activation of deashed samples shows a slight increase in ash content due to carbon burn off. The volatile matter in the UC was 27.7% which increased on deashing.

Surface area is a key property of activated carbons since larger surface area indicates higher adsorption capacity. The surface area of as-is UC is very low ( $29 \text{ m}^2/g$ ). However, deashing increases the surface area. The surface area can be further increased by steam activation. The highest surface area is obtained for 5UC-Ash (604 m<sup>2</sup>/g). Further increase may be possible with steam activation in the presence of salts like cerium nitrate. This aspect is being investigated.

pH of unburnt carbon is 8.52 which indicates its basic nature. Upon deashing with acids, the pH reduces to 3.34. Figure 1.23 shows the particle size distribution of the as-is and deashed unburnt carbon. A major fraction of both carbons has particle size > 425 micron. After deashing, the particle size is marginally reduced.



Figure 1.23. Particle size distribution of unburnt and deashed carbon

The particle size of the unburnt carbon can be easily reduced by ball milling (Figure 1.24). Ball milling for 30min duration is adequate to reduce the entire lot to particle size < 63 micron. The unburnt carbon can thus be readily crushed to powder.



Figure 1.24. Particle size distribution of unburnt carbon after ball milling for different time periods

### Subtask 1.3.2 Performance evaluation for phenolics/pigments recovery

## Recovery of phenolics/pigments from spenwash at Ugar Sugar, Belgaum (TERI)

Batch adsorption experiments were done in a shake flask using humic acid as a model for color compounds (Satyawali and Balakrishnan, 2007); the properties of humic acid are similar to that of melanoidins (the main colouring compounds in distillery spentwash) in terms of their elemental composition, structure, chemical properties and environmental stability. Both are dispersed colloids possessing negative charge due to the dissociation of carboxylic and hydroxyl groups.

The results of humic acid adsorption are shown in Figure 1.25. For the as-is unburnt carbon, % removal at 0.5g adsorbent dosage is negative indicating leaching of coloured compounds from the unburnt carbon. This problem was not encountered with the deashed carbon samples. Maximum removal of humic acid with deashed carbon was 38%. It is expected that removal efficiency can be increased further by using steam activated samples with larger surface area. This aspect is being investigated.



Figure 1.25. Humic acid adsorption results

# Task 1.4: Assessment of treated water quality and its suitability for irrigation (ICRISAT, JISL)

Subtask 1.4.1: Assess effect of untreated and treated wastewater on soil properties (biological and chemical) *in-vitro* studies

# Subtask 1.4.2 Study effect of untreated and treated wastewaters on plant growth in pot culture. *SAB Miller (ICRISAT)*

## Germination bioassay test

Seed germination bioassay was conducted to assess the effects of brewery wastewater on selected crops like mustard, chickpea, pigeon pea, cowpea, green gram, maize, pearl millet and sorghum also to evaluate the suitability of brewery wastewater for irrigation purposes. Untreated (UASB outlet) and Treated (ETP Outlet) samples were collected from SAB Miller factory, Sangareddy, AP. The effluent samples were analysed for Physico-chemical parameters. Different concentrations of effluent (50% & 100%) were prepared using distilled water and tap water was taken as control.

Seeds of eight selected crops were sterilized with ethyl alcohol for 1 minute to remove the microbes, followed with repeated washings by using sterilized distilled water. Each treatment including control was performed in triplicate and in every petriplate 10 healthy sterilized seeds were used. Seeds were

spread on equal distance in each sterilized Petri plate lined with filter paper. Then each Petri plate were irrigated with 5ml of different concentrations of waste water into the respective Petri plate and then incubated at 25+2°C. Different parameters like germination percentage, seedling length, seedling vigour index, fresh and dry weight of seedlings were recorded on different period of growth. First recording of germinated seeds were done after 24h of incubation and subsequent recordings were after 1day interval till 10<sup>th</sup> day of incubation. Radical and plumule length was measured after three days. For biomass of root, shoot and leaves, samples were oven-dried separately at 80°C for 24 hours and dry weight (gm) was determined on a digital balance.

Crop	Treatment	Control	ETP-50%	ETP-100%	UASB-50%	UASB-
Maize	Germination (%)	100	100	87	90	83
	Vigour index	531	521	434	469	373
	Radical (cm)	3.84	3.46	3.41	3.72	3.04
	Plumule (cm)	1.47	1.75	1.58	1.5	1.46
Sunflower	Germination (%)	37	47	20	23	27
	Vigour index	177	432	68	260	48
	Radical (cm)	1.56	2.12	0.98	3.76	0.3
	Plumule (cm)	3.23	7.09	2.45	7.56	1.5
Pigeonpea	Germination (%)	97	90	83	97	83
	Vigour index	822	575	387	746	314
	Radical (cm)	2.65	2.36	2.03	3.14	1.07
	Plumule (cm)	5.83	4.03	2.64	4.56	2.72
Chickpea	Germination (%)	93	97	80	97	83
	Vigour index	1521	1660	920	1710	873
	Radical (cm)	7.41	8.45	4.85	8.19	4.22
	Plumule (cm)	8.95	8.67	6.66	9.44	6.31
Sorghum	Germination (%)	87	67	70	70	60
	Vigour index	1175	647	417	772	394
	Radical (cm)	8.94	5.06	2.53	6.26	3.03
	Plumule (cm)	4.57	4.6	3.43	4.78	3.55
Pearl millet	Germination (%)	70	80	70	47	40
	Vigour index	973	797	223	317	100
	Radical (cm)	10.93	7.05	1.98	4.87	0.97
	Plumule (cm)	2.98	2.92	1.21	1.89	1.54
Greengram	Germination (%)	30	67	43	30	60
	Vigour index	182	582	266	73	427
	Radical (cm)	1.86	2.95	2.21	0.8	2.22
	Plumule (cm)	4.23	5.75	3.98	1.65	4.91
Cowpea	Germination (%)	80	43	70	77	60
	Vigour index	847	199	658	1680	624
	Radical (cm)	5.46	2.74	4.9	11.25	5.46
	Plumule (cm)	5.13	1.89	4.51	10.58	4.95
Mustard	Germination (%)	77	93	83	83	87
	Vigour index	985	1001	591	1402	786
	Radical (cm)	5.13	2.3	4.51	10.58	4.95
	Plumule (cm)	7.67	8.47	2.62	6.32	4.09

Table 1.25. Effect of brewery wastewater on seed germination, vigour index, radical length, and plumule length

Observations for germination bioassay for selected crops are presented in Table 1.25 and Figure 1.26. Germination parameters for all selected crop indicated that 50% diluted effluents were as good as fresh water. However, direct use of effluents without any dilution may have effect of germination and seedling growth. Figure 1.26 shows germinated seed after 5<sup>th</sup> day. In addition to ETP and UASB effluents, germination bioassay test was also conducted for RO reject water (effluent of reverse osmosis plant). Total dissolve salts in RO reject is very high, thus only 10 and 25% solution of RO effluent were used for germination bioassay test. Figure 1.27 clearly indicates that even for 25% solution of RO effluent has negative impact on germination of chickpea. 10% RO effluent solution was as good as fresh water.



Figure 1.26. Germination bioassay test on chickpea for brewery wastewater



Figure 1.27. Germination bioassay test on chickpea for diluted effluent from reverse osmosis water treatment plant

Subtask 1.4.3 Understand changes in soils irrigated with treated and untreated wastewater on biological and nutrient dynamics. *Presented in WP3* 

**Deliverables (brief description and month of delivery)** Detailed characterization of selected wastewaters (month 12)

# 2. Work Package: Bio-treatment of Municipal Wastewater for Reuse and Bioremediation of Degraded Lands

**Objectives:** Selection and optimization of microbial consortium to reclaim degraded lands and biotreatment of municipal wastewater for re-use in agriculture

### Task 2.1a Site selection and wastewater characterization

Following site has been identified for wastewater sources

- Pandherkawada, Nagpur
- ICRISAT, Patancheru, Andhra Pradesh
- Muduvatti, Kolar, Karnataka
- UAS, Dharwad, Karnataka
- Mavanur, Katnur and Gabbur in Dharwad Karnataka

#### Pandherkawada, Nagpur (NEERI)

The site located at sewage treatment plant (STP) of Nagpur Municipal Corporation (NMC) at Bhandewadi, Nagpur was selected for treatment of municipal wastewater using engineered constructed wetland system. The final approval for the demonstration of ECWs at STP, Bhandewadi from the NMC, Nagpur is awaited.

#### ICRISAT, Patancheru, India (ICRISAT)

Location of the experimental site is shown in Figure 2.1. Presently, domestic wastewater from a small community outside the campus is coming in ICRISAT campus through a culvert (shown as inlet point). Preliminary results of wastewater characterization are presented in Table 2.1. This wastewater is being diverted through a series of settling tanks to a small lake (Red Tank). Red Tank is a habitat to several migratory bird species. Wastewater sample at this site were collected and various quality parameter were analyzed. Field experiments to assess the impact of treated wastewater on soil and crop quality will be conducted in nearby fields. These fields are freshly prepared.



Figure 2.1. Plan of wastewater treatment system and field experiment at ICRISAT campus.

#### Musi river area, Hyderabad, India (ICRISAT)

Musi river flows across Hyderabad from east to west. A major portion of the domestic and industrial wastewater of this city containing regular sewage-sludge disposed in it. In this regard, continuous surveillance is necessary to understand the water quality dynamics of Musi. As a part of this continuous process, till now we have completed a phase of water sample collection (with GPS reading) and its analysis. Figure 2.1 shows the different water sampling locations over Musi.



Figure 2.2. Water-sampling from Musi river area, Hyderabad. The locations were: a.Pedha Cheruvu; b. Mughal ka Nala; c. Chadarghat Chota Bridge; d. Tipu Khan Bridge, e. Nagole Bridge, f. Muslim Jung Pool

#### Muduvatti, Kolar, India (ICRISAT)

Mudavatti village is located in Kolar taluk of Kolar district in Karnataka. This village has a masonry drainage canal of about 2000 m, which collects domestic wastewater from about 400 households and the rainwater. Another 500 m length of drain is proposed to be constructed. We identified two farmers who are using untreated wastewater for irrigation. They have constructed water collection pond to collect wastewater. Collected wastewater is being reused for the cultivation of vegetables. One farmer Mr Govindappa has adopted drip irrigation with 3 hp diesel engine pump and cultivated vegetables (bitter gourd, ridge gourd and tomato in one acre of land during the post-rainy season. Another farmer Mr Nagaraj has also constructed a pond (15x20x2 m) and uses sewage water by flood and ridge and furrow method to irrigate one acre of land grown with brinjal, ridge gourd and tomato. We have collected wastewater samples from these water collection ponds and analysed for various parameters (Table 2.1). We propose to convert present water collection pond into constructed wetland in one of two farmers' field.

Other than this location, few more sites were identified in Kolar taluk and wastewater samples were collected for quality check. However, at other sites, wastewater is not being used for irrigation purpose.



Figure 2.3. Wastewater collection pond and wastewater irrigated field at Muduvatti village

Sample Source	ICRISAT			UASD			
	Kolar	Patancheru	Hyderabad	Gabbur	Mavanur	Katnur	Dharwad
рН	7.7-8.0	8.25	7.1-8.1	7.44	7.31	7.4	7.33
EC (mS)	1.8-3.2	1.45	0.98-1.65	1.23	1.19	1.2	1.34
TS (mg/L)	1600-2600	600	400-2000	1130	1080	1090	1020
TDS (mg/L)	400-1600	533	200-1800	380	350	350	200
TSS mg/L	200-1800	67	200-1200	750	730	740	820
NH <sub>4</sub> -N	11.2-19.9	23.82	0-5.1	-	-	-	-
NO <sub>3</sub> -N	0.32-4.74	1.14	0.85-11.38	-	-	-	-
Total N (ppm)	12.5-24.7	24.97	3.48-31.89	31.1	29.6	24.2	17.2
Bacteria CFU/ml	158000-266000	-	-	-	-	-	-
Actinomycetes CFU/ml	200-10000	-	-	-	-	-	-
MPN Index/100 ml	28000-170000	-	-	-	-	-	-
BOD <sub>5</sub>	54.4-112.0	2.18	168-515	640	495	410	390
COD	128-352	30	400-1600	695	634	524	572
Bicarbonate (ppm)	2.8-8.0	3.8	1.6-3.0	7.6	8.4	8.1	6.9
Chloride( ppm)	9-86 me/l	7.0	5-8	8.7	7.8	8.1	7.1
Sulphate (ppm)	-	0.09	-	7.45	7.22	7.52	8.2
S (ppm)	12-196	4.47	11.4-37.3*	-	-	-	-
Boron (mg/L)	0.12-0.28	-	0.09-0.37*	-	-	-	-
Calcium (me/L)	45-86*	3.78	56.3-93.9*	4.45	4.23	4.22	5.34
Magnesium( m.e/L)	28-153*	2.53	41.3-18.9*	4.1	3.75	3.73	4.1
Sodium (m.e/L)	348-4452*	4.55	88.2-151.1*	5.26	5.49	5.13	5.41
Potassium (m.e/L)	65-389*	0.13	13.3-26.8*	1.16	1.19	1.02	0.69
Manganese(mg/L)	0.13-0.85*	0	0.02-0.19*	-	-	-	-
Iron(mg/L)	0.19-2.99*	0	0.06-0.32*	-	-	-	-
Phosphate(mg/L)		-	-	20.2	16.6	19.3	15.3

Table 2.1 Characteristics of wastewater collected from selected site at Kolar, Patancheru, Hyderabad, and Dharwad

\* Unit is ppm

### UAS, Dharwad (UASD)

Domestic wastewater and soil samples were collected from the University campus site and from Mavanur, Katnur and Gabbur sites near Dharwad. The pH was slightly alkaline and remained largely unaffected over time. The sewage water was slightly saline with the salinity load ranging from 1.12 dS/m to 1.28 dS/m. The sewage water of UAS, Dharwad recorded slightly higher salinity over the sewage water collected at three villages near Hubli. Total solids were relatively higher at Gabbur and lower at Katnur. Same trend was observed with respect to total suspended solids. Total dissolved solids did not show such trend. It was important to note high levels of phosphates (Table 2.1). Soil pH was relatively higher at 150 m away from the sewage stream as compared to 50 and 250 m from the stream. Irrespective of proximity to the stream, the soil pH, in general, increased with depth, so also the soluble salt content (Table 2.2).

CL	Distance	Dec			Da			Exch Ca+Ma		
21	Distance	Rar	ige of p⊢	<b>1</b> :2.5	ка ка	nge of EC	1:2.5		Excn. Ca-	+ivig
No.	from the					(dS/m)		[c	mol (p+)	kg⁻¹]
	stream				D	epth (cm	)			
		0-20	20-40	40-60	0-20	20-40	40-60	0-20	20-40	40-60
1	50 m	7.22-	7.06-	8.08-	0.30-	0.31-	0.32-			
		8.09	8.26	8.48	1.53	1.65	0.66	50.7	51.5	46.5
2	150 m	7.79-	8.05-	8.13-	0.23-	0.25-	0.26-			
		7.91	8.22	8.33	0.37	0.41	0.47	51.1	53.3	55.4
3	250 m	7.45-	7.60-	7.71-	0.23-	0.21-	0.23-			
		8.11	8.28	8.44	0.37	0.41	0.37	55.5	55.4	57.5

Table 2.2 : Effect of continuous application of sewage water on pH and EC

## Task 2.1b Biological wastewater treatment (NEERI, TERI, ICRISAT, MSSRF, UASD)

Rationale: Use of wastewater in agriculture in rural as well as peri-urbun area is not uncommon. In fact, wastewater streams serves as perennial source for irrigation. However, most of these users do not follow safe practices to reuse wastewater. We, through this activity, propose to develop decentralized wastewater treatment system, which will improve the quality of wastewater to reuse it safe in agriculture.

# Subtask 2.1.1 Screening and isolation of microorganisms utilizing contaminated wastewater for growth

### Bhandewadi Nagpur

The municipal wastewater from the STP at Bhandewadi were collected from the inlet, primary and secondary treatment plant and analyzed for different microbial groups viz. bacteria, fungi, actinomycete, Azotobacter and Rhizobium present in the wastewater. The results of different microbial groups are presented in Table 2.3. The screening and isolation of microorganism for degradation of pollutants present in the wastewater are under progress.

Table 2.3. Microbiological characteristics of municipal wastewater collected from STP Bhandewadi, Nagpur

Sample Datails		Microbial Count (CFU/ml)								
Sample Details	Bacteria	Fungi	Actinomycetes	Azotobacter	Rhizobium					
Inlet	33×10 <sup>8</sup>	22×10 <sup>4</sup>	55×10 <sup>4</sup>	107×10 <sup>5</sup>	20×10 <sup>3</sup>					
Primary treatment outlet	$100 \times 10^{7}$	24×10 <sup>4</sup>	43×10 <sup>4</sup>	-	20×10 <sup>3</sup>					
Secondary treatment outlet	20×10 <sup>6</sup>	64×10 <sup>4</sup>	140×10 <sup>3</sup>	-	50×10 <sup>3</sup>					

CFU – Colony forming unit

### Subtask 2.1.2 Formation and optimization of microbial consortium for removal of contaminants

# Subtask 2.1.3 Design and demonstration of CWs and HRTS systems *Bhandewadi, Nagpur*

For treatment of municipal wastewater generated at STP, Bhandewadi, Nagpur using ECWs, the investigation on pilot scale for optimization of substrate composition, hydraulic loading rate, vegetation pattern and on delayed clogging in the substrate using Fibre- optic Reinforced Plastic tub (FRP) at CSIR-NEERI, Nagpur is under progress. **Figure 2.4** shows the dimension of FRP procured for treatment of sewage wastewater for crop irrigation. The site proposed for installation of ECWs for treatment of around 3000 litres/day of municipal wastewater at STP, Bhandewadi, Nagpur is shown in Figure 2.5.



Figure 2.4. Dimension of FRP procured for treatment of sewage wastewater for crop irrigation



Figure 2.5. Location of proposed site for treatment of municipal wastewater using Engineered Construction Wetland system at STP Bhandewadi, Nagpur

#### ICRISAT, Patancheru

At ICRISAT campus we propose to construct a wastewater treatment system comprising a wastewater collection tank near inlet, overhead holding tank, constructed wetlands, and treated water storage tank (Figure 2.6).



Figure 2.6. Photographs of wastewater inlet point (right), constructed wetlands (middle) and treated water storage tank (left) being constructed at ICRISAT, Patancheru site.

#### UAS, Dharwad

At UAS Dharwad, Plot No. 14 to 21 ('A' block) and Plot No. 200 ('H' block) were identified as probable sites for Constructed Wetland and wastewater treatment. Daily average wastewater discharge is about 2 lakh litres. Constructed Wetland will be constructed at this site as per the specification given by NEERI.



Figure 2.7. Field visit of Dr. Juwarkar and Prof. Dasog, along with the project team for identification of the site for constructed wetland system

Constructed wetland design for the University domestic waste water discharge was discussed with NEERI, Nagpur and ICRISAT scientists. Initially 50 m<sup>3</sup> capacity constructed wet land will be implemented with provision for further expansion depending on the discharge available and suitability of media for purification of the waste water. The proposed constructed wetland will be ready by September-October 2013. The treated waste water will be reused in crop production. The standard procedures as suggested in kick-off meeting were followed for analysis.

The following is the specifications of the proposed CWL;

- a) Inlet tank L = 8 m, W = 4.5 m and Depth = 0.8 m Below Ground Level (BGL) with bed concrete at the bottom and burnt brick masonry for the walls
- b) Outlet tank L = 4 m, W = 4 m and depth 0.8 m BGL with bed concrete at the bottom and burnt brick masonry for the walls

- c) Wetland tank L = 10 m, W = 8 m BGL and depth = 0.8 m with bed concrete at the bottom and burnt brick masonry for the walls.
- d) Initially wetland tank is designed to accommodate 2 treatments (ie., two types of tree/grass species) with a net treatment of plot size 10 x 3 x 0.8 m
- e) For the filter material, 20 mm down size boulders of thickness 50 cm, sand of thickness 25 cm and biochar of thickness 5 cm will be refilled for the establishment of the selected tree/grass species
- f) Water proofing at the bottom was done to prevent any possible seepage from the bottom.



g) Estate branch entrusted to execute the work

Figure 2.8. Design of constructed wetland at UAS, Dharwad

# Task 2.2: Bio-remedial regeneration of degraded land irrigated with city wastewater for long term (ICRISAT, NEERI, TERI, MSSRF)

#### Subtask 2.2.1: Site selection and characterization

#### Bhandewadi, Nagpur

To study the long term impact of municipal wastewater on soil, the degraded land at Pandherkawada village which is 10-15 kms away from the STP Bhandewadi, Nagpur is selected for bioremediation using biotechnological approach. The degraded land is located at Pandherkawada (2104'52.3" N, 79011'46.3" E), 25Km away from Nagpur. Study area falls under the sub-humid ecosystems of Deccan plateau and central highlands with black soils. The climate of the region is characterized by hot summers and mild winters. The area receives a mean annual rainfall of 100 to 1500mm which covers about 80 % of annual potential evapotranspirative (PET) demand of 1500 to 1600mm. The annual water deficit amounts to 500 to 700 mm. The soils moisture availability (growing) period varies from 150 to 180 days in a year. The soil temperature regime in the area is hypothermic. Accordingly, CSIR-NEERI, Nagpur team visited the site in the month of June 2013 and collected the soil profile samples, irrigated wastewater and groundwater (open dug well and drinking water) samples. The water samples were analyzed as per the Standard Methods for the

Examination of Water and Wastewater (APHA, 2012) for various physico-chemical parameters. The soil samples were analyzed for various physico-chemical and microbiological parameters as per the standard methods (Black et al., 1965) and (Norland, 1991 and Page. et al, 1982).

# Subtask 2.2.2 Baseline data generation with respect to wastewater used for irrigation, soil (Physical, chemical and Biological properties), groundwater quality, cropping pattern

### Bhandewadi, Nagpur

The characteristics of different types of water samples collected in and around the Pandherkawada village, Nagpur are presented in Table 2.4. The results presented in Table 2.4 shows that the pH and EC of the water samples collected from different sources (irrigation water, well water and drinking water) varied from 7.19 to 7.62 and 820 to 3364 µS/cm respectively. The TDS contents in the water samples varied in the range of 460 to 1928 mg L-1. The total alkalinity and suspended solids in the water samples varied in the range of 180 to 230 mg L<sup>-1</sup> and 16 to 60 mg L<sup>-1</sup> respectively. The total hardness in the water samples varied from 196 to 876 mg L<sup>-1</sup> while the corresponding levels of calcium and magnesium varied from 32 to 195.2 mg L-1 and 28.3 to 94.6 mg L-1 respectively. The water samples collected from irrigation water, well water and drinking water shows the magnitude of COD and BOD in the range of BDL to 210 mg L<sup>-1</sup> and BDL to 48 mg L<sup>-1</sup> respectively. The levels of cations with respect to sodium and potassium in the water samples varied from 92 to 310 mg L<sup>-1</sup> and 2.1 to 15.2 mg L<sup>-1</sup> respectively. The concentration of chloride and BOD in the irrigation and well water samples were very high and may pose toxic effects on plant growth. The levels of anions with respect to chloride varied from 25.0 to 724.5 mg L<sup>-1</sup> whereas sulphate varied from 12 to 24 mg L-1 in the water samples respectively. The levels of heavy metals such as zinc, lead, cadmium, nickel, manganese, iron, chromium and copper presented in Table 2.4 indicate that their concentrations were within the permissible limit and do not pose any toxicity to plants species.

Sr.	Parameters	Irrigation water	Well Water	Drinking Water
INO.				
1.	рН	7.62	7.19	7.46
2.	EC, μS/cm	1120	3364	820
3.	TDS, mg L <sup>-1</sup>	742	1928	460
4.	Total Alkalinity, mg L <sup>-1</sup>	220	230	180
5.	TSS, mg L <sup>-1</sup>	60	20	16
6.	Total Hardness, mg L <sup>-1</sup>	240	876	196
7.	Calcium, mg $L^{-1}$	40	195.2	32
8.	Magnesium, mg L⁻¹	33.6	94.6	28.3
9.	COD, mg L <sup>-1</sup>	210	26	BDL
10.	BOD, mg L <sup>-1</sup>	48	9	BDL
11.	Ammonical Nitrogen, mg L <sup>-1</sup>	1.22	1.02	0.24
12.	Nitrate, mg L <sup>-1</sup>	0.53	1.77	4.87
13.	Sodium, mg L <sup>-1</sup>	92	310	105
14.	Potassium, mg L <sup>-1</sup>	15.2	6.2	2.1
15.	Chloride, mg L <sup>-1</sup>	98.5	724.5	25.0
16.	Sulphate, mg L <sup>-1</sup>	16	24	12
17.	SAR			
Heavy	Metals and Oxyanion , mg $L^{-1}$			
18.	Zinc	0.004	BDL	0.14
19.	Lead	BDL	BDL	BDL

 Table 2.4. Physico-chemical characteristics of different types of water samples collected in and around the Pandherkawada village, Nagpur

20.	Cadmium	BDL	BDL	BDL
21.	Nickel	BDL	BDL	BDL
22.	Manganese	0.26	BDL	0.52
23.	Iron	BDL	BDL	0.92
24.	Chromium	BDL	BDL	BDL
25.	Copper	BDL	BDL	BDL
26.	Boron	BDL	7.8	BDL
27.	Arsenic	BDL	BDL	BDL

The water samples (irrigation water and well water) contains appreciable amount of organic and inorganic constituents. Accordingly, CSIR-NEERI team visited the degraded site at Pandherkawada village, Nagpur and collected four soil samples viz. top soil, two profile soil samples from wastewater irrigated field and one from well water irrigated field at a different depths of 0-15 cm, 15-30 cm and 30-60 cm as shown in *Figure 2.9*Figure 2.9 respectively. The physico-chemical and microbiological characteristics of profile soil samples are presented in Table 2.5 to 2.9 respectively.

The particle size distribution presented in Table 14 with respect to percentage of sand, silt and clay in the soil profile samples collected in and around the degraded land varied from 14.0-29.8 %, 22.5-29.2 % and 44.7-60.0 % respectively. These results indicated that the soils belong to textural class "Clay". Similarly, the results of bulk density, porosity and maximum water holding capacity in the soil profile samples varied in the range of 1.25-1.32 mg m<sup>-3,</sup> 53.81-63.61 % and 42.29-59.02 % respectively. There were not much variations observed in wastewater irrigated and well water irrigated soils. The decrease in the bulk density and increase of water holding capacity and porosity in wastewater irrigated soils are due to continuous deposition of suspended solids through wastewater in comparison with well water irrigated soils.



Figure 2.9. Collection of profile soil samples from the degraded site at different depths from the Pandherkawada village, Nagpur

The results presented in Table 2.6 shows that the concentration of pH, soluble salts in terms of electrical conductivity, nutrients (nitrogen, phosphorous and potassium) and organic carbon of the profiles soil samples collected in and around the degraded land at Pandherkawada village, Nagpur. The pH of the soil was neutral to alkaline in reaction (7.40-8.60) with an electrical conductivity (EC) ranging from 2.14-3.70 dS m<sup>-1</sup> respectively. The wastewater irrigated soils are slightly alkaline to alkaline in nature, whereas well water irrigated soils are neutral in reaction. The higher value of pH and EC in the wastewater irrigated soils was due to large amount of inorganics and organics present in the applied wastewater. Similarly, the nutrients status with respect to nitrogen (N), phosphorous (P) and potassium (K) in the wastewater irrigated soils ranged from 0.02-0.08 %, 0.03-0.09 % and

0.42-0.91 % respectively. The organic carbon (OC) content showed higher concentration in top profile soil (0-15 cm), which decreased with the depth of the profile soil.

Sr.	Description	Depth,	Pa Dis	rticle Si stributi	ize on	Texture	Bulk Density.	Porosity,	Maximum Water
No.	lo. of Site o		Sand %	Silt %	Clay %	Class	mg m <sup>-3</sup>	%	Holding Capacity, %
	Red	0-15	18.0	24.0	58.0	Clay	1.25	58.04	59.02
1.	encrustation								
soil	soil								
Mart automation	Mastawatar	0-15	14.0	28.0	58.0	Clay	1.28	63.61	57.58
2.	irrigated field	15-30	20.0	24.0	56.0	Clay	1.29	60.55	56.26
		30-60	18.0	22.5	59.5	Clay	1.30	59.55	55.09
	Masteriater	0-15	14.0	28.0	58.0	Clay	1.27	63.21	58.01
3.	irrigated field	15-30	14.2	25.8	60.0	Clay	1.28	58.82	57.46
	in igated neid	30-60	14.0	28	58.0	Clay	1.30	57.01	55.49
	Well water	0-15	29.8	24.2	46.0	Clay	1.27	55.38	48.37
4.	irrigated field	15-30	23.9	26.8	49.2	Clay	1.30	54.81	44.67
	(control	30-60	26.0	29.2	44.7	Clay	1.32	53.81	42.29

Table 2.5 Particle size distribution of soils collected in and around the Pandherkawada village, Nagpur

Table	2.6.	Chemical	characteristics	of	profile	soil	samples	collected	in	and	around	the
Pandh	erkaw	ada village	, Nagpur									

					Par	ameters		
Sr. No.	Description of Site	Depth, cm	рН	EC, dS m <sup>-1</sup>	Total N, %	Total P, %	Total K, %	Organic Carbon, %
	Red	0-15	8.1	3.70	0.08	0.09	0.91	0.90
1.	encrustation							
	soil							
	Wastewater irrigated field	0-15	8.2	3.52	0.07	0.08	0.83	0.88
2.		15-30	8.3	2.51	0.04	0.06	0.64	0.53
		30-60	8.6	2.21	0.04	0.05	0.49	0.52
	Mastawatar	0-15	8.6	2.91	0.06	0.07	0.75	0.70
3.	wastewater	15-30	8.0	2.56	0.04	0.05	0.46	0.52
	in igated held	30-60	8.5	2.54	0.02	0.03	0.42	0.46
	Well water	0-15	7.6	2.52	0.05	0.06	0.59	0.65
4.	irrigated field	15-30	7.5	2.25	0.05	0.07	0.55	0.58
	(control	30-60	7.4	2.14	0.03	0.04	0.42	0.34

					Ра	rameter	s	
Sr. No.	Description of	Depth,	Са	Mg	Na	К	CEC	ESP
	Site	ciii						
	Red encrustation soil	0-15	35.3	10.7	1.59	0.8	49.0	3.24
1.								
	Wastewater irrigated field	0-15	37.6	9.2	1.24	0.6	46.5	2.67
2.		15-30	35.4	9.2	1.11	0.4	46.0	2.41
		30-60	34.3	8.7	1.06	0. 2	42.0	2.52
		0-15	39.6	11.6	1.22	0.3	52.0	2.35
3.	Wastewater	15-30	37.1	8.9	0.19	0.5	46.0	0.41
	Ingated field	30-60	35.2	7.6	0.09	0.4	42.0	0.21
	Well water	0-15	34.2	11.3	1.43	0.6	49.2	2.91
4.	irrigated field	15-30	32.6	13.9	1.24	0.6	46.0	2.70
	(control	30-60	30.3	7.6	1.18	0.4	44.7	2.64

Table 2.7. Chemical characteristics of soil profile samples collected in and around the Pandherkawada village, Nagpur

The results presented in Table 2.7 indicated the concentrations of exchangeable calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), cation exchange capacity (CEC) and exchangeable sodium percentage (ESP) of the soil profile samples. Amongst the exchangeable cations, calcium was predominant followed by magnesium, sodium and potassium. The concentrations of Ca, Mg, Na and K ranged from 30.3-39.6 cmol (p+) Kg-1, 7.60-13.9 cmol (p+) Kg<sup>-1</sup>, 0.09-1.59 cmol (p+) Kg<sup>-1</sup> and 0.20-0.80 cmol (p+) Kg<sup>-1</sup> respectively. The CEC and ESP in the soils varied from 42-52 cmol (p+) Kg<sup>-1</sup> and 0.21-3.24 respectively. It was observed that none of the soils attained ESP of 15 or more, so as to designate them as saline in nature. The high concentrations of sodium and alkalinity due to presence of carbonates and bicarbonates in the wastewater resulted in the enrichment in the soil exchange complex with sodium ions which reflected the rise in ESP in wastewater irrigated soils.

The concentration of heavy metals estimated in different types of soil profile samples in and around the degraded land at Pandherkawada village, Nagpur is presented in Table 2.8. The results indicated that the concentrations of total heavy metals viz. Cr, Zn, Pb, Cd, Mn, Fe and Cu were within the permissible limit. The slight built up of heavy metals in soils irrigated with wastewater may be attributed to wastewater irrigation.

The estimation of microbial populations in the soil is an important parameter as microbes play an important role in soil's bio-geochemical cycles. The results presented in Table 2.9 showed the development of soil microbial populations in terms of bacteria, fungi, actinomycetes, Azotobacter and Rhizobium. The counts of different microbial groups such as bacteria, fungi and actinomycetes in the wastewater irrigated soils varied from 90x107 - 10x109 CFU/g, 20x102 - 50x102 CFU/g and 65x104 - 12x106 CFU/g respectively. Whereas, the nitrogen fixing bacteria viz. Azotobacter and Rhizobium in the wastewater irrigated soils varied from 90x105 - 10x106 CFU/g and 10x103 - 87x104 CFU/g respectively.

Sr.	Description	Depth	He	avy	Metals	(mg kg	g <sup>-1</sup> )		Micr	onutrie	ents (mg	Kg⁻¹)	
No	of Site	(cm)	Cr	Ni	Pb	Со	Cd	Cu	Zn	В	Mn	Fe	Cu
	Red	0-15	71	63	15	23	0.98	62	87	6477	862	34651	62
1	encrustation												
	soil												
	Wastewater	0-15	71	72	14	26	0.98	43	68	6393	1077	34381	43
2	irrigated	15-30	74	65	16	26	1.01	44	78	6521	1048	34271	44
	field	30-60	72	68	15	25	1.04	44	70	6592	940.3	34171	44
	Wastewater	0-15	73.4	66	16	26	1.07	44	67	6570	1021	33961	44
3	irrigated	15-30	70	65	14	26	1.01	42	68	6544	958.4	33251	42
	field	30-60	72	66	15	26	1.08	43	67	6930	958.4	34066	43
	Well water	0-15	45	40	8.6	15	0.61	30	51	4530	972.5	22361	30
4	irrigated	15-30	45	42	9.2	15	0.68	31	53	4847	994	22322	31
4	field (control	30-60	43.2	41	9.13	14.9	0.58	32.1	54.3	4625	1024	30245	31

Table 2.8. Concentration of heavy metals in soil collected in and around the Pandherkawada village, Nagpur

Table 2.9. Microbiological characteristics of profile soil samples collected in and around the Pandherkawada village, Nagpur

Sr.	Description of	Depth	Microbial groups (CFU/g)								
No.	Site	(cm)	Bacteria	Bacteria Fungi Actinomycetes		Azotobacter	Rhizobium				
	Red	0-15	20×10 <sup>8</sup>	20×10 <sup>2</sup>	65×10 <sup>4</sup>	28×10 <sup>4</sup>	39×10 <sup>4</sup>				
1.	encrustation										
	soil										
		0-15	10×10 <sup>9</sup>	20×10 <sup>2</sup>	23×10 <sup>4</sup>	33×10 <sup>5</sup>	29×10 <sup>3</sup>				
2.	Wastewater irrigated field	15-30	18×10 <sup>8</sup>	10×10 <sup>2</sup>	49×10 <sup>4</sup>	48×10 <sup>5</sup>	10×10 <sup>3</sup>				
		30-60	21×10 <sup>7</sup>	58×10 <sup>1</sup>	19×10 <sup>6</sup>	88×10 <sup>4</sup>	27×10 <sup>2</sup>				
		0-15	90×10 <sup>7</sup>	50×10 <sup>2</sup>	12×10 <sup>6</sup>	15×10 <sup>6</sup>	15×10 <sup>5</sup>				
3.	Wastewater	15-30	13×10 <sup>6</sup>	20×10 <sup>2</sup>	16×10 <sup>5</sup>	10×10 <sup>6</sup>	20×10 <sup>5</sup>				
	ingated held	30-60	80×10 <sup>5</sup>	50×10 <sup>2</sup>	49×10 <sup>4</sup>	20×10 <sup>5</sup>	87×10 <sup>4</sup>				
	Well water	0-15	70×10 <sup>7</sup>	41×10 <sup>3</sup>	27×10 <sup>5</sup>	50×10 <sup>5</sup>	28×10 <sup>5</sup>				
4.	irrigated field	15-30	60×10 <sup>7</sup>	44×10 <sup>3</sup>	21×10 <sup>5</sup>	12×10 <sup>6</sup>	21×10 <sup>4</sup>				
	(control	30-60	52×10 <sup>6</sup>	20×10 <sup>3</sup>	16×10 <sup>5</sup>	90×10 <sup>5</sup>	15×10 <sup>4</sup>				

#### Cropping pattern

The baseline data was collected for the last five years i.e. from 2008 to 2012 with respect to cropping pattern from the Tehsil office, Tarodi, Nagpur. The data showed the reduction in crop yield and soil fertility which might be due to the continuous irrigation of the crops with wastewater. Further, the analysis of the degraded soil showed slight built up of heavy metals and micronutrients in the soil which directly affects the crop yield and quality and hence requires remediation. Further collection of data for remediation of degraded land is under progress.

# Task 2.3: Impact assessment of treated wastewater use in agriculture (ICRISAT, NEERI, TERI, MSSRF)

### Subtask 2.3.1 Assess effect of untreated and treated wastewater on soil properties in-vitro studies

### Subtask 2.3.2 Study effect of untreated and treated wastewaters on plant growth in pot culture

#### ICRISAT, Patancheru

#### Germination bioassay test

Seed germination experiment was conducted for different crops: maize, sorghum, pigeonpea, cowpea, chickpea, greengram, mustard and pearl millet. Two domestic wastewater concentrations (50 and 100%) were used in this test. Wastewater was collected from experimental site at ICRISAT campus. All the seeds showed very good germination percentage, seedling length, and vigour index with 50% dilution of waste water (Table 2.10). The pure municipal waste water also showed effect as 50% but the fungal growth in Petri plates effected the growth.

Reduction in seed germination percentage at higher concentration of effluent may be due to the higher amount of solids present in the effluent, which causes changes in the osmotic relationship of the seed and water. Thus reduction in the amount of water absorption takes place, which results into retardation of seed germination due to, enhanced salinity. The salt concentration, outside of the seed is known to act as limiting factor and it might be responsible for delay in germination (Adraino et al.,1973).

Crop	Treatment	Germination (%)	Vigour index	Radicle	Plumule
				length (cm)	length (cm)
Maize	Control	100	2632	9.34	5.12
	BHEL-50%	90	2388	13.65	6.03
	BHEL-100%	90	2330	12.98	5.97
Pigeonpea	Control	97	1172	2.65	5.83
	BHEL-50%	90	1179	3.68	6.17
	BHEL-100%	80	921	3.60	5.61
Chickpea	Control	93	1916	7.41	8.95
	BHEL-50%	90	2006	7.49	10.91
	BHEL-100%	86	1435	4.77	8.24
Sorghum	Control	87	1582	8.94	4.57
	BHEL-50%	85	1222	6.52	3.91
	BHEL-100%	75	1119	4.52	4.54
Pearl millet	Control	70	1301	9.99	2.94
	BHEL-50%	90	1112	5.91	2.17
	BHEL-100%	80	1138	8.18	1.99
Greengram	Control	30	404	3.89	6.26
	BHEL-50%	78	1702	4.12	11.14
	BHEL-100%	75	1365	3.98	9.47
Cowpea	Control	80	1183	5.46	5.13
	BHEL-50%	70	1549	9.48	8.22
	BHEL-100%	72	1508	9.23	8.68
Mustard	Control	77	1373	5.13	7.67
	BHEL-50%	90	1808	8.22	7.27
	BHEL-100%	83	1926	8.68	8.07

Table 2.10 Effect of domestic wastewater on seed germination, vigour index, radicle length, and plumule length.



Figure 2.10 Germination bioassay test for cowpea (left) and pigeonpea (right)



Figure 2.11 Germination bioassay test for maize (left) and chickpea (right)

# Subtask 2.3.3 Understand changes in soils irrigated with treated and untreated wastewater on biological and nutrient dynamics

#### Effect of domestic waste water on growth and yield of soybean

The effect of continuous discharge of domestic waste water was observed in soybean at the Main Agricultural Research Station, Dharwad. The land was divided into four blocks (with Block 1 - upland or control, Block-2, seepage of waste water at low to moderate rate, Block-3, seepage of waste water at high rate and Block-4, seepage of waste water at very high rate. The plant height (61.9 cm) of soybean was maximum at harvest where low to moderate waste water seepage was discharged (Table 25). Similarly, number of branches at harvest (6.5) and number of pods / plant at harvest (49.2) were higher with low to moderate seepage of waste water discharge as compared to soybean grown in the rest of the blocks. It indicates that where the history of discharge of waste water was

maximum reduction in plant height and number of pods were observed owing to continuous wetting. The yield of soybean and soil is yet to analyse.

						Number		
	Plant	plant	Plant	Number	Number	of		
	height	height	height	of	of	branches	Number of	Number of
	at 30	60	(cm) at	branches	branches	at	pods/plant	pods/plant
	DAS	DAS	harvest	30 DAS	60 das	harvest	at 60 DAS	at harvest
Block 1	41.0	50.0	55.9	3.7	5.20	6.7	38.3	40.6
Block -2	37.8	44.0	61.9	3.3	4.73	6.5	40.3	49.2
Block 3	35.0	48.3	51.9	3.5	4.60	5.1	33.5	39.6
Block 4	28.1	44.8	42.9	3.2	4.73	5.2	30.4	37.1

Table 2.11. Effect of domestic waste water on growth and yield of soybean

Block 1: Upland or control.

Block-2: Seepage of waste water at low to moderate rate.

Block-3: Seepage of waste at high rate.

Block-4: Seepage of waste water at very high rate.

### Deliverables (brief description and month of delivery)

Report on selected strains for microbial consortium (month 12)

## 3. Work Package: Agricultural Water Management

#### Objectives

- 1. Baseline characterization of five benchmark sites with respect to climate, soil, crops and irrigation
- 2. Improving water use efficiency through efficient irrigation systems, strategies and improved agronomic practices
- 3. Assess the impact of waste and low quality water on crop produce, soil and groundwater quality
- 4. Validate simulation models for assessing water use efficiency in the targeted production systems
- 5. Build capacity of community and stakeholders for improving saline wastewater use efficiency through integrated agro-aqua farming system

## Task 3.1: Identification of benchmark sites, baseline data collection and report preparation Subtask 3.1.1: Benchmark sites will be identified based on the representative abundance and severity of industrial and municipal wastewater

JISL

Benchmark Site is identified in the vicinity of Fruit Processing and Onion/Vegetable Dehydration Plant. One acre area is selected for the experiments. Two crops selected to grow at this site maize (0.5 acre) and banana (0.5 acre). Three sources for irrigation are fresh water, treated wastewater from onion dehydration plant and fruit processing plant. Hi Tech drip irrigation system are used with non-pressure compensating and pressure compensating drip. The irrigation system includes fully automatic controlled unit for irrigation and fertigation, soil moisture sensors, and sand and disc filter. Weather parameters observed during 2012 and 2013 are mention in Appendix B.

Soil samples were collected on Grid soil sampling in selected benchmark site. Soil samples collected from 0-10 cm, 10-20 cm, 10-30 cm depth. Collected soil sample were analysed for the various physical (soil texture, moisture retention, bulk density), chemical (pH, electrical conductivity, organic

carbon, N, P, K, Ca & Mg, Fe, Pb, Cd, Zn and other heavy NO3) and biological (pathogenic microorganisms) characters Figure 3.1).



Figure 3.1. Soil sampling at JISL experimental site

Table 3.1 Characteristics of soil at JISL experimental site.

		Depth	
Parameter	10 cm	20 cm	30 cm
Available Nitrogen (kg/ha)	188.16	163.07	137.98
Available Phosphorus (kg/ha)	16.43	2.23	Nil
Exchangeable Potassium (kg/ha)	92.91	126.79	151.62
Exchangeable Calcium (%)	0.14	0.12	0.12
Exchangeable Magnesium (%)	0.08	0.08	0.08
Available Iron (ppm)	4.96	4.77	3.59
Available Manganese (ppm)	2.36	3.52	3.06
Available Zinc(ppm)	0.52	0.37	0.4
Available Copper (ppm)	2.58	2.41	2.52
Available Sulphur (ppm)	8.06	7.06	6.65
Sand (%)	50.25	55.47	50.96
Silt (%)	16.23	33.7	23.96
Clay (%)	33.52	10.83	25.08
Bulk Density (gm/cc)	1.2	1.2	1.28
Boron (ppm)	0.28	Nil	Nil
Texture	Sandy Clay Loam	Sandy Loam	Sandy Clay Loam
Azotobactor species /gm	3x106	3x105	10000
Rhizobium species/gm	4x106	4x105	22x105
Phosphate solubilising bacteria species /gm	1000	1000	<1000
E.coli/gm	Not Detected	Not Detected	Not Detected
Xanthomonas species /gm	Not Detected	Not Detected	Not Detected
Erwinia species /gm	Not Detected	Not Detected	Not Detected
Agrobacter species /gm	<100	<100	<100
Fusarium species/gm	<100	<100	<100

There is deficiency of NPK nutrients in 10, 20, and 30 cm depth of soil layer, only at 30 cm depth K is found in sufficient amount. Calcium and Magnesium is available in sufficient amount throughout the soil profile up to 30 cm depth. There is deficiency of secondary nutrients in the soil except copper up to 30 cm of soil layer.

### ICRISAT, Patancheru, India (ICRISAT)

Location of the experimental site is shown in Figure 3.2. Previously this place was used as dumping site for used soils (soils used for pot culture experiments). The place was occupied by bushes and grasses. The site has been cleared for laying field experiments and constructing wastewater treatment system. Because of dumping different types of soils, there may be variation in soil characteristics across the entire site.



Figure 3.2. Site for field experiments and wastewater treatment system at ICRISAT

Subtask 3.1.2: Inventory of wastewater including quality assessment and status of present usage (ICRISAT, MSSRF, UASD, JISL, SABM)

### JISL

At JISL site, selected sources of wastewater are treated effluent from onion dehydration plant and fruit processing plants. Annual average availability of treated waste water generated from fruit processing is 200000 m<sup>3</sup> and from onion dehydration plant is about 150000 m<sup>3</sup>. Quantity and quality of wastewater from these two plant is mention in Appendix D and E. Table shows that, there is enough availability of treated effluent except in the month of April from fruit processing unit. Fruit processing undergoes annual maintenance to make it ready for Mango season. Similarly, treated effluent may not be available in enough quantity from Onion dehydration plant in months of October and November.

### Subtask 3.1.3: Soil analysis for physical, chemical and biological properties (ICRISAT, UASD) Subtask 3.1.4: Data analysis and report preparation (ICRISAT, UASD, MSSRF, JISL)

#### Selected benchmark sites and wastewater characteristics are mentioned in WP1 and WP2

#### Degraded soil due to long term use of spentwash for irrigation

At Ugar Khurd (Distillery), wastewater samples and soil samples were collected and analysed for different physical, chemical and biological properties. Continuous application of spentwash had no

effect on soil pH at various depths. However, continuous application of spentwash for more than 20 years resulted in relatively lower pH values. Application of spentwash for more than 20 years resulted in accumulation of salt, especially at the surface. The data on exchangeable calcium, magnesium, sodium and potassium as influenced by continuous application of spentwash resulted in higher concentration of these ions with increase in soil depth (Table 4, 5, and 6).



Figure 3.3 View of land affected by continuous application of spentwash for > 20 years

Wastewater			pH <sub>1:2.5</sub>			EC 1:2.5 (dS/m)						
use for	Depth (cm)											
	0-20	0-20 20-40 40-60 60-80 >80 0-20 20-40 40-60 60-80 >80										
5-10 years	8.1	8.0	8.1	8.	8.1	16.2	0.9	0.9	0.9	0.8		
10-15 years	8.1	8.1	8.1	8.1	8.0	0.8	0.8	0.8	0.8	0.9		
15-20 years	8.0	7.9	7.9	7.7	7.9	1.4	1.0	0.9	0.9	0.7		
> 20 years	7.9	7.8	7.9	7.9	7.9	4.7	3.9	3.5	2.9	2.5		

Table 3.2	Effect of	continuous	annlication	of spentwash	on soil nH a	nd FC
Table 3.2.	LITECTOL	continuous	application	UI SPEIILWASH	UII SUII PIT a	

Table 3.3. Effect of continuous application of spentwash on exchangeable Ca and Mg

Wastewater		Exch. Ca	a [cmol (	(p+) kg <sup>-1</sup> ]		Exch. Mg [cmol (p+) kg <sup>-1</sup> ]					
use for					Dept	h (cm)					
		20- 40- 60-									
	0-20	40	60	60-80	>80	0-20	20-40	40-60	80	>80	
5-10 years	43.9	45.4	44.4	45.1	44.1	10.5	8.1	9.5	11.3	9.9	
10-15 years	42.3	41.9	41.7	43.2	41.7	10.2	11.9	10.2	10.6	12.7	
15-20 years	41.8	41.0	43.5	45.2	43.5	4.5	3.0	9.8	13.4	11.0	
> 20 years	47.2	48.3	46.7	48.4	45.8	13.9	11.9	15.9	15.3	17.9	

Table 3.4. Effect of continuous application of spentwash on exchangeable K and Na

Wastewater		Exch. K	[cmol (	o+) kg⁻¹]		Exch. Na [cmol (p+) kg <sup>-1</sup> ]							
use for	Depth (cm)												
	0-20	>-20         20-40         40-60         60-80         >80         0-20         20-40         40-60         60-80         >80											
5-10 years	2.08	2.15	1.63	2.08	1.95	5.25	5.46	6.51	6.83	6.05			
10-15 years	1.63	1.82	1.43	1.56	1.82	6.41	5.88	5.67	5.78	5.04			
15-20 years	2.60	2.08	3.06	1.43	1.89	8.09	5.67	9.45	8.09	8.30			
> 20 years	3.25	3.06	2.47	2.67	2.73	10.40	10.19	9.77	11.13	9.87			

# Task 3.2: Design and implementation of irrigation systems based on crop water requirements

### Subtask 3.2.1: Selection of efficient irrigation systems (JISL)

To study the suitability of different emitter design and flow path geometry a clogging test protocol is prepared. Available emitters will be tested for clogging against effluent from fruit processing and onion dehydration plants. New emitter design and flow path geometry will be done to make the emitter suitable to be used with industrial waste water. Acceptance criteria for emitter are it shall have high emission uniformity (>85%), coefficient of manufacturers variation (CVm) shall be less than 5, it shall have better clog resistance, and components/raw material shall not have any deteriorating effect of chemical present in the water.

Initially to assess the suitability of emitters, selected emitters of different design, teeth geometry and inlet filters will be tested for both treated and untreated effluent from fruit processing unit and onion dehydration unit. Comparative analysis will be done for both Non Pressure Compensating Emitter and Pressure Compensating Emitter. For the clogging test, water before treatment and water after treatment is considered in order to assess the plugging resistance and filtration requirement for selected drip system. This can also give indication about severity of treatment to be done at effluent treatment plant to make it suitable for drip irrigation system.



Figure 3.4 Clogging test setup.

Dimensional	dotails of	h hatsat	rinners are	as follows:
Difficitsional	uctails U	icsicu u	I INNELS ALE	

	Description	Code	Description
Code			
L	Length of Labyrinth Channel	θ	Dentation angle
С	Width of Labyrinth Channel	В	Dentation Spacing
W	Dentation Width	Н	Dentation Height
D	Dentation Depth	Α	Area of Emitter Filter



#### **Dimensional details:**

Dripper	Length,	Width,	Depth,	Theta	В,	Н,	С,	Area of inlet	Filter
	L, mm	W, mm	D, mm		mm	mm	mm	filter, mm2	openings,
									mmxmm
A1.2	90	0.56	0.56	110°	1.94	0.65	1.35	8.2	2.72X0.40
A1.6	90	0.68	0.60	110°	1.94	0.64	1.35	11.6	2.72X0.40
A2.4	58	0.8	0.8	111°	2.13	0.64	1.35	5.4	0.42X2.66
A4.0	58	1.0	0.9	110°	2.54	1.21	2.16	5.4	0.46X1.73
B1.2	17	0.61	0.66	99°	1.39	1.0	2.1	3.4	0.51X0.27
B1.6	18	0.67	0.80	108°	1.52	1.12	2.4	5.4	0.60X0.33
B2.1	18	0.8	0.84	104°	1.74	1.47	2.99	26	0.6x0.3
B4.0	18	1.04	1.05	101°	2.28	1.87	3.89	26	0.6x0.3

Selection of emitter is to be done on basis of clogging resistance as per the protocol mentioned in Appendix H. Validation test includes, Pressure Vs Flow conforming to IS 13488:2008, determination of emitter exponent conforming to IS 13488:2008, clogging test conforming to JISL protocol as given in Appendix H. Eight type of emitters were selected for test. Details of emitters selected for wastewater from onion dehydration plant and fruit processing plant is shown in Table 3.5 and 3.6 respectively.

Table 3.5. S	pecification o	of emitter	selected for	onion deh	vdration wastewa	ter.
10010 01010	peenieation	i chinecci	50100100101	ormorr acri	yaracion masteria	

Sample	Type of Emitter	Tube Diameter mm	Discharge LPH	Class	Dripper spacing cm	Wall thickness, mm	
1	A1.6	16	1.6	2	40	0.72-0.82	
2	A1.2	16	1.2	2	40	0.70-0.74	
3	B1.6	16	1.6	2	40	0.73-0.79	
4	B1.2	16	1.2	2	40	0.71-0.76	

Table 3.6. Specification	of emitter selected	for fruit processing	wastewater.
--------------------------	---------------------	----------------------	-------------

Sample	Type of Emitter	Tube Diameter mm	Discharge LPH	Class Dripper spacing cm		Wall thickness, mm
1	A2.4	16	2.4	2	40	0.72-0.82
2	A4.0	16	4	2	40	0.70-0.74
3	B2.1	16	2.1	2	40	0.73-0.79
4	B4.0	16	4	2	40	0.71-0.76

Untreated waste water from fruit processing unit cannot be used for drip irrigation system without proper filtration. B type of emitter with 2.1 lph discharge has better results for untreated as well treated waste water. B-2.1 lph has finer inbuilt filter (Filter Size: 0.5x0.4mm) as compared to B-4 lph (Filter Size 0.6x0.5mm). Emitter's inbuilt filter plays significant role in performance against clogging. Treated water doesn't have any severe effect on emitter filter clogging, but in case of untreated waste water there is heavy clogging in A4, B4 type of emitter than A2.4, B 2.1. After peeling of emitters from tubes we found that, flow path was not clogged for treated as well as untreated waste water for all types of emitter under test.

	Onion dehydration plant wastewater							Fruit processing plant wastewater					1			
Days	A 1.6	lph	B1.6	lph	B 1.2	2 lph	A 1.2	lph	A 2.4	LPH	B 4	LPH	B 2.1	L LPH	A 4	lph
	U	Т	U	Т	U	Т	U	Т	U	Т	U	Т	U	Т	U	Т
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	6	0	2	0	7	0	3	0	0	0	0	0	0	0	5	1
3	4	0	4	0	6	0	3	0	2	0	2	0	0	0	7	1
4	9	0	9	0	7	0	1	0	2	0	2	0	0	0	8	3
5	4	0	4	0	5	0	1	0	1	0	2	4	0	0	7	2
6	9	0	8	0	7	0	2	0	2	0	3	3	0	0	7	4
7	8	0	8	0	7	0	3	0	2	0	3	5	0	0	6	5
8	8	0	8	0	7	0	4	0	2	0	4	3	0	0	5	4
9	8	0	8	0	9	0	4	0	2	0	6	1	0	0	4	2
10	9	0	8	0	8	0	4	0	2	0	8	0	0	0	7	1
11	10	0	8	0	7	0	6	0	0	1	2	8	0	0	1	5
12	10	0	9	0	6	0	7	0	2	0	6	1	0	0	5	2
13	9	0	8	0	8	0	9	0	4	0	7	2	2	0	5	2
14	9	0	8	0	5	0	8	0	5	0	8	4	1	0	8	2
15	9	0	8	1	8	0	10	1	5	0	5	2	4	0	8	3

Table 3.7. Effect of wastewater on emitter clogging

#### Subtask 3.2.2: Selection of irrigation strategies (ICRISAT, JISL, UASD)

# Task 3.3: Identify improved agronomic practices for enhanced water use efficiency (ICRISAT, UASD, SABM and JISL)

Field experiments were planned at selected site to test the improved agronomic practices for enhancing water use efficiency.

#### SAB Miller Sangareddy

At SAB Miller site a field experiment has been initiated on 6 acre farm (Figure 3.5). During rainy season maize/pigeonpea intercropping system was adopted on these farms. During rainy season crop will be cultivated under rainfed condition without irrigation. The nutrient status in the soil is presented in Table 3.8. Based on these soil test results recommended doses of fertelizers were applied in the field.

Farms	рН	EC	OC	Avail-P	י Exch-K Avail-S Ava		Avail-B	Avail-Zn
		dS/m	%	ppm	ppm	ppm	ppm	ppm
Farm 1	8.66	0.58	0.72	4.76	212	11.54	0.93	0.88
Farm 2	8.53	0.45	0.75	11.75	281	13.01	1.42	0.88
Farm 3	8.58	0.47	1.01	8.49	295	15.52	1.34	0.78

Table 3.8. Nutrient status in soil at SAB Miller Sangareddy farms.



Figure 3.5. Photograph taken while fertilizer application at SAB Miller, Sangareddy farms

#### ICRISAT, Patancheru

A site for field experiment and constructed wetland has prepared near the source of wastewater inside ICRISAT, Patancheru campus (Figure 3.6). The total area of the site is approximately 1.5 ha.



Figure 3.6. Photograph of field experiment site at ICRISAT, Patancheru

Task 3.4: Impact modelling through simulation

Task 3.5: Design and implementation of integrated agro-aqua farming systems using industrial treated water

Subtask 3.5.1: Exploring the quantum of availability of treated water from industries and saline affected land surrounding the industries along Tamil Nadu and Andhra Pradesh coast

K.C.P. Sugar and Industries Corporation Ltd in Vyuru and Laxmipuram was approached and discussed about the Water4Crops project and ascertained the availability of wastewater and land. Major concern is about the colour and odour of the effluent discharged from the distillery.

Proposed integrated agro-aqua farming system (IAAFS)



Figure 3.7. Framework for integrated agro-aqua farming system

Figure 37 depicts the proposed framework for demonstrating integrated agro-aqua farming system (IAAFS) using bio-treated wastewater. Water source for the IAAFS is from constructed wetland (CWL). Outlet of CWL will flow into the aqua farm and from aqua farm the water will be pumped to the agro farm on either side of the aqua farm. The water quality parameters will be closely monitored at regular intervals. The bottom sediment of aqua farm will also be used for crops after characterizing its quality. Tilapia and other suitable species will be cultured in aqua farm while locally cultivated agriculture (sugar cane and paddy) and horticulture crops (timber yielding trees like *Casuarina*, bamboo, subabul, neem fruit yielding trees like sapota, mango, guava and papaya and fodder grass) will be cultures studies and comparison of its growth and quality will be assessed for partially treated wastewater and biologically treated wastewater. The irrigation water from the canals used for agriculture will be used for control plants. In order to enhance water use efficiency appropriate irrigation methods will be adopted. Growth promoters will be used as per the condition and requirement. Pre and post microbial diversity study will be undertaken to assess the impact of wastewater reuse.

#### **Baseline survey**

The project not only aims at providing solutions to treat wastewater but also increase water management practices by enhancing water use efficiency for reusing in agriculture. Therefore it is imperative to ascertain the perception of the end users that is the farmers by conducting baseline survey. Hence both quantitative and qualitative methods of data collection are to be adopted to conduct baseline survey. In order to design the tool a Rapid Rural Appraisal was done in both L1 and L2 sites. The exercise enabled to understand the wastewater reuse practice in the villages surrounding the industries and community perceptions about it. Key observations from different locations and segment of people are presented below:

#### Site L1: Vuyyuru

In Vuyyuru, site L1 interaction with fruits, vegetable and sugarcane growers was undertaken during RRA and the observations are presented below:



Major crops grown by the farmers are turmeric, banana and vegetables like yam, cabbage, lady's finger, musk melon, cauliflower, okra and brinjal. Planting of vegetables is done in June and July while sugarcane is done in March and April. Farmers generally follow crop rotation for better yield. Major source of irrigation is canal supply from Krishna Barrage as well as ground water. Nearly 90% of the farmers have canal water supply however supplement with well water during lean seasons. Farmers' perception on using bio fertilizers is that (i) Bio-fertilizers from KCP are mainly used for annual crop, (ii) Bio fertilizers have increased the yield and (iii) Even though the fertility of the soil is not increased, it is at least maintained. However, farmers expressed that they are facing other issues like (i) red rots - borers and sucking pests, (ii) black gram and green gram which are affected by yellow mosaic disease occurs before flowering stage, (iii) root rot attacks turmeric severely and (iv) shift in canal water supply from June to August. The industry is extending support by providing fertilizers, chemicals, technical inputs, linking with bank for loan to the registered farmers of KCP.

#### Site L2: Lakshmipuram

#### **Farming community**

Major crops grown by Lakshmipuram farmers are sugarcane and paddy. Since sugarcane is water and labour intensive most of the farmers are converting to short term paddy crops. Followed by paddy pulses like green gram, black gram are also cultivated. Those farmers who has ground water source grow maize after paddy. Canal water supply from Krishna Barrage is the main source of irrigation. Wastewater is being used by some farmers during initial stage of paddy and later stages of sugarcane. Using wastewater began recently for sugarcane cultivation and 5 years before for the paddy. Industrial treated wastewater is used during summer season when there is no canal water supply. Farmers perceive that industrial wastewater is rich in organic load which reduces use of fertilizer and crops grow comparatively better in wastewater.

#### **Other issues**

Generally canal water was released from June to November but now it is only between August and January so there is shortage of water. On the other hand ground water is saline beyond 20 feet and soil is also gradually becoming saline

#### **Fishermen community**

Nearly 1500 families from 6 villages (Koththa maajeru, Paatheru, Chennapuram, Gundupaalam, Paladadhippa, Garaladhippa) depend on fresh water drainage canal for fishing. They are registered in Fishery Department as inland fishers and named as Venkateshwara Fishermen Society. Wastewater from sugar unit is released into the drainage canal intermittently which degrades the water quality. Due to water contamination fishes sometime die and float. Cattles if drinks that water gets foot and mouth diseases hence veterinary doctor suggested not allowing cattle to drink water

from it. It also causes skin problems to the people fishing in contaminated water. Livelihoods of fishing families depending on drainage canal are affected.

### Data collection tool and sampling

To accomplish the above objectives interview schedule was designed with along with ICRISAT. Farmers are both men and women and are sharing different roles in agriculture and allied activities. Components of the tool comprises demographic details, economic status, landholding details, cropping pattern, irrigation practices, farmers perception on using waste water, health impacts and institutional practices. However the interview schedule could not capture details on gender dimensions hence it is decided to use gender analysis tools to elicit the gender role and division of labour, access and control over resources as well as women's perception on wastewater reuse. Simple qualitative tools like matrix, problem tree, access and control will be executed in the focus group discussions with women.

Stratified sampling method will be adapted to baseline survey and the key strata would be land ownership, location to canal water supply as well as users of wastewater, press mud bio-fertilizer and fresh water users. If not proportionate sampling from each stratum, purposive sampling technique will be used to draw respondents. Concurrently, the tool will be pilot tested and translated in local language to enable both the field investigator as well as respondents for collecting reliable information.

It is proposed to create database on various aspects as follows:

- 1. Wastewater characterization Distillery and Sugar
- 2. Soil Characterization Demonstration site as well as from farmer's field
- 3. Microbial Diversity Demonstration site as well as from farmer's field
  - 4. Geo & Biophysical analysis
    - Demonstration site - Farmers
  - 5. Socio-economic & gender
- Demonstration site as well as from farmer's field
- Groundwater quality Demons
   Hydrological data All sites
- 8. Bio-fertilizer
- Vuyyuru Unit - All sites
- 9. Plant and fish growth- All sites10. Treated water quality- All sites

Of the above set of database formats so far designed and documentation is initiated for the first four while for the socio-economic aspect master table and coding sheet is drafted. Once data is collected it will be entered and maintained.

#### Mobilising and organising community

Mobilising and organising community and other stakeholders and organising are one of the activities. Though it has to be initiated in first year it continues till end of the project. However mobilization process is started through RRA in second year farmers will be identified and organised according to the need of the project.

#### Capacity building of different stakeholders

Identifying and building capacities of the stakeholders on bio-treatment of industrial wastewater and reusing the treated wastewater in integrated agro-aqua farming system are one of the activities. As of now the industrial partner viz. EID Parry and KCP sugars are providing wastewater and land are identified as major stakeholder. Other stakeholders like local NGO, academic institutes are also identified. Industrial partners, local NGO and University professionals are oriented on the project objectives and process who are sharing their resources and expertise in taking forward this project.
Capacity building of project staff of MSSRF is being done by undertaking exposure visits to successful constructed wetland models of SRM University, NEERI as well as participating in workshops organised by consortium partners. Two SRF is in the process of registering for Ph. D as part of WP 3 – Task 3.5.

### Documenting and disseminating the results

Documenting and disseminating the results is continuous process and so far documented the process such as:

- 1. Identification of industry and land
- 2. Wastewater characterization
- 3. Soil characterization
- 4. Designing of treatment plant and process
- 5. Designing of agro-aqua farming system

### Deliverables (brief description and month of delivery)

3.1 Benchmark sites characterized (month 12)

### 4. Work package: Development of water efficient crop varieties

### Objectives

- 1. Cross-species comparison for biomass production and water use efficiency in maize, sorghum, pearl millet and tomato
- 2. Better understanding of mRNA and mRNA transcriptome of sorghum and pearl millet
- 3. Mapping and characterization of quantitative trait loci (QTL) for drought tolerance related traits in maize, sorghum, pearl millet and chickpea
- 4. Improving drought adaptation using marker-assisted breeding and trait-based selection approaches in maize, sorghum, pearl millet and chickpea
- 5. Capacity building on NARS in research on drought adaptation of crops and integrated breeding for drought adaptation

# Task 4.1a: Analyze comparative abilities of maize, sorghum and millet association panel genotypes for biomass production and water use efficiency (ICRISAT)

Exchange of maize, sorghum and pearl millet material between EU and India (**Task 4.1 & 4.2**) is taking place. The initial considerations were the choice of genetic material and it has been agreed with colleagues at INRA to exchange only inbred materials (and not hybrids), since the main purpose is to explore genetic variation for common traits across species. The list of genotypes being distributed is given in Table 4.1 List of entries being distributed. Maize materials (germplasm) have now been received in India. Inbred materials of pearl millet (all FAO designated) are about to go as well as sorghum materials. The reason for the delay is because of the close scrutiny imposed on the export of genetic material from India. The maize germplasm are also about to be planted in the lysimetric system, alongside sorghum and pearl millet germplasm.

	5	
Maize	Sorghum	Pearl millet
MBS847	IS 393 (411) 659	Okashana 1 (ICMV 88908)
B73	IS 8347	IP 6179
FV-252	IS 20743	IP 13520
KY21	IS 25910	IP 20349
M017	SSM 275	IP 3110
W117U	IS 20763	IP 14311

Table	11	Lict	٥f	ontrioc	hoing	distributed
rable	4.L	LISU	0T	entries	being	aistributea

A188	IS 30619	IP 7953
W64A	IS 14276	IP 15857
CH10	IS 27791	IP 8647
EA1197	IS 29472	IP 6125
EP1	IS 31693	IP 6891
FC16	IS 16044	IP 9651
FV-2	IS 16173	IP 3471
FV-76	IS 8348	IP 9391
LP1233	IS 14556	IP 13363
CLA17	IS 15428	IP 12395
KUI3	IS 10876	IP 9351
CML69	IS 3583	IP 4542
LPSC7-F86	IS 10978	IP 4979
CML254	IS 3147	Check
CML287		
CML312		
CML341		
CML344		
CML444		
CZL04006		
DTPYC9-F74		
CML245		
SCMALAWI		
ZN6		

A prototype comparison of 10 genotypes of maize, 16 of sorghum and 10 of pearl millet entries has been carried out to design the stress conditions to apply to these crops in the lysimetric system, to design adequate water treatments in the lysimetric system. So far, we have used a water stress treatment by stopping irrigation at the time of flowering, which was then chronologically different in the three crops. A repeat trial has now been concluded in which we have submitted the three crop species to four water regimes, i.e. a fully irrigated control and three water stress treatments, each imposed by stopping irrigation at the time each species reached flowering. This trial has been harvested recently (late September / early October and the results will be discussed in the next mid-Year 2 report. The Figure 4.4 indicates the plant water use across a range of maize, sorghum and pearl millet genotypes, under both well-watered conditions (WW) and water stress (WS), from the initial trial carried out in the post-rainy season 2012-13.



Figure 4.1. Plant water use in maize, sorghum and pearl millet across a range of genotypes under wellwatered (WW) and water-stressed (WS) conditions.

### Task 4.2a: Characterization and response of maize, energy-dedicated sweet sorghum and pearl millet isogenic lines to water deficits (ICRISAT)

The testing of the pearl millet inbred germplasm association panel (PMiGAP) has been carried out and shows large range of variation for TE (see figure below – about 50% range of variation). This variation is lower than what was earlier identified in sorghum (See Vadez et al., 2011 – Crop and Pasture Science 62 (8) 1-11), or what is shown below in Figure 4.2.

Contrasting line have been identified for further investigation and also part of the most contrasting have been selected for sharing with INRA partners. A response of transpiration to a ladder of increasing VPD conditions has been carried out with the most contrasting 20 entries for TE. A large range of variation was identified in these response, some germplasm being capable of restricting transpiration under high VPD (low response slope), whereas other had an un-restricted response (high slope). However, different from what has been found in other pearl millet material (recombinant inbred lines), there was no direct relationship between the response to VPD and the differences in transpiration efficiency.



Figure 4.2. Variation in transpiration efficiency in 260 genotypes of pearl millet



Figure 4.3. Variation in transpiration efficiency in 15 sweet sorghum entries

### Task 4.2b: Mapping of genomic regions controlling traits related to drought tolerance/WUE in tomato (UASB)

This sub-task is aimed at assessing genetic diversity among wild and cultivated tomato germplasm lines for traits related to water use efficiency (WUE) and yield, standardizing protocols for root studies, and mapping genomic regions controlling traits related to WUE and fruit yield using linkage mapping and association mapping.

A total of 403 germplasm accessions of different species were obtained from different institutions (Table 4.2).

S. No.	Species	Institute	No. of germplasm
1	Solanum lycopersicum	Regional Station, NBPGR, Hyderabad	150
2	Solanum sp.	NBPGR, New Delhi	50
3	Solanum pimpinellifolium	Regional Station, NBPGR, Hyderabad	19
4	Solanum peruvianum	AVRDC Taiwan	8
5	Solanum lycopersicum × Solanum pimpinellifolium	AVRDC Taiwan	1
6	Solanum lycopersicum	AVRDC Taiwan	20
7	FRESH MARKET TOMATO	AVRDC Taiwan	7
8	PROCESSING/FRESH MARKET TOMATO	AVRDC Taiwan	6
9	CHERRY TOMATO	AVRDC Taiwan	1
10	HIGH BETA CAROTENE TOMATO	AVRDC Taiwan	3
11	<i>Solanum lycopersicum</i> (VI series)	Regional Station, AVRDC, Hyderabad	7
12	S.cecareforme	IIVR, VARANASI	1
13	S.peruvianum	IIVR ,VARANASI	2
14	Solanum pimpinilifolium	IIVR , VARANASI	2
15	Cultivated Variety	IIVR , VARANASI	10
16	Solanum sp.	AVRDC, Taiwan	37
17	Solanum habrochaites	TGRC, USA	6
18	Solanum pimpinellifolium	TGRC, USA	17
19	Solanum lycopersicum	TGRC, USA	26
20	Solanum galapagense	TGRC, USA	3
21	Solanum pennellii	TGRC, USA	10
22	Solanum chmielewskii	TGRC, USA	3
23	Solanum huaylasense	TGRC, USA	1
24	Solanum arcanum	TGRC, USA	5
25	Solanum chilense	TGRC, USA	8
TOTAL			403

 Table 4.2 Details of tomato germplasm obtained from different institutes

Drought is a major constraint to tomato production in semi-arid tropics and subtropics. Stress avoidance characters such as water use efficiency (WUE), leaf characteristics to conserve tissue water, stomatal and cuticular characteristics, root characteristics their extraction efficiency, which favours maintenance of higher tissue water content under receding moisture stress, only postpone the immediate effect of moisture stress. Therefore, under severe moisture stress conditions, the

intrinsic tolerance mechanism becomes more relevant. Under rainfed situations, where the crop is subjected to cycles of stress, survival at the end of stress and recovery on alleviation is important. In view of this, the present investigation was planned to identify variability in different drought adoptive mechanisms among different tomato genotypes with the following objectives.

- To screen the tomato germplasm for drought tolerance or to assess genetic diversity among wild and cultivated tomato germplasm lines for traits related to WUE and fruit yield.
- To assess the variability among root traits for identification of water use efficient genotypes.
- Standardization or characterization of sampling day for root traits among different species of tomato.
- Development of mapping population and Identification of DNA markers polymorphic to parents of mapping population.
- Genotyping F<sub>2</sub> and Phenotyping F<sub>3</sub> mapping populations for traits related to WUE and yield and Mapping of genomic regions controlling traits related to Water Use Efficiency and fruit yield.
- Phenotyping and Genotyping of cultivated and wild germplasm accessions with informative markers to establish association with traits related to WUE and fruit yield.

### 4.2b.1 Evaluation of tomato germplasm for drought tolerance

### 4.2b.1.1 Details of experiment:

**Experimental site:** The experiments were conducted in  $K_1$ -block University of Agricultural Sciences (UAS), GKVK, Bangalore, Karnataka. UAS is situated in zone-5 of Eastern dry zone of Karnataka state at an altitude of 930 m above mean sea level 12° 58' North and 77°35' East latitude and longitude, respectively. The meteorological data during the period of experiments are recorded at the meteorological observatory of Agricultural Research Station, Bangalore. The mean temperature during first experiential period varied from 30.9°C (March 2013) to 37.9°C (May 2013). The experimental site consisted of medium red sandy loam soil. The experiment received 5mm rainfall after 20 days of water stress (60-80 days after sowing).

**Experimental material and methods:** The material for the present study comprised of 116 indigenous and exotic germplasm accessions of tomato (*Solanum lycopersicum* L.) and 3 check entries (Arka ahuti, Arka vikas and Pusa ruby) procured from the National Bureau of Plant genetic resources (NBPGR) New Delhi and Asian Vegetable Research and Development Centre (AVRDC) Taiwan. The check entries are popular released varieties in India.

One hundred and sixteen germplasm accessions and three check entries were sown in Augmented design (Federer, 1956) during *summer* 2013, under stress and control conditions. The stress experiment consisted of three blocks and each block with 31 germplasm accessions, three checks and two border entries. While in control experiment four blocks and each block with 29 accessions, three checks and two border entries. Each entry was transplanted in a single row of 5 meters length with a row spacing of 0.60 m and 0.3 m between plants within a row. The total area of the experimental site was 820 m<sup>2</sup>. Ridges were prepared at a spacing of 60 cm. Healthy and uniform seedlings were transplanted on one side of the ridges with a spacing of 60 cm between the plants and recommended agronomic and plant protection practices were followed during the crop growth period to raise a healthy crop.

Fertilizers at the rate 60 kg N, 50 kg  $P_2O_5$  and 30 kg  $K_2O/ha$  were applied uniformly to all the genotypes. Half the dose of nitrogen and entire dose of phosphorous and potassium were applied before transplanting of seedlings and remaining 50 *per cent* of nitrogen was applied four weeks after

transplanting. Three weeks after transplanting, plants were supported to galvanized wire which was tied to bamboo poles. Other cultivation operations including plant protection measures were carried out as per recommended package of practices of University of Agricultural Sciences, Bangalore (Anon., 2007).

Observations on morphological parameters viz., days to fifty percent flowering, days to first fruit set, plant height, number of branches per plant, fruit traits like average fruit weight, fruit length and width, fruit number, fruit yield per plant and traits related to WUE like SPAD chlorophyll meter reading(SCMR), Specific leaf area(SLA) and SCMR was measured using SPAD chlorophyll meter, SLA was computed by measuring leaf area and leaf dry weight (SLA =leaf area (cm<sup>2</sup>)/ leaf dry weight (g)) fruit length and width measured using verniar caliparse.

**Treatment imposition**: Drought was imposed 60 days after sowing or 30 days after transplanting to all the genotypes by withholding irrigation in stress plot for twenty days. The control plot was given normal irrigation at weekly intervals.

**Collection of Experimental Data:** Five randomly chosen plants in each genotype were labeled and used for recording different morphological, physiological and fruit yield parameters at appropriate stage of crop growth. The mean of five plants was taken for analysis.

- The characters studied and techniques adopted to record the observation are given below
  - Morphological characters: Plant height (cm), Number of branches per plant.
  - Drought tolerance/WUE related physiological characters: Specific leaf area (SLA), SPAD Chlorophyll Meter Reading (SCMR).
  - Fruit Yield parameters: Number of fruits per plant, Yield (kg.plant<sup>-1</sup>) Average fruit weight (g), fruit length(cm), fruit width(cm)

### 4.2b.1.2 Experimental results

### Analysis of variance of tomato germplasm accessions for quantitative traits.

The analysis of variance indicated significant amount of variation among all the genotypes for most of the traits studied except days to fifty percent flowering, number of branches per plant and fruit parameters.

### Identification of drought tolerant tomato genotypes based on various methods of screening.

- > Mean of control and stress values for genotypes.
- > Per cent reduction in stress plot over control
- Correlation co-efficient ('r' value)
- > Yield at stress plot which yields more than check (Pusa ruby) 1.06 kg/plant

### Mean of control and stress values for genotypes.

**Mean value for fruit yield related traits:** The mean value for average fruit weight among the genotypes ranges from 168.48g (EC 677 068) to 19.79g (EC 610661) and genotypes EC 677068, EC 582 611, EC 686703, EC 586980 and EC 582631 were found superior for average fruit weight. The range of mean values for number of fruits per plant varies from 63.90 (EC 582 629) to 12.70 (EC 676771) and the genotypes EC 582629, EC 677078, EC 608468, EC 676794 and L- 02831were observed to have higher number of fruits per plant. The mean fruit yield per plant ranged from 2.70 kg (EC 676794) to 0.34kg (BC-1198) and genotypes EC 676 794, EC 677 123, EC 676780, EC 582629 and EC 608411 were found promising for higher fruit yield per plant.

		Morpholog	ical Paramet	ers		Physiolog	ical	Fruit Parar	neters			
Source of	Degrees	Days to	Days to	Plant	No of	SPAD	SLA	Average	Fruit	Fruit	No of	Fruit
variation	of	50 %	first fruit	height	branches		(cm <sup>2</sup> g <sup>-1</sup> )	fruit	length	width	fruits	yield/pl
	freedom	flowering	set	(cm)	/plant			weight	(cm)	(cm)	plant⁻¹	ant(kg)
Block	3	11.26	4.39	157.65	3.53	343.60	1105.57	1844.80	0.90	5.64	238.10	0.36
Genotypes	118	8.88	8.71	171.14	1.61	302.96	1185.14	419.58	0.61	0.66	135.54	0.34
Checks	2	0.59	0.58	17.16	0.28	0.59	312.42	5.83	0.03	0.08	31.11	0.01
Varieties	115	9.35	8.87	167.62*	1.73	280.90*	1230.64*	477.90*	0.64	0.73	136.27*	0.36*
Check vs	1	-28.02	6.28	884.08	-9.41	3444.45	-2302.98	-5460.66	-0.86	-5.74	261.05	-0.93
varieties												
Error	6	10.47	8.69	31.86	1.24	1.62	550.44	47.57	0.49	0.51	43.47	0.03

Table 4.3. Analysis of variance for morphological, fruit yield and physiological traits under control condition

Table 4.4. Analysis of variance for morphological, fruit yield and physiological traits under stress condition

		Morphologic	cal Paramet	ers		Physiolog	ical	Fruit Para	meters			
Source of	Degrees	Days to	Days to	Plant	No of	SPAD	SLA	Average	Fruit	Fruit	No of	Fruit
variation	of	50%	first fruit	height	branche		(cm <sup>2</sup> g <sup>-1</sup> )	fruit	length	width	fruits	yield/
	freedom	flowering	set	(cm)	s/plant			weight	(cm)	(cm)	plant⁻¹	Plant
												(kg)
Block	2	6.66	1.20	298.55	4.1491	493.23	488.42	508.45	1.44	3.82	331.72	0.11
Treatments	116	6.56	6.44	197.26	1.6550	323.00	974.07	418.17	0.77	0.72	121.59	0.23
Checks	2	10.11	1.33	95.34	1.2825	4.12	87.82	115.62	0.12	0.2171	2.93	0.09
Varieties	113	6.62	6.32	198.21*	1.7168	331.03*	951.38*	436.06*	0.79*	0.74	124.07*	0.23 *
Check vs	1	-7.94	30.83	294.76	-4.5782	53.47	5310.32	-998.36	-1.08	-0.47	78.71	-0.10
varieties												
Error	4	6.78	6.33	35.38	1.00	31.27	135.86	78.11	0.22	0.40	6.70	0.02

**Mean value for drought related physiological traits:** The mean SPAD chlorophyll meter reading (SCMR) values ranged from 89.10(L-02846) to 17.14(L- 02831) and genotypes L-02846, EC 686 98, EC 676 770, EC 677 068 and EC 608 468 were having higher mean chlorophyll content indicating their tolerance to drought. The mean Specific leaf area (SLA) values ranged from 87.65 cm<sup>2</sup>g<sup>-1</sup> (EC 582 632) to 264.06 cm<sup>2</sup>g<sup>-1</sup> (EC 608 381) and genotypes EC 686 529, EC 608 377, EC 608 391, EC 677 095, EC 608 381 with lower mean SLA were found promising and drought tolerant.

#### Per cent reduction in stress plot over control plot.

*Per cent* reduction of fruit yield in stress over control condition ranges from 1.93% (EC 677 105) to 81.89% (EC 671 599) and genotypes EC 677 105, EC 676 804, EC 677 100, EC 676 922 and EC 677 071 with lower fruit yield reduction found tolerant to drought due their inherit mechanism to withstand water stress. While, the genotypes EC 671599, EC 677051, LA-01049, BC-1198 and EC 676730 with higher *per cent* fruit yield reduction were found highly drought susceptible.

#### Correlation between fruit yield with morphological and WUE related traits.

The fruit yield per plant was found to be significantly positive association with number of fruits per plant, number of branches per plant, fruit length, fruit width and plant height. While significant negative association with days to fifty *per cent* flowering and days to first fruit set. SPAD chlorophyll meter reading (SCMR) was found to be significantly negative association with Specific leaf area (SLA) exhibiting that genotypes with higher SCMR and lower SLA would be drought tolerant. The genotypes (EC 677 078, EC 608 468, EC 686703 and EC 58 629) which had maximum number of fruits per plant and branches per plant were able to produce more yield both under normal and water stress condition.

The fruit yield per plant was found to have significant positive association with number of fruits per plant, fruit length, fruit width and significant negative association with days to fifty *per cent* flowering and days to first fruit set. The trait SCMR had negative association, while character SLA had positive association with fruit yield per plant both under control and stress condition indicating genotypes with higher SCMR and lower SLA to be drought tolerant with higher yield. The genotypes (EC 582 629, EC 608 468, BC-CLN-44, EC 676 794 and EC 676 750) which had maximum number of fruits per plant were able to produce more yield both under normal and water stress condition. Hence, selection for the character fruit number under stress condition may increase fruit yield.

	Days to	Days to	SPAD	SLA	No of	Average	Fruit	Fruit	Plant	No of	Fruit
	50%	TIrst		(cm²/g)	Truits	truit	length	width	neight	branche	yield
	flowering	fruit set			plant	weight(g)	(cm)	(cm)	(cm)	S	(kg)
Days to 50% flowering	1.000	0.816 <sup>**</sup>	0.126	-0.097	-0.116	-0.212*	-0.298 <sup>**</sup>	-0.220 <sup>*</sup>	-0.039	0.045	-0.262**
Days to first fruit set		1.000	0.094	-0.080	-0.103	-0.184 <sup>*</sup>	-0.371**	-0.300**	-0.054	-0.065	-0.258 <sup>**</sup>
SPAD			1.000	-0.185 <sup>*</sup>	-0.119	0.064	0.033	-0.152	-0.072	0.107	-0.027
SLA(cm <sup>2</sup> g <sup>-1</sup> )				1.000	0.023	-0.036	0.116	0.031	0.012	0.039	0.042
No of fruits plant <sup>-1</sup>					1.000	0.077	-0.093	-0.040	0.131	0.370 <sup>**</sup>	0.637**
Average fruit weight(g)						1.000	0.179	$0.281^{**}$	0.145	0.084	0.252**
Fruit length(cm)							1.000	.449 <sup>**</sup>	0.085	-0.058	0.250 <sup>**</sup>
Fruit width(cm)								1.000	0.229 <sup>*</sup>	0.085	0.302**
Plant height (cm)									1.000	0.393**	0.268 <sup>**</sup>
No of branches/plant										1.000	0.382**
Fruit yield/plant(kg)											1.000

Table 4.5. Correlation between fruit yield with morphological and drought related traits under stress condition

Table 4.6. Correlation between fruit yield with morphological and drought related traits under stress condition

	Days to	Days to	SPAD	SLA	No of	Average	Fruit	Fruit	Plant	No of	Fruit
	50%	first fruit		(cm <sup>2/</sup> g)	fruits	fruit	length	width	height	branch	yield
	flowering	set			plant <sup>-1</sup>	weight(g)	(cm)	(cm)	(cm)	es	(kg)
Days to 50% flowering	1.000	0.693**	0.120	-0.147	-0.215 <sup>*</sup>	-0.174	-0.301**	-0.183	0.029	0.008	-0.379 <sup>**</sup>
Days to first fruit set		1.000	0.077	-0.207 <sup>*</sup>	-0.291**	-0.005	-0.300**	-0.231*	0.012	-0.074	-0.357**
SPAD			1.000	-0.111	-0.125	0.027	-0.061	-0.126	-0.074	0.105	-0.124
SLA(cm <sup>2</sup> g <sup>-1</sup> )				1.000	0.103	-0.044	0.184 <sup>*</sup>	-0.080	-0.004	0.071	0.105
No of fruits plant <sup>-1</sup>					1.000	0.010	0.091	0.067	0.109	0.212*	0.615**
Average fruit						1 000	0 1 1 7	0 227*	0.150	0 1 2 6	0 1 9 2
weight(g)						1.000	0.117	0.257	0.159	0.120	0.162
Fruit length(cm)							1.000	0.354**	0.088	-0.006	0.306**
Fruit width(cm)								1.000	0.146	0.011	0.285**
Plant height (cm)									1.000	0.328 <sup>**</sup>	0.010
No of branches										1.000	0.041
Fruit yield(kg)											1.000

Based on the above results it may be concluded that the genotypes EC 676 728, EC 676 794, EC 676 783, EC 686 703 and EC 677 078 were identified as high yielding genotypes under well irrigated condition.

Genotypes	Fruit yield/ plant (kg)	Number of branches/plant	Number of fruits /plant	Average fruit weight(g)
EC 676 728	3.30	8.40	42.40	55.25
EC 676 783	3.03	7.80	50.80	67.83
EC 676 794	2.99	7.80	58.60	74.69
EC 686 703	2.27	9.60	45.60	98.00
EC 677 078	2.03	8.80	71.00	70.40

Table 4.7. High yielding genotypes under well irrigated condition.

The genotypes EC 676794, EC 677123, EC 676780, EC 608 411, EC 676783 and EC 582629 were identified as drought tolerant source based on fruit yield, SLA and SCMR may be included in the future crop improvement program

Sl.no		Average fruit	Number of branches per	Number of fruits per	Fruit yield per
	Genotype no	weight(g)	plant	plant	plant(kg)
1	EC 676 794	68.55	6.40	54.40	2.42
2	EC 582 629	52.82	4.00	60.00	2.29
3	EC 677 123	52.49	5.75	40.50	2.20
4	EC 676 780	43.77	5.80	28.40	2.04
5	EC 608 411	45.10	4.67	47.33	1.87
6	EC 676 922	40.00	5.00	29.40	1.87
7	H - 1996	19.64	4.00	42.00	1.79
8	EC 676 808	45.90	5.20	30.00	1.76
9	EC 676 750	55.41	3.50	54.00	1.74

Table 4.8 List of top high yielding drought tolerant tomato germplasm accessions



Figure 4.4. Tomato plot at harvest stage (top) and Solanum pimpinellifolium (bottom)

### 4.2b.2 Studies on assessment of variability among root traits for identification of water use efficient genotypes

With the prediction of extreme events due to climate change, world over now there is more emphasis on breeding varieties and hybrids that can tolerate drought. The prerequisite for such breeding programme is identification of genotypes with one or more such traits that impart tolerance. Among many traits that associated with drought tolerance, root characters and water use efficiency (WUE) related are considered to be most relevant as the former is related to acquisition of water from soil and latter referring to efficient use of absorbed water. Therefore, an experiment was conducted to identify genotypes with better root growth and WUE traits.

### **Materials and methods**

Thirty diverse germplasm lines from NBPGR, New Delhi and AVRDC, Taiwan were transplanted during summer 2013 in two specially constructed root structures of 20 m length, 1.5 m height and 2.4 m width on either side of central 30 cm permanent wall. Thirty lines of six genotypes each were planted per structure and were replicated twice. The spacing adopted was 60 x 30 cm and recommended dose of fertilizers were applied.

Plants were grown for 70 days and then root structures were dismantled partly and only three plants out of six along with roots were separated using water. Observations on morphological parameters

viz., days to fifty *per cent* flowering, number of branches per plant, shoot length, shoot dry weight, WUE related traits like SPAD chlorophyll meter reading (SCMR), Specific leaf area (SLA) and root characters ie., root length, root dry weight, root volume and root: shoot ratio. SCMR was measured using SPAD chlorophyll meter. SLA was computed by measuring leaf area and leaf dry weight (SLA =leaf area (cm<sup>2</sup>)/ leaf dry weight (g)). Root volume was measured by quantifying the amount of water displacement method.

SI.	Accession								
no	number								
1.	EC-608438	7.	EC-677204	13.	EC-68698	19.	EC-677034	25.	EC-608394
2.	EC-608468	8.	EC-676730	14.	EC-686702	20.	EC-677076	26.	EC-608419
3.	EC-610643	9.	EC-610654	15.	EC-686703	21.	EC-677080	27.	EC-676816
4.	EC-685705	10.	EC-521038	16.	EC-588221	22.	EC-676770	28.	H-7996
5.	EC-677041	11.	EC-686531	17.	EC-677065	23.	EC-608271	29.	CLN-2070A
6	EC 676742	12		10	EC 677079	24	EC 609277	20	Sankranthi(C
0.	EC-070745	12.	EC-06067	10.	EC-077078	24.	EC-008577	50.	heck)

Table 4.9 List of tomato germplasm accession used for root studies

Analysis of variance revealed highly significant variation among germplasm accessions for all the traits studied. The mean, range and standard deviation for different shoot and root characters is presented in Table 4.11. Larger genotypic variation is seen for these characters. The range for days to fifty *per cent* flowering among tomato accessions varied from 45 to 60.5 days with an average value of 49.08. The genotypes EC 588221, EC 677034, EC 677076 and EC 608271 were of early flowering hence provide mechanism of drought escape. The number of branches per plant varied from 4.5 to 9.5 and lines EC 677065, H 1996, EC 588221 and EC 677041 had more branches per plant. The genotypes EC 608419, EC 677076, EC 676743 and EC 677078 had higher mean values for fruits per plant and range for number of fruits was 16.5 to 118. The fruit yield per plant is the most important trait and genotypes EC 676743, EC 677076, EC 610654 and EC 608419 were found to be superior with maximum fruit yield.

Plant root system plays an important role in regulation of water uptake and extraction from deep soil layers. A positive linear relationship between root and shoot length was noticed among genotypes EC676743 and EC677034 and these had higher mean value for shoot and root length. The range of values for shoot length and root length are 48.25 to 990 cm and 42.15 to 98.8 cm, respectively. Different germplasm lines recorded root volume ranging from 10.5 to 32.5 cm<sup>3</sup> and average being 4.59 cm<sup>3</sup>. The genotypes EC676743, EC677065, EC68698 and EC608271 had higher mean value for root volume. Root dry weight varies from 3.2g to 16.0g and genotype EC676743, EC608438, EC676730 and EC610643 exhibited higher dryweight. Root length and dry weight showed significant positive correlation with fruit yield under stress. Alternate approaches for measuring WUE include measuring SLA and SCMR. SLA is used as alternate method for estimation of genetic variability for transpiration efficiency or water use efficiency (WUE) and Similarly SCMR can also provide a good estimate of leaf chlorophyll content. Variation for SCMR is from 14.57 - 41.60 and for SLA it ranged from 99.43- 209.72 cm<sup>2</sup>g<sup>-1</sup>. The genotypes EC608419, EC676770,



Figure 4.5. (1&2) Structures for root studies, (3) Uprooting of tomatoes for root studies, (4) Tomato plants uprooted for root studies, (5) Variations for root length, and (6) A field view



Figure 4.6 Variation for root length (top) and field staff recording observations (bottom)

Source of variation	Degrees of freedom	Days to 50% flowering	No of branche s plant <sup>-1</sup>	No of fruits plant <sup>-1</sup>	Fruit yield plant <sup>-1</sup>	Shoot length (cm)	Shoot dry weight (g)	SCMR	SLA (cm <sup>2</sup> g <sup>-1</sup> )	Root length (cm)	Root : Shoot ratio	Root volume (cm <sup>3</sup> )	Root dry weight (g)
Treatment	29	19.52		1170.57				78.67*	1797.40	287.10			
			2.63*	*	3.49*	223.35*	332.18*		*	*	0.05*	42.23*	11.46*
Replication	1	2.82	0.61	220.42	0.24	80.27*	72.38	2.68	167.77*	14.11	0.01	6.06	1.21
Error	29	0.95	0.80	49.14	0.12	25.9	25.35	6.33	51.69	45.10	0.01	4.05	0.72
SE±M		0.69	0.63	4.96	0.25	3.61	3.56	1.78	5.08	4.75	0.08	1.42	0.59
SE±D		0.98	0.89	7.01	0.35	5.09	5.03	2.52	7.19	6.72	0.11	2.01	0.85
CD @ 0.05		1.99	1.83	14.34	0.72	10.43	10.29	5.15	14.70	13.73	0.23	4.12	1.73
CV		1.99	13.92	11.84	11.47	7.55	11.11	11.02	4.66	10.50	11.74	10.97	14.45

Table 4.10. Analysis of variance for morphological, fruit yield and root traits among tomato germplasm accessions

Table 4.11. Mean, range and standard deviation for different root and shoot characters in tomato germplasm accessions

Characters	Days to 50% flowering	No of branches	No of fruits	Fruit yield per plant(kg)	Shoot length(cm)	SCMR	SLA (cm <sup>2</sup> g <sup>-1</sup> )	Root length(cm)	Root: Shoot ratio	Root volume(c m³)	Shoot dry weight(g)	Root dry weight(g)
Range	45 - 60.5	4.5 -9.5	16.5-	0.58 -	48.25-	14.57 -	99.43-	42.15-98.8	0.65 -	10.5 -	20.5 –	3.2 – 16.0
			118	5.48	99.00	41.60	209.72		1.31	32.5	79.0	
Mean	49.08	6.43	59.22	3.06	67.50	22.84	154.13	63.955	0.96	18.35	45.32	5.86
Standard												
deviation	3.12	1.15	24.19	1.32	10.56	6.27	29.98	11.98116	0.16	4.59	12.88	2.39

EC677065 and EC677034 with lower SLA found to have higher water use efficiency. While for SCMR the genotypes EC677041, EC610654, EC521038 and EC676770 had higher chlorophyll content. Thus, the lines EC676730, EC686531, EC677076, EC677080, CLN2070A and EC686703 were identified as high fruit yielding and Water use efficient genotypes based on root and shoot traits

SI no	Genotypes	SCMR	SLA (cm <sup>2</sup> g <sup>-1</sup> )	No of fruits per plant	Fruit yield per plant	Root length (cm)	Root : shoot ratio	Root volume (cm <sup>3</sup> )
1	EC676730	15.70	181.16	93.0	5.48	98.80	1.00	32.5
2	EC686531	33.43	131.80	70.5	4.89	73.80	1.14	15.0
3	EC677076	22.14	159.50	89.0	3.94	77.35	1.15	14.6
4	EC677080	16.98	183.96	50.0	3.83	81.85	0.95	15.4
5	CLN2070A	21.12	99.43	118.0	4.83	63.15	1.02	14.15
6	EC686703	16.02	129.71	68.0	4.80	77.25	1.18	26.25

Table 4.12 List of water use efficient and high fruit yielding tomato germplasm accessions

### 4.2b.3 Evaluation of wild and cultivated genotypes to identify water use efficient lines

During *kharif* 2013 two experiments were carried out with an objective of evaluating wild and cultivated genotypes to identify water use efficient lines and also standardization of root characters of diverse species for root studies. The major objective of these experiments was to assess genetic variability for water use efficiency and fruit yield characters among the cultivated and wild accessions of tomato.

Total of 122 tomato germplasm accessions of six different species collected from IIVR (Varanasi), AVRDC (Taiwan), TGRC (USA) and some superior genotypes identified in summer experiment were used for sowing during *kharif* 2013. The experimental material was sown and transplanted in augmented design (seven blocks, 24 genotypes, 3 checks and 2 boarder rows in each block) in experimental plot of University of Agricultural Sciences, Bangalore. The observations on days to 50% flowering, days to first fruit set, fruits per cluster, flowers per cluster, SCMR, SLA and fruit parameters have been recorded. The experiment is in progress.

### 4.2b.4 Characterization or standardization of root characters among different species of tomato

**Material and methods**: Fifteen diverse tomato genotypes were transplanted in root structures for characterization and standardization for sampling day for root traits related to water use efficiency. Since in the previous experiment there was lot of variability observed for root traits and for an indeterminate crop there are no references for sampling day. So the present experiment is planned to standardize the day of sampling.

Sl.no	Accession no	Species	Sl.no	Accession no	Species
1	LA 1316	S. chmielwaskii	9	EC 771596	S. lycopersicum
2	LA 1246	S.pimpinellifolium	10	CLN 2070A	S. lycopersicum
3	EC - 514101	S.pimpinellifolium	11	EC 771607	S.pimpinellifolium
4	EC-514109	S.pimpinellifolium	12	LA 1713	S. lycopersicum
5	WIR 131706	S. cerasiformae	13	Arka vikash	S. lycopersicum
6	WIR 13708	S. cerasiformae	14	Arka meghali	S. lycopersicum
7	WIR 3957	S.peruvianum	15	Arka abha	S. lycopersicum
8	LOO 887	S.peruvianum			

Table 4.13 Tomato genotypes used for root studies

The root parameters were characterized among diverse tomato accessions at time of transplanting, 25 days after transplanting and 50 days after transplanting. The experiment is in progress.



Kharif 2013 tomato germplasm evaluation plot



Figure 4.7. A veiw of genotypes evaluated during kharif 2013 (top) and variability for root traits among tomato accessions

Female parents	Characters	Male parents	Characters
EC 676790	Dought susceptable,	LA 2963	S.pennelli, High WUE
	high average fruit weight		and drought tolarent
EC 25265	Fruit borer resistant,	WIR 3957	S.pimpinellifolium
	High fruit weight		
EC 109754	Dought susceptable,	LA 4343	S. cersiformae
	More fruit number		
EC 676596	Dought susceptable	LA 2828	S. cersiformae
Arka meghali	Dought susceptable,	RED TOM	S.pimpinellifolium, high
	high yielding		lycopene content local
			germplasm
EC 771594	Dought susceptable		

Table 4 14 Crosses	made for develop	ment of manning	nonulatiion tor	drought/W/LIF	studies
10010 4.14. 0100000	made for acveropi	inche of mapping	population for		Juanco

The above crosses have been affected to develop mapping populatiion for drought/WUE studies

# Task 4.3: Deep sequencing of mRNA and smRNA transcriptome of sorghum and pearl millet for identification of genes and smRNAs functioning in abiotic stress tolerance, with a focus on drought and salinity (MSSRF)

This tasks involves comparative analysis of the stress responsive transcriptome of sorghum and pearl millet accessions with control unstressed transcriptome to identify genes reponsible for abiotic

stress tolerance. The team have completed the activities for the first six months of the project, and are well into the rest of the activities.

### Identification of study material

**Sorghum** – based on Gholipoor et al 2010, and Mutava et al 2011, sorghum accessions for the study were selected. This includes sorghum germplasm varying in their leaf temperature and yield. The seeds were obtained from USDA GRIN.

Accession	Grin No.	Water status	Leaf temperature	Yield
SC 782	PI 576364	irrigated	High leaf temperature (37.3)	High yield (3944)
SC 803	PI 533964	irrigated	High leaf temperature (37.1)	High yield (5354)
SC 60	PI 533962	Irrigated	Low leaf temperature (33.9)	High yield (6656)
SC 110	PI 533794	Irrigated	low leaf temperature (33.4)	High yield (5233)
SC 25	PI 656092	Irrigated	low leaf temperature (34.0)	low yield (851)
SC 15	PI 534124	Irrigated	low leaf temperature (33.5)	low yield (587)
SC 1076	PI 597960	Irrigated	High leaf temperature (36.8)	low yield (93)
SC 1439	PI 656081	Irrigated	High leaf temperature (36.6)	low yield (801)

Table 4.15. List of sorghum accessions selected

**Pearl millet** – The study selected one representative from each of the wild relatives (*Pennisetum sieberianum* (PI 532675, Mali) and *P. violaceum* (PI 564586, Zimbabwe), which can easily hybridize with cultivated pearl millet and are reported to be more drought tolerant than it. In addition, two cultivars reported to be drought tolerant developed for different geographical locations (PI 591068 (developed for India), PI 586660 (recommended for Burkina faso, Mali and other neighboring countries) were also selected. The study plans to do comparative transcriptomics analysis of these accessions under stress and control conditions. The seeds for the germplasm were obtained from USDA GRIN.

### Protocol used for seed treatment with fungicide and germination

- 1. Place seeds in clean, labeled tube. For large seeds, 15 mL tubes can be used, for smaller seeds, 1.5mL microfuge tubes can be used.
- 2. Add fungicide (2 drops per 5 small seeds or 1 drop per large seed) to tube. Labcoat, goggles, and gloves must be used when handling fungicide. Shake tube until seeds are well coated (can use vortexer if desired).
- 3. Let seeds air dry on labeled paper towel.
- 4. Set up labeled petri dishes with larger lid of the dish on top. Label both lids with the accession, the date, and number of seeds.
- 5. Place seeds in petri dish (no more than 6 seeds per dish) between two sheets of 90 mm filter paper. Moisten paper until saturated, but do not overwater (water should not pool if you tip the dish to one side).
- 6. Place petri dishes in germination box (large Tupperware) in growth chamber. Check on germination/water regularly.



Figure 4.8. Sorghum and pearl millet germination in petridishes

**Plant growth and stress treatments:** After three days, transfer the seedlings to 0.5 × Hoagland's solution and allow to grow for an additional 7 days in a controlled environment chamber (Grow in broad shallow trays, hydroponically, supported by styrofoam sheets). Change the nutrient medium every 3-4 days.

**Preparation of Nutrient Solution:** The modified Hoagland's solution after Epstein (1972) as described by Taiz and Zeiger (2002) was prepared and used for the hydroponics experiment in the present study. Composition of the modified Hoagland's solution is given in Table 4.16. The stock solution of each macro nutrient was prepared separately and appropriate volumes were mixed to make up nutrient solution of final volume and concentration. A combined stock solution is made with all micro nutrients except iron. Iron was added separately. The pH of the final solution was adjusted to 6-7 using 0.1 N HCl or NaOH.

Compound	Molecular weight	Concentration of stock solution	Concentration of stock solution	Volume of stock solution per litre of final solution	Final concentration of element	
	g mol <sup>-1</sup>	mM	g l <sup>-1</sup>	ml	μM	ppm
Macronutrients			n and an and			10.10
KNO3	101.10	1,000	101.10	6.0	16,000	224
Ca(NO <sub>3</sub> ). 4H <sub>2</sub> O	236.16	1,000	236.16	4.0	6,000	235
NH4H2PO4	115.08	1,000	115.08	2.0	4,000	160
MgSO <sub>4</sub> . 7H <sub>2</sub> O	246.48	1,000	246.49	1.0	2,000	62
				10.00	1,000	32
				-	1,000	24
Micronutrient						
KCl	74.55	25.0	1.864		50.0	1.77
H <sub>3</sub> BO <sub>3</sub>	61.83	12.5	0.773		25.0	0.27
MnSO <sub>4</sub> . H <sub>2</sub> O	169.01	1.0	0.169		2.0	0.11
ZnSO <sub>4</sub> . 7H <sub>2</sub> O	287.54	1.0	0.288	2.0	2.0	0.13
CuSO <sub>4</sub> .5H <sub>2</sub> O	249.68	0.25	0.062		0.5	0.03
H <sub>2</sub> MoO <sub>4</sub> (85% MoO <sub>3</sub> )	161.97	0.25	0.040	the state of the	0.5	0.05
NaFeEDTA (10% Fe)	558.50	53.7	30.00	0.3-1.0	16.1-53-7	1.00- 3.00

Table 4.16. Composition of the modified Hoagland's solution

Adopted from Taiz and Zeiger (2002)

### Growth chamber conditions

Day length -	12 hrs
Humidity -	65/70 %
Day/night temperatures -	31°C/25°0

### **Plant stress treatments**

- 1. On 10<sup>th</sup> day, subject the plants to salt and drought stress by transferring them to fresh Hoagland's solution containing one of the following
  - 1). 15% PEG-8000
  - 2). 150 mM NaCl
  - 3). milliQ water (control)
- 2. Check for visible symptoms of stress, check Relative Water Content (RWC) of leaf at 0, 12h, 24, and 36h of stress.
- 3. Repeat plant growth, stress treatments and relative water content measurements *three* times.
- 4. Repeat plant growth and stress a fourth time, and harvest leaf and root tissue separately for RNA isolations.



Figure 4.9. Plant growth and stress treatments

**Measurement of RWC: L**eaf discs are removed using a leaf punch about 15 cm from the tip of first and second fully expanded leaves, taking care to avoid the mid-vein. Each sample is placed in a pre-weighed airtight tube and weighed to obtain the fresh weight (FW). The samples are then hydrated in distilled water with gentle shaking at 4°C overnight and weighed to obtain the turgid weight (TW). Water is then discarded and the samples are finally dried overnight in an oven and weighed again to obtain the dry weight (DW). The RWC was calculated according to the formula: RWC (%) = [(FW-DW) / (TW-DW)] x 100. (Humbert et al 2012)

**Determination of tissue harvest time after stress treatments:** Based on the relative water content measurements, 36 hours of salt/drought stress was selected as the time point for tissue harvesting for library construction (moderate stress)

**Bulking of samples for RNA isolation:** Each sample for RNA isolation was bulked from tissue from six seedlings.

Isolation of mRNA and lower molecular weight RNA from leaf and root tissues of sorghum and pearl millet: The selected Sorghum and pearl millet accession were germinated on petri dishes, and

grown in controlled conditions hydroponically in Hoagland's nutritional medium. The ten day old seedlings were subjected to control no-stress and drought/salt stress conditions. The leaf and root tissues at 0, and 36 hours of drought/salt stress were harvested and kept frozen. Total RNA and small RNA from samples were isolated using a protocol from Ravikumar et al 2012, with in house modifications.

Library No.	Species	Accession	Sample and stress		
1			S1-root -control		
2			S1-leaf -control		
3		S1	S1-root –drought -36h		
4		PI576364	S1-leaf -drought -36h		
5			S1-root –salt -36h		
6			S1-leaf –salt -36h		
7			S2-root -control		
8			S2-leaf -control		
9		S2	S2-root -drought-36h		
10		PI533964	S2-leaf -drought-36h		
11			S2-root -salt-36h		
12			S2-leaf -salt-36h		
13			S3-root -control		
14			S3-leaf -control		
15		S3	S3-root -drought-36h		
16		PI533962	S3-leaf -drought-36h		
17			S3-root -salt-36h		
18			S3-leaf -salt-36h		
19			S4-root -control		
20		S4	S4-leaf -control		
21	Sorghum		S4-root -drought-36h		
22	Joighum	PI533794	S4-leaf -drought-36h		
23			S4-root -salt-36h		
24			S4-leaf -salt-36h		
25			S5-root -control		
26			S5-leaf -control		
27		S5	S5-root -drought-36h		
28		PI656092	S5-leaf -drought-36h		
29			S5-root -salt-36h		
30			S5-leaf -salt-36h		
31			S6-root -control		
32			S6-leaf -control		
33		S6	S6-root -drought-36h		
34		PI534124	S6-leaf -drought-36h		
35			S6-root -salt-36h		
36			S6-leaf -salt-36h		
37			S7-root -control		
38			S7-leaf -control		
39		S7	S7-root -drought-36h		
40		PI597960	S7-leaf -drought-36h		
41			S7-root -salt-36h		
42			S7-leaf -salt-36h		

 Table 4.17. Plant Tissue frozen for making illumina, differential expression libraries

43			S8-root -control		
44			S8-leaf -control		
45		S8	S8-root -drought-36h		
46		PI656081	S8-leaf -drought-36h		
47			S8-root -salt-36h		
48			S8-leaf -salt-36h		
49			P1-root -control		
50			P1-leaf -control		
51		P1	P1-root -drought-24h		
52		PI564586	P1-leaf -drought-24h		
53			P1-root -salt-24h		
54			P1-leaf -salt-24h		
55			P2-root -control		
56			P2-leaf -control		
57	Doorl millot	P2	P2-root -drought-24h		
58	reall lillet	PI586660	P2-leaf -drought -24h		
59			P2-leaf -salt-24h		
60			P2-root -salt-24h		
61			P3-root -control		
62			P3-leaf -control		
63		Р3	P3-root -drought-24h		
64		PI591068	P3-leaf -drought-24h		
65			P3-root -salt-24h		
66			P3-leaf -salt-24h		

### Task 4.4: Improving drought adaptation in chickpea through marker-assisted breeding and trait based selection (ICRISAT)

### (A) Improving drought adaptation in chickpea through marker-assisted breeding

A "QTL-hotpsot" on Linkage Group 4 (CaLGO4), that harbors several quantitative trait loci (QTLs) for root traits and other drought tolerance related traits, contributing up to 58.6% of the phenotypic variation, has been identified in chickpea (Varshney et al., unpublished results). The QTL-hotspot was identified from the drought tolerant line ICC 4958.

We proposed to transfer this QTL-hotspot from ICC 4958 into two popular chickpea cultivars JAKI 9218 and JG 14 using marker-assisted backcrossing (MABC). The simple sequence repeat (SSR) markers linked to QTL-hotspot were not polymorphic between JG 14 and ICC 4958. Thus, we replaced JG 14 with JG 16. Now, JAKI 9218 and JG 16 are being used for improvement of drought tolerance.

Eight SSR markers linked to the QTL-hotspot were analysed on the parental lines to identify polymorphic markers between parents of a cross for use in monitoring the introgression (foreground selection) of the QTL-hotspot (Table 4.18). The markers CaM1903, ICCM0249, NCPGR 21 and TA 170 were polymorphic between parents of both the crosses (JAKI 9218 x ICC 4958 and JG 16 x ICC 4958), the marker NCPGR 127 was polymorphic only in the first cross (JAKI 9218 x ICC 4958), the marker TA 130 was polymorphic only in the second cross (JG 16 x ICC 4958), while the markers GA 24 and STMS 11 were monomorphic in both the crosses. Thus, five potential markers for foreground selection were found polymorphic for each cross.

Markar	JAKI 9218 x I	CC 4958	JG 16 x ICC 4958			
Warker	JAKI 9218 ICC 4958		JG 16	ICC 4958		
CaM 1903	274	278	274	278		
ICCM0249	161	191	182	191		
GA24	219	219	219	219		
NCPGR127	218	216	216	216		
NCPGR21	148	151	153	151		
STMS11	231	231	231	231		
TA130	221	221	232	221		
TAA170	264	242	254	242		

Table 4.18 Parental polymorphism for ikers linked to a QTL-hotspot that contains several QTLs for root traits and other drought tolerance related traits in chickpea.

Note: Highlighted cells indicate polymorphism for the marker between the parents

Two crosses (JAKI 9218 x ICC 4958, JG 16 x ICC 4958) were made during the 2012-13 crop season (Oct 12 – Feb 13). The  $F_1$  plants were grown during the off-season (Mar 13 – Jun 13) in greenhouse and the hybridity was confirmed using molecular markers. Confirmed five F1 plants from the first cross (JAKI 9218 x ICC 4958) and three F1 plants from the second cross (JG 16 x ICC 4958) were backcrossed with the cultivars (JAKI 9218 and JG 16) using the cultivar as female parent. Eighty-six BC1F1 seeds have been harvested from the first cross and 90 F1 seeds from the second cross. The BC1F1 plants from these crosses will be grown in the field during 2013-14 crop season (Oct 13 – Feb 14) for second cycle of backcrossing.

### (B) Improving drought adaptation in chickpea through trait based selection

The most commonly used selection criterion for drought tolerance is the yield performance under drought stress condition. But, the low heritability and high genotype x environment interaction for grain yield reduces efficiency of selection based on grain yield. A trait-based selection approach can achieve further gains in drought tolerance that has been achieved through direct selection for grain yield. A recent study conducted at ICRISAT showed that selection for greater partitioning efficiency (p) will confer greater tolerance to terminal drought in chickpea as the terminal drought reduces length of the reproductive period (Krishnamurthy et al. 2013, Field Crops Research, in press).

This activity under Task 4.4 is aimed at developing drought tolerant chickpea breeding lines by selecting for high partitioning efficiency. A total of 300 advanced breeding lines, including both desi and kabuli types, were evaluated under two growing conditions, rainfed (terminal drought stress) and irrigated (no drought stress), at ICRISAT-Patancheru during the post-rainy season (Oct-Feb) 2012-13. The observations were recorded on days to 50% flowering, days to maturity, plant height (cm), biological yield (g), seed yield (g) and 100-seed weight (g) under both irrigated and rainfed conditions. Harvest index (HI), duration of growth before the start of 50% flowering °Cd (Dv), duration of growth after the start of 50% flowering °Cd (Dr), crop growth rate (c) and rate of partitioning coefficient (p) were calculated from the observations recorded.

Breeding lines were selected on the basis of crop growth rate (c) and partitioning coefficient (p). Under irrigated condition, the top 10 lines with high c and p included ICCV 03406, ICCV 04304, ICCV 06302, ICCV 00108, ICCV 01103, ICCV 03201, ICCV 03211, ICCV 04104, ICCV 07102, and ICCV 07103 (Table 4.19). These lines had plant height in the range of 49-65cm; hundred seed weight 17-41g;

harvest index 52-59%; biological yield 270-351g per plot and seed yield 149-195 g per plot. Under rainfed condition, the top 10 lines having high c and p included ICCV 00402, ICCV 01103, ICCV 03106, ICCV 07101, ICCV 08101, ICCV 08103, ICCV 09101, ICCV 09109, ICCV 10108 and ICCV 10116. These lines had plant height in the range of 34-55cm; hundred seed weight 19-32 g; harvest index 46-56%; biological yield 318-359 g per plot and seed yield 155-181 g per plot.

	Irrigated						Rainfed					
S. No	Name of entries	РН	HSW	н	BY	SY	Name of entries	РН	HSW	н	BY	SY
	High crop gro	owth r	ate (c) a	and h	igh parti	tioning	coefficient (p)	•	•	•	•	
1	ICCV 03406	54	38	55	270	149	ICCV 00402	44	27	49	359	176
2	ICCV 04304	62	39	55	309	171	ICCV 01103	52	25	49	344	168
3	ICCV 06302	50	41	58	288	168	ICCV 03106	51	27	47	330	156
4	ICCV 00108	49	25	59	313	184	ICCV 07101	50	21	50	358	177
5	ICCV 01103	64	24	52	317	166	ICCV 08101	55	21	46	358	166
6	ICCV 03201	65	23	56	333	186	ICCV 08103	45	22	50	335	165
7	ICCV 03211	51	24	59	332	195	ICCV 09101	34	19	52	324	168
8	ICCV 04104	61	25	52	351	183	ICCV 09109	40	32	47	337	159
9	ICCV 07102	57	17	56	299	168	ICCV 10108	40	22	49	318	155
10	ICCV 07103	54	19	52	307	158	ICCV 10116	37	25	56	326	181
	Low crop gro	wth r	ate (c) a	ind hi	igh parti	tioning	coefficient (p)					<u> </u>
1	ICCV 01305	54	26	61	154	95	ICCV 07302	37	34	50	241	122
2	ICCV 03405	52	35	62	141	87	ICCV 09302	33	47	59	147	86
3	ICCV 07309	50	35	58	155	90	ICCV 10307	34	29	57	194	111
4	ICCV 08305	51	36	61	166	102	ICCV 97301	38	44	47	261	122
5	ICCV 09304	49	43	61	148	91	ICCV 01103	52	25	49	344	168
6	ICCV 96321	56	33	61	172	105	ICCV 04105	34	20	51	183	93
7	ICCV 97308	43	31	56	165	93	ICCV 07101	50	21	50	358	177
8	ICCV 04101	34	14	63	133	84	ICCV 08111	41	31	55	300	164
9	ICCV 05110	49	24	63	181	114	ICCV 09101	34	19	52	324	168
10	ICCV 88202	52	18	56	154	86	ICCV 10116	37	25	56	326	181

Table 4.19 Top ranking chickpea lines with a combination of high c and high p and a combination of low c and high p under Irrigated and rainfed conditions

Where, PH= Plant height (cm), HSW= Hundred Seed Weight (g), HI=Harvest Index (%), BY= Biological yield (g), SY= Seed Yield (g).

Correlation coefficients were calculated among nine morpho-physiological traits (Table 4.20). Plant height, hundred seed weight, harvest index, biological yield, crop growth rate and partitioning coefficient were positively correlated with seed yield under both irrigated as well as rainfed environment. Plant height was positively correlated with biological yield and duration of growth before the start of 50% flowering (Dv), while hundred seed weight was negatively correlated with these two traits. Harvest index was positively correlated with partitioning coefficient. Biological yield was positively correlated with duration of growth before the start of 50% flowering and crop growth rate under both irrigated and rainfed environments. The most important traits associated with grain yield under water-stressed condition included harvest index, biological yield and portioning efficiency. Thus, these traits can be used in selection for identification of drought tolerant genotypes.

Table 4.20 Correlation coefficient among metric and physiological traits under normal-irrigated and normal-rainfed conditions in Chickpea.

Traits	Environment	HSW	н	BY	Dv	Dr	С	Р	SY
РН	Irrigated	0.30**	-	0.22**	0.28**	-	0.01	0.11*	0.16**
			0.15**			0.19**			
	Rainfed	0.05	-0.07	0.29**	0.37**	-0.02	0.07	0.02	0.16**
	Irrigated		0.08	-	-	-0.01	-0.11*	0.18**	0.30**
нс\//				0.35**	0.38**				
113 VV	Rainfed		-0.02	-	-	-0.06	-0.10	-0.05	0.13*
				0.18**	0.20**				
	Irrigated			-	-	0.13*	-0.09	0.53**	0.27**
н				0.16**	0.20**				
	Rainfed			0.07	-0.03	-0.02	-0.01	0.95**	0.71**
DV	Irrigated				0.44**	-0.08	0.23**	0.17**	0.91**
ы	Rainfed				0.11*	0.04	0.29**	0.08	0.74**
	Irrigated					-	0.10	0.68**	0.34**
Dv						0.67**			
	Rainfed					-	0.01	0.24**	0.05
						0.29**			
Dr	Irrigated						-0.03	-	-0.01
								0.65**	
	Rainfed						-0.05	-	0.01
								0.25**	
С	Irrigated							0.01	0.18**
	Rainfed							-0.01	0.20**
Р	Irrigated								0.39**
	Rainfed								0.69**

Where, \*, \*\*= Significant at 5% and 1% probability level (table value of correlation with 298 degree of freedom at 5% 0.11 and 1% 0.14). Dv=Duration of growth before the start of 50% flowering °Cd and Dr=Duration of growth after the start of 50% flowering °Cd.

# Task 4.5: Capacity building of NARS in research on drought adaptation of crops and integrated breeding for drought adaptation (ICRISAT)

The training of a number of young scientists is an on-going activity. During the post rainy season we have had two trainees, one from Senegal and one from Spain, working on the assessment of sorghum germplasm for the response of transpiration to high VPD.

# 5. Work Package: Enabling Green Growth using Water Treatment and Reuse Innovations

### Objectives

- To identify boundary conditions and perspectives for enabling green economy
- To facilitate a trans-disciplinary co-creation process and identification of agri-business opportunities to increase the use of bio-treatment
- To stimulate the cross-fertilization and knowledge transfer between the individual work packages and activities in Europe and India
- To evaluate and optimize the proposed combinations of bio-treatment and wastewater reuse from a perspective of supporting green growth

During the project Kick –off meeting in January 2013, it was decided that EIRC will lead this WP and supported by the partners. EIRC is in continuous communication with the EU WP5 counterpart and carrying out the activities simultaneously with them.

### Task 5.1: Stakeholder mapping (EIRC, TERI)

EIRC after discussing with EU WP5 partners have decided to work jointly on certain activities and have implemented the common strategies to perform various tasks. This task is further divided into following sub tasks:

### Establishment of Innova paltform Members-

EIRC identified the key stakeholders and practitioners from knowledge (intrinsic and explicit) sector, enterprises, governmental and non-governmental entities. Experts were selected from each sector and a wish list of INNOVA platform members was prepared. This wish list was sent to the coordinator for feedback and it will be sent to all partners in the coming months. From this list 10-15 members will be selected for 1st Indian INNOVA platform meeting which is planned to be organized in the first half of Year 2..

### Creation of digiinnova Platform- LinkedIn Group -

EIRC and EU WP 5 partners decided to use online LinkedIn Forum as digiinnova platform. The water4crops group profile was created and EIRC sent mailers and invited all the Indian partners to join the group. The same is been carried out at the EU counterpart. Further task would be to enhance the group and encourage Indian partners to actively participate in the discussions that are already being held. This is a common platform for both the EU and Indian consortiums to exchange and share thier experiences about project activities they are undertaking.

### Develop a database of stakeholders-

EIRC prepared a database template and sent to all the Indian partners to collect information about relevant key stakeholders which was identified from different sub-sectors of water and waste water treatment and organizations working on water use efficiency. Information from all the partners have been collated and final deliverable D5.1 "Database of stakeholders" will be submitted to DBT by end of November 2013.

### Task 5.2: Future trends and boundary conditions (all partners)

EIRC and EU WP5 partners discussed and jointly developed 5 factsheet templates with feedback from the coordinators of both sides. The factsheet templates have been finalized in the first year. Factsheet will be mailed to all the relevant Indian and EU partners and information will be gathered in the first half of Year 2 and the factsheet deliverable will be developed in the second half of Year 2. This activity is in progress.

# Task 5.3: Co-creation process of identifying innovation potentials to enable green economy (all partners)

In order to prepare for the first Innova platform meeting a questionnaire was jointly prepared by the EU Partner – Alterra and EIRC and the Indian coordinator provided feedbacks and suggestions which was incorporated by the EU partner and the questionnaire was finalized. This questionnaire was designed to collate the information from each EU and Indian partner regarding the technological development they are involved in and new innovative technologies that will be developed under Water4Crops. The questionnaire was sent to all the partners and the answers were collated. Based on the questionnaire inputs, relevant experts (from within the consortium and also stakeholders from outside) will be selected to be invited for the 1st INNOVA platform meeting. The technologies and issues that have been mapped in the questionnaires will be discussed during the INNOVA platform meetings. The first EU Innova Platform meeting is scheduled to be held on 5th December 2013 at Bari, Italy subsequent to the Year 1 Annual Project Meeting of the EU consortium, planned from 3rd to 5th December. The EU Annual Meeting and the Innova Platform meeting will be attended by all the Indian consortium partners. The Indian Innova Platform meeting will follow suit, in the first half of Year 2.

### Task 5.4: Evaluation of shortlisted business opportunities (all partners)

Quick scan of expected socio-environmental aspects; Quick scan of unwanted socio-economic aspects; Quantification of sustainable production thresholds.

### Task 5.5: Synthesis of results and initiation of an implementation process (EIRC)

A "list of technical innovations" template was prepared to summarize all the technologies under development in Water4Crops, with the aim to create an overview of those technologies which could lead, in one way or another, to (marketable) innovations. This document was circulated to all the Indian partners to get their inputs. The information from all the partners was collected and the final list of EU and Indian technical innovations was prepared and analyzed. This analysis is also intended to provide input for the Innova Platform meeting. This list of technologies was used to identify partners to answer the questionnaire survey.

### Deliverables

5.1 Database of stakeholders (Month 12)

### 6. Work Package: Dissemination and Technology Exchange

### Objectives

- To disseminate local entrepreneur demands within the projects
- To disseminate technology offers to entrepreneurs
- To disseminate and exchange the experience between India and Europe on advancing Green Economy in cooperation with EBTC
- To disseminate project results to EBTC, the scientific and wider public community, ensuring maximum use of the project results by a broad audience (scientists, policymakers, planners)
- To provide tailor made capacity building to support the identification of green Growth solutions

During the first project period (month 1 to 12) the main objectives of WP6 was to design the Dissemination and communication strategy for the project and to focus on the activities like developing tools that will be used for effective dissemination of the project results and for enabling technology transfers and exchange of knowledge between India and Europe. The progress and activities undertaken under each task of WP6 are as follows

### Task 6.1: Exchange of experiences and results within the innovation platforms (IP's) (EIRC)

EIRC and Alterra (EU partner in-charge of WP6) have initiated the Water4Crops LinkedIn joint account which is at the moment open only for the consortium partners to start discussing the project related activities and at a later stage it may be made open for the stakeholders that will participate in the Innova Platform Meetings. This LinkedIn account will serve as the Online Innovation Platform, And supplements the Water4Crops website which is partly integrated in it. On the platform upcoming events, meetings, syntheses of specific newsletters and reports are being posted. It is also designed to host discussion on upcoming Water4Crops drafts, e.g. on the WP5 factsheets and on several overarching Water4Crops topics like legislation and cost-benefits of waste water treatment and reuse technologies.

This discussion will provide input to the innovation process in WP5.

The Water4Crops discussion portal via LinkedIn group is available under the link: <u>http://www.linkedin.com/groups/Water4Crops-4799081</u>

Another part of this task was the coordination and exchange of experience between the EU and Indian counterparts in setting up an INNOVA innovation process. Several meetings (EU Kick-off Rome 2012, Indian Kick off meeting Hyderabad 2013, and visit to EBTC Delhi in July 2013 by EIRC and Alterra partners) and regular Skype calls and email exchanges have led to a common understanding and synchronised approach by the EU and Indian partner's in-charge of dissemination and communication activities. (EIRC and ICRISAT (coordinator) from India, Alterra, STEP-Forward and IRSA-CNR, Italy (coordinator) from EU)

# Task6.2:OrganizationofspecialentrepreneurandSMEknowledgebrokerageevent(establishment of the Science Practice interface (EIRC)

EIRC discussed with the coordinator and is planning to have the SME brokerage event along with the First Indian INNOVA platform meeting during the first 6 months of Year 2. The preparatory work for creation and organisation of brokerage events is under process and the brokerage event will be added as special session at the INNOVA meeting in order to promote the project results effectively.

EIRC and EU WP6 Partners had several discussions and Skype meetings about developing the Dissemination strategy/ Plan. After taking suggestions from both EU and Indian coordinators the final dissemination plan was prepared within the time period. A separate dissemination plan was

prepared with EBTC and discussed the future activities with EBTC officials in Delhi. On 1st July 2013, Dr Siderius Christian from Alterra and Ms Sourabha Rani from EIRC held a meeting with the EBTC Environment Sector Expert Dr Monish Verma in the EBTC head office on promoting Water4Crops activities and results through EBTC platforms and events

### Task 6.3: Providing Mass Media dissemination (EIRC)

### Establishment and maintenance of joint project website and project document store:

During the kick off meeting in Hyderabad (India), from 28-30 January 2013, it was decided that one common website (www.water4crops.org/) for the both projects (Water4Crops-India & Water4Crops-EU) should be established. This conveys to outsiders that W4Cs is a joint project between EU & India and both sides are working together. Moreover, it enables effective connections among both projects partners. Every partner gets the same information, and all are informed about the progress going on in India and Europe.

The project website is not only the main tool for information exchange among the partners and between the 2 parallel EU-India consortia, it is one of the important tools for disseminating and promoting project results to the stakeholder communities around the world. Several versions of the website templates were created & finalized the present one after reviews and suggestions from all the project partners.

The content for the website was framed by the EIRC team and the website designed by the technical manager of EIRC. Detailed description about the project objectives, project structure, and brief information about the project partners is made available in the website. There are separate sections for events - a list of organized events, participated events and upcoming events are covered.

There is a separate section called "Downloads" where all the project promotional materials like brochure, project presentations and logo is uploaded for distribution and promotion.

An email to the project partners to provide project related information and event participated details is sent regularly and all the information related to the project activities, related articles and events are regularly published on the website.

**Project Store:** The "Project Document Store" or the "Intranet" has been developed by EIRC in the time frame of the 1<sup>st</sup> reporting period. The tool will be presented at the EU Annual Project Meeting in December 2013 at Bari, Italy and EIRC will seek all the partners' feedback and inputs before making it online. The online store will help both the Indian and EU partners share documents and files, locally or remotely, in groups or privately in a project centric environment. Partners can easily get access to all the deliverables stored in the intranet. Separate Indian and EU folders are created to avoid confusions and mishandling of documents. This project store will soon be made online and will be connected to the project website. The log in window will be provided on the homepage of the project website.

**Project Brochure-** Two sets of brochures were developed during the first half of Year 1. One set of brochures was designed for Indian kickoff meeting held in January 2013. After the kick off meeting both the consortiums decided to have a common brochure and the final version of the common Brochure was designed and developed after reviews and suggestions from both project partners. A project event brochure was developed for the W4C workshop on "Phytosystems for Wastewater Treatment and Reuse in Agriculture" organized by CSIR-NEERI on 23rd October 2013 at NEERI Nagpur. The snapshots of all the brochures are attached in this document. Ms Sourabha Rani from EIRC participated in the event and made a presentation on the project overview.

### **Project Directory**

An Indian project directory was developed for the Indian kick off meeting. The directory contains the list of all the Indian partners, coordinators, team members and their contact information, including their photographs, the email addresses and contact numbers. This will be available in the website under restricted area accessible only for project partners. The same is been developed for EU partners having both EU and Indian directories available in the website.

### Project LOGO

Initial project logo was developed for the kick off meeting. Later EIRC enhanced the logo by making color changes and designed a high resolution logo in Vector format. The logo will be made available in the project store for partners' use.

### YouTube Clipping

EIRC created a YouTube channel for Wwater4Crops project. The kick off meeting and related videos was uploaded to the Water4Crops website and is also disseminated through social media networks. <u>http://www.youtube.com/watch?v=tOCC7z2fUdQ</u>

### Elaboration of Annual Newsletters for the wider public

EIRC and EU partner STEP have discussed about the Common Annual Newsletter. The common Newsletter template is being created by EIRC. Later EIRC and STEP will jointly prepare the draft and send out to partners for review. The first newsletter will be prepared immediately after the 1<sup>st</sup> INNOVA platform meeting at Bari.

### Mass media and press releases, information to social media with project progress statements

A huge promotion and announcement of the project commencement was carried out during the kick off meeting in India by the ICRISAT partners. Many newspapers and local televisions reported the event and the project information.

### List of press releases

- <u>SABMiller India Print Media Coverage</u> (The Hindu Business Line)
- International Crop Research Institute for the Semi-Arid Tropics to lead global water recycling project (The Times of India)
- ICRISAT-led consortium in pact with EU nations (BusinessLine)
- <u>EU, India kick off 6 million Euro 'Water 4 crops' (The Hindu)</u>
- Water4Crops-India kicks off at ICRISAT (Sky News, UK)
- India, EU working on bio-treated waste water project (Business Standard)
- India, EU plan Rs 80 crore recycled wastewater project (Indian Water Review)
- ICRISAT leads consortium for project on wastewater reuse in EU & India (F&B News)
- <u>Wastewater recycling will improve agricultural production in India, EU: ICRISAT (</u>Commodity Online)
- <u>EU, ICRISAT unveil rural development project (Deccan Herald)</u>
- <u>Recycling of wastewater to improve agricultural production (New Kerala News)</u>
- <u>Water4Crops-India kicks off at ICRISAT</u> (Popular science writing Blog)
- <u>EU, ICRISAT unveil rural development project (Newr, India)</u>
- EU, ICRISAT unveil rural development project (RR Features)
- <u>Water4Crops-India kicks off at ICRISAT (UNI News)</u>
- ICRISAT to lead global water recycling project (I4U News)
- ICRISAT to lead global water recycling project (Indian Technology)
- EU and India kick-off 6 million Euros 'Water 4 crops' (Blogspot)
- India, EU sign pact to recycle waste water (IBEF News)

- India, EU sign pact to recycle waste water (BusinessLine)
- <u>http://www.icrisat.org/newsroom/news-releases/icrisat-pr-2013-media3.htm</u>
- <u>http://wle.cgiar.org/blog/2013/04/30/water4crops-exploring-the-opportunities-of-wastewater-use/</u>
- http://foodtank.org/news/2013/03/icrisat-launches-water4crops-india
- <u>http://www.livemint.com/Companies/3cbPG07ZtIIU4EUSdS654I/Beer-major-SABMiller-joins-Indias-Water4Crops-project.html</u>
- <u>http://www.wageningenur.nl/en/show/Water4Crops-integrating-biotreated-wastewater-reuse-and-valorization-with-enhanced-water-use-efficiency-to-support-the-Green-Economy-in-Europe-and-India.htm</u>
- <u>http://www.thehindu.com/todays-paper/tp-national/tp-andhrapradesh/eu-india-kick-off-6-</u> <u>million-euro-water-4-crops/article4363377.ece</u>
- <u>http://www.indiawaterreview.in/Story/News/india-eu-plan-rs-80-crore-recycled-wastewater-project/1046/1#.UQuGgh12w7k</u>
- <u>http://www.deccanherald.com/content/308543/eu-icrisat-unveil-rural-development.html</u>
- <u>http://www.indiawaterreview.in/Story/News/india-eu-plan-rs-80-crore-recycled-wastewater-project/1046/1#.UQuGgh12w7k</u>
- http://news.webindia123.com/news/articles/India/20130131/2147627.html

### Task 6.4: Dialogue with EU delegations on Green Economy (EIRC, TERI)

### Task 6. 5: Capacity building (all partners)

During the first project activity reporting period EIRC, STEP and ALTERRA have worked in collaboration on the demand for capacity building and started to develop necessary tools for providing workshops (one day event) to offer the systematic tailor made tools. At the Kick-off meeting in Hyderabad in India, STEP presented the main idea of SAT (Strategic Approach to Training) to the Indian and European participants. In the next steps, the general SAT tools will be adapted to Water4Crops demand of capacity building. The demonstration of the utilisation of these new training tools will be presented to participants in workshops at INNOVA meetings and project meetings in India and in Europe. These tools will also be used for implementation of an online training for distance learning

### Deliverables

6.1. Internal report on customer / entrepreneur demands and technological offer (Month 12)

6.2. Webpage and Public Dissemination material (Month 6, 12, 24, 36, 42)

### 7. Work Package: Coordination and Management

### Objectives

- To co-ordinate and supervise, jointly with the Indian consortium, activities to be carried out;
- To carry out the overall administrative and financial management of the project;
- To manage the Grant Agreement with DBT and the Consortium Agreement;
- To manage the Coordination Agreement with the Indian consortium;
- To manage the foreground generated by the project and IPR;
- To manage contacts with the DBT;
- To monitor quality and timing of project deliverables;
- To establish effective internal communication procedures

### Task 7.1: Communication and coordination (EIRC, ICRISAT)

**Consortium formation:** The process of consortium formation started with identifying the partners across different disciplines that are suited for water4crops project across the country. The first meeting of the consortium partners was held at ICRISAT to discuss the modalities of the proposal preparation and follow-up procedures. All the partners were present and indicated possible opportunities in the water4crops project and expressed consensus over working together in the project. After consortium formation and process meeting, research/academic ideas were exchanged and it is a continuous process between Indian and European partners and within Indian partners on different academic as well as management aspects of the projects through e-mails, telephones, etc.

### Joint Euro-India Kick off meeting (30-31 October 2012):

In this event Water4Crops project was launched. During this event members from India and EU discussed about managerial and scientific structure of project.

### EU-India STI cooperation days 2012 at NGRI (8-9 November 2012)

The third edition of the EU-India STI Cooperation Days was held in Hyderabad on 8 and 9 November 2012, with a thematic focus on water-related challenges. This event was jointly organized by New INDIGO, INDIA Gate, EURAXESS links India and EBTC and hosted by the National Geophysical Research Institute (CSIR-NGRI) and the Indo-French Center for Groundwater Research (IFCGR/CEFIRES), which was celebrating 11 years of fruitful and daily collaboration between France and India.

# Pre-planning meeting (India-side) (8-9 January 2013): All members of Indian consortium partners were

A pre-planning meeting of consortium partners of the water4crops project was held on 8-9 January at the ICRISAT headquarters in Patancheru, Andhra Pradesh, India. The EU-Indian consortium comprises of 14 partners state agricultural universities, national institutes like TERI, NEERI, nongovernment organizations (NGOs), and private industrial partners.



India-side kick-off meeting (28-30 January 2013):

The two-day kick-off meeting, attended by EU and Indian consortia partners from state agricultural universities, national research institutes, nongovernment organizations (NGOs), and private industrial partners, aimed to discuss India's side of the workplans; prepare detailed activity plans with milestones and timelines; identify opportunities for collaboration among the Indian partners and develop package-wise ties with EU partners; build the consortium team; and discuss the project's dissemination and communication strategy.



EU-India STI Cooperation Days 2013 on Affordable Health at Paris (10-11 October 2013)

This event was jointly organised by New INDIGO, EBTC and the European Commission and brought together 150 stakeholders from the business and research communities on Health. With a high attendance rate from the Indian side and more than 17 European countries represented, the event mobilised the research and business communities from both regions and was a tremendous opportunity for partnering. Water4crops project represented by project coordinator at this meeting.

### Joint EU-India review meeting (3-5 December 2013)

First joint meeting of consortium partners of the project "Integrating bio-treated wastewater reuse with enhanced water-use efficiency to support the green economy in EU and India", also known as Water4Crops (India-EU Project), was held on 3-5 December 2013 at the Bari, Italy. The project brings together an Indo-European consortium of 36 organizations [14 Indian and 22 European] belonging to research institutions, universities, large industries and SMEs. From India side Drs Suhas Wani (ICRISAT), Alok Adholeya (TERI), GS Dasog (UAS, Dharwad), JD Sophia (MSSRF), Asha Juwarkar (NEERI, Nagpur), DL Savithramma (UAS, Bangalore), and Mr Sanjeev Singh (NEERI, Nagpur) and Ms Deepthi Prasad (ERIC) had participated in joint meeting and represented various work packages in the project.



Subtask 7.1.1: Communication and exchange with the DBT

Subtask 7.1.2: Establishment and management of communication procedures and tools for facilitating exchange among partners and joint consortia.

Subtask 7.1.3: Organisation and follow up of co-ordination meetings among the partners

Subtask 7.1.4: Monitoring of project activities and work progress, reports from the partners, quality control of project deliverables

Task 7.2 Administrative management of the project (EIRC)

Task 7.3 Management of knowledge generated by the project (EIRC, ICRISAT)

### Deliverables

7.1 First year annual report to DBT (Month 12)

### Appendix A

Sl. No.	Institute	RA	SRF/JRF	Project Assistant	LT/FA	Graduate Assistants and Students (M.Sc.)	Total
1	ICRISAT	3	3**		3	5	14
2	UASB						
3	JISL		2				2
4	NEERI						
5	UASD	1	1	1	2	3*	8
6	MSSRF		1				1
7	TERI	2	2	2			6
	Total						

Table 1. List of Staff recruited for consortium partners

\* These are supported out of Recurring expenses (Miscellaneous). 5 students admitted during this academic year and going to be supported from 2014-15 financial year. \*\*One JRF left the job

Table 2. List of equipment purchased by partners

SI. No.	Name	Status	Institute
1	Moisture measuring system (Neutron probe)	In process	ICRISAT
2	Oven for plant samples	Purchased	ICRISAT
3	Leaf area meter	Purchased	ICRISAT
4	Infrared thermometer	Purchased	ICRISAT
5	Computer and UPS	Purchased	ICRISAT
6	Digital camera	Purchased	ICRISAT
7	Auto analyzer	Purchased	ICRISAT
8	Moisture measuring system	In process	SABM
9	Computer with Printer & UPS	In process	SABM
10	Digital camera	Purchased	SABM
11	Touch Down PCR		UASB
12	Electrophoresis Units		UASB
13	Computer with printer and UPS		UASB
14	Camera		UASB
15	SPAD Chlorophyll meter		UASB
16	Gel Rocker		UASB
17	Wastewater analysis kit		NEERI
18	Double ring infiltrometer		NEERI
19	Pipette equipment		NEERI
20	Sieve shaker		NEERI
21	Reciprocating shaker		NEERI
22	Bailer sampler (Stainless steel) 1000 ml		NEERI
23	Soil sampler		NEERI
24	Soil moisture meter		NEERI
25	TOC Analyzer		NEERI
26	Pressure plate extractor 15 bar & 1/3 Bar		NEERI
27	Computer with printer and UPS		NEERI
28	Centrifuge	Purchase order	TERI
		released	

29	Jars & Spares for Ball Mill	Purchased	TERI
30	FTIR Spares	Purchase order	TERI
		released	
31	Computer with Printer & UPS	Computer	TERI
		except Printer	
		& UPS	
		purchased	
32	Moisture measuring system	Tendered	UASD
33	Infrared thermometer (Everest)	Insufficient	UASD
		Fund, not yet	
		purchased	
34	Computer with Printer & UPS	1 purchased	UASD
35	Digital camera	1 Purchased	UASD
36	Computer with Printer & UPS		MSSRF
37	Computer printer & UPS		ERIC

### Appendix B

### Mean Monthly Meteorological for JISL site

								-
Month	Maximum	Solar Rad.	Temperature	Humidity	Wind	Wind	Wind	Rain Fall
	Minimum	Watts/m2	Degree C	%	Degree	Km/hr	Km/hr	mm
Jan-12	Max.	794.6	29.6	62.6		16.4	8.6	0
	Min.	3	15.5	21.3		2	2	0
Feb-12	Max.	885.1	33.7	40.7			17.6	0
	Min.	3.2	18.1	11.6			2.2	0
May 12	Max.	1041.2	37.9	28.2			16.7	0
IVIAI-12	Min.	1.8	21.2	6.8			2	0
Apr 17	Max.	1067	40.8	42		17.6	6.6	0
Abi-12	Min.	3.1	25.3	9.1		2	2	0
May 12	Max.	1069.03	41.47	61.46	23.23		9.84	0
IVIdy-12	Min.	2.19	25.93	13.09	2.42		1.97	0
lup 12	Max.	797.2	32.7	96	19.9		6.6	168.4
Juli-12	Min.	2.4	24.2	54.6	2		2	168.4
1.1.1.2	Max.	797.2	32.7	96	19.9		6.6	168.4
Jui-12	Min.	2.4	24.2	54.6	2		2	168.4
Aug 12	Max.	887.4	32.5	95.3		16.3	6.4	80.1
Aug-12	Min.	2.5	23.7	53.2		2	2	80.1
Son 17	Max.	1030	34.3	95.7		15	4.7	64.3
Seb-15	Min.	2.2	23.4	44.6		2	2	64.3
Oct 12	Max.	962.3	35.5	65.5		17.3	7.1	16.1
001-12	Min.	2.03	22.25	20.27		2	2	16.1
Nov 12	Max.	832.7	33	49.9		19	8	0
NOV-12	Min.	1.8	18.6	16.2		2	2	0
Dec 12	Max.	760.6	31.6	49.3		21	10.4	0
Det-12	Min.	2.4	18.1	17.1		2.4	2	0
lan 12	Max.	765.1	30	47.4			22.9	0
Jail-12	Min.	1.6	16.2	17.5			3.6	0
Fab 12	Max.	1010	37.4	99.8		55	21	20.6
Feb-13	Min.	1	13.9	5.9		2	1	20.6
Mar 12	Max.	1078	40.2	62.2		40	21	0
IVIdI-15	Min.	1	19.5	5.9		0	0	0
Apr 12	Max.	1049.2	41	43.7			16.4	0
Apr-13	Min.	2.1	25	8.3			2	0
May 12	Max.	1030	42.1	53.7	357	40	18	0
iviay-13	Min.	0	28	19	1	2	0	0
Jun-13	Max.	827.1	33.2	90.9			7.3	0
	Min.	0	24.7	47			0	0
Jul-13	Max.	1205	100	35			10	313.1
	Min.	0	39.6	22.8			0	313.1
Aug-13	Max.	1304	100	33.7			10	202.6
	Min.	0	37.6	22.1			0	202.6
Con 12	Max.	1243	100	36.7			11	202.5
Sep-13	Min.	0	33.1	21.4			0	202.5
# Appendix C

	Rainfall (mm)			Max.	temper	ature	Min. t	empera	iture	Relative Humidity		
Month			-		(∘C)	-		(∘C)			(%)	
WORth	Average			Ave-			Ave-			Ave-		
	(30 years *)	2012	2013	rage	2012	2013	rage	2012	2013	rage	2012	2013
Jan	1.6	0	0.0	28.7	29.8	31.2	14.07	13.9	14.4	64.8	62	68
Feb	3.1	0	2.2	31.6	32.8	32.5	16.56	16.1	16.0	54.4	44	62
Mar	11.9	0	42.0	34.9	35.8	34.9	19.71	18.5	19.1	64.2	43	70
Apr	40.4	56.6	10.0	36.6	35.7	36.9	20.11	21.2	20.5	78.1	57	75
May	64.0	3.8	124.2	35.2	35.7	35.3	20.95	21.5	21.8	75.8	57	84
Jun	116.2	43.4	73.9	30.2	30.2	28.0	21.68	21.2	20.7	86.3	76	93
Jul	129.7	111.6	177.2	27.3	27.3	25.4	20.85	20.8	20.4	89.2	83	95
Aug	104.0	92.4	97.2	27.2	27	26.6	20.16	20.5	19.9	88.6	85	93
Sep	103.1	87	84.7	27.9	28.2	27.8	19.96	19.7	20.2	86.7	80	94
Oct	107.9	89.2	27.8	29.5	29.7	20.7	18.65	18.2	13.9	79.4	65	65
Nov	32.2	35.7	-	28.9	29.2	-	15.93	17	-	73.6	63	-
Dec	6.7	19.6	-	27.8	30	-	13.2	14.4	-	69.1	58	-
Total	720.8	539.3	639.2									

Mean Monthly Meteorological data of Main Agricultural Research Station, University of Agricultural Sciences Dharwad

\*Average of 30 years (from 1982 to 2012)

## Rainfall data (mm) of Ugar khurd from 2005 to 2013

Sr.	Month/Year	2005	2006	2007	2008	2009	2010	2011	2012	2013
1	January	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
2	February	Nil	Nil	Nil	5.0	Nil	Nil	Nil	Nil	Nil
3	March	Nil	52.0	Nil	91.0	0.0	Nil	Nil	Nil	Nil
4	April		5.0	14.0	13.0	0.0	5.0	30.0	57.0	3.0
5	Мау	92.0	66.0	19.0	8.0	87.0	Nil	25.0	3.0	7.0
6	June	103.0	54.0	188.0	74.0	71.0		57.0	9.0	51.0
7	July	118.0	90.0	29.0	15.0	68.0	70.0	34.0	56.0	62.0
8	August	103.0	62.0	85.0	56.0	26.0	136.0	77.0	49.0	20.0
9	September	65.0	139.0	99.0	108.0	242.0	136.0	7.0	59.0	123.0
10	October	128.0	4.0	16.0	34.0	56.0	94.0	66.0	131.0	34.0 (upto 24.10.2013
11	November	Nil	83.0	Nil	63.0	68.0	58.0	Nil	16	Nil
12	December	Nil	Nil	Nil	4.0	2.0	Nil	Nil	Nil	Nil
	Total	690	555	450	471	624	633	296	380	305

# Appendix D

## Waste Water Details:

Untreated Waste Water Details On Monthly Basis - 2012:

Month	Fruit - Untreated Waste Water – m <sup>3</sup>	Onion- Untreated Waste Water – m <sup>3</sup>					
Jan	NA	NA					
Feb	NA	NA					
Mar	NA	NA					
April	4805	20345					
Мау	22524	16373					
June	32083	14479					
July	14619	16267					
August	14952	3585					
September	33174	4265					
October	16431	1867					
November	13420	2613					
December	15952	16566					

Untreated Waste Water Details on Monthly Basis - 2013:

Month	Fruit - Untreated Waste Water -	Onion- Untreated Waste Water -
	m3	m3
Jan	12068	14447
Feb	15795	14109
Mar	11290	15215
April	3244	12879
May	10119	13148
June	29020	14225
July	16288	13351
August	15500	12650
September	17392	12807
October		
November		
December		

# Appendix E

## Untreated Waste Water From Fruit (Banana) 2013:

Parameters	Jan	Feb	Mar	April	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
рН												
EC (ms/cm)	1.827	NA			16.2	1.627	1.185	1.388	1.652			
TDS (ppm)	4966	3206	2400		3150	1434	3436	NA	2320			
BOD (ppm)	7245	1815	1575		2625	1785	2835	NA	1500			
Carbonates (ppm)	Nil	Nil	NA		5064	Nil	Nil	120	57			
Bicarbonates (ppm)	458.72	525.2			Nil	475.19	488	329.4	278.16			
Chlorides (ppm)	236.45	113.11			2909.6	339.25		212.91	139			
Sulphates (ppm)	80.1	14.54	90.1		46.8	82.5		54.16	130			
Phosphates (%)	1.01	Nil	NA		Nil	84.73		0.46	3.98			
Sodium (ppm)	235.5	95.6	375		3148.3	143.02		188.27	87.17			
Potassium (ppm)	150	24.1	100.55		28.34	84.73		14.46	98.23			
Ca(ppm)	96.19	41.68	NA		40.08	49.05		92.18	216.43			
Mg (ppm)	40.78	0.07	44.85		36.43	59.46		14.54	33.92			
Arsenic (ppm)	Nil	NA	0.01		Nil	Nil		Nil	NA			
Iron (ppm)	3.59	NA	4.88		0.107	1.265		Nil	2.566			
Lead (ppm)	Nil	NA	Nil		Nil	Nil		Nil	Nil			
Cadmium (ppm)	Nil	NA	Nil		Nil	Nil		Nil	Nil			
ZInc (ppm)	0.136	0.04	0.506		Nil	0.184		Nil	1.1			
Pathogenic microbes												
Nitrates (ppm)	0.31	NA			22.75	0.65		Nil	NA			
Physical Impurities												

Treated Waste Water From Fruit (E	Banana) 2013:
-----------------------------------	---------------

Parameters	Jan	Feb	Mar	April	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
рН												
EC	3.17	NA	NA		3.77	2.19	3.13	2.11				
TDS		1212	1818		2050	1876	1638					
BOD	60	60	90		30	75	120					
Carbonates &	110	20.16	NA		34.2	Nil	120	72				
Bicarbonates	1629.92	796.8	NA		1205.6	1066.28	1683	939.4				
Chlorides	257.01	133.29	NA		251.7	224.67	211.42	197.7				
Sulphates	49.1	10.6	35.9		62.6	80.66	11.83	34.75				
Phosphates	168	Nil	NA		0.008	1.38	1.28	1.66				
Sodium	468	162.6	264.39		512.91	287.71	338.8	360.39				
Potassium	168	22.3	118.05		9.37	41.92	53.9	34.32				
Ca	59.32	54.5	NA		67.33	42.51	76.15	49.54				
Mg	49.1	35.94	36.6		47.6	53.46	41.27	45				
zinc	Nil	ND	NIL		Nil	0.048	Nil	Nil				
Arsenic (ppm)	NIL	NA	NIL		Nil	Nil	Nil	NA				
lron (ppm)	NIL	ND	NIL		Nil	1.241	Nil	0.146				
Lead (ppm)	NIL	NA	NIL		Nil	Nil	Nil	Nil				
Cadmium (ppm)	NIL	NA	NIL		Nil	Nil	Nil	Nil				
Pathogenic microbes												
Nitrates	2.43	NA	NA		Nil	0.041	0.38	NA				
Physical Impurities												

# Appendix F

Table	B1: Guidelines fo	or interpretations	of wate	er qualit	y for irri	gation		
Doton	tial Irrigation Dra	blom		Unite	Degree	e of Restriction on Use		
Poten	tial imgation Pro	biem		Units	None	Slight to Moderate	Severe	
Salinit	y(affects crop wa	ater availability) <u>2</u>						
	ECw			dS/m	< 0.7	0.7 – 3.0	> 3.0	
	(or)							
	TDS			mg/l	< 450	450 - 2000 > 2000		
Infiltra	ation(affects infil	tration rate of wat	ter into	the soil.	Evaluate	e using ECw and SAR tog	ether)	
SAR	= 0 - 3	and ECw	=		> 0.7	0.7 – 0.2	< 0.2	
	= 3 – 6		=		> 1.2	1.2 – 0.3	< 0.3	
	= 6 – 12		=		> 1.9	1.9 – 0.5	< 0.5	
	= 12 – 20		=		> 2.9	2.9 – 1.3	< 1.3	
	= 20 - 40		=		> 5.0	5.0 – 2.9	< 2.9	
Specif	ic Ion Toxicity (af	ffects sensitive cro	ps)					
	Sodium (Na)							
	Surface irrigat	tion		SAR	< 3	3 – 9	> 9	
	Sprinkler irrig	ation		me/L	< 3	> 3		
	Chloride (Cl)							
	Surface irrigat	tion		me/L	< 4	4 - 10	> 10	
	Sprinkler irrig	ation		me/L	< 3	> 3		
	Boron (B)			mg/L	< 0.7	0.7 – 3.0	> 3.0	
	Trace Elemen	ts (see Table 21)						
Misce	llaneous Effects (	affects susceptible	e crops)					
	Nitrogen (NO	3 - N)		mg/L	< 5	5 – 30	> 30	
	Bicarbonate (	HCO₃)						
	overhead spri	nkling only		me/L	< 1.5	1.5 – 8.5	> 8.5	
	рН				Norma	l Range 6.5 – 8.4		

Table	<b>B2: Laboratory</b>	determinations	needed	to	evaluate	common	irrigation	water	quality
proble	ems								

Water parameter	Symbol	Units	Usual range in irrigation water		
SALINITY					
Salt Content					
Electrical Conductivity	ECw	dS/m	0-3	dS/m	
(or)					
Total Dissolved Solids	TDS	mg/L	0 – 2000	mg/L	
Cations and Anions					
Calcium	Ca <sup>++</sup>	me/L	0 – 20	me/L	
Magnesium	Mg <sup>++</sup>	me/L	0 – 5	me/L	
Sodium	Na⁺	me/L	0 - 40	me/L	
Carbonate	CO <sup></sup> <sub>3</sub>	me/L	0 – .1	me/L	
Bicarbonate	HCO <sub>3</sub> <sup>-</sup>	me/L	0 - 10	me/L	
Chloride	Cl	me/L	0 – 30	me/L	
Sulphate	SO4	me/L	0 – 20	me/L	
NUTRIENTS2					
Nitrate-Nitrogen	NO <sub>3</sub> -N	mg/L	0 - 10	mg/L	
Ammonium-Nitrogen	NH <sub>4</sub> -N	mg/L	0 – 5	mg/L	
Phosphate-Phosphorus	PO <sub>4</sub> -P	mg/L	0 – 2	mg/L	
Potassium	K <sup>+</sup>	mg/L	0 – 2	mg/L	
MISCELLANEOUS					
Boron	В	mg/L	0 – 2	mg/L	
Acid/Basicity	рН	1-14	6.0 - 8.5		
Sodium Adsorption Ratio3	SAR	me/L	0 – 15		

## Table B3: Recommended maximum concentrations of trace elements in irrigation water

Element	Recommended Maximum Concentration2 (mg/L)	Remarks		
Al (Aluminium)	5	Can cause non-productivity in acid soils (pH < 5.5), but more alkaline soils at pH > 7.0 will precipitate the ion and eliminate any toxicity.		
As (Arsenic)	0.1	Toxicity to plants varies widely, ranging from 12 mg/l for Sudan grass to less than 0.05 mg/l for rice.		
Be (Beryllium)	0.1	Toxicity to plants varies widely, ranging from 5 mg/l for kale to 0.5 mg/l for bush beans.		
Cd (Cadmium)	0.01	Toxic to beans, beets and turnips at concentrations as low as 0.1 mg/l in nutrient solutions. Conservative limits recommended due to its potential for accumulation in plants and soils to concentrations that may be harmful to humans.		
Co (Cobalt)	0.05	Toxic to tomato plants at 0.1 mg/l in nutrient soluti Tends to be inactivated by neutral and alkaline soils.		
Cr (Chromium)	0.1	Not generally recognized as an essential growth element. Con-servative limits recommended due to lack of knowledge on its toxicity to plants.		
Cu (Copper)	0.2	Toxic to a number of plants at 0.1 to 1.0 mg/l in nutrient solutions.		
F (Fluoride)	1	Inactivated by neutral and alkaline soils.		
Fe (Iron)	5	Not toxic to plants in aerated soils, but can contribute to soil acidification and loss of availability of essential phosphorus and molybdenum. Overhead sprinkling may result in unsightly deposits on plants, equipment and buildings.		
Li (Lithium)	2.5	Tolerated by most crops up to 5 mg/l; mobile in soil. Toxic to citrus at low concentrations (<0.075 mg/l). Acts similarly to boron.		
Mn (Manganese)	0.2	Toxic to a number of crops at a few-tenths to a few mg/l, but usually only in acid soils.		
Mo ( Molybdenum)	0.01	Not toxic to plants at normal concentrations in soil and water. Can be toxic to livestock if forage is grown in soils with high concentrations of available molybdenum.		
Ni (Nickel)	0.2	Toxic to a number of plants at 0.5 mg/l to 1.0 mg/l; reduced toxicity at neutral or alkaline pH.		

Pd (Lead)	5	Can inhibit plant cell growth at very high concentrations.
Se (Selenium)	0.02	Toxic to plants at concentrations as low as 0.025 mg/l and toxic to livestock if forage is grown in soils with relatively high levels of added selenium. An essential element to animals but in very low concentrations.
Ti (Titanium)		Effectively excluded by plants; specific tolerance unknown.
V (Vanadium)	0.1	Toxic to many plants at relatively low concentrations.
Zn (Zinc)	2	Toxic to many plants at widely varying concentrations; reduced toxicity at pH > 6.0 and in fine textured or organic soils.

# Appendix G

## Hi tech irrigation component details

Controller : It is a device to control irrigation system or may be called as an irrigation monitoring unit. Operational role of controller for W4C will be as noted below:1.Pump operation,2. Sand filters cleaning, 3. Water meter operation, 4. On/Off of field control valves, 5. Receiving input data from Tensiometer 6. Monitoring EC / pH of fertilizer by means of Fertigation machine

**Filter**:-Sand filter is used as a filtering device for three different kinds of water as mentioned in the task 3.1. Operation of back flushing will be done automatically to improve filtration efficiency.

<u>FERTIGATION MACHINE</u>: Fertijet is a hi tech Fertigation machine which monitors EC and controls pH as per requirement. Fertilizer application will be done precisely through this machine/instrument.

<u>TENSIOMETER</u>: Tensiometer is a device used to measure water tension in the soil. Input data from the Tensiometer will be provided to controller and accordingly the operations will be done. Tensiometer will be buried at a depth of 30 cm below the soil.

<u>COMPLETE METEOROLOGICAL STATION</u>: Weather station will help to provide day to day climate data for irrigation scheduling. This will provide parameters like temperature, humidity, Rainfall, wind speed, wind direction, sunshine hours/ radiations.

<u>WATER METER</u>: Water meter help to check amount of water pass through it. It will pass signal to controller for each new operation on volumetric basis to On / Off sectional valve.

<u>SOLENOID VALVE</u>: These are field control valves/sectional valves used on main/sub main lines pass the water into irrigation system.

<u>EMITTING PIPES:</u> Two different emitting pipes are used for trail purpose. Pressure compensating (PC) and Non pressure compensating (NPC) its technical details are mentioned.













## Appendix H

## Protocol for Clogging Test Using Food Processing Water Aim:

To test the capability of emitters to let pass processed water from Food Processing Industry, with a view to approach minimum size of internal aperture within emitter and further help establish guidance for system filtration size.

## Purpose of the test method:

The test method has been developed for testing capability of emitters either to let pass or to prevent entry of solid particles of a given size, with a view to approach minimum size of internal aperture within emitter, and possibly to further help establish guidance for system filtration size.

## Requirements for layout and equipment of the clogging test facility: General layout principles

The layout of the clogging test facility should be very simple to avoid, as much as can be done, all occurrences of zones of low water velocity or of other potential trapping of suspended particles throughout the test bench, and to allow for convenient and easy cleaning of all parts with or without dismantling the installation. In particular the design of hydraulic lines should be simplified at maximum, by minimizing lengths of pipes, numbers of fittings elbows, tees, valves, controllers, filters, by discarding devices with sharp angles, complicated internal geometry, sharp restrictions in cross section, by favoring no- or low-obstruction devices and installation rules, and more generally by avoiding devices or constructive dispositions offering potential traps for particles. Indeed, excessive use of such devices and dispositions might have dramatic consequences into causing a too poor capability for controlling the required stability of concentration and particle size distribution during the progress of the test, and might result in impossibility for tester to advance the test to its normal end, conforming the test specifications and requirements set out in this standard.



**Clogging Test Setup** 

## Tank:

The tank volume can be as small as necessary to ensure constancy of load during test phases. An efficient agitation system shall be installed to maintain in suspension the clogging materials suspended in the test water.

## Pump:

Preferably resistant to processed water (from Mango / Guava / Onion Food Processing Industry), flow rate as required to operate at test pressure 20 emitters and maintain in addition a velocity of 1 m/s at the end of test lines.

## Pump pressure control:

Motor rotation speed controller (Variable Frequency Drive) can be used with its appropriate pressure sensor to be recorded.

## Agitation system in tank:

Propeller, water nozzles, high pressure water nozzles, air nozzles, ultrasonic cleaning system, as appropriate for keeping particles in suspension and maintaining constant concentration (if present in processed water) in the reservoir of test water, with continuous operation 24h/24.

## Disposal of water from end of line:

Water is returned to the tank.

## Disposal of water from emitter test sample:

Water is return to the tank.



## Settings and Accuracy: Table: test parameter settings for "medium term" emitter clogging test

Test sample	20 emitters
Number of test lines	4, horizontal, with valves at both ends, water conserved in line when non pressurized no repetition line requested
Number of flow measurements from tested sample	14
Number of individual flow measurements from tested emitters	14 x 20 = 280 nos
Test pressure	<ul> <li>nominal pressure of emitters, or</li> <li>pressure mid-range of regulation range of emitters</li> <li>tolerance +/- 5%</li> </ul>
Temperature of water	27 °C +/- 3 °C
Velocity of water at end of line	1 m/s tolerance +/- 20%
Cycle duration	480 min
Duration of line pressurization within cycles	240 min
Duration of line non- pressurization within cycles	240 min
Number of cycles per day	3
Number of days per phase	7
Number of phases per test	2
Cumulated duration of line pressurization in a phase	10080 min
Cumulated duration of line non- pressurization in a phase	10080 min
Detection of clogging	The sample is not declared to be clogged whenever: Vcc / Vrc ≥ 70% where - Vcc: sample Volume of water emitted as measured for the current cycle Vrc: sample Volume of reference calculated for current cycle, by multiplying value of reference average "clear water" emission rate established and duration of line pressurization as measured for the current cycle
End of test	End of last phase (2) or whenever Vcc / Vrc < 30%

## Accuracy:

Measurements shall be made with an accuracy of +/- 10% of the initial values at the beginning of test.

## Testing procedure:

- Test the water (treated / untreated) from laboratory before start of testing
- Set the initial required flow rate to pass through Inline tube.
- Check for inlet and outlet pressure.
- Set velocity of flow in the each line at 1 m/s (tolerance +/- 20%).
- Allow source water (e.g. Mango / Guava / Onion etc. from Food Processing Industry; treated / untreated) to pass through Inline tube with mentioned rate (as in below result table).
- After running each number of phases (for 6 days), shut down system for 24hrs and then restarted testing on next day.
- Agitation is continuously kept in running condition.
- CV in the context of this report is used for statistic clarification and not meant to represent coefficient of manufacturing variability
- Repeat this test once for validation.
- Testing is carried out at temperature 27 °C +/- 3 °C.

## Data to be collected mandatorily during test phases progress:

The types of data and frequency of measurements specified in Table are mandatory and shall be included in the testing report.

Type of Data	Periodicity of measurement
Temperature, EC, pH and Hardness (ppm) of test water	Once at the end of each phase
Pressure at beginning of line	Once at the beginning and at end of phase
Flow rate at the end of line	Once at the beginning of each test
Individual emission rate of every emitter in test sample	Once at the end of each phase

## Analysis of emitter:

- At the end of testing check the flow rates of each emitter.
- If Vcc / Vrc < 30%, then peel out the emitter from tube.
- Check the plug point and cause of plugging of emitter.
- If the emitter is plugged because of biological impurity then check the biological matter.

## Appendix I

## **Snapshots of the Official Website and Inner Pages**



#### Water4Crops LinkedIn group



## First Water4 Crops Brochure (Indian side)



## First Water4Crops brochure (Indian side) Inner page



#### Second Water4Crops Common Brochure



### Second Water4Crops Common brochure Inner page



## Water4Crops Brochure for CSIR NEERI Workshop

This workshop is organized under the Indo-EU

09:30 Hrs	Inaugural session
10:00 Hrs	Technical session-I
11:30 Hrs	Health break + Photo session
12:00 Hrs	Technical session-II
13:00 Hrs	Lunch break
14:00 Hrs	Technical session-III
15:30 Hrs	Health break
16:00 Hrs	Technical session-IV
17:30 Hrs	Concluding remarks followed by High Tea

#### ORGANIZING COMMITTEE

Dr. Mrs. Asha A. Juwarkar, CSIR-NEERI Dr. Tapas Nandi, Chief Scientist, CSIR-NEERI Dr. Pawan K. Labhasetwar, Pr. Scientist, CSIR-NEERI Mr. S.K. Singh, Senior Scientist, CSIR-NEERI Mrs. H. P. Jambhulkar, Sr. Technical Off., CSIR-NEERI Dr. P. R. Thawale, Sr. Technical Off., CSIR-NEERI



CARSEN & TOURRO

The Ugar Sugar Works Ltd.

#### CONTACT

Dr. Mrs. Asha A. Juwarkar Chief Scientist & Head, CSIR-NEERI, Nagpur-20, India email : aa\_juwarkar@neeri.res.in Phone : 0712-2249764, 9765251513

## Workshop

## Phytosystems for wastewater treatment and reuse in agriculture

23" October, 2013



## Water4Crops Brochure for CSIR NEERI Workshop Inner page



"Phytosystems for wastewater treatment and reuse in agriculture"

Plant based systems (Phylosystems) are widely used low cost method for treating domestic wastewater workdwide. The use of Phylosystems in the form of Engineered Constructed Wetland systems (ECWs) in water pollution control has been a matter of considerable interest and research from the early eighties. While most of the work has focused on the use of wetlands as polishing systems and on removal of nutrients, metals and pathogens, research has also revealed their application for primary wastewater treatment.

Reuse of domestic and industrial wastewater for nonpotable purposes has become necessary due to increasing demand on high quality water. ECWs have proven to reliably achieve efficient treatment processes, satisfying non potable reuse requirements. This is of extreme importance in the Indian context, with rapid expansion of cities and domestic water supply, quantity of wastewater is increasing in the same proportion. Almost 90% of total water supplied for domestic use gets generated as wastewater which could be diverted for agriculture purpose.

ECWs is now a well-established technology. There are several thousand wetland systems treating municipal,

wetland systems have a wide variety of engineering designs, wetted areas, flow rates, influent and effluent quality, hydraulic properties and monitoring requirements. Research is necessary in areas of system longevity, pollutant removal process dynamics and system modeling.

The major aim of the workshop is to bring together researchers and professionals to discuss new developments and exchange experiences in the field of ECWs and the health aspects of the crops and soils with the application of treated and untreated wastewater.

#### THEMES

- Importance of constructed wetlands in treating wastewater under Indian scenario.
- Design and optimization of constructed wetland system for treating domestic and industrial wastewaters.
- Implications on crop and soil health due to imigation of constructed wetland treated wastewater.
- Socio-economic aspects Vs. wastewater irrigation of crops.

#### CSIRANEERI

CSIR-National Environmental Engineering Research Institute (CSIR-NEERI), a constituent laboratory of CSIR endeavors to provide Leadership in environmental science and engineering for sustainable development. CSIR-NEERI dedicates itself in the service of humankind by providing innovative and effective solutions to environmental and natural resource problems. The institute is known for low cost wastewater treatment technologies, especially CSIR-NEERI has demonstrated first constructed

### **Snapshot of Project Directory**

On 23<sup>st</sup> October, 2013 at CSIR-NEERI Auditorium, Nagpur. The city is synonymously known as Orange city. During October Nagpur's weather is pleasant with the onset of winter season.



Dr. Mrs. Asha A. Juwarkar Chief Scientist & Head Ecosystem Division CSIR-National Environmental Engineering Research Institute, Nehru Marg, Nagpur - 440020 Phone: 0712-2249764, 9765251513 emait aa\_juwarkar@neeri.res.in

#### ADVISORY COMMITTEE

- Prof. M. S. Swaminathan, Emeritus Chairman, MSSRF Dr. S. R. Wate, Director, CSIR-NEERI Dr. Panitosh Tyagi, Ex. Chairman, CPCB Prof. C. R. Babu, University of Delhi Prof. Kasturi Datta, JNU Dr. Chandra Shekhar Naultyal, Director, CSIR-MBRI Dr. Tapan Chakrabarti, Emeritus Scientist, CSIR-MERI Dr. K. S. Charak, Scientist G, DBT, Govt. of India Prof. Vinod Tare, IIT, Kanpur Dr. Antonio Lopez, Head of Unit, CNR-IRSA, Italy Dr. S. P. Wani, Asst. Research Program Director, ICRISAT Prof. G. S. Dasog, UAS, Dharwad Dr. Meenakshi Sharma, Vice President, SABMiler,
- Dr. J. H. Kulkarni, Manager Special Duty, Ugar Sugar
- Mr. Nitin Vaidya, Deputy General Manager, MVML
- Shri, N.K. Saha, CEO, OPM, Amlai
- Shri. Prakash Urade, Executive Engineer, NMC, Nagpur



## Partners

## EU CONSORTIUM PARTNERS



## INDIA CONSORTIUM PARTNERS



# About ICRISAT

## ICRISAT International Crops Research Institute Science with a human face for the Semi-Arid Tropics

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a non-profit, non-political organization that conducts agricultural research for development in Asia and sub-Saharan Africa with a wide array of partners throughout the world. Covering 6.5 million square kilometers of land in 55 countries, the semi-arid tropics have over 2 billion people, of whom 644 million are the poorest of the poor. ICRISAT innovations help the dryland poor move from poverty to prosperity by harnessing markets while managing risks – a strategy called Inclusive Market-Oriented Development (IMOD).

ICRISAT is headquartered in Patancheru near Hyderabad, Andhra Pradesh, India, with two regional hubs and five country offices in sub-Saharan Africa. It is a member of the CGIAR Consortium. CGIAR is a global research partnership for a food secure future. ICRISAT-Patancheru (Headquarters) Patancheru 502 324 Andhra Pradesh, India Tel +91 40 30713071

ICRISAT-Bamako (Regional hub WCA) BP 320 Bamako, Mali

1 204 ISAT-Bulawayo Matopos Research Station PO Box 776 Bulawayo, Zimbabwe



ICRISAT-Liaison Office CG Centers Block NASC Complex Dev Prakash Shastri Marg New Delhi 110 012, India

ICRISAT- Kano PMB 3491, Sabo Bakin Zuwo Road Tarauni, Kano, Nigeria

ICRISAT-Lilongwe Chitedze Agricultural Research Station PO Box 1096 Lilongwe, Malawi ICRISAT is a member of the CGIAR Consortium

> ICRISAT-Nairobi (Regional hub ESA) PO Box 39063, Nairobi, Kenya

ICRISAT-Niamey BP 12404 Niamey, Niger (Via Paris)

ICRISAT-Maputo c/o IIAM, Av. das FPLM No 2698 Caixa Postal 1906 Maputo, Mozambique www.icrisat.org