Annual Report
(2014-15)

Integrating Bio-treated Wastewater Reuse With Enhanced Water Use Efficiency to support the green economy in EU and India (India side)

Water4Crops

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

Department of Biotechnology
Ministry of Science and Technology

ICRISAT
Science with a human face

International Crops Research Institute for the Semi-Arid Tropics

This work is being undertaken as part of the

CGIAR RESEARCH PROGRAM ON Water, Land and Ecosystems

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Summary

The simultaneous effects of agricultural growth, industrialization and urbanization are further increasing pressure on limited water resources. Water resources are depleting at faster rate than the rate of recharge, thus the world is experiencing moderate to severe water shortages. In fact, one third of global population will face water scarcity by 2025, which will be largely in the developing world. Current and future fresh water demand could be met by enhancing water productivity. Three basic principles for improving water productivity are reduce the water use, substitute the good quality water with marginal quality water, and recycle the wastewater. 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 11 research partners including private companies along with the research institutions and similarly EU consortium consists of 22 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 work package 1, work has been conducted at four locations: SAB Miller India (Sangareddy, Telangana), Jain Irrigation System Ltd (Jalgaon, Maharashtra), Ugar Sugar Works (Belgavi, Karnataka), and K.C.P. Sugar and Industries Corporation Ltd (Laksmhipuram), Tamilnadu. The physic-chemical characteristics of the wastewaters from these sites suggested that these wastewaters may not be suitable for direct reuse in agriculture. Moreover, the impact of long term application of wastewater for irrigation on soil health and crop productivity is also assessed. At SAB Miller India and Laksmhipuram site of K.C.P. Sugar and Industries Corporation Ltd, constructed wetland were prepared to treat the effluent coming from effluent treatment plant of the factories. At Lakshmipuram site, agro-aqua system was demonstrated using treated water coming from constructed wetland. The constructed wetlands at both sites were able to reduce Chemical Oxygen Demand (COD) by 30-92%. Dark colour of the industrial effluent is also one limiting factor for reuse of wastewater. In this package, an indigenous bacterial consortium is developed that reduced color by 32%. The total colour removal of 58% at the site is due to the cumulative treatment effect of adapted bacteria and algal consortium followed by activated charcoal, whereas in the lab study, only 8.8% of colour was reduced by 2nd generation adapted bacterial consortium. Algal treatment was also studied to remove the COD from distillery wastewater. Four studies of bacterial treated distillery effluent carried out using free cells of algae, growing algal cells, Strychnos potatorum seed, moringa seeds observed reduction in COD by 4000, 4666, 933, and 15066 mg/L respectively. The bacterial and subsequent algal treatment of distillery wastewater couldn't reduce the salt content to meet the irrigation standard (EC: 0 to 3 mS/cm). Hence, one of the research leads is to use the halophytes for phyto-remediation in constructed wetland. The study has to be carried out to evaluate the uptake of salinity and other organic contaminants by different sp. of halophytes.

The research in implementation work related to domestic wastewater (work package 2) is being conducted at multiple locations: NEERI and Pandherkawada (Nagpur), ICRISAT and Kothapally (Telangana), UAS, Dharwad (Karnataka), and Mavanur, Katnur and Gabbur in Dharwad (Karnataka). The wastewaters at all of these sites are not suitable for direct reuse in agriculture. However, farmers are using these wastewaters as it is. The wastewater samples were also characterized for different microbial groups *viz.* Bacteria, Fungi, *Actinomycetes*, *Azotobacter* and *Rhizobium*. These microbial isolates were tested to treat the wastewater. The water4crops teams have constructed wetland as a decentralized wastewater treatment system at these locations. The regular monitoring of the performance of constructed wetland has indicated high treatment efficiency of contaminants. For example, COD removal efficiency from field scale wetlands constructed at ICRISAT and Kothapally is about 30- 60% and from pilot scale constructed wetland at NEERI is highest 90-95%. Apart from wastewater treatment, remediation of degraded soil due to longterm application of wastewater is also studied under this work package. Microbial consortium of *Enterobacter aerogenes, Azospirillum irakense, Enterobacter cloacae,* and *Pseudomonas sp*. was used to reclaim the degraded land as Ugar Sugar site.

Efficient use of treated wastewater is major goal of third work package. In this package, impact of wastewater reuse in agriculture on crop and soil is assessed through laboratory and field experiments. The experimental sites for the impact assessments are SAB Miller India, Ugar Sugar, KCP Sugar Industry, ICRISAT, Jain Irrigation, and UAS Dharwad. At each site, farms are selected for conducting filed experiments. One of the tasks in this package is developing efficient irrigation system. The experiments were conducted by Jain Irrigation System Ltd to assess the feasibility of treated wastewater from food processing plant in agriculture through drip irrigation system. Different configurations of emitters for micro irrigation system were tested and suitable emitters were identified. Laboratory and field experiments were conducted to assess the effect of wastewater irrigation on crop and soil. Apart from irrigation, the scope of this package is also extended to agro-aqua farming system. One of the expected outcomes of the integrated approach of reusing bio-treated distillery effluent first in aquaculture and then in agriculture is for fertilizer savings.

Comparative physiological studies on pearl millet, sorghum and maize provided useful information on common and crop specific mechanisms of drought tolerance in these crops. The studies on chickpea confirmed introgression of the genomic region controlling drought tolerance traits. Screening of tomato germplasm for stress tolerance based on fruit yield and physiological characters was found effective. The studies led to better understanding of genetic mechanisms and interrelationships of these traits. These traits were found useful surrogates in breeding for water use efficient (WUE) genotypes. Root studies in different tomato species observed the positive and significant association of root traits (root length, root dry weight) and root to shoot ratio with fruit yield.

The fourth work package is about improving crop cultivars that use water effectively. The genetic material of crop exchanged between Indian and EU consortium. Crop specific mechanisms of drought tolerance in pearl millet, sorghum and maize were assessed through comparative physiological studies in lysimeters. The studies on chickpea confirmed introgression of the genomic region controlling drought tolerance traits. Screening of tomato germplasm for stress tolerance based on fruit yield and physiological characters was

found effective. The studies led to better understanding of genetic mechanisms and interrelationships of these traits. These traits were found useful surrogates in breeding for water use efficient (WUE) genotypes. In tomato, high yielding and drought tolerant genotypes were identified and hybridization was under taken to introgress drought tolerance traits from two wild species (S. pennellii and S. galapagenes) into the cultivated species.

A common online platform prepared for both the EU and the Indian consortium to exchange and share their experiences about project activities they are undertaking. It is designed to host discussion on upcoming factsheets especially on the topics like legislation and cost-benefits of waste water treatment and reuse technologies. This discussion will provide inputs to the innovation process in WP5. The external stakeholders from Innova Platforms were also invited to the group. On 28th May 2014, first Indian INNOVA meeting was organized. The meeting brought together the Industry experts from CII (Confederation of Indian Industry), EBTC (European Business Technology Center), Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) Germany and EnviroTech Water Management Pvt. Ltd. in the field of wastewater treatment and water use efficiency. These experts were challenged to explore business opportunities for the new technologies in the domains of wastewater reuse and valorization, and water use efficiency that are being developed in Water4Crops project. The EU-India Joint water4crops website is the main dissemination tool to showcase significant results and outcomes and project events. The website is regularly updated with information from both EU and Indian side. Apart from project activities, the news, events and related articles are also posted in the website. This conveys to outsiders that W4Cs is a joint project between India & EU and both sides are working together.

The water4Crops-India consortium partners are working to develop and implement the technologies to tackle the wastewaters from industries as well as small communities and provide solutions for emerging water, energy and related problems for achieving sustainable development in the country. Wastewater treatment and its reuse in agriculture are also complementing the Swachha Bharat Mission by govt. of India. In brief, the consortium project Water4Crops has made good progress up to second year and works under each deliverable is on track. Numbers of manuscript are in pipe line and partners are taking initiative to take the project learning's to policy maker for scaling-up of key activities of project. The consortium team is working as one team and substantial progress has been achieved and plans for strengthening and expanding the work during the third year are already in place.

This annual report is structured in sequence of Work Packages and Deliverables. Please note that all deliverables completed in a given year and some of them may not be completed during third year.

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^3 in 1951 to

1820 m^3 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^3 in 2025 and 1140 m^3 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.

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.

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, and 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 bio-treatment 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 0-1. 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 are 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 India, Ugar Sugar are working towards developing and demonstrating integrated treatment processes for bio-refinery effluents. Another industry Jain Irrigation Systems Limited (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 is developing water efficient crop variety for selected crops and on integrated mangrove-fishery farming system to optimise use of saline wastewater.

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 ICRISAT (Telangana 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 0-2) (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.

Table 0-1. List of consortium members from India and EU

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

Table 0-2. List of work packages and work package leader.

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

Table 0-3. List of deliverable during project period.

Delive-rable No.	Deliverable name	WP No.	Delivery date
1.1	Detailed characterization of selected wastewaters	WP1	Month 12
1.2	Demonstration of CW and HRTS systems	WP1	Month 30
1.3	Demonstration of fungal decolourization system	WP1	Month 42
1.4	Demonstration of algal treatment system	WP1	Month 48
1.5	Carbons and membranes for the recovery of phenolics / pigments	WP1	Month 48
1.6	Impact of treated and untreated wastewater use on soil, crop and groundwater quality	WP1	Month 48
2.1	Report on microbial consortium formed using available strains	WP2	Month 12
2.2	Optimized microbial consortium for remediation of degraded land	WP2	Month 24
2.3	Demonstration of CWs and HRTS systems	WP2	Month 30
2.4	Bio-remedial measures tested to improve degraded lands due to use of wastewater	WP2	Month 36
2.5	Report on impact assessment of wastewater use in agriculture	WP2	Month 48
3.1	Benchmark sites characterized	WP3	Month 12
3.2	Efficient irrigation system evaluated	WP3	Month 36
3.3	Impact assessment of wastewater on crops, soil and groundwater documented	WP3	Month 48
3.4	Validated models for enhancing WUE at field and micro-watershed level	WP3	Month 48
3.5	Increased land and saline wastewater productivity in 20 ha	WP 3	Month 48
3.6	Replicable model demonstrated for integrated saline wastewater use and livelihood options	WP 3	Month 48
3.7	Package of agro-aqua farming system available for	WP 3	Month 48

Table 0-3. List of deliverable during project period.

Delive-rable No.	Deliverable name	WP No.	Delivery date
	replication		
3.8	Enhanced capacity of community, other stakeholders and MSSRF staff on saline wastewater farming	WP 3	Month 48
3.9	Availability of tool kit on agro-aqua farming system in print and multimedia format	WP 3	Month 48
4.1	Information on the most adequate combinations of species/genotypes x environment x management for different drought scenarios in India and EU	WP4	Month 36
4.2	Information on QTL (QTL combination) underlying the drought adaptation traits in maize, sweet sorghum, pearl millet and tomato at particular drought stress environments	WP4	Month 36
4.3	Mechanisms for improved water use efficiency and salinity tolerance characterized across crop species	WP4	Month 48
4.4	Chickpea breeding lines with improved drought adaptation	WP4	Month 48
4.5	Trained human resources in research on drought adaptation of crops and integrated breeding for drought adaptation	WP4	Month 48
5.1	Database of stakeholders	WP5	Month 12
5.2	Report of agribusiness opportunities	WP5	Month 24
5.3	Position papers on wastewater topics	WP5	Month 48
6.1	Internal report on customer / entrepreneur demands and technological offer	WP6	Month 12
6.2	Webpage and Public Dissemination material	WP6	Months 6,12,24,36, 42
6.3	Report on training course including online curricula	WP6	Month 36
7.1	Workshop to workout common protocols to be adopted by the partners in the project	WP7	Month 12
7.2	First year annual report to DBT	WP7	Month 12
7.3	Second year annual report to DBT	WP7	Month 24
7.4	Third year annual report to DBT	WP7	Month 36
7.5	Fourth year annual report to DBT	WP7	Month 48

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.

1.1 Detailed Characterization of Selected Wastewaters

Four sites were selected in WP1 to study potential of industrial wastewater recycling in agriculture. Profile of the wastewater and degraded soil collected from these sites were presented in the first annual report. The results from regular monitoring of the wastewater quality are mention in this section.

Table 1-1. Selected sites for reuse of industrial wastewater

Sl No.	Site	Industry type
1	SAB Miller India, Sangareddy, Telangana	Brewery
2	Ugar Sugar Works, Belgavi, Karnataka	Distillery
3	K.C.P. Sugar and Industries Corporation Ltd, Chennai, Tamilnadu (**Lakshmipuram and Vuyyuru**)	Distillery
4	Jain Irrigation System Ltd, Jalgaon, Maharashtra	Food processing

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

Reverse osmosis (RO) plant

The pH of the RO reject water was consistently in the alkaline range. The chemical oxygen demand was in the range of 128 mg/L and a total inorganic nitrogen concentration of 4.68 mg/L was observed (Table 1-2). The total alkalinity of the water was very high at 1644 mg/L as $CaCO_3$ exhibiting strong acid assimilation capacity of the water. The presence of high concentrations of alkali metal ions such as sodium (> 1000 mg/L), calcium (143 mg/L), potassium (176 mg/L) and magnesium (72.44 mg/L) can be correlated with these extremely high levels of total alkalinity. Furthermore the presence of other ionic species such as chloride (955.27 mg/L) and sulphate (200 mg/L) reflected with a high electrical conductivity (EC) of 6 mS/cm. Not surprisingly the wastewater was in the very hard category (> 180 mg/L as CaCO3) with a total hardness level of 660 mg/L. The overall SAR of the wastewater was found to be 18. The wastewater was analysed for eight heavy metals viz. arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), nickel (Ni) and zinc (Zn). Presence of all the heavy metals was detected in the inductively coupled plasma mass spectrometry (ICP-MS) analysis. According to the Food and Agriculture Organization (FAO-UN) standards, the permissible limits for application of waters containing As, Cr, Cd, Zn, Pb, Co, Cu and Ni for irrigating fields are 0.1 mg/L, 0.1 mg/L, 0.01 mg/L, 2 mg/L, 0.1 mg/L, 0.05

mg/L, 0.2 mg/L and 0.2 mg/L respectively. The heavy metal concentration was found to higher the prescribed standards for irrigation for seven out of these eight heavy metals baring zinc.

Table 1-2. Wastewater characterization (composite data, August 2014 - June 2015) for SAB Miller India

Sl No.	Parameter	Unit	RO reject	ETP	UASB
1	Arsenic	mg/L	0.690	0.329	0.126
2	Boron	mg/L	0.167	0.083	0.085
3	Calcium	mg/L	143.04	73.05	72.14
4	Cadmium	mg/L	0.363	0.193	0.185
5	Chemical oxygen demand	mg/L	128	96	320
6	Chloride	mg/L	955.27	322.38	301.22
7	Chromium	mg/L	0.306	0.150	0.142
8	Cobalt	mg/L	0.434	0.189	0.181
9	Copper	mg/L	0.208	0.014	0.000
10	Electrical conductivity	mS/cm	6.04	1.38	1.6
11	Iron	mg/L	1.660	0.535	1.215
12	Potassium	mg/L	176.46	41.14	50.08
13	Lead	mg/L	0.445	0.209	0.227
14	Magnesium	mg/L	72.44	21.45	23.28
15	Sodium	mg/L	1064.22	465.44	465.15
16	Inorganic nitrogen	mg/L	4.68	14.89	50.68
17	Nickel	mg/L	0.662	0.256	0.235
18	pH at 25 ° C		8.02	7.66	7.68
19	Phosphate	mg/L	1.39	1.42	1.91
20	Sulphate	mg/L	200.45	24.95	22.70
21	Sulphur	mg/L	76.37	9.287	9.944
22	Total dissolved solids	mg/L	8630	2130	2320
23	Total alkalinity	(mg/L as CaCO3)	1644	588	762
24	Total hardness	(mg/L as CaCO3)	600	170	230
25	Zinc	mg/L	0.480	0.055	0.070
26	Sodium adsorption ratio (SAR)		18.02	12.27	12.15

Effluent treatment plant (ETP)

The average pH of the ETP effluents was found to be 7.66 which are close to neutral. The average COD value for the wastewater was is 96 mg/L which is less than the CPCB permissible limit of 250 mg/L. The average inorganic nitrogen concentration in the wastewater was found to be 14.89 mg/L without much deviation throughout the year exhibiting limited fertilizer value for this wastewater in terms of irrigation. The alkali metal concentrations viz. Na, K, Ca and Mg were high at 465 mg/L, 41 mg/L, 73 mg/L and 21 mg/L respectively. The high alkali metal ion concentration was reflected in high total alkalinity

value of 588 mg/L as $CaCO_3$ and was also responsible for the high SAR of 12.27. The wastewater was of very hard category as the total hardness value was at 282 mg/L as $CaCO_3$. The presence of anionic species particularly chloride and bivalent sulphate in high concentration was reflected with EC value of 3.78 for the wastewater. Heavy metal concentrations were found to be higher than the standards prescribed for irrigation (as discussed above) for six of the eight chemicals it was tested for barring only zinc and copper.

Upflow anaerobic sludge blanket (UASB) unit

The COD concentration of 320 mg/L in the UASB effluent is much higher than CPCB limit of 250 mg/L. The high inorganic nitrogen concentration is indicative its high fertigation potential for this wastewater if the salinity and SAR can be reduced via remediation. Though the alkali metal concentration in the wastewater is similar to the ETP effluent the combination of slightly higher potassium concentration (50 mg/L) and relatively higher inorganic nitrogen and phosphate concentrations have resulted in a higher EC for this wastewater at 4.12. The total alkalinity of the wastewater is very high though at 762 mg/L as $CaCO_3$. A higher ammonium ion concentration and as mentioned above the slightly higher potassium concentration, may be the attributing factor behind this higher total alkalinity compared to ETP effluents. The pH of the wastewater was found to be 7.68 and did not show much fluctuation throughout the year.

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. Please see Section 3.2

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

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^3/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.

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 % treated water 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 is 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 is 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 is 7.0 to 8.0.

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 (SWWAnP) 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 $4^{o}C$ till use.

1.2 Demonstration of CW and HRTS systems

SAB Miller India, Sangareddy, India

In order to explore and evaluate the bioremediation potential for the treatment of industrial wastewater, a field scale constructed wetland (CW) was commissioned at the Sangareddy campus of SAB Miller India, a large brewery. The CW was comprised of two chambers one sub-surface cell with dimensions of 20 m X 20 m X 1 m and one holding tank for the treated wastewater of the same dimension. The sub-surface cell was having a 20 cm large (40 mm) gravel layer at the bottom, covered with successive 20 cm layers of medium (20 mm) and small gravel (10 mm) each. A 15 cm layer of coarse sand (1.5 mm) covering the small gravel layer constituted the top layer. Vegetation introduced initially were Napier (*Penisetum purpurem*) and Bamboo (*Bambuseae spp.*). Figure 1.1 gives an overview of different phases of construction of the CW at Sangareddy.

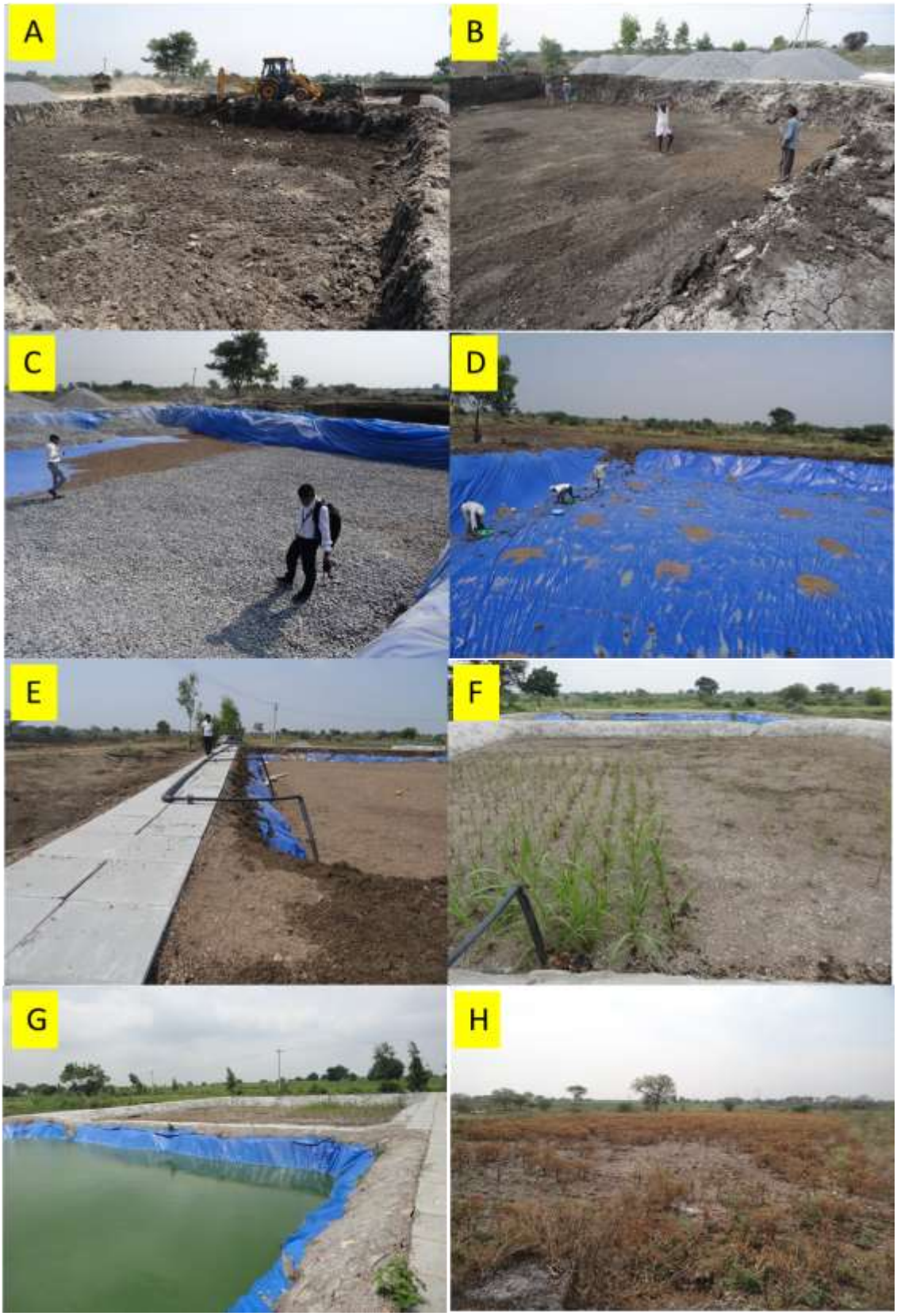

Figure 1-1. Different phases of construction of CW at Sangareddy : A) Excavation; B) Compacting; C) Layering of media; D) Liner in holding tank; E) Pipe fittings at the inlet side; F) Plantation; G) Stabilizing phase; H) Safflower fields irrigated with treated wastewater

Performance of constructed wetland

The inlet and outlet samples were collected on a monthly basis from February 2015 onwards and were analysed in ICRISAT laboratory. The flow as well as the wastewater constituents both may change diurnally as well as seasonally (peak season and lean season of the plant operation). Thus the performance of CW can only be assessed via the composite datasets for inlet and outlet water quality over a period. Likewise, Table 1-3 represents the performance of the CW during Feb 2015 to June 2015. The pH of the wastewater remained in the alkaline range throughout the months both at the inlet as well as the outlet of the CW. The COD as well as the inorganic nitrogen removal efficiency (RE) were moderate at 33.33 % reported for each. The treated wastewater COD of 64 mg/L is less than the CPCB limit of 250 mg/L and suitable for irrigation. We expect these Res to increase once the vegetation as well as its root microbiology reaches stabilization. The phosphate removal efficiency observed was 37.77 %. The sulfate level was rather low at 2.83 mg/L in the inlet wastewater; the marginal RE of 12.37 % can be further increased with introduction of Typha which has higher sulfate uptake capacity. The wetland showed a removal efficiency of 11. 48 % for total hardness. Among the macro elements sodium concentration was particularly very high at 439 mg/L. The high salt concentration was reflected by an electrical conductivity above 3 ms/cm in inlet as well as outlet (irrigation standard as per CPCB standards of 2010 is below 4 ms/cm). The calcium, magnesium as well as sodium removal efficiency was found to be around 10 %. This removal however may be abiotic rather than biotic. Plant analysis data in the inlet wastewater in subsequent months will be helpful in assessing this aspect. The overall sodium adsorption ratio (SAR) does not show much change with inlet and outlet values of 12.8 and 12.17 respectively. This water however can be used in irrigation as per Central Pollution Control Board of India which sets the SAR limit at 26. The treated wastewater has 166 mg/L chloride which is less than the FAO standard of 354 mg/L. Alkalinity is the measure of dissolved carbonates, bi-carbonates, and hydroxides concentrations in the wastewater. The total alkalinity in the inlet and outlet wastewater is much greater than the desirable limit for agriculture (100 mg/L). The combination of high alkalinity and high pH reduces the irrigation potential of the treated wastewater.

Table 1-3. Wetland performance based on Feb 2015 data sets (SAB Miller India sangareddy)

SNo	Parameter	Unit	Inlet	Outlet	Efficiency (%)
1	Calcium	mg/L	75.69	68.45	9.57
2	Chemical oxygen demand	mg/L	96	64	33.33
3	Chloride	mg/L	174	166	4.60
4	Electrical conductivity	ms/cm	3.39	3.12	-
5	Potassium	mg/L	58.89	58.72	0.29
6	Magnesium	mg/L	25.23	22.82	9.57
7	Sodium	mg/L	439	397	9.57
8	Inorganic nitrogen	mg/L	18	12	33.33
9	pH at 25 ° C	-	7.98	8.78	-
10	Phosphate	mg/L	1.88	1.17	37.77
11	Sulfate	mg/L	2.83	2.48	12.37
12	Total dissolved solids	mg/L	2019.50	1432	29.09
13	Total Alkalinity	(mg/L as CaCO3)	606	515	15.02
14	Total hardness	(mg/L as CaCO3)	610	540	11.48
15	Sodium adsorption ratio (SAR)	-	12.80	12.17	-

Lakshmipuram site

The Lakshmipuram sugar unit is one among the most modern plants with a crushing capacity of 4500 TCD. It has a cogeneration power plant with 5 MW capacities being in operation since January 2006. The effluent generated is subjected to a sequential treatment *viz.,* aeration, anaerobic, contact filter and final aeration processes. Floating, foreign materials as well as oil and grease are removed through bar screens and grid beds before passing it to aeration tank. Raw effluent is initially mixed with lime solution and passed on to the anaerobic treatment process where cow dung and urea is added to mainly reduce biochemical oxygen demand (BOD) and chemical oxygen demand (COD). The primary treated effluent is filtered through contact filter medium to remove total suspended solids (TSS) and total dissolved solids (TDS) and finally let into aeration tank.

The water from final aeration tank is the source for Water4Crops project. The water characterization indicates that BOD, COD, TSS and TDS values are 120, 220, 65 and 1545 mg/l respectively. The standards to discharge effluent for irrigation is 30, 90, 50-100 and 2000 mg/l for BOD, COD, TSS and TDS respectively. For aquaculture use the permissible limit of water quality is BOD 30-60 mg/l, total alkalinity 80-120 mg/l, total hardness 120-200 mg/l, NH_3N 0.05 mg/l, NO_2N 0.001 mg/l, NO_3N 0.005 mg/l and Total Phosphorous 0.25 mg/l. Using untreated water for agro-aqua farming systems will lead to reduction in hydraulic conductivity, reduction in soil porosity, and decrease in the infiltration rate, reduced soil permeability and increased bulk density which will affect the soil and groundwater in the long run. Therefore to meet the standards of agriculture and aquaculture needs the effluent should be further treated. Bio-treatment using constructed wetland is proposed for the treatment as a cost effective technology.

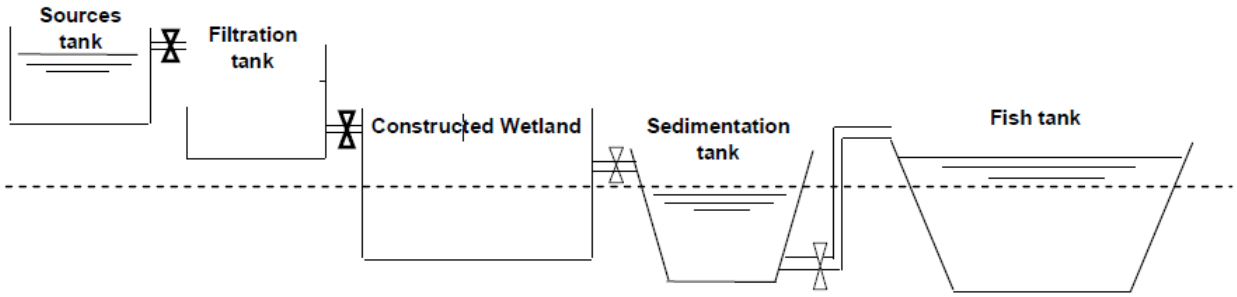

Figure 1-2. Schematic diagram of sugar effluent treatment process at Lakshmipuram

As depicted in the schematic diagram (Figure 1-2) secondary treatment of anaerobic treated sugar effluent is sequentially treated through pre-treatment in filtration tank, constructed wetland followed by sedimentation before discharging into fish tank which also contributes for treatment process. The design specification and the purpose are discussed below. The source water is a partially treated sugar effluent drawn from the aeration tank established by the industry in the upstream. Pre-treatment facility was created to filter the large coarse materials before allowing the influent into wetland, which would otherwise reduce the hydraulic conductivity and performance of wetlands in the long- term. The filtration tank with dimensions of 3.6mx3.6mx1m was constructed enabling the flow through gravity. A barrier with a height of 0.3 m is constructed in the middle of the tank and filled with 40-60 mm gravel in one part of the barrier enabling filtration of large coarse materials.

The rich biological productivity in terms of floral and faunal diversity makes the wetlands unique among all other ecosystems. By the virtue of its land area coupled with ecological dynamism like plants, animals and soil, sun and wind wetlands has high potential to transform pollutants in wastewater into beneficial nutrients that enhances biological productivity. Hence to augment the treatment potential, artificial wetlands depicting the characteristics of natural wetland ecosystem is constructed with different hydrologic approach. There are two types of constructed wetland system with surface flow and subsurface flow. This project adopted subsurface flow type with hybrid model of vertical and horizontal flows to treat the anaerobic treated sugar effluent in Lakshmipuram site.

A wetland comprising of 5 beds were constructed with the dimension of 21.85m×11.10mx1.0m covering an area of 242.53 m^2. The bed has regular rectangular shape with 1% slope. The wetland dimension is designed with an aspect ratio of 2:1. Dimension of each bed along with different medium are as follow: (i) 1st Bed is of 3.24 m length, (ii) 2nd Bed is of 2.10 m length, (iii) 3rd Bed of 11.10 m length is the main bed consisting different substrates of organized layers (iv) 4th Bed is of 2.10 m length and the (v) 5th Bed is of 3.24 m length.

Hybrid model of vertical and horizontal flow constructed wetland

Water mass balance in constructed wetland

Table 1-4 depicts the water balance in the constructed wetlands. The out flow from filtration tank is the inflow to CWL which 19.44 m^3/day. The coarse aggregate volume in the CWL is 145.72 m^3 which is calculated by multiplying the length x width x bed depth. Based on the experiment conduct in the field porosity of different substrates were derived and presented in the above table and the average porosity of the CWL is 49.8%. Coarse aggregate volume multiplied by the actual porosity of the each bed gives the volume of water in the void i.e. 73.67m^3. Volume of water above the aggregate was calculated simply by multiplying length x width x 0.2m depth which constitutes 48.57 m^3. Summation of volume of water in the void and volume of water above the aggregate gives the total volume of water (122.24 m^3) in the CWL. To estimate net volume of water in the wetland evaporation and ET losses from respective beds were deducted from the total volume of water in the CWL which is 120.74 m^3.

Table 1-4. Hydrology balance in constructed wetlands

CWL Beds	Length of CWL beds (m)	Width of CWL (m)	Coarse aggregate volume (m^3)	Porosity	Volume of water in the void (m^3)	Volume of water above aggregate (m^3)	Total volume of water in the CWL (m^3)	ET losses (m^3)	Precipitation (m^3)	Net volume of water in CWL (m^3)
C1	3.24	11.10	21.59	0.53	11.36	7.20	18.55	0.23	0.707	18.31
C2	2.11	11.10	14.04	0.49	6.94	4.68	11.62	0.15	0.460	11.46
C3	11.18	11.10	74.46	0.50	37.08	24.82	61.90	0.71	2.439	61.18
C4	3.24	11.10	21.59	0.53	11.36	7.20	18.55	0.23	0.707	18.31
C5	2.11	11.10	14.04	0.49	6.94	4.68	11.62	0.15	0.460	11.46
Total	**21.88**	**----**	**145.72**	**----**	**73.67**	**48.57**	**122.24**	**1.49**	**4.773**	**125.55**

Hydraulic retention time was derived by dividing the net volume of water in CWL by volume of inflow into CWL i.e. 120.75/19.44 is 6.2 days. Hydraulic loading rate is 2.91m^3/day/m^2 and the number of days taken to lead the entire wetland is estimated to be 7.5 days. Nominal velocity (V_1) is 2.189 m/day which was arrived by dividing inflow volume of water by width x depth of water (.8m). Actual velocity (V) is calculated by dividing nominal velocity by average porosity (0.5) which is 4.378m/day.

Water quality and treatment mechanism at Lakshmipuram

Active treatment process was in witnessed since October 2014 and the results presented here are for five months (October 2014 to February 2015). Filtration tank was constructed as a pre-treatment facility mainly to remove coarse particles from the sugar effluent and avoid clogging of wetland system. Despite very less retention time removal potential is though not significant it is contributing in the reduction of pH, total alkalinity and COD which may be due to the presence of microbes in the surface area 40 to 60 mm gravel filled partially. Constant monitoring of other important parameters and the corresponding removal mechanism is initiated.

For the months from August to November 2014 water quality monitoring was done for inlet to filtration tank and the outlet of constructed wetland for some of the parameters. And the changes in water quality during these months show that pH was reduced by 0.12 to 1.43. The improvement in the reduction of pH is mainly attributed to the increase in the density of planted emergent macrophyte i.e. *Typha angustifolia*. However, the natural development of floating macrophytes like duck weeds and algae in the months October and November also played a vital role in increased reduction of pH. During the growth stage of the emergent and floating macrophytes the uptake of available materials like phosphate, chloride and sulphate led to decrease in the total alkalinity concentration which in turn had an impact in the reduction of pH. To sustain the effectiveness of the treatment, free floating macrophytes are removed on a regular basis at two days interval. Observation of EC indicated that there is no significant change during the above four months.

The total dissolved solid (TDS) of the raw water was very high (1046.5 mg/L) in the month of September. This was reduced to 684 mg/L after treatment through CWL. The reduction of TDS is a significant attribute of wetland. In the month of October and November, the TDS of raw water was very low compared to the month of September. Also, there was no significant change in the TDS after treatment. In the physical removal process higher initial concentration of TDS was a driving force for its significant removal compared to the lower initial concentration. The total hardness was reduced moderately in the month of August and September. There was no change in the total hardness in the month of October which also coincides with very less reduction of salts in the same month. In the month of November the total hardness was reduced moderately.

Physico chemical properties

pH

As presented in Figure 1-3 the raw water had a basic pH of 8.6. The pH level of the water is important for reusing in integrated aqua and agro purposes. Hence, it is important to reduce the pH to desired level. The pH of the water from filtration tank was 8.54. An insignificant

reduction of pH was observed which may be due to the removal of ions by sedimentation and filtration. The pH was reduced to 8.3 and 8.2 in the C1 and C2 beds respectively. This reduction was facilitated by the removal of contaminants from the water by the algae present on the substrates. In C3 bed, the pH was reduced to 7.5. This is due to the breakdown of organic contaminants by the aerobic organism which produces an acidic end product. The acidic end product helps in the reduction of pH significantly. In C4 bed there was no change in pH and there was an insignificant reduction in the C5 bed. It is evident that the reduction in pH was facilitated by both biological and chemical processes in the wetland.

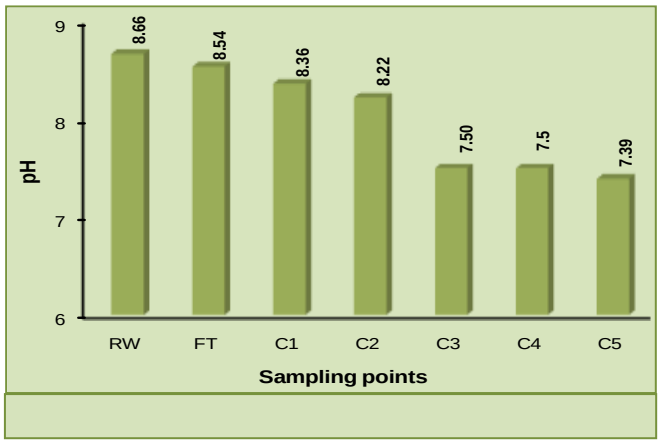

Figure 1-3. Changes in pH levels

Conductivity (EC)

The conductivity of water is due to the presence of salts. The conductivity of raw water was 1.59 mS this may be due to the high amount of salts added to the effluent by the industry to increase the pH. The conductivity was reduced to1.57, 1.55 and 1.47 mS in the FT, C1 and C2 respectively which was insignificant. The reduction in conductivity to 1.34 mS was observed in the C3 bed followed by insignificant removal in the C4 & C5 beds. The key biological process is that the salts assimilated by microbes adhered to the plant roots was up-taken by the emergent macrophytes which contributed for reduction of conductivity.

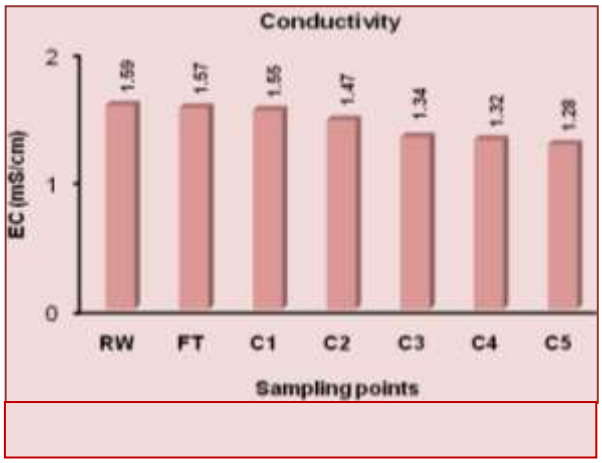

Figure 1-4. Changes in conductivity (EC)

Total Dissolved Solids (TDS)

Total dissolved solids are the solids in water that can pass through a filter (usually with a pore size of 0.45 micrometers). TDS is a measure of materials dissolved in water which includes carbonate, bicarbonate, chloride, sulfate, phosphate, nitrate, calcium, magnesium, sodium, organic ions, and other ions. Water quality is determined by the presences of TDS and a certain level of these ions in water is necessary for aquatic life. A high concentration of TDS not only reduces water clarity also plays role in decreasing photosynthesis, increasing water hardness which could result in a laxative effect.

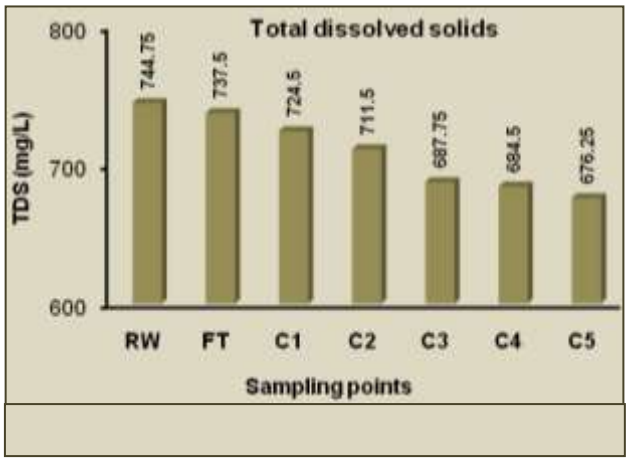

Figure 1-5. Changes in total dissolved solids (TDS)

From the treatment of sugar effluent concentration of TDS in RW had an average of 744.75 mg/L which was reduced to 737.5 mg/L in the filtration tank. In the C1 bed it was further reduced to 724.5 mg/L. In the consecutive beds, C2, C3, C4 & C5, the TDS concentration was 711.5, 687.75, 684.5, and 676.25 respectively. The reduction of TDS in the filtration tank, C1, C2, C4 & C5 is due to the physical process like sedimentation and filtration by the substrate. The reduction of TDS in the C3 bed was significantly higher compared to the other beds. This is due to physical and biological processes such as sedimentation, filtration, bacterial decomposition and adsorption. The bacterial decomposition is favored by the aerobic bacteria present on the soil surface. The adsorption and uptake of various ions by both floating and emergent macrophytes play a vital role in reducing TDS.

Phosphate (PO_4)

Phosphorus is a nutrient required by all organisms for the basic processes of life. Phosphorus clings tightly to soil particles and is used by plants. As the source water for bio-treatment is sugar effluent concentration of phosphorus is found to be high 4.375 mg/L. The phosphate concentration was reduced to 4.125 mg/L in the filtration tank. In C1 bed it was reduced to 3.87 and was further reduced to 3.3, 2.17, 1.95 & 1.65 in the subsequent beds C2, C3, C4 & C5 respectively. Around 62.28% of phosphate is removed by the wetland treatment. The removal in the filtration tank followed by C1, C2, C4 & C5 is due to the uptake by algal biomass formed on the surface of the substrates which is a biological process. Also, physical process like sedimentation and filtration would have played an important role. The phosphate removal was notably high in the C3 bed which is due to the chemical processes such as phosphate adsorption, complexation and precipitation. Phosphate uptake by both emergent and floating macrophytes and biotic assimilation by

the microbes present in the wetland soil and *Typha* roots played an important role in the enhanced removal of phosphate from sugar effluent.

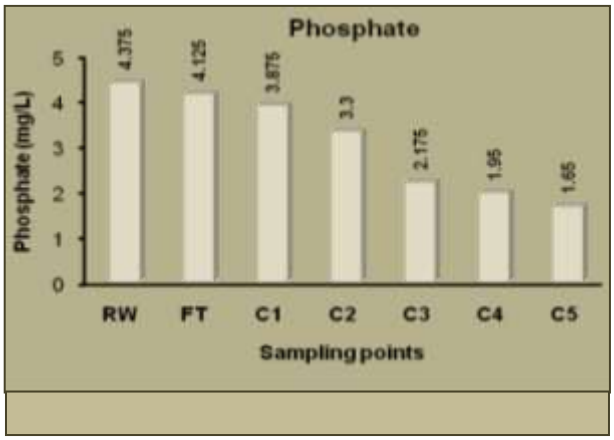

Figure 1-6. Changes in phosphate concentration

Total Hardness

Hardness is the measure of polyvalent cations (ions with a charge greater than +1) in water. Hardness generally represents the concentration of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions, because these are the most common polyvalent cations. Other ions, such as iron (Fe^{2+}) and manganese (Mn^{2+}), may also contribute to the hardness of water. Waters with high hardness values are referred to as "hard," while those with low hardness values are "soft". The total hardness of the RW was 342.25 mg/L which is due to the addition of quick lime by the industry during primary treatment of sugar effluent and the changes in total hardness in the treatment process is depicted in

Figure 1-7. The hardness of the effluent from filtration tank was 330 mg/L while in the subsequent beds of wetland from C1 to C5 were estimated as 326.25, 314, 285, 280.5 & 272.5 respectively. The reduction of total hardness observed in the C1, C2, C4 & C5 beds can be corroborated to the decrease in dissolved solids like salts due to physical process such as sedimentation and filtration. The reduction of total hardness was relatively high in C3 bed than the other beds which is due to the biological mediated chemical processes. As attributed for EC reduction similar biological process was observed in the reduction of total hardness by uptake microbial degraded salts by plant roots.

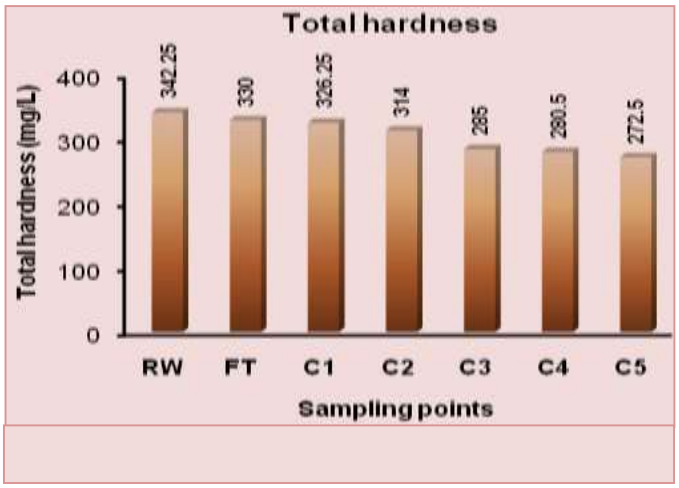

Figure 1-7. Changes in total hardness

Total Alkalinity

The release of hydrochloric acid during the extraction of sugar from sugar cane acidifies the effluent water this is then treated with quick lime to increase the pH. Hence, as given in *Figure 1-8* the total alkalinity of RW is very high which is 699.25 mg/L compared to the desirable level of 25-100 mg/L for aquaculture. High total alkalinity in water will resist any change in pH. Therefore in order to bring the pH to neutral it is mandatory to reduce the total alkalinity of water. The total alkalinity of the effluent was reduced to 672.5 mg/L in the filtration tank. Further reduction to 644.25, 609.25, 513, 491.25, & 467.75 mg/L were estimated in the C1, C2, C3, C4 & C5 beds respectively. The removal is confirmed with change in pH levels (Refer section: pH). Reduction in total alkalinity is due to the adsorption by microbes present on the surface of substrates. Also, the exopolysaccharide produced by these microbes help in adsorption and sedimentation of the carbonate salts present in the effluent which is a combined effect of biological and physical process. Cumulative effect of physical, biological and chemical processes reduced a considerable amount of the total alkalinity in the C3 bed. The biological process was facilitated by the uptake of carbonates by the emergent and floating macrophytes. The microbes helped in the absorption and sedimentation which are physical processes. The sediments on reaction with the enzymes in the rhizosphere region helped in further reduction of total alkalinity.

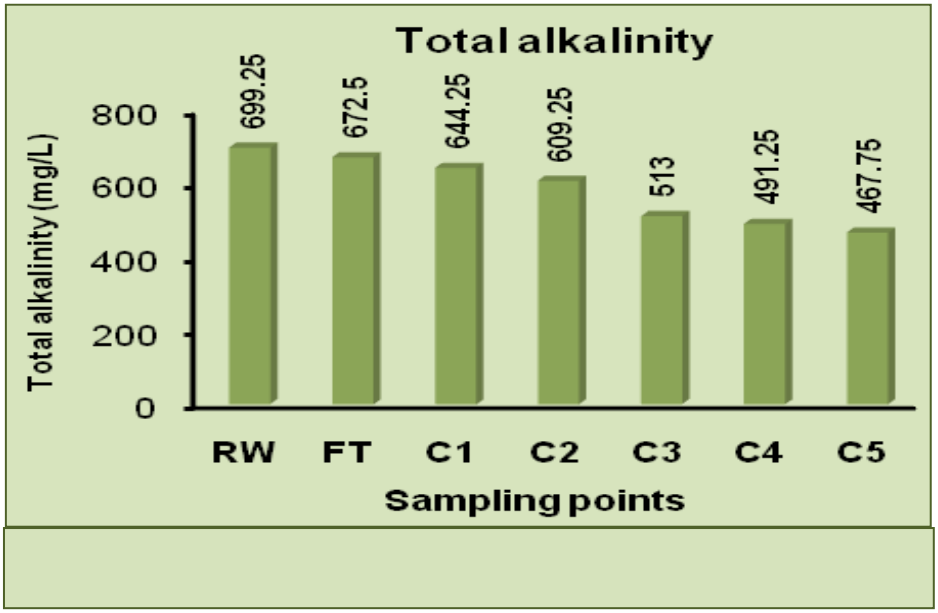

Figure 1-8. Changes in total alkalinity

Chemical Oxygen demand (COD)

COD analysis is carried out to measure the amount of organic compounds present in water. It indicates the amount of oxygen consumed per liter of water to oxidize the organic compounds. Organic pollution occurs when there are large quantities of organic compounds, which act as substrate for microorganisms. During the decomposition process the dissolved oxygen in the water is used at a greater rate than it can be replenished, causing oxygen depletion and having severe consequences for the biota hence it is mandatory to reduce COD.

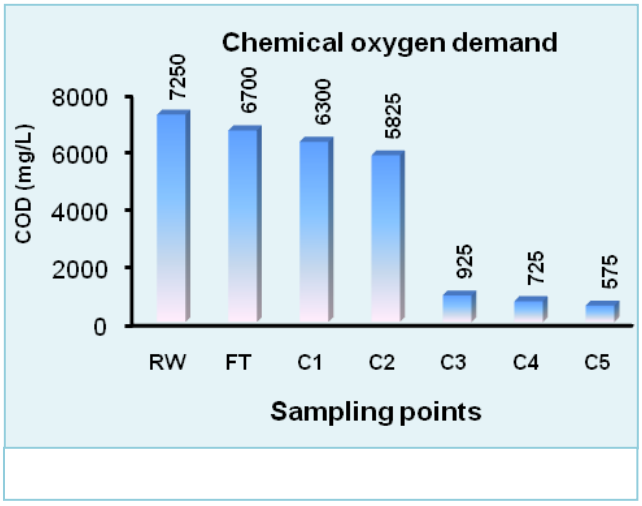

Figure 1-9. Changes in chemical oxygen demand

Levels of COD in different stages of treatment are presentes in *Figure 1-9* and COD of the raw water was 7250 mg/L, which shows that it is not suitable for reuse. The COD was reduced to 6700 mg/L in the filtration tank. Also a continuous reduction was observed in the consecutive beds C1 to C5 which was 6300, 5825, 925, and 575 mg/L respectively. The

reduction of COD in different beds is due to degradation of organic pollutants by the algal biomass on substrate surfaces. On the other hand rapid growth of algae and saturation will hinder the removal of COD hence algal biomass from wetland is taken away. This continuous removal of algae helps to maintain the reduction of COD in these beds. In C3 bed, alone is contributing 67.5% (4900 mg/L) removal of COD. This enhanced removal of COD can be attributed to the enhanced supply of oxygen by the emergent macrophytes. Though the floating macrophytes like algae and duckweeds are removing organic compounds it largely depends on the emergent macrophytes for its oxygen demand in water. Here, the removal of COD is facilitated by biological and chemical process.

Table 1-5. Monthly water quality in settling tank

Parameters	Nov	Dec	Jan	Feb
pH	7.82	7.26	7.34	7.1
EC (mS)	1.1	1	1.27	1.2
Temp	28.5	28	27.4	28.6
TDS (mg/L)	718	674	700.5	553
Total hardness (mg/L)	375	300	293	287
Chloride (mg/L)	240	189	210	244
Phosphate (mg/L)	2.5	1.1	1.7	1.2
Sulphate (mg/L)	1.7	1.4	1.3	1.3
Total alkalinity (mg/L)	320	325	445	370
COD (mg/L)	265	428	455	410

Treated water from the wetland was collected through gravity and stored with 14 days retention time in the settling tank which has a permeable bottom layer. To use the stored water productively fingerlings of less than 100 g is stocked as fish culture seed for the next season and fed with de-oiled rice bran (DOB). As given in Table 1-5, in settling tank, the pH was 7.82 in the month of Nov which decreased in the subsequent months and was found to be 7.1 in the month of Feb. This decrease may be attributed to the acidic end products released by fingerlings growing in the settling tank. The concentration of TDS was found to decrease significantly from 718 mg/L in Nov to 553 mg/L in Feb which is attributed to the sedimentation process and another reason may be the uptake by fish which has to be studied in future. The total hardness of the water was 375 mg/L in the month of Nov, which gradually decreased in the following months and 287 mg/L was present in Feb. The decrease in total hardness would be due to longer retention time enabling particle settlement which in turn decreases carbonates and bicarbonates. The phosphate concentration decreased from 2.5 to1.2 mg/L which is well within the desirable range for reuse in integrated aqua-agro farming system. Phosphate is an important nutrient for the fish to grow which may be the reason for the reduction in phosphate concentration. The less COD concentration in Nov was 265 mg/L due to the use of previous year's effluent stored in aeration tank. In the month of Dec, the fresh effluent was used as source leading to an increase in the COD. Although, the COD concentration in the month of Dec to Jan was high in the settlement tank, they were less than the wetland discharge. This shows that the physical sedimentation process is reducing COD in the settling tank.

Table 1-6. Monthly water quality in fish tank

Parameter	Nov	Dec	Jan	Feb
pH	7.91	7.1	7.12	7.06
EC (mS)	0.7	0.6	0.9	0.7
Temp	29.2	28.6	28.1	29.3
Dissolved Oxygen (mg/L)	4.5	4.8	5.0	4.8
Parameter	**Nov**	**Dec**	**Jan**	**Feb**
TDS (mg/L)	461.5	438	457	338
Total hardness (mg/L)	265	227	218	180
Chloride (mg/L)	170	163	174	84
Phosphate (mg/L)	2.5	1	1.6	0.34
Sulphate (mg/L)	1.4	1.3	1.15	1.1
Total alkalinity (mg/L)	280	278	400	208
COD (mg/L)	228	337	375	323

Treated water from the settling tank is pumped to the fish tank which is an earthen pond and stocked with Catla and Rohu fish of size ranging from 150 to 250 g. As data presented in the Table 1-6 the pH of water in fish tank shows a decline trend from the month of Nov to Feb. The decrease is due to the acidic fish excreta present in the fish pond. The DO is maintained by establishing water circulation facility and ensured its level ranging between 4.5 and 5.0 mg/L. The TDS was high (461.5 mg/L) in the month of Nov and decreased gradually to 338 mg/L in the month of Feb which may be due to the aggregation and sedimentation of dissolved solids. The decrease in total hardness from 265 to 180 mg/L over four months is attributed to the settlement of carbonates and bicarbonate and the chloride concentration in the fish tank was low (84 mg/L) during February as it was reduced due to fish activity. The phosphate concentration decreased to 0.34 mg/L in Feb from 2.5 mg/L in Nov. In February, the sulphate and total alkalinity concentration was 1.1 mg/L and 208 mg/L respectively in fish tank which is lower than the previous months. This is due to the effective remediation by grown fishes in the later month. The COD concentration in Nov was 228 mg/L which is low due to the use of previous year's effluent stored in aeration tank. In the month of Dec, the fresh effluent was used as source leading to an increase in the COD. Although, the COD concentration in the month of Dec to Jan was high in the fish tank, they were less than the settling tank which may be due to the physical sedimentation process.

Biological properties

Baseline status

In the beginning sample from sugar effluent was collected at anaerobic and final aeration tanks to determine the microbial and pathogenic loads to establish a baseline and compare the changes after treatment. The culturable microbial population *viz*., bacteria and fungi were isolated from these samples using nutrient agar (NA) for bacteria and potato dextrose agar (PDA) for fungi respectively. About 10 ml from each sample were diluted in 90 ml of sterile dis. H_2O and incubated at 150 rpm to ensure proper dispersion of the sample. Serial dilution was done and 100µl of inoculum from 10^{-3} and 10^{-4} tubes were spread plated on NA to determine the bacterial load in the sample. The fungal population was determined in 10^{-2} and 10^{-3} PDA. The plates were incubated at room temperature and the bacterial and fungal colonies were counted after 24 hours and 76 hours respectively and represented as log cfu/ml (Figure 1-10). In both the samples total bacterial load was ≥log 6 cfu/ml which is

considered to be moderate in Indian scenario. The fungal population as not observed in the isolation plates.

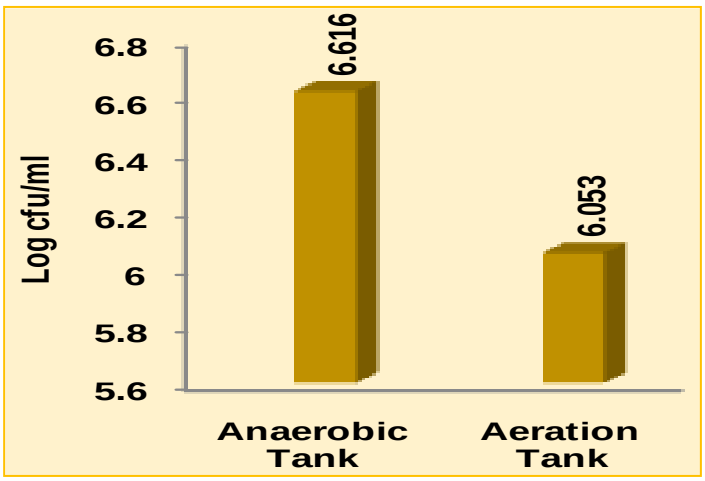

Figure 1-10. Total microbial load in sugar effluent

The pathogenic population in the samples from anaerobic and final aeration tanks was determined using selective medium as listed in previous reports. It was identified that three pathogens such as *Yersinia* sp. *Staphylococcus* sp. and *Shigella* sp. were present in anaerobic tank at a load of <log 5 cfu/ml whereas in the final aeration tank *Staphylococcus* sp., *Vibriocholera* sp. and *Shigella* sp. are found at a load of <log 6 cfu (*Figure 1-11*). The presence of *Staphylococcus* sp. and *Shigella* are found in both anaerobic and aerobic tanks at a density <log 6 cfu/ml which is not a risk to human health. However, it is proposed to undertake studies to determine virulence of these pathogens by screening for virulent genes in these strains for in depth understanding of its implications.

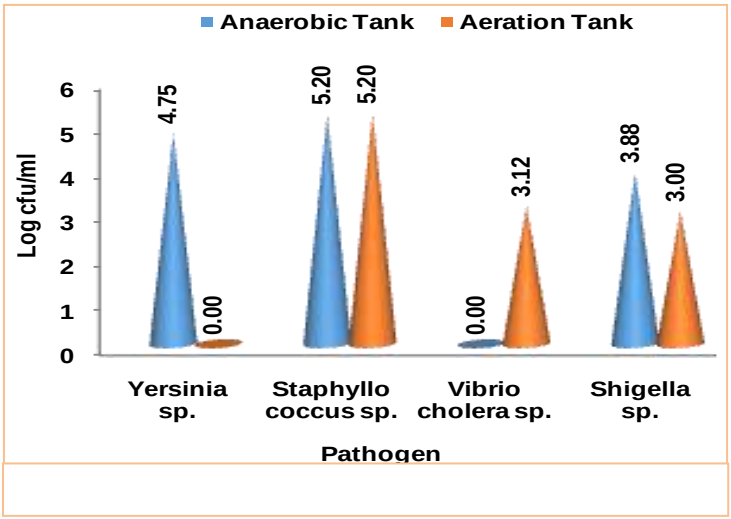

Figure 1-11. Pathogenic populations in sugar effluent

Current status

The total microbial load in the different beds remained constant at log 4 cfu/ml. The baseline data of the source water from final aeration tank had a microbial load of log 6.053 cfu/ml. Around 2 cfu/ml of total microbial load was reduced after treatment in the CWL.

Determination of pathogenic bacterial population as on November 2014 (*Figure 1-12*) revealed that *Yersinia* sp. was >4 cfu/ml in the inlet to CWL while it is between 1 and 4 cfu/ml in the filtration tank, CWL beds, sedimentation tank and fish tank. Irrespective of the stages of treatment *Staplylococcus* sp., is observed to be <4 cfu/ml. In the inlet to CWL the *Shigella* sp. was found to be > 4cfu/ml while in other locations it is < 4 cfu/ml. The *Enterobacter* sp. is < 2 cfu/ml which is an indicator of better water quality. Almost same pathogenic populations are observed in ST and FT this may be due to continuous human interaction with water during fish feeding.

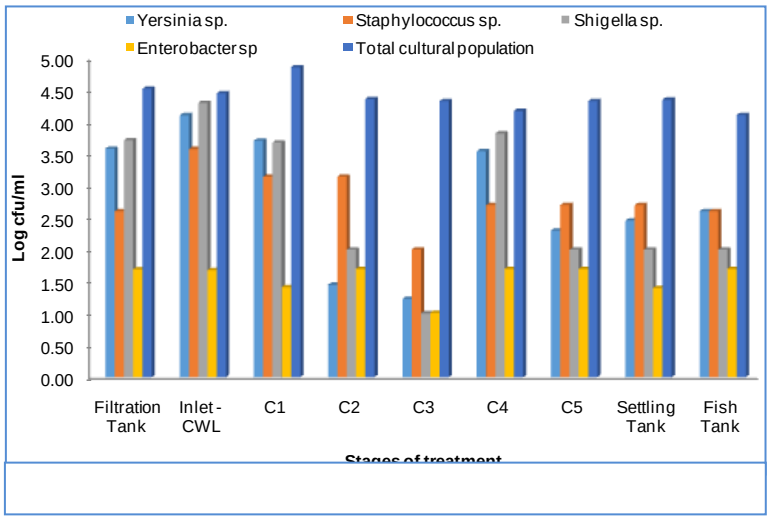

Figure 1-12. Pathogenic populations in different stages of treatment in Nov 2014

Total culturable bacterial population was almost ≥ 4 cfu/ml throughout the system without much variation compared to November 2014 results (*Figure 1-13*). Both the results indicate that the pathogenic bacterial population in the treated sugar effluent is well within the desired concentration in Indian scenario free from risk to humans and animals. Comparison of results between Nov 2014 and Jan 2015 indicate a mixed trend where there is reduction in certain population while certain are maintained as given in *Figure 1-14*. Except *Enterobacter* sp. other three pathogens showed a declined trend of >1.5 log cfu/ml almost in all the stages of treatment. Increase in levels of all is seen in C3 and C4 which may be due to rhizospheric association of these pathogens with *Typha* sp. Except *Staplylococcus* sp. other pathogens are increased in ST and FT at <2log cfu/ml. As mentioned before it may be due to human interaction with water.

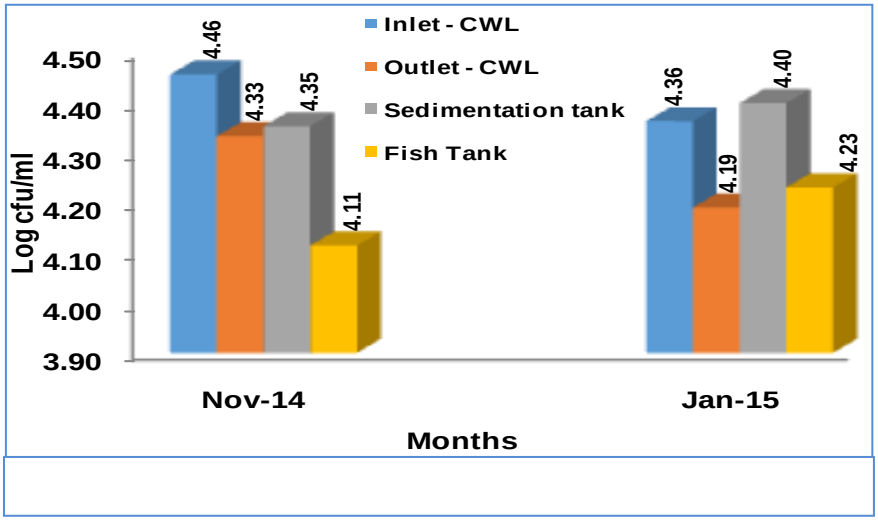

Figure 1-13. Microbial load during Nov 2014 & Jan 2015

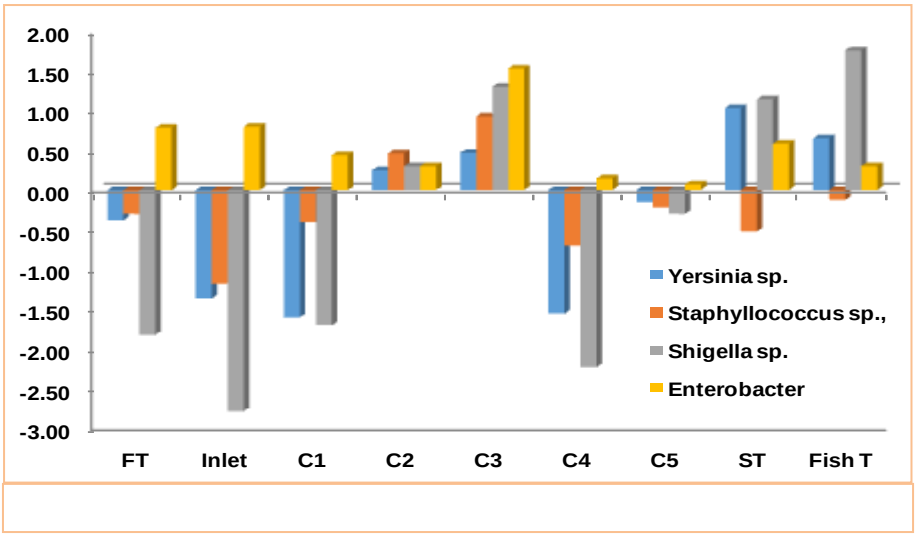

Figure 1-14. Pathogenic populations in different stages of treatment in Jan 2015

1.3 Demonstration of microbial decolourization system (MSSRF)

Decolourization efficiency of the bacterial isolates

The efficiency of four bacterial isolates Terribacillus sp. MSSRFH36, E. indicum MSSRFH1.1, B. enclensis MSSRFW 20, and P. putida MSSRF D41 to decolourize anaerobic treated distillery effluent was carried out. The bacterial culture were grown individually in sterilized Luria-Bertani (LB) broth and 2 ml of overnight grown cultures were inoculated in 10% of anaerobic treated distillery effluent amended LB medium and adapted to grow for 60 h and the decolourising efficiency of the adapted cultures were measured at 12 h interval at 475 nm in a Spectrophotometer (Shimadzu UV 1800). Around ≤ 10% decolorization was observed for all the isolates and the consortium. The isolates which had decolourisation potential in the first generation were selected for the subsequent adaption and decolourisation studies. Since the cultures that had the potential for decolourizing

anaerobic treated effluent were unstable in the first generation, sequential adaptation was carried out in the 10% anaerobic treated distillery effluent amended minimal media to achieve stable growth along with enhanced colour reduction. The color reduction was determined every 24 hours. It was observed that P. putida and the consortium decolourised 8.8% and 8% respectively in 36 h which is higher compared to other three isolates. The decolourization efficiency of these second generation adapted isolates enhanced and stable decolourization activity was observed compared to the first generation. Further, these isolates will be sequentially adapted for at least five consecutive generations to determine if the decolourization efficiency is enhanced due to adaptation.

Decolourization of the consortia in the treatment tank

In the field study, the decolourization of anaerobic treated effluent was studied and it was found that 32% of colour was removed by adapted indigenous bacterial consortium. After the treatment in settlement tank, around 51% of colour was removed and finally 58% of colour removal was observed after passing it through the activated charcoal. The total removal of 58% at the site is due to the cumulative treatment effect of adapted bacteria and algal consortium followed by activated charcoal, whereas in the lab study, only 8.8% of colour was reduced by 2nd generation adapted bacterial consortium. The increase in colour removal in the field is due to sequential adaptation to higher concentration and using culture nth generation culture has improved the adaptation and colour removal potential. Hence, sequentially adapting the consortium to high concentration is necessary to understand the role of consortium and adaptation in colour removal.

1.4 Demonstration of algal treatment system

Vuyyuru site

Status of bio-treatment of distillery effluent

During inception of project water inventory was done for all the parameters and compared with the irrigation standards given by ICRISAT and are found to be high than standards including salinity. Based on which the treatment process was designed and executed. However, in order to improvise the treatment process an assessment was carried out. And the current status of treatment stages is presented below.

Aeration tank (AT)

The effluent from the anaerobic digester was diluted 50% in the aeration tank and aerated by water circulation. The aeration process takes place for 8 h/day for 3 days which provides oxygen supply for the adapted aerobic bacterial consortium which concurrently mixes bacterial population enabling its growth. A sample was collected after three days from the aeration tank for the analysis of key physico-chemical parameters. As and when required the biological parameters are also analysed.

Settlement tank (ST1 & ST2)

After third day the bacterial treated water was transferred from aeration tank to the settlement tank using an electrical motor pump. In settlement tank (ST1 & ST2), the adapted algae from the previous treatment were added and allowed to grow in the bacterial treated

effluent for 3 days. The algal cells enabled the treatment and settlement of contaminants in the effluent.

Charcoal tank (CT)

On the 6th day of the process, the effluent was passed from the settlement tank to ET through charcoal filter. The samples were collected in clean bottles before and after charcoal filtration and analysed.

Constructed wetland

The treated effluent from ET was passed through the CWL. The final treated effluent was passed on to a tank and from there it was used for agricultural purposes.

Changes made in bio-treatment process

Since, the amount of COD removed by the existing process was very low, few technical changes were made in the microbial process to enhance the removal of COD. Following were the changes made to overcome the problems.

A very good growth of bacterial cultures should be more than 1 OD @ 600 nm. The bacterial growth in the microbial tank was very poor (0.3 to 0.4 OD @ 600 nm). This was mainly due to two reasons,

- The usage of unadapted bacterial consortium had a slow log phase resulting in the reduced removal of COD. To overcome this problem, the microbial consortium grown at each batch in the microbial tank was used as the seed for next batch continuously which allowed the bacterial consortium to adapt and yield efficient results.
- Initially a loop full of inoculum was added directly into six liter broth was insufficient to achieve the required bacterial growth to inoculate in the aeration tank which in turn affected the remediation process. In order to overcome this problem the consortium was pre inoculated in 100 ml broth as a primary culture and added into culture flasks which subsequently enhanced the bacterial growth and used as inoculum for the aeration tank.

  These changes in the aeration tank had yielded improvement in the removal of COD. Adding a well grown initial inoculum had increased the removal of COD to 7000 mg/L by bacterial consortium and gradually increased by 10000 mg/L, 12000 mg/L in the subsequent batches. The increase in pH from mild acidic to neutral and above was observed after treatment with the adapted microbial consortium. The adapted bacterial consortium also degraded melanoidin the colouring agent which is confirmed by the reduction in colour. The Eh of 50% diluted anaerobic digested effluent was positive which was decreased to negative after microbial treatment. This was due to oxidation of organic content by the adapted bacterial consortium. Conductivity in the aeration tank increased than anaerobic treated effluent during the initial cycles of treatment process. Later, conductivity decreased as the bacterial consortium was adapted and used for treatment.

Experiments

Batch experiments were carried out to identify a potential biological material other than bacteria to enhance the treatment process after bacterial treatment. Four studies of

bacterial treated effluent were carried out using free cells of algae, growing algal cells, *Strychnos potatorum seed, moringa seeds.*

Main thrust was given to COD as the distillery effluent contains more sugar compounds made of nitrogen, phosphate, sulphate and other ions responsible for contamination. Reduction of COD indicates reduction in other parameters including BOD, TDS, TSS etc. hence changes in the COD from different experiments are highlighted below.

- 4000 mg/L of COD was removed by free cells of algae
- 4666.67 mg/L of COD was removed by the seeds of *Strychnos potatorum*
- 933.33 mg/L of COD was removed by the seeds of Moringa
- 15066.67 mg/L of COD was removed by the growing cells of algae

Physico Chemical properties

pH

As presented in the pH of anaerobic treated effluent was 6.7 which on treatment with adapted bacterial consortium in aeration tank (AT) were increased to 7.2. The increase in pH is due to the growth of adapted bacteria feeding on the organic contaminants. The algal consortium growing in the settlement tank (ST) further increased the pH to 7.4 by degrading the contaminants followed by their settlement. The effluent passed through the activated charcoal filter (CT) had an insignificant raise in pH which could be attributed to the filtration process. Bio-mediated reduction reaction has facilitated the increase in pH from weak acidic to neutral and above.

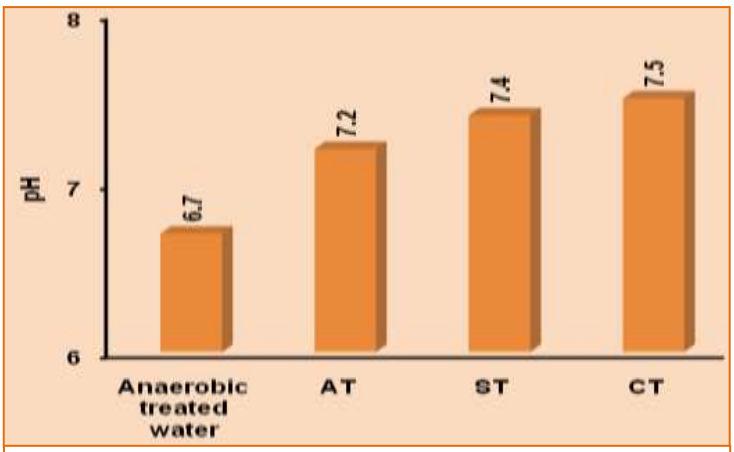

Figure 1-15. Increase of pH levels in distillery effluent

Conductivity (EC)

The conductivity of the anaerobic treated effluent decreased from 18.6 mS/cm to 17.9 mS/cm which is due to the removal of salts by the bacterial population in AT. Further as indicated in *Figure 1-16*. It was reduced to 17.3 mS/cm in the ST which is due to removal of salts by algal cells and settlement of contaminants. Insignificant decrease in conductivity (17.2 mS) was observed after filtration by activated charcoal.

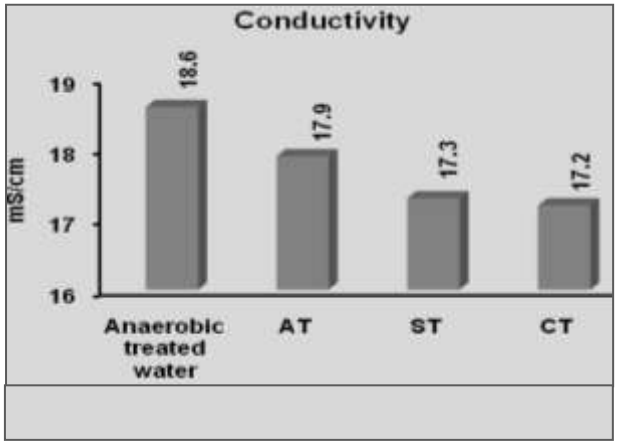

Figure 1-16. Decreasing conductivity in distillery effluent

Salinity

In aeration tank, the salinity of anaerobic treated water decreased from 9.8 to 9.5 ppt due to the uptake of salts by bacteria (*Figure 1-17*). This was further reduced to 9.2 ppt by the algal consortium in the ST. The charcoal filter helped in an insignificant removal of salinity from the effluent. The reduction of salinity by the current treatment process was not significant. Further studies are being carried out to enhance the reduction of salinity from the effluent through halophytes for phytoremediation in CWL.

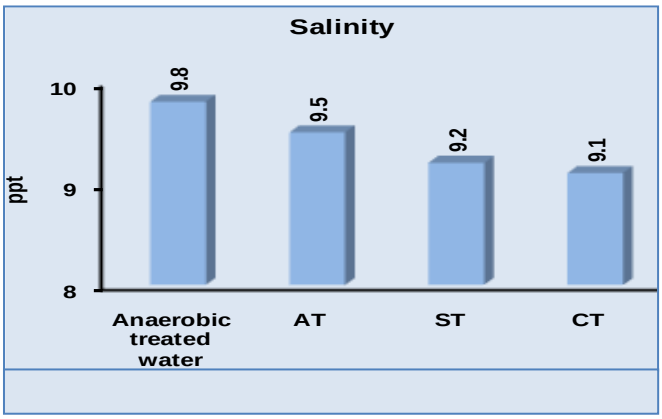

Figure 1-17. Salinity reduction in distillery effluent

Eh

The potential difference (Eh) decreased from 19.2 to −7 mV in AT due to bacteria which evidently show that the bacterial mediated reduction has taken place. The reduction is due to the acceptance of electrons from bacterial cells facilitating a biochemical redox reaction enabling a decrease in the positive charged contaminants such as melanoidin. The Eh was further reduced to −23 mV in the ST which is due to the growing algal cells. The negative surface charge in the ST facilitates aggregation and the settlement of the degraded contaminants due to provision of an undisturbed period of 3 days. After filtration through CT, there was no significant change in Eh as it was only a physical process.

Chemical Oxygen Demand (COD)

The anaerobic treated effluent discharged into the aeration tank had 52000 mg/L of COD was decreased to 18200 mg/L due to the redox reaction facilitated by adapted bacterial consortium. The reduction of COD is achieved due to the reduction of organic contaminants,

like sugar, phosphate, TDS, TSS and BOD. The growing algal cells played a vital role in the degradation, uptake of organic contaminants and also, adsorbed the contaminants to the biomass surface and increased the settlement of degraded contaminants which cumulatively facilitated a further decrease in COD to 11600 mg/L (Figure 1-18). The activated charcoal filtered the algal treated effluent which enabled the reduction of COD to 9425 mg/L.

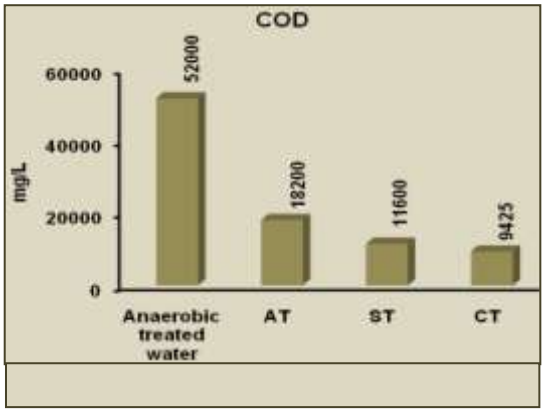

Figure 1-18. COD reduction in distillery effluent

Colour removal

The colour in the distillery effluent is due to the presence of a polymer called melanoidin. The degradation of melanoidin is important to avoid the further increase in COD and BOD in the effluent as melanoidin has the ability to adhere to the contaminants present and cause problem to the remediation process. The degradation of melanoidin is indicated by the colour of the effluent. As depicted in *Figure 1-19*, in AT 32% of colour was removed by the bacterial consortium and a further reduction to 51% and 58% was facilitated by algal cells in the ST and activated charcoal respectively. This confirms that 58% of melanoidin was degraded by the current treatment process.

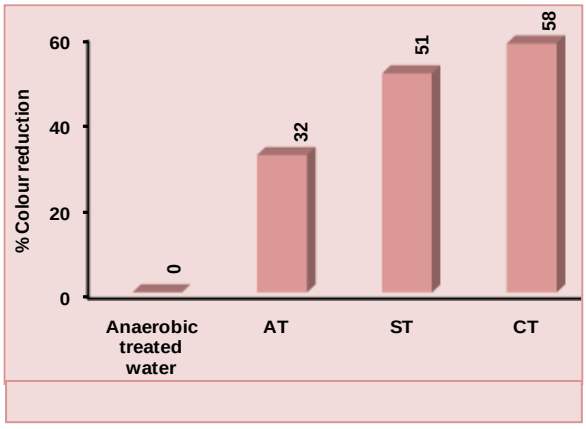

Figure 1-19. Percentage of colour reduction in distillery effluent

In the current study, a systematic & novel approach of subsequently treating the anaerobic treated distillery effluent with adapted bacterial consortium followed by adapted algal consortium and activated charcoal has played a vital role in enhanced remediation of contaminants from distillery effluent. Preparation of consortium using indigenous bacterial isolates and sequential adaptation to distillery effluent enhanced the bioremediation potential of indigenous bacterial isolates. This enabled a significant removal of contaminants in the aeration tank. A novel approach to sequentially adapt indigenous algae in batch

reactor enabled an efficient growth in bacterial treated distillery effluent which in turn enhanced the remediation potential in settlement tank. Also, these algal isolates enhanced the sedimentation rate of contaminants present in the bacterial treated distillery effluent.

Biological properties

Baseline status

To determine baseline data of bacterial population in the anaerobic treated distillery effluent similar methodology followed above for Lakshmipuram samples were adopted. Insignificant population of pathogens were present in the anaerobic treated distillery effluent whereas, the sample collected from the aeration pond in the industry had the pathogens like *Staphylococcus* sp., *Shigella* sp. *Legionella* sp. *Yersinia* sp. and *Klebsella* sp. at >log 5 cfu/ml. Since, the pathogenic population in the anaerobic treated distillery effluent was less than in the aeration pond, the former was chosen as the source for the treatment process.

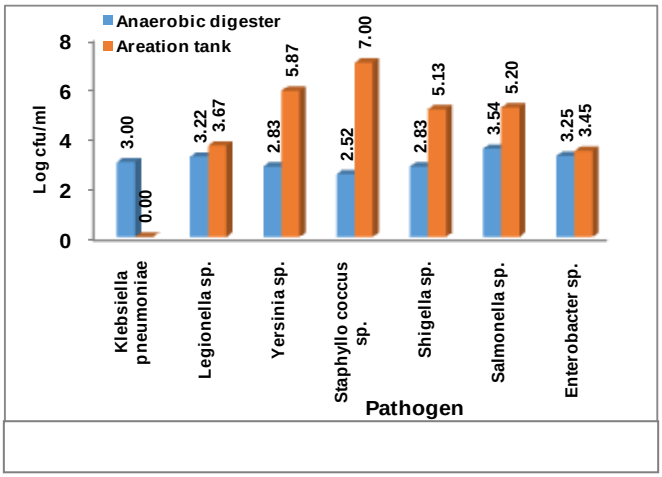

Figure 1-20. Pathogenic population in distillery effluent

Current status

Comparison of results between Nov 2014 and Jan 2015 indicates an absolute reduction in all the pathogenic population except for *Yersinia* sp. and *Salmonella* sp. which was >1 log cfu/ml which is less than the initially population. All the pathogenic population viz., *Yersinia* sp. *Staphylococcus* sp. *Salmonella* sp. *Shigella* sp and *Enterobacter* sp. were in the range between 1 to >5 log cfu/ml in Nov 2014 batch. The efficiency of the sequential treatment exhibited greater reduction of the pathogens, which is reflected in the total bacterial population.

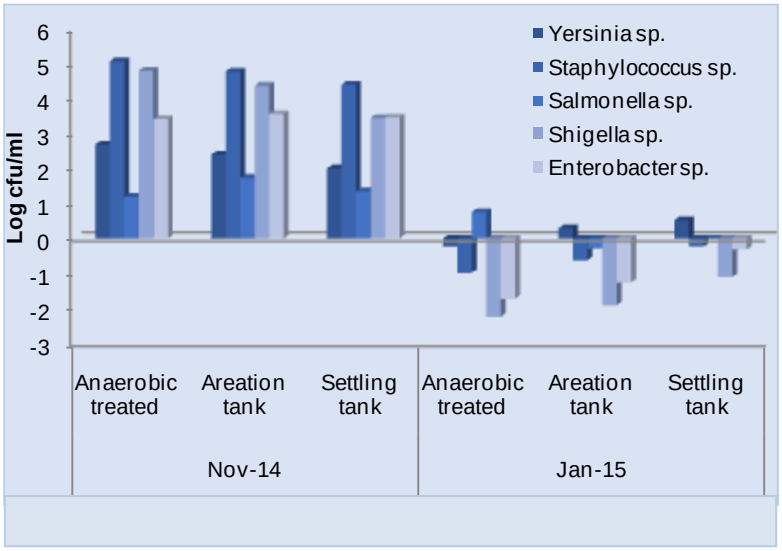

Figure 1-21. Pathogenic population in treatment process during Nov -14 & Jan-15

1.5 Carbons and membranes for the recovery of phenolics / pigments

The modified unburnt carbon from bagasse ash is suited to adsorption of low molecular weight fractions from distillery wastewater which have significant antioxidant property. Mixed matrix membranes (MMMs) prepared by incorporation of bio-nanocomposites in PSF polymer matrix and surface sealed with PVA is promising for melanoidins recovery. Further work on process development for recovery of melanoidins/phenolics from distillery wastewater is ongoing.

Preparation and characterization of separation media

Adsorbent based on unburnt carbon from bagasse ash

Work on the modification of the unburnt carbon (to enhance mesoporosity and adsorption of large molecules) was continued. Selected carbons were further characterized for pore size, pore volume, multipoint surface area and CHN analysis as shown in Table 1-7. It is observed that the amount of fixed carbon increased in all the modified carbons as compared to the unburnt carbon (UC). Furthermore, 3UC-Ash-740 showed the highest increase in the pore volume and surface area.

Table 1-7. Advanced characterization of selected carbons

Sample	Pore size (Å)	Pore volume (cm^3/g)	Elemental analysis			Multipoint BET surface area (m^2/g)
			Carbon (%)	Hydrogen (%)	Nitrogen (%)	
UC	22.27	0.1413	39.78	1.33	1.15	282
UC-Ash	24.14	0.1945	72.70	2.10	1.70	394
3UC-Ash-740	21.51	0.3849	72.80	2.20	1.69	673
3UC-Ash-740-0.5	21.40	0.3354	74.35	1.69	1.65	634

Development of mixed matrix membranes

Polysulphone (PSF) membranes are widely used in microfiltration (MF) and ultrafiltration (UF) applications due to low cost, superior film forming ability, good mechanical and anti-compaction properties, strong chemical and thermal stabilities and acid/alkaline resistance[1]. In recent years, mixed matrix membranes (MMMs) involving dispersion of inorganic particles in a polymer matrix have been prepared with polysulphone by phase inversion technique. Addition of these materials improves both overall and surface porosity. In this work, we have incorporated bio-nanocomposites into PSF membrane and tested it for melanoidins recovery. The focus was on development of bio-nanocomposites/PSF MMMs that operate at lower pressure (1-2 bar) with high melanodins retention. Such a membrane is expected to have significant application in fermentation wastewater treatment.

The details of the membrane preparation were as follows. Polysulphone (PSF) Udel® P-3500 was purchased from Solvay Specialty Polymers, India, and non-woven polypropylene/ polyethylene fabric Novatexx 2471 from Freudenberg, Germany. N-Methyl-2-Pyrrolidone (NMP) was obtained from Sigma Aldrich, India, and glutaraldehyde (GA) solution (25% aqueous) from Merck, India. Polyvinyl alcohol LR (MW 125000) and dibutyl maleate (DBM) were procured from S.D. Fine Chemicals, Bangalore, India. Chitosan was obtained from Marine Chemicals, Cochin, India with 85% deacetylation. Tapioca starch was obtained from Natsyn Catalysts, Bangalore, India, MWCNTs with diameter ranging from 50-70 nm and lengths ranging from 1-2 μm were purchased from I-Can Nano, Kolkata. India. All other reagents were analytical grade and purchased locally.

A detailed description on the preparation of bio-nanocomposites and their characterization is mentioned described by Deepthi et al (2014)[2]. MWCNTs were functionalized with acid mixture (H_2SO_4/HNO_3) by sonication followed by heating at 50^oC in microwave reactor. It was then washed with water and oven dried at 100^oC, to obtain f-MWCNTs. Chitosan was cross-linked with glutaraldehyde (25% v/v) and filtered, followed by oven drying at 50^oC. The product was referred as X-CTS. Thermoplastic starch (X-TPS) was prepared using a mixture of starch, glycerol and water. The mixture was cross-linked with glutaraldehyde. The bio-nanocomposites were prepared by mixing equal amount of X-CTS and X-TPS in 4% f-MWCTs.

The mixture was mixed in a kitchen mixer and sonicated using ultrasonicator (Branson, Model 2510 E/DTH) for 30 min. DBM (10% v/v) was then added in the mixture as coupling agent in order to improve the interfacial interaction of blend composites with f-MWCNTs. The final mixture was again mixed for 10 min in a kitchen mixer. The bio-nanocomposites powder was stored at room temperature for further use.

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[1] N. Ma, J. Wei, R. Liao, C.Y. Tang, Zeolite-polyamide thin film nanocomposite membranes: Towards enhanced performance for forward osmosis, J. Membr. Sci. 405–406 (2012) 149–157.

[2] M. V. Deepthi, R. R. N. Sailaja, G. S. Ananthapadmanabha, G. S. Avadhani, Cross-linked Chitosan/Thermoplastic Starch Reinforced with Multiwalled Carbon Nanotubes Using Maleate Esters as Coupling Agent: Mechanical, Tribological and Thermal Characteristics Polymer-Plastics Technology and Engineering 53: 1476–1486, 2014

PSF membranes of different concentrations (12%, 14%, 16%, and 18%) with NMP were prepared by phase inversion method using an automatic film applicator (A J Carsten Co. Ltd., Canada). The mixtures were stirred for 24 h in a mechanical stirrer followed by overnight degassing at room temperature. PSF films of controlled thickness (250 µm) were cast on non-woven polypropylene support and coagulated by immersion in a water bath at room temperature. After 10 min, the membranes were immersed in a fresh water bath for an hour and finally preserved in water at 4^{o}C. Similarly, MMMs were prepared by adding different amounts of bio-nanocomposites (1%, 2%, 5%, 10%, 15%, and 20%) in the PSF (18 wt%)/NMP (82 wt%) mixture. The bio-nanocomposites were dispersed in NMP for 24 h before adding the required PSF in the mixture. PVA layer of different concentrations (0.1%, 0.25%, 0.5%, 1%, and 2%) on top of MMMs was applied by dip coating. PVA was dissolved in hot aqueous solution (90^{o}C) under stirring for 8 h and the solution was cooled to room temperature before coating. Membranes were attached on glass a plate and the ensemble was dipped in the PVA solution for a specified time (1 h-4 h). Excess PVA solution was drained by holding the ensemble vertically. This was followed by crosslinking with glutaraldehyde (2.5% v/v). The membranes were dried at room temperature for 24 h and stored in water at 4^{o}C.

The morphology of the bio-nanocomposites, PSF membranes and PSF/ bio-nanocomposites MMMs was determined by scanning electron microscope (SEM) (Zeiss- EVO/MA10 instrument). The membrane samples were prepared by freeze fracturing under liquid nitrogen. After chemical drying with hexamethyldisilazane, the samples were coated with palladium in an argon atmosphere using a vacuum evaporator and examined. The porosity and the pore size of the membranes were determined by gravimetric method and by filtration velocity method respectively[3].

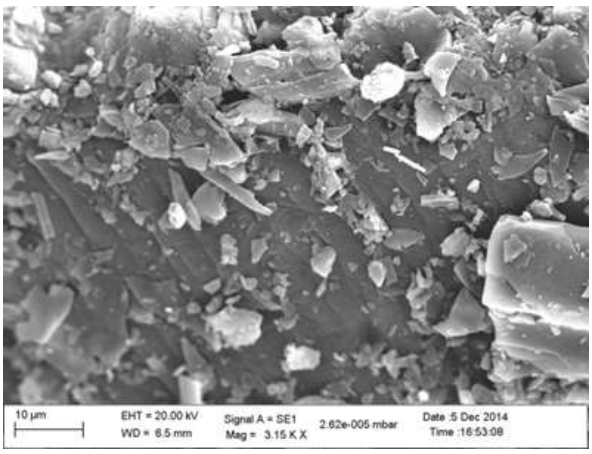


Figure 1-22 shows the SEM image of the bio-nanocomposites. The bio-nanocomposites appear as a mixture of particles of different shapes and sizes.

[3] Basri, H., Ismail, A. F., & Aziz, M. 2011. Polyethersulphone (PES)- silver composite UF membrane: Effect of silver loading and PVP molecular weight on membrane morphology and antibacterial activity. *Desalination*, 273, 72-80.

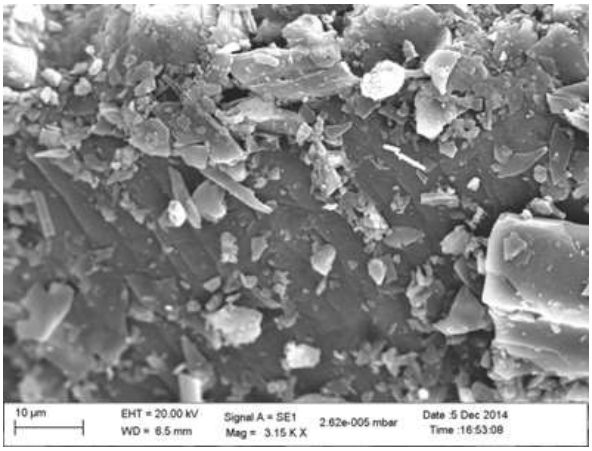


Figure 1-22. SEM image of bio-nanocomposites.

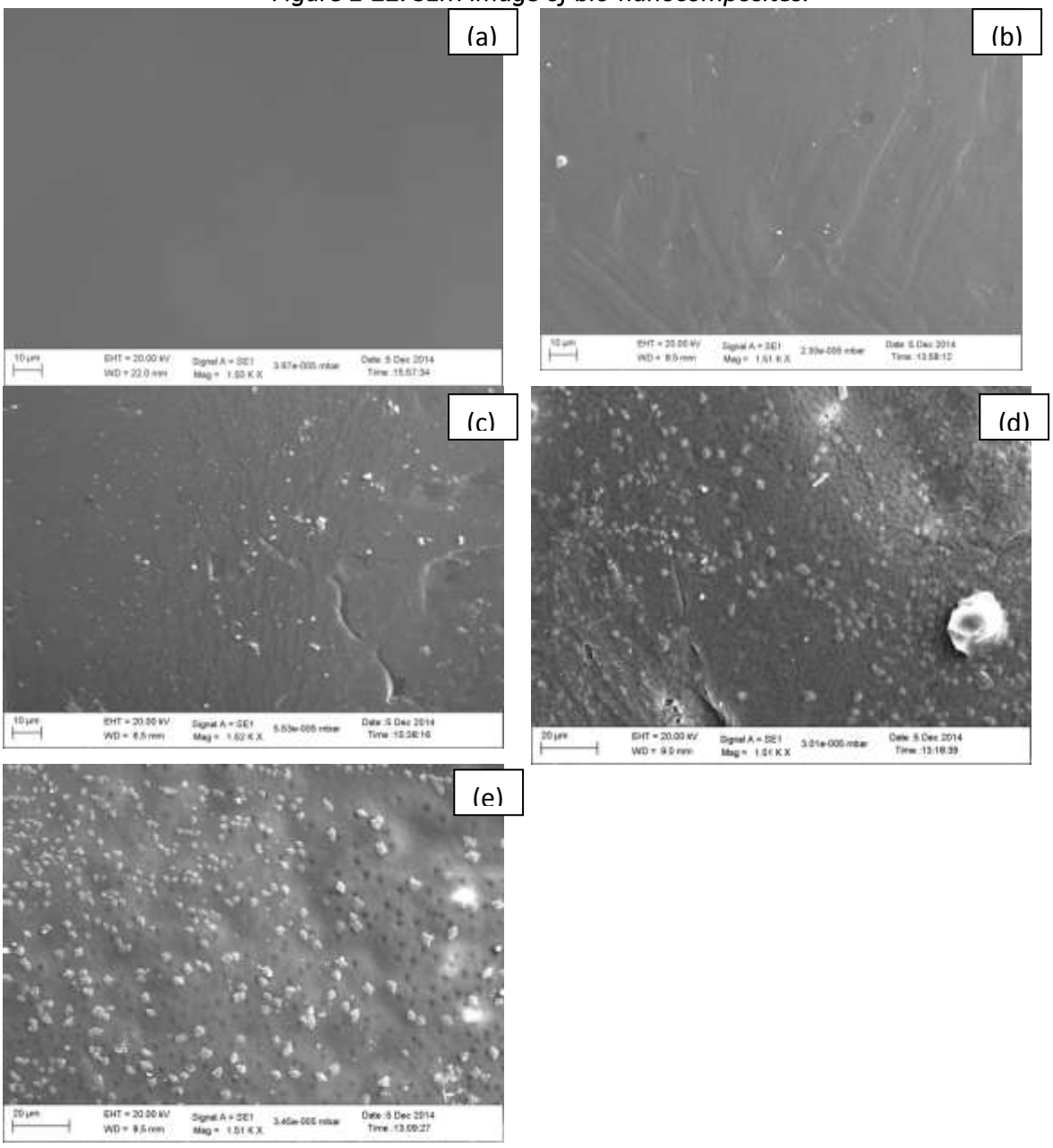


Figure 1-23. SEM image (planar view) of (a) PSF, and MMMs with bio-nanocomposites content of (b) 5%, (c) 10%, (d) 15%, (e) 20% (magnification 1.5KX).

Figure 1-23 shows the distribution of bio-nanocomposites on the PSF polymer matrix. The bio-nanocomposites are well dispersed in the PSF matrix of MMMs (Figure 2b-e). No aggregation was observed. This indicates that the bio-nanocomposites and the PSF are compatible to each other. The MMM sample with 20% bio-nanocomposites (Figure 1-23e) shows some pitted marks on the surface. These are due to the loss of the bio-nanocomposites during freeze-fracturing of the samples for SEM analysis.

Performance evaluation for phenolics/pigments recovery

Melanoidins recovery using mixed matrix membranes (MMMs)

This study was performed with synthetic melanoidins. Melanoidins were synthesized in the laboratory by mixing 1M D-glucose, 1M glycine (both obtained from Sigma Aldrich, India) and 0.5M sodium carbonate in 1L distilled water[4]. The solution was autoclaved at 121^{o}C for 20 min, cooled to room temperature (28±2^{o}C) and the pH adjusted to 7 using 1N NaOH. Chemical oxygen demand (COD) of the solution was 30,000 mg/L and it was dark brown in colour. The resulting stock solution was dialyzed for 72 h in distilled water using cellulose dialysis tube (Sigma Aldrich, USA) that retains molecules ≥ 12 kDa. The dialysate (distilled water) was replaced every 24 h. The dialyzed fraction was stored at 4˚C and was used in all further experiments.

Adsorption, desorption and filtration experiments were carried out in triplicate with 5% melanoidins solution. Different amount of bio-nanocomposites (0.5%, 1%, 2.5%, 5%, 10%, 15%, 20%, 25% w/v) were added in 5% (v/v) melanoidins solution. Adsorption was carried out at room temperature in a 250 mL shake flask (Scigenics Biotech, India) at 120 rpm. Samples at different time intervals (1, 2, 3, 4 h) were analyzed after centrifugation (Thermo Fisher Scientific, India) at 7000 rpm for 20 min. The melanoidins retention percentage was calculated at 475 nm as given in equation (i)

Retention (%) = $[(C_i-C_f)/C_i] \times 100$……. ……..(i)

where, C_i = Initial melanoidins concentration (mg/L)

C_f = Final melanoidins concentration (mg/L)

The melanodins adsorbed on to bio-nanocomposite were recovered by desorption using different solvents (e.g. 25% pyridine, 5N HCl, 5N NaOH, hot water 90^{o}C, acetone, 10% ethanol, NaCl) . The reaction was carried out in shake flask (4g/L) at 120 rpm, 24h, at room temperature. The melanodins recovered was calculated using equation (ii)[5]

Desorption (%) = $[(C_{ed}/(C_o-C_e)] \times 100$ ……..(ii)

where C_{ed}= Melanoidins concentration in the liquid phase after desorption (mg/L)

C_o= Initial melanodins concentration in adsorption experiment (mg/L)

C_e= Melanodins concentration in the liquid phase after adsorption (mg/L)

Filtration was conducted using a dead-end filtration cell (Millipore, India) with 0.00152 m^2 active membrane area. The feed solution was poured into the cell and pressurized with

[4] Dahiya, J., Singh, D., & Nigam, P. 2001. Decolourisation of molasses wastewater by cells of *Pseudomonas fluorescens* immobilised on porous cellulose carrier. Bioresource Technology, 78 (1), 111-114.

[5] F. Ferri , L. Bertin, A. Scoma, L. Marchettia, F. Fava, Recovery of low molecular weight phenols through solid-phase extraction, Chem. Eng. J. 166 (2011) 994-1001.

nitrogen to the desired pressure at room temperature (27°C). Permeate was collected under atmospheric pressure. Sampling was done after 1 h of filtration at constant pressure. The feed solution was stirred by a teflon lined magnetic stirrer at 700 rpm. Permeability was calculated using equation (iii),

Permeability (L/m^2 h bar) = V/ (A x P x t) ……..(iii)

where, V = Permeate volume (L)

A = Membrane filtration area (m^2)

ΔP = Transmembrane pressure (bar)

t = Filtration time (h)

Figure 1-24a shows the adsorption of melanoidins using bio-nanocomposites. Different concentrations of bio-nanocomposites (0.5% to 25%) were studied for different time intervals (1h to 4h). Melanoidins recovery (as retention) increases with increased concentration of the bio-nanocomposites (0.5% to 25%) and contact time (1 h to 4 h). More than 95% adsorption was obtained with 5% bio-nanocomposites and 4 h contact time. High removal is observed for bio-nanocomposites concentration in the 10% to 25% range. Such high and rapid adsorption is due to the high surface area of the bio-nanocomposites. Similar adsorptions studies have been reported with chitin fiber and activated carbon, but no desorption studies have been reported in the literature. [9,10] In the present work, 70-75% melanoidins was recovered using 25% pyridine as desorbent, but < 5% was desorbed using rest of the solvents.

The porosity and pore radius of membranes with different PSF concentrations (12% to 18%) is summarized in Table 1-8. The pore size is in the ultrafiltration range and porosity decreases with increasing PSF concentration. Figure 1-24b shows the filtration performance for these membranes over duration of 4 h. Melanoidins retention increased with increasing membrane PSF content. However, for all membranes, retention decreased with increasing filtration time, possibly due to swelling of the membranes. PSF16% and PSF18% membranes showed comparatively higher retention (80% over 30 min), with PSF16% being marginally higher than PSF18%. Thus, PSF16% and PSF18% were selected for preparing MMMs. However, below 18% the polymer matrix could not hold the bio-nanocomposites in suspension. So, further studies were done with PSF18%.

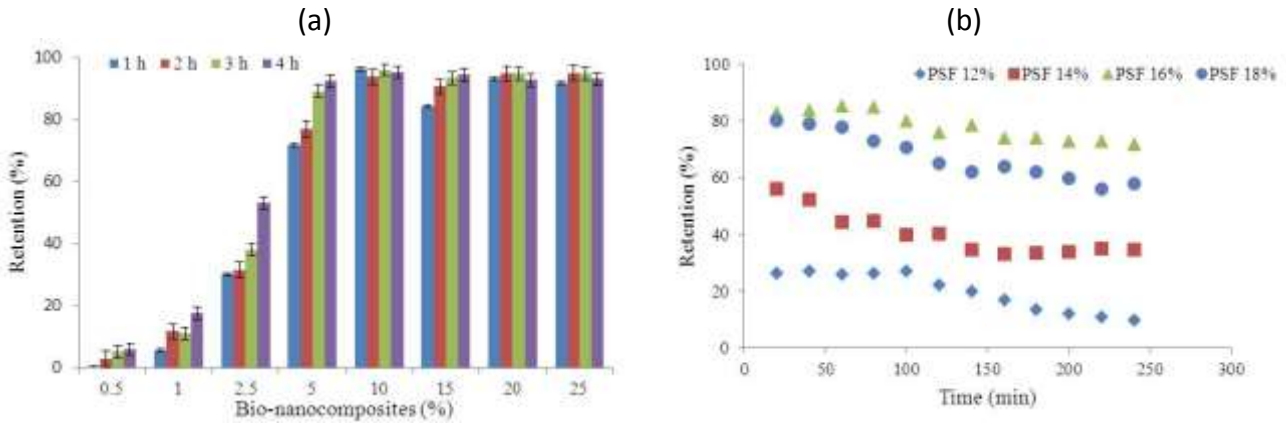


Figure 1-24. Melanoidins recovery by (a) bio-nanocomposites adsorption, and (b) PSF membrane filtration at room temperature.

Table 1-8. Porosity and pore radius of PSF membranes.				
	PSF 12%	**PSF 14%**	**PSF 16%**	**PSF 18%**
Porosity (%)	41.7	39.4	32.7	32.8
Pore radius (µm)	0.019	0.02	0.022	0.022

Figure 1-25 shows the filtration performance of MMMs containing 1% to 20% bio-nanocomposites in PSF18%. PSF18% with 1% bio-nanocomposites shows a permeability of 45 L/m^{2}hbar which is similar to the value for the control (PSF18% with 0% bio-nanocomposite). However, further increase in bio-nanocomposites content reduced the permeability. The decrease in the permeability with increased bio-nanocomposites in the polymer matrix could be due to the increase in the density of the mixture resulting in a tighter membrane. The average melanoidins retention for 0% PSF membrane is 71% which increased to 74% with the addition of 2% to 5% bio-nanocomposites. Further increase in bio-nanocomposite content in the MMM reduced the melanoidins retention to 67% (10% content), and 57% (15% and 20% content). The decrease in retention indicates the possibility of interfacial voids between the polymer and the bio-nanocomposites at higher concentrations. Thus, for further improvement of the MMM properties, 5% bio-nanocomposites in PSF18% was selected.

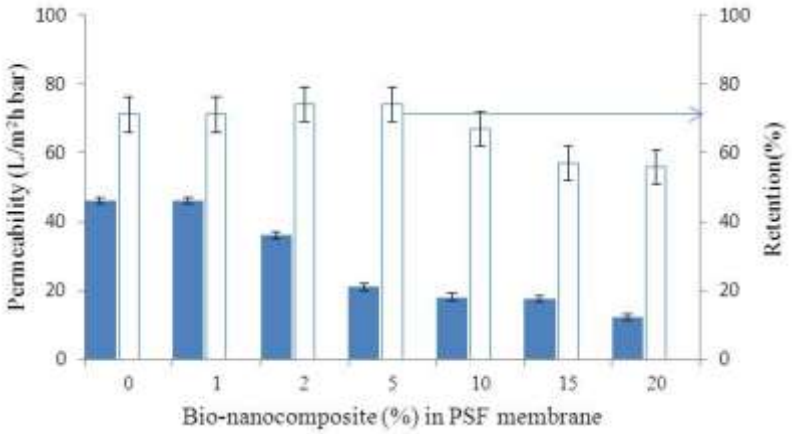


Figure 1-25. Melanoidins permeability and retention in MMMs with varying bio-nanocomposites content.

Figure 1-26 shows the optimization of PVA coating conditions on the MMM surface. Different concentrations of PVA (0.1% to 2%) were coated for different times (1 min to 4 min). The optimum condition was selected based on the permeability and retention of melanoidins.

Figure *1-26*a shows the permeability trends. At lower PVA concentrations (0.1% and 0.25%), the permeability increased on increasing coating time while the reverse was observed at higher PVA concentration (≥ 0.5%). For any particular coating time, the permeability decreased with increasing PVA concentration. Higher PVA concentration and coating time results in formation of thick and dense seal over the asymmetric MMMs. Figure 4b shows the retention of melanoidins. At lower PVA concentration (0.1%), retention increased from 70% to 91% with increasing the coating time from 2 min to 4 min. However, at all other higher concentrations and coating time, the retention was almost similar at 90%. Based on the permeability and retention trends, 0.25% PVA concentration and 2 min coating time was

selected for further studies. Under these conditions, the melanoidins permeability was 4 L/m^2 h bar and retention was 92%.

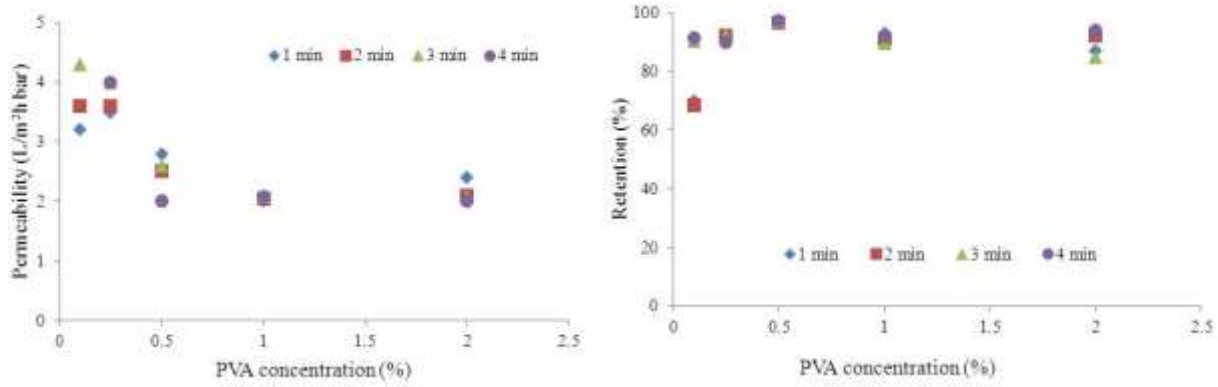


Figure 1-26. (a) Permeability and (b) retention of melanoidins at different PVA coating time and concentrations on PSF 18% membrane.

Figure 1-27a shows the SEM image of the PSF membrane, which is asymmetric with a thin separation layer at the top of a porous support. Addition of PVA coating (Figure 1-27b) forms another layer on top of the separation layer and thereby seals the probable membrane defects formed on addition of the bio-nanocomposites in the PSF matrix.

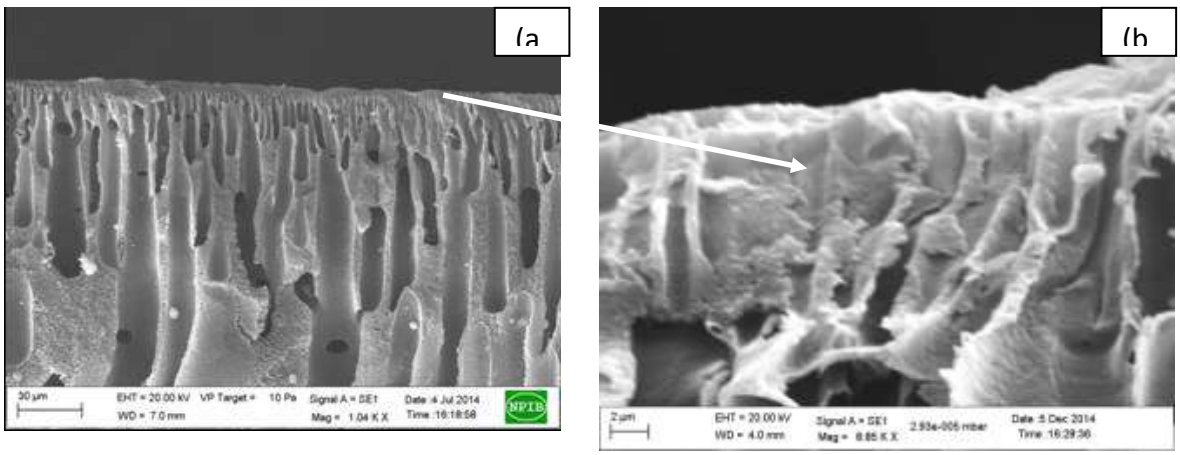


Figure 1-27. SEM image (a) without PVA coating (b) with PVA coating

Figure 1-28a shows melanoidins retention and permeability for different bio-nanocomposites content in PSF18% matrix with 0.25% PVA coated for 2 min. 98% melanoidins retention was obtained for 1% and 2%, 95% for 5% and 10%, and 90% for 15% of bio-nanocomposites content in the MMMs. Further increase in bio-nanocomposites content reduces the melanoidins retention with poor retention (40%) at 20% content. There is a sharp increase in permeability at 20% bio-nanocomposites content indicating that surface defects resulting from high bio-nanocomposites content in the PSF matrix are not completely sealed by the 0.25% PVA coating. Based on these results, PSF18% with 1% bio-nanocomposite content and 0.25% PVA coating was examined further for its fouling tendency of the membranes. Figure 1-28b shows the filtration over a period of 8h. Interestingly, the permeability was almost constant throughout this period maintaining 98% melanoidins retention.

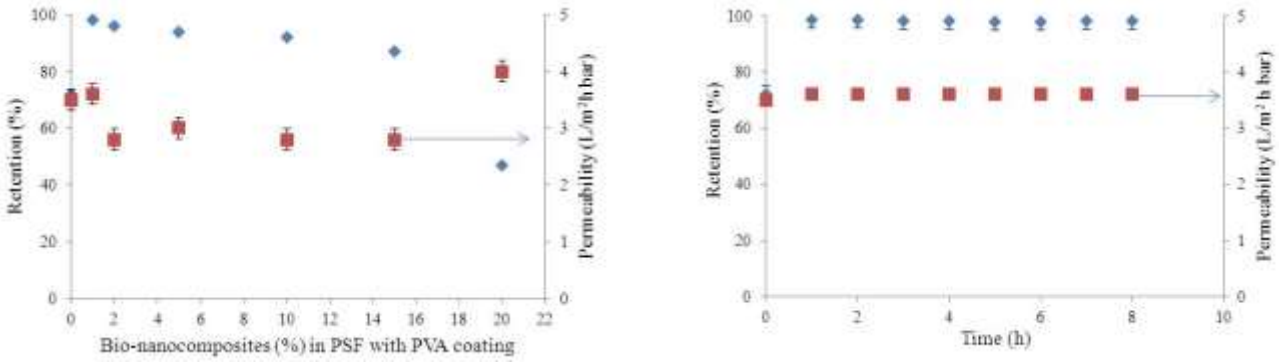


Figure 1-28. Retention and permeability of (a) PVA coated MMMs and (b) long term performance of MMMs.

Membrane filtration based process for melanoidins/phenolics recovery from distillery wastewater

Studies on the recovery of melanoidins from distillery wastewater through ultrafiltration was continued. Distillery wastewater was collected from Brajnathpur distillery unit of Simbhaoli Sugars Limited, Ghaziabad district, Uttar Pradesh. This stream, generated from the distillation column, was used as-received. In the previous report, two different procedures viz. ultrafiltration and isopropanaol fractionation followed by ultrafiltration were examined. Based on the results, serial ultrafiltration of as-is distillery wastewater through polymeric 100kD and 10 kD membranes (procured from Sterlitech, Mumbai) was selected for all further work. The ultrafiltration was conducted in a cross-flow unit from Rayflow (France). Studies are also ongoing to develop suitable ceramic membrane filters from waste sugarcane bagasse ash for distillery wastewater fractionation.

The retenetate and permeate fractions obtained after ultrafiltration was analyzed by gel permeation chromatography (results presented in previous report). The 10 kD retentate was further incubated overnight in 2M NaCl and subsequently ultrafiltered through 10 kDa membrane to recover the <10 kDa ionically bound melanoidins fraction and the >10 kD pure melanoidins core. All fractions were further analyzed for antioxidant activity.

Two different procedures for antioxidant assay were standardized as described below.

<u>2,2'-Azobis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) Assay</u>

The antioxidant capacity of the fractions was estimated in terms of radical scavenging activity in aqueous solution following the procedure described by Delgado-Andrade et al, 2005[6] with some modifications. Chemicals for the assay procedure viz. 2,2'-Azobis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), potassium persulphate, N,N-Dimethylformamide (DMF) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, (Trolox) were procured from Sigma, New Delhi. $ABTS^{+}$ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12 -16 hours before use. The $ABTS^{+}$ (stable for 2 days) was

[6] Delgado-Andrade, C.; Rufian-Henares, J.A.; Morales, F.J. (2005).Assessing the antioxidant activity of melanoidins from coffee brews by different antioxidant methods. Journal of agriculture and food chemistry 53, 7832-7836.

diluted with 5 mM phosphate buffered saline (PBS) (pH 7.4) to an absorbance of 0.70± 0.02 at 734 nm. 5 mM stock solution of trolox was prepared in phosphate buffered saline (PBS) after dissolving it in DMF. 3ml of $ABTS^+$ was used to record the baseline absorbance ($A_{baseline}$) using spectrophotometer (Aquamate, India). After addition of 50µl of sample (melanoidins fractions), or trolox standards to 3ml of $ABTS^+$ solution, absorbance reading (A_{sample}) was taken after 2 min. Absorbance of 50µl PBS in 3ml $ABTS^+$ after 2 min was taken as control

$$\% \text{ Radical scavenging activity} = 100 - [(A_{sample} / A_{baseline}) * 100]$$

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

DPPH radical scavenging activity of was evaluated according to method of Cheng and Ho (1995)[7], as modified by Xu and Chang (2007)[8]. DPPH and methanol were procured from Sigma, New Delhi. 125 µl sample of melanoidins fractions was added to 3.8 ml of methanol solution of DPPH (0.1 mM). The mixture was shaken for 1 min and left to stand in the dark at room temperature for 30 min. Thereafter, the absorbance of sample was measured using spectrophotometer at 517nm against methanol as blank. The percent of DPPH discolouration of the sample was calculated according to the equation:

$$\% \text{ Radical scavenging activity} = [1 - (A_{sample}/A_{control})] \times 100$$

Trolox solutions were prepared at concentrations ranging from 150-1150 µM for calibration purpose. The antioxidant activity of melanoidins fractions for both ABTS and DPPH assays was expressed as an equivalent of that of Trolox using the equation derived from the respective calibration curve of Trolox. Corresponding equation from the calibration curve obtained for ABTS and DPPH assays (Figure 1-29 and Figure 1-30) can be used to obtain the antioxidant activity for the ultrafiltered fractions in terms of Trolox Equivalent Antioxidant Capacity (TEAC). Hence, TEAC is equal to the millimolar concentration of Trolox solution having the antioxidant capacity equivalent to 1mM solution of substance under investigation.

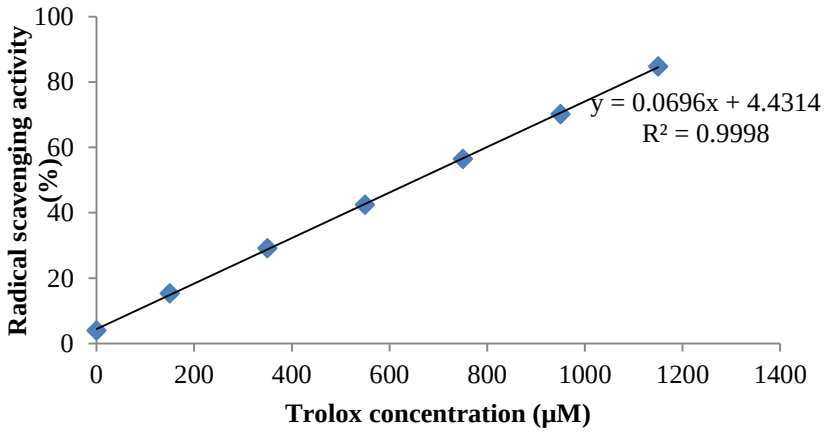


Figure 1-29. Calibration curve of Trolox for ABTS assay.

[7] Chen, C.W. and Ho, C.T. (1995). Antioxidant properties of poly- phenols extracted from green and black teas. Journal of Food Lipids, 2,(1), 1995, 35-46.

[8] Xu, B.J. and Chang, S.K.C. (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. Journal of Food Sci- ence, 72, (2), 160-161.

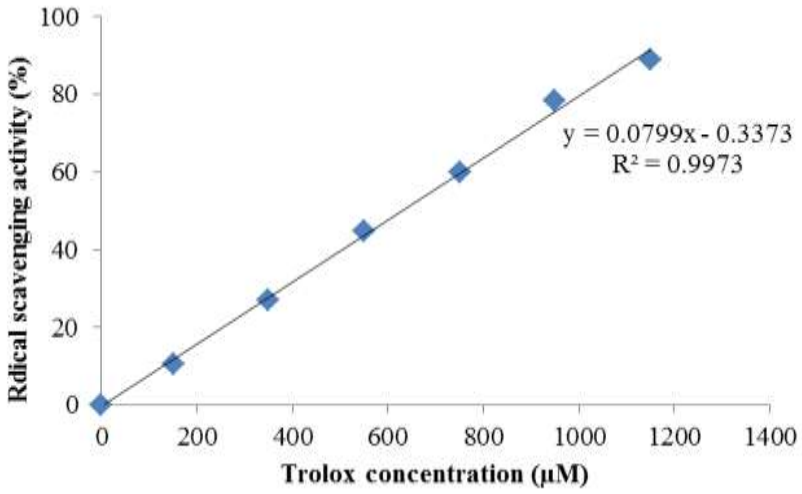


Figure 1-30. Calibration curve of Trolox for DPPH assay.

The results obtained for antioxidant activity for ABTS and DPPH are shown in Table 1-9. Antioxidant values obtained with DPPH assay are lower than the ABTS assay values, likely due to different reaction media (aqueous and methanolic for ABTS and DPPH, respectively), hence indicating subdued antioxidant behaviour in methanol. The trends with both assays are however similar.

Table 1-9. Antioxidant activity of ultrafiltered distillery wastewater

Sample Code	Description	ABTS Assay*(TEAC)	DPPH Assay*(TEAC)
RF100	Retentate fraction (100 kD)	319	106
FF100	Filtrate fraction (100 kD)	1383	723
RF10	Retentate fraction (10 kD)	464	95
FF10	Filtrate fraction (10 kD)	1067	476
IBMF	Ionically bound melanoidin fraction (from retentate of 10 kD)	443	176
MC	Melanoidin core (from retentate of 10 kD)	125	100

*All fractions were 20 fold diluted

From Table 1-9 it is evident that for the high molecular weight fractions, RF100 and RF10 antioxidant activity was found to be lower, (319 and 464 µmol equivalent of Trolox respectively) than their respective low molecular weight fractions FF100 and FF10 (1383 and 1067 µmol equivalent of Trolox respectively) as measured by ABTS assay. Additionally, the melanoidins core itself exhibited low antioxidant behaviour. Thus it appears that the low molecular weight compounds linked non- covalently to the melanoidins skeleton make an important contribution to the overall antioxidant activity of the high molecular weight melanoidins fraction (Delgado-Andrade and Morales, 2005; Delgado-Andrade et al , 2005; Rufian-Henares and Morales, 2007[9]). This is contrary to the observations of Del Castillo et al,

[9] Rufian-Henares, J.A. and Morales, F.J. (2007). Functional properties of melanoidin: In vitro antioxidant, antimicrobial and antihypertensive activities. Food Research International 40, 995-1002.

2006[10] who have pointed out that bread derived melanoidins has a stronger peroxyl radical scavenging activity than the low molecular weight compounds which has been previously reported to be bound to the melanoidin skeleton (Cammerer et al, 2002; Delgado-Andrade et al, 2005) and contributes to the antioxidant activity. The mechanism of the antioxidant effect of melanoidins is however still unclear as the chemical structure of melanoidins is not fully understood.

The RF100 and FF100 fractions were diluted to concentrations ranging from 1% to 10%, in order to determine the maximal inhibitory concentration (IC_{50}). IC_{50} represents the concentration of a substance (or a mixture of substances) required to scavenge 50% of the radicals. As evident from Figure 1-31, 10% of FF100 scavenged upto 95% of ABTS radical. The inhibitory concentration, $IC_{50,}$ was found to be lower for FF100 (<2% of FF100), as compared to RF100 (>10%).

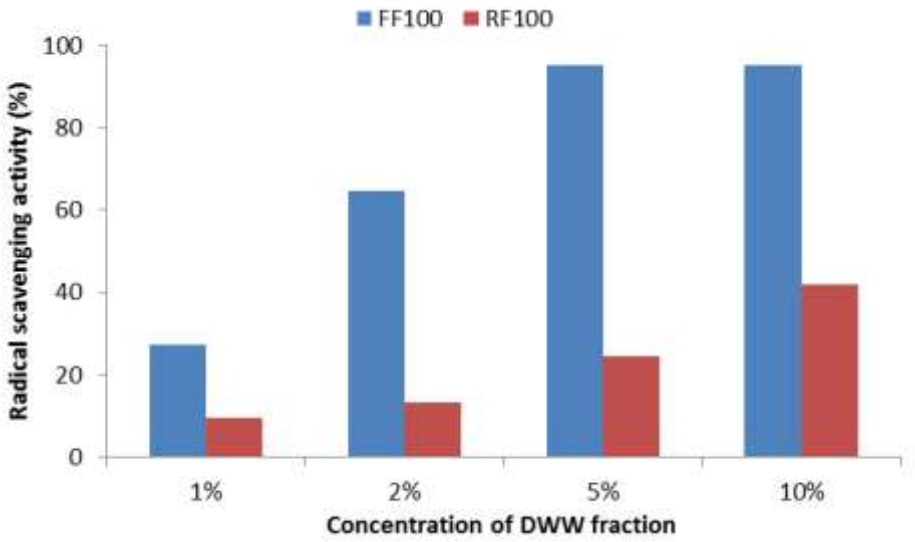


Figure 1-31. IC50 value of distillery wastewater fractions (ABTS assay values)

Adsorptive recovery of melanoidins from distillery wastewater using modified unburnt carbon

Batch adsorption experiments were done with as received distillery (As-is DWW) as well as the retentate and filtrate fractions of the wastewater ultrafiltatred through 100kD and 10 kD membranes.

For a given adsorbate, 50 ml solution was mixed with 0.2g of carbon in 100 ml flasks. The mixture was kept in a shaker (Orbitek, Scigenics Biotech, India) for 24h at 25˚C at 160 rpm. One set of flasks without carbon addition was kept as control. After 24h, the mixture was vacuum filtered through 0.45 µm filter paper (Millipore). The filtrate was analyzed for melanoidins concentration at 475 nm using spectrophotometer (Aquamate, India). All batch adsorption experiments were done in duplicate.

[10] Del Castillo, M.D.; Ferrigno, A.; Acampa, I.; Borrellt, R.C.; Olano, A.; Martinez-Rodriguez, A. (2007). In vitro release of angiotensin-converting enzyme inhibitors, peroxyl radical scavengers and antibacterial compounds by enzymatic hydrolysisof glycated gluten. Journal of Cereal Science, 45(3) 327–334.

Among the different modified unburnt carbons evaluated, maximum color removal of 38% was obtained with 3UC-Ash-740 for low molecular weight fraction, FF100 (Figure 1-32). This sample shows a marginally higher adsorption of 43% with FF10. Thus the modified unburnt carbon is better suited to adsorption of low molecular weight fractions in distillery wastewater. The adsorption process has to be further optimised.

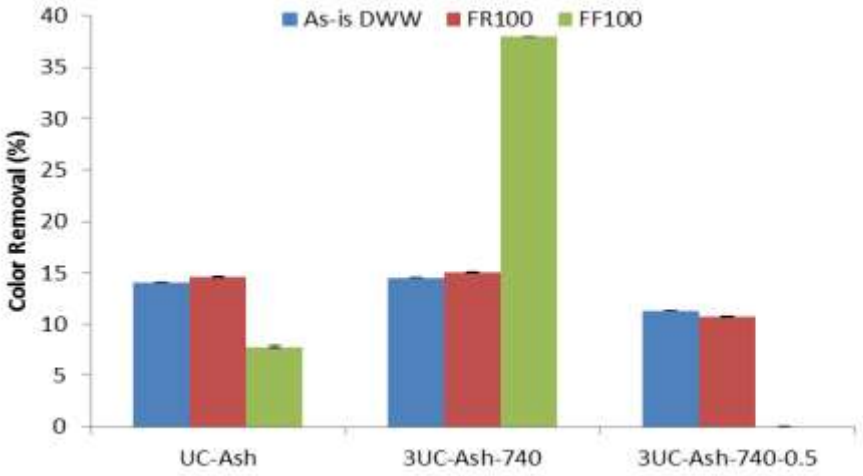


Figure 1-32. Adsorption of distillery wastewater fractions on modified unburnt carbons (4g/L adsorbent)

1.6 Impact of treated and untreated wastewater use on soil, crop and groundwater quality

Ugar sugar site

Spentwash, an agro-industrial effluent is a light brown colored non-toxic liquid containing residual nutrients from sugarcane and yeast cells with rotten jaggery smell. Distillery effluent is characterized by neutral pH, high EC, high biological oxygen demand and chemical oxygen demand and contains a high percentage of essential nutrient elements. Application of the primary treated spentwash on land offers a promising alternative as irrigation water and as a source of plant nutrients while offering solutions to the disposal problem. However, due to continuous and indiscriminate use of spentwash, the sugarcane yield has been drastically reduced over years. The area known for a potential cane yield of 100 t/ha has come down practically to 10 -15 t/ha, due to continuous use of spentwash. Sugarcane being a moderately sensitive crop to soil salinity was affected much and the yield level has gone below the economic threshold. This study was aimed at characterizing soils affected by long-term application of spentwash under semi-arid climate of Northern Dry Zone of Karnataka.

The mean characteristics of raw and secondary aerated spentwash are given in Table XX. The secondary aerated spent wash was further treated in effluent treated plant (ETP) and then diluted with river water (usually 1:10) and used for ferti-irrigation till 2008. Soils from the experimental site were categorized into P_0 as control, P_1 for irrigation of 5 to 10 year, P_3 for irrigation of 10-15 years, P_4 for irrigation of 15-20 year, and P_5 for soils irrigated with spentwash water for more than 20 years.

Table 1-10. Characteristics of Raw spentwash and Secondary aerated spent wash			
Sl.No	**Parameters**	**Raw Spent wash**	**Secondary aerated spent wash**
1.	pH	3.68	7.25
2.	C.O.D	100000	26560
3.	B.O.D	42260	7225
4.	Total Solids	65255	27672
5.	Total dissolved solids	57245	20836
6.	Suspended solids	8010	6836
7.	Chlorides	7384	6567
8.	Total hardness	10400	9100
9.	Conductivity	12890	10666
10	Oil & Grease	Nil	Nil

Note: All results are in mg/l except pH. Source: ugar sugar Factory, Ugar Kurd, Belagavi, Karnataka

Impact on soil characteristics

The long-term application of spentwash, besides serving as a good source of nutrients and organic matter, also greatly influenced the soil physico-chemical properties and the microbial population. Under each spentwash application treatment (i.e., periods of spentwash application: P_0 to P_5) soil samples from five depths (0-20, 20-40, 40-60, 60-80 and > 80 cm) were collected at five places (designated by corresponding latitude and longitude) and analyzed for chemical characterization. The physical properties (0-20 cm depth) were estimated on triplicates at each of these application sites.

The long-term application of spentwash resulted in significant improvement in physical properties of soil like bulk density, aggregate stability, maximum water holding capacity, infiltration rate, dispersion ratio and erosion index.

Bulk density of the soil was significantly influenced by the long-term spentwash application. The lower bulk density was observed in the treatment that received spentwash for more than 20 years. The reduction in bulk density was due to addition of high organic matter through spentwash.

Long-term application of spentwash significantly influenced per cent water stable aggregates, maximum water holding capacity and infiltration rate. The higher value of per cent water stable aggregates, maximum water holding capacity and infiltration rate were observed in the treatment that received spentwash for 15 to 20 years. However, long-term spentwash application for > 20 years lead to slight reduction in these parameters which might be due to clogging of pores with high particulate organic carbon. The application of spentwash for limited periods increased aggregate stability which might be due to the salts present in the spentwash. The fresh application of effluent might also had stimulated the microbial activity and secretion of microbial polysaccharides, which helped in stabilization of soil aggregates. Higher concentration of Ca in soil solution and on exchange sites in spentwash applied plots might have improved aggregation and its strength. Increase in

infiltration rate in spentwash treated plots was ascribed to higher organic matter content and changed distribution of pore sizes of the soil. An increase in maximum water holding capacity was due to increased number of small pores caused by better and fine grade aggregation. Water holding capacity was related to the number and size distribution of soil pores and consequently increased with soil organic matter level.

The dispersion ratio and erosion index values were higher in control plot and application of spentwash for varying periods significantly reduced erosion index and dispersion ratio. The spentwash applied fields were physically better with respect to water stable aggregates and infiltration rate as compared to control. Apparently, this was due to higher organic carbon content and improved aggregate stability which were evident in this study.

Long term spentwash application brought major changes in the chemical properties of soil. Marginally higher soil pH was observed in spentwash applied fields compared to control. This was due to long-term application of bio-methanated spentwash which was neutral to basic in reaction. Further, in case P_3 (15 to 20 years of spentwash application) and P_4 (>20 years of spentwash application) there was slight decrease in pH which might be due to release of organic acids from accumulated organic matter.

Electrical conductivity is an indicator of total soluble salts present in soil. The long-term application of spentwash significantly increased the EC of experimental soil. This might be due to the fact that the effluent contained higher amounts of soluble salts (17.32 dS m^{-1}). Soil organic carbon increased with increasing duration of spentwash application. Organic carbon was highest in the treatment that received spentwash for more than 20 years.

There was a significant influence of long-term spentwash application on available nitrogen status of soil. Available nitrogen was significantly higher in the treatment that received spentwash for more than 20 years. Even though spentwash contained only small quantity of nitrogen, its long-term application increased the available nitrogen status of the soil. This might be due to its direct contribution to N supply as well as the increased microbial activity due to added organic matter which might have increased the available N pool. Spentwash acted as a slow releasing liquid nitrogen fertilizer. The control field recorded lower available nitrogen compared to the spentwash applied plots.

The available phosphorus content in soil varied from low (10.09 kg ha^{-1}) to high (34.42 kg ha^{-1}). Soils irrigated with spentwash recorded higher phosphorus content than un-irrigated field which might be due to addition of phosphorus through spentwash in the organic form that mineralized to inorganic P. The soil organic matter acted as a substrate for microorganisms which in turn mineralized the organic forms of phosphorus. The available potassium was significantly higher in the treatments which received spentwash. This was due to presence of higher amount of potassium (6213.12 mg L^{-1}) in the spentwash.

The available micronutrients *viz*., Fe, Mn, Zn and Cu increased with increased periods of spentwash application and the availability was maximum with the application of spentwash for more than 20 years. The increased availability was due to the direct contribution from the effluent and might be due to solubilisation and chelation effect of organic matter. The

availability of these micronutrients decreased with depth in treatments possibly due to increased $CaCO_3$ content and pH with depth.

The higher ESP was recorded in spentwash irrigated fields than the control. This increased ESP was attributed to accumulation of sodium in soils due to spentwash irrigation. The ESP decreased with depth due to greater accumulation of sodium in surface.

The soil in the present study was calcareous in nature. The fields irrigated with spentwash for various periods recorded higher values of $CaCO_3$ than unirrigated one. Among spentwash irrigated fields, those irrigated for more than 20 years recorded the highest value. The increase in $CaCO_3$ content in soil was due to increased addition of carbonates and calcium through spentwash.

The soils irrigated with spentwash for different periods recorded more CEC and base saturation than unirrigated field. Long-term application of spentwash> 20 years recorded higher CEC and base saturation values than other treatments. This was mainly due to high content of cations and organic matter in spentwash. Both CEC and base saturation decreased with depth. The exchangeable bases in all the treatments were in the order of Ca^{+2}> Mg^{+2}> Na^{+} > K^{+} on the exchange complex. From the distribution of Ca^{+2} and Mg^{+2}, it was clear that Mg^{+2} was present in low amount than Ca^{+2} because of its higher mobility.

The population of bacteria, fungi and actinomycetes was higher in the spentwash irrigated fields compared to control. This was attributed to higher organic load present in the spentwash, which might have served as source of energy for the growth and multiplication of micro-organisms and also for various enzyme activities in soil. The increase was maximum in case of bacteria, followed by fungi and actinomycetes. Further in P_4 (more than 20 years spentwash application) there was marginal reduction in the population of bacteria, fungi and actinomycetes which might be due to buildup of salinity and chloride.

Table 1-11. Effect of spentwash on soil pH, EC and OC

Periods of spent was application (years)	**pH $_{2.5}$**	**$EC_{2.5}$ (dS m^{-1})**	**OC (g kg^{-1})**
Control	7.84	0.39	2.79
5 to 10	8.08	0.70	4.74
10 to 15	8.11	0.83	6.40
15 to 20	8.04	1.03	7.40
> 20	7.90	3.10	8.10

Table 1-12. Effect of spentwash on soil micronutrients status

Periods of spent wash applications (year)	**Zn (mg kg^{-1})**	**Fe (mg kg^{-1})**	**Mn (mg kg^{-1})**	**Cu (mg kg^{-1})**
Control	0.26	1.17	1.29	1.59
5 to 10	1.05	5.2	3.30	2.64
10 to 15	1.17	5.43	4.06	3.86
15 to 20	1.32	5.45	4.38	3.20
>20 years	1.34	5.96	5.05	3.36

Lakshmipuram site

Sugarcane was selected for field trials as was requested by the industry because the Chellapalli regions is mostly saline affected area and majority of the farmers are growing sugarcane. A saline tolerant variety suggested by the industry was used in the study to assess the reuse potential of bio-treated distillery effluent compared to the fresh water as control.

The volume of water required by sugarcane and the timing of irrigation were matched with the volume and timing of effluent discharge from the fish tank. Water from fish tank is pumped out for agriculture purpose. Sugar effluent is widely used in agriculture as a source of plant nutrients and irrigation. The beneficial effects of sugar industry wastewater on crop and soil are well established however, it is imperative to assess its suitability to the local soil condition and native crops grown by farmers. In this regard, field experiments are conducted to study the effectiveness of bio-treated sugar effluent through constructed wetland and aquaculture. The efficacy of the treated sugar effluent will be compared with fresh water, with difference in the soil type as well.

Biological properties of soil

Soil biota is a significant component of soil quality and the soil microbial communities play a vital role in soil ecosystem functioning related to soil fertility and primary production through organic matter decomposition and nutrient cycling. Hence it is important to assess the microbial load and pathogenic population in the soil. Soil samples were collected on 21st November 2013 from agriculture field and aquaculture pond by transact Z walk at Lakshmipuram. One kg of soil collected from five points were mixed and divided into four quadrants. Two opposite quadrants were taken as sample for analysis. These samples were packed in poly bags, labelled and stored at 4°C until analysis was carried out.

The pathogenic population in the soil was determined using different media as described earlier which would be the baseline data to monitor and compare the changes after field trials demonstrate using bio-treated sugar effluent. Comparison of results generated two consecutive years i.e. Jan 2013 and 2014 indicates that the soil samples harbours pathogenic organisms such as *Legionella* sp., *Staphylococcus* sp. and *Shigella* sp. At ≤log 5cfu/ml whereas *Yersinia* sp. *Enterobacter* sp. were detected at log 3cfu/ml and < log 1cfu/ml (fig 23). Overall observation of both the years portrays that there is a slight increase

in pathogenic population at < log 1.5cfu/ml which is within the permissible limits of Indian standards.

Vuyyuru site

Sweet corn cultivation using bio-treated wastewater

Experimental details

Sweet corn, one type of maize which is largely grown in AP was selected to study the reuse potential of bio-treated distillery effluent compared to anaerobic treated distillery effluent keeping fresh water as control. A common variety i.e. F1 Hybrid Sweet Gold 95 used by local farmers was selected for the experiment.

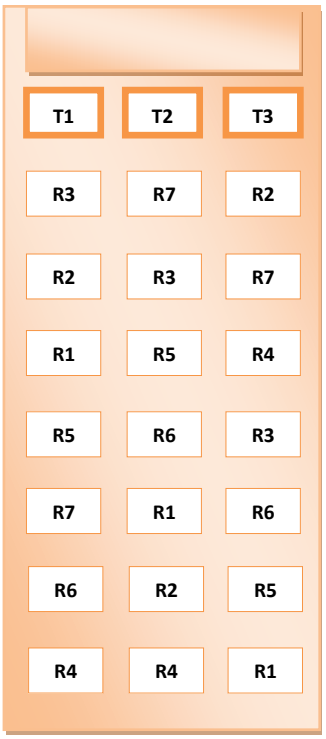


Figure 1-33. RBD of sweet corn trials

Design and layout

As given in Figure 1-33 the experiment was carried out in randomized block design (RBD) with 7 replicates in each of the three treatments where T1 is bio-treated distillery effluent, T2 anaerobic treated distillery effluent and T3 fresh water as control. Each replicate was of 5m x 2.5 m = 12.5 m^2. And the net replicate size for each treatment was 87.5 m^2.

Details of cultural operations

The experimental field within the industrial premise was prepared using tractor mounted disc plough and leveling was done before laying down the replicates as per the plan. Seeds were dibbled at a depth of 2 to 3 cm in the soil followed by slight irrigation using fresh water across the treatments to ensure proper and uniform germination. About 1120g of each urea, Potash and Super phosphate were given to all the three treatments on the 20 days after sowing (DAS). The same amount of Potash and Urea was applied on the 50th DAS. Chlopyrifos 50% was sprayed on 40th DAS and subsequently on 55th day to control shoot borer. In addition, 5 g of Forg 3 was applied on 40th DAS for each crop. Hand weeding was done as and when required.

Alternate day wetting was done by adopting localized irrigation method, which is applying water around each plant to wet locally and root zone only. The application rate was adjusted based on the crop growth where 2 L/day was applied till 50th DAS after which it was increased to 4 L/day. Harvesting was done by observing maturity signs like full size green cobs with tight husk, dry brown silks, smooth and plumpy kernels which exude milky liquid when punctured. The cobs were harvested and the biometric observations were recorded.

Experimental observation on crop

To monitor and record periodical observations on growth character, yield attributes and crop yield three plants per replicate were tagged randomly in each treatment. Plant height was recorded at 30, 60 and 90 DAS from the base of the plant to the ligule of the last leaf before tasseling and up to the tip of the tassel after its emergence. Average plant height in T1, T2 and T3 are 199 cm, 180 cm and 218 cm respectively. In fresh water and bio-treated water there were 12 leaves on 60 DAS while it was only 9 leaves in anaerobic treated water. Whereas in 90 DAS one leaf each was dried in T1 and T3 on the other hand one new leaf emerged in the T2. The tagged plants were uprooted after harvest and were sun dried initially and subsequently dried in hot air oven at 60° C. The weight of dried shoot and root were measured and observed to be 114.8 and 44.6 g in T1, 92.4 and 35.8g in T2, 140.8 and 46.2 in T3. The shoot and root weights of sweet corn irrigated with bio-treated distillery effluent was 16% and 19.1% higher than the anaerobic treated distillery effluent.

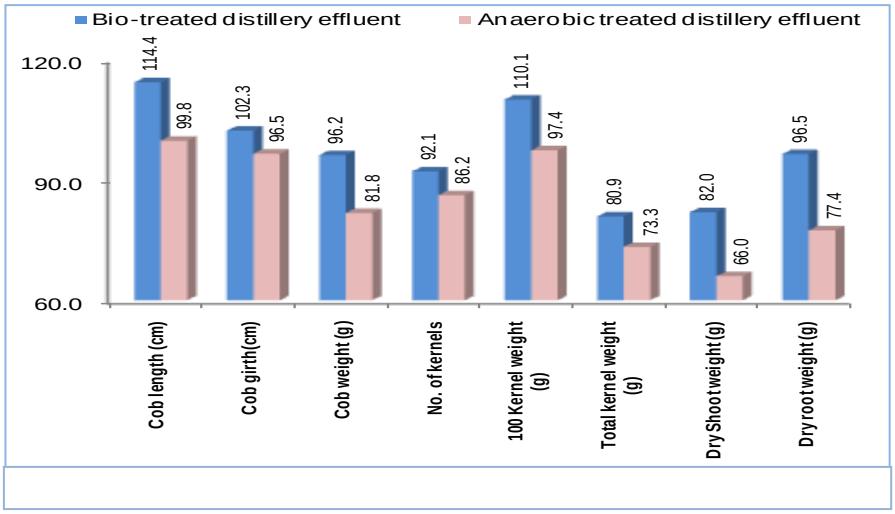


Figure 1-34. Biometrics of sweet corn crop

The data on the yield attributes is given in Figure 1-34. The number of cobs per plant did not show any significant variation in different treatments. However, significant differences were observed in cob length, cob width, cob weight, No. of kernels, 100 kernels weight and total kernels weight in varied sources of irrigation. In the experiment the variation in yield attributes from T1 and T2 were compared with T3 which is the control. The cob length in T1 was 14.4% higher than the control while in T2 there was no significant variation. Similar trend was observed in width of the cob with 2.3% higher in T1 and 3.5% lesser in T2. There was a 3.8% decrease in the cob weight in T1 compared to control while 18.2% reduction was found in T2. Though No. of kernel and total kernel weight showed a decreasing trend i.e. T3 > T1 > T2, the 100 kernel weight portray 10.1% increase in T1 whereas 2.6%fall was observed in T2.

Biometric monitoring *T1- cobs* *T2- cob* *T3- cob* *Damaged cobs*

Total yield from all the treatment were 256 cobs of which 76 were destroyed by monkeys, crows and parrots. And the total cobs harvested were 74, 38 and 68 in treatments irrigated with bio-treated, anaerobic treated distillery effluent and fresh water respectively. It is evident that the yield is higher in T1 compared to treatments irrigated with other sources. *Further studies shall be carried out to explore the feasibility of supplementing bio-treated distillery effluent to fresh water for irrigating sweet corn.* Around 8 plants in T2 died between 60 and 90 DAS and monkey destroyed 3 each from T1 and T2 which also affected the yield in addition to cob loss as specified above.

The water quality of T1 was improved after treating it with adapted bacterial consortium, adapted algal consortium followed by filtration through activated charcoal chamber. The pH was changed from weak acidic to neutral. Around 80.88% of COD was reduced in the treated water. Both these parameters would have played a role in better crop growth and yield as compared to T2. Despite there was no greater difference in salinity levels of T1 (9.36 ppt) and T2 (10.4 ppt) the crop is resistant to salinity which is evident from the growth and yield attributes.

Economics

A rough economics were worked out and presented in Table 1-13 indicates that the total cost incurred towards experimenting sweet corn cultivation using various sources of water is Rs.4142.12. Labour cost includes sowing, fertilizer and pesticide application, irrigation, weeding and harvesting. Local whole sale market value is Rs.15/cob. Out of total yield 180 was harvested while 76 was destroyed by monkeys, crow and parrot. Total value of the yield is Rs.3840. After deducting the crop value from total cost there is a gap. This may be attributed to the lesser yield as well as plant mortality from T2. As this is an experimental plot certain parameters were not taken for calculation. The land, water and electricity were provided by the industry which is also not taken.

Table 1-13. Economics of sweet corn crop		
Sl. No	**Description**	**Cost (Rs.)**
1	Land preparation	400.00
2	Seed	200.00
3	Fertilizer	57.12
4	Pesticide	580.00
5	Labour cost	2905.00
Total cost		**4142.12**
6	Harvested crop value	2700.00
7	Value of crop lost	1140.00
Total crop value		3840.00
Net balance		-302.12

Microbial load in soil

The culturable microbial population viz., bacteria, fungi and pathogens in the soil samples were determined using nutrient agar (NA) and potato dextrose agar (PDA) respectively by serial dilution. The fungal population was determined in 10^{-3} in PDA plates. The plates were incubated at room temperature and the pathogenic population in the soil samples were determined using specific media at 10^{-3} dilution. From the fig 33 the total microbial load is detected at ≥7 log cfu/g. The presence of pathogens looks like a ladder where *Enterobacter* sp. was found to be least with <5 log cfu/g while *Yersinia* sp., *Clostridium* sp. and *Shigella* sp. are in the range between log 5 and 6 cfu/g and the *Staphylococcus* sp. at ≤log 6 cfu/ml. This is the baseline data to monitor and compare changes in soil microbial diversity due to reuse of treated distillery effluent.

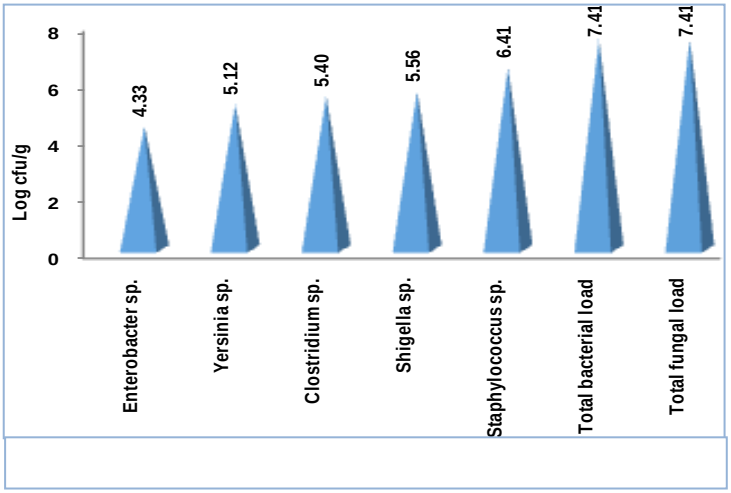


Figure 1-35. Microbial load of Vuyyuru soil

Microbial load in sweet corn cultivated plots

The microbial load assessment from standing crop of sweet corn soil showed that the plots irrigated with treated distillery effluent was on par with fresh water irrigated soil. But the bacterial load of the soil irrigated with anaerobic treated distillery effluent is higher than T1 and T3 irrigated soils. However the fungal population was almost equal to T3 and lesser than the T1 irrigated soils. Microbial load assessment after crop harvest showed that there is no significant change in bacterial population but a slight decrease in fugal population is evident.

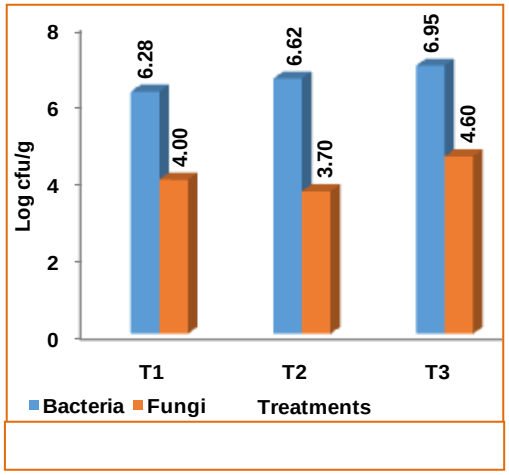


Figure 1-36. Microbial load in crop harvested soil

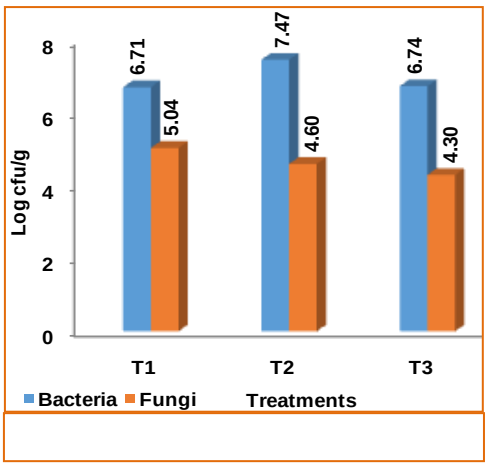


Figure 1-37. Microbial load in soil from standing crop

Pathogenic population in sweet corn cultivated plots

Assessment of pathogenic population as given in Figure 1-38 indicates that in T1 *Yersinia* sp. *Shigella* sp. *Proteus* sp. and *Salmonella* sp. were observed at ≥ log 4 cfu/ml whereas *Staphylococcus* sp. *Legionella* sp. and *Enterobacter* sp. were detected at ≥ log 5cfu/ml. In the T2 all the pathogenic population except *Salmonella* sp. and *Shigella* sp. were determined at ≥ log 6cfu/ml while the former were detected at ≥ log 3 cfu/ml. In T3 treatment the pathogenic population was less compared to T1 and T2 except for *Staphylococcus* sp. which was detected at ≥ log 6cfu/ml. Overall observation is that the T2 plots comprises of higher pathogenic population compared to T1 and T3 indicating that the treatment process is effective. After harvest of sweet corn a decline in the pathogenic population was observed except for few groups as depicted in *Figure 1-36*, which needs to be monitored and assessed in the subsequent trials. The age of the crop determines the rhizoshperic microbial association. Since the age of the standing crop was 25 DAS which represents higher pathogenic population that declined at the later stage of crop harvest. However, there is significant reduction in the pathogenic population.

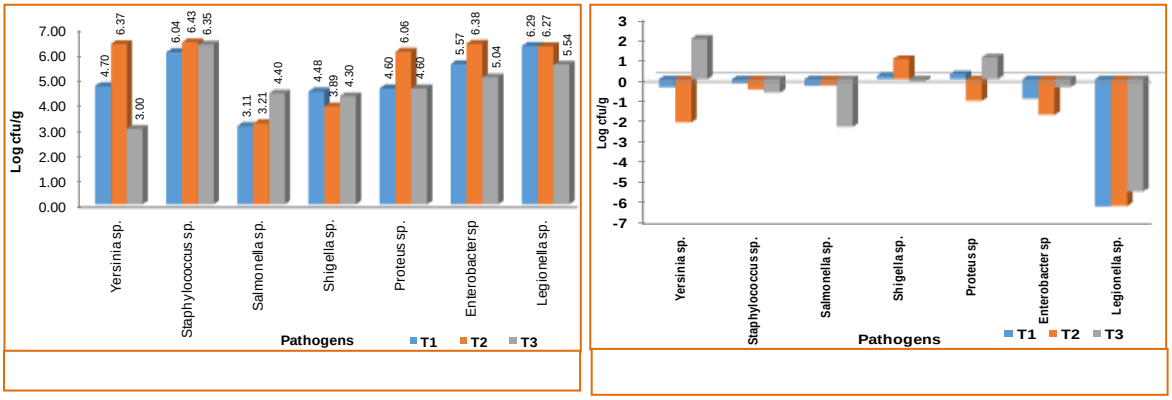


Figure 1-38. Pathogenic population in soil standing sweet corn (left) and after crop harvesting

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 bio-treatment of municipal wastewater for re-use in agriculture

2.1 Report on microbial consortium formed using available strains

Characterization of wastewater collected from the CSIR-NEERI campus

The domestic wastewater generated within the CSIR-NEERI campus was selected for its treatment using ECWs at pilot scale. The characteristics of wastewater are presented Table 2-1. The wastewater sample was analyzed as per the Standard Methods for the Examination of Water and Wastewater (APHA, 2012) for various physico-chemical parameters. Results show that the colour of the wastewater is pale yellow having 19 – 21 hazen colour unit. The pH and electrical conductivity (EC) of the wastewater samples collected during different intervals of time varied from 7.28 to 7.32 and 0.60 to 0.63 dS m^{-1} respectively. The total dissolved solids (TDS) contents in the wastewater samples varied in the range of 298 to 328 mg L^{-1}. The total alkalinity and suspended solids in the water samples varied in the range of 320 to 340 mg L^{-1} and 109 to 120 mg L^{-1} respectively. The total hardness in the water samples varied from 240 to 256 mg L^{-1} while the corresponding levels of calcium and magnesium varied from 40 to 48 mg L^{-1} and 34 to 36 mg L^{-1} respectively. The chemical oxygen demand (COD) and biochemical oxygen demand (BOD) varied in the range of 200 to 250 mg L^{-1} and 127 to 156 mg L^{-1} respectively. The levels of cations with respect to sodium and potassium in the wastewater samples varied from 28 to 33 mg L^{-1} and 2 to 4 mg L^{-1} respectively. The levels of anions with respect to chloride varied from 48 to 54.8 mg L^{-1} whereas sulphate varied from 2.0 to 2.4 mg L^{-1} in the wastewater samples respectively.

According to the CPCB standards for the discharge of effluent on land for irrigation, the wastewater complies with the standard values, except for the BOD and colour. The levels of heavy metals such as zinc, lead, cadmium, nickel, iron, chromium and copper presented in indicate that their concentrations were within the toxicity limit and do not pose any toxicity to plants species as per Indian Standards (IS:10500,1993) and Food and Agricultural Organization guidelines for wastewater use in agriculture (FAO, 1985). The major concern is to treat BOD, COD, colour and odor of wastewater for its use in agricultural purposes.

Table 2-1. Physico-chemical characteristics of domestic wastewater samples collected at CSIR-NEERI campus, Nagpur

Sl. No.	Parameters	Domestic Wastewater	FAO Wastewater quality Guidelines for Agricultural Use (1985)	Indian Standards for discharge of effluent on land for irrigation (IS:10500, 1993)
1.	Colour, Pt Co Hazen	19 – 21	--	--
2.	pH	7.28 - 7.32	6.50 to 8.00	5.5 to 9.0
3.	EC, dS m^{-1}	0.60 -0. 63	< 0.70	--
4.	TDS, mg L^{-1}	298 - 328	< 450	2100
5.	Total Alkalinity, mg L^{-1}	320 - 340	--	200
6.	TSS, mg L^{-1}	109 - 120	--	--
7.	Total Hardness, mg L^{-1}	240 - 256	--	--
8.	Calcium, mg L^{-1}	40 - 48	--	--
9.	Magnesium, mg L^{-1}	34 - 36	--	--
10.	COD, mg L^{-1}	200-250	--	--
11.	BOD, mg L^{-1}	127-156	--	100
12.	Ammonical Nitrogen, mg L^{-1}	1.25 – 1.78	--	--
13.	Nitrate, mg L^{-1}	0.60 – 0.86	--	--
14.	Sodium, mg L^{-1}	28 - 33	--	--
15.	Potassium, mg L^{-1}	2 - 4	--	--
16.	Chloride, mg L^{-1}	48 – 54.8	142	600
17.	Sulphate, mg L^{-1}	2.0 – 2.4	--	1000
18.	Sodium Absorption Ratio (SAR)	0.78 – 0.80	< 3.0	--
Heavy Metals and Oxyanion , mg L^{-1}				
19.	Zinc	0.02	2.0	--
20.	Lead	BDL	5.0	--
21.	Cadmium	BDL	0.01	--
22.	Nickel	BDL	0.20	--
23.	Manganese	0.01	0.20	2.0
24.	Iron	BDL	5.0	3.0
25.	Chromium	BDL	0.10	--
26.	Copper	BDL	0.20	--
27.	Boron	BDL	0.70	--
28.	Arsenic	BDL	0.10	0.2

BDL: Below Detection Limit

Screening and isolation of microorganisms for degradation of pollutants utilizing contaminated wastewater for growth (Completed)

The wastewater samples collected from CSIR-NEERI campus were characterized for different microbial groups *viz.* Bacteria, Fungi, *Actinomycetes*, *Azotobacter* and *Rhizobium* present in the wastewater. In total, 6 bacterial cultures were isolated from the wastewater. To assess the efficiency of isolates/consortium for removal of pollutants, the sewage wastewater was collected from open nullah near Dhantoli area, Nagpur. The collection of sewage wastewater from Dhantoli area was selected based on the nullah / other drainage system

were joining to the Nag River passing through that area and in future the ECWs will be demonstrated at Pandherkawada village for treatment of the same sewage wastewater at pilot scale. The wastewater was collected in bulk quantity and batch experiments were conducted to assess the performance of six identified bacterial isolates towards removal of pollutants present in the wastewater. The bacterial cultures were grown at 37^0C on an orbital shaker incubator at 120 rpm in nutrient broth till mid log phase (OD ~1). The cultures were then centrifuged at 8000 rpm and pellet obtained was re-suspended in autoclaved distilled water to form a load of 10^8 cells/ml. 10ml of individual culture was inoculated in 1L flask containing 600ml of sewage wastewater with mineral salt medium (MSM) nutrients. Another flask containing sewage wastewater with MSM nutrients was kept as control. The entire flasks were incubated at 37^0C on an orbital shaker incubator at 120 rpm. The samples were analyzed for different parameters after every 24hrs for a period of 5 days. The efficiency of cultures was evaluated for parameters such as pH, EC, TDS, BOD and dehydrogenase activity.

Table 2-2. Microbiological characteristics of domestic wastewater collected within the CSIR-NEERI campus, Nagpur

Microbiological Parameters	**Microbial Count (CFU/ml)**
Bacteria	20×10^5
Fungi	13×10^1
Actinomycetes	10×10^2
Azotobacter	15×10^2
Rhizobium	12×10^2

CFU – Colony forming unit

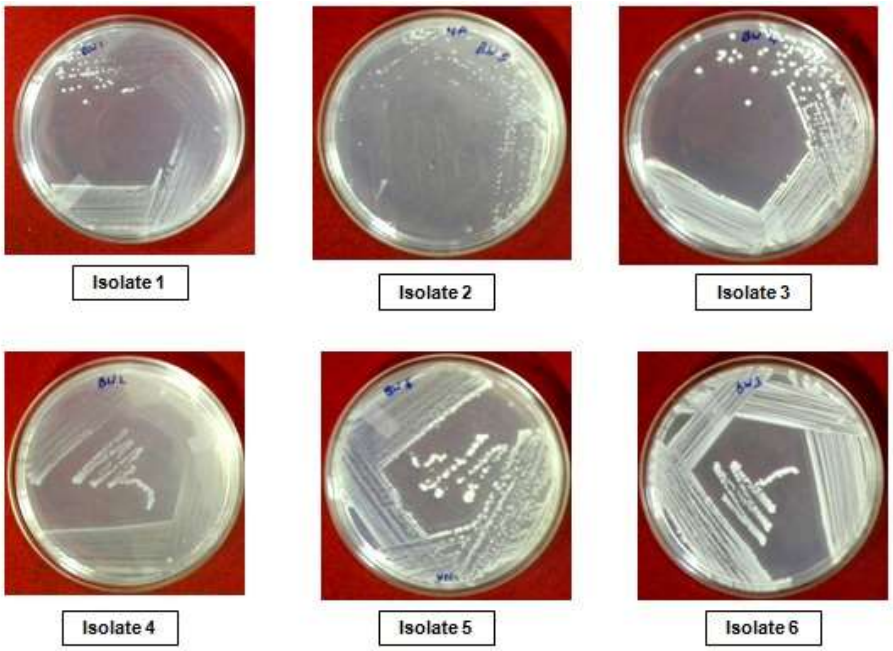


Figure 2-1. Bacterial cultures isolated from the domestic wastewater collected from CSIR-NEERI campus

The results presented in Table 2-3 show the ability of six bacterial isolates on parameters such as pH, EC and TDS. It was found that from 2nd day onwards there was a consistent and systematic decrease in pH during the biological treatment that is associated with the presence of biomass. Decrease in pH may be due to physical oxidation of organic compounds and dissolution of carbon dioxide in wastewater. As the wastewater contains various organic acids, the bacteria will secrete organic acids, enzymes, antioxidants and metallic chelates during the degradation activities may also be the cause of pH reduction towards acidic environment. The other two parameters i.e. EC and TDS also follow the similar pattern. Reduction in EC was observed using all the six bacterial isolates. EC has a positive correlation with TDS and hence it was also monitored during the study. A steady decrease in TDS was observed in all the samples treated with bacterial isolates. The decrease in TDS might be due to utilization of dissolved solids by the degrading bacterial isolates, both indigenous and augmented. The overall results indicate that the bacterial isolates 3, 5 and 6 show better performance towards reduction of selected parameters as compared to other bacterial isolates 1, 2 and 4 respectively.

Similarly the results presented in Table 2-4 show the ability of six bacterial isolates towards BOD reduction and on dehydrogenase activity. BOD represents only the organic matter which is degraded / oxidized by microbes whereas dehydrogenase activity is an index of microbial population. The maximum reduction of 86.4%, 90.9% and 81.8% of BOD were shown by bacterial isolates 3, 5 and 6 respectively. While the other bacterial isolates 1, 2 and 4 showed BOD reduction in the range of 50-60% respectively. In case of dehydrogenase activity, sudden increase in activity was observed till 2nd day of incubation after which a gradual decrease was noted. Increase in dehydrogenase activity indicates increase in metabolically active microbial population. The organic components of wastewater were utilized by bacterial isolates as carbon source initially which justifies the increase in activity during initial days of study. Also, a drop in count accounts for scarcity of nutrients/organic matter which were used by the bacterial isolates for their growth and multiplication. Hence, it can be concluded that the bacterial isolates 3, 5 and 6 were more effective among all the six bacterial isolates towards removal of pollutants present in the wastewater.

Table 2-3. Variation in pH, EC and TDS in the sewage wastewater treated with various bacterial isolates

	0th day	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**
Control						
pH	7.42	8.02	8.00	7.52	7.24	6.82
EC	540	522	453	442	438	459
TDS	350	343	297	290	287	301
Isolate 1						
pH	7.42	7.88	7.32	7.16	7.05	6.92
EC	540	527	475	441	429	427
TDS	350	347	312	289	281	280
Isolate 2						
pH	7.42	7.85	7.38	7.22	7.17	6.54
EC	540	527	487	450	442	438
TDS	350	347	320	295	290	287

	0th day	1st day	2nd day	3rd day	4th day	5th day
Isolate 3						
pH	7.42	7.89	7.46	7.22	7.12	6.16
EC	540	514	465	435	362	356
TDS	350	338	305	285	236	232
Isolate 4						
pH	7.42	7.84	7.48	7.21	7.12	6.28
EC	540	520	499	465	442	427
TDS	350	342	328	305	290	280
Isolate 5						
pH	7.42	7.83	7.51	7.23	7.13	6.02
EC	540	505	472	270	240	234
TDS	350	332	310	270	240	234
Isolate 6						
pH	7.42	7.78	7.39	7.24	7.14	6.33
EC	540	495	442	405	375	365
TDS	350	325	290	265	245	238

Table 2-4. Variation in BOD and dehydrogenase activity in the sewage wastewater treated with various bacterial isolates

	0th day	1st day	2nd day	3rd day	4th day	5th day	%BOD Reduction
Control							
BOD	220	216	210	192	178	156	29.0
Dehydrogenase Activity	293	563	541	193	110	38	
Isolate 1							
BOD	220	200	170	140	110	90	59.0
Dehydrogenase Activity	293	380	256	85	44	13	
Isolate 2							
BOD	220	205	165	130	120	110	50.0
Dehydrogenase Activity	293	449	749	331	220	49	
Isolate 3							
BOD	220	190	120	80	50	30	86.4
Dehydrogenase Activity	293	556	833	234	39	21	
Isolate 4							
BOD	220	210	190	145	105	90	59.0
Dehydrogenase Activity	293	330	183	143	63	14	
Isolate 5							
BOD	220	180	120	60	30	20	90.9
Dehydrogenase Activity	293	392	333	150	86	28	
Isolate 6							
BOD	220	170	110	70	50	40	81.8
Dehydrogenase Activity	293	428	318	103	40	14	

Comparative study to assess the performance of bacterial isolates 3, 5 and 6 and its consortium

A comparative study was carried out to assess the performance of bacterial isolates 3, 5 and 6 and their consortium together for parameters such as pH, BOD and dehydrogenase activity. The results presented in Table 2-5 indicate similar trend i.e. decrease in pH and BOD was observed as found in the previous study. The maximum reduction of 94.5% of BOD was shown by consortium of screened bacterial isolates as compared to individual isolates 3, 5 and 6 respectively. Similarly, in case of dehydrogenase activity, sudden decrease after 2nd day of incubation was observed. The maximum dehydrogenase activity was shown by bacterial isolates as consortium together as compared to individual bacterial isolates. These results indicate that the bacterial isolates 3, 5 and 6 work more effectively as consortium together rather than individually. Further studies with respect to performance of these selected bacterial isolates and their consortium together at pilot scale are under progress.

Table 2-5. Variation in pH, BOD and dehydrogenase activity in the sewage wastewater treated with bacterial isolates 3, 5 and 6 and through consortium

	0th day	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**% BOD Reduction**
Control							
pH	7.72	7.26	7.18	7.14	7.09	7.09	29.5
BOD	220	196	180	165	160	155	
Dehydrogenase Activity	293	318	564	974	139	24	
Isolate 3							
pH	7.72	7.50	6.90	6.38	6.18	6.10	84.0
BOD	220	160	110	70	55	35	
Dehydrogenase Activity	293	682	780	960	247	50	
Isolate 5							
pH	7.72	7.48	7.28	6.89	6.08	5.90	89.0
BOD	220	120	80	40	30	24	
Dehydrogenase Activity	293	579	815	1061	271	60	
Isolate 6							
pH	7.72	7.08	6.92	6.54	6.42	6.30	80.0
BOD	220	110	75	55	45	42	
Dehydrogenase Activity	293	810	1011	980	324	67	
	0th day	**1st day**	**2nd day**	**3rd day**	**4th day**	**5th day**	**% BOD Reduction**
Consortium							
pH	7.72	7.36	7.17	6.87	5.93	5.80	94.5
BOD	220	100	60	30	15	12	
Dehydrogenase Activity	293	1018	1682	1886	497	55	

2.2 Optimized microbial consortium for wastewater treatment

Reported in deliverable 2.1

2.3 Demonstration of CWs and HRTS systems

CSIR-NEERI, Nagpur

Engineered Constructed Wetland Systems has been a proven technology for treatment of domestic wastewater. The major concern with ECWs is to understand its mechanisms, and to optimize the performance and durability (delayed clogging) of the system. CSIR-NEERI, Nagpur is conducting series of experiments to establish the rate kinetics of pollutant degradation occurring in the ECWs and to arrive at rationalized design criteria. The experiments are carried out in pilot scale and column lysimeters. The output of the same will help in implementation of ECWs at large scale field application. To assess the performance of ECWs at pilot scale in terms of substrate composition, hydraulic loading rate, vegetation pattern, biofilm formation and on delayed clogging, column lysimeter experiment was conducted.

Column lysimeter experiments are being setup to investigate the trend of pollutant removal, biofilm formation and hydraulic conductivity. These columns were used as a prototype of subsurface flow of constructed wetland system, which operates in batch mode. In all, twelve experimental columns consist of HD PVC pipe of 20 cm diameter and 80 cm height attached with cap at the bottom were designed and installed at CSIR-NEERI, Nagpur. An outlet pipe is provided at the bottom of the column as shown in Figure 2-2**.** In total, four different treatments were screened under column lysimeter experiments. The details of substrate material used in different treatments are as follows:

- T1: Gravel (20%) + Sand (80%)
- T2: Gravel (20%) + Sand (70%) + Soil (10%) + *Typha latifolia*
- T3: Gravel (20%) + Marble Chips (80%)
- T4: Gravel + Marble Chips (70%) + Soil (10%) + *Typha latifolia*

The details of substrate materials used under column lysimeters experiments are given below:

- Sand (< 2mm)
- Gravel (50-100 mm)
- Marble Chips (10-15 mm)

Initially, all the columns were treated with tap water for 90 days so that the system becomes stable and the plant can grow well within the column lysimeters. Then the columns were filled with domestic wastewater collected from CSIR-NEERI premises and appropriate volume of leachate samples was collected daily. Each cycle was conducted for specific retention time. First, second and third cycles were conducted for three, six and seven days. Each treatment has one control set up.

Figure 2-2. Column lysimeter experiments to study the removal trend of pollutants, clogging and biofilm formation installed at CSIR-NEERI, Nagpur

It was found that maximum removal was observed in three days of retention time and the experiment was continued for one year.

The relative importance of either sand or marble chips or *Typha latifolia* with treatments of domestic wastewater has been studied using column lysimeter for the major parameters such as BOD, COD, sulphate, phosphate and turbidity which are considered prior to disposal of domestic wastewater onto the surface / land or ground water. The results presented in Table 2-6 shows the decreasing trend in all the treatments with respect to various parameters. The treatment T2 (i.e. Gravel (20%) + Sand (70%) + Soil (10%) + *Typha latifolia*) shows better removal for all the parameters as compared to other treatments except for sulphate. Whereas, the treatment T4 (i.e. Gravel (20%) + Marble chips (70%) + Soil (10%) + *Typha latifolia)* greatly contributes for the sulphate removal. This might be due to the action of chemical reactions with the formation of calcium sulphate, which needs to be understood in further studies. The ratio of outlet to inlet concentration of pollutants in wastewater was plotted with the time spent in the column. Figure 2-3 shows the removal trend of BOD, COD, sulphate, phosphate, turbidity and nitrate with respect to retention time.

The nitrate removal was monitored because the removal of nitrogen is mostly attributed to nitrification-denitrification followed by plant removal. The overall results indicated that the treatment T2 (i.e. Gravel (20%) + Sand (70%) + Soil (10%) + *Typha latifolia*) plays a significant role in removal of organic pollutants.

In addition to analyze the pollutant removal efficiencies, biofilm formation and reduction in hydraulic conductivity were also studied. It was found that the microbes which habitat in between the substrate macro pores helps in formation of biofilm, also the organic particulate get stuck in the pores to make the slimy shape of biofilm. Increased formation of biofilm will eventually restrict the free passage of wastewater through the substrate thereby reducing the hydraulic conductivity of the filter media. The results presented in Figure 2-4 shows that with increase in biofilm formation the filter media loses its hydraulic conductivity.

Table 2-6. Percentage removal of pollutants present in wastewater using column lysimeter experiment				
Parameter	**% Removal of pollutants**			
	T1	**T2**	**T3**	**T4**
BOD	82	91	70	85
COD	80	98	56	65
Sulphate	52	56	86	90
Phosphate	93	97	4	7
Turbidity	93	96	82	86

T1: Gravel (20%) + Sand (80%)
T2: Gravel (20%) + Sand (70%) + Soil (10%) + *Typha latifolia*
T3: Gravel (20%) + Marble Chips (80%)
T4: Gravel + Marble Chips (70%) + Soil (10%) + *Typha latifolia*

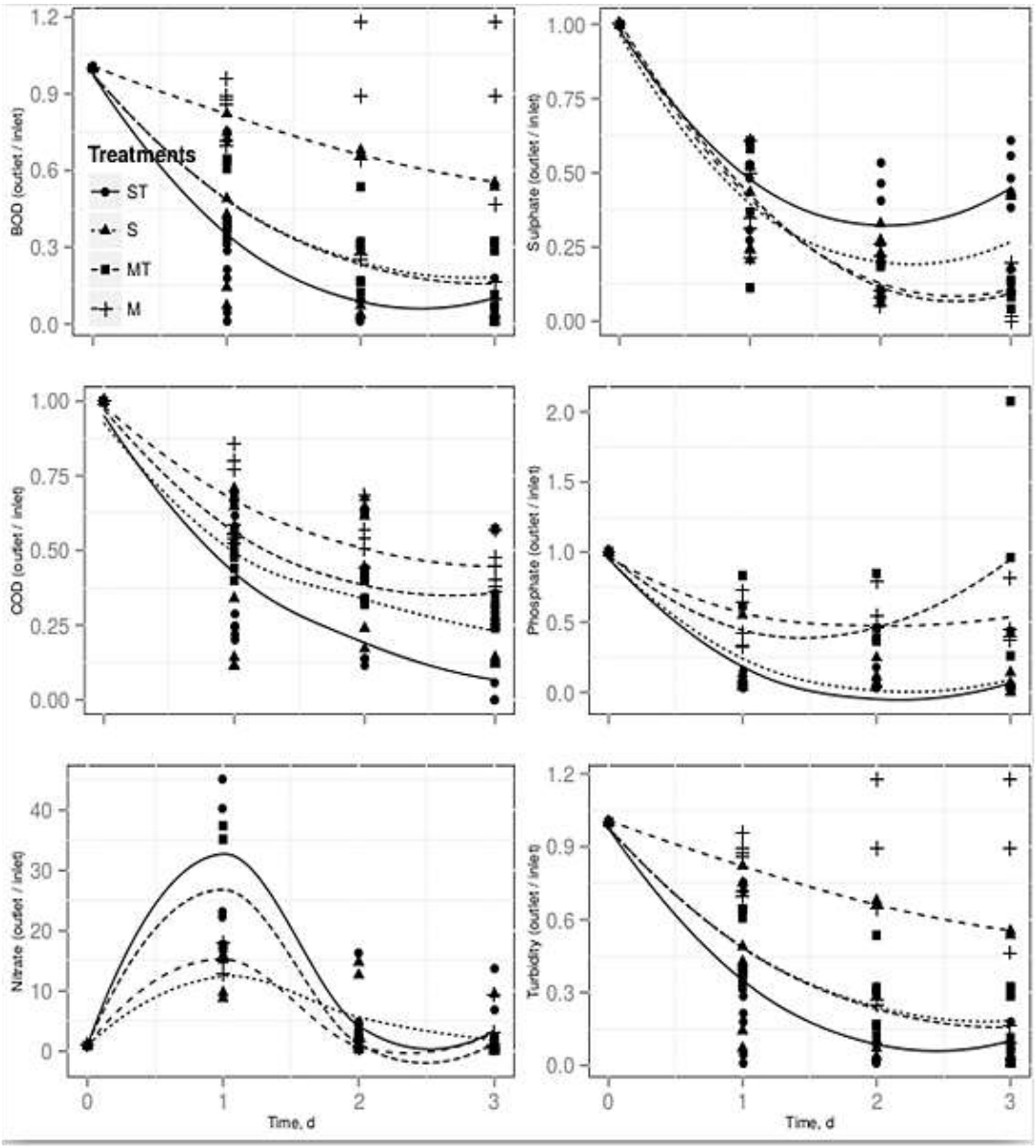


Figure 2-3. Removal trends of BOD, COD, Sulphate, Phosphate, Turbidity and Nitrate with respect to retention time

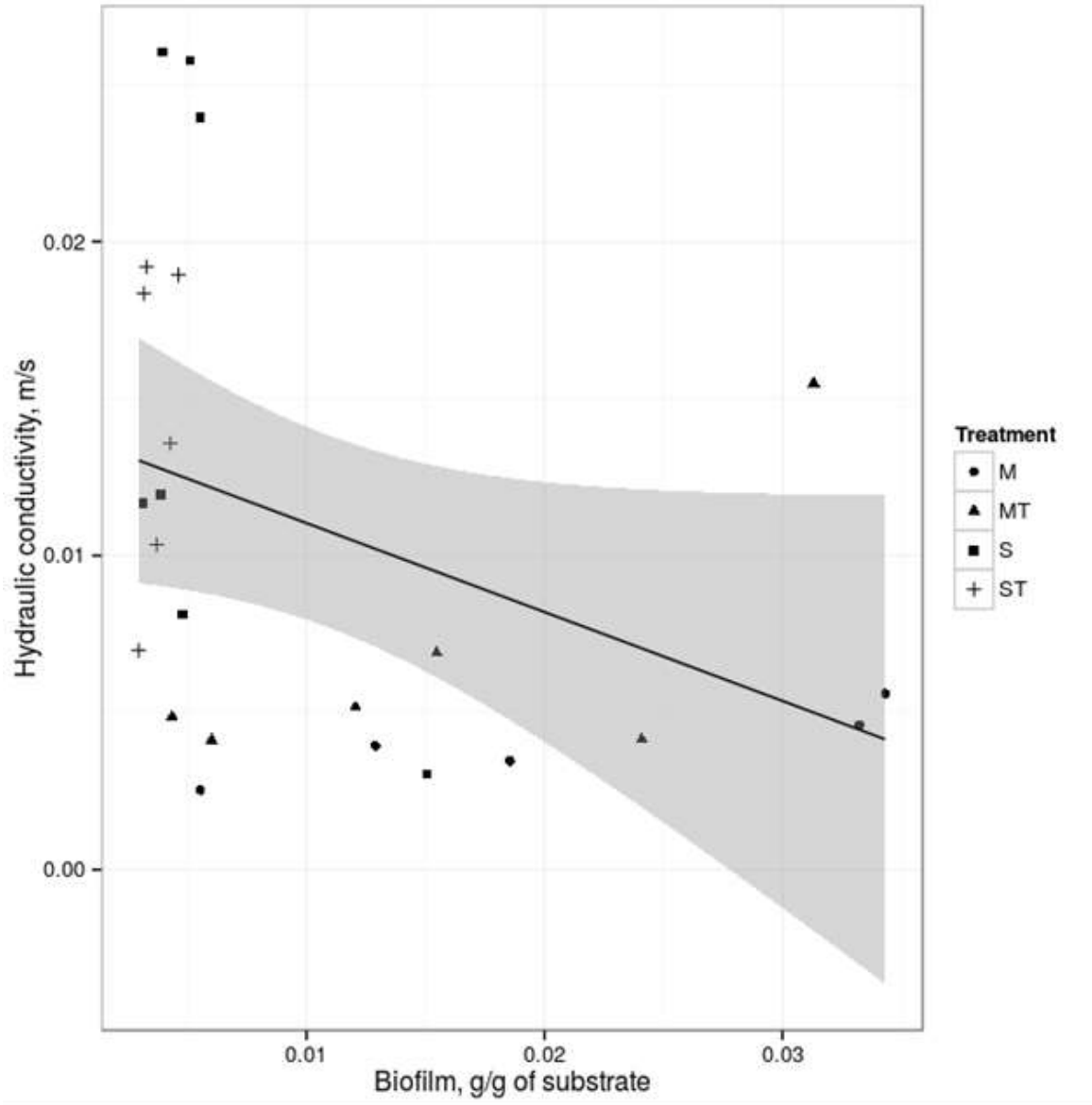


Figure 2-4. Negative correlation between Hydraulic conductivity and Biofilm formation

Column lysimeter experiment to evaluate the effect of substrate and vegetation on removal of human parasitic nematode ova, protozoan cry and pathogens from sewage wastewater

To investigate the effect of different substrates and vegetation on parasites removal capacity, column lysimeter experiment was conducted in green net house at CSIR-NEERI, Nagpur as shown in *Figure 2-5***.** The experimental column set up consists of HD PVC pipe of 20 cm diameter and 100 cm height attached with caps at the bottom of the column. An outlet pipe is provided at the bottom of the column. The details of substrate materials used under column lysimeters experiments are given below:

- Sand (< 2mm)
- Gravel (50-70 mm)
- Marble Chips (15-20 mm)
- Plants: *Typha latifolia* and *Cyperus rotundus*

The different treatments screened under column lysimeter are as follows:

Treatments	Details of Substrate Materials
ST	Gravel + Sand + *Typha latifolia*
S	Gravel + Sand
MT	Gravel + Marble chips + *Typha latifolia*
M	Gravel + Marble chips
SC	Gravel + Sand + *Cyperus rotundus*
MC	Gravel + Marble chips + *Cyperus rotundus*
SMT	Gravel + Sand + Marble chips+ *Typha latifolia*
SMC	Gravel + Sand + Marble chips+ *Cyperus rotundus*

Initially, all the columns were treated with tap water for 90 days so that the system becomes stable and the plant can grow well within the column lysimeters. The wastewater used for this experiment was collected from Nag River at Dhantoli area where many nullah / streams containing domestic wastewater were joining at one place.

Figure 2-5. Column lysimeter experiments to estimate the removal efficiency of parasites and pathogens using different substrate materials and vegetation

Hence, for this study the wastewater was regularly collected from the Nag River at Dhantoli area for the experiment. The analysis of the wastewater collected from Nag River shows *Entamoeba histolytica, Strongyloides strcoralis* and *Ascaris lumbricoides,* the three most common parasites are shown in *Figure 2-6***.** Thus, the studies were focused on the removal efficiency of various substrate materials used under column lysimeter experiment. After 90 days, the columns were filled with wastewater collected from Nag River and appropriate volume of leachate samples was collected daily. Each cycle was conducted for specific retention time. The concentration of cysts and eggs in the wastewater samples was done by the Téléman–Rivas technique. Microscopic observation was done in a neubauer chamber at 400 X magnification for protozoan cysts and in MacMaster counting cell at 100X magnification for helminth eggs.

The results on removal efficiency of various substrate materials and vegetation for *Entamoeba histolytica, Strongyloides strcoralis* and *Ascaris lumbricoides* are presented in Table 2-7**.** It was found that the maximum removal was observed at 48 hrs retention time. The treatment SC (Gravel + Sand + *Cyperus rotundus*) showed maximum removal of parasites followed by treatment ST (Gravel + Sand + *Typha latifolia)* and mix treatments. The parasites removal was mainly due to mechanism of filteration and adheration to roots. As the *Cyperus rotundus* has greater root surface area and more number of adventitious roots as compared to *Typha latifolia,* thus it shows better removal efficiency of the respective parasites respectively.

The results on pathogen removal by different substrate materials and vegetation under column lysimeter are depicted in Table 2-8**.** The results on pathogen removal indicate that the treatment ST (Gravel + Sand + *Typha latifolia*) was capable of completely removing *fecal coliform and Salmonella* (100%) and also other pathogens such as *Total coliform, E. coliform and Shigella* in the range of 65-70% respectively. This reduction might be a result of interaction of pathogens present in wastewater with that of plant roots and biofilm developed in the rhizosphere. Sand, biofilm formation and antibiosis by plant can be responsible for removal of pathogenic bacteria during filtration process.

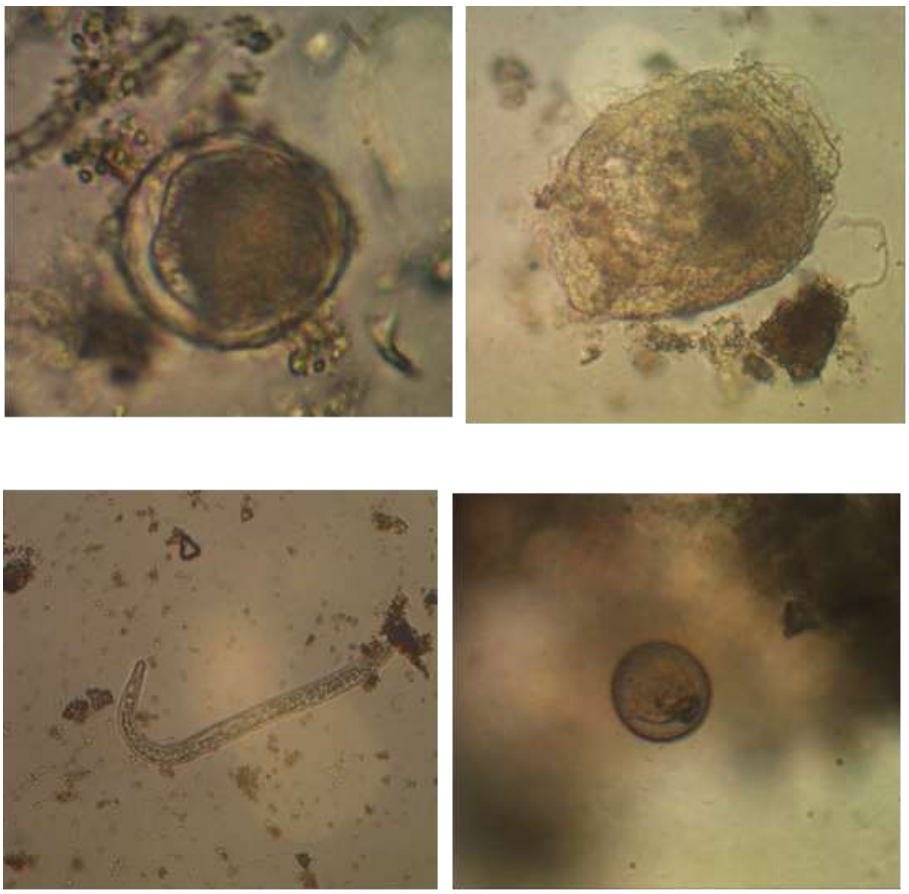


Figure 2-6. Nematode ova and protozoan cyst found in wastewater collected from Nag River. A. Lumbricoides fertilized egg (top left), A. Lumbricoides Unfertilized egg (top right), S. Stercolaris Larvae (bottom left), E. Hystolytica Cyst (bottom right)

Table 2-7. Removal percentage of parasites in different treatments screened under column lysimeter experiment

Treatments	Mean concentration of different parasites					
	Entamoeba histolytica (cyst/l)	**% Removal**	***Ascaris lumbricoides (ova/l)***	**% Removal**	***Strongiloides stercoralis (larvae/l)***	**% Removal**
Inlet	550	-	133	-	40	-
ST	7.0	98.7	1.3	99.0	2.0	95.0
S	8.7	98.4	2.3	98.2	3.3	91.7
MT	21.0	96.2	4.7	96.5	4.7	88.3
M	29.0	94.7	5.3	96.0	5.7	85.9
SC	6.7	98.8	1.0	99.4	2.0	95.0
MC	18.3	96.7	4.3	96.7	4.0	90.0
MXT	9.0	98.4	2.7	98.0	3.0	92.5
MXC	8.7	98.4	2.7	98.0	2.3	94.2

ST : Gravel + Sand + *Typha latifolia*
S : Gravel + Sand
MT : Gravel + Marble chips + *Typha latifolia*
M : Gravel + Marble chips
SC : Gravel + Sand + *Cyperus rotundus*
MC : Gravel + Marble chips + *Cyperus rotundus*
SMT : Gravel + Sand + Marble chips+ *Typha latifolia*
SMC : Gravel + Sand + Marble chips+ *Cyperus rotundus*

Table 2-8. Removal percentage of pathogens in different treatments screened under column lysimeter experiment

Treatments	***% Pathogen Removal***				
	TC	**EC**	**FC**	***Salmonella***	***Shigella***
S	39.0	46.1	85.8	100	53.1
M	42.5	48.6	55.9	81.4	41.5
ST	64.9	67.9	100	100	68.9
SC	51.3	58.1	58.3	100	37.2
MT	54.3	63.2	65.8	100	54.9
MC	39.4	48.1	50.9	100	38.3
MXT	64.6	59.5	100	100	64.3
MXC	54.9	58.0	79.8	100	48.8

TC: Total Coliform, **EC:** E. Coli, **FC:** Fecal Coliform

*The analysis was performed in triplicates and above mentioned results are averages of triplicates.

Also, high copepods concentration in wastewater may be a reason for decrease in pathogen number owing to copepods predation mechanism. The results indicate that as compared to other treatments, the treatment ST (Gravel + Sand + *Typha latifolia*) was more efficient in removal of pathogenic bacteria. Hence, the leachate after treatment with ST (Gravel + Sand + *Typha latifolia*) can be used for agricultural practices as per FAO guidelines.

Demonstration of ECWs at pilot scale

The pilot scale setup of Engineered Constructed Wetland system (ECWs) for the treatment of domestic wastewater has been installed at CSIR-NEERI campus as shown in Figure 2-7. The domestic wastewater collected within the CSIR-NEERI's campus has been fed to the ECWs for its subsequent treatment. The treated wastewater is then used for crop irrigation. The experiments on ECWs at pilot scale consisted of two treatments, one with substrate configuration of sand, marble chips and gravel and another with marble chips and gravel for treatment of domestic wastewater.

Figure 2-7. Pilot scale demonstration of ECWs for the treatment of domestic wastewater installed at CSIR-NEERI campus, Nagpur

To assess the performance of ECWs at pilot scale, the field experiment was laid out in randomized block design with three main treatments;

- T1 - Field irrigated with raw domestic wastewater
- T2 - Field irrigated with treated wastewater (ECWs)
- T3 - Field irrigated with tap water

Each treatment has three replications. The size of the experimental plot was 3 x 3 meter and standard agronomic practices as per the local area were considered. The first field trial was carried out during February 2014 to April 2014 with Tomato (*Solanum lycopersicum*) crop and the second field trial during July 2014 to January 2015 with Red gram *(Cajanas Cajan)* crop variety, Ankur – Prabha. Recommended dose of farm yard manure was given to each crop at the time of land preparation to boost the growth of the plant initially. The domestic wastewater collected from CSIR-NEERI campus was used for the experiment. The characteristics of domestic wastewater and treated wastewater through ECWs are presented in Table 2-9**.** The four samples of domestic wastewater, treated wastewater through ECWs and tap water were collected at monthly interval through the crop irrigation period and characterized for parameters such as BOD, COD, TSS, ammonical nitrogen and sulphate respectively. The results indicated that the BOD and COD removal through ECWs varied in the range of 80-85% and 90-95% respectively. The TSS removal varied in the range of 90-95% while the removal of ammonical nitrogen and sulphate varied in the range of 55-60% and 50-70% respectively. Normally, in ECWs, the pollutant removal flow of vertical

ECWs is higher than the horizontal flow constructed wetland system. Figure 2-8 shows the growth performance of Tomato (*Solanum lycopersicum*) and Red gram *(Cajanas Cajan)* crops at the experimental site.

Effect on soil properties:

The soil from the experimental plot were collected and analyzed at the time of transplanting and after harvesting of the crop. The results are presented in Table 2-10 and Table 2-11.

Table 2-9. Physic-chemical characteristics of domestic wastewater collected at CSIR-NEERI campus and treated wastewater through ECWs

Sl. No.	Parameters	Domestic Wastewater	Treated wastewater through ECWs	% Reduction
1.	pH	7.28 – 7.32	7.20 – 7.35	
2.	EC, mS/cm	0.60 – 0.63	0.22 – 0.25	
3.	TDS, mg L^{-1}	298 - 328	180 - 198	
4.	BOD, mg L^{-1}	127-156	10.2 – 11.8	90-95
5.	COD, mg L^{-1}	200-250	25-50	80-85
6.	TSS, mg L^{-1}	109 - 120	10-15	90-95
7.	Sulphate, mg L^{-1}	2.0 – 2.4	0.8-1.3	50-70
8.	Ammonical Nitrogen, mg L^{-1}	1.25 – 1.78	0.68-1.06	55-60

Table 2-10. Physico-chemical characteristics of soil before and after irrigated with domestic wastewater, treated wastewater through ECWs and tap water at the experimental field at CSIR-NEERI, Nagpur

Season	Treatment	Depth (cm)	pH	EC (mS/cm)	Organic Carbon (%)	Total N (%)	Total P (%)	Total K (%)	Na ($Cmol^+$ /kg soil	K ($Cmol^+$ /kg soil	Ca ($Cmol^+$ /kg soil	Mg ($Cmol^+$ /kg soil	CEC (meq/ 100gm)	ESP (%)	BD (gm/cc)	WHC (%)	POR (%)
At the beginning	T1	0-15	8.23	0.17	0.58	0.06	0.07	0.28	0.42	0.68	28.89	6.70	42.69	1.13	1.25	58.16	49.14
		15-30	8.25	0.18	0.43	0.05	0.07	0.30	0.37	0.63	28.62	7.05	42.66	1.00	1.26	55.37	47.63
		30-45	8.21	0.15	0.36	0.04	0.07	0.29	0.25	0.76	29.09	5.95	42.05	0.69	1.28	51.92	46.11
	T2	0-15	8.24	0.12	0.91	0.09	0.07	0.25	0.36	0.47	29.26	7.94	44.02	0.93	1.24	58.55	48.10
		15-30	8.15	0.12	0.61	0.07	0.07	0.28	0.29	0.75	28.26	5.98	42.28	0.81	1.26	54.07	47.53
		30-45	8.14	0.13	0.58	0.05	0.07	0.30	0.26	0.87	27.27	4.94	40.35	0.79	1.27	53.88	47.29
	T3	0-15	8.38	0.14	0.49	0.07	0.07	0.27	0.38	0.51	30.03	8.32	45.23	0.96	1.25	57.97	48.91
		15-30	8.32	0.13	0.45	0.06	0.07	0.25	0.34	0.68	27.61	7.10	42.73	0.95	1.27	54.05	46.20
		30-45	8.20	0.14	0.44	0.05	0.07	0.26	0.29	0.60	26.84	5.73	41.46	0.85	1.29	52.33	44.75
Season 1 (Tomato)	T1	0-15	8.46	0.20	0.88	0.09	0.09	0.37	0.51	0.86	28.79	7.13	42.28	1.43	1.23	57.99	46.94
		15-30	8.58	0.20	0.87	0.09	0.08	0.32	0.46	0.92	28.53	7.21	43.13	1.25	1.27	55.09	43.14
		30-45	8.66	0.20	0.86	0.07	0.08	0.28	0.47	0.88	28.91	7.54	43.80	1.23	1.29	52.21	41.36
	T2	0-15	8.51	0.16	0.87	0.09	0.08	0.34	0.36	0.87	25.85	5.34	39.41	1.12	1.25	58.62	46.81
		15-30	8.36	0.17	0.86	0.08	0.08	0.32	0.34	0.89	27.31	6.00	41.54	1.00	1.28	54.36	42.70
		30-45	8.25	0.17	0.72	0.07	0.09	0.32	0.32	0.90	27.07	6.25	41.54	0.93	1.29	54.19	41.41
	T3	0-15	8.34	0.15	0.78	0.05	0.07	0.30	0.30	0.77	25.06	6.69	41.83	0.93	1.25	56.10	45.52
		15-30	8.31	0.15	0.71	0.06	0.07	0.25	0.31	0.74	26.51	6.46	41.02	0.91	1.28	53.23	41.84
		30-45	8.31	0.15	0.59	0.05	0.08	0.26	0.30	0.75	26.40	6.24	41.70	0.90	1.30	51.58	40.61
Season2 (Red gram)	T1	0-15	8.21	0.23	0.85	0.09	0.10	0.44	0.59	1.49	31.22	10.43	47.74	1.36	1.27	53.73	46.22
		15-30	8.20	0.21	0.76	0.08	0.08	0.47	0.51	1.39	28.31	9.10	45.32	1.31	1.34	48.04	40.10
		30-45	8.21	0.19	0.65	0.07	0.09	0.43	0.49	1.25	30.30	8.73	46.77	1.21	1.42	42.61	40.31
	T2	0-15	8.19	0.19	0.68	0.07	0.08	0.43	0.47	1.39	30.57	10.23	47.67	1.11	1.34	56.12	52.96
		15-30	8.08	0.18	0.65	0.07	0.08	0.41	0.47	1.19	29.34	9.54	45.53	1.15	1.41	51.61	48.15
		30-45	8.20	0.18	0.60	0.06	0.08	0.41	0.46	1.26	28.18	7.47	42.37	1.30	1.34	51.19	46.70
	T3	0-15	8.09	0.16	0.73	0.07	0.07	0.32	0.33	0.85	27.57	6.91	42.66	0.92	1.35	57.28	52.23
		15-30	8.12	0.16	0.61	0.06	0.07	0.26	0.33	0.86	26.99	6.69	41.87	0.95	1.38	54.95	49.23
		30-45	8.07	0.17	0.60	0.05	0.07	0.22	0.33	0.84	26.08	5.84	40.09	1.00	1.39	51.95	45.57

Table 2-11. Total heavy metals concentration of soil before and after irrigated with domestic wastewater, treated wastewater through ECWs and tap water at the experimental field at CSIR-NEERI, Nagpur

Season	Treatment	Depth (cm)	Co	Cr	Cu	Fe	Mg	Mn	Ni	Pb	Zn
At the beginning	T1	0-15	30.0	48.3	83.0	32443.7	5205.0	1023.1	42.3	9.8	114.6
		15-30	27.5	50.0	84.0	32557.0	4831.5	1033.6	40.3	9.8	141.6
		30-45	25.6	58.00	87.9	31330.3	5161.5	1047.3	42.8	12.2	132.6
	T2	0-15	31.9	36.4	91.5	32103.7	4690.3	1122.2	34.3	8.7	135.3
		15-30	27.5	57.6	90.5	32530.3	4918.3	1083.7	41.8	11.7	148.7
		30-45	24.1	54.1	71.0	29777.0	4269.7	1123.4	34.9	15.0	149.9
	T3	0-15	30.2	41.8	62.7	31950.3	5023.7	1048.1	31.4	11.4	115.0
		15-30	31.8	38.6	86.7	33243.7	4655.0	1106.1	30.6	12.2	132.4
		30-45	29.0	40.3	87.5	30737.0	4977.0	1086.8	37.9	12.6	112.3
Season 1 (Tomato)	T1	0-15	29.4	63.8	89.4	40014.4	7339.1	1183.5	53.9	14.2	130.0
		15-30	28.0	65.3	84.7	38414.6	6321.8	1199.3	51.5	11.0	124.9
		30-45	28.3	61.2	92.5	37824.1	6827.1	1231.2	54.1	12.6	119.4
	T2	0-15	27.4	58.1	85.4	35282.2	6403.5	1073.9	48.8	10.8	125.6
		15-30	26.0	55.6	82.9	33275.5	5785.0	1195.1	45.5	12.1	122.1
		30-45	26.2	53.0	79.6	34071.8	5565.6	1186.7	44.5	13.9	118.3
	T3	0-15	24.1	56.7	64.8	33024.5	5489.8	968.0	45.6	10.2	111.6
		15-30	22.5	47.3	63.7	31367.0	5445.2	925.3	37.9	11.2	118.1
		30-45	24.7	49.1	73.8	33070.2	5349.2	950.5	39.4	8.8	106.6
Season2 (Red gram)	T1	0-15	30.6	90.0	93.3	38327.4	7227.0	1129.2	82.3	9.9	118.8
		15-30	28.4	103.7	89.0	37125.0	6864.1	1104.4	108.2	11.1	116.9
		30-45	27.0	103.2	81.3	34906.8	6001.0	1036.2	111.7	17.2	112.6
	T2	0-15	25.7	92.4	85.2	33536.7	6684.4	953.0	72.3	9.1	106.7
		15-30	26.0	93.2	85.0	33002.5	6330.3	1053.5	70.0	9.8	110.8
		30-45	25.3	98.2	83.0	32826.5	6294.7	1090.9	86.3	12.2	116.6
	T3	0-15	24.8	71.6	79.4	32063.0	5983.3	945.6	63.2	4.9	98.8
		15-30	24.4	58.8	81.9	32284.3	5870.3	1019.0	64.3	5.7	105.8
		30-45	25.0	72.4	82.6	32113.9	6045.5	955.9	63.7	6.3	109.5

The results indicated that not much variation was observed in physical properties with respect to bulk density, maximum water holding capacity and porosity of the soil treated with raw domestic wastewater, treated wastewater through ECWs and tap water respectively. The pH of soil samples collected and analyzed for the plots showed alkaline pH. There was slight increase in EC was observed in first layer of the soil in all the treatments during the study period. The domestic wastewater irrigated soil showed slight increase in EC than the soil irrigated with treated wastewater of ECWs and tap water irrigated soil respectively. Similarly, the organic carbon and nutrient with respect to nitrogen, phosphorous and potassium were comparatively higher in T1 and T2 treatments and significantly low in T3 treatment. No significant trend was observed in case of exchangeable cations and cation exchange capacity in the soil in all the treatments. Also, no significant difference in heavy metals concentration was observed in the soil irrigated with domestic wastewate, treated wastewater of ECWs and tap water respectively. Since, the domestic wastewater itself contains very low concentration of heavy metals that could be the reason for triviality in the results. The overall results of the soil indicated that no significant difference was observed in physico-chemical properties even treated with domestic wastewater. But the long time assessment of soil irrigated with domestic wastewater and ECWs are required to develop a suitable ECWs technology with suitable substrate material and vegetation.

Effect on crop growth:

The plant growth parameters such as germination (%), height, branches/plant and yield were monitored during the growing period. The results are presented in Table 2-12**.** The crops i.e. Tomato (*Solanum lycopersicum*) crop and Red gram *(Cajanas Cajan)* irrigated with domestic wastewater and treated wastewater through ECWs resulted no significant

difference with respect to height and branches/plant. The yield of the Tomato (*Solanum lycopersicum*) crop in terms of Q/ha as influenced by various treatments varied in the range of 79.6 Q/ha to 137.4 Q/ha respectively. Similarly, the yield of the Red gram *(Cajanas Cajan)* crop in terms of Q/ha as influenced by various treatments varied in the range of 9.1 Q/ha to 21.3 Q/ha respectively.

Table 2-12. Crop growth parameters of Tomato (*Solanum lycopersicum*) crop and Red gram *(Cajanas Cajan)* at the experimental site

Season	Treatment	Germination (%)	Height at the time of harvesting (cm)	Branches /plant	Yield (Quintal/ hectare)
Season 1 (Tomato)	T1	80	24.45	11.02	137.48
	T2	80	23.13	10.31	112.77
	T3	85	20.15	9.56	79.56
Season 2 (Red gram)	T1	75	165.21	8.12	21.28
	T2	78	153.89	7.88	18.5
	T3	80	134.75	6.11	9.12

The uptake of nutrients by Tomato (*Solanum lycopersicum*) and Red gram *(Cajanas Cajan)* through the enzymatic effect in metabolic processes may account for higher yield. Not much variation was observed in the yield shown by Tomato (*Solanum lycopersicum*) crop and Red gram *(Cajanas Cajan)* crop when irrigated with domestic wastewater and treated wastewater through ECWs respectively. The treatments differences were also found to be significant as observed by the significant increase in grain yield. The crops irrigated with treated wastewater through ECWs not only increasing the yield of the crop but also maintain the soil fertility.

ICRISAT Patancheru

The construction of the constructed wetland and plantation of wetland vegetation were done during Feb 2014 to May 2014. The wastewater was pumped to a overhead tank (2 m head) from a stabilization pond receiving wastewater from a nearby urban housing colony. The Capacity of the overhead tank is about 100 m^3. The design outline of the wetland during this period is given in Figure 2-8. The wetland was allowed to stabilize by passing through wastewater which served as sole source of nutrients in the wetland. The root zone microbiology got established for the various plant species grown in the wetland during this period. The activities during June 2014 to June 2015 may be broadly categorized as i) interventions; ii) performance monitoring and iii) maintenance. The wetland performances were studied from June 2014 onwards, with weekly wastewater analysis and monthly soil and plant analysis.

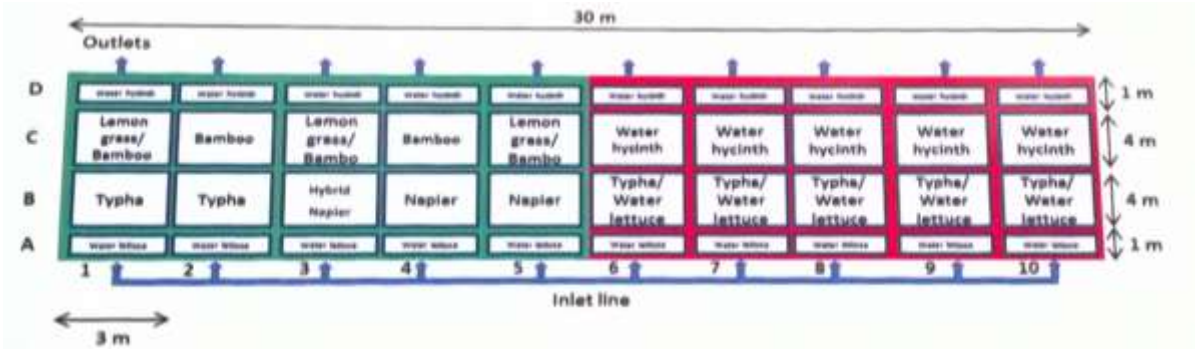


Figure 2-8. The overall layout of the constructed wetlands as on June 2014.

The first major intervention was weeding out the opportunistic plants which mainly grew in cells with relatively slow growing plant species such as Bamboo. It was observed that a weed of a particular species is growing luxuriantly in cell 3 C and 4 C. Further wastewater and plant analysis identified the species *Ageratum conyzoides (*Billygoat weed*),* highly efficient in nutrient removal. Hence, two cells were dedicated for this particular weed species two further explore its performance.

As the wastewater analysis data indicated a need for higher hydraulic resident time (HRT), flow was adjusted to 2 liter per min (which corresponds to 5 day HRT). Flow meters as well as regulators were installed to ensure consistent flow among the different constructed wetlands. Moreover, a U-shape bend (*Figure 2-9*) with silt and grit drain was provided at the inlet side to prevent clogging of the inlet pipes at this reduced flow rate. A picture of the constructed wetlands at this stage is given as Figure *2-10*.

Figure 2-9. The U-bend and sludge drain at the wetland inlet

Figure 2-10. The constructed wetlands at ICRISAT, Patancheru

The wastewater treatment efficiency of different constructed wetlands were studied and compared. The wastewater characteristic did show some seasonal variation, particularly for parameters such as chemical oxygen demand (varied between 64 mg/L to 228 mg/L), ammoniacal nitrogen concentration (varied between 50 to 100 mg/L) and sulphate (varied

between 20-35 mg/L). The pH, total hardness total alkalinity, concentration of sodium, chloride, fluoride, potassium, and calcium and were among the parameters stayed more or less consistent throughout the study period. The year on average of concentrations observed different wastewater parameters for the inlet wastewater is presented in Table 2-13. The heavy metal concentrations were found to be very low and for cadmium, lead nickel and zinc were below detectable limit (BDL) consistently in inductively coupled plasma mass spectrometry (ICP-MS) analysis. The average SAR of the inlet wastewater was 2.67.

The CWs showed significant difference in 11 out of the 30 parameters analyzed. The performances of the ten different CWs for these key parameters are presented in Table 2-14. The COD (chemical oxygen demand) removal efficiency was about 45 – 63 % between different cells. The removal efficiency was impacted by inlet concentration as well as growth phase of the plant species. Typically, the after harvesting maximum removal efficiency was observed. In terms of removal efficiency for total inorganic nitrogen, the subsurface cells performed better than the free surface cells consistently. The higher per square meter dry biomass generation compared to the macrophytes may be over the same period of time may be attributed to this higher removal efficiency. Also the subsurface species were found relatively robust to activities such as harvesting compared to the macrophytes. The phosphate removal was better in the free surface cells in general. Both water hyacinth as well as water lettuce showed high phosphate removal capacity. *Conyzoides ageratum*, Marigold as well as para grass too showed significant phosphate removal efficiency among the sub-surface plant species. Plant sample analysis revealed that among the plant species typha and water hyacinth were having the maximum sulphate uptake capacity, the same was reflected by the performances CWs. CWs without this two species showed lower removal efficiency for sulphate.

Marigold was introduced in the cell 5 C in the month of December 2015. As seeds sowed directly in the wetland bed did not germinate, small saplings were grown separately in a seed bed and were then transplanted (about 7 cm in height) to the cell 5 C. In total 112 plants were planted. During plant analysis the marigold plants were found to have significant heavy metal uptake capacity. In particular heavy metals such as chromium, lead and arsenic, which were present in the wastewater at a concentration of 0.01 to 0.02 mg/L, got accumulated to a tissue concentration of 6.58, 0.79 and 1.84 mg/kg respectively. About 450 flowers were harvested every week from these plants during December and January. The fresh weight of the flowers harvested over a 45 day period was 12.768 kg (dry weight of 1.021 kg). Though growing marigold was found to be economically beneficial apart from its heavy metal uptake capacity, the plants were very sensitive to ponding. In real environmental condition with minimal maintenance growing marigold in CWs may be a challenge. The plants were severely damaged during a spell of heavy non seasonal rain during mid-October. Moreover, as the total plant biomass per square area is far less compared to species like Typha, Napier etc. for marigold, limiting nutrient uptake, formation of algal mat on the surface of the wetland bed was observed.

Table 2-13. Inlet wastewater characteristics of the constructed wetlands at ICRISAT, Patancheru

Sl. No.	Parameters	Inlet
1	Arsenic (mg/L)	0.04
2	Boron (mg/L)	0.04
3	Cadmium (mg/L)	BDL
4	Calcium (mg/L)	75.48
5	Chlorides (mg/L)	59.75
6	Chromium (mg/L)	0.01
7	Cobalt (mg/L)	0.05
8	Chemical oxygen demand (mg/L)	176
9	Copper (mg/L)	0.04
10	Detergents (mg/L)	1.59
11	Electrical Conductivity (ms)	2.43
12	Fluorides (mg/L)	1.70
13	Lead (mg/L)	BDL
14	Magnesium (mg/L)	32.75
15	Manganese (mg/L)	0.48
16	Ammoniacal nitrogen (mg/L)	61.81
17	Nickel (mg/L)	BDL
18	Nitrate nitrogen (mg/L)	2.65
19	pH	7.68
20	Phosphates (mg/L)	14.72
21	Potassium (mg/L)	18.49
22	Sodium (mg/L)	78.51
23	Sulfates (mg/L)	24.83
24	Sulfur (mg/L)	8.54
25	Total dissolved solids (mg/L)	1214
26	Total Alkalinity (mg/L)	294
27	Total Hardness (mg/L as CaCO3)	370
28	Total iron (mg/L)	0.15
29	Total suspended solids (mg/L)	44
30	Zinc (mg/L)	BDL

Table 2-14. Performance of different CWs for key wastewater parameters

Parameter	Unit	Inlet	CW-1	CW-2	CW-3	CW-4	CW-5	CW-6	CW-7	CW-8	CW-9	CW-10
Chemical Oxygen Demand	mg/L	176	96.0	96.0	64.0	128.0	64.0	64.0	96.0	64.0	96.0	96.0
Detergent	mg/L	1.59	0.7	0.3	0.6	0.3	0.3	1.0	0.7	0.5	0.6	0.7
Nitrogen-ammoniacal	mg/L	61.81	20.5	28.0	22.6	30.0	22.0	41.7	48.6	40.8	46.9	39.3
Nitrogen-nitrate	mg/L	2.65	5.0	1.9	1.7	2.0	2.1	1.4	1.5	1.4	1.7	1.9
Inorganic Nitrogen	mg/L	64.46	25.5	29.9	24.3	31.9	24.1	43.1	50.1	42.2	48.6	41.2
Phosphate	mg/L	14.72	9.0	5.7	4.6	8.2	4.5	3.5	5.2	4.9	3.9	3.9
Sulfate	mg/L	24.83	8.7	8.8	10.2	11.3	12.7	10.7	4.6	13.4	13.5	5.0
Total Dissolved Solids	mg/L	1213.5	1030.0	839.3	951.1	909.2	1002.0	1048.0	1186.0	1109.0	1187.0	1094.0
Total Alkalinity	(mg/L as CaCO3)	294	216.0	210.0	210.0	222.0	228.0	240.0	276.0	264.0	276.0	282.0
Total Hardness	(mg/L as CaCO3)	370	350.0	310.0	300.0	280.0	330.0	300.0	360.0	330.0	340.0	310.0
Total Suspended Solids	mg/L	44	12.0	20.0	20.0	12.0	20.0	28.0	40.0	20.0	24.0	12.0

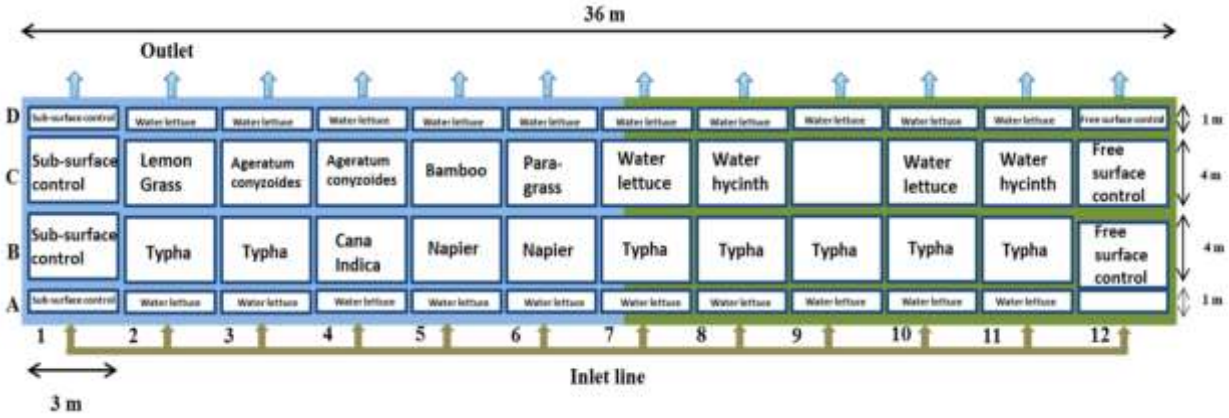


Figure 2-11. Marigold plants in cell 5 C of the constructed wetlands at ICRISAT, Patancheru

In the end of February para-grass was introduced in cell 5 C. About 30 cm cutting were collected from the banks of nearby water bodies. Like marigold about 112 paragrass plants were planted. In the initial days after plantation the growth was minimal; however the growth peaked up after about a period of 45 days, and even in peak summer significant amount of biomass was generated.

Figure 2-12. Paragrass in cell 5 C of the constructed wetlands at ICRISAT, Patancheru

Figure 2-13. Phases of new cell construction at the constructed wetlands at ICRISAT, Patancheru

As we monitored the wastewater treatment efficiency, it was felt that an abiotic control cell for both free surface as well as sub-surface type is important to assess the performance of different plant combinations. Likewise on the both ends of the existing structure a control was constructed, i.e. a similar physical structure without any vegetation. The hydraulic loading for the newly constructed cells were kept identical to the rest of the CWs. The construction work was finished between 2md March and 23rd March 2015. The harvesting activities carried out periodically is depicted in Figure 2-14.

Figure 2-14. Harvesting activity at the constructed wetlands at ICRISAT, Patancheru

Kothapally, India (ICRISAT)

A field scale constructed wetland (CW) was commissioned in the Kothapally village of Telengana. The construction was carried out by renovating an existing village drain. The wastewater of approximately 200 households was treated by this CW. The wetland was consisting of four equal sized and inter-connected chambers each with a dimension of 15 m X 4 m X 0.8 m. The construction was complete by December 2014 and additional two months were allowed for the CW to stabilize. Napier (*Penisetum purpurem*), *Cana indica* and bamboo (*Bambuseae spp*) were the plant species As the wastewater flow as well as quality was varying widely seasonally as well as diurnally, the performance of the wetland was estimated by comparing the average inlet wastewater characteristics and the average outlet wastewater characteristic of the last six months. The removal efficiencies for different wastewater parameters are presented in Table 2-15.

Figure 2-15. Constructed wetland at the Kothapally village, Telengana, India.

The overall TSS removal efficiency was 62 %. Resuspension of bio-particles from different plants near the outlet decreases the actual efficiency though. The removal of inorganic nitrogen and phosphate were found to be 34.93 % and 21.56 % respectively. In absence of periodic harvesting COD removal efficiency of the CW dropped steadily from initial values of around 65 % to about 30% at present. The absence of weeding severely affected the growth of slow growing plants like bamboo very much restricted. Average sulphate removal observed was about 24.75 %. Significantly however, about 38 % sodium removal was observed along with 11.3 % chloride removal. If the trend persists it may be attributed to some particular weed species yet to be recognized.

Table 2-15. Performance of the CW at Kothapally location-1

Parameter	Unit	Average	Average	Ruduction in %ge
Chloride	mg/L	184.80	163.92	11.30
Chemical Oxygen Demand	mg/L	294.00	206.50	29.76
Detergents	mg/L	12.34	7.44	39.76
Electrical conductivity	ms/cm	2.94	2.46	16.24
Fluoride	mg/L	1.67	1.65	1.19
Nitrogen-ammoniacal	mg/L	33.73	21.66	35.79
Nitrogen-nitrate	mg/L	6.14	4.28	30.24
pH	-	7.52	7.54	
Phosphate	mg/L	1.65	1.30	21.56
Potassium	mg/L	21.88	18.40	15.91
Sodium	mg/L	142.04	87.68	38.27
Sulphate	mg/L	20.19	15.19	24.75
Total Dissolved Solids	mg/L	1799.40	1511.63	15.99
Total Alkalinity	mg/L	382.40	340.50	10.96
Total Hardness	mg/L	684.00	598.75	12.46
Total Suspended Solids	mg/L	80.20	30.50	61.97

UAS, Dharwad

During 2014 engineered constructed wetland of size 10 m x 8 m has been constructed. Typha and paragrass has been established. The general view of the constructed wetland is shown in Table 2-16.

Figure 2-16. Constructed wetland at UAS, Dharwad for treating domestic wastewater

Construction of another wetland is under progress at “H” Block at UAS, Dharwad campus with the technical specification mention in Table 2-16.

Table 2-16. Dimension of planed wetland to be constructed in UAS Dharwad			
Inlet tank		**Length**	**Width**
	Top	8 m	6 m
	Bottom	5.6 m	3.6 m
	Side slope	1:1	1:1
	Depth	1.2 m	1.2 m
Outlet		**Length**	**Width**
	Top	6 m	6 m
	Bottom	4 m	4 m
	Side slope	1:1	1:1
	Depth	1 m	1 m
Wet land		**Length**	**Width**
	Top	15 m	14 m
	Bottom	12 m	11 m
	Side slope	1:1	1:1
	Depth	1.5 m	1.5 m

2.4 Bio-remedial measures tested to improve degraded lands due to use of wastewater

Remediation of land previously loaded with biorefinery wastewater through biological means

Out of 57 isolates, 12 isolates have shown salt tolerance upto 10% and 4 isolates have shown salt tolerance upto 15%. Amongst 12 salt tolerant bacteria, all the isolates showed siderophore and IAA production, 8 isolates have also showed phosphate solubilizing activity and 10 isolates also showed acid production. Antagonistic and synergistic activities of these salt tolerant bacteria were also checked. 16S rDNA sequencing was carried out for molecular confirmation of isolates. 12 salt tolerant bacterial isolates are selected for green house experiment on sweet sorghum as model plant in soil with effluent from 15 years. Evaluation of Plant biomass, nutrient uptake and soil properties in different treatment under greenhouse experiments was established. Based on observation from greenhouse experiments, sugarcane field trials will be initiated with best consortia in August 2015.

Growth Curves of bacterial isolates

Growth curve studies were conducted on 57 isolated bacteria form sugarcane rhizosphere using spectrometric (OD) measurement and CFU count (*Figure 2-17*). These growth curves along with CFU counts generated at different phases of an isolate's growth was used to accurately conduct functional and biochemical characterization tests. The idea was to inoculate all the isolates in a particular test at the same phase of their life cycle as well as the same cell count. This provided an accurate and reliable comparison of PGPR activity among the isolated bacterial strains.

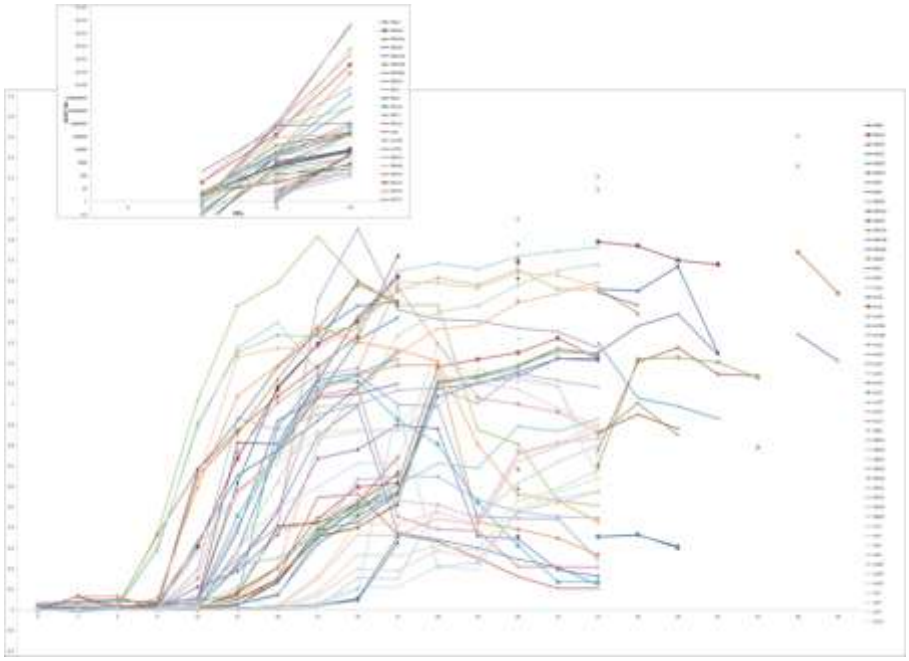


Figure 2-17. Curve depicting growth and CFU for the bacterial isolates obtained from sugarcane rhizosphere

Phosphate solubilization: Qualitative and quantitative analysis

For the qualitative assay cultures were spot inoculated onto the plates containing Pikovaskaya's Agar and NBRIP medium and incubated at 30°C. The results were interpreted based on the colour change due to solubilisation of phosphate. The presence of clear zone around bacterial colonies after incubation was used as an indicator for phosphate solubilisation.

Table 2-17. Screening and estimation of phosphate solubilizing activities of isolated bacteria

Isolates	Activity	Isolates	Activity
SRA1	-VE	SRA18	-VE
SRA2	-VE	SRA19	-VE
SRA3	-VE	SRA20	-VE
SRA4	-VE	SRA21	-VE
SRA5	-VE	SRA22	-VE
SRA6	+VE	SRA23	-VE
SRA7	-VE	SRA24	+VE
SRA8	-VE	SRA25	+VE
SRA9	+VE	SRA26	-VE
SRA10	+VE	SRA27	-VE
SRA11	-VE	SRA28	-VE
SRA12	+VE	SRA29	-VE
SRA13	+VE	SRA30	+VE
SRA14	-VE	SRA31	+VE

Isolates	Activity	Isolates	Activity
SRA15	-VE	SRA32	+VE
SRA16	-VE	SRA33	+VE
SRA17	+VE	SRA34	+VE
SRC1	+VE	SRC13	-VE
SRC2	+VE	SRC14	+VE
SRC3	-VE	SRC15	+VE
SRC4	-VE	SRC16	-VE
SRC5	+VE	SRC17	-VE
SRC6	-VE	SRC18	-VE
SRC7	-VE	SRC19	-VE
SRC8	+VE	SRC20	-VE
SRC9	+VE	SRC21	-VE
SRC10	-VE	SRC22	-VE
SRC11	+VE	SRC23	+VE
SRC12	-VE		

Estimation of solubilised phosphate using Barton's reagent

Bacteria were inoculated in 5 ml NBRIP medium in test tubes in triplicates. Uninoculated test tubes were treated as control. The test tubes were incubated at 30 deg C for 48 hours at 140 rpm. Culture was harvested by centrifugation at 2000X g for 2 mins to separate out the TCP and then at 6000Xg for 10 mins to pellet down cells. Supernatant were diluted to 1:30 with RO water and phosphate concentration estimated using Barton's reagent. 1 ml of diluted culture incubated with 3 ml of Bartons reagent for 30 minutes. The concentration of phophates released was determined by standard curve of KH_2PO_4 (0-15µg/ml). OD was taken at 410 nm. Estimation of phosphate solubilizing activities of bacteria graphically presented in *Figure 2-18*.

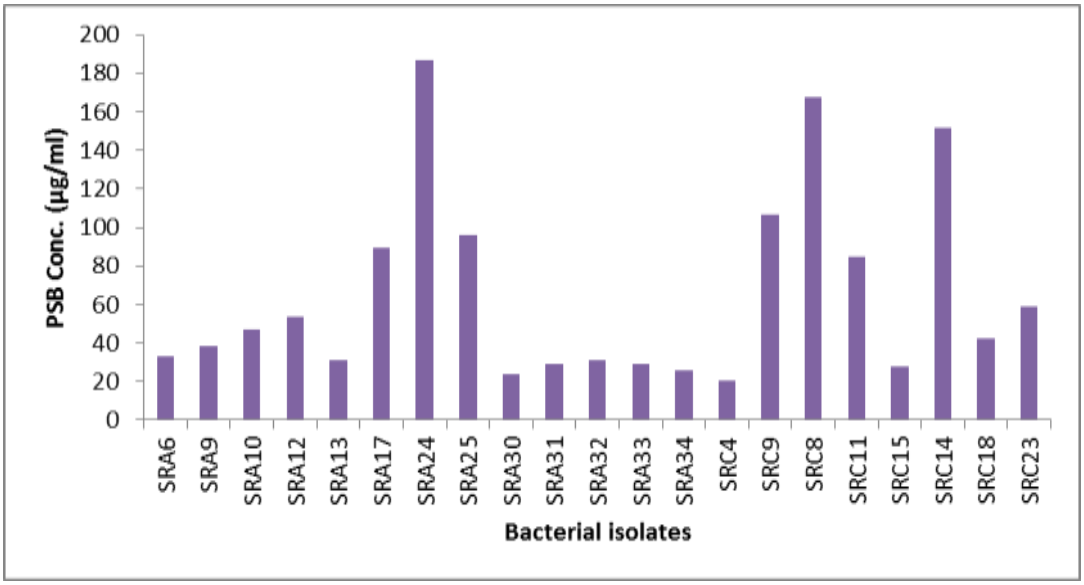


Figure 2-18. Estimation of phosphate solubilizing activities of bacteria

Test of bacterial growth in salt stress:

Since bacterial isolates were to be used for inoculating plant seedlings to test their effect on plant growth under salt stress, bacterial growth under given stress conditions was evaluated. NaCl incorporated into Nutrient agar medium and various concentrations (3%, 6%,8%,10%15% and 20%) were amended to the medium and the test bacterial strains were streaked. Out of 57 isolates, 12 isolates have shown salt tolerance upto 10% and 4 isolates have shown tolerance upto 15%. Result of 12 bacterial isolates growth at different concentration (5%, 10%, 15%) of NaCl has been given in *Figure 1-19*. Optimum growth temperature for most of the isolates was 30 ^{o}C. Bacterial growth decreased with increase in salt concentration. Highest growth in salt stress condition was observed in SRA33, SRC3, SRA25 (*Figure 2-19*).

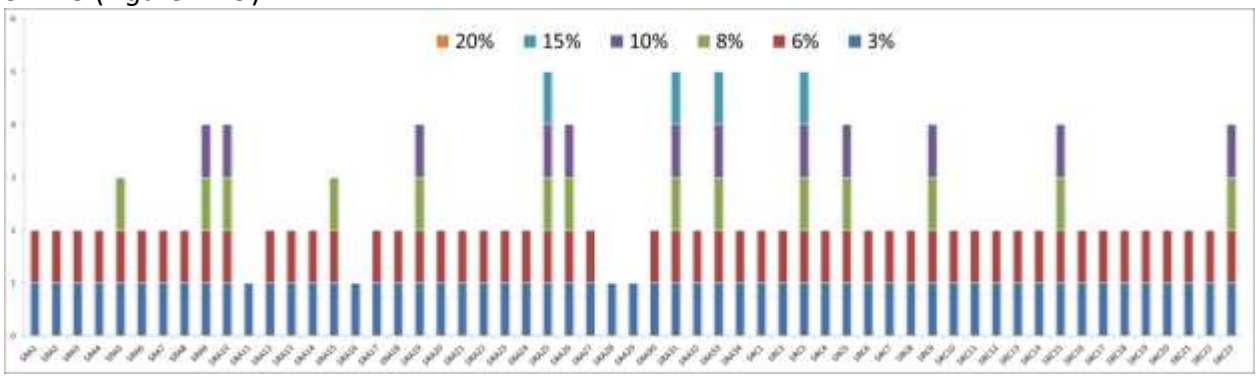


Figure 2-19. NaCl (3%, 6%,8%,10%15% and 20%) tolerant activity among 57 bacteria isolated from sampling site A and C

Table 2-18. Test for growth of bacteria at various salt concentration.

Bacteria	NB	NB + 5%Nacl	NB + 10%Nacl	NB + 15%Nacl
SRA9	2.314	2.014	0.065	0.008
SRA10	2.164	2.142	0.014	0.011
SRA19	1.889	0.96	0.016	0.009
SRA25	1.486	1.306	0.011	0.013
SRA26	1.175	1.842	1.72	0.016
SRA31	1.592	1.611	0.725	0.018
SRA33	1.241	1.555	0.831	0.331
SRC3	2.634	2.376	1.651	0.179
SRC5	2.344	1.675	0.115	0.012
SRC9	2.144	1.005	0.071	0.019
SRC15	2.101	1.770	0.105	0.057
SRC23	2.274	2.062	0.02	0.009
CONSORTIA	2.322	2.331	0.007	0.011

IAA production of bacterial isolates

IAA production was checked by use of Salkowski reagent. Color development was first visible at the highest IAA concentration within minutes and continued to increase in intensity for a period of 30 min. L-Tryptophan is generally considered as an IAA precursor. Effect of addition of L-tryptophan on IAA enhancement was found in bacterial cultures. 10^8 CFU/ml of 12 salt tolerant isolates were inoculated and incubated for early log phage of

each in the peptone broth enriched with tryoptophan broth to check for the production of indole acetic acid, a precursor of auxin which is an important plant hormone. The quantitative estimation of IAA is performed by using Salkowski method by using the reagent, 1 ml of FeCl3, 0.5 mM in 35% $HClO_4$. Mixtures were incubated at room temperature for 25 min and observed for pink colour production and read colorimetrically and compared with standards. Results are graphically presented in *Figure 2-20*.

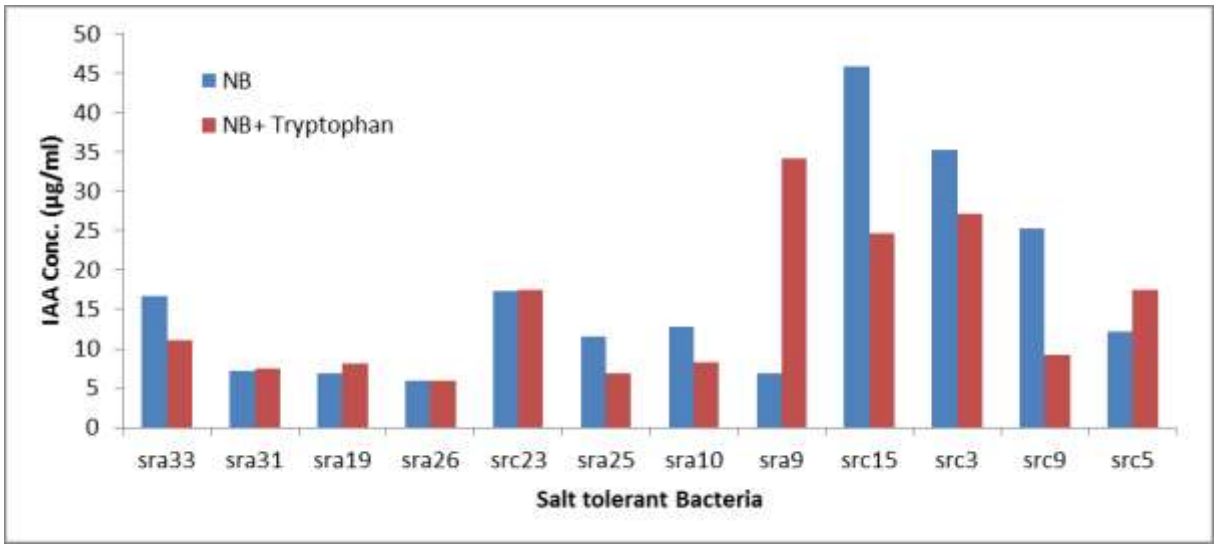


Figure 2-20. IAA production of salt tolerant bacteria isolated from sampling site A and C

Acid production

All isolates were streaked on Tryptone soy agar having 0.5% BTB(bromo thymol blue).Change in colour of dye(bluish green to yellow) in medium due to bacterial growth indicated positive results for acid production (*Figure 2-21*).

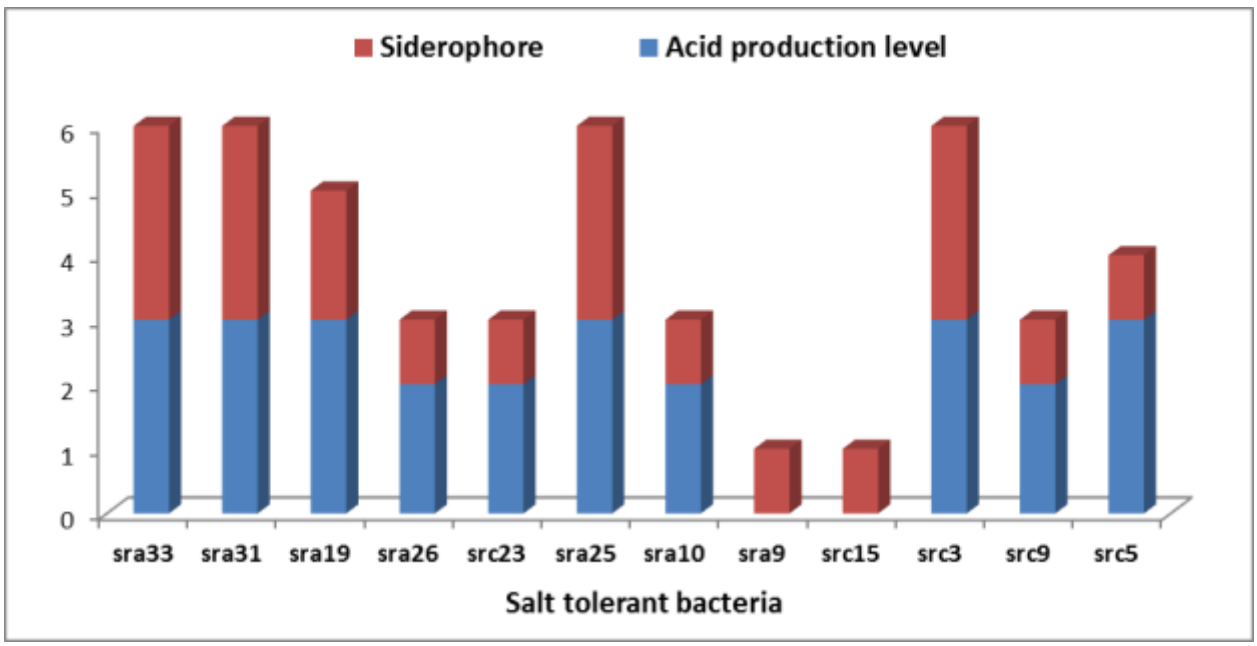


Figure 2-21. Siderophore and acid production of salt tolerant bacteria isolated from sampling site A and C

Siderophore production

Siderophore production was tested qualitatively using chrome azurol S medium (CAS-medium. The cultures of 6 isolates were spot inoculated on the surface of CAS agar medium and incubated at room temperature for 2 to 4 days. Siderophore production was indicated by orange halos around the colonies after the incubation, and this test was done in two replications (Figure 2-22).

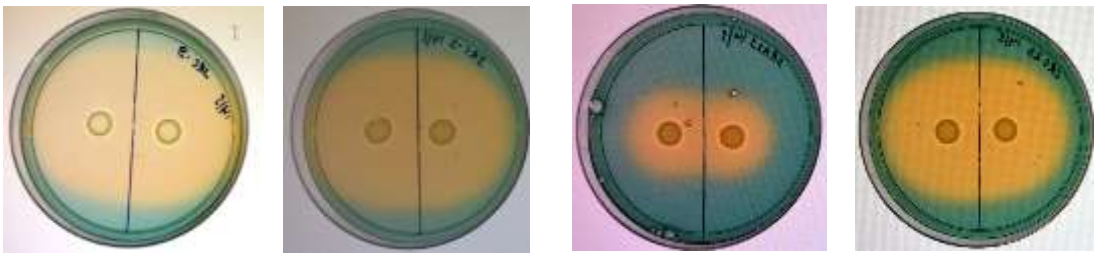


Figure 2-22. Siderophore production of salt tolerant bacteria isolated from sampling site A and C

Quantitative estimation of growth of salt tolerant bacteria on medium amendment with soil (S1, S2, S3) extraction

100gm of soil was dissolved in 1000 ml of autoclaved water. This solution was then kept for shaking at 200 rpm for 48 hrs. Solution was then filtered using filter paper. Then this extract was used for preparing media (Nutrient broth and Nutrient Agar).Media containing soil extract were inoculated with selected bacterial isolates (Figure 2-23).

Table 2-19. Quantitative estimation of growth of salt tolerant bacteria on medium amendment with soil (S1,S2,S3) extraction

ISOALTE	OD (530) WITH SOIL (S1) EXTRACTION	OD (530) WITH SOIL (S2) EXTRACTION	OD (530) WITH SOIL (S3) EXTRACTION
SRA9	1.740	2.411	2.152
SRA10	2.737	2.667	2.752
SRA19	2.837	2.652	2.712
SRA25	1.041	1.540	1.708
SRA26	1.825	2.169	2.274
SRA31	0.815	1.184	1.361
SRA32	0.311	0.211	0.750
SRA33	2.254	1.770	2.314
SRC3	2.846	2.734	2.828
SRC5	1.876	1.834	1.854
SRC9	2.549	2.503	2.556
SRC15	2.516	2.449	2.460
SRC23	2.206	2.028	1.405

Site No.	No. of year spent wash applied	Type	RS No.	Code
S1	Above 15 years	Company plot	242	S1
S2	15-20 years		239/4	S2
S3	Above 15 years	Vithal dev trust	319	S3

Figure 2-23. Qualitative assay of growth of salt tolerant bacteria on medium amendment with soil (S1, S2, S3) extraction

Antagonistic & Synergistic Activities

A culture of bacterial isolate was sprayed on quadrants nutrient agar medium plates. Then the plates were spot inoculated with 20 l of half strength molten nutrient agar (sterilized) containing culture of another four bacterial isolates. The plates were incubated for 24 hr at 30±2ºC. The plates were observed for the inhibition zone after 24-48 hour of incubation at 35±2º C and experiment was replicated thrice.

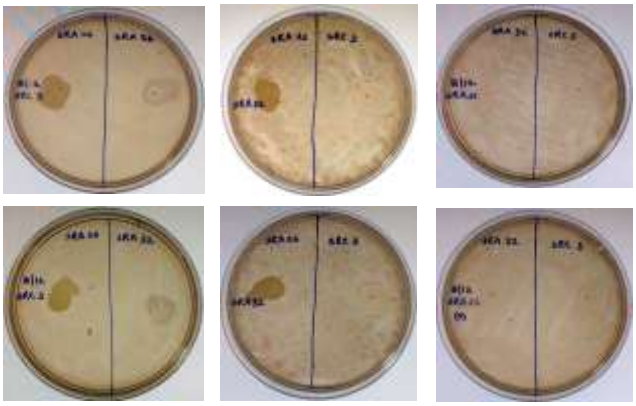


Table 2-20. Antagonistic and synergistic activities of selective salt tolerant bacteria

	SRA9	**SRA10**	**SRA25**	**SRA31**	**SRA33**	**SRC3**	**SRC9**	**SRC15**	**SRC23**
SRA9	-	syn	syn	syn	syn	syn	syn	syn	anta
SRA10	syn	-	syn	syn	syn	syn	syn	Syn	syn
SRA25	syn	syn	-	syn	syn	anta	syn	syn	syn
SRA31	syn	syn	syn	-	syn	anta	syn	syn	syn
SRA33	syn	syn	syn	syn	-	syn	syn	syn	syn
SRC3	syn	syn	anta	syn	syn	-	syn	syn	syn
SRC9	syn	syn	syn	syn	syn	syn	-	syn	syn
SRC15	syn	syn	syn	syn	syn	Syn	syn	-	syn
SRC23	anta	syn	syn	syn	syn	syn	syn	syn	-

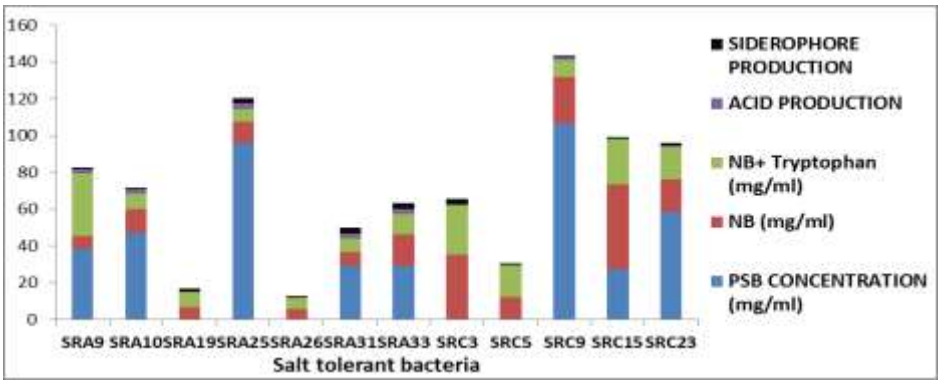


Figure 2-24. Overall functional screening of 12 salt tolerant bacteria to determine best consortia for greenhouse experiment on sweet sorghum

Microcosm (experimental ecosystem) setup for determination of best consortia under green house condition

Microcosm (experimental ecosystem) experimental setup was carried out under green house condition for determining the effectiveness of the 12 isolated bacteria and their Synergistic isolates as bioinoculants. 5ml microtips filled with 10g soil samples [1. Above 15 years Company plot 242 (Normal) 2. Above 15 years Company plot 242 (autoclaved), 3. Normal Soil (autoclaved)] Seeds of sweet Sorghum (*Sorghum bicolor* L. Moench) were sown and irrigated daily (*Figure 2-25*). Evaluation of Plant biomass, nutrient uptake and soil properties in different treatment under greenhouse experiments are ongoing. Based on observation from greenhouse experiments field trials will be initiated with best consortia

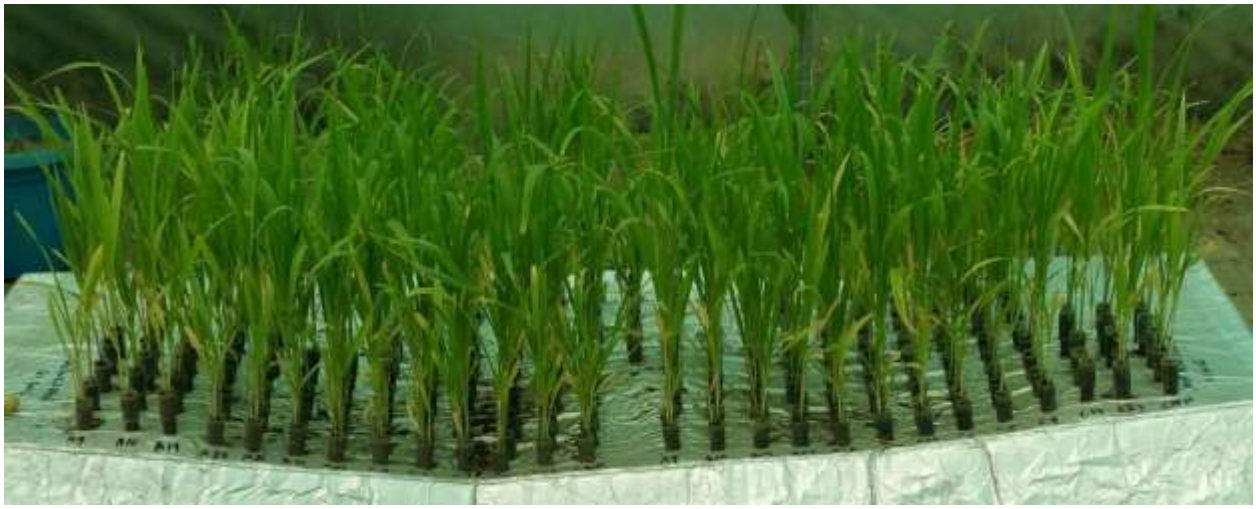


Figure 2-25. Microcosm (experimental ecosystem) setup for determination of best consortia under green house condition

Identification of bacterial strains by 16 S rDNA sequencing

Genomic DNA of bacterial isolates were prepared and their 16S rDNA was amplified using universal primers. The primers were used to amplify nearly full-length 16S rDNA sequences yielded 1.5kb product. Genomic DNA was amplified by mixing the template DNA (50 ng), with the polymerase reaction buffer, dNTP mix, primers and Taq polymerase. Polymerase Chain Reaction was performed in a total volume of 100 µl, containing 78 µl deionized water, 10 µl 10 X Taq pol buffer, 1 µl of 1 U Taq polymerase enzyme, 6 µl 2 mM dNTPs, 1.5 µl of 100 mM reverse and forward primers and 1 µl of 50 ng template DNA. PCR was programmed with an initial denaturing at 94oC for 5 min. followed by 30 cycles of denaturation at 94 ^{o}C for 30 s, annealing at 55 ^{o}C for 40 s and extension at 70 ^{o}C for 90 s and the final extension at 72 ^{o}C for 7 min in a Primus 96 advanced gradient Thermocycler. PCR product (20 µl) was mixed with loading buffer (8 µ1) containing 0.25 % bromophenol blue, 40 % w/v sucrose in water, and then loaded in 0.8% Agarose gel with 0.1 % ethidium bromide for examination with horizontal electrophoresis. The PCR products were purified from agarose gels with the PCR Clean-up Gel. Extraction Kit and were sequenced. The nucleotide sequences were compared using the Blast N program and the closest match of known phylogenetic affiliation was used to assign the isolated strains to specific taxonomic groups.

Table 2-21. Blast N sequence analysis of bacterial sequences

Isolates	Bases	Homology	%age	Acc No.
SRC14	1200	*Enterobacter*	96	KJ184887.1
SRC12	1200	*Bacillus sp.*	98	KJ184903.1
SRA5	1171	*Brevibacterium epidermis*	97	EU046495.1
SRC4	1200	*Brevibacterium epidermis*	98	EU046495.1
SRC7	1200	*Alcaligenes faecalis*	97	JX975452.1
SRA32	1200	*Bacillus licheniformis*	98	KC342874.1
SRA34	1200	*Bacillus licheniformis*	97	JN391533.1
SRA26	1200	*Bacillus marisflavi*	98	KC414706.1
SRA22	1200	*Alcaligenes faecalis*	96	KF925435.1
SRA21	1200	*Alcaligenes faecalis*	97	JX849036.1
SRA8	1200	*Alcaligenes faecalis*	97	KF641850.1
SRC3	1096	*Brevibacterium linens*	96	KJ019204.1
SRC30	1200	*Bacillus licheniformis*	98	JN391533.1
SRA9	1000	*Bacillus pumilus*	92%	KF217253.1
SRC23	1000	*Bacillus pumilus*	95%	KF217253.1
SRA10	937	*Bacillus pumilus*	97%	JN210575.1
SRA31	917	*Bacillus licheniformis*	98%	FJ493053.1
SRA33	917	*Bacillus cereus*	97%	KJ729602.1
SRA19	917	*Brevibacterium iodinum*	91%	KF424671.1
SRA32	917	*Bacillus licheniformis*	91%	JN411571.1
SRC3	917	*Brevibacterium sp*	92%	GQ250446.1
SRC15	930	*Achromobacter xylosoxidans*	98%	KJ659368.1

Bio-remedial regeneration of degraded land irrigated with city wastewater for long term (Pandherkawada village, Nagpur)

To study the long term impact of municipal wastewater on soil, the degraded land at Pandherkawada village which is approximately 25 km away from Nagpur; is selected for bioremediation using biotechnological approach. *Figure 2-26* shows the degraded land site selected at Pandherkawada village ($21^0$4'52.3'' N, $79^0$11'46.3'' E) for bioremediation. The study area falls under the sub-humid ecosystems of Deccan plateau and central highlands with black soils (Vertisol). The climate of the region is characterized by hot summers and mild winters. The area receives a mean annual rainfall of 100 to 1500 mm 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 experience anustic soil moisture regime and the moisture availability (growing) period varies from 150 to 180 days in a year. The soil temperature regime in the area is hypothermic. *Figure 2-27* shows the aerial view of bioremediation site at Pandherkawada village, Nagpur. Accordingly, CSIR-NEERI, Nagpur, team visited the site in the month of June 2013 and collected the profile soil samples, irrigation water and groundwater (open dug well) samples. The irrigation water samples were collected from the stream that contains Nagpur city municipal wastewater and the well water sample was collected from the well nearer to the degraded land.

Figure 2-26. Location of degraded land selected for bioremediation at Pandherkawada village, Nagpur

Figure 2-27. Locations of bioremediation site at Pandherkawada village, Nagpur

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.

Characteristics of water samples

The characteristics of different types of water samples collected in and around the Pandherkawada village, Nagpur are presented in Table 2-22. The results presented in Table 2-22 shows that the pH of the water samples collected from different sources (irrigation water and well water) was 7.19 and 7.62 and EC were 1.12 and 2.30 dS m^{-1} respectively. The TDS contents in the water samples were 742 and 1140 mg L^{-1}. The total alkalinity in the water samples were 220 and 230 mg L^{-1} and suspended solids 20 to 60 mg L^{-1} respectively.

The total hardness in the water samples analyzed were 240 and 876 mg L^{-1} while the corresponding levels of calcium were 40 and 195.2 mg L^{-1} and magnesium 33.6 and 94.6 mg L^{-1} respectively. The water samples collected from irrigation water and well water shows the magnitude of COD 26 and 250 mg L^{-1}, BOD 9 and 160 mg L^{-1} respectively. The levels of cations with respect to sodium in the water samples were 92 and 310 mg L^{-1}, potassium 6.2 to 15.2 mg L^{-1} 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 were 98.5 and 724.5 mg L^{-1} whereas sulphate being 16 and 24 mg L^{-1} in the water samples respectively. The SAR of the well water is high as compared to irrigation water respectively. According to the Indian Standard (IS: 10500, 1993) the wastewater used for irrigation has high chloride and BOD concentration.

Table 2-22. Physico-chemical characteristics of different types of water samples collected in and around the Pandherkawada village, Nagpur

Sl. No.	**Parameters**	**Irrigation water**	**Well Water**	**FAO Wastewater Guidelines for Agricultural Use* (1985)**	**Indian Standards for discharge of effluent on land for irrigation (IS:10500, 1993)**
1.	pH	7.62	7.19	6.50 to 8.00	5.5 to 9.0
2.	EC, dS m^{-1}	1.12	2.30	0.7-3.0	--
3.	TDS, mg L^{-1}	742	1140	450-2000	2100
4.	Total Alkalinity, mg L^{-1}	220	230	--	200
5.	TSS, mg L^{-1}	60	20	--	--
6.	Total Hardness, mg L^{-1}	240	876	--	--
7.	Calcium, mg L^{-1}	40	195.2	--	--
8.	Magnesium, mg L^{-1}	33.6	94.6	--	--
9.	COD, mg L^{-1}	250	26	--	--
10.	BOD, mg L^{-1}	160	9	--	100
11.	Ammonical Nitrogen, mg L^{-1}	1.22	1.02	--	--
12.	Nitrate, mg L^{-1}	0.53	1.77	--	--
13.	Sodium, mg L^{-1}	92	310	--	--
14.	Potassium, mg L^{-1}	15.2	6.2	--	--
15.	Chloride, mg L^{-1}	98.5	724.5	142-355	600
16.	Sulphate, mg L^{-1}	16	24	--	1000
17.	SAR	2.59	4.56	3.0-9.0	--
Heavy Metals and Oxyanion , mg L^{-1}					
18.	Zinc	0.004	BDL	2.0	--
19.	Lead	BDL	BDL	5.0	--
20.	Cadmium	BDL	BDL	0.01	--
21.	Nickel	BDL	BDL	0.20	--
22.	Manganese	0.26	BDL	0.20	2.0
23.	Iron	BDL	BDL	5.0	3.0
24.	Chromium	BDL	BDL	0.10	--
25.	Copper	BDL	BDL	0.20	--
26.	Boron	BDL	7.8	0.70	--
27.	Arsenic	BDL	BDL	0.10	0.2

BDL: Below Detection Limit

*FAO Guidelines “Slight to Moderate” degree of restriction on use

According to the FAO guidelines on the restriction of use of wastewater in agriculture, the irrigation water used in Pandherkawada village belongs to "**slight to moderate"** degree of restriction on its use for crop irrigation. The levels of different heavy metals namely zinc, lead, cadmium, nickel, iron, chromium and copper presented in Table 2-22 indicate that their concentrations were within the toxicity limit and do not pose any toxicity to plants species. But, the concentration of manganese in the irrigation water is slightly high, which may pose toxic effect on various crops.

Characteristics of profile soil samples

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 (Site-I and Site-II) and one from well water irrigated field at a different depths of 0-15 cm, 15-30 cm and 30-60 cm. The physico-chemical and microbiological characteristics of profile soil samples are presented in Table 2-23, Table 2-24, Table 2-25, and Table 2-27, respectively.

The particle size distribution presented in Table 2-23 with respect to percentage sand, silt and clay in the profiles soil samples collected in and around the degraded land at varied from 14.0-29.8 %, 22.5-29.2 % and 44.7-60.0 % respectively. These results indicated the soils belong to textural class "Clay". Similarly, the results of bulk density, porosity and maximum water holding capacity in the profiles soil samples varied in the range of 1.25-1.32 g/cc, 53.81-63.61 % and 42.29-59.02 % respectively. There was not much variations was observed wastewater irrigated soil and well water irrigated soil. 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.

Table 2-23. Particle size distribution of soils collected in and around the Pandherkawada village, Nagpur

Sl. No.	Description of Site	Depth, cm	Particle Size Distribution			Texture Class	Bulk Density g/cc	Porosity %	Maximum Water Holding Capacity, %
			Sand %	Silt %	Clay %				
1.	Red encrusted soil	Top soil	18.0	24.0	58.0	Clay	1.25	58.04	59.02
2.	Wastewater irrigated field (Site-I)	0-15	14.0	28.0	58.0	Clay	1.28	63.61	57.58
		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
3.	Wastewater irrigated field (Site-II)	0-15	14.0	28.0	58.0	Clay	1.27	63.21	58.01
		15-30	14.2	25.8	60.0	Clay	1.28	58.82	57.46
		30-60	14.0	28	58.0	Clay	1.30	57.01	55.49
4.	Well water irrigated field (control	0-15	29.8	24.2	46.0	Clay	1.27	55.38	48.37
		15-30	23.9	26.8	49.2	Clay	1.30	54.81	44.67
		30-60	26.0	29.2	44.7	Clay	1.32	53.81	42.29

The results presented in Table 2-24 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^{-1} 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.

Table 2-24. Chemical characteristics of profile soil samples collected in and around the Pandherkawada village, Nagpur

Sl No.	Description of Site	Depth, cm	Parameters					
			pH	EC, dS m^{-1}	Total N, %	Total P, %	Total K, %	Organic Carbon, %
1.	Red encrusted soil	Top Soil	8.1	3.70	0.08	0.09	0.91	0.90
2.	Wastewater irrigated field (Site-I)	0-15	8.2	3.52	0.07	0.08	0.83	0.88
		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
3.	Wastewater irrigated field (Site-II)	0-15	8.6	2.91	0.06	0.07	0.75	0.70
		15-30	8.0	2.56	0.04	0.05	0.46	0.52
		30-60	8.5	2.54	0.02	0.03	0.42	0.46
4.	Well water irrigated field (control	0-15	7.6	2.52	0.05	0.06	0.59	0.65
		15-30	7.5	2.25	0.05	0.07	0.55	0.58
		30-60	7.4	2.14	0.03	0.04	0.42	0.34

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.

The results presented in Table 2-25 shows the concentrations of exchangeable calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), cation exchange capacity (CEC) and exchangeable sodium percentage (ESP) of the profiles soil 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^{-1}, 0.09-1.59 cmol (p^+) Kg^{-1} and 0.20-0.80 cmol (p^+) Kg^{-1} respectively. The CEC and ESP in the soils varied from 42-52 cmol (p^+) Kg^{-1} and 0.21-3.24 respectively. It was observed that none of the soil attained ESP of 15 or more, so as to designate the soil, 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 profile soil samples collected in and around the degraded land at Pandherkawada village, Nagpur are presented in Table 2-25. The results indicated that the concentrations of most of the heavy were

within the toxicity limit, except Cu and Zn, according to B. J. Alloway (1995). The slight built up of heavy metals in soils irrigated with wastewater may be attributed to long term irrigation of raw wastewater.

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 2-27 showed the development of soil microbial populations in terms of Bacteria, Fungi, *Actinomycetes*, *Azotobacter* and *Rhizobium.* Not much variations was observed in the counts of different microbial groups *viz.* Bacteria, Fungi and *Actinomycetes, Azotobacter* and *Rhizobium* in the wastewater and well irrigated soils respectively.

Table 2-25. Chemical characteristics of profile soil samples collected in and around the Pandherkawada village, Nagpur

Sl. No.	Description of Site	Depth, cm	Parameters					
			Ca	Mg	Na	K	CEC	ESP
			cmol (p^+)kg^{-1}					
1.	Red encrusted soil	Top soil	35.3	10.7	1.59	0. 8	49.0	3.24
2.	Wastewater irrigated field (Site-I)	0-15	37.6	9.2	1.24	0. 6	46.5	2.67
		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
3.	Wastewater irrigated field (Site-II)	0-15	39.6	11.6	1.22	0. 3	52.0	2.35
		15-30	37.1	8.9	0.19	0.5	46.0	0.41
		30-60	35.2	7.6	0.09	0. 4	42.0	0.21
4.	Well water irrigated field (control	0-15	34.2	11.3	1.43	0. 6	49.2	2.91
		15-30	32.6	13.9	1.24	0. 6	46.0	2.70
		30-60	30.3	7.6	1.18	0. 4	44.7	2.64

Table 2-26. Concentration of heavy metals in soil collected in and around the Pandherkawada village, Nagpur

Sr. No.	Description of Site	Depth, cm	Heavy Metals, mg kg^{-1}					Micronutrients, mg Kg^{-1}				
			Cr	Ni	Pb	Co	Cd	Cu	Zn	B	Mn	Fe
1.	Red encrusted soil	Top soil	71.00	63.00	15.00	23.00	0.98	62.00	87.00	6477.00	862.00	34651.00
2.	Wastewater irrigated field (Site-I)	0-15	71.00	72.00	14.00	26.00	0.98	43.00	68.00	6393.00	1077.00	34381.00
		15-30	74.00	65.00	16.00	26.00	1.01	44.00	78.00	6521.00	1048.00	34271.00
		30-60	72.00	68.00	15.00	25.00	1.04	44.00	70.00	6592.00	940.30	34171.00
3.	Wastewater irrigated field (Site-II)	0-15	73.40	66.00	16.00	26.00	1.07	44.00	67.00	6570.00	1021.00	33961.00
		15-30	70.00	65.00	14.00	26.00	1.01	42.00	68.00	6544.00	958.40	33251.00
		30-60	72.00	66.00	15.00	26.00	1.08	43.00	67.00	6930.00	958.40	34066.00
4.	Well water irrigated field (control	0-15	45.00	40.00	8.60	15.00	0.61	30.00	51.00	4530.00	972.50	22361.00
		15-30	45.00	42.00	9.20	15.00	0.68	31.00	53.00	4847.00	994.00	22322.00
		30-60	43.20	41.00	9.13	14.90	0.58	32.1	54.3	4625.00	1024.00	30245.00

Table 2-27. Microbiological characteristics of profile soil samples collected in and around the Pandherkawada village, Nagpur

Sl. No.	Description of Site	Depth, cm	Microbial groups (CFU/g)				
			Bacteria	Fungi	*Actinomycetes*	*Azotobacter*	*Rhizobium*
1.	Red encrusted soil	Top layer	23×10^5	9×10^3	80×10^3	17×10^1	30×10^1
2.	Wastewater irrigated field (Site-I)	0-15	10×10^7	20×10^2	23×10^3	45×10^2	29×10^2
		15-30	18×10^6	5×10^1	49×10^2	38×10^2	10×10^1
		30-60	21×10^6	3×10^1	19×10^2	88×10^1	8×10^1
3.	Wastewater irrigated field (Site-II)	0-15	90×10^7	50×10^2	14×10^3	15×10^3	30×10^2
		15-30	13×10^6	20×10^1	16×10^2	10×10^2	28×10^1
		30-60	80×10^5	10×10^1	49×10^1	20×10^1	20×10^1
4.	Well water irrigated field (control)	0-15	70×10^7	41×10^3	27×10^3	50×10^3	28×10^2
		15-30	60×10^7	30×10^2	21×10^2	50×10^2	21×10^1
		30-60	52×10^6	10×10^2	16×10^2	25×10^2	15×10^1

Whereas in case of red encrusted soil (Top layer), lower microbial counts was observed as compared to wastewater and well irrigated soils. This may be due deposition of heavy metal and other inhibitors present in the wastewater.

The overall analysis of the soil showed accumulation of heavy metals and micronutrients in the wastewater irrigated soil. Figure 2-28 shows the red encrustation formed over the top soil layer due to continuous application of wastewater. The formation of red colour on the top soil layer might be due to formation of oxides of heavy metals present in the wastewater. The preliminary investigation on the soil characteristics resulted that the long term application of wastewater had influenced the soil quality which ultimately affects the growth of the crops.

Information about cropping pattern at degraded site at Pandherkawada village, Nagpur

The baseline data was collected for the last five years i.e. from 2008 to 2012 with respect to cropping pattern from the Tahasil office, Tarodi, Nagpur. 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 is required for remediation of degraded land.

Figure 2-28. The red encrustation formation over the top soil layer, due to the long term application of wastewater at Pandherkawada village, Nagpur.

Table 2-28. Baseline data of cropping pattern collected from the Pandherkawada village, Nagpur

Sl. No.	Year	Crops	Area under each crop (Acre)	Yield (Quintal)
1.	2008	Soybean	8	55
		Wheat	2	42
		Gram	5	19
		Chilli (without wastewater)	1	10
2.	2009	Soybean	8	42
		Wheat	2	43
		Gram	5	17
		Chilli (without wastewater)	1	11
3.	2010	Soybean	8	38
		Wheat	2	41
		Gram	5	16
		Chilli (without wastewater)	1	9
4.	2011	Soybean	8	12-13
		Wheat	2	41
		Gram	5	16
		Chilli	1	4
5.	2012	Wheat	2	40
		Gram	5	19
		Chilli	1	10-12

* From 2012, the cultivation of Soybean crop has been stopped due to decline in its yield.

Bio-remediation of lands previously loaded with bio-refinery waste water.
Treatment details
Main plot (Drainage methods)
D_1 – Surface drainage
D_2 – Sub-Surface drainage
D_3 – Control

Sub plot (Soil fertility)
S_1 – Green manuring in-situ (Dhaincha – wheat)
S_2 – Use of pressmud (Soybean- wheat)
S_3 – Microbial culture (Soybean-wheat)
S_4 – S_1 + microbial culture (Dhaincha-wheat)
S_5 – S_2+ microbial culture (soybean – wheat)
S_6- Control

The experiment was initiated during *kharif* 2014 at Ugar khurd. The yield of maize did not differ significantly with drainage methods. However yield of maize ranged from 6422 to 7099 kg /ha (with stover yield of 8.03 to 11.86 t/ha). Whereas soil fertility management practices had significant effect on yield of maize. The grain (7060 kg/ha) and stover (10.52 t/ha) yields were higher with use of organics (Pressmud) as compared to the rest of the soil fertility management practices (Table 2-29 and Table 2-30). The sequence crop of wheat is at grain filling stage

Figure 2-29. General view of Maize at Ugar khurd

Table 2-29. Effect of drainage practices and microbial culture inoculation on yield (kg ha^{-1}) of Maize				
Soil fertility management	**Drainage system**			
	D_1	**D_2**	**D_3**	**Mean**
S_2	7459	7488	6233	7060
S_3	7827	7001	6058	6962
S_5	6647	5499	7143	6429
S_6	6464	5702	6740	6302
Mean	7099	6422	6543	
	SEm±		CD (p=0.05)	
Main (M)	180		NS	
Sub (S)	245		NS	
MXS	490		1402	

Table 2-30. Effect of drainage practices and microbial culture inoculation on stover yield (t ha^{-1}) of maize				
Soil fertility management	**Drainage system**			
	D_1	**D_2**	**D_3**	**Mean**
S_2	11.82	9.31	10.42	10.52
S_3	10.56	9.13	7.57	9.09
S_5	11.82	8.48	6.94	9.08
S_6	13.21	5.20	8.75	9.05
Mean	11.86	8.03	8.42	
	SEm±		CD (p=0.05)	
Main (M)	0.70		NS	
Sub (S)	0.57		1.63	
MXS	1.14		3.27	

Effect of bio-remedial measures and management practices on growth, yield and quality of soybean-wheat sequence cropping system

Treatment details
Main plot (land management practices)

M_1 – Land leveling and grading
M_2 – Land leveling, grading and compartment bunding
M_3 – Big compartment bunds
M_4 – Control

Sub plot (Soil fertility management practices)
S_1 – Green manuring *in-situ* (Dhaincha – wheat)
S_2 – Use of pressmud (Soybean- wheat)
S_3 – Microbial culture (Soybean-wheat)
S_4 – S_1 + microbial culture (Dhaincha-wheat)
S_5 – S_2+ microbial culture (soybean – wheat)
S_6- Control

Consortia of Microbes used

Enterobacter aerogenes (S63 (1) R): A free-living N_2 fixing bacteria tolerating 15%NaCl
Azospirillum irakense (S173E): An associative N_2 fixing bacteria tolerating 10% NaCl
Enterobacter cloacae (S125R): A phosphate solubilizing bacteria tolerating 15% NaCl
Pseudomonas sp. (S4 (1) S): A fluorescent *pseudomonas* bacteria tolerating 17.5% NaCl

The results of 2014-15 indicated that, sowing of soybean in areas loaded with distillery spent wash failed to respond due to high salinity (4.50 dS/m) and water logging. The area received excess rainfall of 352.1 mm with 45 number of rainy days in the month of July and August (Soybean was sown on 26-06-2014), hence the experiment was vitiated. However, the sequence crop of wheat is at flowering stage and the results will be presented after the harvest.

Figure 2-30. General view of wheat at Ugar khurd

Microbial load

Both land management practices and soil fertility management practices showed to have significant influence on microbial population at harvest of soybean crop. Among the land management practices, treatments such as control (M4) and land leveling and grading (M1) recorded significantly higher bacterial population over all other treatments. Among soil fertility management practices, treatment receiving green manure and microbial culture (S4) was found to record higher bacterial population. However, the observed bacterial population was on par with those observed in green manure with microbial culture (S4). The interaction effect of land management practice and soil fertility management practices it is evident that land levelling grading and formation of compartment bund and application of pressmud @ of 2.5 t ha^{-1} along with microbial culture (M2S5) resulted in higher bacterial populations than the other, although the difference in population was not significant in M1S1, M4S5, M4S4 and M4S3 (Table 2-31).

The fungal population was also significantly influenced due to management practices. Land management practices such as land levelling, grading and compartment bunding (M2) and that under control (M4) recorded significantly higher fungal population, which were at par with each other, but significantly superior fungal population over land levelling and grading (M1) and big compartment bunds (M3). The population of fungi did not vary in treatment receiving green manuring (S1), use of press mud (S2) and microbial culture (S3). While

fungal population in treatment with only green manuring + microbial culture (S4) and use of pressmud@ of 2.5 t ha^{-1} + microbial culture (S5) were significantly higher than green manuring alone (S1). The actinomycetes population in treatments receiving green manure along with microbial culture (S4) was also significantly superior over green manuring (S1). Rest of the treatments were on par. Land leveling and grading (M1) reduced the actinomycetes population significantly. While use of big compartment bunds (M3) significantly increased the actinomycetes population.

All land levelling management practices significantly reduced the population of phosphorus solubilising microorganisms (PSM) compared to control. The population of PSM was higher in control (S6) followed by treatment with press mud @ of 2.5 t ha^{-1} + microbial culture (S5) and microbial culture alone (S3) which were on par with each other. While population recorded with rest of the treatments were significantly lower (Table 2-32). In general from the data it may be concluded that land management practices tend to reduce the microbial population which might be due to disturbance of top soil. Among the soil fertility management practices, use of microbial culture tends to restore the microbial population.

Table 2-31. Effect of management practices and microbial culture inoculation on bacteria and fungi population in Soybean

Land management practices	**Bacteria (CFU X 106 ml-1)**							**Fungi (CFU X 104 ml-1)**						
	Soil fertility management													
	S1	S2	S3	S4	S5	S6	Mean	S1	S2	S3	S4	S5	S6	Mean
M1	282	182	122	144	203	97	171	2	4	6	1	5	3	3
M2	56	66	171	169	289	139	148	4	2	2	25	26	133	32
M3	167	57	85	165	160	153	131	1	5	3	1	5	10	4
M4	75	122	237	242	267	231	195	1	2	3	64	74	78	37
Mean	145	107	154	180	230	155		2	3	3	22	27	56	
	SEm+				CD (p=0.05)			SEm+				CD (p=0.05)		
Main (M)	13				32			3				7		
Sub (S)	20				61			6				18		
MXS	24				50			7				15		

Main plot (Land management practices)

M_1 – Land leveling and grading

M_2 – Land leveling, grading and compartment -bund

M_3 –Big compartment bunds

M_4 – Control

Sub plot (soil fertility management practices)

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

S_2 – Use of press mud (Soybean- wheat)

S_3 – Microbial culture (Soybean-wheat

S_4 – S_1 + microbial culture (Dhaincha-wheat)

S_5 – S_2+ microbial culture (soybean – wheat)

S_6- Control

Table 2-32. Effect of management practices and microbial culture inoculation on Actinomycetes and PSM population in Soybean														
Land management practices	**Actinomycetes (CFU X 103 ml-1)**							**PSM (CFU X 105 ml-1)**						
	Soil fertility management													
	S1	S2	S3	S4	S5	S6	Mean	S1	S2	S3	S4	S5	S6	Mean
M1	94	60	79	79	108	105	87	20	17	19	1	22	21	16
M2	54	164	65	116	109	106	102	18	17	36	17	35	26	24
M3	85	105	225	268	147	150	163	8	8	5	7	36	7	12
M4	57	137	105	173	128	111	118	4	19	59	39	75	135	55
Mean	72	116	118	159	123	118		12	15	29	16	42	47	
	SEm+				CD (p=0.05)			SEm+				CD (p=0.05)		
Main (M)	7				17			3				7		
Sub (S)	28				84			9				27		
MXS	32				69			10				22		

Main plot (Land management practices)

M1 – Land leveling and grading

M2 – Land leveling, grading and compartment -bund

M3 – Big compartment bunds

M4 – Control

Sub plot (soil fertility management practices)

S1 – Green manuring in-situ (Dhaincha – wheat)

S2 – Use of press mud (Soybean- wheat)

S3 – Microbial culture (Soybean-wheat)

S4 – S1 + microbial culture (Dhaincha-wheat

S5 – S2+ microbial culture (soybean-wheat

S6-Control

3 Work package: Agricultural water management

Objectives

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

3.1 Benchmark sites characterized

Socio-economic survey (Lakshmipuram and Vuyyuru village)

Farming household was the study unit for socio-economic survey. Landholding households were stratified as marginal, small and larger farmers and those using wastewater for irrigation, press mud, farmyard manure and bio-fertilizer for soil amendment were identified through purposive sampling. Total sample covered is 111 and 135 in Lakshmipuram and Vuyyuru sites respectively of which 20% of the households were selected for collecting soil and water samples. The implications of using waste water for irrigation and soil amendment products on physico chemical and biological parameters of soil and water were analysed and presented in the subsequent sections.

Soil analysis from farmer's fields

Soil samples were collected from farmers' field at Vuyyuru and Lakshmipuram villages of Vijayawada, Seema Andhra. Farmers used some organic amendments viz., farm yard manure, press mud, bio fertilizer and industrial wastewater such as sugar industry and distillery industry wastewater to their field. Soil samples were collected at the depth of 0-15 cm at different stages of crops and analysed for physico-chemical (pH, EC, organic carbon, phosphorus, potassium and physical properties viz., bulk density, particle density, water holding capacity and porosity) parameters.

Effect of amendments application to soil at Lakshmipuram

The change observed in soil physico-chemical properties due to amendment application at Lakshmipuram is presented in Table 3-1. The pH of the soil varied from 7.07 (13^{th} no sample) to 8.51 (4^{th} sample), EC ranged from 0.18 (7^{th} no) to 2.91 dS/m (16^{th} no), organic carbon content recorded from 0.65 (6^{th} no) to 1.21% (23^{rd} no), available phosphorus content ranged from 12.0 (8^{th} no) to 46.8 kg/ac (2^{nd} no), available potassium content varied from 200 (5^{th} no) to more than 500 kg/ac (6^{th} no and 22^{nd} no). Soil physical properties viz., bulk density, particle density, water holding capacity and porosity were also impacted due to amendment application. The bulk density of the soil varied from 0.89 (20^{th} no) to 1.28 g/cc (11^{th} no), particle density ranged from 1.46 (5^{th} no) to 2.08 g/cc (3^{rd} no), water holding capacity recorded from 46.8 (15^{th} no) to 83.5 % (20^{th} no) and porosity ranged from 55.3 (28^{th} no) to 65.8 % (17^{th} no).

Table 3-1. Physico-chemical properties of soil samples collected from farmers' field at Lakshmipuram										
Sl No.	**BF,PM, FYM & effluent applied field***	**pH**	**EC (dS/m)**	**OC (%)**	**P (kg/ac)**	**K (kg/ac)**	**BD* (g/cc)**	**PD* (g/cc)**	**WHC* (%)**	**Porosity (%)**
1	BF, PM, FYM	7.58	0.46	0.90	36.1	280	1.16	1.85	65.4	58.6
2	BF, PM, FYM	7.44	0.48	0.84	46.8	378	1.04	1.94	68.4	60.8
3	BF, PM, FYM	7.51	0.58	0.96	39.9	361	1.06	2.08	74.8	57.4
4	BF, PM, FYM	8.51	0.37	0.80	30.0	210	1.04	1.54	80.6	56.8
5	BF, PM, FYM	7.32	0.32	1.02	35.5	200	0.92	1.46	56.6	55.3
6	BF, PM, FYM	8.19	0.52	0.65	27.5	>500	0.96	1.80	68.7	60.4
7	BF, PM, FYM	7.68	0.18	0.71	18.0	235	1.12	1.68	55.7	59.2
8	FYM	7.53	0.53	0.93	12.0	255	1.24	1.76	48.2	60.5
9	FYM	8.01	0.34	1.02	19.5	352	1.15	1.94	54.4	58.5
10	BF, PM, FYM	7.60	0.28	0.99	36.5	402	1.15	1.82	52.8	61.2
11	BF, PM, FYM	7.85	0.42	0.87	35.5	386	1.28	1.95	65.6	62.6
12	FYM	7.89	0.27	0.93	29.5	455	1.25	1.82	78.4	58.8
13	FYM	7.07	0.78	1.02	20.5	343	1.1	1.78	74.5	63.4
14	FYM	7.81	1.07	0.90	23.8	361	1.25	1.65	67.4	56.4
15	FYM	7.89	0.49	0.87	12.9	270	1.24	1.98	46.8	56.3
16	BF, PM, FYM	7.92	2.91	0.68	36.6	473	0.98	1.85	68.3	63.6
17	BF, PM, FYM	7.43	0.57	1.05	20.0	305	1.04	1.9	71.3	65.8
18	BF, PM, FYM	7.75	0.52	0.74	25.4	382	1.12	2.04	55.6	59.5
19	BF, PM, FYM	8.05	0.43	0.90	37.3	225	0.92	1.67	61.7	55.7
20	BF, PM, FYM	8.11	0.58	0.93	31.7	464	0.89	1.74	83.5	58.3
21	FYM	7.56	0.45	0.90	36.8	235	0.94	1.7	79.4	64.7
22	BF, PM, FYM, Effluent	8.07	0.94	0.96	17.8	>500	1.14	1.82	70.3	65.6
23	BF, PM, FYM, Effluent	8.04	1.91	1.21	27.5	469	0.97	1.90	71.2	63.5
24	BF, PM, FYM, Effluent	7.79	0.61	1.18	31.7	410	1.11	2.07	54.9	57.1

* BF-Biofertilizers, PM-Pressmud, FYM- Farm yard manure and effluent applied field.

*BD-Bulk density, PD-Particle density, WHC-Water holding capacity

Effect of amendments application to soil at Vuyuuru

The soil physico-chemical properties due to amendment application at Vuyyuru were analysed and presented in Table 3-2. The pH of the soil varied from 6.87 (2nd no) to 8.72 (24th no), EC ranged from 0.23 (7th no) to 1.11 dS/m (17th no), organic carbon content recorded from 0.62 (11th no) to 1.21% (1st no), available phosphorus content ranged from 9.3 (20th no) to 43.2 kg/ac (1st no), available potassium content varied from 148 (1st no) to more than 428 kg/ac (24th no). The bulk density of the soil varied from 0.88 (9th no) to 1.32

g/cc (24th no), particle density ranged from 1.42 (2nd no) to 2.12 g/cc (24th no), water holding capacity recorded from 47.6 (5th no) to 82.8 % (13th no) and porosity ranged from 53.4 (4th no) to 65.4 % (23rd no).

Inference

The soil sample was neutral to alkalinity in reaction, salt content was non saline to slightly saline, organic carbon and soil available phosphorus content was medium to high in range and available potassium content was very high (> 500 kg/ac). Based on salt content, farmers' can grow different crop to their field. For example for maize crop, they will get 100-90% yield, paddy crop, get 100% yield, for onion, 75-100% yield, cowpea crop, 75-100% and tomato crop, 100% yield they will get.

Table 3-2. Physico-chemical properties of soil samples collected from farmers' field at Vuyyuru

SL. No	BF,PM, FYM & effluent applied field*	pH	EC (dS/m)	OC (%)	P (kg/ac)	K (kg/ac)	BD* (g/cc)	PD* (g/cc)	WHC* (%)	Porosity (%)
1	BF, FYM	7.57	0.63	1.24	43.2	270	0.98	1.67	60.5	55.7
2	BF, PM, FYM	6.87	0.37	0.90	42.6	265	1.18	1.97	55.8	58.5
3	BF, PM, FYM	7.68	0.63	0.93	34.4	310	1.28	1.92	48.7	59.6
4	BF, PM, FYM	7.74	0.40	0.84	34.2	245	1.16	1.73	51.4	53.4
5	BF, PM, FYM	7.85	0.41	0.84	17.0	235	1.18	1.96	47.6	59.4
6	BF, FYM	7.52	0.48	0.77	27.1	428	1.12	1.77	53.8	55.8
7	BF, PM, FYM	8.38	0.23	0.80	28.2	325	1.21	1.85	54.6	56.8
8	BF, FYM	8.28	0.25	0.71	21.8	225	1.05	1.93	52.8	60.4
9	BF, PM, FYM	8.72	0.52	0.90	12.6	245	0.88	1.71	82.4	56.3
10	BF, PM, FYM	8.12	0.38	0.68	13.3	265	1.08	1.88	67.8	62.5
11	BF, PM, FYM	8.44	0.29	0.62	12.1	225	1.26	1.99	70.5	63.5
12	BF, PM, FYM	7.95	0.25	0.74	28.8	250	1.16	2.12	76.8	59.6
13	BF, FYM	8.06	0.46	0.84	25.8	245	1.05	1.55	82.8	58.4
14	BF, FYM	8.15	1.04	0.90	18.4	220	0.97	1.42	64.7	57.4
15	FYM	8.07	0.51	0.80	24.1	230	1.15	1.82	51.1	54.6
16	BF, FYM	7.93	0.32	0.74	25.6	190	1.04	1.72	58.7	61.5
17	BF, FYM	8.23	1.11	0.93	36.6	153	1.17	1.76	49.6	62.7
18	BF, FYM	7.81	0.50	1.02	27.0	170	1.08	1.84	55.8	56.9
19	BF, PM, FYM	8.19	0.31	0.77	9.9	148	1.05	1.8	54.9	64.8
20	BF, FYM	7.66	0.67	0.84	9.3	235	1.20	1.92	67.9	64.9
21	BF, FYM	6.99	0.74	0.84	24.1	176	1.27	1.78	80.5	62.8
22	PM, FYM	7.92	0.68	0.71	27.0	255	0.89	1.68	78.5	62.1
23	PM, FYM	8.24	0.28	0.99	27.8	180	1.18	1.74	78.4	65.4
24	FYM	7.61	0.27	0.93	10.6	200	1.32	1.62	70.6	58.7

* BF-Biofertilizers, PM-Pressmud, FYM- Farm yard manure and effluent applied field.

*BD-Bulk density, PD-Particle density, WHC-Water holding capacity

Table 3-3. Biological properties of soil samples collected from farmers' field at Lakshmipuram						
BF,PM, FYM & effluent applied field*	**Log cfu/g**					
	Total Bacatarial Load	***Shigella* sp./ *Salmonella paratyphi A***	***Enterobacter* sp.**	***Proteus mirabilis***	***Staphylococcus* sp.**	**Fungi**
BF, PM, FYM	6.009	3.699	BDL	3.301	4.447	3.602
BF, PM, FYM	6.193	BDL	BDL	3.477	4.556	3.699
BF, PM, FYM	6.064	3.301	BDL	4.431	4.322	3.845
BF, PM, FYM	5.964	BDL	3.301	BDL	3.903	3.301
BF, PM, FYM	5.531	BDL	3.000	4.079	3.699	3.903
BF, PM, FYM	6.107	BDL	3.602	3.301	4.279	3.477
BF, PM, FYM	5.580	BDL	4.041	3.301	3.699	4.204
FYM	5.914	4.919	5.045	BDL	4.079	BDL
FYM	6.033	4.663	4.623	4.724	5.152	4.857
BF, PM, FYM	6.072	3.000	5.004	BDL	4.000	3.000
BF, PM, FYM	5.929	4.740	4.875	BDL	4.792	3.699
FYM	5.982	4.813	4.863	BDL	4.806	3.778
FYM	6.340	4.613	BDL	4.708	5.307	4.813
FYM	6.320	4.544	BDL	4.681	5.276	4.653
FYM	6.297	4.505	BDL	4.591	5.250	4.591
BF, PM, FYM	6.021	BDL	BDL	BDL	5.057	4.041
BF, PM, FYM	5.978	BDL	BDL	BDL	4.991	3.954
BF, PM, FYM	5.949	BDL	BDL	BDL	4.892	3.845
BF, PM, FYM	5.544	BDL	BDL	BDL	4.531	4.230
BF, PM, FYM	5.613	BDL	BDL	BDL	4.477	4.041
FYM	5.580	BDL	BDL	BDL	4.447	3.954
BF, PM, FYM, effluent	6.320	BDL	3.000	BDL	5.210	4.716
BF, PM, FYM, effluent	6.401	3.477	3.301	3.699	5.276	4.613
BF, PM, FYM, effluent	6.199	BDL	3.301	BDL	5.217	4.505

Note: BF – Bio Fertilizer, PM – Press Mud, FYM – Farm yard Manure and effluent applied soil

Biological properties of soil samples from farmer's of Lakshmipuram villages

In the 24 soil samples collected from farmer's field at Lakshmipuram, pathogens *Proteus* sp., *Salmonella* sp. and *Shigella* sp were detected in 45.8% of samples with more than 3 cfu/g. *Enterobacter* sp (Table 3-3). was detected in 50 % of the soil samples with a load more than 3 cfu/g. Whereas, *Staphylococcus* sp. and *was* detected in 100% of the soil samples with a bacterial load ranging from 3 to 5.3 cfu/g. Around 95.8% of samples had fungi at a load raninging between 3 to 4.8 cfu/g. The total culturable bacterial load in the farmer's field ranged from 5.53 to 6.4 cfu/g.

Table 3-4. Biological properties of soil samples collected from farmers' field at Vuyyuru						
BF,PM, FYM & effluent applied field*	**Log cfu/g**					
	Total Bacatarial Load	***Shigella* sp./ *Salmonella paratyphi A***	***Enterobacter* sp.**	***Proteus mirabilis***	***Staphylococcus* sp.**	**Fungi**
BF, FYM	5.531	BDL	BDL	BDL	4.898	4.000
BF, PM, FYM	5.447	BDL	BDL	BDL	4.708	3.699
BF, PM, FYM	5.279	BDL	BDL	BDL	4.672	3.903
BF, PM, FYM	5.580	BDL	BDL	BDL	4.663	3.602
BF, PM, FYM	5.544	BDL	BDL	BDL	4.716	3.699
BF, FYM	5.613	BDL	BDL	BDL	4.690	3.477
BF, PM, FYM	5.556	3.477	BDL	BDL	4.663	3.699
BF, FYM	5.544	BDL	BDL	BDL	4.591	3.602
BF, PM, FYM	5.447	BDL	BDL	BDL	4.491	3.699
BF, PM, FYM	5.505	BDL	BDL	BDL	5.196	3.699
BF, PM, FYM	5.613	BDL	BDL	BDL	5.037	3.602
BF, PM, FYM	5.681	BDL	BDL	BDL	5.152	3.699
BF, FYM	5.591	BDL	BDL	BDL	4.940	3.477
BF, FYM	5.531	BDL	BDL	BDL	4.914	4.041
FYM	5.653	BDL	BDL	BDL	5.029	4.176
BF, FYM	5.785	BDL	BDL	BDL	4.964	4.613
BF, FYM	5.544	BDL	BDL	BDL	5.033	3.903
BF, FYM	5.580	BDL	BDL	BDL	4.987	3.954
BF, PM, FYM	6.476	5.332	5.477	5.477	5.316	4.114
BF, FYM	5.944	4.176	4.322	4.255	4.813	4.041
BF, FYM	5.740	4.041	3.845	3.954	4.708	3.903
PM, FYM	5.415	BDL	BDL	3.903	4.146	BDL
PM, FYM	5.342	BDL	BDL	3.699	4.041	3.301
FYM	5.491	BDL	BDL	3.602	4.204	3.000

Note: BF – Bio Fertilizer, PM – Press Mud, FYM – Farm yard Manure and effluent applied soil

Biological properties of soil samples from farmer's of Vuyyuru villages

In the 24 soil samples collected from farmer's field at Vuyyuru, pathogens *Salmonella* sp. and *Shigella* sp were detected in 16.6 % of samples with 3.4 to 5.3 cfu/g. *Enterobacter* sp. and *Proteus* sp (Table 3-4). was detected in 3 % and 25% of the soil samples respectively with a load more than 3.6 cfu/g. Whereas, *Staphylococcus* sp. and *was* detected in 100% of the soil samples with a bacterial load ranging from 4 to 5.3 cfu/g. Around 95.8% of samples had fungi at a load ranging between 3 to 4.6 cfu/g. The total culturable bacterial load in the farmer's field ranged from 4 to 6.4 cfu/g.

Groundwater analysis from farmer's field

Physico-chemical properties of ground water samples from Lakshmipuram villages

The well water were collected for analysis from the sites at Lakshmipuram where farm yard manure (FYM), Bio-fertilizer (BF), Press mud (PM) and effluent are used separately or together for agriculture. The results are given in the Table 3-5. The pH of the water from

FYM was 7.81 which increased to 8.15 in the water from lands using BF, PM and FYM. The pH had decreased to 7.66 in the land using BF, PM, FYM and effluent. The conductivity of water from FYM was 0.93 mS/cm which increased to 1.24 mS/cm as BF & PM were used along with FYM. This increase in EC may be due to the use of press mud. The EC was comparatively low (0.79 mS/cm) when the effluent water was used with BF, PM and FYM. The chemical oxygen demand (COD) of the well water in the lands where FYM is used was 400 mg/L, which increased to 2400 mg/L when press mud was used additionally along with BF. COD was maximum (3000 mg/L) when effluent and PM were used with BF & FYM. BOD, TSS and TDS of the water was 182, 6.7 & 100 mg/L respectively where FYM alone was used and it increased to 1091, 400 & 600 mg/L respectively when press mud and BF were added which was further increased to 1364, 500 and 750 mg/L respectively on adding effluent.

Table 3-5. Physico-chemical properties of water samples collected from farmers' field at Lakshmipuram

Sl. No	BF,PM, FYM & WW applied field*	pH	E_C (mS/cm)	E_h mV	COD mg/L	BOD mg/L	TSS mg/L	TDS mg/L
1	BF, PM, FYM	8.03	1.13	-60	320	145	53.3	80
2	BF, PM, FYM	8.15	1.24	-71	0	0	0	0
3	BF, PM, FYM	7.31	0.48	-17	320	145	53.3	80
4	BF, PM, FYM	7.98	1.09	-55	400	182	66.7	100
5	BF, PM, FYM	8.03	1.13	-60	560	255	93.3	140
6	BF, PM, FYM	7.15	0.33	-6	400	182	66.7	100
7	BF, PM, FYM	7.32	0.49	-18	400	182	66.7	100
8	FYM	7.65	0.79	-35	400	182	66.7	100
9	FYM	7.02	0.21	0	80	36.4	13.3	20
10	BF, PM, FYM	6.38	0.37	38	560	255	93.3	140
11	BF, PM, FYM	7.48	0.63	-10	1400	636	233	350
12	FYM	7.7	0.83	-42	240	109	40	60
13	FYM	7.81	0.93	-46	200	90.9	33.3	50
14	FYM	6.76	0.02	17	320	145	53.3	80
15	FYM	7.51	0.66	-29	400	182	66.7	100
16	BF, PM, FYM	7.56	0.7	-31	2000	909	333	500
17	BF, PM, FYM	7.42	0.58	-23	1320	600	220	330
18	BF, PM, FYM	7.43	0.59	-23	2400	1091	400	600
19	BF, PM, FYM	7.37	0.53	-20	1280	582	213	320
20	BF, PM, FYM	7.01	0.2	-1	2160	982	360	540
21	FYM	7.21	0.39	-11	200	90.9	33.3	50
22	BF, PM, FYM, effluent	7.65	0.79	-37	1600	727	267	400
23	BF, PM, FYM, effluent	7.66	0.79	-38	2800	1273	467	700
24	BF, PM, FYM, effluent	7.51	0.66	-28	3000	1364	500	750

Note: BF – Bio Fertilizer, PM – Press Mud, FYM – Farm yard Manure and effluent

Physico-chemical properties of ground water samples from Vuyyuru villages

The well water from Vuyyuru were collected for analysis from the lands where farm yard manure (FYM), Bio-fertilizer (BF), Press mud (PM) and effluent are used separately or together for agriculture. The results are provided in theTable 3-6. The pH of the water from FYM was 7.61 which increased to 8.2 in the water from lands using PM and FYM whereas it was 8.06 in the water from lands using PM with FYM. The pH of the water from the land

using BF, PM, FYM and effluent was 8.03 which is similar to the pH of water from BF and FYM used land. The conductivity of water from FYM was 0.75 mS/cm which increased to 1.3 mS/cm as PM & FYM and reduced to 1.16 when BF was used instead of PM which also decreased when all BF, PM and FYM were used together. This increase in EC may be attributed to the use of press mud. The chemical oxygen demand (COD) of the well water in the land where FYM is used was 100 mg/L, which increased to 1200 mg/L when press mud was used additionally along with FYM and to 600 mg/L when BF was used additionally with FYM. COD was 600 mg/L when BF was used with PM & FYM which shows that COD had decreased due to the microbes present in BF. BOD, TSS and TDS of the water was high where PM was used with FYM and it decreased when BF was added to PM & FYM. They were less when was used alone and also when used with BF.

Table 3-6. Physico-chemical properties of water samples collected from farmers' field at Vuyyuru

Sl. No.	BF,PM, FYM & WW applied field*	pH	E_C (mS/cm)	E_h mV	COD mg/L	BOD mg/L	TSS mg/L	TDS mg/L
1	BF, FYM	7.4	0.56	-22	560	255	93.3	140
2	BF, PM, FYM	7.39	0.55	-21	800	364	133	200
3	BF, PM, FYM	7.32	0.49	-17	640	291	107	160
4	BF, PM, FYM	7.59	0.73	-33	420	191	70	105
5	BF, PM, FYM	8	1.1	-58	300	136	50	75
6	BF, FYM	7.61	0.75	-35	560	255	93.3	140
7	BF, PM, FYM	7.71	0.84	-43	320	145	53.3	80
8	BF, FYM	8.06	1.16	-65	400	182	66.7	100
9	BF, PM, FYM	7.62	0.76	-36	320	145	53.3	80
10	BF, PM, FYM	7.75	0.88	-46	480	218	80	120
11	BF, PM, FYM	7.2	0.38	-12	400	182	66.7	100
12	BF, PM, FYM	7.34	0.5	-20	560	255	93.3	140
13	BF, FYM	7.8	0.92	-49	100	45.5	16.7	25
14	BF, FYM	7.8	0.92	-49	220	100	36.7	55
15	FYM	7.61	0.75	-36	40	18.2	6.67	10
16	BF, FYM	7.84	0.96	-51	300	136	50	75
17	BF, FYM	7.79	0.91	-48	320	145	53.3	80
18	BF, FYM	7.84	0.96	-50	360	164	60	90
19	BF, PM, FYM	8.03	1.13	-62	400	182	66.7	100
20	BF, FYM	7.33	0.49	-19	180	81.8	30	45
21	BF, FYM	7.89	1	-54	620	282	103	155
22	PM, FYM	8.22	1.3	-74	1200	545	200	300
23	PM, FYM	7.3	0.47	-16	40	18.2	6.67	10
24	FYM	7.33	0.49	-18	100	45.5	16.7	25

Note: BF – Bio Fertilizer, PM – Press Mud, FYM – Farm yard Manure

Biological properties of ground water samples from Lakshmipuram villages

In the 24 groundwater samples collected from farmer's field at Lakshmipuram, pathogens *Shigella* sp were detected in 66.6 % of samples with a load of 2.3 to 4.3 cfu/g. *Enterobacter* sp. and *Proteus* sp (Table 3-7) was detected in 33.3 % and 75% of the water samples respectively with a load more than 2 cfu/g. Whereas, *Staphylococcus* sp. and *was* detected

in 37.5% of the water samples with a bacterial load ranging from 2.4 to 5.4 cfu/g. The total culturable bacterial load in the ground water samples from farmer's field ranged from 3.3 to 6.1 cfu/g.

Table 3-7. Biological properties of groundwater samples collected from farmers' field at Lakshmipuram

BF,PM, FYM & WW applied field*	**Log cfu/ml**				
	Total Bacterial Load	***Shigella* sp.**	***Enterobacter* sp.**	***Proteus* sp.**	***Staphylococcus* sp.**
BF, PM, FYM	4.996	3.113	BDL	2.477	BDL
BF, PM, FYM	4.934	3.204	BDL	BDL	BDL
BF, PM, FYM	4.944	BDL	2.602	2	BDL
BF, PM, FYM	4.602	2.301	BDL	2	BDL
BF, PM, FYM	5.336	2.602	BDL	2.699	BDL
BF, PM, FYM	4.663	BDL	BDL	2.301	BDL
BF, PM, FYM	4.763	3.556	BDL	3.322	BDL
FYM	3.301	BDL	2.699	2.602	BDL
FYM	5.230	BDL	BDL	BDL	BDL
BF, PM, FYM	6.076	BDL	BDL	5.477	5.461
BF, PM, FYM	3.903	BDL	BDL	BDL	BDL
FYM	5.270	3.079	3.544	3.934	2.477
FYM	5.248	2.699	2	2.301	2.477
FYM	4.778	2	BDL	2.699	2.477
FYM	5.303	4.336	4.012	4.272	3.9139
BF, PM, FYM	5.294	2.903	BDL	3.672	BDL
BF, PM, FYM	5.117	2.477	2.301	BDL	BDL
BF, PM, FYM	5.225	3.799	2.301	3.924	BDL
BF, PM, FYM	4.322	BDL	BDL	BDL	3.279
BF, PM, FYM	5.556	2.301	BDL	3.301	BDL
FYM	4.785	3.255	2.778	3.881	3.447
BF, PM, FYM, Effluent	5.134	3.342	BDL	3.322	2.845
BF, PM, FYM, effluent	4.477	2.903	BDL	2.477	BDL
BF, PM, FYM, effluent	6.107	BDL	BDL	BDL	4.342

Biological properties of ground water samples from Vuyyuru villages

In the 24 groundwater samples collected from farmer's field at Vuyyuru, pathogens *Shigella* sp was detected in 58.3 % of samples with a load of 2.3 to 4.3 cfu/g (Table 3-8). *Enterobacter* sp. and *Proteus* sp. was detected in 29.1 % and 70.8% of the water samples respectively with a load more than 2 cfu/g. Whereas, *Staphylococcus* sp. was detected in 16.6% of the water samples with a bacterial load ranging from 2 to 2.6 cfu/g. The total culturable bacterial load in the ground water samples from farmer's field ranged from 3.3 to 5.4 cfu/g.

Table 3-8. Biological properties of groundwater samples collected from farmers' field at Vuyyuru					
BF,PM, FYM & WW applied field*	**Log cfu/ml**				
	Total Bacterial Load	***Shigella* sp.**	***Enterobacter* sp.**	***Proteus* sp.**	***Staphylococcus* sp.**
BF, FYM	3.477	BDL	BDL	BDL	BDL
BF, PM, FYM	5.057	3.146	3.940	4.057	BDL
BF, PM, FYM	5.190	4.260	3.982	4.455	BDL
BF, PM, FYM	5.417	3.362	BDL	3.398	BDL
BF, PM, FYM	4.833	3.447	3.881	3.944	BDL
BF, FYM	5.121	2.301	BDL	2.602	BDL
BF, PM, FYM	4.740	3.770	2.699	3.82	2
BF, FYM	4.505	3.361	BDL	3	BDL
BF, PM, FYM	3.301	BDL	BDL	BDL	BDL
BF, PM, FYM	5.188	4.296	2	4.477	BDL
BF, PM, FYM	5.107	2.477	3.643	3.851	2.954
BF, PM, FYM	4.447	BDL	BDL	2.301	BDL
BF, FYM	4.851	BDL	BDL	2.602	BDL
BF, FYM	4.892	BDL	BDL	BDL	BDL
FYM	4.886	BDL	BDL	2.477	BDL
BF, FYM	3.602	BDL	BDL	BDL	BDL
BF, FYM	4.519	4.033	BDL	4.350	BDL
BF, FYM	5.320	3.0791	BDL	3.204	BDL
BF, PM, FYM	5.083	3.204	BDL	3.398	2.602
BF, FYM	3.301	BDL	BDL	BDL	BDL
BF, FYM	4.322	2.477	BDL	BDL	2.477
PM, FYM	5.330	BDL	BDL	BDL	BDL
PM, FYM	5.029	3.431	4.086	3.763	BDL
FYM	3.301	BDL	BDL	2.477121	BDL

Implications of wastewater use in agriculture on socio-economics in Karnataka (Dharwad study)

Sampling Procedure

Three villages namely, Gabbur, Katnur and Mavanur of Hubli taluk wherein farmers extensively use sewage water generated from the Hubli-Dharwad Municipal Corporation for irrigation were chosen considering volume and the extent of use of sewage water for agriculture under diverse production patterns in the peri-urban area. For comparison Parasapur village adjacent to the above villages where fresh water is used for irrigation was chosen as a control village. For the purpose of study, sample of 30 farmers using sewage water for irrigation in each of these villages were selected randomly. Another 45 sample farmers were selected randomly from control village where fresh water was used for irrigation.

Nature and Sources of Data

Primary data relating to demographic profile, assets position, pattern of land ownership, cropping pattern, input utilization pattern, yield, costs and returns for crops, sources and quality of water and extent of irrigation, problems associated with use of sewage water for irrigation and its impact on soil, water and human health, etc were collected using a pre-tested and well-structured schedule.

Results

Ownership pattern of landholding

Pattern of land ownership by farmers in the sewage and fresh water/control villages indicated that total operated land among sewage water villages was found to be less at 1.75 ha and was almost was twice at 3.37 ha in the fresh water control village (Table 3-9). The status on area irrigated by farmers across seasons implied higher irrigation intensity among sewage water villages over fresh water village. This was mainly attributed to lifting of sewage water for irrigation round the year in addition to own groundwater source. The farmers in the sewage water villages also had the tendency of taking irrigated land on lease for cultivation. Similar trend of leasing of irrigated land was observed even in fresh water village.

Table 3-9. Land ownership pattern among farmers (in hectares)

Villages	Type of land	*Kharif*		*Rabi*		Total land holding
		Irrigated cultivated	Dry land cultivated	Irrigated cultivated	Dry land cultivated	
Sewage water villages	Total owned	1.26	0.36	1.26	0.30	1.48
	Total operated	1.66	0.36	1.66	0.30	1.75
Fresh water/ Control Village	Total owned	1.58	1.19	1.58	1.19	2.77
	Total operated	2.18	1.19	2.18	1.19	3.37

Note: Operated land also includes leased in land

Impact of sewage water on health status

The health status of members of the sample respondents in the sewage and fresh water used indicated large number of family members suffered due to heath related problems in case of sewage water used villages over that of fresh water used village (Table 3-10). This implied a majority of the family members suffered from diarrheal diseases (22.6%), cholera (4.5%), malaria (2.6%) and typhoid (4.0%) in the sewage water villages. Higher incidence of health related problems/diseases could be due to the increased mosquito menace in these villages, greater chance of contamination of drinking water sources, consistent use of sewage water for crops that could have caused a greater chance of contamination of food and could be attributed to lack of sanitation measures and unhygienic practices among the farmers in the management of waste water for irrigation without taking any precautionary measures. This eventually led to higher per capita annual expenditure of Rs. 1917 incurred for treatment of health related problems against an expenditure of only Rs.835/person in case of control/fresh water village.

Table 3-10. Impact of sewage water use on health status of farmers		
Health particulars	**Sample category (%)**	
	Sewage water villages	**Fresh water villages**
Total no. of households suffering from health related problems/diseases	36.7	26.7
Total No. of family members suffering from health problems	33.7	18.8
Type of Disease		
1. Diarrheal diseases	22.6	12.3
2. Cholera	4.5	2.6
3. Malaria	2.6	2.0
4. Typhoid	4.0	2.0
Total household members	**100.0**	**100.0**
Annual per capita health expenditure (Rs.)	1917.0	835.0
Source of finance		
Own fund	29.2	14.0
Hand loan	2.6	2.9
SHG Loan	1.9	2.0
Distance to health facility (in km)	8.0	6.0

Treatment of drinking water

Majority of the farmers in sewage water (71.1%) villages and fresh water (66.7 %) village adopted water treatment by straining through cloth when water supplied was turbid, whereas, 30 per cent and 20 per cent in respective villages boiled water before drinking (Table 3-11). Among the sewage water villages, by virtue of location close to city, a large number of households of Gabbur village have settled in Hubli city and hence are more health conscious. The use of water filters was very rare (2.2% and 8.9%, respectively). About 31.1 per cent farmers in sewage water villages expressed that the colour of the drinking water was not normal and had colour problems w.r.t. groundwater upon its supply indicating possible contamination of ground water which was not noticed in case of fresh water village.

Sources of irrigation water

The tube wells was the major source of water for irrigating crops both in the experimental and control villages. Experimental sewage water villages majority (47.8%) households were having tube wells where as in the fresh water used village, 80 per cent farmers owned tube wells as irrigation source (Table 3-12). Tube wells are functional despite varied water yields. Complete drying up of tube wells in both experimental and control villages hasn't been noticed, tube wells are productive from last 11 to 12 years which can be ascribed to nearly stable rainfall pattern in the area which falls in transitional belt.

The water table depth in the experimental sewage water villages was observed to be at 228 feet and was found to be much closer to the surface when compared to that in the control village which was found to be at 275 feet. The average depth of tube wells dug was also less in sewage water villages (273 ft) over fresh water (335 ft) village. This could be due to the

percolation of the perennial sewage water round the year into the soil surface which influenced depth of tube wells and thereby on investment cost of tube wells.

Table 3-11. Treatment of drinking water by households (Per cent)

Category/ Treatment	Sewage water villages	Fresh water village
Boil water	30.0	20.0
Strain through cloth	71.1	66.7
Use of water filters	2.2	8.9
Problems in ground water		
Colour problems	31.1	-
Average time required to fetch/collect water		
15 min	3.3	4.4
30 min	5.6	-
>30 min	11.1	28.9
No. of households having access to water at their premises		
No time	80.0	66.7

Table 3-12. Status and sources of irrigation water

Irrigation particulars	Sewage water villages	Fresh water village
Number of tube wells (%)	47.8	80.0
Investment cost of tube well (Rs)	90,000	1,10,000
Functioning of tube wells	Functioning	Functioning
No. of productive years of tube wells	12	11
Average depth of tube wells (ft)	273	335
Average water table depth (ft)	228	275
Problem of drying up of tube wells	Not observed	Not observed

Cropping pattern and its intensity

It is evident from the cropping pattern that maize, soybean, cotton were being cultivated in these villages during *kharif* whereas during *Rabi* season maize and sorghum were the predominated crops grown. The farmers from the sewage and fresh water villages have an advantage of the availability of Hubli and Dharwad markets and hence allocated considerable area under leafy vegetables and tomato crops (25 % and 27%, respectively) during summer season **(**Table 3-13**)**. Interestingly, it could be observed from the cropping pattern results that major owned/cropped area was under perennial fruit crops such as Guava (15%) and Sapota (10%) due to proximity to perennial sewage water availability round the year and also the availability of market while, the seasonal crops were cultivated by the farmers as inter crops. Contrarily, there was no area allocation under fruit crops in the fresh water village.

Among the seasonal crops, maize occupied the largest area (12.8%) in *Kharif* in sewage water villages as well as in fresh water control village (16.4%) followed by cotton (9.9% and

13.9%, respectively) and soybean (7.4% and 13.1%, respectively). During *Rabi* season, sorghum (11% and 20%) dominated as major crop in these villages followed by maize (about 9%). Thus, proportion of cropped area allocated during *kharif* season was 30 per cent and 43.4 per cent, respectively in the sewage and fresh water villages. The corresponding areas allocated were 20.1 and 29.5 per cent, respectively during *Rabi* season. This has resulted in a higher cropping intensity of 296 per cent in sewage water villages when compared to intensity of 230 per cent in fresh water control village.

Methods and composition of irrigation water

In the sewage water used villages, the farmers' practiced providing irrigations using both sewage and fresh water and they have found out the ratios of sewage water irrigation to fresh water irrigation on their own through field observations and the same varied from crop to crop (Table 3-13**)**. For field crops the ratio of sewage to fresh water practiced by the farmers, approximately ranged from 1:1 to 1:4 while, for fruit crops the ratio practiced is 1:4. They have found out that by using sewage water alone would damage the soil structure and the quality of the output. For instance, especially in case of plantations Guava and Sapota, irrigating only with sewage water, they have witnessed, has caused fruit drops, thereby contributing to decreased yields. Also it has been found to affect the keeping quality especially of highly perishable products such as fruits and vegetables.

Table 3-13. Cropping pattern adopted by farmers during 2013-14 (Per farm in Hectares)

Season	Crop	Variety	Sewage water villages	% area to the gross cropped area	Fresh water irrigated village	% area to the gross cropped area
Kharif	Soybean	JS 355	0.38	7.4	1.02	13.1
	Maize	CP 818	0.66	12.8	1.28	16.4
	Cotton	Kanaka	0.51	9.9	1.08	13.9
	Sub-total		**1.56**	**30.1**	**3.37**	**43.4**
Rabi	*Rabi* sorghum	M 35 1	0.57	11.0	1.59	20.5
	Maize	CP 818	0.47	9.1	0.70	9.1
	Sub-total		**1.04**	**20.1**	**2.29**	**29.5**
Summer	Leafy Vegetables	Local	0.90	17.4	0.00	0.0
	Tomato	Hybrid	0.39	7.5	2.10	27.1
	Sub-total		**1.29**	**24.9**	**2.10**	**27.1**
Plantation Crops	Guava	Lucknow / Sardar	0.78	15.0	-	0.0
	Sapota	Cricket Ball	0.52	10.0	-	0.0
	Sub-total		**1.30**	**25.0**	**-**	**0.0**
	Gross Cropped Area		**5.19**	**100.0**	**7.76**	**100.0**
	Net Sown Area		1.75		3.37	
	Cropping intensity (%)		**296.35**		**230.40**	

Table 3-14. **Methods and composition of irrigation water management**			
Crop	**Methods of irrigation**	**No. of Irrigation**	
		Sewage water villages	**Fresh water Village**
No. of irrigations: Sewage and fresh water			
Soybean	Flooding	01	03
Maize	Flooding	03	06
Cotton	Flooding	03	09
Sorghum	Flooding	01	01
Guava	Basin method	03	13
Sapota	Basin method	03	12
Tomato	Flooding	2	4
No. of irrigations: Fresh water			
Soybean	Flooding	0	3
Cotton	Flooding	0	13
Maize	Flooding	0	10
Sorghum	Flooding	0	2
Tomato	Flooding	0	7

Crop yield performance under sewage and fresh water use

Crops like soybean, maize, cotton, *Rabi* sorghum, guava, sapota, methi and tomato were the crops grown in experimental villages, the farmers in the control village also cultivated these crops except guava, sapota and methi. The perceptions of the farmers that they get more yields by using sewage water for irrigation compared to fresh water. This was reflected by higher crop yield levels in the sewage water over fresh water irrigated crops (Table 3-15 and *Figure 3-1***)**. While, an increase in yield was by 21.7 per cent in case of soybean in sewage water villages over the control village, the corresponding yield increases in maize, cotton, sorghum and tomato were 30.1 per cent, 16.3 per cent, 26.0 per cent and 30.7 per cent, respectively.

Table 3-15. Crop yield performance under sewage and fresh water use (q per ha)				
Villages	**Crops**	**Main product yield (q per ha)**	**Per cent increase in main yield under sewage water irrigation over fresh water**	**By product yield (q per ha)**
Sewage water villages				
	Soybean	28.8	21.7	42.1
	Maize	59.0	30.1	90.1
	Cotton	26.9	16.3	-
	Guava	258.5	-	-
	Sapota	131.9	-	-
	Sorghum	31.3	26.0	52.5
	Methi	132.4	-	-
	Tomato	260.6	30.7	-
Fresh water/Control village				
	Soybean	22.5	-	30.6
	Maize	41.3	-	61.3
	Cotton	22.5	-	-
	Sorghum	23.1	-	37.5
	Tomato	180.6	-	-

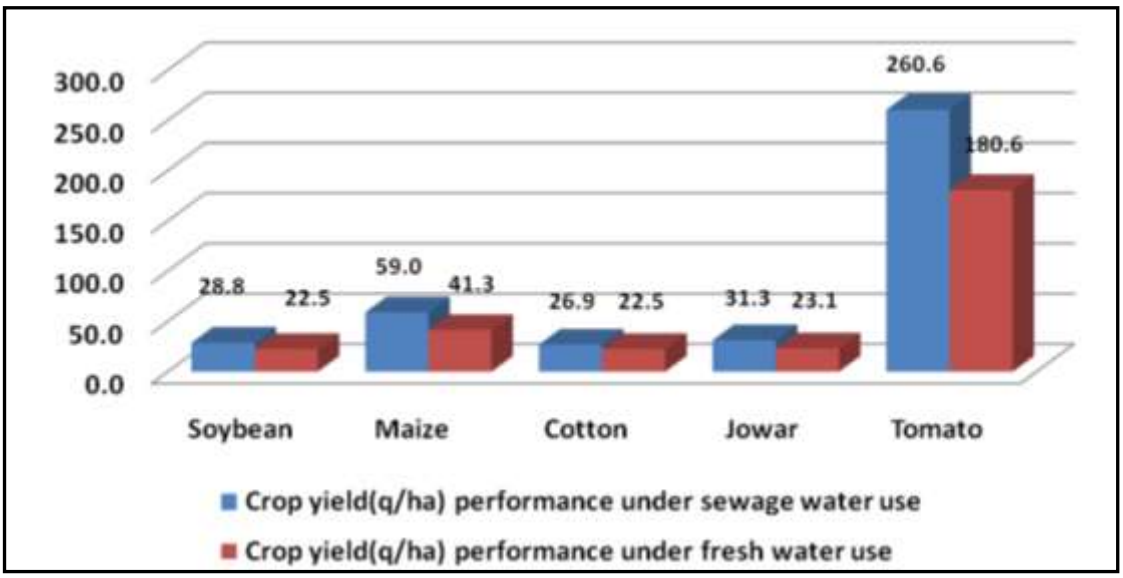


Figure 3-1. Crop yield performance under sewage and fresh water muse (q per ha)

Cost and returns structure for major crops

The results on input costs in the cultivation of crops and their composition and returns for major crops in sewage and fresh water irrigated conditions through light on pattern, behaviour and extent of outlay of funds spent in the cultivation of crops and in turn on the extent of gross and net returns realised. The composition of different input costs as a proportion of total cost is presented in Table 3-16, Table 3-17, and *Figure 3-2***.** The analysis of cost structures in respect of major crops was made using variable and fixed cost components. In case of seasonal crops cultivated using sewage water, the variable cost accounted a largest share in the total cost and varied between 70 and 82 per cent. The corresponding variable cost composition in case of fresh water production varied between 74 and 84 per cent. In case of maize, soybean, cotton and sorghum under sewage water production, out of the total cost incurred for crops (Rs.53593 per ha, Rs.53070, Rs.71610 and Rs.43658 per ha, respectively), the variable costs respectively were Rs.40458, Rs.39935, Rs.58475, and Rs.30523 per ha in that order. The cost sharing among different variable inputs used across crops under sewage water production implied that a sizable expenditure was made on labour component and it ranged between 31.0 and 39.0 per cent of the total cost. While, the cost spent on bullock and machine labour components together was between 9.5 and 20.6 per cent, respectively. Similar trend of cost sharing was observed w.r.t. human labour (29.0% and 39.0%) and bullock and machine labour (9.2% and 20.0 %) even in case of crops under fresh water production. Thus, it could be ascertained from the results that cost of labour component (human, bullock and machine labour together) constituted a vital cost category which substantially influenced the total cost of cultivation of crops.

Consequently, the farmers in the recent years are more inclined towards the use of machineries in farm operations to increase cost efficiency. In addition to variable costs, the fixed cost components as land revenue paid, rental value of land as opportunity cost and depreciation cost were accounted to arrive at net profit.

A critical analysis of returns realised for major crops was made to know the extent of gross and net returns realised under sewage and fresh water conditions. In case of maize, soybean, cotton

and sorghum under sewage water production, it was evident that per acre returns obtained by farmers were found to be substantially higher (Rs.102065, Rs.113283, Rs.122765 and Rs.53375 per ha, respectively) than in fresh water irrigated condition due to higher (Rs.71155, Rs.88683, Rs.100193 and Rs.39345 ha) productivities in that order.

The crop wise net returns for maize, soybean, cotton and sorghum were also found to be higher under sewage water irrigated condition. The corresponding per acre net returns were Rs. 48473, Rs. 60210, Rs.51155, and Rs.9718 in case of sewage water irrigation as against Rs.23045, Rs.40733, Rs.35023, and a negative returns of Rs.-1863 per ha. Thus, higher production efficiency was observed in the nutrient rich sewage water when irrigated with fresh ground water, and thereby, per quintal cost of production of all crops were found to be lower than in exclusively fresh water irrigated control village. The corresponding benefit-cost ratios for major crops in sewage water irrigation were higher at 1.90, 2.13, 1.71, and 1.22 compared to 1.48, 1.85, 1.54 and 0.95 in that order.

Guava and sapota were the major plantation corps in sewage water villages and the trend in costs depicted highest share w.r.t. variable cost components (88 to 90%) and the labour cost was found to be highest. The labour components (human, bullock and machine labour) together accounted to have been shared about 52 and 68 per cent cost of the total cost respectively, in case of guava and sapota. The gross returns realised in case of sapota was more (Rs.369250per ha) than in case of guava (Rs.310230 per ha) and the net returns of Rs.241500 per ha and Rs.197220 per ha respectively. The benefit cost ratios were 2.89 and 2.75 in case of sapota and guava.

The critical input costs for major crops incurred as a proportion of total cost and their comparison provide key information about the differences in the pattern of input usage as influenced by sewage water use against the use of fresh water for irrigation**.** It could be very well ascertained from the results that sewage water, a rich source of major nutrients supplier seemed to have influenced the farmers towards the lesser application of fertilizers for seasonal crops when compared to the farmers who used fresh water for irrigation to a almost negligible proportion of total cost spent on fertilizers evident in fruit crops namely, guava and sapota. Crop wise inference indicated that in case of maize, its application was less both by magnitude and as proportion of total cost under sewage water (Rs.6288 per ha and about 12%) compared to fresh water (Rs.7538 per ha and 16%) use. Similar trend of lower application of fertilizer was noticed in respect of all major crops viz, soybean at Rs.3500/acre and 6.6% in sewage water against fresh water (Rs.4950 per ha and 10.3 %) use, followed by considerable difference in cotton between sewage water (Rs.8313 per ha and about 12 %) and fresh water (Rs.14875 per ha and about 23%) use and the same results witnessed even in case of sorghum. A similar observation was made where the farmers applied micro-nutrients for crops namely maize, soybean and cotton only under fresh water irrigated condition and the proportion of the cost spent was between 1.9 per cent and 2.9 per cent of the total cost. On the extent of use of plant protection chemicals, it was found that farmers applied more of PPCs in cotton towards pest management under sewage water (8.2%) condition compared to farmers of fresh water (3.8%) used village. Similarly, the use of PPCs was only marginally more under sewage water use over their counter parts in control village in respect of maize and soybean crops with no PPCs used for sorghum.

In case of weedicide application for management of weeds in these villages, it was found that the farmers of sewage water villages applied weedicides in case maize (3.8%), soybean (1.9%) and cotton (2.9%) due to higher intensity of weed infestation as against none in fresh water irrigated village.

Table 3-16. Cost and returns structure for major crops under sewage and fresh water irrigation (Rs per ha)

Particulars	Maize		Soybean		Cotton		Sorghum		Guava	Sapota
	Sewage water	Fresh water	Sewage water	Fresh water	Sewage water	Fresh water	Sewage water	Fresh water	Sewage water	Sewage water
Seed (Kg)	2000	2000	3750	3750	5500	5250	350	400	8000	4000
FYM (t)	1965	1750	1313	1750	2375	1310	918	1255	9563	7438
Fertilisers (Kg)	6288	7538	3500	4950	8313	14875	4560	6588	7425	4505
Micronutrients (Kg)	0	1250	0	1375	0	1250	0	0	6230	4125
PPCs (l)	1230	825	2533	1965	5875	2500	0	0	875	875
Weedicides (l)	2060	0	1030	0	2060	0	0	0	2125	0
Female Human labour (mandays)	12375	10500	8625	6750	15375	12375	9000	8250	13125	15750
Male Human labour (mandays)	8500	8000	8000	7000	9500	9000	6000	5000	40000	65000
Total Human Labour (mandays)	20875	18500	16625	13750	24875	21375	15000	13250	53125	80750
Bullock labour	3000	3000	6000	4500	4500	4500	4500	3750	6000	6000
Machine labour (hours)	2118	1625	4500	4500	3000	1500	4500	4500	0	0
Interest on Working capital (7%)	923	853	688	640	1978	1840	695	695	6535	7538
Total Variable Cost	**40458**	**37340**	**39935**	**37180**	**58475**	**54400**	**30523**	**30438**	**99875**	**115230**
Land revenue	113	113	113	113	113	113	113	113	113	113
Rental value of land	10500	8750	10500	8750	10500	8750	10500	8750	10500	10500
Interest on fixed Capital (12%)	1353	1043	1353	1043	1353	1043	1353	1043	1353	1043
Depreciation cost	1170	865	1170	865	1170	865	1170	865	1170	865
Total Fixed Cost	**13135**	**10770**	**13135**	**10770**	**13135**	**10770**	**13135**	**10770**	**13135**	**12520**
Total Cost (TVC+TFC)	**53593**	**48110**	**53070**	**47950**	**71610**	**65170**	**43658**	**41208**	**113010**	**127750**
Yield (q/acre)	59	41	29	23	27	23	31	23	259	132
Gross Return	102065	71155	113283	88683	122765	100193	53375	39345	310230	369250

Table 3-17. Composition of costs under sewage and fresh water irrigation for major crops (Per cent)

Particulars	Maize		Soybean		Cotton		Sorghum		Guava	Sapota
	Sewage water	Fresh water	Sewage water	Fresh water	Sewage water	Fresh water	Sewage water	Fresh water	Sewage water	Sewage water
Seed (Kg)	3.7	4.2	7.1	7.8	7.7	8.1	0.8	1.0	7.1	3.1
FYM (t)	3.7	3.6	2.5	3.7	3.3	2.0	2.1	3.1	8.5	5.8
Fertilizers (Kg)	11.7	15.7	6.6	10.3	11.6	22.8	10.4	16.0	6.6	3.5
Micronutrients (Kg)	0.0	2.6	0.0	2.9	0.0	1.9	0.0	0.0	5.5	3.2
PPCs (l)	2.3	1.7	4.8	4.1	8.2	3.8	0.0	0.0	0.8	0.7
Weedicides (l)	3.8	0.0	1.9	0.0	2.9	0.0	0.0	0.0	1.9	0.0
Female Human labour (mandays)	23.1	21.8	16.3	14.1	21.5	19.0	20.6	20.0	11.6	12.3
Male Human labour (mandays)	15.9	16.6	15.1	14.6	13.3	13.8	13.7	12.1	35.4	50.9
Total Human Labour (mandays)	39.0	38.5	31.3	28.7	34.7	32.8	34.4	32.2	47.0	63.2
Bullock labour	5.6	6.2	11.3	9.4	6.3	6.9	10.3	9.1	5.3	4.7
Machine labour (hours)	4.0	3.4	8.5	9.4	4.2	2.3	10.3	10.9	0.0	0.0

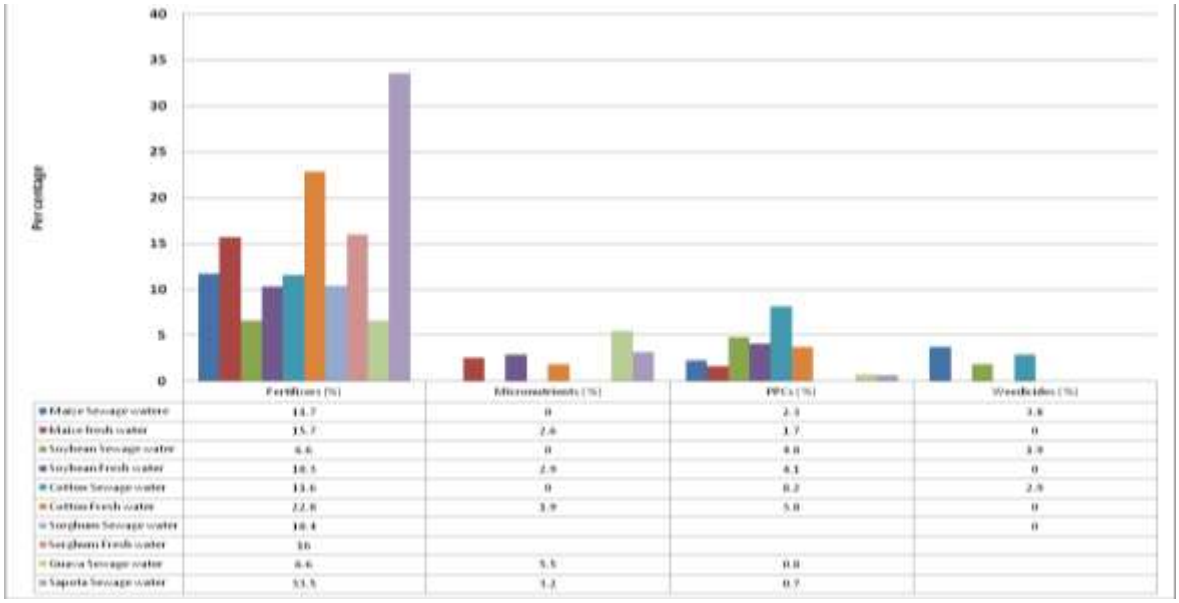


Figure 3-2. Comparison of critical input cost of major crops (% of total cost)

Farm and non-farm sources of income

The households also derived their income from various non-farm including from farm enterprises. Thus, average per family income derived from other sources including agriculture is presented in Table 3-18. They included income from faming, livestock, labour, service, pension and business. The farm income accounted the largest share in the total income in both categories of farmers and it was highest in case of farmers of sewage water villages at Rs. 314357 per farm with a share of 86 per cent followed by Rs. 180975 per farm having a share of 75.3 per cent for farmers' fresh water villages. The off-farm labour income was the second highest accounted 6.7 and 8.4per cent of the total income, respectively among sewage and fresh water villages and was almost on par across villages. Income from service was the next highest source across villages and it was more among the households of fresh water villages (12.9%) compared to households of sewage water villages (5.3%) and was mainly derived from private service in the Hubli city besides government service. An additional income from petty businesses was another source of income to the farmers in these villages such as from vegetable vending, grocery business, tailoring, etc. Thus, the average household annual income among sewage water villages was Rs. 365004 per farm as against Rs. 240573.51 per farm in case fresh water village farmers. Thus, it could be concluded that the annual average income from farming was more among the farmers of sewage water villages compared to fresh water village owing to sustained availability of nutrient rich irrigation water round the year coupled with substantial income contributed from plantation and vegetable crops on considerable area in these villages. Thereby, the families in the sewage water villages were economically considered to be better-off than their counterparts in control fresh water village.

Perceptions on quality of fruits and vegetables

The availability of sewage water for irrigation round the year in the peri-urban villages has changed way in which the farming is being done. They have been observing since decades the impact of using the sewage water and acknowledge it as boon for them as they could irrigate their crops throughout the year. While they know that in some ways it may be a boon, in some other aspects; it brings disadvantages along with it. The farmers have been

observing keenly the structure, texture, colour, keeping quality, taste, marketability, and the price dynamics of the produce using sewage waste water. All these parameters are critical in realising good returns on sale of their produce. The study attempted to understand the perceptions of the households on these parameters. Table 3-19 throws the comparison of the fruits and vegetables on the aforementioned parameters when they are grown using sewage water vis-a-vis fresh water. The information shared by farmers using sewage water plus the observations of the other fellow farmers and farmers using fresh water for fruits and vegetables is discussed below.

Table 3-18. Total annual farm and non-farm income of farmers (Rs. /farm/annum)

Source	Average for sewage water Villages (n=90)		Fresh water/Control Village (n=45)	
	Amount (Rs.)	%	Amount (Rs.)	%
Agriculture	314357	86.1	180975	75.2
Livestock	847	0.2	2110	0.9
Labour	24500	6.7	20222	8.4
Service	19500	5.3	31000	12.9
Pension	3667	1.0	3067	1.3
Business	2133	0.6	3200	1.3
Total	**365005**		**240574**	

When asked about their perceptions on the quality of fruits and vegetables grown using sewage water vis-a vis fresh water, the farmers of both the sewage water villages and fresh water village shared some common observations. The keeping quality of fruits in case of use of sewage water was reported to be lesser (1-2 days) than in case of use of fresh water (3-4 days). The colour of the fruits and vegetables grown using the sewage water was reported to be darker than in case of fresh water. Similarly, the size of the fruits generally was bigger under sewage water condition while it was small to medium as a result fresh water fruit fail to attract better market price where, the farmers using sewage water have been getting higher prices for their fruits and vegetables compared to the farmers using fresh water. In case of vegetables, the vegetables are characterized by broader leaves compared to fresh water vegetable. They also felt that fresh water output has better taste than one grown in sewage water. Majority of the farmers sell the fruits to the middlemen and generally on contract basis while in case of vegetables sale was both through the middlemen and as well as directly to the consumers.

Table 3-19. **Perceptions on quality of fruits and vegetable produced**

Particulars	Quality of fruits		Quality of Vegetables	
	Sewage Water	Fresh water	Sewage Water	Fresh water
Colour	Dark and shining	Light in colour	Dark green colour and shining	Light green colour
Size	Big	Small to Medium	Broader leaves	Narrow leaves
Taste	Less tasty	More tasty	Less tasty	More tasty
Keeping quality	1-2 days	3-4 days	Few hours/1-2 days	One day/2-3 days
Appearance	Attractive	Less attractive	Attractive	Less attractive
Price	Fetch high price	Fetch low price	Fetch high price	Fetch low price
Mode of selling	Middlemen	Middlemen	Middlemen/directly to Consumer	Middlemen/ directly to consumer

Consequence on human health

An attempt was made to capture the perceptions of the households in the sewage water irrigated villages on the health related problems they are facing and their reactions have been depicted in *Figure 3-3***.** According to farmers of these villages, the use has increased the incidence of skin diseases (19.0%) among the farmers and the serious risk of suffering from skin ailments in the years to come likely to increase. The sewage water forms a solid stratum for mosquitoes to grow and multiply. A large majority of the farmers shared their perceptions on the sewage water use and opined that the use of sewage water has been contributing multi fold increase in the mosquitoes' population which is affecting both the human health (91.1%) and animal health (70.0%).

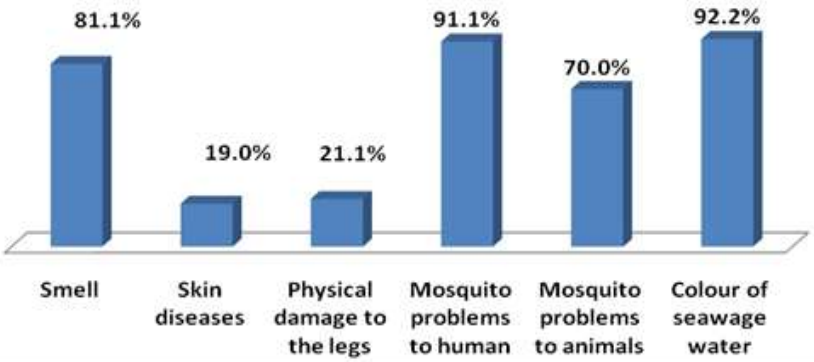


Figure 3-3. Consequence of sewage water use on human health

While, 81.1 per cent farmers reported that the sewage water use has increased foul smell emission, where as 92.2 per cent farmers inferred that the colour of sewage water was not normal and turning into greyish/dark greenish in colour. The debris that included harmful solids like used surgical instruments, nails, hacksaw blade, etc often have been found to be harming the farmers physically and shared by 21.1 per cent of sample farmers. The waste water that the farmers use also carries grece, lubricants, detergents, chemicals, acids, etc. that pose skin problems to the farmers and implied (19.0%) incidence of skin diseases such as rashes, hardening of hand skin due to direct handling of such water while irrigating crops.

Problems and prospectus of sewage water use

The farmers perceived to have been facing many problems in agriculture due to waste water use and also prospects and the same are presented in Figure 3-4. According to 94.4 per cent farmers the waste water is rich in nutrients and provides surplus nutrients to the crop and this resulted in excessive vegetative crop growth, which means that the excessive vegetative growth happens at the cost of the reproductive growth. Obviously this factor has a bearing on the ultimate productivity. As high as 72.2 per cent farmers agreed increased pest and disease incidence with the use of sewage water for irrigation, The supply of nutrients in high doses would render the leaves, stems and other parts of crops more succulent there by attracting more pest and diseases attack. Also the sewage waste water flowing through the canal might carry numerous eggs of pests that enter the fields of the farmers when this water gets pumped into the farms lands. Majority (66.7%) of the respondents mentioned the severe menace of snails in the study villages as the sewage water canal serve as

perennial breeding ground and depositing large cache of snails onto the farmers' fields during the season which damage the standing crops. Another important problem associated with use of sewage water for irrigation was increased weed infestation. As large as 81.1% farmers shared that they witnessed many weed species that they never saw before. The weed and pest infestation have risen phenomenally since last 10-15 years thereby increased the cost of plant protection measures and weeding operation.

In spite of the problems posed by sewage water use in agriculture and quantitatively captured the farmers consider sewage water availability as boon. On the positive impact of its use in agriculture, 92.2 per cent farmers each shared that by virtue of using the sewage water, they have been harvesting higher crop yields and have succeeded in saving considerably on fertilizer cost.

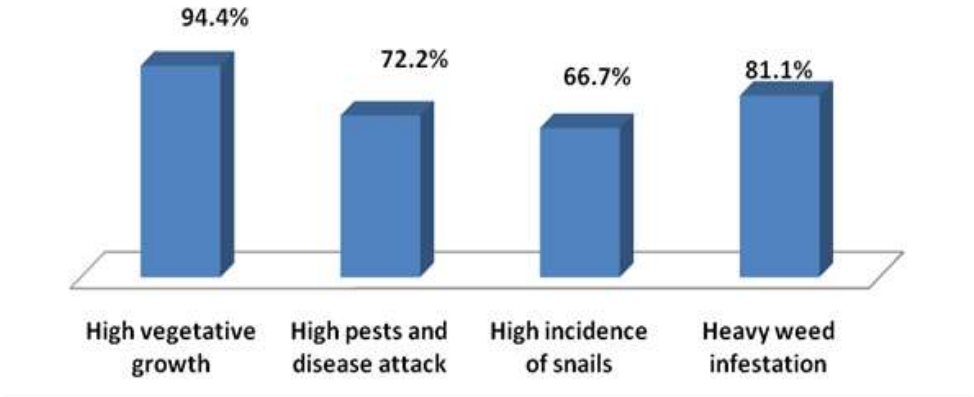


Figure 3-4. Problems of sewage water use in agriculture

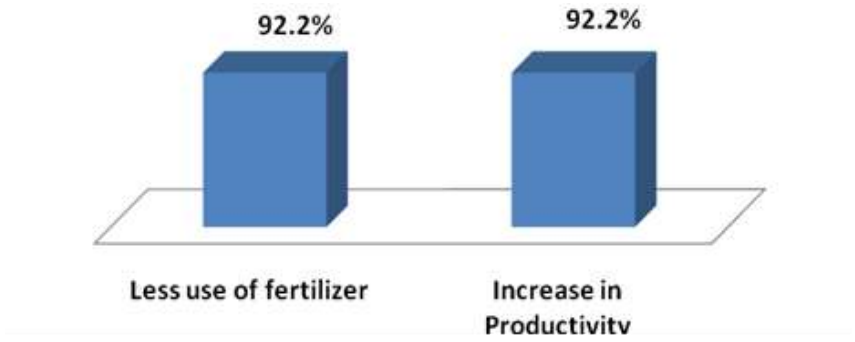


Figure 3-5. Advantages of sewage water use in agriculture

Effect of sewage water use on soil properties

The perceptions of the households on the sewage water impact on soil properties have been quantified and presented in *Figure 3-6*. About 76.0 per cent farmers observed that the sewage water had worsened the soil properties while, 11.0 per cent of them said there was no effect on soil properties due to its use. A majority of the farmers (58.9%) using sewage water gave feedback of debris getting accumulated on their farms and reported that they observed (54.4%) their soil being hardened due to the persistent/continuous use of sewage water. Only 26.7 per cent reported cracking of soil due use of sewage water. However, none

of the farmers could infer any possibility of improvement in the soil properties and only 13.3 per cent felt that they were unaware of having noticed any changes in the properties of the soil.

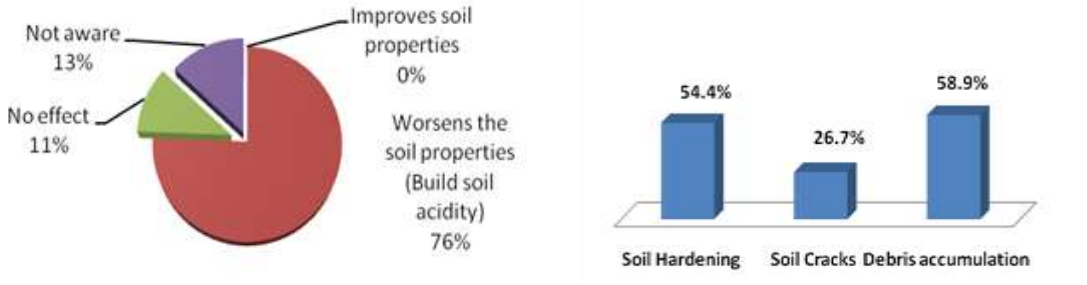


Figure 3-6. Farmer's perceptions on sewage water use on soil properties

Lessons learnt and feed back

Rapid urbanization places immense pressure on the fragile and dwindling fresh water resources and over-burdened sanitation systems, leading to environmental degradation. Thus, at this point of time wastewater is a resource of increasing importance. However, use of such sewage water without proper management poses high risks to human health and cause environmental degradation. The present investigation on the impact of sewage water in agriculture provided an opportunity to learn various dimensions-positive and negative externalities associate with the phenomenon. Some of the important lessons learnt were outlined below.

- Availability of sewage water according to farmers is a boon as a water source of irrigation water and provides opportunity to expand irrigated area and thereby explore potential benefits from its use.
- Sewage water is a rich source of essential macro nutrients-nitrogen, phosphorus, potash and there by contributed towards increased crop productivity and income of farmers and thereby induced either reduced or no use of fertilizer for crops.
- Heavy weed infestation and of diverse varieties of weed seeds observed due to sewage water irrigation amounting to increased cost on weeding operation.
- Direct handling of hazardous, contaminated and untreated sewage water without any precautions by farmers.
- Use of sewage water for long has left resulted in several heath related problems such as diarrheal diseases, cholera, malaria and typhoid among the farmers. A continuous flow of sewage water has created unhygienic environment and offered breeding ground for the mosquitoes to multiply resulting in increased health expenditure.
- Farmers acknowledged the contamination of groundwater as evident through the tube well water colour and its turbidity.
- Farmers recognized lower keeping quality and poor taste in case of fruits and vegetables when produced under sewage water contrarily produce attracted premium price for bigger size, and attractive colour.

3.2 Efficient irrigation system evaluated

Location of study area

The experiment was conducted on two different locations Jain Plastic Park and Jain Valley, Jain Irrigation Systems Ltd., Jalgaon (Maharashtra). The Jain Plastic Park lies between 75°32'55" E to 75°33'20" E longitude and 21°00'05" N to 21°00'20" N latitude. It is about 227 m above mean sea level. Jain Valley lies between 21° 05' N latitude, 75° 40'E longitude and at an altitude of 209 m above mean sea level. The climate of the area is semi - arid with 690 mm mean annual rainfall. The laboratory test was carried at the Jain Plastic Park and field evaluation was done in Jain Valley. Three water sources were selected for the experiment are Treated Fruit Waste Water (TFWW), Treated Onion Waste Water (TOWW), and Bore Well Fresh Water (BWFW). The sample of irrigation water was collected in 1000 ml of polythene bottle from each source. The parameters like TDS, EC, pH, BOD, COD, TH, Nitrate-N, Total-P, K, Na, Ca, Mg, Chloride and Sulphate-S were analysed in the laboratory. The water analysis was done at 01 DAS, 30 DAS, 60 DAS, 90 DAS and120 DAS. The results of monthly analysis of TFWW, TOWW and BWFW are presented in Table 3-20, Table 3-21, and Table 3-22.

Table 3-20. Monthly analysis of treated fruit waste water (TFWW)

Parameters	01 DAS	30 DAS	60 DAS	90 DAS	120 DAS	Average
TDS (ppm)	820.5	780	770.6	789.1	893.1	810.67
EC (dS/m)	1.142	1.165	1.362	1.224	1.388	1.22
Ph	7.20	7.50	7.10	7.30	7.25	7.27
BOD (ppm)	9.2	8.43	8.87	7.45	7.90	8.83
COD (ppm)	65.5	57.8	110.78	78.77	72.43	78.03
TH (ppm)	210	225	278	221	265	237.67
Nitrate - N (ppm)	5.55	0.36	0.78	2.33	0.66	2.23
Total - P (ppm)	0.092	1.04	0.81	0.78	0.90	0.65
K (ppm)	14.70	62	56	72	64	44.23
Na (ppm)	60	221	246	249	233	175.67
Ca (ppm)	96	80	88	92	86	88.00
Mg (ppm)	78	40	43	44	54	53.67
Chloride (ppm)	132	165	128	120	134	141.67
Sulphate - S (ppm)	43	18	82	87	97	47.67

Table 3-21. Monthly analysis of treated onion waste water (TOWW)

Parameters	01 DAS	30 DAS	60 DAS	90 DAS	120 DAS	Average
TDS (ppm)	928	1050	1112	960	987	1030.00
EC (dS/m)	1.681	1.484	1.62	1.53	1.45	1.60
pH	7.73	7.42	7.74	7.67	7.69	7.63
BOD (ppm)	10.2	18.43	16.87	13.45	19.90	15.17
COD (ppm)	105.5	97.8	112.78	158.77	98.43	105.36
TH (ppm)	324	285	378	281	295	329.00
Nitrate - N (ppm)	0.37	1.04	0.43	0.56	0.46	0.61
Total - P (ppm)	18	0.52	0.26	0.36	1.23	6.26
K (ppm)	27.6	49.8	57.87	66.8	73.20	45.09
Parameters	**01 DAS**	**30 DAS**	**60 DAS**	**90 DAS**	**120 DAS**	**Average**
Na (ppm)	71.8	182.6	41.98	56.7	84.30	98.79
Ca (ppm)	153	121	96.99	90.12	93.67	123.66
Mg (ppm)	123.5	58.74	36.31	43.24	56.89	65.45
Chloride (ppm)	53.45	128.6	130.65	122.0	116.67	104.23
Sulphate - S (ppm)	15.6	49.25	56.75	65.45	63.67	40.53

Table 3-22. Monthly analysis of bore well fresh water (BWFW)						
Parameters	**01 DAS**	**30 DAS**	**60 DAS**	**90 DAS**	**120 DAS**	**Average**
TDS (ppm)	788.68	760.50	780.80	735.55	875.88	788.30
EC (dS/m)	1.03	1.08	1.12	1.18	1.09	1.10
pH	6.54	6.77	6.99	6.79	6.58	6.734
BOD (ppm)	0.80	0.40	0.60	0.40	0.60	0.56
COD (ppm)	1.04	0.80	0.97	0.86	0.98	0.93
TH (ppm)	130	118	185	153	166	135.50
Nitrate - N (ppm)	0.35	0.43	0.52	0.86	0.21	0.48
Total - P (ppm)	0.92	1.30	1.60	1.93	1.67	1.48
K (ppm)	31.50	48.50	63.98	58.67	52.04	50.94
Na (ppm)	19.50	21.50	24.54	23.45	27.83	23.36
Ca (ppm)	48	165.50	71.2	89.67	103.86	95.65
Mg (ppm)	48.5	23.5	36.64	32.43	30.67	34.35
Chloride (ppm)	0	0	0	0	0	0
Sulphate - S (ppm)	0	0	0	0	0	0

Laboratory study

Emitter exponent prior to the clogging test

Emitter exponent was determined by measuring the discharge of 25 emitters at different pressure levels for Model A2.0, Model A4.0 , Model B2.0, Model B4.0, Model D1.1, Model D1.7 types of emitters were 0.5, 1.0, 1.2, 1.5 and 2.0 kg cm^{-2} and for Model C1.6 and Model C2.0 types of emitters were 1.0, 1.2, 1.5, 1.8 and 2.0 kg cm^{-2}. To obtain emitter exponent, four emitters were selected for measurement of discharge having position 7, 12, 13 and 21 from the first catch can or emitter and its values are presented in Table 3-23. The highest emitter exponent was found in Model A2.4 and Model A4.0 emitter *i.e.* 0.52 and lowest emitter exponent was found in Model D1.1 *i.e.* 0.01. Higher the emitter exponent greater a variation in the emitter discharge and vice versa.

Table 3-23. Emitter exponent obtained by catch can method		
Emitter Type	**Technical Details**	**Emitter Exponent**
Model A2.4	Non pressure compensating (NPC)	0.52
Model A4.0	Non pressure compensating (NPC)	0.52
Model B2.0	Non pressure compensating (NPC)	0.48
Model B4.0	Non pressure compensating (NPC)	0.49
Model C1.6	Pressure compensating, compensating non leakage, anti-syphon (PC CNL AS)	0.02
Model C2.0	Pressure compensating, compensating non leakage, anti-syphon (PC CNL AS)	0.02
Model D1.1	Pressure compensating and anti-syphon (PCAS)	0.01
Model D1.7	Pressure compensating and anti-syphon (PCAS)	0.02

Table 3-24. Effect of TFWW on the clogging resistance of emitters																	
Emitter type	**Number of emitter clogged within 15 days of test (test sample containing 10 emitters)**															**Number of emitter clogged at end of test**	**Percent of emitter clogged during test**
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Model A2.4	0	0	0	0	0	1	0	0	1	1	1	2	2	2	2	2	20
Model A4.0	0	0	0	1	1	0	0	0	0	1	2	2	2	2	3	3	30
Model B2.0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	10
Model B4.0	0	0	0	0	0	0	1	0	0	1	1	2	2	2	2	2	20
Model C1.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Model C2.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Model D1.1	0	0	1	0	2	2	3	4	5	6	7	7	8	8	8	8	80
Model D1.7	0	0	1	1	1	2	2	3	5	6	7	8	8	9	9	9	90
Avg. pH								**7.27**									
Avg. EC (dS/m)								**1.22**									
Avg. TDS (ppm)								**810.67**									

Table 3-25. Effect of TOWW on the clogging resistance of emitters																	
Emitter type	**Number of emitter clogged within 15 days of test (test sample containing 10 emitters)**															**Number of emitter clogged at end of test**	**Percent of emitter clogged during test**
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Model A2.4	0	0	0	1	1	2	2	2	1	1	1	2	2	3	3	3	30
Model A4.0	0	0	0	1	1	1	1	1	1	2	2	2	2	2	3	3	30
Model B2.0	0	0	0	0	0	0	0	0	1	0	0	1	1	2	2	2	20
Model B4.0	0	0	0	0	1	1	1	1	1	1	1	2	2	2	2	2	20
Model C1.6	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Model C2.0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Model D1.1	0	0	1	0	2	2	3	4	6	6	8	8	9	9	8	8	80
Model D1.7	0	0	1	1	1	2	2	3	5	6	7	9	8	10	10	10	100
Avg. pH								**7.63**									
Avg. EC (dS/m)								**1.60**									
Avg. TDS (ppm)								**1030.00**									

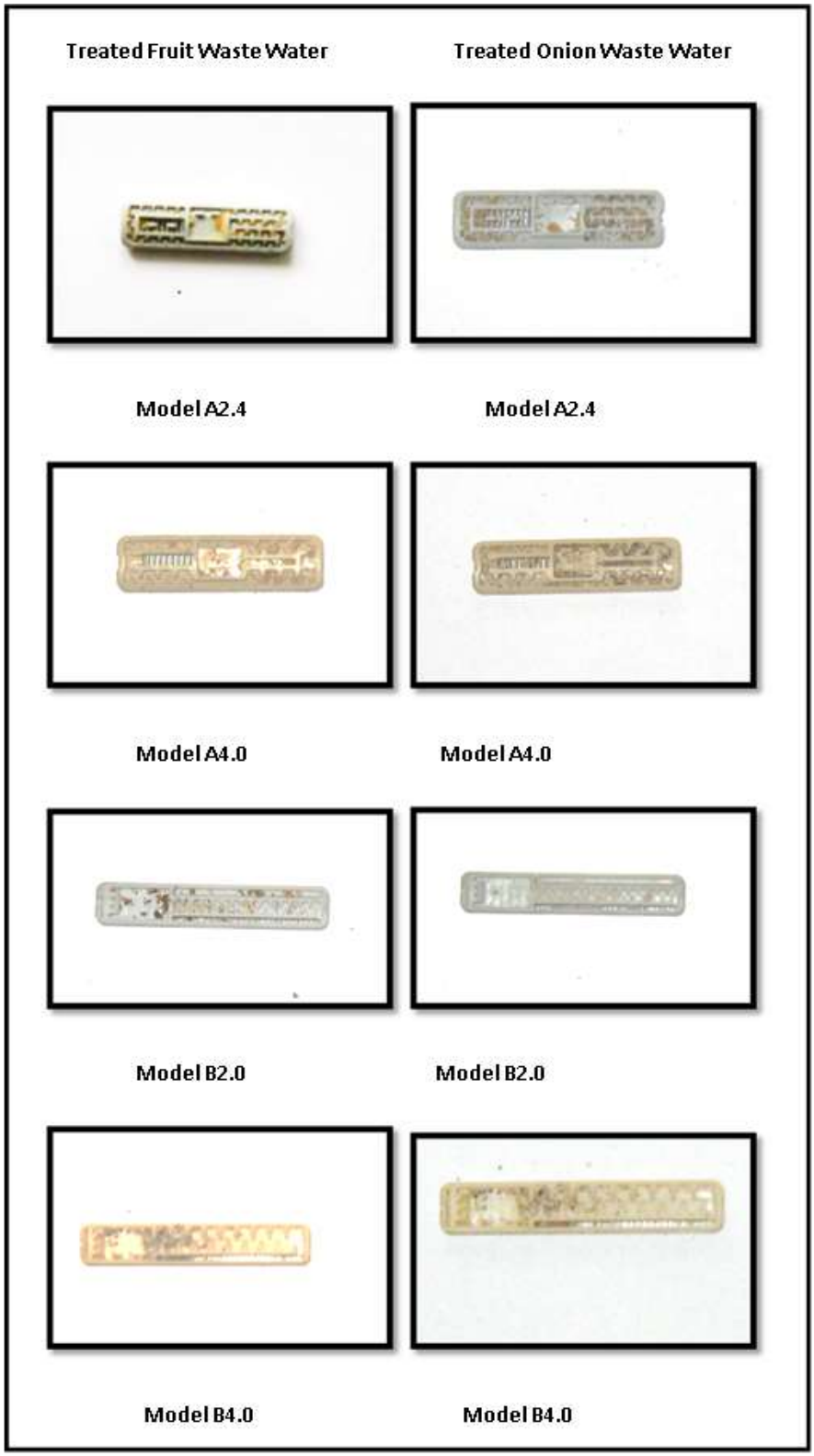


Figure 3-7. Clogging resistance of NPC emitters against TFWW and TOWW

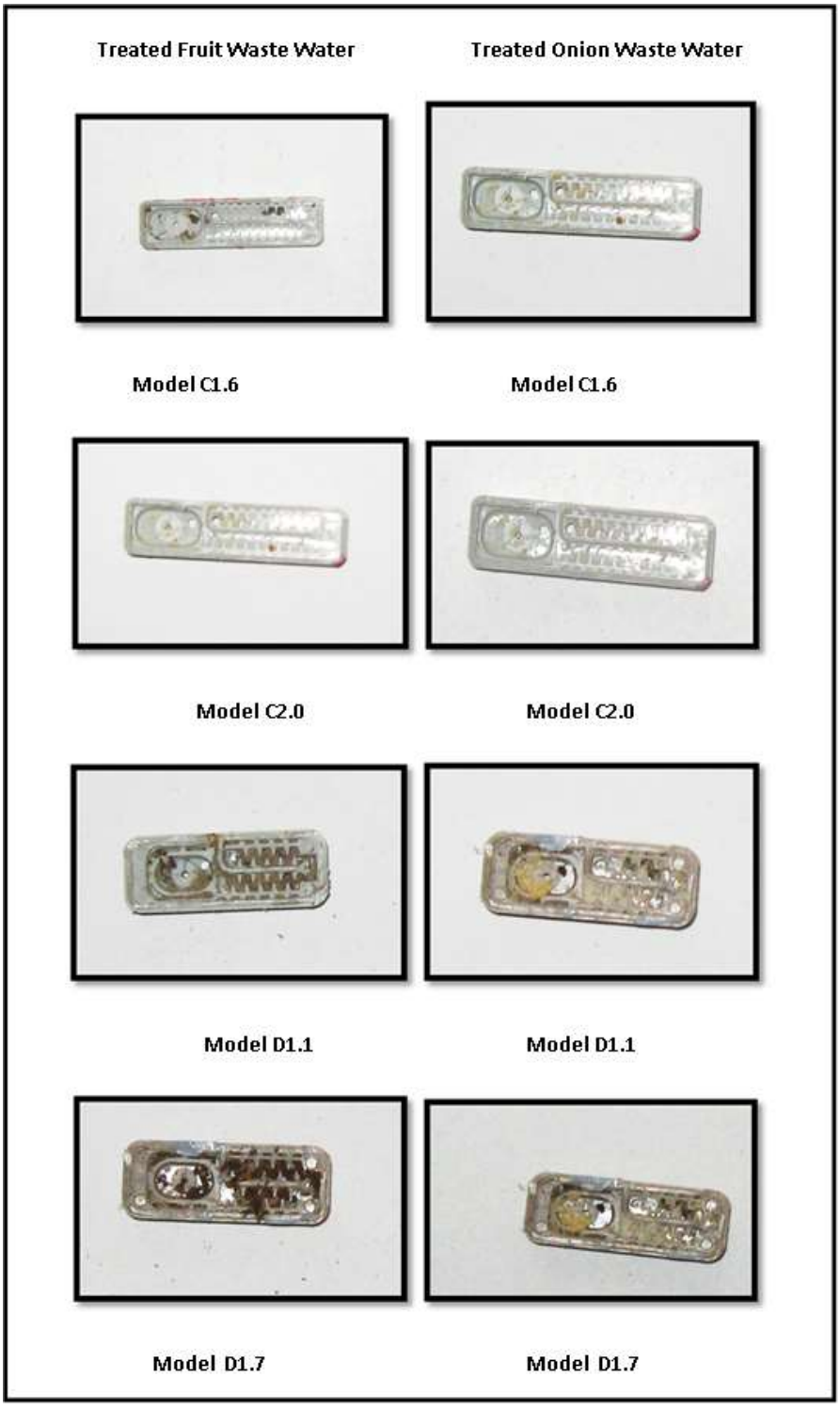


Figure 3-8. Clogging resistance of PC, CNL, AS emitters against TFWW and TOWW

Selection of suitable emitter geometry by using clogging test method

Eight different types of emitter were tested to find out the suitable emitter geometry under TFWW and TOWW. Emitter clogging percentage of all the selected emitters after 15 days of test is presented in Table 3-24 and Table 3-25. To find the clogging points in emitters, emitters were peeled out through lateral as presented in the *Figure 3-7* and *Figure 3-8*. It was found that flow path of Model B2.0, Model B4.0, Model C1.6, Model C2.0 type of emitter were

having high clogging resistance for TFWW and TOWW than Model D1.1 and Model D1.7 type of emitters which was severely clogged for same water. Model A2.4 and Model A4.0 type emitters were also clogging moderately against TFWW and TOWW except Model A2.4 type emitter has shown better resistance to TFWW.

Field experiments

The crop was sown on 30th January 2014. The mean maximum and minimum temperature was about 37^{o}C and 20^{o}C, respectively, with mean maximum and minimum relative humidity was 68 per cent and 32 per cent were recorded during the period of experimentation. The crop was harvested on 30th May 2014. There was no precipitation during the entire crop growth period. So, the crop was irrigated frequently as and when required on the basis of daily evapotranspiration. Selection of suitable emitter geometry for field experiment was based on clogging test of emitter in the laboratory. On the other hand, analysis of soil and water helped to understand the necessary changes occurred into it during the experiment. Effect of different irrigation treatments and sub treatment on irrigation scheduling, uniformity coefficient, distribution uniformity, water use efficiency and maintenance scheduling are explained below:

Irrigation scheduling

It was observed that, water requirement of maize crop was 12.60 mm / day in the month of May and 2.66 mm / day in month of February. This may be the attributed to growth stage of crop, the higher ambient temperature and higher evaporation losses during this month. Total water requirement of maize crop during its growing season is 855.46 mm.

Determination of uniformity coefficient

The data pertaining to uniformity coefficient of drip irrigation system at different stages (01, 30, 60, 90 and 120 DAS) of crop growth as influenced by different irrigation treatments and different emitter types as well as their interactions are presented in the Table 3-26. There was significant effect of different irrigation treatments on the uniformity coefficient throughout the experiment, except at the end of experiment i.e. 120 DAS, there was not much difference in the uniformity under each treatment due to maintenance of drip system. The highest uniformity coefficient about was 96.07 per cent (01 DAS) was observed under TFWW. On the contrary, lowest uniformity coefficient of 90.07 per cent (90 DAS) was observed under TOWW.

There was no any significant effect among the different emitter types, hence the highest uniformity coefficient of 96.04 per cent was observed under Model C2.0 type emitter (01 DAS). On the contrary, lowest uniformity coefficient of 88.60 per cent (90 DAS) was observed under Model B2.0 type emitter. Only after 90 DAS, the statistically significant uniformity coefficient was observed in Model C2.0 type of emitter (95.09 per cent) than Model B2.0type emitter (88.60 per cent).

Interaction effect was significant among different irrigation sources and emitter types on uniformity coefficient except TFWW was having non-significant effect on 01 DAS, 30 DAS and 60 DAS. The Model C.20 type emitter was having higher uniformity coefficient than Model B2.0 type emitter.

Determination of distribution uniformity

The data pertaining to distribution uniformity of drip irrigation system at different stages (01, 30, 60, 90 and 120 DAS) of crop growth as influenced by different irrigation treatments and different emitter types as well as their interactions are presented in Table 3-16. Significant distribution uniformity was observed in each treatment during the experiment except at the end of experiment i.e. 120 DAS. The highest distribution uniformity 92.99 per cent (01 DAS) was observed under BWFW. On the contrary, lowest distribution uniformity of 78.80 per cent (90 DAS) was observed under TOWW.

Among the different emitter types, the highest distribution uniformity of 92.57 per cent was observed under Model C2.0 type emitter (01 DAS). On the contrary, lowest distribution uniformity of 78.61 per cent (90 DAS) was observed under Model B2.0 type emitter.

There was no significant effect among the interaction between different irrigation sources and emitter types on distribution uniformity except TOWW. The Model B2.0 type emitter under TOWW had a significant result to clogging at 90 DAS and 120 DAS but the Model C2.0 type emitter was having good clogging resistance throughout the experiment. The Model C.20 type emitter was having higher distribution uniformity than Model B2.0 type emitter.

Table 3-26. Effect of different water treatments on uniformity coefficient of emitters.

M: Different irrigation sources	**Uniformity coefficient (%)**				
	01 DAS	**30 DAS**	**60 DAS**	**90 DAS**	**120 DAS**
M1	96.07	95.95	95.74	91.72	94.07
M2	95.14	94.80	94.74	90.07	94.31
M3	95.28	95.14	95.06	93.74	94.39
S.Em ±	0.14	0.12	0.17	0.28	0.40
C. D. (5%)	0.42	0.36	0.51	0.86	1.24
CV	0.46	0.39	0.55	0.97	1.35
S : Different emitter types					
S1	96.04	95.89	95.85	95.09	95.61
S2	94.95	94.71	94.51	88.60	92.90
S.Em ±	0.46	0.54	0.56	1.13	0.54
C. D. (5%)	1.40	1.65	1.72	3.48	1.66
CV	0.67	0.80	0.83	1.74	0.81
Interaction					
MxS					
S.Em ±	0.29	0.34	0.35	0.71	0.34
C. D. (5%)	0.89	1.04	1.09	2.20	1.05
CV	0.67	0.80	0.83	1.74	0.81

Where,

M1: Treated fruit waste water (TFWW)

M2: Treated onion waste water (TOWW)

M3: Bore well fresh water (BWFW)

S1: Pressure compensating, compensating non leakage and anti-syphon (PC CNL and AS) emitter.

S2: Non pressure compensating (NPC) emitter

Table 3-27. Effect of different water treatments on distribution uniformity of emitters.

M: Different irrigation sources	Distribution uniformity (%)				
	01 DAS	30 DAS	60 DAS	90 DAS	120 DAS
M1	92.24	92.11	91.54	84.53	89.29
M2	91.61	90.82	90.28	78.80	89.80
M3	92.99	92.77	92.03	88.01	89.02
S.Em ±	0.18	0.38	0.53	1.38	0.97
C. D. (5%)	0.56	1.19	1.62	4.25	2.99
CV	0.63	1.33	1.83	5.20	3.43
S : Different emitter types					
S1	92.57	92.50	92.56	88.95	91.54
S2	91.99	91.30	89.98	78.61	87.20
S.Em ±	1.10	1.38	1.78	3.35	2.20
C. D. (5%)	3.39	4.25	5.48	10.32	6.78
CV	1.69	2.12	2.76	5.66	3.48
Interaction					
MxS					
S.Em ±	0.70	0.87	1.13	2.12	1.39
C. D. (5%)	2.14	2.69	3.47	6.53	4.29
CV	1.69	2.12	2.76	5.66	3.48

Where,

M1: Treated fruit waste water (TFWW)

M2: Treated onion waste water (TOWW)

M3: Bore well fresh water (BWFW)

S1: Pressure compensating, compensating non leakage and anti-syphon (PC CNL and AS) emitter.

S2: Non pressure compensating (NPC) emitter

Effect on soil

Soil analysis prior to sowing of crop and after the treatments (60 DAS and 120 DAS) is presented in the Table 3-28. The soil analysis was carried after every two months to check the effect of different water treatments on soil parameters. This data was necessary to know the changes arose in the macro and micro nutrient level under each water treatment on soil parameters.

Effect on crop

At germination stage, there was no significant effect of different irrigation treatments and emitter types on plant population. In different irrigation treatments plants population varied between 59100 (TOWW) to 59300 (TFWW) and in different emitter type plant population varied between 59160 Model B2.0 type emitter to 59266.67 Model C2.0 type emitter. The interaction effects due to different irrigation treatment and emitter types on plant population during germination were found to be non- significant.

At harvesting stage, there was no significant effect of different irrigation treatments and emitter types on plant population. In different irrigation treatments plants population

varied between 57540 (TOWW) to 57900 (BWFW) and in different emitter type plant population varied between 57546.67 Model B2.0 type emitter to 57853.33 Model C2.0 type emitter. The interaction effects due to different irrigation treatment and emitter types on plant population during harvesting were found to be non- significant except TFWW. The Model C2.0 type emitter was having significantly plant population (58280) than Model B2.0 type emitter (57040).

Table 3-28. Effect of different water treatments on soil properties after 60 and 120 DAS of maize.

Parameters	Before Treatment	TFWW		TOWW		BWFW	
		60 DAS	120 DAS	60 DAS	120 DAS	60 DAS	120 DAS
N (kg/ha)	188.16	325.21	481.88	313.41	444.61	261.68	265.58
P (kg/ha)	16.43	102.06	260.49	131.10	254.02	89.19	108.91
K (kg/ha)	92.91	505.0	865.71	649.70	731.84	397.07	585.88
Ca (%)	0.14	0.17	0.64	0.16	0.71	0.17	0.59
Mg (%)	0.08	0.12	0.18	0.14	0.16	0.12	0.14
Fe (ppm)	4.96	10.17	12.01	9.98	11.20	8.85	10.65
Mn (ppm)	2.36	6.78	12.20	7.46	10.52	7.77	9.84
Zn (ppm)	0.52	1.26	1.83	0.854	1.48	1.13	2.854
Cu (ppm)	2.58	3.62	4.73	4.082	5.58	3.734	4.014
S (ppm)	8.06	10.65	13.03	9.05	9.85	8.63	8.95
Bulk density (gm/cc)	1.30	1.32	1.31	1.30	1.33	1.31	1.30
Field capacity (%)	33.24	32.66	32.68	32.52	33.05	32.67	32.62
Permanent wilting point (%)	21.76	22.18	21.10	21.97	22.01	21.32	21.04
Texture	Clay loam	Clay loam	Clay loam	Clay loam	Clay loam	Clay loam	Clay loam

Table 3-29. Effect of different water treatments on plant population per hectare.

M: Different irrigation sources	Plant population per hectare	
	Germination	Harvesting
M1	59300.00	57660.00
M2	59100.00	57540.00
M3	59240.00	57900.00
S.Em ±	149.56	244.82
C. D. (5%)	460.83	754.34
CV	0.80	1.34
S : Different emitter types		
S1	59266.67	57853.33
S.Em ±	464.40	376.39
C. D. (5%)	1430.96	1159.76
CV	1.11	0.92
Interaction		
MxS		
S.Em ±	293.72	238.05
C. D. (5%)	905.02	733.50
CV	1.11	0.92

Where,
M1: Treated fruit waste water (TFWW)
M2: Treated onion waste water (TOWW)
M3: Bore well fresh water (BWFW)
S1: Pressure compensating, compensating non leakage and anti-syphon (PC CNL and AS) emitter.
S2: Non pressure compensating (NPC) emitter

Grain yield

The data pertaining to grain yield influenced by different irrigation treatments and different emitter types as well as their interactions are presented in the Table 3-30. Significantly highest grain yield was observed under BWFW was about 9.92 t ha^{-1}. On the contrary, lowest grain yield 7.15 t ha^{-1} was observed under TOWW but among treated waste water TFWW (8.34 t ha^{-1}) has significant effect on grain yield. Among the different emitter types, significantly highest grain yield of 9.04 t ha^{-1} was observed under Model C2.0 type emitter. On the contrary, lowest grain yield was 7.15 t ha^{-1} under Model B2.0 type emitter.

Table 3-30. Effect of different water treatments on maize grain yield.

M: Different irrigation sources	**Grain yield (t/ha)**
M1	8.34
M2	7.15
M3	9.92
S.Em ±	0.10
C. D. (5%)	0.30
CV	3.63
S : Different emitter types	
S1	9.04
S2	7.89
S.Em ±	0.17
C. D. (5%)	0.54
CV	2.90
M: Different irrigation sources	**Grain yield (t/ha)**
Interaction	
MxS	
S.Em ±	0.11
C. D. (5%)	0.34
CV	2.90

Where,
M1: Treated fruit waste water (TFWW)
M2: Treated onion waste water (TOWW)
M3: Bore well fresh water (BWFW)
S1: Pressure compensating, compensating non leakage and anti-syphon (PC CNL and AS) emitter.
S2: Non pressure compensating (NPC) emitter

Interaction effects due to different irrigation treatments and emitter types on grain yield were found to be significant. The more difference in the grain yield was observed in the BWFW followed by TFWW and TOWW. The maximum grains yield was observed in the Model C2.0 type emitter (10.56 t ha^{-1}) in BWFW and minimum in Model B2.0 type emitter (6.62 t ha^{-1}) in TOWW. The influence of different irrigation treatments and different emitter types as well as their interactions on quality parameters of maize such as protein, carbohydrates, fats, ash, crude fiber and energy are presented in the following Table 3-31. Cost economics of maize crop under drip irrigation by using different irrigation treatments and sub treatments are presented in Table 3-32.

Table 3-31. Effect of different water treatments on the quality parameter of maize

Sl. No.	Parameters	TFWW	TOWW	BWFW
1	Protein (%)	7.82	7.62	4.92
2	Carbohydrates (%)	82.48	82.24	87.70
3	Fat (%)	3.01	3.21	1.28
4	Ash (%)	1.67	1.74	2.12
5	Crude Fiber (%)	1.36	2.15	1.46
6	Energy (kcal)	388.31	388.34	382.03

Table 3-32. Economics of maize under treated waste water by using drip irrigation system.

Treatments	Yield (kg ha^{-1})	Cost of cultivation(Rs)	Gross returns (Rs)	Net returns (Rs)	Returns per rupee of investment
M1S1	8901	133950	284832	186582	1.39
M1S2	7770	133950	248640	150390	1.12
M2S1	7675	133950	245600	147350	1.10
M2S2	6618	133950	211776	113526	0.85
M3S1	10559	133950	337888	239638	1.79
M3S2	9278	133950	296896	198646	1.48

Where,
M1: Treated fruit waste water (TFWW)
M2: Treated onion waste water (TOWW)
M3: Bore well fresh water (BWFW)
S1: Pressure compensating, compensating non leakage and anti-syphon (PC CNL and AS) emitter
S2: Non pressure compensating (NPC) emitter

Field water use efficiency (WUE)

The data pertaining to WUE of crop growth as influenced by different irrigation treatments and different emitter types as well as their interactions are presented in the Table 3-33. Significantly highest WUE 11.60 kg$ha^{-1}mm^{-1}$ was observed under BWFW. On the contrary, lowest WUE 8.36 kg $ha^{-1}mm^{-1}$was observed under TOWW. Among the different emitter types, significantly highest water use efficiency 10.58 kg $ha^{-1}mm^{-1}$was observed under

Model C2.0 type emitter. On the contrary, lowest WUE of 9.23 $kgha^{-1}mm^{-1}$ was observed under Model B2.0 type emitter. Interaction effect was significant among different irrigation sources and emitter types on WUE. The Model C2.0 type emitter was having higher WUE than Model B2.0 type emitter in each treatment. Significantly highest WUE was observed in BWFW 12.35 kg $ha^{-1}mm^{-1}$under Model C2.0 type emitter and lowest in TOWW 7.74 kg $ha^{-1}mm^{-1}$in Model B2.0 type emitter.

Table 3-33. Effect of different water treatment on water use efficiency of maize.

M: Different irrigation sources	**WUE (kg/ha/mm)**
M1	9.75
M2	8.36
M3	11.60
S.Em ±	0.11
C. D. (5%)	0.35
CV	3.63
S : Different emitter types	
S1	10.58
S2	9.23
S.Em ±	0.20
C. D. (5%)	0.63
CV	2.90
Interaction	
MxS	
S.Em ±	0.13
C. D. (5%)	0.40
CV	2.90

Where,
M1: Treated fruit waste water (TFWW)
M2: Treated onion waste water (TOWW)
M3: Bore well fresh water (BWFW)
S1: Pressure compensating, compensating non leakage and anti-syphon (PC CNL and AS) emitter
S2: Non pressure compensating (NPC) emitter

3.3 Impact assessment of wastewater on crops, soil and groundwater documented

Assessing Suitability of Brewery Wastewater as Irrigation in Field Crops Using In-vitro bioassay and pot culture

Pot culture

A pot culture experiments were conducted in glasshouse at International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India (17.53° N, 78.27° E) to study the effects of wastewater on soil and crop growth. The soil taken for pot culture was medium black clayey (Vertisols). The initial characteristics of soil are mention in Table 1. Pearl millet (ICMV221), pigeon pea (Asha), maize (kauvery 235), okra (MH10), tomato (lakshmi) and sorghum (CSV15) were grown during the experiments.The experiment consisted of four

treatments with three replications in a Completely Randomized design (CRD). Four treatments of source of irrigation water were good quality groundwater (T_1), untreated domestic wastewater (T_2), treated effluent from brewery (T_3) and partially treated effluent from brewery (T_4). Seventy two pots containing 10 kg of soil was taken for the experiment. Out of seventy two pots, twelve pots were assigned for each crop. Five seeds were dibbled in soil at the depth of 3-5 cm in each pot. Fertilizer scheduling was followed as per the soil test based recommendation specific to each crop. Full dose of P, K, Zn, B, S and 50% of N dose was applied basally before sowing and remaining 50 % N dose was top dressed.

Two different sources of water are used for conducting the experiment. One water source was taken from SAB Miller India factory at Sangareddy particularly Effluent Treatment Plant (ETP) and UASB (Up flow Anaerobic Sludge Bed) reactor and the other source of water was drawn from Bharat Heavy Electrical Limited (BHEL).Irrigation plays a vital role in global food security. About 40% of global food production consumes 70% of fresh water therefore when wastewater is used for irrigating the land by altering the existing irrigation infrastructure and scheduling will totally reduce the amount of fresh water and this was the strategy expectation. Irrigation water quality can be measured by total salt content, sodium, pH (alkalinity-Carbonate and bicarbonate), and specific ions such as chloride, sulfate, boron and nitrate. In first season of pot culture experiment CROPWAT 8.0 was used for scheduling the irrigation and in the second season of pot culture experiment irrigation scheduling was done by alternate wet and dry method.

Crop parameters such as biomass and height were measured. Biomass was done at postharvest stage of the crop and height was measured from peak vegetative stage (30DAS) to preflowering stage (45DAS -60DAS) respective to the specific crop duration.In each treatment and replication, three plants were selected and their respective shoot and root mass was recorded. In each treatment and replication, three plants were selected and the weight of seeds per plant was recorded. The grains were dried in oven at 60^0 C for 24 hours and 100 grain weight for each treatment (in three replications) was recorded. The average was calculated and expressed in grams. The yield of pods per pot^{-1} of each treatment and replication was recorded and expressed as gram per pot.

Soil samples are drawn at the vegetative (30DAS), flowering (45-60DAS) and post harvest stage respective to the crop duration. The soil samples were collected from 15-20 cm depth before the conduct of the experiment and after the harvest of the crop. Samples were air dried, sieved through 2 mm mesh and used for the soil nutrient estimation. pH and EC was measured with soil water extract (Jackson, 1973) and particle size analysis performed by hydrometer method (Day, 1965). The organic carbon was estimated by the method proposed by Walkley and Black (1934). Available soil phosphorus was estimated by the procedure outlined by Bray *et al* (1954), available potassium by neutral ammonium acetate by flame photometric method (Stanford and English, 1949) and available sulphur by 0.01M $CaCl_2$ extract (Randall, 1988). Available micronutrients (Zn, Fe, Mn, Cu was estimated by DTPA extract outlined by (Lindsay and Norvell, 1978).To determine the plant nutrient uptake parameters such as dry weight and nutrient content are required. Dry weight was measured after the postharvest of the crop and nutrient content was estimated by selenium sesquioxide method (Sahrawat, 2002).

Effect of wastewater on soil

The soil samples were collected from surface (0-15 cm) of the pots irrigated with good quality ground water (T_1), untreated domestic wastewater (T_2), treated effluent from brewery (T_3) and partially treated effluent from brewery (T_4). The chemical characteristics of the wastewater on soil in different crops are presented in Table 3-34. The results indicated that the pH of the soil varied in the range of 7.21 to 7.84 in first pot culture experiment and 7.4 to 7.8 in second pot culture experiment with an electrical conductivity ranging from 287 to 559.5 µS in first pot culture experiment and 6730 to 17600 µS in second pot culture experiment respectively. The pH and EC of the wastewater irrigated soil were slightly higher than control due to the presence of sodium ions in wastewater which is used as a supply of irrigation source. As per the data the pH values tends to vary from neutral to slightly alkaline in condition and therefore falls within the permissible limits ranging from 6.0 to 9.0. Similarly the electrical conductivity of wastewater irrigated soils are higher but found to be within the toxic limit as per the USSL (1954). The organic carbon content of surface soils irrigated with wastewater in first pot culture experiment varies from 0.51 per cent to 0.63 per cent and 0.43 to 0.49 per cent in second pot culture experiment. Based on low (<0.5 per cent), medium (0.5-0.75 per cent) and high (> 0.75 per cent) status, all values in first pot experiment fell under medium level of organic carbon and second pot experiment values fell under low level of organic carbon. This decrease in the level of organic carbon content may be due to utilization of carbon by the crop as a source of nitrogen.

Table 3-34. Effect of wastewater irrigation on chemical properties of sandy clay loam soil in different crops (sorghum bicolor & Solanum lycopersicum)

Crop	Treatment	pH		EC(µS)		OC(%)	
		P-I	P-II	P-I	P-II	P-I	P-II
Sorghum	**Control-T_1**	7.21	7.4	292.1	6900	0.57	0.46
	BHEL untreated-T_2	7.24	7.6	517.3	9050	0.51	0.45
	ETP-T_3	7.30	7.8	387.4	11200	0.51	0.45
	UASB-T_4	7.31	7.6	394.6	11000	0.54	0.47
Tomato	**Control-T_1**	7.21	7.6	336.8	6730	0.59	0.46
	BHEL untreated-T_2	7.44	7.7	287.0	8070	0.54	0.49
	ETP-T_3	7.84	7.7	559.5	12500	0.63	0.43
	UASB-T_4	7.44	7.6	514.9	17600	0.54	0.46

The available phosphorous content in soil varies from 2.77 to 57.7 ppm. In first pot culture experiment in sorghum and tomato crop maximum phosphorous value was reported in untreated domestic wastewater (T_2). But in second pot culture experiment in tomato crop highest value was observed in partially treated effluent from brewery (T_4) and in sorghum highest value was obtained in untreated domestic wastewater (T_2).All the values fell under the very high. In first pot experiment in sorghum crop $T_1$and T_4 falls under the category of very low level (0-3 ppm),T_2 and T_3 fell under low level (4-7 ppm).In tomato all the treatments falls under low level (4-7 ppm).In such cases build up recommendation will benefit the crop production. In second pot experiment in sorghum and tomato crop all the values falls under the category of very high range (16+ ppm).Therefore maintenance recommendation should be taken up to maintain the soil quality and health. Application of domestic wastewater along with the recommended NPK dose increases the phosphorous content in soil.

The exchangeable potassium content in soil varies from 162 to 298 ppm. In first pot culture experiment in sorghum and tomato crop maximum potassium value was reported in treated effluent from brewery (T_3). But in second pot culture experiment in tomato and tomato crop highest value was observed in partially treated effluent from brewery (T_4).In first and second pot experiment in tomato and sorghum crop all the values got from all the treatments falls under very high range of concentrations (161+ ppm).In general there is no need to add fertilizer to the soil. The available calcium content in soil varies from 6914 to 8168 ppm. In first and second pot culture experiment in sorghum crop highest value was obtained in untreated domestic wastewater (T_2) and in tomato crop maximum value was observed in ground water application (T_1).All the values obtained falls under the category of very high range of concentration (4500+ ppm).The available sodium content in soil varies from 219 to 2429 ppm. In first pot culture experiment in sorghum and tomato crop highest value was obtained in treated effluent from brewery (T_3). In second pot culture experiment in sorghum highest value was obtained in partially treated effluent from brewery (T4) but in tomato highest value was observed in treated effluent from brewery (T_3).

Table 3-35. Effect of wastewater irrigation on macro and micronutrient status in vertisol in different crops(sorghum bicolor & Solanum lycopersicum)

Crop	Treatment	Avail-P (ppm)		Exch-K (ppm)		Avail-Zn(ppm)		Avail-B (ppm)		Avail-S(ppm)		Na(ppm)	
		P-I	P-II	P-I	P-II	P-I	P-II	P-I	P-II	P-I	P-II	P-I	P-II
Sorghum	Control-T_1	2.77	28.3	201	162	1.52	234.0	0.7	3.51	20.7	43	219	395
	BHEL untreated-T_2	5.65	22.4	205	145	3.24	207.1	1.3	4.58	31.0	22	334	541
	ETP-T_3	3.73	22.3	209	247	1.62	253.7	0.8	3.71	26.2	42	621	2281
	UASB-T_4	2.89	29.6	200	262	0.90	114.1	0.7	3.06	23.7	43	463	2440
Tomato	Control-T_1	4.48	36.9	174	186	1.66	147.3	0.6	4.44	25.6	66	256	479
	BHEL untreated-T_2	5.87	57.7	187	228	1.86	125.8	1.0	4.43	24.3	60	358	710
	ETP-T_3	4.84	36.5	229	256	1.80	230.5	1.0	4.32	26.8	37	1519	2429
	UASB-T_4	4.70	42.5	207	298	1.26	211.7	0.9	6.80	24.6	67	1160	2254

Table 3-36. Effect of wastewater irrigation in nutrient uptake of different crops (sorghum bicolor & Solanum lycopersicum)

Crop	Treatment	N (mg)		P(mg)		K(mg)		Zn(mg)		B(mg)		S(mg)	
		P-I	P-II	P-I	P-II	P-I	P-II	P-I	P-II	P-I	P-II	P-I	P-II
Sorghum	Control-T_1	2.29	1.33	0.12	0.08	2.49	1.23	54.69	73.54	17.69	18.38	1638.49	899.06
	BHEL untreated-T_2	2.41	1.90	0.12	0.25	2.34	1.34	56.75	73.19	20.49	21.61	1505.69	1135.99
	ETP-T_3	1.43	1.11	0.06	0.08	2.23	1.21	26.55	74.83	11.4	2.25	1143.77	720.46
	UASB-T_4	2.24	1.30	0.11	0.11	2.16	0.82	51.65	55.22	14.24	5.72	1528.29	790.20
Tomato	Control-T_1	1.66	4.21	0.12	0.21	3.25	3.32	74.41	183.16	64.03	89.08	4839.57	6148.09
	BHEL untreated-T_2	2.03	6.74	0.14	0.39	3.67	4.77	69.31	339.48	58.82	102.54	4822.49	9016.14
	ETP-T_3	1.69	6.19	0.09	0.28	2.61	3.34	95.95	204.47	61.63	72.05	3952.81	6865.92
	UASB-T_4	2.76	5.51	0.13	0.25	3.02	4.01	92.72	200.18	57.65	65.38	5484.38	4646.71

The available sulphur content in soil varies from 20.7 to 67 ppm. In first pot culture experiment in sorghum crop highest value was obtained in untreated domestic wastewater (T2) and in tomato maximum value recorded in treated effluent from brewery (T3).In second pot experiment maximum value was observed in ground water application (T1) and partially treated effluent from brewery (T4).In both the pot experiment all values falls under very high concentration (15+ ppm).Therefore external source of addition of fertilizer is not needed and in turn reduces the fertilizer cost and its application.

Effect of wastewater on crops

Plant samples were collected and dry weight and nutrient content was measured and further used for the calculation of nutrient uptake. The nitrogen uptake in crop ranges from 1.11 to 6.74 mg. In first pot experiment in sorghum maximum N uptake obtained in untreated domestic wastewater (T_2) and minimum uptake observed in Treated effluent domestic wastewater (T_3).In tomato maximum uptake observed in partially treated effluent from brewery (T_4) and minimum uptake in ground water application. But in second pot experiment for both sorghum and tomato crop maximum uptake was observed in untreated domestic wastewater (T_2) and in sorghum and tomato minimum uptake recorded in treated effluent domestic wastewater (T_3) and ground water respectively.

The phosphorous uptake in crop ranges from 0.06 to 0.39 mg. In first and second pot experiment for both tomato and sorghum crops maximum P uptake was observed in untreated domestic wastewater (T_2) and minimum uptake was recorded in treated effluent domestic wastewater (T_3). The potassium uptake in crop ranges from 0.82 to 4.77 mg. In first pot experiment in sorghum maximum K uptake observed in ground water (T_1) and minimum in partially treated effluent from brewery (T_4) but in case of tomato crop maximum K uptake was recorded in untreated domestic wastewater (T_2).In sorghum and tomato minimum K uptake was obtained in partially treated effluent from brewery (T_4) and ground water (T_1) respectively.

Wastewater irrigation effect on aboveground biomass:

Generally there is an increase in plant shoot biomass was observed when wastewater is used as an irrigation source consequently for two years (2013-2014). In first pot culture experiment in tomato and sorghum maximum shoot biomass was obtained in ground water (T_1) followed by treated effluent from brewery (T_3). But in the case of second pot culture experiment in tomato and sorghum highest biomass recorded in untreated domestic wastewater (T_2) followed by treated effluent from brewery (T_3).Comparison between the biomass of 2013 and 2014 there was a significant increase in biomass was observed in 2014.This may be due to the enrichment of soil with nutrients when wastewater is used as an irrigation source and in turn enables the plant growth.

Influence of wastewater on crop yield

From the data it was inferred that in first pot culture experiment tomato and sorghum obtained maximum yield in partially treated effluent from brewery (T_4) followed by ground water treatment (T_1). But in second crop season in tomato and sorghum crop highest yield was obtained in ground water (T_1) followed by untreated domestic wastewater (T_2).The use of wastewater significantly increased the yield of the crop. This may be due to continuous supply of wastewater over a year results in build up of nutrients in the top layers of the soil.

Among the two crops (sorghum bicolor & solanum lycopersicum) used for the study tomato was observed to have obtained higher yield than sorghum. Decrease in yield in sorghum may be due to the phytotoxicity effect of some heavy metal accumulation.

Field scale experiment to assess effect of wastewater reuse on crop and soil

The experiment conducted to assess the effect of wastewater irrigation on crop and soil quality over the three consecutive seasons (kharif, rabi and summer) during 2014-15. The present investigation was carried out at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. The site is geographically situated at 17^0 53' N Latitude, 78^0 27' E Longitude and at an altitude of 542.3 m above sea level. It falls under Southern Telangana Agro-climatic zone of Andhra Pradesh (Now Telangana State). The experimental soil is Alfisol. Initial soil samples were collected prior to layout of the experiment at 0-15 cm depth. Composite soil sample were prepared by quartering technique. These samples were dried in shade, pounded and passed through 2 mm sieve and preserved in polythene bags after proper labelling. These samples were analysed for various physic-chemical properties by adopting standard methods and the data is presented in Table 3-37.

Table 3-37. Physical and chemical properties of soil in the experimental field

Particulars	Results	Method adopted
Physical properties		
Mechanical composition		Bouyoucos hydrometer method
Sand (%)	64.6	(Piper , 1966)
Silt (%)	19.4	
Clay (%)	16.0	
Soil texture	Sandy loam	USDA method
Chemical properties		
a. Soil reaction (pH)	7.3	1:2.5 soil water suspension by using Elico pH meter (Jackson, 1967)
b. Electrical conductivity(dSm^{-1})	0.21	1:2.5 soil water suspension by using Elico Conductivity meter (Jackson, 1967)
c. Organic carbon (%)	0.20	Walkley and Black rapid titration method (Walkley and Black, 1934)

The main aim of the field experiment was to study the effect of wastewater and treated water on crop and soil properties. Based on the objective of experiment the field is divided into the 24 plots. The main treatment of water quality i.e. Fresh water, Treated wastewater and untreated wastewater and two method of application of water i.e. drip and furrow irrigation each treatment were replicated 4 times.

In kharif season maize crop variety Hybrid-5401 were grown.

Treatments

T1- Fresh water application with furrow irrigation

T2- Treated wastewater application with furrow irrigation

T3- Untreated wastewater application with furrow irrigation

T4- Fresh water application with Drip irrigation
T5- Treated wastewater application with Drip irrigation
T6- Untreated wastewater application with Drip irrigation
Crop was harvested plot-wise at by removing the cobs from the plants. The cobs were sun dried, threshed using machine. Seeds were cleaned and weighed. Seed yield and stover yield from each plot was recorded after drying.

Effect of sources of irrigation on growth, yield, quality, soil properties and water productivity of tomato-palak sequence cropping
Treatment details:
Main plot: Irrigation sources (I) at 30 per cent moisture depletion
I_1: Treated domestic wastewater
I_2: Fresh water (bore well water)
I_3: Wastewater altered with fresh water
I_4: Farmers practice (untreated wastewater)

Sub Plot: Fertilizer levels (F)
F_1: 50% N, P_2O_5 and K_2O + Biofertilizer
F_2: 75% N, P_2O_5 and K_2O + Biofertilizer
F_3: RDF alone (As per POP)
F_4: Control (No fertilizer)

The experiment was initiated during summer 2013-14 with tomato-palak sequence cropping with horizontal surface treatment. The second year trail is continued during 2014-15 and the pooled data will be presented after the harvest.

Figure 3-9. General view of Tomato at MARS, Dharwad

Effect of sources and methods of irrigation on growth, yield, quality and water use efficiency of okra-leafy vegetable cropping sequence
Treatment Details
Main Plot: Sources of irrigation (I)
I_1- Treated waste water

I_2- Fresh water
I_3- Sewage water alternated with Fresh water
I_4-Farmers practice (untreated sewage water)
Subplot: Methods of Irrigation (M) at 30 per cent depletion.
M_1-Ridge and furrow (Farmers practice)
M_2- Alternatively alternate furrow irrigation
M_3-Ridge and furrow at 50% depletion of soil moisture
M_4-Basin irrigation

The experiment was initiated during summer 2013-14 with Okra-leafy vegetable sequence cropping. The second year trail is continued during 2014-15 and the pooled data will be presented after the harvest.

Figure 3-10. General view of Okra at MARS, Dharwad

***In-situ* soil and water conservation practices on productivity of Maize-wheat cropping sequence**
Treatment Details

(i)Main plot : Conservation practices (C)
C_1- Broad bed and furrow
C_2 – Zero tillage
C_3 – Minimum tillage
(ii) Sub plot: Cover crops and weed management practices
W_1 – Sole Maize (no weed management and no mulching)
W_2 – Maize + cowpea- 1:1 and round up is sprayed at 40 – 45 DAS
W_3 – Maize +beans-1:1 and round up is sprayed at 40-45 DAS
W_4- Maize with atrazine
W_5 – Maize with atrazine – 2, 4-D

The experiment was initiated during *kharif* 2014. Maize yield differed significantly with conservation practices. Broad bed and furrow practices recorded significantly higher maize grain (5821 kg/ha) and stover (7.39 t/ha) yields than zero tillage. However it was on par with minimum tillage practices (5580 kg/ha and 6.12 t/ha grain and stover yields respectively). Maize with cover crops and weed management practices did not differ significantly with respect to yield. However the interaction effect due to broad bed and furrow with atrazine followed by 2,4-D spray recorded significantly higher grain (6525 kg/ha) and stover (7.96 t/ha) yields as compared to zero tillage and cover crops with weed management practices. The results of succeeding wheat will be presented after the harvest.

Table 3-38. Effect of tillage and weed management practices on yield (kg ha-1) of Maize

Weed management (W)	**Conservation tillage practices (C)**			
	C_1	**C_2**	**C_3**	**Mean**
W_1	6084	5336	3699	5040
W_2	5454	5958	3683	5032
W_3	5305	5619	3723	4882
W_4	5738	4982	3731	4817
W_5	6525	6005	3809	5446
Mean	5821	5580	3729	
	SEm±	**CD (p=0.05)**		
Main (C)	170	589		
Sub (W)	386	NS		
CXW	772	2207		

Table 3-39. Effect of tillage and weed management practices on stover yield (t ha-1) of maize

Weed management (W)	**Conservation tillage practices (C)**			
	C_1	**C_2**	**C_3**	**Mean**
W_1	7.03	5.62	5.70	**6.11**
W_2	7.59	7.41	4.53	**6.51**
W_3	6.93	6.32	5.39	**6.21**
W_4	7.45	4.58	6.43	**6.15**
W_5	7.96	6.70	4.69	**6.45**
Mean	**7.39**	**6.12**	**5.35**	
	SEm±	**CD (p=0.05)**		
Main (C)	0.24	NS		
Sub (W)	0.43	1.24		

Effect of sources of irrigation and levels of fertilizer on growth, yield and quality of sunflower

(i) Main plot: Sources of irrigation (I)

I_1 – Treated waste water (TWW)

I_2 –Domestic Waste Water

I_3 – Fresh water (Borewell)

(ii) Sub plot: fertilizer levels

M_1 – Control (No NPK)
M_2 – 50 % NPK
M_3 - 75 % NPK
M_4 – 100 % NPK

The experiment was initiated during summer 2014-15. Presently the sunflower is at grain filling stage.

Figure 3-11. General view of Okra at MARS, Dharwad

Effect of sources of irrigation on growth, yield and quality of cotton.
Treatment Details

Sources of irrigation (I)

I_1: Treated domestic wastewater
I_2: Fresh water (bore well water)
I_3: Wastewater altered with fresh water
I_4: Farmers practice (untreated wastewater)

The experiment was initiated during summer 2014-15. Presently harvesting of cotton is in progress. The performance of cotton and maize roots is initiated as pot culture studies.

Figure 3-10. Root architecture in Cotton and Maize with respect to different sources of water

A study on assessment of quality of sewage irrigated vegetables, post-harvest practices and health status of handlers of sewage irrigated villages

A study on assessment of quality of sewage irrigated vegetables, post-harvest practices and health status of handlers of sewage irrigated villages has been initiated to study the nutritional quality of vegetables grown in sewage and non-sewage water. Microbial analysis of edible parts of vegetables grown in sewage and non-sewage water and assessment of the health status of handlers involved in post-harvest practices of vegetables is progress.

Sampling: Vegetables (Palak, methi leaves, coriander leaves, tomato and brinjal) collected randomly from 5 farmers each from control and experimental village at the time of marketing (farm gate). Random sampling of 30 households with two members from each household was done for health status assessment. The information on family history, anthropometry, clinical examination and hemoglobin status in both the village was collected.

Sample preparation: Moisture content was estimated for fresh sample and then the sample were dehydrated and used for nutritional quality assessment viz. ash content (Socsplus instrument), protein and crude fibre (fibraplus instrument). The sample will also be analyzed for total sugars (Nelson somogyi's method) and trace elements (micro nutrients and heavy metals). Microbial enumeration from fresh sample for bacterial, fungi, actinomycetes and *E-coli* count will be done by using standard plating technique.

Health status assessment: General information on family characteristics, health history, family schedule, anthropometric observations, socio-economic scoring, environmental scoring encompassing type and nature of house, occurrence of human pests and house refuse disposal details were collected using questionnaire. General clinical examination including haemoglobin status (Sahli's method) was assessed. Socio-economic classification and environmental status are also included in the scheme of assessment. Statistical analysis and the laboratory analysis are under progress. The data pertaining to mentioned programme will be presented on completion of the analysis.

Effect of domestic waste water on growth and yield of soybean (UASD)

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 3-40). 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.

Table 3-40. Effect of domestic waste water on growth and yield of soybean								
	Plant height at 30 DAS	**plant height 60 DAS**	**Plant height(cm) at harvest**	**Number of branches 30 DAS**	**Number of branches 60 das**	**Number of branches at harvest**	**Number of pods/plant at 60 DAS**	**Number of pods/plant 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

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.

3.4 Validated models for enhancing WUE at field and micro-watershed level

Water Impact Calculator

Agriculture is the largest consumer of the water. Inappropriate management of water resources resulted in low crop yields and poor water use efficiency. Conservation and efficient use of water resources both at micro and meso scale (farmers' field and watershed scale) is essential for enhancing crop yield, productivity and income. To utilize the water resources more efficiently, there is an urgent need to enhance WUE through enabling farmers to adopt need-based irrigation scheduling and efficient irrigation methods.

There are number of decision making tools capable of doing water balance and irrigation scheduling for different cropping systems. Use of these modeling tools is mainly limited to the scientific community due to complex parameterization. Currently available software/models either are data demanding or require high-quality subject expertise. Moreover, these tools provide irrigation scheduling based on single time run and there is no other means to modify recommendations with follow-up rainfall events and actual farmers' practices in due course. Availability of a decision making tool which is simple to use and technically robust will help farmers for applying irrigation as per need rather than adopting the calendar-based irrigation application. Moreover, using such tool, farmers and other practioners (stakeholders) should be able to decide suitable cropping system and cultivation intensity for their field and watershed or community scale and also could be potentially useful in large scale irrigation planning and management (for example, canal water release and water allocation).

Model

While developing WIC, it is primarily considered that tool should be simple and user-friendly in terms of data requirement. User should quickly enter input data relating to their farm; quickly understand the main water related impacts and get irrigation scheduling. Microsoft Excel is found a suitable computational platform for developing WIC. Different hydrological components (modules) were developed in Excel sheets separately and integrated together using logical functions [for examples, *if(), sumif(), countif()*]. Moreover, soil and weather parameters such as field capacity, permanent wilting point, ET_0 and crop growth parameters (crop coefficient and root growth) is used from the default values stored in the back-up files based on farmers' input about soil type, crop grown and site location.

Irrigation scheduling

The moment water availability in root zone reaches below the defined threshold, WIC calculates i) total crop water requirement for following one week period by considering ET_0 and crop growth stage and ii) analyze moisture holding capacity of elongated root zone at the given stage and choose minimum among i) and ii). Irrigation efficiency is an important parameter which describes that how much extra irrigation to be applied to cover-up field completely, has also been considered during the calculation. Moreover, user is allowed to enter actual irrigation practices and amount of rainfall received during subsequent crop growth stages. Based on such information, WIC re-analyze water balance and modifies follow up recommendations.

Field scale experiment to compare different micro irrigation system and irrigation strategy based on water impact calculator

A field scale experiment is planned on a six hectare land to compare different drip and sprinkle irrigation system at ICRISAT. Two cropping pattern sorghum-chickpea and groundnut-millet are selected for cultivation.

3.5 Increased land and saline wastewater productivity in 20 ha

Cultivation of halophytes reusing bio-treated distillery effluent

Demonstration of reusing bio-treated industrial saline water in agriculture is one of the key objectives of the project. The bacterial and subsequent algal treatment of anaerobic treated distillery effluent couldn't reduce the saline content to meet the irrigation standards (EC: 0 to 3 mS/cm). Hence an attempt to grow halophytes likes *Sesuvium portulacastrum* and *Suaeda maritima* using the bio-treated distillery effluent was made. The purpose was to observe the growth and survival of these species under the irrigation of bio-treated distillery effluent which has the salinity level of 9.1 ppt (EC Value?).

Field trials on *Sesuvium portulacastrum and Suaeda maritima* was conducted in the premises of KCP sugar industry located in Vuyyuru from mid September 2014. Treatments using bio-treated distillery effluent (T1) and anaerobic treated distillery effluent (T2) were established with 8 replicates each for both species. Plot area of each replicate was 2.5 m x 2.5m and the total area of each treatment for *Sesuvium portulacastrum* and *Suaeda maritima* is 50 m^2 respectively.

Suaeda maritima

Each treatment was planted with 48 vegetative fragments of height between 10 and 15 cm. Around 192 L of bio-treated distillery effluent and anaerobic treated distillery effluent was irrigated respectively to T1 and T2 on every alternate day. Localized irrigation method was

adopted where water was applied around each plant to wet only the root zone. Periodical monitoring and recording of growth and survival of *Suaeda maritima* was done. Though there is no mortality in both the treatments stunted growth was observed in T2. Plant height was taken on 30 and 60 and 150 days after planting and found that the average height was 23.4 cm, 58.7 cm and 112cm in T1 whereas in T2 the height was 14.3 cm, 26 cm and 90 cm respectively. Flowering was observed to be good in T1 replicates as compared to the T2 replicates. Circumference of the plant is 5.2 m in T1 while it is 4 m in T2.

Sesuvium portulacastrum

Each treatment was planted with 48 vegetative fragments with equal spacing. Irrigation quantity and method adopted were similar to that of *Suaeda* as mentioned above. There was no mortality in this species also. Measurement of shoot length of *Sesuvium* as on 150 days after plantation indicates that in T1 the shoot length is 200 cm while in T2 it is 60 cm. The circumference in T1 is 9.5 m and in T2 it is 1.14 m.

Future plan

The field trials indicate that the *Sesuvium portulacastrum and Suaeda maritima* survive and grow luxuriantly in the bio-treated distillery effluent. It is proposed to conduct in depth studies to explore the phytoremediation potential of these species by using it in the constructed wetlands in comparison with control. Secondly, cultivation of these species as well as other suitable halophytes including some grasses will be done using bio-treated and anaerobic treated distillery effluent as irrigation source. Systematic observations will be done to determine the biometrics, chlorophyll content, flowering, yield, plant uptake and other characteristics.

Microbial load and Pathogens in halophyte cultivated plots

Total microbial load assessment in halophytes grown plots showed (*Figure 3-12*) no variation between *Suaeda* sp. and *Sesuvium* sp. irrigated with T1 and T2 source of water both during 25 DAS and 100 DAS. This may be an indication of the positive association of the rhizospheric microbes with halophytes. Similarly assessment of the pathogenic population showed that the different species ranges from ≥ log 1 to ≥ log 6 cfu/g. Data in *Figure 3-13* indicates that a significant reduction in the population was observed on the 100 DAS irrespective of the pathogens. This indicates the negative association of halophytes with the pathogens in the rhizosphere region which can be confirmed by further assessments. It also evident that there halophyte species can be a potential candidate for reducing the pathogenic population in the effluent irrigated soils. Though reduction in the pathogenic population was observed the total load remains stable indicating the association of beneficial microbes in the halophyte rhizosphere.

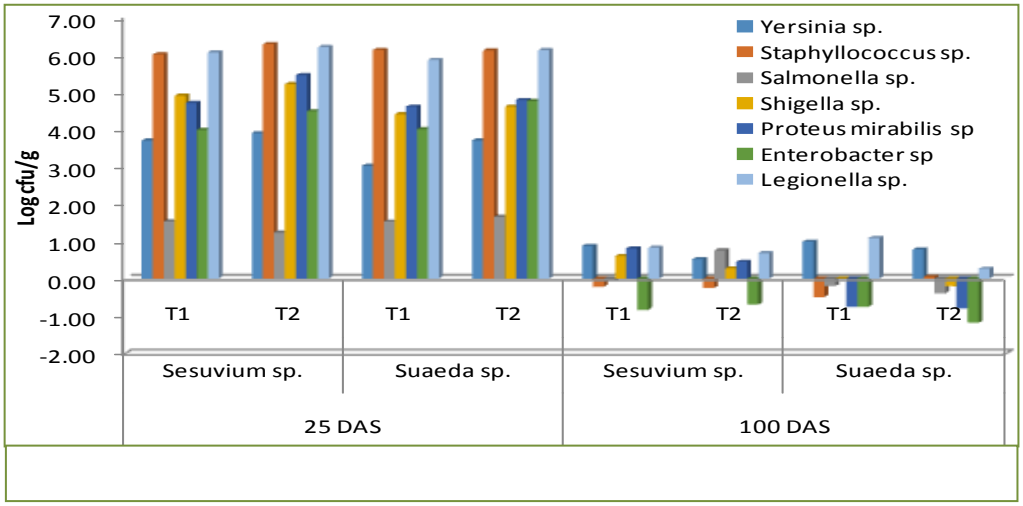


Figure 3-12. Pathogenic population in halophyte cultivated plots

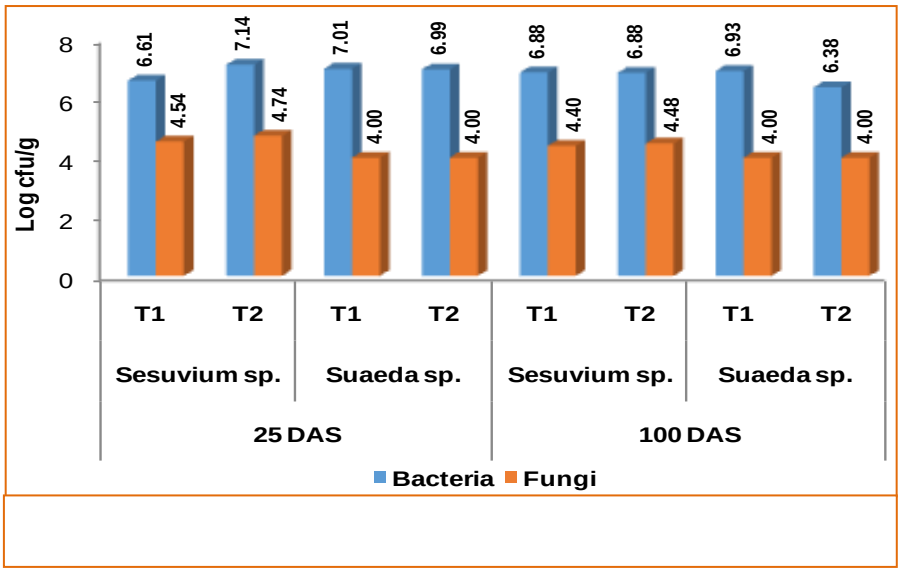


Figure 3-13. Microbial load in halophyte cultivated plots

3.6 Replicable model demonstrated for integrated saline wastewater use and livelihood options

Work is in progress

3.7 Package of agro-aqua farming system available for replication

The benefits of treating sugar effluent by bioremediation and phytoremediation process is a simple and low-cost technology. As mentioned in the earlier sections sequentially treated sugar effluent is the source of water for fish culture in an earthen tank. A trapezoid shaped tank was excavated with the dimensions 45mx15mx2 m for culturing fish. Total volume of the tank is 1449 m_3 with the retention time of 14 days at the rate of 50% water replenishment from sedimentation tank where the fish seeds are grown. In order to maintain the level of dissolved oxygen a motor operated water circulation facility is established. Indian Major Carp viz., Rohu (*Labeo rohita*) and Catla (*Catla catla)* are released in 80:20 ratio. Main purpose was to observe the survival, growth and health of fishes in the first season. Hence extensive farming system is adopted by stocking very low density (350

Rohu and 50 Catla) of initial weight ranging from 150 to 200 g. Based on the observations the stocking density is to be increased in the next season to monitor productivity. Fish are fed with rice bran and groundnut oil cake with proximate protein content ranging from 16 to 18%. Feeding is done on daily basis depending on the 2% of body weight so as to allow them to harness optimum primary productivity. The average length and weight ranges from 30 to 37 cm and from 450 to 650 g in a period of 150 days respectively.



Water quality monitoring is done on fortnightly basis and analysed the key physico chemical parameters and the results are presented in tables 4 and 5 indicates that the quality is maintained as per the standards of fish culture. One of the expected outcomes of the integrated approach of reusing bio-treated sugar effluent first in aquaculture and then in agriculture is for fertilizer savings. To ascertain whether the fish culture contributes towards fertilizer savings and to plan appropriate fertigation for sugarcane crop water sample from fish tank was analysed and the results are presented below.

Primary treated sugar effluent was the source for the bio-treatment. The source was bio-treated through different stages such as Filtration, Wetland, Sedimentation, and Fish tanks for irrigation purpose. The quality of the treated water for irrigation is evaluated both physically and chemically. The physical properties such as appearance, colour, odour, turbidity, TDS and EC were analyzed and the results are presented in Table 3-41. The source water was turbid, dark brown colored with an unpleasant smell, whereas after the bio-treatment process, the water in the FT was clear, colour less, odour less. For aquatic and irrigation purpose the turbidity of water should not exceed 10 NTU (nephelometric turbidity units) and in this study, the turbidity of the treated water was 1 NTU which shows that the quality of the water is excellent. The TDS of treated water was 338 mg/L which is good as the desired level of 0 – 2000 mg/L for irrigation. EC_w of treated water in FT was within the desirable range for irrigation.

Table 3-41. Physical parameters of water quality in fish tank

Sl. No	Parameters	Result
1.	Appearance	Clear
2.	Colour	Colour less
3.	Odour	Nil
4.	Turbidity NT Units	1.0
5.	Total dissolved Solids (mg/L)	500
6.	Electrical Conductivity (micro mho/cm)	714

Table 3-42. Chemical parameters of water quality in fish tank

Sl. No.	Parameter	Primary treated sugar effluent	Water in FT	Irrigation Standard
1	pH	8.2	7.2	6 - 8.05
2	Alkalinity as $CaCO_3$ (mg/L)	-	BDL	
3	Total alkalinity as $CaCO_3$ (mg/L)	700	208.0	
4	Total hardness as $CaCO_3$ (mg/L)	310	180.0	
5	Calcium as Ca (me/L)	2.1	2.7	0-20
6	Magnesium as Mg (me/L)	2.7	0.8	0-5
7	Sodium as Na (me/L)	7.3	2.9	0-40
8	Potassium as K (me/L)	72.9	8.0	0-2
9	Iron as Fe (mg/L)	2.5	0.3	5
10	Manganese (mg/L)	ND	BDL	0.2
11	Free Ammonia as NH_3 (mg/L)	ND	0.5	0-2.5
12	Nitrite as NO_2 (mg/L)	9.1	0.7	0-10
13	Nitrate as NO_3 (me/L)	1.1	5.0	0-10
14	Chloride as Cl (me/L)	6.6	2.4	0-30
15	Fluoride as F (mg/L)	ND	0.7	1
16	Sulphate as SO_4 (me/L)	2.1	0.02	0-20
17	Phosphate as PO_4 (mg/L)	732.9	0.3	0-2

About 17 chemical parameters of water quality from fish tank was analysed and the results are presented in Table 3-42. The pH of water in the FT was 7.2 which is reduced from 8.2 due to treatment and is in the desirable level. The total alkalinity of the treated water has been significantly reduced by 70.3% from 700 mg/L to 208 mg/L. Also, total hardness of water has reduced from 310 mg/L to 180 mg/L which is good for both aquaculture and agriculture. The concentration of calcium in the primary treated sugar effluent was 2.1 me/L whereas, in fish tank it had increased to 2.7 me/L and it is well within the desired level for irrigation. The magnesium content of treated water has reduced from 2.7 to 0.8 me/L which is a very low concentration and is in the desired level for irrigation to avoid infiltration problems. A significant reduction of sodium from 7.3 me/L to 2.9 me/L indicates that the bio-treatment process in efficient and the water is good for irrigation. In the primary treated sugar effluent, the concentration of potassium was 72.9 me/L which is very high for irrigation than the desired level of 0-2 me/L. After bio-treatment, around 82% of potassium was removed and finally 0.8 me/L of potassium is present in FT which is well within the range for irrigation. Around 88% of iron was removed from the primary treated sugar effluent water enabling the water to be suitable for irrigation. Manganese was below detection limit and free ammonia was 0.5 mg/L in the bio-treated water in FT which is in the desired range. Nitrite reduction from 9.1 to 0.7 mg/L and increase in nitrate concentration from 1.1 to 5 me/L. A very less concentration of Ammonia, nitrite and an increased concentration of nitrate in treated water shows that the water is suitable for fish culture and agriculture which would have been facilitated by the wetland treatment of sugar effluent. In wetland, ammonia ion was oxidized in the presence of oxygen by nitrosomonas bacteria to form nitrite. The nitrite was then oxidized to nitrate in the absence of oxygen (anaerobic) by the nitrobacter bacteria in the wetland.

Reduction of chloride concentration from 6.6 to 2.4 me/L is due to the biological process and removal of salt ensures a good quality of water of fish and subsequently for agriculture too. This result corroborates with the decrease in conductivity observed after treatment. The concentration of toxic fluoride is less than the permissible limit which confirms the enhanced quality of water. Around 99% of sulfate was removed from the primary treated sugar effluent ensuring it to be within the desirable level. This is achieved by both biological and physical process in the bio-treatment. Adsorption is the most important phosphorus removal process in the wetlands.

The concentration of phosphorus was 366 times higher than the desired level of 0-2 mg/L in the primary treated sugar effluent. Phosphorus in the water causes algae to grow faster than ecosystems can handle. Significant increases in algae will harm water quality, food resources, habitats, and decrease the oxygen that fish and other aquatic life need to survive. Algal blooms can severely reduce or eliminate oxygen in the water, leading to illnesses in fish and the death of large numbers of fish. Around 99.95% of phosphorus was removed from sugar effluent and a final concentration of 0.3 mg/L was observed in FT which is well within the irrigation limit. In the current treatment system, it is evidently noticed that the algae growth is observed in the wetland but not in the ST and FT which shows that the phosphorus removal has been facilitated by the algae grown in the wetland. Also, phosphorus removal in the wetland occurs due to adsorption reactions with iron, calcium and magnesium which are present in the water. Adsorption of phosphorus to iron ions takes places under aerobic and neutral conditions to form stable complexes. Adsorption to calcium ions takes place under basic to neutral pH conditions. Thus adsorption of phosphorus to the ions removes it from the wastewater. The pH of water is important to be neutral and above to avoid a reversible process which will be harmful. The phosphorus removal in wetland has concurrently played a significant role in the removal of iron, calcium and magnesium. Thus the results observed in the bio-treated water reveals that the current treatment process in efficient to provide excellent quality water for irrigation purpose.

3.8 Enhanced capacity of community, other stakeholders and MSSRF staff on saline wastewater farming

Vuyyuru and Lakshmipuram sites of KCP

- Around 15 officials at different levels and professional background from Vuyyuru and Lakshmipuram sites of KCP were oriented on the project concept and processes.
- Other stakeholders like local NGO PPSS, academic and research institutes like Anna University and Central Institute for Freshwater Aquaculture are working in partnership along with the Industrial partner and sharing their resources and expertise in taking forward this project
- Around 400 farmers who are the primary stakeholder and expected end users of the innovations were oriented on the integrated and hybrid approach of treating industrial wastewater and reusing it in the aqua and agro farming systems
- Two day programme for the aquaculture and agriculture farmers was conducted. Around 80 farmers from 10 villages participated. Representatives from KCP sugar industries and PPSS the local partner NGO participated as resource persons both in technical session and at field site.

Exposure visit to farmers

Exposure visit for aquaculture and agriculture farmers was organised to the Lakshmipuram site, where constructed wetlands as a treatment system and reuse of bio-treated sugar effluent in an integrated aqua-agro farming system is demonstrated. Two day programme separately for the aquaculture and agriculture farmers was conducted on 17 and 18 March 2015. In which 38 farmers from 6 villages and 39 aquaculture farmers from 4 villages participated. Representatives from KCP sugar industries and PPSS the local partner NGO participated as resource persons both in technical session and at field site. The programme comprised of a class room session and exposure to the field site. In the class room session, community was oriented on the concept of treatment and reuse of wastewater as an emerging water management practice not only to reduce fresh water demand in agriculture but also to help in protecting groundwater contamination and water quality at downstream water bodies as well as its implications on health and environmental degradation. The constructed wetland used as a treatment technology and its process were explained using power point presentation. Also the design and process of integrated aqua-agro farming system was detailed to them. In the afternoon they were taken to the field site at Lakshmipuram.

Key observations and feedback by the famers

- Appreciated the constructed wetland system as it is biological and low cost technology needed to large industries
- Requested similar type of small scale and low cost technology for treating groundwater with high salinity and hardness – demonstration of pilot model at farmers field
- Commended the improved water quality as well as reduction in colour and odour
- Admired the integrated aqua-agro farming system and the luxuriant growth of fish and fertilizer saving
- Asked about the feasibility of replicating this model in farmers field
- Suggested if the algal growth is reduced in fish tank other than C*atla* sp. & *Rohu s*p shall be cultured
- Expressed that there is no difference between the crops irrigated with fresh water and treated water

3.9 Availability of tool kit on agro-aqua farming system in print and multimedia format

Work is in progress

4 Work package: Development of water efficient crop varieties

Objectives

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

4.1 Information on the most adequate combinations of species/genotypes x environment x management for different drought scenarios in India and EU

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

Activity 4.1.a.1. Test the transpiration response to changing VPD conditions in 40 entries of pearl millet, sorghum and maize using standard protocols.

10 genotypes of maize, 16 of sorghum, and 10 of pearl millet were tested for the capacity to restrict transpiration under high VPD. Below are the summary tables reporting the data on the response curve, especially the breakpoint values and whether there was, or not (linear response), a breakpoint in the transpiration response. If there is a breakpoint, the response of transpiration is then characterized by a two-linear-segments response, with two slopes one per each segment.

Table 4-1. Analysis of the transpiration response to VPD in 10 genotypes of maize. The analysis provides breakpoint, if any, and then slope 1 and slope 2 (in case of breakpoint).

Maize	Genotype	Breakpoint	Slope 1	SE	Slope 2	SE	R2
M1	**9424780**	3.29	12.77	2.66	-1.14	2.43	0.62
M2	**783527**	4.14	13.83	1.98	0.93	8.49	0.74
M3	**4695575**	4.00	11.30	1.94	0.80	5.84	0.68
M4	**18270413**	Linear	7.56	1.39	-	-	0.47
M5	**22525674**	3.27	10.84	3.17	-0.90	2.91	0.45
M6	**8315622**	Linear	10.83	1.05	-	-	0.76
M7	**14746185**	3.03	17.69	4.31	-4.85	3.69	0.42
M8	**30V92**	3.67	11.27	2.81	1.17	4.77	0.55
M9	**900MG**	Linear	10.31	1.54	-	-	0.58
M10	**Public check**	Linear	-0.98	1.78	-	-	**0.01**

Table 4-2. Analysis of the transpiration response to VPD in 16 genotypes of of sorghum. The analysis provides breakpoint, if any, and then slope 1 and slope 2 (in case of breakpoint).

Sorghum	Genotype	Breakpoint	Slope 1	SE	Slope 2	SE	R2
S1	BTx623	3.82	13.31	2.19	3.10	5.13	0.73
S2	IS18551	3.30	11.81	2.78	1.58	2.73	0.62
S3	296B	4.42	10.63	1.52	-1.72	9.28	0.71
S4	E 36-1	3.82	11.95	1.52	7.71	3.44	0.86
S5	N13	3.72	13.11	2.69	2.64	5.46	0.63
S6	IS9830	4.23	17.72	2.64	-13.61	12.60	0.66
S7	ICSV745	Linear	11.73	1.13	-	-	0.77
S8	PB15220-1	Linear	13.43	8.31	-	-	0.74
S9	PB15881-3	3.03	8.78	3.46	6.40	2.96	0.54
S10	PVK 801-P23	Linear	10.90	0.64	-	-	0.8968
S11	ICSV93046-P1	Linear	10.18	1.209	-	-	0.7394
S12	S35	Linear	12.84	0.9582	-	-	0.8693
S13	ICSR93024	Linear	12.31	1.023	-	-	0.8144
S14	ICSV1	Linear	10.18	0.8568	-	-	0.8106
S15	ICSV700-P10	Linear	11.08	0.9621	-	-	0.8007
S16	M 35-1	Linear	12.01	1.17	-	-	

Table 4-3. Analysis of the transpiration response to VPD in 10 genotypes of pearl millet. The analysis provides breakpoint, if any, and then slope 1 and slope 2 (in case of breakpoint).

Pearl Millet	Genotype	Breakpoint	Slope 1	SE	Slope 2	SE	R2
PM1	H77/833-2	Linear	16.73	2.12	-	-	0.65
PM2	PRLT	3.15	11.92	2.03	1.90	1.91	0.74
PM3	841B	4.35	8.78	2.00	-1.31	11.23	0.52
PM4	863B	2.67	9.80	9.92	3.42	4.18	0.14
PM5	GB8735	Linear	12.71	1.53	-	-	0.68
PM6	ICMV-IS 92222	Linear	12.31	1.60	-	-	0.64
PM7	PT732B-P2	2.67	7.91	4.73	5.49	1.99	0.49
PM8	841B x PPMI 301 (Pusa 322)	3.22	11.15	2.42	2.50	2.22	0.67
PM9	ICMP 451-P6	Linear	6.86	1.33	-	-	0.50
PM10	Tift 238D1-P158	Linear	12.07	2.33	-	-	0.46

Below are typical responses displaying presence, or absence of a breakpoint in the three crops. The top case illustrates two well-known maize hybrids, i.e. Monsanto 900MG, bred as a typically rainy season hybrid, and which displays unlimited transpiration response under high VPD. The second maize hybrid is 30V92, a drought tolerant hybrid released by Pioneer, and which displays a transpiration restriction under high VPD conditions. Below are contrasting cases in sorghum and pearl millet. It should be noted that genotype PRLT is the donor parent of a terminal drought tolerance QTL, which also co-locates with a QTL for the capacity to restrict transpiration under high VPD.

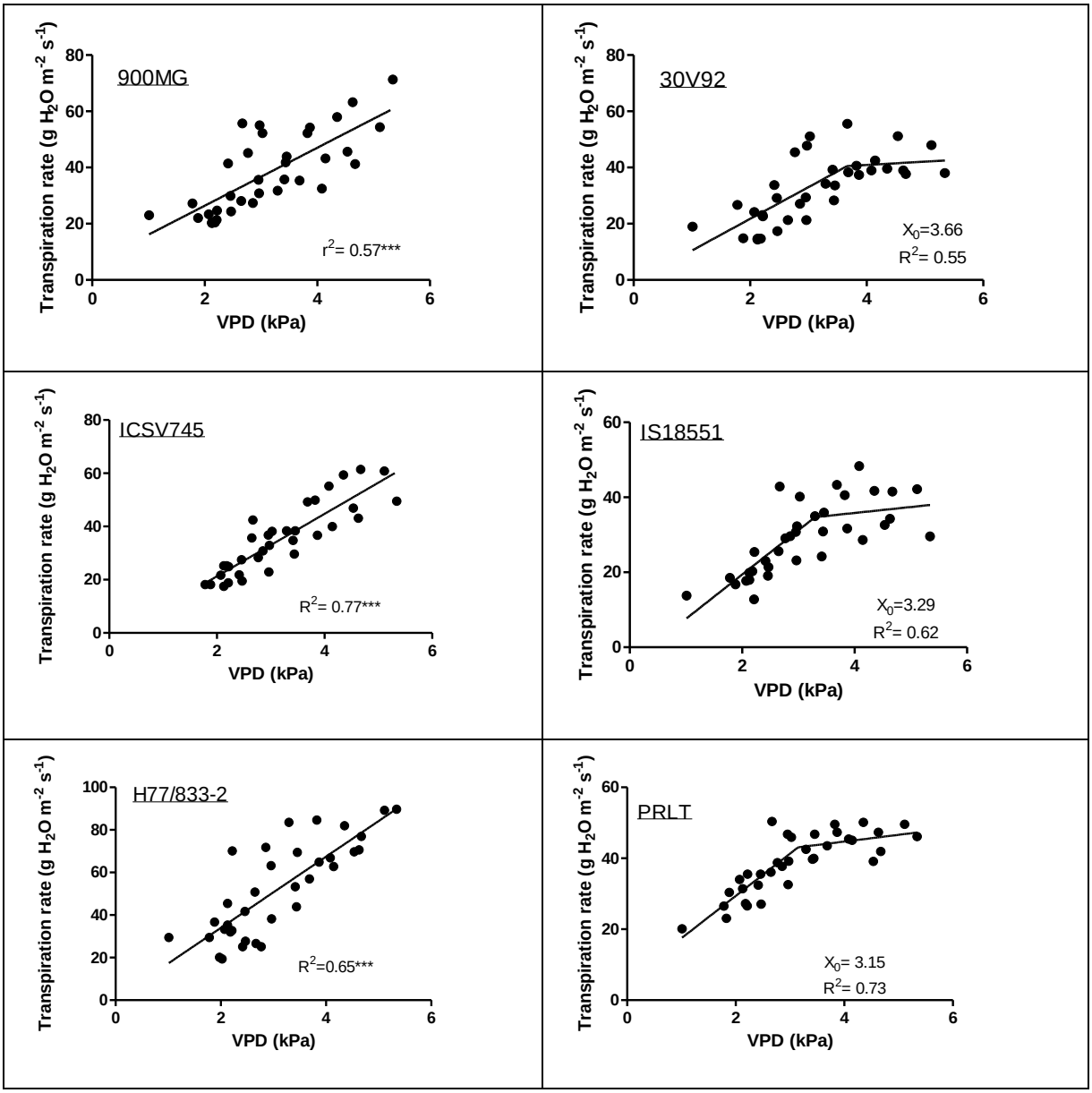


Figure 4-1. Typical transpiration response to high VPD in genetic materials of maize, pearl millet and sorghum.

In summary of the current status of that activity, we have shown that the capacity of restricting transpiration under high VPD operates in three different C4 species, where it discriminates well entries that are bred for environment with high water availability and then characterized by the absence of a restriction of transpiration (eg 900MG, H77/833-2, or ICSV745), in contrast to entries that have drought adaptation characteristics (eg 30V92, PRLT). Of course, this is also based on other work carried out in the group in the scope of other projects, in which we have also demonstrated a clear link between the capacity to restrict transpiration under high VPD and higher transpiration efficiency (TE), thereby our great interest in that trait. In the scope of that activity, we will now be able to screen selected germplasm of maize, sorghum and pearl millet for that phenotype.

Activity 4.1.a.2. Test the transpiration response to changing VPD conditions in NILs of B73 and Gaspe Flint

This experiment has been carried out in the February-March period. The trial included 77 NIL lines (=74 NILs + 3 B73 recurrent), along with the assessment of 18 maize germplasm entries. It consisted in growing individual plants in large pots containing 11 kg of Alfisol under outdoors conditions. The plants were maintained under fully irrigated condition during the entire growth period. The sowing was done in a staggered manner, sowing two replications per day and leaving a 2-days gap between each sowing. This way, plants were assayed for the response of transpiration to high VPD at the same physiological age. At 4 weeks after sowing, the pots of the first two replications were brought to field capacity and covered with a plastic sheet and a 2-cm layer of plastic beads to prevent most of the soil evaporation. The pots were left to drain the excess water overnight. The following day, the pots were weighted three times, early in the morning, in the mid-morning, and early afternoon. Prior to that, we had monitored the evolution of the VPD in the course of several days. We had then fixed the mid-morning measurement to that time when the VPD crosses a threshold of 2 kPa. Therefore, the transpiration, measured gravimetrically from the first two measurements reflected a period of time with low VPD, whereas the transpiration, measured gravimetrically from the last two measurements reflected a period of time with high VPD.

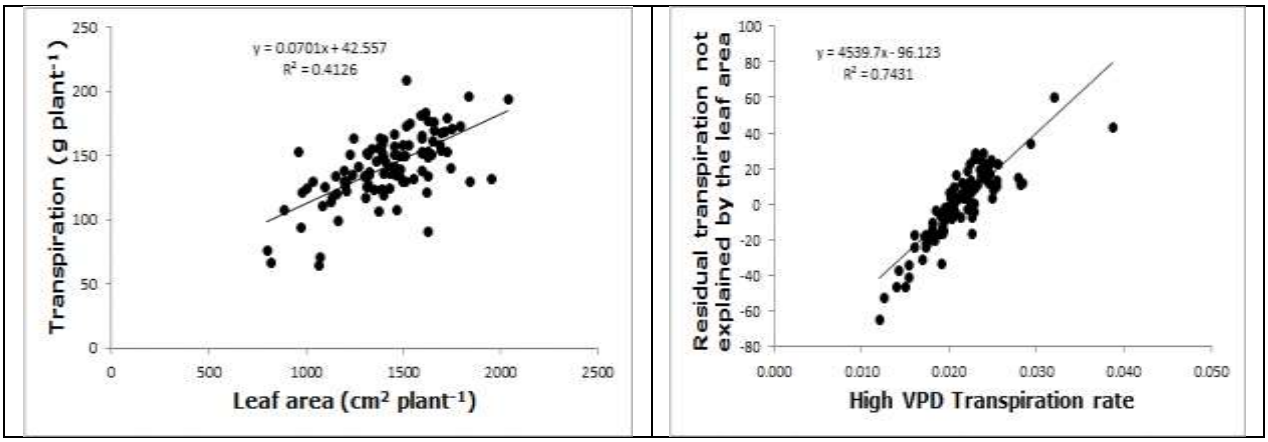


Figure 4-2. Relationships between transpiration and leaf area in a set of maize germplasm and in a set of B73 introgression lines (left) and relationship between the residual variation in transpiration not explained by the leaf area (right). Residual were calculated as the difference between the predicted transpiration (from the equation on left panel) and the observed transpiration. Data are the mean of six replicated pots per genotype.

Differences in leaf area among the lines tested explained about 40% of the transpiration differences under these conditions of high evaporative demand. This meant that at any given leaf area, there were lines transpiring quite contrasting amounts of water. The residual transpiration unexplained by the leaf area corresponded to the distance between each data point and the regression line from the left panel of Figure 4-2, and was then computed as the observed transpiration minus the transpiration predicted by the leaf area. Positive residuals indicated that the transpiration was above what could be expected from the leaf area, whereas negative residuals had transpiration below what could be expected from the leaf area. There was a large variation in the residuals (from about -80 g up until +40 g plant-1),

confirming that there was a substantial part of the transpiration variation that was unexplained by the leaf area. These residuals showed a very tight relationship with the transpiration rate under high evaporative demand, indicating that lines with high residual transpired high amount under high VPD conditions.

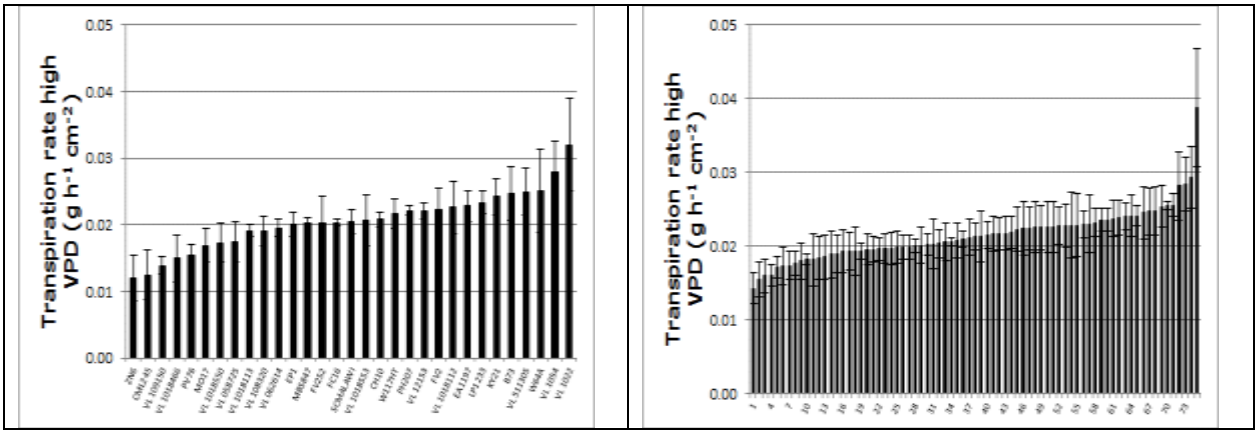


Figure 4-3. Range of variation in the transpiration rate under high VPD in a set of maize germplasm (left) and in a set of introgression lines in B73 background (right). Data are the mean of six replicated pots per genotype (± SE).

Genetic variation in the transpiration rate under high VPD (Figure 4-3) was about 3-folds in the set of germplasm (left panel on Figure 4-3) and only 2-folds on the set of introgression lines in B73 background.

Activity 4.1.a.3. Test key staygreen QTL (likely Stg1, StgB and Stg3) introgression lines of sorghum for traits putatively underlying these QTL, towards refining the QTL interval responsible for the trait

This activity has been concluded. The experimental approach was similar to Activity 4.1.a.2. above, except that because the number of plants was lower, more transpiration measurements could be done in the course of the day. Here also, we have acquired evidences of differences in the transpiration response to VPD in introgression lines containing staygreen QTL. Figure 4 below shows that both Stg3 and StgB restrict transpiration under high VPD, compared to recurrent parent R16. This results agrees closely with our field testing (in the scope of other projects) revealing a grain and stover yield advantage of StgB and Stg3 introgressions over recurrent R16.

There is also the case of Stg1 introgressions where the restriction is even more severe than in Stg3 and StgB introgression. In this case, the restriction likely confers a yield reduction (as shown by the yield penalty in our field trials). In any case, this trait appears to be conferred by the B35 donor parent which also shows a transpiration restriction.

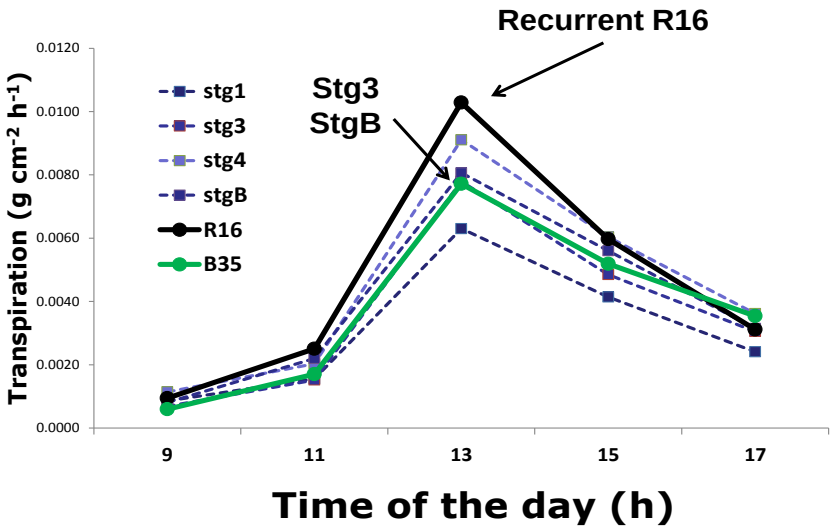


Figure 4-4. Transpiration response to high VPD (where the time of the day is taken as a proxy for VPD) in introgression lines of staygreen QTL in the background of R16 recurrent (senescent) parent. Each data point for the introgression lines is the mean of 4 to 5 individual introgressions.

We have also tested the transpiration response to high VPD in similar staygreen QTL introgressions in other backgrounds, to find out that in S35 background, we had no effect of the introgression. These results suggest that the recurrent S35 already possesses the VPD-response trait.

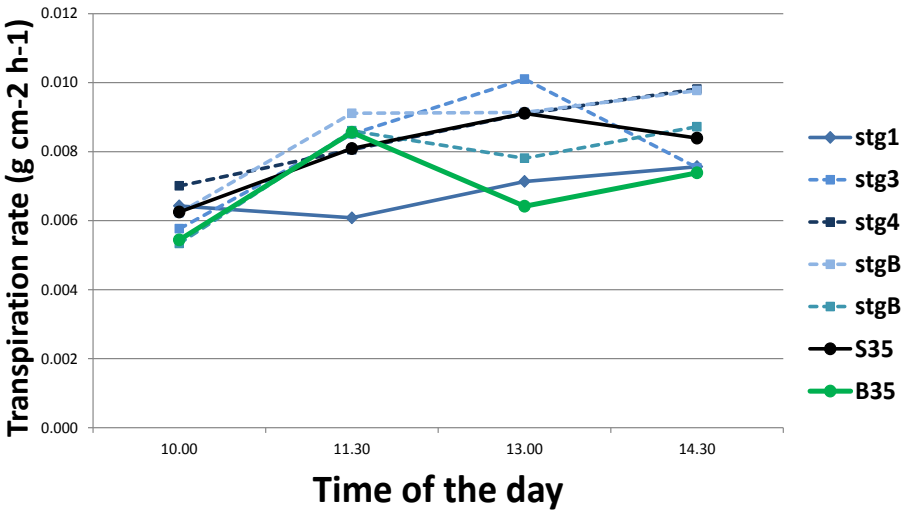


Figure 4-5. Transpiration response to high VPD (where the time of the day is taken as a proxy for VPD) in introgression lines of staygreen QTL in the background of S35 recurrent (senescent) parent. Each data point for the introgression lines is the mean of 4 to 5 individual introgressions.

4.2 Information on QTL (QTL combination) underlying the drought adaptation traits in maize, sweet sorghum, pearl millet and tomato at particular drought stress environments

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

Activity 4.2.a.1. Test about 40 entries of pearl millet, sorghum and maize germplasm in lysimeters

This summarizes several trials that have been carried out and partially reported in the Year 1 report. Here we report the rainy season trial and compare to the post rainy season trial reported in the Y1 report. During the rainy season, we have tested the same 10 genotypes of maize, 16 genotypes of sorghum and 10 genotypes of pearl millet in the lysimeters. The overall idea was to assess the plant water requirement of these three C4 species across season varying in the evaporative demand. The 2012-13 season data, reported in Year 1, was characterized by a high evaporative demand. The rainy season was of course characterized by a much lower plant water requirement.

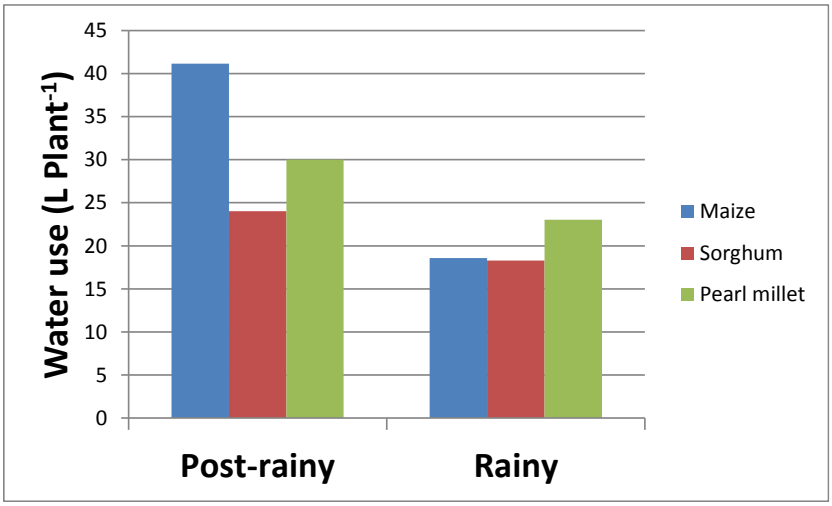


Figure 4-6. Plant water use in maize, sorghum and pearl millet in the post-rainy and rainy season. Data points are means of 10, 16, and 10 entries in maize, sorghum and pearl millet respectively

The results show that under high evaporative demand, the water requirement of maize was very large compared to sorghum and pearl millet, whereas under low evaporative demand, the water requirement of the three species was very similar. This may suggest a better capacity of sorghum and pearl millet to curb water use under high VPD conditions, making them species better adapted to semi-arid tropical areas.

During the rainy season, we have used four water regimes, i.e. a fully irrigated control and three water stress treatments, imposed at the time of flowering of each of the crops. Therefore, DS3 was imposed at the time of pearl millet flowering, DS2 at the time of sorghum flowering (about two weeks after DS3) and DS1 at the time of maize flowering (one week after DS2). The graph below report yield data across the water regimes. The yield

decreases of maize from a fully irrigated situation to increasing conditions of stress are gradual. In all cases though, the grain yield of maize was above sorghum, although the difference is small under the most severe stress. For sorghum and pearl millet, which require less water across their cycle, the yield decrease are very mild across treatment, except the most severe DS1. Sorghum and pearl millet use similar water than maize in the rainy season but reach lower yield than maize, which probably is explained by the production of unproductive tillers.

By contrast, in the postrainy season, the yield decrease caused by drought was dramatic in maize (>60%), whereas that in sorghum and pearl millet is a lot less (about 20-30%). It should be noted that the yield data reported from these lysimetric trial can be extrapolated to field conditions. Because we use a planting density in the lysimeters of 10 plants m^{-2}, the 110 g $plant^{-1}$ of maize under WW conditions is equivalent to 11 t ha^{-1}, which is equivalent to field observations in the same season.

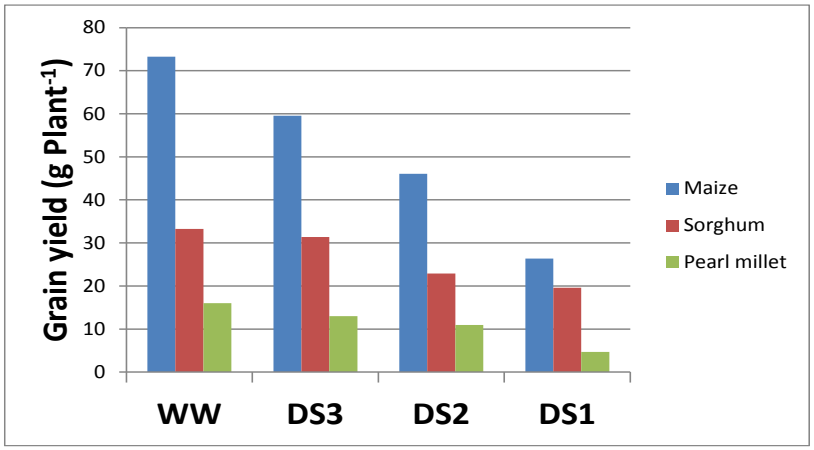


Figure 4-7. Grain yield in maize, sorghum and pearl millet in the rainy season 2013. Data points are means of 10, 16, and 10 entries in maize, sorghum and pearl millet respectively. Four water regimes were used, i.e. well-watered treatment (WW) and three drought stress imposed at the time of pearl millet, sorghum and maize flowering (DS1, DS2, DS3 respectively).

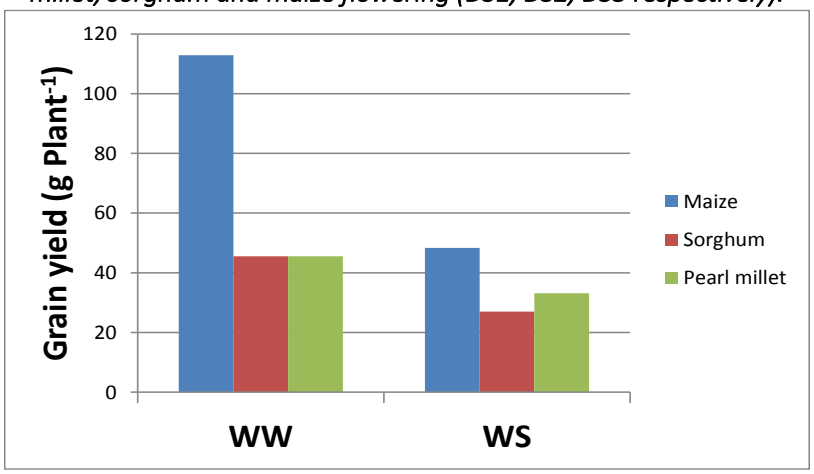


Figure 4-8. Grain yield in maize, sorghum and pearl millet in the post-rainy season 2012-14. Data points are means of 10, 16, and 10 entries in maize, sorghum and pearl millet respectively. Four water regimes were used, i.e. well-watered treatment (WW) and three drought stress imposed at the time of pearl millet, sorghum and maize flowering (DS1, DS2, DS3 respectively).

Re-assessment of TE in the pearl millet germplasm - During the summer 2014 season, we have also re-assessed the pearl millet inbred germplasm association panel (PMiGAP). In the previous years, we had tested the testcross hybrid version of the PMiGAP. However, in our collaboration with INRA-France, it was decided to only assess inbred versions of the three species. Therefore, there was a need to re-assess the PMiGAP in its inbred versions. We did so in the 2014 season, under water stress conditions (and are repeating it during the summer of 2015). This consisted of 238 inbred entries, which we completed with 42 of the most contrasting PMiGAP testcross hybrids for transpiration efficiency (TE). These latter 42 entries had been tested in Year 1 (and reported).

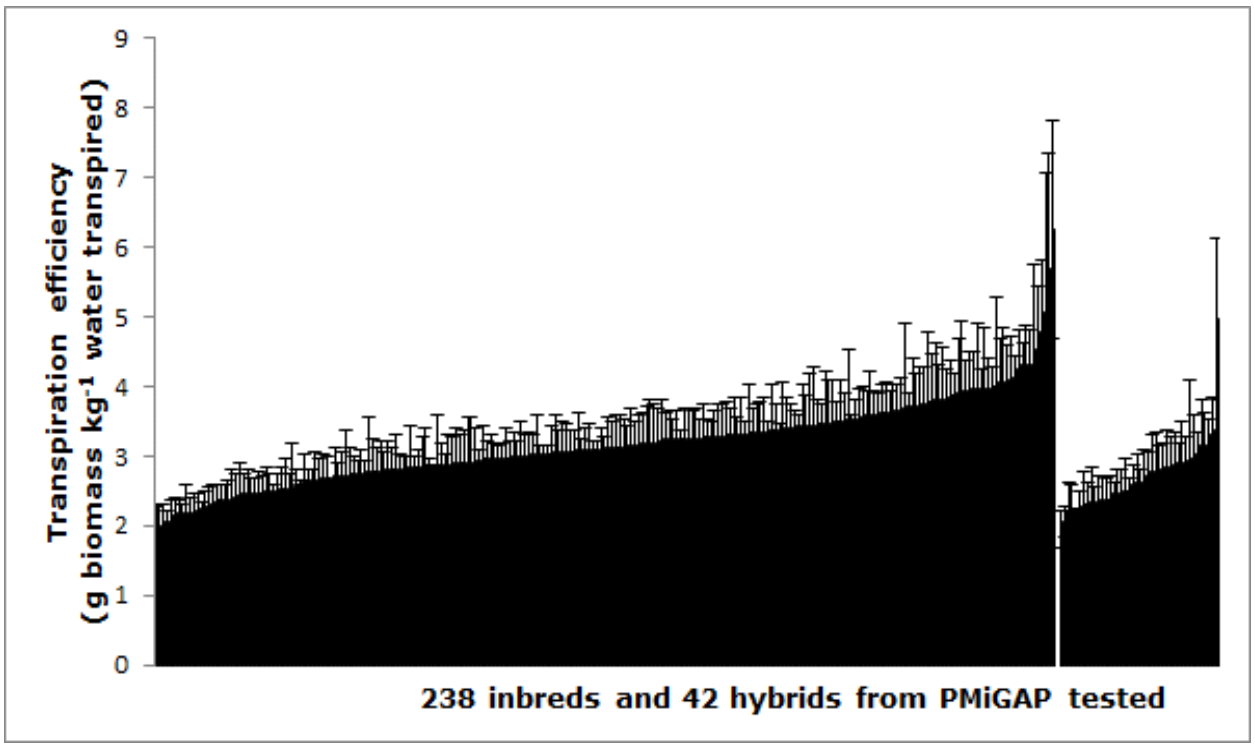


Figure 4-9. Transpiration efficiency (TE, in g biomass kg^{-1} water transpired) in 238 inbred lines from the PMiGAP and 42 testcross hybrids of the PMiGAP. Data are means of 5 replicated lysimeter per genotype (±SE).

Results showed a large range of variation for TE among the genotype. This range was large in the inbred (3-folds from about 2 to 6 g kg^{-1}) than in the testcross hybrids (about 1.5-folds from 2 to 3 g kg^{-1}). A repeat of this trial has been carried out in the summer season of 2015 and will be reported in Year 3 report. From this, the highest TE variants will be used to develop BCNAM population in the breeding program of pearl millet.

Activity 4.2.a.2. Test ILs of B73 background in lysimeters

We have received the introgression lines from Italy late in 2013 and could not include them in the 2013-14 lysimetric trials. These were then tested during the rainy season 2014.

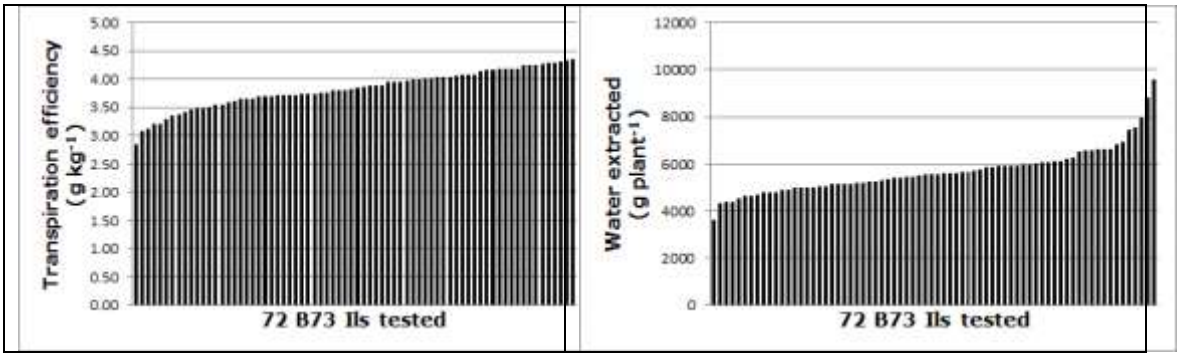


Figure 4-10. Transpiration efficiency (TE, in g biomass kg-1 water transpired) in 72 introgression lines in B73 background (Gaspe Flint as donor parent).

This trial was carried out in the rainy season, although in a "dry" rainy season with fairly high evaporative demand. Nevertheless, the variation for TE was relatively small (3.00-4.50 g kg-1, i.e. about 1.5 fold variation) (Figure 4-10, left panel), which is relatively small. By contrast, the range of variation for the water extraction was really large (about 2-folds, from 4 to more than 8 L plant-1) (Figure 4-10, right panel). On the one hand, the amount of water extracted from the profile was small compare to other experiments with maize in a similar system. This owed in large part to the fact that these ILs were of temperate background (B73), and indeed suffered the tropical temperature conditions of South India. On the other hand, it was expected to find large differences in the water extraction since these introgression vary for the introgression of segments responsible for root attribute differences (depth / angle).

Activity 4.2.a.3. Test ideotypes of pearl millet having contrasting "dosage" of QTLs involved in plant water use traits, across a gradient of water stress treatments in the lysimeters

The testing of pearl millet ideotypes containing different QTLs controlling water use traits has continued in 2014. We have repeated a field trial of 2013 where 4 water regimes (one fully irrigated control and three water deficit treatments had been used). A similar trial has just been harvested we again 4 water regimes were used (again one fully irrigated treatment and 3 water deficit treatments). Here the rationale is that the drought tolerance QTL which was identified around 14 years ago was specific of the long terminal stress conditions it was exposed to in the set of trials that were carried out then. Most of these indeed were done during the summer season at the ICRISAT headquarter, in deep and high clay content soils, allowing the slow development of stress conditions. The purpose of these experiments was then to go beyond the stress conditions that were used when the QTL was identified, by also exposing the lines to intermittent forms of stress and then to generate a large pool of phenotypic responses across different environments and water regimes. Testcross hybrids (DM-resistant 843A tester) of the RIL population (H77/833-2 x PRLT2/89-33) were then assessed under field conditions across water regimes that were designed to mimic the rainfall distribution variation of the A1 zone (terminal stress and different kinds of intermittent stresses).

The QTL analysis of the results of the first year is reported in the ppt presentation (Annex 1 – QTL analysis Year 1) to that report. QTL analysis of the second year is in progress. The

overall objective will be to assess the extent of co-mapping of yield QTL with trait QTL, but also the extent of "interaction QTLs" (QTTL found in specific environment) and co-mapping of such QTL with specific traits would tell us about the importance of such and such mechanisms in specific environments.

Table 4-4. Evaluation of the parental lines in different water regimes, varying in intensity.

Parental lines	Treatment	Irrigation	Svdwm2	pndwm2	grdwm2	HI %	PnHI%
H 77/833-2	control	450 mm	652.16	427.62	286.04	26.89	67.88
PRLT 2/89-33			583.93	398.84	313.13	33.18	80.74
H 77/833-2	DS1	300 mm	440.92	299.63	174.85	23.67	57.88
PRLT 2/89-33			330.78	278.97	218.81	35.93	79.42
H 77/833-2	DS2	150 mm	210.55	389.20	223.39	37.06	57.36
PRLT 2/89-33			164.17	294.85	196.58	43.76	67.78
H 77/833-2	DS3	150 mm	210.87	367.53	246.25	41.52	64.93
PRLT 2/89-33		terminal stress	179.17	341.64	259.16	49.81	76.20

Svdwm2	Stover dry weight per sqm
pndwm2	Panicle dry weight per sqm
grdwm2	Grain dry weight per sqm
HI %	Harvest index percentage
PnHI%	Panicle harvest index percentage

The data showed that there was a GxE interaction in that the parents did not perform equally well across all water regimes, especially the drought stress ones (Table 4-4).

Table 4-5. QTL for grain yield across the different water regimes

Trait	control						DS1						DS2						DS3					
Grdwt persqm	LG	Pos	Left Mark	LOD	add	R^2	LG	Pos	Left Mark	LOD	add	R^2	LG	Pos	Left Mark	LOD	add	R^2	LG	Pos	Left Mark	LOD	add	R^2
	LG1	96	P_7875	2.54	16.964	8.2							LG1	138	P_9433	3.44	-9.698	16.7						
	LG1	110	m01/1088	7.24	-32.21	17.5																		
	LG2	328	psmp2059	3.71	-13.52	5.5													LG2	338	P_7330	3.14	13.099	**18.5**
	LG3	2	ipes0095	4.48	14.673	12.6	LG3	2	ipes0095	7.9	11.5	15	LG3	0	ipes0095	5.83	13.681	20.4	LG3	2	ipes0095	5.21	13.386	25
	LG4	122	P_10552	6.73	-26.851	15.8																		
	LG6	56	psmp2270	2.82	12.333	10.9							LG6	54	P_13164	5.99	14.941	16.6						
																			LG5	36	psmp2274	4.86	12.381	10.2

Under the typical terminal stress conditions (DS3) under which the QTL (for yield) was initially identified in that population, the same QTL was again identified (on LG2, Table 2). By contrast, in the other water regimes (intermittent stress DS1 and DS2), other QTLs for grain yield understress were identified, in other linkage groups (LG3 and LG6). The QTL on LG3 was then common to both stress conditions (DS1 and DS2).

Task 4.2b: Mapping of genomic regions controlling traits related to drought tolerance/WUE in tomato (Lead Institute: UAS-B; Task Leader: DL Savithramma)

Phenotyping of fruit yield and WUE related traits among different tomato species

The experiments were conducted at K_1-block University of Agricultural Sciences, GKVK, Bangalore, Karnataka. It is situated in zone-5 of Eastern dry zone of Karnataka state at an altitude of 930 m above mean sea level with a 12˚58' North and 77˚35' East latitude and longitude, respectively. The experimental site consisted of medium red sandy loam soil.
One-hundred indigenous and exotic germplasm accessions of six tomato species along with three check entries (Arka abha, Arka vikas and Arka meghali) procured from National Bureau of Plant genetic resources (NBPGR), New Delhi and Asian Vegetable Research and Development Centre (AVRDC), Taiwan.

One hundred germplasm accessions and three check entries were sown in Augmented design (Federer, 1956) during *summer* 2014, under stress and control conditions. Each entry was transplanted in a single row of 5.00m in length with a spacing of 75cm (between rows) x 50cm (between plants in each row). Healthy and uniform seedlings were transplanted on one side of the ridges and recommended agronomic and plant protection practices were followed during the crop growth period to raise a healthy crop. Five weeks after transplanting, plants were supported by galvanized wire which was tied to wooden poles.

Drought was imposed at 90 days after sowing or 60 days after transplanting to all the accessions by withholding irrigation in stress plot for twenty days. The control plot was given normal irrigation two times a week using drip irrigation system.

Five randomly chosen plants in each accession were labeled and used for recording fourteen different morphological parameters, *viz*., days to fifty percent flowering (DFF), days to first fruit-set (DFFS), plant height (PH), number of branches per plant (BN), number of flowers per cluster (FLPC), number of clusters per plant (CPP), number of fruits per cluster (FNPC), number of fruits per plant (FPP), fruit traits like average fruit weight (AFW), fruit volume (FV), fruit flesh thickness (FT), locule number per fruit (LN), total soluble solids (TSS), fruit yield per plant, FYPP (Y_N denoting for control yield and Y_S depicting for stress yield) and five WUE related traits including SPAD chlorophyll meter reading (SCMR), specific leaf area (SLA), stem girth (STG), leaf rolling (LR), relative water content (RWC). SCMR was measured using SPAD chlorophyll meter, SLA (cm^2/g) was computed by dividing fresh leaf area (cm^2) and their dry weight (g) and stem girth was measured by venier-caliper.

Statistical analysis
Fifteen drought tolerant indices including tolerance index (TOL), mean productivity index (MP), geometric mean productivity (GMP), harmonic mean (HAM), stress tolerance index (STI), relative drought index (RDI), abiotic tolerance index (ATI), stress susceptibility percentage index (SSPI), stress non-stress production index (SNPI), yield index (YI), yield stability index (YSI), modified stress tolerance index (K_1STI for control condition and K_2STI for stress condition), drought resistance index (DI) and stress susceptibility index (SSI) were calculated and adjusted based on fruit yield under stress (Y_S) and control (Y_N) conditions to screen drought tolerant tomato germplasm accessions.

Table 4-6. Drought tolerance indices used to identify drought tolerant tomato accessions

Sl. No.	Name	Full form	Formula	Author
1	**TOL**	Tolerance index	= Y_P-Y_S	Rosiele and Hamblin,1981
2	**MP**	Mean productivity	= (Y_P+ Y_S)/2	Rosielle and Hamblin,1981
3	**GMP**	Geometric mean productivity	= √(Y_P xY_S)	Fernandez, 1992
4	**HM**	Harmonic mean	= 2*(Y_P x Y_S)/(Y_P+ Y_S) $\sqrt{(Y_S \times Y_P}$	Kristin *et al.,* 1997
5	**STI**	Stress tolerance index	= (Y_P x Y_S)/(Y_P)^2	Fischer and Maurer, 1978
6	**RDI**	Relative drought index	= (Y_S/Y_P)/(Y_S/Y_P)	Fischer and Wood, 1979
7	**ATI**	Abiotic tolerance index	= [(Y_P-Y_S)/ (Y_P/Y_S)] x √(Y_P x Y_S)	Moosavi *et al*., 2008
8	**SSPI**	Stress susceptibility percentage index	= 100 x [(Y_p-Y_s)/2Y_p) $\sqrt{(Yp \times Ys)}$	Moosavi *et al*., 2008
9	**SNPI**	Stress non-stress production index	= $[\sqrt[3]{(Yp+Ys)/(Yp-Ys)}] \times [\sqrt[3]{Yp \times Ys \times Ys}]$	Moosavi, 2008
10	**YI**	Yield index	= Y_S/Y_S	Gavuzzi, 1997
11	**YSI**	Yield stability index	= Y_S/Y_P	Bouslama and Schapaugh, 1984
12	**KSTI**	Modified stress tol. index	**K_1STI** = Y_P^2/Y_P^2 **K_2STI** = Y_S^2/Y_S^2	Farshadfar and Sutka, 2002
14	**DI**	Drought resistance index	= [Y_S x(Y_S/Y_P)]/Y_S)	Lan, 1998
15	**SSI**	Stress susceptibility index	= (1-Ys/ Y_P)/SI *Where,* SI = 1- (Y_S/Y_P)	Fischer and Maurer, 1978

Where,
Y_P: Fruit yield under control condition
Y_S: Fruit yield under stress condition
Y_P: Fruit yield mean under control condition
Y_S: Fruit yield mean under stress condition

ANOVA table and genetic variability, correlation analysis and principal component analysis (PCA), based on the rank correlation matrix and biplot analysis were performed by INDOSTAT, SPSS ver. 16 and XLSTAT 2014, respectively.

Table 4-7. List of tomato germplasm of six species (Solanum spp.) used for variability and drought tolerance studies								
S.N	**Name**	**Species**	**S.N**	**Name**	**Species**	**S.N.**	**Name**	**Species**
1	LA 1255	*S. habrochaites*	38	LA 1479	*S. cerasiforme*	75	CLN 2070 A	*S. lycopersicum*
2	LA 1353	*S. habrochaites*	39	EC 771616	*S. cerasiforme*	76	CLN 13149	*S. lycopersicum*
3	LA 2976	*S. habrochaites*	40	LA 2138 B	*S. cerasiforme*	77	EC 771593	*S. lycopersicum*
4	L 00673	*S. peruvianum*	41	LA 2138 A	*S. cerasiforme*	78	EC 771598	*S. lycopersicum*
5	L 00882	*S. peruvianum*	42	LA 1713	*S. cerasiforme*	79	EC 771610	*S. lycopersicum*
6	L 00671	*S. peruvianum*	43	WIR 13708	*S. cerasiforme*	80	EC 771597	*S. lycopersicum*
7	L 00887	*S. peruvianum*	44	WIR 3957	*S. cerasiforme*	82	EC 771593	*S. lycopersicum*
8	EC 771608	*S. peruvianum*	45	EC 771588	*S. cerasiforme*	83	EC 776581	*S. lycopersicum*
9	EC 771609	*S. peruvianum*	46	IC 45	*S. cerasiforme*	84	EC 771584	*S. lycopersicum*
10	EC 771607	*S. peruvianum*	47	EC 677191	*S. cerasiforme*	85	EC 771585	*S. lycopersicum*
11	EC 771603	*S. peruvianum*	48	EC 608394	*S. cerasiforme*	86	EC 771612	*S. lycopersicum*
12	EC 520044	*S. cheesmanii*	49	H 7996	*S. cerasiforme*	87	EC 771601	*S. lycopersicum*
13	WIR 3969	*S. cheesmanii*	50	EC 676732	*S. lycopersicum*	88	EC 771580	*S. lycopersicum*
14	EC 54109	*S. pimpinellifolium*	52	EC 68687	*S. lycopersicum*	89	EC 771591	*S. lycopersicum*
15	EC 541101	*S. pimpinellifolium*	53	EC 677034	*S. lycopersicum*	90	EC 771614	*S. lycopersicum*
16	LA 1246	*S. pimpinellifolium*	54	EC 686531	*S. lycopersicum*	91	EC 771594	*S. lycopersicum*
17	LA 1245	*S. pimpinellifolium*	55	EC 608275	*S. lycopersicum*	92	EC 771582	*S. lycopersicum*
18	LA 1478	*S. pimpinellifolium*	56	EC 676819	*S. lycopersicum*	93	EC 771589	*S. lycopersicum*
19	EC 541109	*S. pimpinellifolium*	57	EC 610654	*S. lycopersicum*	94	EC 771611	*S. lycopersicum*
20	EC 677049	*S. pimpinellifolium*	58	EC 676796	*S. lycopersicum*	95	LA 1545	*S. cerasiforme*
21	LA 0114	*S. pimpinellifolium*	59	EC 676809	*S. lycopersicum*	96	EC 771590	*S. cerasiforme*
22	LA 0121	*S. pimpinellifolium*	60	EC 109762	*S. lycopersicum*	97	LA 2205 B	*S. cerasiforme*
23	LA 0400	*S. pimpinellifolium*	61	EC 677091	*S. lycopersicum*	98	WIR 13706	*S. cerasiforme*
24	LA 0369	*S. pimpinellifolium*	62	EC 676779	*S. lycopersicum*	99	LA 1311-18	*S. cerasiforme*
25	LA 0373	*S. pimpinellifolium*	63	EC 608391	*S. lycopersicum*	100	EC 25265	*S. cerasiforme*
26	LA 1468	*S. cerasiforme*	64	EC 677123	*S. lycopersicum*	check	Arka Meghali	*S. lycopersicum*
27	LA 1206	*S. cerasiforme*	65	EC 676730	*S. lycopersicum*	check	Arka Vikas	*S. lycopersicum*
28	LA 1632	*S. cerasiforme*	66	EC 677076	*S. lycopersicum*	check	Arka Abha	*S. lycopersicum*
30	EC 771615	*S. cerasiforme*	67	EC 677079	*S. lycopersicum*			
31	LA 0475	*S. cerasiforme*	68	VRTC-17	*S. lycopersicum*			
32	LA 0168	*S. cerasiforme*	69	Pusa Ruby	*S. lycopersicum*			
33	HAT- 121	*S. cerasiforme*	70	EC 676778	*S. lycopersicum*			
34	LA 0292	*S. cerasiforme*	71	EC 676745	*S. lycopersicum*			
35	LA 1311-19	*S. cerasiforme*	72	EC 676596	*S. lycopersicum*			
36	LA 1311-16	*S. cerasiforme*	73	EC 109754	*S. lycopersicum*			
37	EC 514100	*S. cerasiforme*	74	LA 4345	*S. lycopersicum*			

Results

To assess genotypic variability among tomato germplasm for traits related to WUE and fruit yield. The analysis of variance indicated significant anout of variation among all the accessions for the traints studied in both conditions, except locule number in stress conditon

(Table 2. and Table 3). The characters *viz.,* PH, FNPC, FLPC, CPP, FPP, AFW, FV and FT recorded wide range and also high in GCV, PCV, h^2 (broad sense) and GAM indicating substantial amount of genetic variability that provide scope for selection. Some superior accessions across the species for important specific traits were identified as the following based on their *per se* performance**:**

Among the *S. lycopersicum* accessions, EC 677123 with relative early DFFS had high fruit yield whereas EC 771594, EC 771580, EC 771584 and CLN 13149 were late flowering and lower fruit yield in both conditions. While among the cherry tomato accessions, WIR 3957 and LA 1632 were early flowering type with higher fruit yield. Mean values for fruits per plant among *S. lycopersicum* ranged from 4.61 to 72.39 in control condition and 1.88 to 89.77 in stress condition and accessions EC 608275, EC 677123, Pusa Ruby, CLN 2070 A, EC 771598 and EC 771611 recorded higher fruits per plant. The mean values for fruits per plant among cherry types ranged from 44.28 to 617.49 in control condition and from 24.28 to 472.05 in stress condition and accessions LA 1206, LA 0168, EC 514100, LA 1713, WIR 3957 and IC 45 were found promising with higher fruits per plant. In the species *S. pimpinellifolium* number of fruits per plant varied from 969.52 to 2450.32 in control condition and from 660.93 to 1923.26 in stress condition. The superior accession for fruits number in other species were L 00882 and EC 771609 *(S. peruvianum),* LA 1353 and LA 2976 *(S. habrochaites)* and WIR 3969 (*S. cheesmanii*).

Fruit yield per plant ranged from 0.23 to 2.60kg in control condition and from 0.10 to 1.58kg in stress condition with the superior accessions were EC 677123 and EC 771598(*S. lycopersicum*). Among the cherry types it varied from 0.82 to 2.78 kg in control condition and from 0.41 to 2.21 kg in stress condition with superior accessions *viz.,* LA 1632, WIR 13706, EC 25265 and WIR 3957. In *S. pimpinellifolium* yield per plant varied from 1.12 to 2.56 kg in control condition and 0.89 to 1.93 kg in stress condition. Among the *S. peruvianum* it ranged from 0.82 to 1.63 kg in control condition and from 0.39 to 1.60 kg in stress condition with the best accessions were L 00882, EC 771609 and EC 771603. The superior accessions for in other species were LA 1353 and LA 2976 *(S. habrochaites)* and WIR 3969 (*S. cheesmanii*).

Mean SCMR values in the control and stress condition ranged, from 45.36 to 62.9 and 43.1 to 55.3 for *S. lycopersicum* respectively*,* from 39.3 to 55.7 and 37.2 to 53.1 for cherry types respectively, from 43.10 to 53.3 and 40.8 to 52.3 for *S. pimpinellifolium*, from 45.7 to 50.5 and 45.3 to 47.5 for *S. peruvianum,* from 42.3 to 47.8 and 40.9 to 44.4 for *S. habrochaites* respectively. The accessions EC 677123, VRTC-17, EC 771598 and EC 676596 for *S. lycopersicum*, EC 676790, EC 771590 and EC 25265 for the cherry types, EC 541101, EC 541109 and EC 677049 for the *S. pimpinellifolium*, L 00673, EC 771608 and EC 771609 for the *S. peruvianum* and LA 2976 for *S. habrochaites* were superior for this trait. Mean SLA values under the control condition and stress condition, varied from 154.98 to 265.82cm^2g^{-1} and 125.08 to 197.30cm^2g^{-1} for *S. lycopersicum,* from 185.10 to 397.19cm^2g^{-1} and 120.27 to 258.36cm^2g^{-1} for cherry types, from 197.15 to 332.49cm^2g^{-1} and 143.50 to 216.67cm^2g^{-1} for *S. pimpinellifolium*, from 214.84 to 345.39cm^2g^{-1} and 138.08 to 267.22cm^2g^{-1} for *S. peruvianum,* from 244.35 to 294.49cm^2g^{-1} and 197.62 to 209.88cm^2g^{-1} for *S. habrochaites* respectively. The accessions EC 677123, VRTC-17, EC 771598 (*S. lycopersicum)*, LA 0168, WIR 13706 (cherry types), EC 54109, EC 541101, EC 541109, EC 677049 (*S. pimpinellifolium)*, EC 771609

(*S. peruvianum)* and LA 1255 (*S. habrochaites*) were found superior for this trait with relative lower Specific leaf area.

Identification of drought tolerant tomato accessions based on tolerance indices

Under control condition: Among *S. lycopersicum* accessions, EC 771585, EC 677123, EC 771612, Pusa Ruby and EC 608275 recorded higher fruit yield per plant. In cherry tomato, accessions LA 0475, LA 1632, EC 676790, WIR 13706 and WIR 3957 and in *S. pimpinellifolium* EC 677049, EC 541101 and LA 0400 recorded high mean yield under control condition. While for *S. peruvianum,* L 00882, EC 771609 and for *S. habrochaites* LA 2976 accession were superior for fruit yield per plant.

Under stress condition: In *S. lycopersicum* accessions, EC 109762, EC 608391, EC 677123, EC 771598 and EC 771597 were found to be drought tolerant with higher fruit yield under stress condition. Among the cherry tomato accessions, WIR 3957, LA 0168, HAT- 121, EC 25265 and LA 1632 were found to be superior with stress tolerance. Among *S. pimpinellifolium*, accessions LA 0400, EC 541101 and EC 54109 were promising for fruit yield under stress condition. The accessions L 00882 and EC 771609 *S. peruvianum* and LA 2976 of *S. habrochaites* were the promising for fruit yield under stress condition.

Thus, the accessions EC 677123, WIR 3957, LA 1632, LA 0400 and EC 541101 were promising for fruit yield under both conditions.

Table 4-8. Per cent reduction of yield (PRY) of fruit yield in stress over control condition among 100 tomato germplasm accessions

Accession	PRY	**Accession**	PRY	**Accession**	PRY	**Accession**	PRY	**Accession**	PRY
LA 1255	22.45	LA 0114	20.05	WIR 13706	35.45	EC 686531	1.31	LA 4345	27.92
LA 1353	15.23	LA 0121	20.54	LA 1311-18	22.34	EC 608275	54.92	CLN 2070 A	50.75
LA 2976	5.31	LA 0400	40.79	EC 25265	15.18	EC 676819	57.83	CLN 13149	55.87
L 00673	3.14	LA 0369	4.78	EC 771613	29.22	EC 610654	**65.03**	EC 771593	46.64
L 00882	1.69	LA 0373	17.83	LA 0384	44.51	EC 676796	**69.19**	EC 771598	22.13
L 00671	**64.72**	LA 1468	48.88	LA 1479	17.02	EC 676809	34.37	EC 771610	24.30
L 00887	3.83	LA 1206	17.70	EC 771616	14.55	EC 109762	2.21	EC 771597	2.54
EC 771608	56.05	LA 1632	8.17	LA 2138 B	28.20	EC 677091	39.92	EC 771593	47.68
EC 771609	1.84	EC 676790	48.68	LA 2138 A	53.25	EC 676779	21.69	EC 776581	49.90
EC 771607	25.25	EC 771615	**66.32**	LA 1713	38.62	EC 608391	3.36	EC 771584	43.86
EC 771603	11.19	LA 0475	**66.92**	WIR 13708	15.26	EC 677123	34.18	EC 771585	**82.40**
EC 520044	48.31	LA 0168	7.44	WIR 3957	34.95	EC 676730	66.81	EC 771612	72.79
WIR 3969	50.32	HAT- 121	9.18	EC 771588	31.43	EC 677076	17.17	EC 771601	24.20
EC 54109	2.29	LA 0292	41.51	IC 45	38.60	EC 677079	57.25	EC 771580	45.83
EC 541101	12.63	LA 1311-19	30.04	EC 677191	16.99	VRTC-17	42.36	EC 771591	42.78
LA 1246	17.94	LA 1311-16	44.60	EC 608394	38.28	Pusa Ruby	48.41	EC 771614	22.92
LA 1245	6.43	EC 514100	55.11	H 7996	29.54	EC 676778	9.14	EC 771594	37.67
LA 1478	22.58	LA 1545	44.98	EC 676732	14.17	EC 676745	46.97	EC 771582	27.44
EC 541109	25.95	EC 771590	5.08	EC 68687	49.21	EC 676596	**61.96**	EC 771589	22.59
EC 677049	26.73	LA 2205 B	0.46	EC 677034	37.98	EC 109754	50.61	EC 771611	21.45

***Per cent* reduction of fruit yield in stress over control condition**

Per cent reduction of fruit yield among all the accessions was 32.24 *per cent* (), while the traits LR and TSS increased by 123.70% and 15.14%, respectively. *Per cent* reduction of fruit

yield in stress over control condition ranges from 0.46% (LA 2205 B) to 82.40% (EC 771585) and accessions, LA 2976 (*S. habrochaites*) L 00673, L 00882, L 00887, EC 771609 (*S. peruvianum),* EC 54109, LA 0369 (*S. pimpinellifolium)*, LA 2205 B , EC 771590 (cherry types), EC 686531, EC 109762, EC 771597, EC 608391 (*S. lycopersicum)* with lower per cent values observed to be drought tolerant, due their inherit mechanism to withstand water stress. Whereas, accession L 00671 (*S. peruvianum),* EC 771615, LA 0475 (cherry types), EC 610654, EC 676796, EC 676730, EC 676596, EC 771585 (*S. lycopersicum*) with higher *per cent* fruit yield reduction are highly drought susceptible (Table 4-8).

Correlation between fruit yield with other related traits under control condition

The FYPP was found to be significantly positive association with PH, BN, SCMR, RWC, STG, CPP and FPP, whereas it was significantly negative association with DFF, DFFS, SLA, LR and FT. The SCMR was found to be significant negative association with SLA. The accessions, *viz.,* EC 677123, EC 771598, EC 771611, EC 608391 and EC 771612 (S. *lycopersicum*), LA 1713, WIR 13706, LA 1479 and EC 25265 (cherry type), EC 54109, EC 541101 and EC 541109 (*S. pimpinellifolium)* and L 00673, L 00882 and EC 771609 (*S. peruvianum)* with higher mean values for SCMR, STG, RWC, BN, CPP and lower SLA were found to be useful for breeding drought tolerant and or water use efficient genotypes.

Correlation between fruit yield with other related traits under stress condition

The FYPP having significant and positively associated with PH, BN, RWC, STG, FNPC, FLPC, CPP, FPP and TSS, while it was significant negatively associated with DFF, DFFS, SLA, LR, AFW, FV, FT and LN. The SCMR had negative association with SLA. The selection for accessions, *viz.,* EC 677123, EC 771598, EC 608391, EC 771611 and Pusa Ruby from *S. lycopersicum,* LA 1479, LA 1713 and LA 0168 from cherry type, EC 54109, EC 541101 and EC 541109 from *S. pimpinellifolium* and L 00673, L 00882 and EC 771609 from *S. peruvianum* with higher mean values of STG, RWC, BN and CPP were able to produce more yield under water stress condition.

Drought indices for identification of drought tolerant accessions

Drought indices provide a measure of drought, based on loss of yield under drought-conditions in comparison to control conditions have been used for screening drought-tolerant accessions (Mitra, 2001). Various researchers have used different drought tolerance indices for evaluating genetic differences in drought tolerance and for identification of drought tolerant accessions. To investigate suitable stress resistance indices, a suitable index must have a significant correlation with fruit yield under both the conditions (Mitra, 2001). Correlation analysis between fruit yield and drought tolerance indices can be a good criterion for screening the best cultivars and indices used. Fruit yield in stress condition (Y_S) was significantly and positively correlated with MP, GMP, HAM, STI, RDI, SNPI, YI, K_1STI, K_2STI, YSI and Y_N, and negatively correlated with TOL, SSPI and SSI. Yield in non-stress condition (Y_N) was significant and positively correlated with all drought indices, except RDI, YSI and SSI. The drought indices, TOL, MP, GMP, HAM, STI, SSPI, SNPI, YI, K_1STI, K_2STI and DI were correlated with both Y_N and Y_S indicating that these indices were more effective in identifying high yielding cultivars under moisture-stress condition.

Principal component and biplot analysis for screening drought tolerance indicators

The relationships among different indices are graphically displayed in a biplot of PCA1 and PCA2 (*Figure 4-11*). The PCA 1 and PCA 2 axes which justify 96.43% of total variation, mainly distinguish the indices in different groups. In PCA, the PCs axes divided the indices into four groups. Group 1 (G1) included only the parameter SSI and in group 2, TOL, SSPI and ATI. In group 3 consisted of major indices K_1STI, MP, GMP, HAM, STI, SNPI, YI, K_2STI and DI and were strongly correlated with yield under both conditions indicating that these criteria are suitable for identification of drought tolerant genotypes. Indices RDI and YSI were separated as group 4 (G4). In general indices in the same group distinguish drought tolerant genotypes in the same manner.

Table 4-9. ANOVA for traits related to growth, water use efficiency, fruit yield and its attributes under control condition

Traits	Source of variation	Block	Treatments	Checks	Varieties	Check *vs* varieties	Error
Growth traits	**DF**	3	102	2	**99**	1	6
	DFF	23.6	57.5**	3.2	**58.0****	115.9**	6.81
	DFFS	29.88	105.4**	11.08	**106.9****	150.4*	13.64
	PH	48.04	4300.9**	77.8*	**3940.7****	48412.3**	13.14
	BN	1.103*	10.6**	1.5**	**10.6****	33.4**	0.13
WUE traits	**SCMR**	2.8	15.32*	0.67	**14.9***	84.7**	3.03
	SLA	134	1652.6**	365.5	**1583.3****	11095.8**	135.03
	RWC	7.18	60.2*	22.3	**59.5***	205.6**	12.226
	LR	0.61	1.79*	0.98	**1.8**	0.05	0.47
	STG	0.11	4.18*	0.14	**4.3***	0.07	0.91
Yield related traits	**FNPC**	0.16	7.22**	0.09	**6.8****	57.0**	0.18
	FLPC	0.04	16.3**	1.18**	**15.4****	138.6**	0.04
	CPP	10.42	8775.6**	25.847*	**8591.1****	44541.2**	2.86
	FPP	11.99	361011.4**	59.7	**354994.7****	1678568.0**	18.57
	AFW	88.15	1508.4**	840.6**	**1321.1****	21392.1**	92.39
	FV	95.94	1698.9**	708.40*	**1531.7****	20053.1**	82.458
	FT	0.16	3.46**	0.45	**3.35****	29.43**	0.35
	TSS	0.12*	1.73**	0.05	**1.59***	18.83**	0.02
	LN	0.01	2.03**	3.19**	1.45*	57.44**	0.28
	FT	0.17	0.31*	0.02	0.32*	0.02	0.07

***. Significant at the 0.01 level and *. Significant at the 0.05 level probability.*

Biplot diagram

Selection based on combination of indices may provide a more useful criterion for improving drought resistance but study of correlation coefficients is useful in finding out the degree of overall linear association between any two attributes. Thus a better approach than correlation analysis and principal component is needed to identify the superior genotypes for both stress and non-stress environments. Relationship between accessions and tolerance to drought was used as a biplot for identification of drought tolerant accessions. Biplot diagram showed that the first component was higher (59.29%) and the

second component was lower (37.05%) for accessions like WIR 13706, WIR 3957, EC 25265, LA 1632, HAT- 121, WIR 13708, EC 771590 and LA 0168 (cherry), EC 541101 and EC 54109 (*S. pimpinellifolium),* EC 771609 (*S. peruvianum*), LA 1353 and LA 2976 (*S. habrochaites),* EC 109762, EC 608391, EC 677123, EC 771597 and EC 771598 (*S. lycopersicum*). Thus, selection of these accessions with high PC1 and low PC2 are suitable for both control and stress conditions.

Ranking method

To determine the most desirable drought tolerant accessions according to all indices, rank mean, standard deviation of ranks and rank sum of all drought indices were calculated. In consideration to all indices, accessions LA 1353, LA 2976, EC 54109, LA 1632, EC 541101, LA 0168, HAT- 121, EC 25265, WIR 13708 and EC 771598 showed the best rank mean, rank sum and low standard deviation of ranks in stress condition, hence they were identified as the most drought tolerant accessions, while accessions, L 00671, EC 771615, LA 1545, CLN 13149, EC 771584, EC 771585, EC 771601, EC 771580, EC 771614 and EC 771594 as the most sensitive.

Table 4-10. ANOVA for traits related to growth, water use efficiency, fruit yield and its attributes under stress condition

Traits	Source of variation	Block	Treatments	Checks	Varieties	Check *vs* varieties	Error
Growth traits	**DF**	3	102	2	**99**	1	6
	DFF	41.5	53.8*	10.3	**52.1***	308.5**	10.556
	DFFS	27.7	94.6*	25	**95.1***	188.4*	14.19
	PH	13.27*	2069.0**	77.5**	**2398.4****	28522.6**	2.4
Traits	**Source of variation**	**Block**	**Treatments**	**Checks**	**Varieties**	**Check *vs* varieties**	**Error**
WUE traits	**BN**	0.03	8.85**	0.61	**8.9****	11.9**	0.25
	SCMR	2.37	26.62*	4.36	**26.73***	60.32*	5.2
	SLA	76.59	674.9**	48.38	**680.0****	1415.5**	49.92
	RWC	11.0**	88.6**	85.1**	**89.5****	0.97	1.06
	LR	0.36	1.2**	0.3	**1.2****	0.19	0.09
	STG	1.15*	3.8**	0.05	**3.9****	0.25	0.14
Yield related traits	**FNPC**	0.002	6.113**	0.053	**5.887****	40.630**	0.026
	FLPC	0.01	11.6**	0.5**	**10.9****	100.5**	0.05
	CPP	9.67	7513.3**	21.96	**7442.4****	29512.5**	6.5
	FPP	30.15*	209123.8*	48.8*	**206955.0****	841975.1**	4.97
	AFW	38.25	1059.0**	479.72	**908.3****	17138.5**	127.628
	FV	79.74	947.1**	568.3*	**758.4****	20390.7**	52.201
	FT	0.33	2.7**	1.1	**2.5****	27.1**	0.34
	TSS	0.05	2.5**	0.08	**2.3****	25.1**	0.057
	LN	0.07	1.2	1.96	**0.63**	55.9**	0.39
	FY	0.08	0.2**	0.06	**0.22****	0.11	0.02

***. Significant at the 0.01 level and *. Significant at the 0.05 level probability.*

Table 4-11. Genotypic variability for fruit yield and related traits among tomato germplasm accessions from six species under control condition											
Species	**Parameters**	**DFF**	**DFFS**	**PH**	**BN**	**SCMR**	**SLA**	**RWC**	**LR**	**STG**	**FNPC**
S. lycopersicum	Mean ± SE	57.07 ± 1.00	63.00 ±1.27	68.39 ±3.10	7.31 ± 0.25	52.14 ± 0.58	202.55 ± 3.88	84.12 ±1.23	3.16 ±0.20	12.20 ±0.24	2.41 ±0.08
	Range	46.00-77.00	50.00-85.00	35.20-110.20	4.60-11.60	45.30-62.90	154.98-265.82	66.40-95.74	1.00-5.40	9.40-15.50	1.40-3.30
S. lycopersicum var. cerasiforme	Mean ± SE	53.78±0.77	60.28±1.26	127.70±6.35	11.05±0.41	49.79±0.58	231.92±7.15	85.00±1.23	1.29±0.10	13.15±0.25	4.05±0.18
	Range	46.00-65.00	45.00-75.00	55.40-200.00	5.20-17.80	39.30-55.70	185.10-397.19	66.15-96.40	1.00-2.70	9.60-15.00	2.20-5.60
S. pimpinellifolium	Mean ± SE	46.42±0.91	52.25±1.25	206.40±14.40	14.25±0.46	47.13±0.77	236.22±13.60	83.13±2.56	1.28±0.19	12.20±0.58	7.54±0.28
	Range	43.00-50.00	47.00-60.00	147.00-296.00	11.60-18.20	43.10-53.30	197.15-332.49	68.25-93.75	1.00-2.70	9.60-16.40	5.70-8.80
S. peruvianum	Mean ± SE	60.33±0.73	72.67±1.92	172.00±2.27	12.27±0.63	46.90±0.52	230.32±14.14	86.06±3.04	1.87±0.33	10.73±0.25	9.40±0.29
	Range	57.00-63.00	69.00-83.00	155.80-176.00	9.80-15.00	45.70-50.50	214.84-345.39	66.80-91.32	1.00-3.60	9.80-12.00	9.00-11.70
S. cheesmanii	Mean ± SE	44.50±1.50	49.50±0.50	121.20±15.80	11.70±2.30	49.50±1.70	236.58±28.08	83.03±1.64	1.85±0.85	12.20±1.40	6.10±1.00
	Range	43.00-46.00	49.00-50.00	105.40-137.00	9.40-14.00	47.80-51.20	208.50-264.65	81.39-84.68	1.00-2.70	10.80-13.60	5.10-7.10
S. habrochaites	Mean ± SE	77.67±2.03	91.67±1.76	280.67±19.50	15.93±0.70	45.23±1.60	272.86±14.88	90.45±3.30	1.57±0.57	19.91±0.75	9.47±0.64
	Range	74.00-81.00	89.00-95.00	243.20-308.80	14.80-17.20	42.30-47.80	244.35-294.49	84.25-95.53	1.00-2.70	18.80-21.33	8.20-10.20
	GCV (%)	12.36	14.81	50.26	30.80	6.58	16.32	7.81	52.77	13.98	55.80
	PCV (%)	13.22	15.95	50.35	31.00	7.44	17.13	8.84	61.99	15.90	56.65
	h^2_{bs} (%)	87.35	86.25	99.64	98.67	78.25	90.77	78.00	72.48	77.24	97.03
	GAM	23.80	28.34	103.35	63.02	11.99	32.03	14.20	92.56	25.30	113.24

Species	**Parameters**	**FLPC**	**CPP**	**FPP**	**AFW**	**FV**	**FT**	**TSS**	**LN**	**Y_N**
S. lycopersicum	Mean ± SE	5.11±0.15	16.29±1.12	32.76±2.60	66.76±5.40	71.06±5.40	5.20±0.15	5.09±0.05	3.72±0.20	1.38±0.09
	Range	3.40-8.00	3.00-33.60	4.61-72.39	23.40-07.40	27.00-212.00	3.10-7.00	4.28-5.90	2.00-6.80	0.23-2.60
S. lycopersicum var. cerasiforme	Mean ± SE	6.33±0.33	79.62±6.84	274.76±29.80	12.94±1.66	14.79±2.02	2.53±0.16	6.01±0.11	2.47±0.12	1.80±0.09
	Range	4.00-12.10	14.00-151.00	44.28-617.49	2.40-42.20	2.80-50.00	1.08-4.90	4.94-7.64	2.00-4.20	0.82-2.78
S. pimpinellifolium	Mean ± SE	10.88±0.49	286.52±23.92	1744.68±126.25	1.75±0.54	1.87±0.57	1.13±0.09	7.52±0.29	2.03±0.03	1.71±0.12
	Range	6.80-13.75	179.80-420.00	969.52-2450.32	0.80-7.60	0.80-8.00	0.80-1.90	5.64-8.76	2.00-2.40	1.12-2.56
S. peruvianum	Mean ± SE	16.60±0.63	132.80±12.11	888.86±123.08	2.97±0.15	3.53±0.15	2.23±0.09	7.93±0.28	2.00±0.00	1.26±0.10
	Range	15.10-19.50	70.40-167.00	596.62-1651.01	2.90-4.20	3.20-4.40	1.60-2.40	6.84-9.24	2.00-2.00	0.82-1.63
S. cheesmanii	Mean ± SE	10.30±3.70	155.50±21.50	741.51±266.24	4.10±0.30	4.70±0.10	1.55±0.05	6.31±0.87	2.00±0.00	1.97±0.27
	Range	6.60-14.00	134.00-177.00	475.27-1007.75	3.80-4.40	4.60-4.80	1.50-1.60	5.44-7.18	2.00-2.00	1.70-2.24
S. habrochaites	Mean ± SE	10.83±2.12	136.47±6.31	1031.34±26.01	2.60±0.31	2.67±0.27	1.03±0.28	8.82±0.17	2.00±0.00	2.19±0.18
	Range	6.60-13.10	124.20-145.20	995.33-1081.85	2.20-3.20	2.40-3.20	0.70-1.60	8.48-9.06	2.00-2.00	1.96-2.54
	GCV (%)	50.36	106.36	133.567	102.09	101.33	48.75	19.87	35.68	30.19
	PCV (%)	50.43	106.37	133.571	106.19	104.43	51.76	19.98	40.14	34.37
	h^2_{bs} (%)	99.72	99.96	99.99	92.42	94.16	88.71	98.88	79.01	77.14
	GAM	103.59	219.05	275.14	202.17	202.55	94.58	40.70	65.34	54.62

Table 4-12. Correlation coefficients of traits related to growth, water use efficiency and fruit attributes with fruit yield under stress condition (Ys)

Traits	DFF	DFFS	PH	BN	SCMR	SLA	RWC	LR	STG	FNPC	FLPC	CPP	FPP	AFW	FV	FT	TSS	LN	Ys
DFF	1	0.895**	-0.049	-0.209*	-0.017	0.191	-0.110	0.118	0.179	-0.126	-0.105	-0.442**	-0.324**	0.330**	0.310**	0.224*	-0.274**	0.395**	-0.286**
DFFS		1	0.096	-0.112	-0.129	0.291**	-0.097	0.104	0.217*	0.056	0.089	-0.304**	-0.165	0.196*	0.182	0.085	-0.097	0.278**	-0.289**
PH			1	0.860**	-0.391**	0.229*	0.145	-0.485**	0.453**	0.799**	0.691**	0.671**	0.744**	-0.676**	-0.684**	-0.726**	0.767**	-0.490**	0.505**
BN				1	-0.339**	0.060	0.212*	-0.522**	0.481**	0.694**	0.522**	0.733**	0.752**	-0.649**	-0.651**	-0.733**	0.715**	-0.416**	0.622**
SCMR					1	-0.600**	0.219*	0.176	-0.079	-0.385**	-0.299**	-0.301**	-0.346**	0.372**	0.370**	0.457**	-0.428**	0.266**	**0.196***
SLA						1	-0.280**	-0.065	-0.019	0.274**	0.260**	-0.023	0.074	-0.241*	-0.233*	-0.258**	0.220*	-0.166	**-0.453****
RWC							1	-0.190	0.227*	0.021	0.070	0.105	0.071	-0.086	-0.095	-0.055	0.015	-0.081	**0.453****
LR								1	-0.302**	-0.351**	-0.234*	-0.435**	-0.442**	0.359**	0.357**	0.374**	-0.404**	0.242*	**-0.480****
STG									1	0.114	-0.094	0.140	0.173	-0.135	-0.131	-0.174	0.088	-0.068	**0.512****
FNPC										1	0.889**	0.621**	0.766**	-0.616**	-0.621**	-0.683**	0.816**	-0.468**	0.353**
FLPC											1	0.545**	0.661**	-0.547**	-0.551**	-0.584**	0.774**	-0.436**	0.228*
CPP												1	0.944**	-0.542**	-0.546**	-0.644**	0.726**	-0.383**	0.459**
FPP													1	-0.521**	-0.526**	-0.641**	0.779**	-0.373**	0.435**
AFW														1	.989**	0.819**	-0.650**	0.815**	-0.350**
FV															1	0.832**	-0.645**	0.789**	-0.349**
FT																1	-0.688**	0.479**	-0.321**
TSS																	1	-0.493**	0.348**
LN																		1	-0.283**
Ys																			1

***. Correlation is significant at the 0.01 level and *. Correlation is significant at the 0.05 level probability.*

Table 4-13. Correlation coefficients of different drought tolerance indices with fruit yield under control (YN) and stress condition (YS).

Drought indices	**TOL**	**MP**	**GMP**	**HAM**	**STI**	**RDI**	**ATI**	**SSPI**	**SNPI**	**YI**	**YSI**	**K_1STI**	**K_2STI**	**DI**	**SSI**	**Y_N**	**Y_S**
TOL	1	0.244*	0.124	0.020	0.085	-0.843**	0.918**	1.000**	-0.456**	-0.211*	-0.841**	0.604**	-0.229*	-0.496**	0.842**	**0.585****	**-0.211***
MP		1	0.991**	0.971**	0.959**	0.233*	0.518**	0.244*	0.579**	0.896**	0.238*	0.893**	0.858**	0.707**	-0.236*	**0.929****	**0.896****
GMP			1	0.994**	0.972**	0.339**	0.421**	0.123	0.632**	0.942**	0.343**	0.836**	0.902**	0.776**	-0.341**	**0.876****	**0.942****
HAM				1	0.971**	0.426**	0.333**	0.020	0.671**	0.970**	0.430**	0.779**	0.929**	0.827**	-0.428**	**0.820****	**0.969****
STI					1	0.324**	0.397**	0.085	0.612**	0.928**	0.329**	0.838**	0.940**	0.774**	-0.327**	**0.834****	**0.928****
RDI						1	-0.626**	-0.844**	0.757**	0.620**	1.000**	-0.171	0.576**	0.820**	-1.000**	-0.126	0.621**
ATI							1	0.917**	-0.246*	0.102	-0.623**	0.821**	0.067	-0.222*	0.624**	0.782**	0.102
SSPI								1	-0.456**	-0.211*	-0.841**	0.603**	-0.229*	-0.496**	0.842**	**0.585****	**-0.212***
SNPI									1	0.793**	0.759**	0.259**	0.777**	0.894**	-0.757**	**0.311****	**0.792****
YI										1	0.624**	0.624**	0.970**	0.939**	-0.622**	**0.670****	**1.000****
YSI											1	-0.166	0.579**	0.822**	-1.000**	-0.121	0.625**
K_1STI												1	0.619**	0.354**	0.168	**0.976****	**0.623****
K_2STI													1	0.928**	-0.578**	**0.631****	**0.969****
DI														1	-0.821**	**0.402****	**0.939****
SSI															1	0.123	-0.623**
Y_N																1	**0.669****
Y_S																	1

***. Correlation is significant at the 0.01 level and *. Correlation is significant at the 0.05 level probability.*

Table 4-14. Top 10 drought tolerant accessions based on drought tolerance indices

Code	Accession	TOL	MP	GMP	HAM	STI	SSPI	SNPI	YI	K_1STI	K_2STI	DI	Y_N	Y_S	R_{avr}	SDR	RS
3	LA 1353	0.39	2.35	2.34	2.33	2.19	30.56	5.23	2.03	2.58	4.13	1.72	**2.54**	**2.15**	17.12	**22.47**	39.59
4	LA 2976	0.11	2.02	2.01	2.01	1.63	8.69	6.63	1.85	1.72	3.42	1.75	**2.07**	**1.96**	15.65	**20.40**	36.05
20	EC 54109	0.05	1.95	1.95	1.95	1.52	3.58	8.58	1.82	1.56	3.31	1.78	**1.97**	**1.93**	15.12	**21.89**	37.00
21	EC 541101	0.28	2.05	2.05	2.04	1.68	21.87	4.92	1.81	1.92	3.26	1.58	**2.19**	**1.91**	18.88	**18.09**	36.98
35	LA 1632	0.20	2.31	2.31	2.31	2.14	15.56	6.52	2.09	2.33	4.36	1.92	**2.41**	**2.21**	13.82	**20.68**	34.51
39	LA 0168	0.15	1.94	1.94	1.94	1.51	11.85	5.66	1.76	1.63	3.10	1.63	**2.02**	**1.87**	19.47	**18.76**	38.23
40	HAT- 121	0.19	2.01	2.01	2.01	1.62	15.28	5.43	1.81	1.78	3.26	1.64	**2.11**	**1.91**	18.06	**18.37**	36.43
50	EC 25265	0.36	2.19	2.19	2.18	1.92	28.48	4.89	1.90	2.26	3.61	1.61	**2.37**	**2.01**	18.47	**20.54**	39.01
58	WIR 3708	0.32	1.95	1.94	1.94	1.51	25.47	4.34	1.69	1.79	2.85	1.43	**2.11**	**1.79**	22.53	**16.54**	39.07
108	EC 771598	0.41	1.63	1.62	1.60	1.05	32.02	3.11	1.35	1.34	1.81	1.05	**1.83**	**1.43**	32.53	**12.43**	44.95

Table 4-15. Top 10 drought susceptible accessions based on drought tolerance indices

Code	Accession	TOL	MP	GMP	HAM	STI	SSPI	SNPI	YI	K_1STI	K_2STI	DI	Y_N	Y_S	R_{avr}	SDR	RS
10	L 00671	0.72	0.75	0.66	0.58	0.17	56.86	0.71	0.37	0.50	0.14	0.13	**1.11**	**0.39**	87.53	**19.09**	106.62
37	EC 771615	0.81	0.81	0.71	0.61	0.20	63.68	0.74	0.39	0.59	0.15	0.13	**1.22**	**0.41**	85.76	**19.83**	105.60
45	LA 1545	0.37	0.64	0.62	0.59	0.15	29.55	0.84	0.43	0.28	0.19	0.24	**0.83**	**0.46**	82.71	**20.15**	102.85
106	CLN 13149	0.13	0.17	0.15	0.14	0.01	10.13	0.17	0.10	0.02	0.01	0.04	**0.23**	**0.10**	88.94	**30.54**	119.48
113	EC 771584	0.14	0.26	0.25	0.24	0.02	11.41	0.33	0.17	0.04	0.03	0.10	**0.33**	**0.18**	84.88	**30.23**	115.11
114	EC 771585	1.89	1.35	0.96	0.69	0.37	149.64	0.82	0.38	2.12	0.15	0.07	**2.30**	**0.40**	77.76	**35.80**	113.57
116	EC 771601	0.17	0.62	0.62	0.61	0.15	13.55	1.13	0.51	0.20	0.26	0.38	**0.71**	**0.54**	74.35	**28.44**	102.80
117	EC 771580	0.22	0.37	0.35	0.34	0.05	17.38	0.47	0.25	0.09	0.06	0.13	**0.48**	**0.26**	85.35	**26.47**	111.83
119	EC 771614	0.12	0.45	0.44	0.44	0.08	9.13	0.85	0.37	0.10	0.13	0.28	**0.50**	**0.39**	77.47	**32.47**	109.94
120	EC 771594	0.23	0.50	0.48	0.47	0.09	18.16	0.73	0.36	0.15	0.13	0.22	**0.61**	**0.38**	81.76	**26.45**	108.22

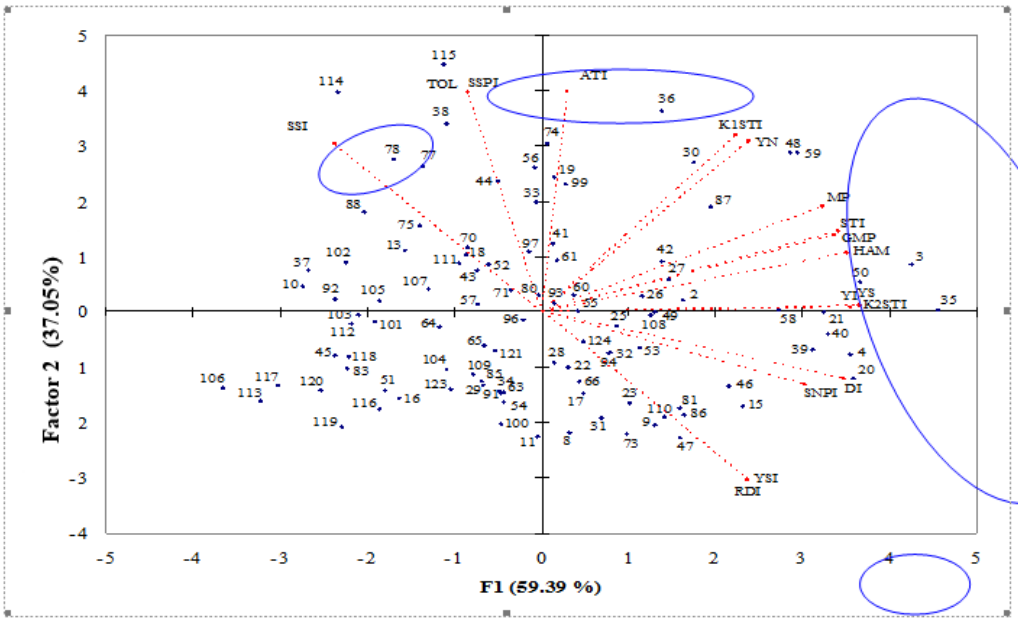


Figure 4-11 Screening drought tolerant tomato accessions using biplots analysis from drought indices

Figure 4-11. Screening drought tolerant tomato accessions using biplots analysis from drought indices

Drought Tolerance Analysis of selected Tomato Germplasm based on quantitative indices

Evaluation and systematic study of diverse germplasm species is of great importance for current and future crop improvement. Understanding the physiological basis of drought adaptation or water use efficiency can aid in determining the importance of wild species and the suitability of traits related for crop improvement. Most commercial cultivars of S. *lycopersicum* are sensitive to abiotic stresses during all stages of plant development. The cultivated species of tomato has a very narrow diversity in the germplasm and the best genetic sources for drought are coming from other species indigenous to arid and semi-arid environments in South America (Peralta and Spooner, 2005). Breeding for drought resistance is complicated by the lack of fast, reproducible screening techniques and the inability to routinely create defined and repeatable water stress conditions.

Various quantitative criteria have been proposed for selection of genotypes based on their yield performance in stress and non-stress environments. Several drought tolerance indices(Yield index (YI), Yield stability index (YSI), Tolerance(TOL), Mean productivity (MP) Harmonic mean(HM), Stress susceptibility index (SSI), Geometric mean productivity (GMP), Stress tolerance index (STI), Drought resistance index (DRI), modified stress tolerance index (K1STI and K2STI), Relative drought index(RDI), Abiotic tolerance index (ATI), Stress susceptibility percentage index (SSPI) and Stress non-stress production index (SNPI), which were used for screening quantitative indicator of drought tolerance in experiment 1. are also used to identify drought-tolerant tomato genotypes among eighteen germplasm. Hence, present study was undertaken to assess the extent of drought tolerance in tomato germplasm.

From the *kharif 2013* experiment, eighteen promising genotypes were identified based on Specific leaf area (SLA), SPAD chlorophyll meter reading (SCMR), stem girth (mm) and fruit yield. These genotypes were evaluated for drought tolerance in RCBD design with two replications each, under control and stress condition during *summer* 2014. Each genotype was transplanted in 5 meters length with a spacing of 0.75 m x 0.5 m. Observations were recorded for eighteen characters *viz*., Plant height (cm), number of primary branches, days to 50% flowering, days to first fruit set, number of fruits per clusters, number of clusters per plant, number of fruits per plant, fruit length (cm), fruit width (cm), fruit weight, fruit volume, Locules number, pericarp thickness, fruit yield per plant (kg), TSS (ºBrix) and Water use efficiency traits like Specific leaf area(SLA), SPAD chlorophyll meter reading(SCMR) and stem girth(mm).

Water stress consistently lowered the yield of tomato genotypes in moisture-stress rather than non-stress condition. Based on the stress tolerance index (STI), modified stress tolerance index (K2STI) and fruit yield, genotypes WR 3969, EC 541101, EC 520044 and EC 514109 were found drought tolerant with high STI and fruit yield under stress and irrigated conditions, while genotypes LA 0384, EC 771612, EC 771584, EC 771597 and EC 771608 displayed the lowest amount of STI, MSTI and fruit yield under stress and irrigated conditions. Talebi *et al.,* (2009) also reported that cultivars producing high yield in both drought and well watered conditions can be identified by STI, GMP and MP values.

The relationships among different indices are graphically displayed in a biplot of PCA1 and PCA2 (*Figure 4-12*). The first and second components justified 94.99% of the variations between criteria. The PCA1 and PCA2 mainly distinguish the indices in to different groups. The PCs axes separated ATI, TOL, SSI, and SSPI, in group 1 (G1). The parameters YP, MP, HM, GMP, STI, and K1STI were separated as groups 2 (G2). The PCs axes separated YS, YI, DI, RDI, YSI, SNPI and K2STI in group (G3). The cosine of the angle between the vectors of two indices approximates the correlation between them and the angles are informative enough to allow a whole picture about the interrelationships among the stability estimates.

To determine the most desirable drought tolerant genotypes according to all indices mean rank and standard deviation of ranks of all criteria were calculated. Based on these biplot and rank mean, the most desirable drought tolerant genotypes were identified. In consideration to all indices, genotypes LA 1311-18, EC 514109, and WR 3969 showed the best rank mean and low standard deviation of ranks in stress condition; hence these were identified as the most drought tolerant genotypes. While genotypes LA 0384, Arka abha, EC 771612 and EC 771597 as the most sensitive, so they are recommended for use in hybridization programme and genetic analysis of drought tolerance or WUE surrogate traits.

Table 4-16. Drought tolerance indices of tomato genotypes under stress and non-stress condition

SN	Genotype	Yp	YI	YSI	TOL	MP	HM	SSI	GMP	STI	DI	RDI	ATI	SSPI	SNPI	K1STI	K2STI	Ys	R	SDR
1	Arka abha	1.74	0.68	0.32	1.19	1.14	0.84	1.40	0.98	0.37	0.17	0.62	1.49	37.24	1.54	0.44	0.17	0.55	8	3.74
2	EC 771612	1.62	0.63	0.31	1.11	1.07	0.78	1.40	0.91	**0.32**	0.16	0.62	1.29	34.82	1.47	0.33	0.13	0.51	8	4.80
3	EC 771584	1.25	0.63	0.41	0.74	0.88	0.73	1.20	0.80	**0.25**	0.21	0.81	0.76	23.03	1.46	0.16	0.10	0.52	9	4.89
4	EC 771597	1.65	0.76	0.37	1.04	1.14	0.90	1.28	1.01	**0.40**	0.23	0.73	1.34	32.43	1.65	0.43	0.23	0.62	9	2.33
5	EC 771610	1.73	1.03	0.49	0.89	1.28	1.13	1.05	1.20	0.57	0.41	0.95	1.37	27.81	2.03	0.66	0.60	0.84	10	1.21
6	Arka alok	2.10	1.22	0.47	1.11	1.54	1.35	1.08	1.44	0.81	0.47	0.92	2.05	34.73	2.26	1.40	1.20	0.99	10	4.42
7	EC 676596	1.86	1.05	0.46	1.01	1.36	1.17	1.11	1.26	0.62	0.39	0.90	1.63	31.63	2.05	0.85	0.68	0.85	9	2.25
8	VRCT 17	**2.82**	0.95	0.27	2.05	1.80	1.22	1.48	1.48	0.85	0.21	0.54	3.88	64.20	1.97	2.68	0.77	0.77	8	6.30
9	EC 514109	1.27	1.50	0.97	0.04	1.24	1.24	0.07	1.24	0.60	1.18	1.89	0.07	1.32	5.67	0.38	1.36	**1.22**	12	5.42
10	EC 541101	1.94	1.48	0.62	0.74	1.57	1.49	0.78	1.53	**0.91**	0.75	1.22	1.44	23.06	2.71	1.35	**1.99**	**1.20**	11	4.95
11	WR 3957	2.28	1.06	0.38	1.41	1.57	1.25	1.27	1.40	0.77	0.33	0.74	2.54	44.33	2.06	1.57	0.87	0.87	9	4.86
12	LA 0384	1.90	0.56	0.24	1.45	1.18	0.73	1.55	0.93	0.34	0.11	0.47	1.72	45.28	1.41	0.48	0.10	0.45	7	5.96
13	EC 771590	1.50	0.71	0.39	0.92	1.04	0.84	1.25	0.93	0.34	0.22	0.76	1.10	28.89	1.58	0.30	0.17	0.58	9	2.90
14	LA 1311-18	1.08	1.27	0.96	0.04	1.06	1.06	0.07	1.06	0.44	1.00	1.89	0.05	1.19	4.98	0.20	0.71	1.04	12	5.16
15	WR 3969	**1.81**	2.16	0.97	0.05	1.78	1.78	0.06	1.78	**1.24**	1.71	1.90	0.11	1.57	7.68	**1.60**	**5.80**	**1.76**	12	7.21
16	EC 520044	1.39	1.48	0.87	0.19	1.30	1.29	0.27	1.30	0.66	1.05	1.70	0.31	5.83	3.61	0.50	1.45	**1.21**	11	4.47
17	LOO 882	0.40	0.44	0.89	0.04	0.38	0.37	0.22	0.37	0.05	0.32	1.75	0.02	1.31	1.72	0.00	0.01	0.35	11	6.69
18	EC 771608	0.39	0.40	0.84	0.06	0.36	0.35	0.32	0.36	0.05	0.27	1.65	0.03	1.93	1.43	0.00	0.01	0.33	10	7.04

Table 4-17. Association between drought tolerance indices with fruit yield under control (Yp)and stress(Ys).

	YP	YI	YSI	TOL	MP	HM	SSI	GMP	STI	DI	RDI	ATI	SSPI	SNPI	K1STI	K2STI	YS
YP	1.000	0.317	-0.612**	0.803**	0.897**	0.634**	0.614**	0.775**	0.698**	-0.076	-0.614**	0.863**	0.803**	-0.027	0.837**	0.235	0.314
YI		1.000	0.501*	-0.312	0.704**	0.926**	-0.497*	0.842**	0.873**	0.907**	0.498*	-0.141	-0.312	0.869**	0.468*	0.882**	1.000**
YSI			1.000	-0.929**	-0.225	0.158	-1.000**	-0.022	0.093	0.786**	1.000**	-0.788**	-.929**	0.726**	-0.246	0.464	0.503*
TOL				1.000	0.456	0.053	0.929**	0.247	0.150	-0.647**	-0.929**	0.952**	1.000**	-0.575*	0.544*	-0.318	-0.314
MP					1.000	0.907**	0.227	0.973**	0.930**	0.366	-0.227	0.581*	0.456	0.385	0.846**	0.587*	0.702**
HM						1.000	-0.154	0.980**	0.972**	0.682**	0.154	0.219	0.053	0.653**	0.687**	0.782**	0.925**
SSI							1.000	0.026	-0.090	-0.784**	-1.000**	0.787**	.928**	-0.725**	0.246	-0.463	-0.499*
GMP								1.000	0.975**	0.546*	-0.025	0.398	0.247	0.539*	0.779**	0.706**	0.840**
STI									1.000	0.614**	0.090	0.330	0.15	0.599**	0.812**	0.811**	0.872**
DI										1.000	0.784**	-0.504*	-.647**	0.969**	0.156	0.842**	0.908**
RDI											1.000	-0.787**	-0.929**	0.724**	-0.246	0.463	0.499*
ATI												1.000	0.952**	-0.450	.735**	-0.188	-0.144
SSPI													1.000	-0.574*	0.544*	-0.318	-0.314
SNPI														1.000	0.184	0.831**	0.870**
K1STI															1.000	0.471*	0.466
K2STI																1.000	0.881**

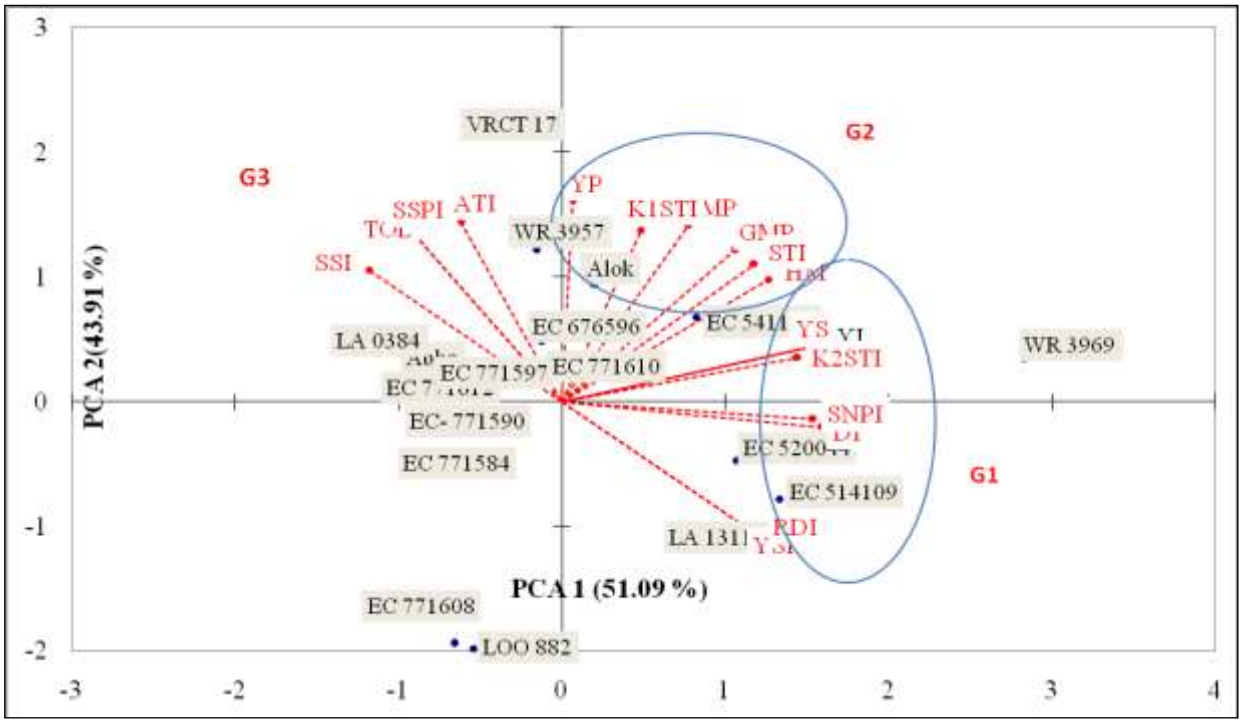


Figure 4-12. Biplot analysis for drought tolerance indices and tomato germplasm

Assessment of genetic variability for root traits and its association with the fruit yield in tomato

Eighty-five germplasm accessions of six tomato species were transplanted during *rabi*-2014 in specially constructed root structures of 20 m length, 1.5 m height and 2.4 m width having at central and either side a 20 cm permanent wall. Six plants of each genotype were sown per structure and were replicated twice. The spacing adopted was 60 x 30cm and recommended dose of fertilizers were applied.

Tomato accessions of all the species were grouped in to two sets based on the earlier experiment of "*Standardization for days to root sampling among different species of tomato*" with findings of days to root sampling for *S. lycopersicum* and *S. pimpinellifolium* at 90 DAS and *S. cerasiforme, S. peruvianum, S.cheesmani and S. habrochaites* at 120 DAS. Hence, The present tomato accessions was split into two sets, first one consists **of *S. lycopersicum* and *S. pimpinellifolium* (set 1) and** the second one is ***S. cerasiforme, S. peruvianum, S.cheesmani and S. habrochaites (set 2*).** Plants were grown for 90 days and 120 days for set 1 and for set 2, respectively and then root structures were dismantled partly and the plants along with roots were separated using water. Observations on morphological parameters *viz*., shoot length (SHL), shoot dry weight (SHDW), WUE traits like root characters *viz.*, root length (RL), root dry weight (RDW), root volume (RV) and root: shoot ratio (RL/SHL, RDW/SHDW). Root volume was measured by quantifying the amount of water displacement method.

Analysis of data for genetic variability for all the traits studied were independently within each set 1, set 2 and combining the both sets to overview for all the accessions by using Excel programme. The Pearson correlation coefficients were computed between various

shoot and root traits and control fruit yield obtained at field condition to identify superior accessions by using SPSS ver 16.

Results

Analysis of variance revealed highly significant variation among germplasm accessions for all traits studied indicating the sufficient amount of variability hence provide scope for selection. Wide range, high h^2 (broad sense) and moderate PCV and GCV were recorded for RDW, SHDW and RV among all the germplasm accessions and SHDW for *S. lycopersicum* and *S. pimpinellifolium* accessions. High GAM was recorded for RL/SHL and RDW/SHDW. Thus, selection of accessions for these traits is more advantage.

Table 4-18. ANOVA for shoot and root traits among tomato germplasm of all species of 85 accessions

Source of variation	RL	SHL	RL/SHL	RV	RDW	SHDW	RDW/SHDW
Treatments	48344.14*	213355.82*	15.463*	45503.78*	2113.46*	662188.42*	0.092*
Replication	207.33	117.43	0.085*	18.01	2.08	4.02	0.000
Error	14761.06	9336.89	1.680	8204.05	242.52	40704.22	0.016
SEM±	9.37	7.45	0.100	6.99	1.20	15.57	0.010
CD @5%	26.17	20.81	0.279	19.51	3.35	43.45	0.027
CV (%)	15.10	11.14	13.69	27.28	23.10	17.42	19.57

**. Significant at 5% level*

Table 4-19. ANOVA for shoot and root traits among tomato germplasm S. lycopersicum and S. pimpinellifolium (set 1)

Source of variation	RL	SHL	RL/SHL	RV	RDW	SHDW	RDW/SHDW
Treatments	18350.89*	95862.82*	9.273*	10456.74*	435.72*	262961.55*	0.028*
Replication	265.48*	2.38	0.050	15.49	0.04	1394.92*	0.000
Error	2421.36	5088.98	0.768	1054.85	43.71	15984.33	0.003
SEM±	5.25	7.60	0.093	3.46	0.70	13.48	0.006
CD @5%	14.94	21.66	0.266	9.86	2.01	38.39	0.017
CV (%)	9.02	13.84	11.16	15.56	16.85	17.42	13.393

**. Significant at 5% level*

Table 4-20. ANOVA for shoot and root traits among S. cerasiforme, S. peruvianum, S.cheesmani and S. habrochaites species

Source of variation	RL	SHL	RL/SHL	RV	RDW	SHDW	RDW/SHDW
Treatments	26541.96*	72630.52*	3.103*	32174.25*	1357.20*	372136.23*	0.050*
Replication	77.07	198.62	0.001	4.26	0.07	1302.17	0.000
Error	3259.94	4188.36	0.406	3523.97	89.00	22616.47	0.004
SEM±	6.31	7.15	0.070	6.56	1.04	16.61	0.007
CD @5%	18.00	20.40	0.201	18.71	2.97	47.40	0.019
CV (%)	9.59	9.08	11.27	22.54	16.71	16.36	13.88

Table 4-21. Genotypic variability for shoot and root traits among 85 tomato germplasm							
Source of variation	**RL**	**SHL**	**RL/SHL**	**RV**	**RDW**	**SHDW**	**RDW/SHDW**
Mean	**87.81**	**94.60**	**1.033**	**36.22**	**7.36**	**126.38**	**0.071**
Range	48.42-126.00	35.33- 203.33	0.560- 2.021	10.63-81. 67	1.78- 16.83	35.39-329.02	0.026-0.148
PCV	22.07	38.49	30.93	**49.36**	**50.91**	**51.18**	35.89
GCV	16.10	36.84	27.73	**41.14**	**45.37**	**48.13**	30.08
h^2_{bs}	53.22	91.61	80.40	**69.45**	**79.41**	**88.42**	70.26
GAM	1.25	1.99	60.34	3.95	22.24	1.44	49.04

Table 4-22. Genotypic variability for shoot and root traits among set 1 tomato accessions							
Source of variation	**RL**	**SHL**	**RL/SHL**	**RV**	**RDW**	**SHDW**	**RDW/SHDW**
Mean±SE	**82.28**	**77.73**	**1.178**	**31.47**	**5.92**	**109.39**	**0.067**
Range	48.42-122.83	35.33-165.04	0.603-2.021	10.63-52.50	1.78-11.10	35.39-263.52	0.034-0.112
PCV	18.67	43.58	28.55	36.36	39.46	**51.47**	29.56
GCV	16.35	41.32	26.28	32.86	35.68	**48.43**	26.35
h^2_{bs}	76.69	89.92	84.71	81.67	81.76	**88.54**	79.47
GAM	1.92	2.38	47.51	5.35	28.48	1.67	52.44

Table 4-23. Genotypic variability for shoot and root traits among set 2 tomato germplasm							
Source of variation	**RL**	**SHL**	**RL/SHL**	**RV**	**RDW**	**SHDW**	**RDW/SHDW**
Mean±SE	**92.98**	**111.28**	**0.884**	**41.13**	**8.82**	**143.56**	**0.074**
Range	61.71-126.00	50.54-203.33	0.560-1.648	11.25-81.67	2.48-16.83	30.43-329.02	0.026-0.148
PCV	20.50	27.51	23.41	**50.73**	**47.63**	**48.33**	37.21
GCV	18.12	25.96	20.52	**45.44**	**44.60**	**45.48**	34.52
h^2_{bs}	78.12	89.10	76.83	**80.26**	**87.69**	**88.54**	86.09
GAM	1.73	1.65	19.08	4.02	20.49	1.27	25.73

Plant root system plays an important role in regulation of water uptake and extraction from deep soil layers. Among the total accessions, **SHDW, RDW and** RV recorded higher GCV and PCV indicating possible scope for improvement of these traits upon selection. A positively significant relationship was observed for RL, RDW with fruit yield in all germplasm accessions. The mean values for RL varied from 48.42 to 126.00 cm and accessions IC 45, LA 0400, LA 1479, LA 1632 and LA 0292 had higher mean values for this trait. The range of mean for RL/SHL ranged from 0.560 to 2.021 and accessions EC 771582, EC 608391, EC 771598, EC 676790 and EC 77612 were superior. The range of mean for RV ranged from 10.63 to 81.67cm^3 and accessions, LA 1311-16, LA 0168, LA 0475, EC 514100 and EC 771608 were best for this trait. The mean values for RDW varied from 1.78 to 16.83g and accessions, LA 0168, LA 1479, L00 671, LA 0475 and EC 771608 were promising for this trait. For RDW/SHDW ratio, it ranges from 0.026 to 0.148 with a mean value of 0.071 and accessions, EC 771609, EC 771608, L 00882, EC 677191 and L 00673 were superior.

Table 4-24. Correlation coefficients for shoot and root traits with the fruit yield under control (YN) among all germplasm

Traits	RL	SHL	RL/SHL	RV	RDW	SHDW	RDW/SHDW	Y_N
RL	1	0.694**	-0.259*	0.711**	0.758**	0.733**	-0.044	**0.273***
SHL		1	-0.808**	0.512**	0.690**	0.771**	-0.187	0.149
RL/SHL			1	-0.219*	-0.416**	-0.532**	0.267*	-0.019
RV				1	0.861**	0.649**	0.177	0.200
RDW					1	0.720**	0.219*	**0.247***
SHDW						1	-0.396**	0.168
RDW/SHDW							1	0.172
Y_N								1

***. Correlation is significant at the 1% level and *. Correlation is significant at the 5% level.*

Among the set 1 accessions, positively significant linear relationship between RL and SHL and between RDW, SHDW with control fruit yield was recorded (Table 4-25). The mean values for RL was from 48.42 to 122.83cm and accessions, LA 0400, EC 676809, EC 676779, LA 0373 and EC 541101 had higher mean. The range of mean values for RDW was from 1.78 to 11.10g and LA 0400, EC 54109, Pusa Ruby, EC 676809 and EC 677049 were good for this trait. The range of mean values for RDW/SHDW varied from 0.034 to 0.112 with its mean value was 0.067 and the accessions Arka vikas, EC 771598, EC 677123, EC 771612 and VRTC-17 were superior.

Table 4-25. Correlation coefficients for shoot, root traits with fruit yield (YN) among set 1 germplasm

Traits	RL	SHL	RL/SHL	RV	RDW	SHDW	RDW/SHDW	Y_N
RL	1	0.682**	-0.222	0.594**	0.780**	0.739**	-0.312*	0.229
SHL		1	-0.797**	0.314*	0.677**	0.852**	-0.545**	0.262
RL/SHL			1	-0.090	-0.378*	-0.611**	0.576**	-0.104
RV				1	0.786**	0.637**	-0.078	0.248
RDW					1	0.856**	-0.177	**0.439****
SHDW						1	-0.585**	**0.314***
RDW/SHDW							1	0.232
Y_N								1

***. Correlation is significant at the 0.01 level and *. Correlation is significant at the 0.05 level*

Among the set 2 accessions, a positively significant correlation between RL and SHL and between RL/SHL ratio with fruit yield was observed. The mean values for RL was from 61.71 to 126.00cm and accessions IC 45, LA 1479, LA 0292, LA 1632 and LA 2138 B had higher mean values for this trait. The range of mean values for RL/SHL was from 0.560 to 1.648 and accessions EC 676790, EC 77590, IC 45, EC 677191 and Arka abha found to promising for this trait.

Table 4-26. Correlation coefficients for shoot and root traits with fruit yield (YN) among set 2 germplasm

Traits	RL	SHL	RL/SHL	RV	RDW	SHDW	RDW/SHDW	Y_N
RL	1	0.631**	0.028	0.737**	0.717**	0.675**	0.023	0.220
SHL		1	-0.688**	0.553**	0.630**	0.660**	-0.086	-0.187
RL/SHL			1	-0.077	-0.223	-0.304	0.191	**0.461****
RV				1	0.870**	0.636**	0.208	0.097
RDW					1	0.644**	0.302	0.038
SHDW						1	-0.395**	-0.061
RDW/SHDW							1	0.073
Y_N								1

Thus, the best accessions with specific root trait were listed in Table 4-27 and the accessions EC 771598, EC 771609, LA 0168, LA 0292 and LA 1632 were identified as best genotypes with superior good root traits and high fruit yield.

Table 4-27. Top ten superior tomato accessions for various specific root traits

Traits	Accession									
RL	IC 45	LA 0400	LA 1479	**LA 1632**	LA 0292	LA 2138 B	LA 1311-16	LA 1545	LA 0475	LA 1353
RL/SHL	EC 771582	EC 608391	**EC 771598**	EC 676790	EC 771612	VRTC 17	EC 676778	EC 676779	EC 771601	EC 677079
RV	LA 1311-16	**LA 0168**	LA 0475	EC 514100	EC 771608	LA 0292	LA 1479	L 00673	LA 1353	EC 520044
RDW	**LA 0168**	LA 1479	L 00671	LA 0475	EC 771608	LA 0292	L 00673	LA 1311-16	L 00882	LA 1353
RDW/ SHDW	**EC 771609**	EC 771608	L 00882	EC 677191	L 00673	Arka vikas	**EC 771598**	EC 677123	EC 771612	VRTC 17

Table 4-28. Top five superior tomato accessions for specific root traits and control fruit yield

Accession	RL	RL/SHL	RV	RDW	RDW/SHDW	Fruit yield
EC 771598	80.25	**1.802**	32.50	6.24	**0.106**	1.83
EC 771609	109.67	0.922	53.75	10.20	**0.148**	1.63
LA 0168	104.33	0.913	**81.04**	**16.83**	0.075	2.02
LA 0292	**121.17**	0.837	**67.50**	**14.85**	0.073	1.94
LA 1632	**117.50**	1.041	30.33	10.07	0.087	**2.41**

The promising genotypes for root length and root volume (drought avoidance traits) from above table (Table 4-28) can be utilized in hybridization programme for improvement of drought tolerance in tomato.

Evaluation of inter - specific crosses for traits related to Water use efficiency and fruit yield
The high yielding low WUE parents selected from previous studied are used as female and crossed in Line x Tester mating Design with High WUE male lines to develop 14 F_1s or crosses. These hybrids were evaluated in RCBD design with two replications for traits related to Water use efficiency and fruit yield

Table 4-29. List of parents used for hybridization and to develop mapping population

SL.NO.	Male parents	*S. Pennellii*	Female parents	*S. lycopersicum*
1	LA 1926	High WUE(low SLA, High SCMR, low $\Delta^{13}C$) and low yielding green fruited genotypes	VRCT- 17	Drought susceptible, low WUE, high $\Delta^{13}C$ high yielding red fruited genotype
2	LA 1946		EC- 771597	
3	LA 2657		EC- 771612	
4	LA 1360	*S. cheesmanii*	Arka alok	

Table 4-30. List of fourteen crosses evaluated for fruit yield and WUE attributes

SN	cross	SN	cross	SN	cross	SN	cross
1	EC 771612 X LA 1926	5	EC 771597 X LA 1926	9	VRCT 17 X LA 1926	13	EC 771597 X LA 2963
2	EC 771612 X LA 1946	6	EC 771597 X LA 1946	10	VRCT 17 X LA 1946	14	ARKA ALOK X LA 1360
3	EC 771612 X LA 2657	7	EC 771597 XLA 2657	11	VRCT 17 X LA 2657		
4	EC 771612 X EC 517605	8	EC 771597 X EC 517605	12	VRCT 17 **X** EC 517605		

Results:
ANOVA revealed highly significant difference among genotypes for all the traits under study except locule number and per carp suggesting presence of substantial amount of variability for all the characters in 16 genotypes.

The crosses Arka alok x LA 1360(3.14 kg), EC 771612 X LA 2657(2.48kg) and VRCT 17 x LA 1946(2.02kg) recorded higher mean fruit yield with higher SCMR and low SLA. These three crosses have been advanced to F_2 generation.

Most of the hybrids/crosses showed lower SLA than their parents and fruit colour was light yellow. The hybrids were vigorous than their parents in growth habit.

Evaluation of F_2 generation/ mapping population(s) for traits related to Water use efficiency and fruit yield
It is often observed appearance of individuals in segregating populations that fall beyond their parental phenotypes. In the progeny derived from interspecific matings. Interspecific transgression is also significant with respect to crop improvement since it represents a potential source of novel genetic variation. While many of the transgressive phenotypes observed in progeny of wide crosses are of no obvious practical value, there are a number of

documented cases where novel characters of agronomic importance have appeared in offspring from the mating of crop plants with their wild relatives.
Among the above 14 F_{1s} or crosses, only three crosses are advanced to next generation (F_2) based on contrasting trait values for water use efficiency (SLA, SCMR, $\Delta^{13}C$) and fruit yield attributing traits but only one population will be used for mapping.

Table 4-31. List of F2 populations evaluated for WUE and fruit yield attributes in tomato

Sl. No	Gen-eration	Female parent	$\Delta^{13}C$	SCMR	SLA (cm^2/mg)	Male parent	$\Delta^{13}C$	SCMR	SLA (cm^2/mg)
1	F_2	EC 771597 *(S.lycopersicum)*	28.551	53.50	198.10	LA2657 *(S. pennellii)*	29.87	40.00	23.32
2	F_2	VRCT 17 *(S. lycopersicum)*	28.815	51.00	157.55	LA 1946 *(S. pennellii)*	29.22	40.60	25.17
3	F_2	Arka Alok *(S. lycopersicum)*	27.194	53.50	150.20	LA 0438 *(S.galapagense)*	27.70	41.20	98.46

The observation on traits related to WUE and fruits yield attributes were recorded and harvesting is going on.

Status: still crop is at final harvesting stage and research is under progress Molecular work:
DNA of parents and its F_2 mapping population for QTL mapping was extracted by CTAB method. DNA of tomato germplasm for Association mapping was also extracted by CTAB method.

Screening parents of mapping population is in progress and so-for few polymorphic SSR markers have been identified.

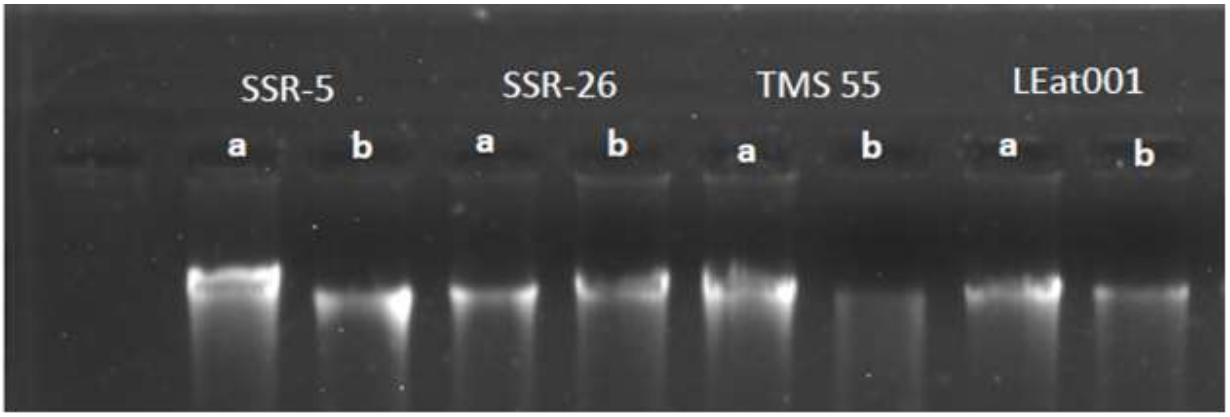


Figure 4-13. Parental Polymorphism for SSR markers. Parents: (a) EC 771597, (b) LA 2657; SSR Markers: (1) SSR-5, (2) SSR-26, (3) TMS 55 and (4) LEat001

Figure 4-14. Variation in fruit shape and colour among tomato germplasm

Figure 4-15. Phenotypic variation in plant growth under control and stress condition

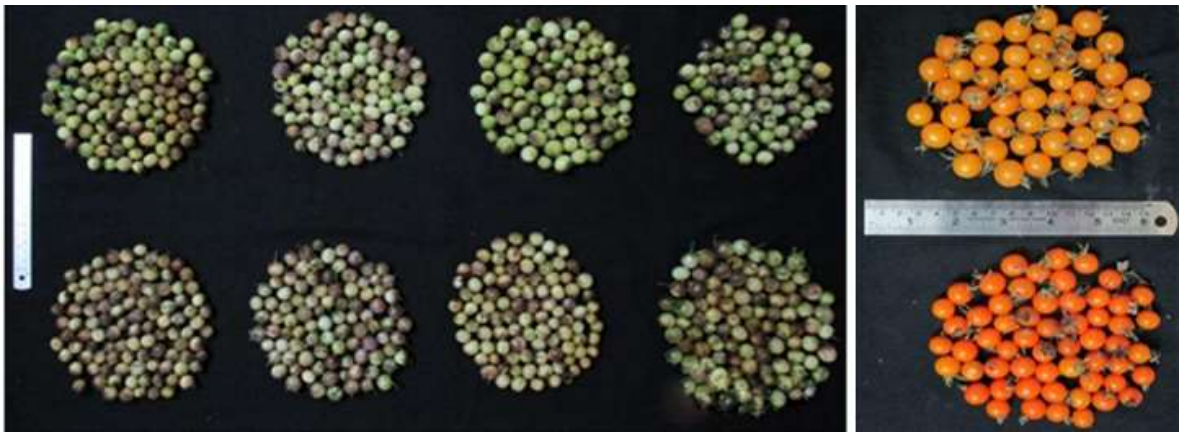


Figure 4-16. Fruit size and colour variation among S. peruvianum, S. habrochaites and S. cheesmanii species

Figure 4-17. Phenotypic appearance of drought sensitive tomato genotypes EC 771597 and EC 771612 (S.lycopersicum) and EC 514109 (S.pimpinellifolium)

Figure 4-18. Experimental plot view of drought and stress plot of tomato germplasm

4.3 Mechanisms for improved water use efficiency and salinity tolerance characterized across crop species

This task involves transcriptome profiling of leaf and root tissue of *Sorghum bicolor* and *Pennisetum typhoides* genotypes under drought and salt stresses to identify genes and small RNAs differentially expressed under stress conditions compared to control no-stress conditions.

Brief description of first year's work

In the year 1, eight genotypes of *Sorghum bicolor* were selected based on data from an irrigated field, varying in leaf temperature and grain yield potential (Mutava et al, 2011). These genotypes belonged to a collection of 300 photoperiod insensitive Sorghum genotypes. Two genotypes were selected from four categories such as 'high leaf

temperature and high yield (HT_HY)', 'high leaf temperature and low yield (HT_LY)', 'low leaf temperature and high yield (LT_HY)', 'low leaf temperature and low yield (LT_LY)'.

For Pearl millet, genotypes PI586660 (drought tolerant cultivar developed for Burkina, Faso), PI591068 (drought tolerant cultivar developed for India), and PI564586 (*Pennisetum violaceum* a wild relative of *P.typhoides*) were selected.

10 day old uniform healthy seedlings were transferred to fresh Hoagland's solution with; a) milliQ water (control), b) 15% PEG-8000 (drought stress), c) 150 mM NaCl (salt stress) and leaf and root tissues of each genotype were frozen at 0h (control) and 36h of stress. To reduce plant-to-plant variability, tissue samples from six randomly selected seedlings were pooled before library construction.

Construction of cDNA and small RNA libraries and deep sequencing

Six cDNA libraries per genotype were constructed as previously reported with minor in house modifications. Library quality and integrity was confirmed with an Agilent Bioanalyzer 2100 (Agilent technologies, Palo Alto, CA) and the concentration of each individual library was calculated using qPCR. For Sorghum, the 48 bar coded libraries were pooled together in equimolar concentrations and sequenced to generate 100 bp single-end raw nucleotide sequences in fastq format. For pennisetum, the 18 barcoded libraries were pooled in equimolar concentrations and sequenced to generate 100bp paired-end raw nucleotide sequences in fastq format. A minimum of 30 million reads were generated per library. For small RNA library construction, only one Sorghum and one Pearl millet genotype were selected. Total RNA from leaf and root tissues were combined in equimolar concentration to create 3 small RNA libraries per genotype (control, drought stressed, salt stressed). All the six Sorghum and Pearl millet small RNA libraries were pooled together and sequenced to generate 50bp single-end raw nucleotide sequences. A minimum of 10 million reads were generated per miRNA library. The sequencing was carried out at the DNA Technologies Core in Genome Center at University of California, Davis, using Illumina HiSeq 2500 platform.

Processing of raw sequence reads

Only reads from Sorghum RNAseq libraries (48) were further analyzed in the last year.

Read trimming and mapping

Because of the in-line barcodes used to prepare the libraries, the raw reads were de-multiplexed into individual samples using sabre (https://github.com/ucdavis-bioinformatics/sabre) by allowing only one mismatch in the sequence of barcode.

Each de-multiplexed library was processed to eliminate the contamination of the adapter sequence using Scythe (https://github.com/ucdavis-bioinformatics/scythe), followed by removing low quality nucleotide bases (phred score < 25) from 3' location using sickle (https://github.com/ucdavis-bioinformatics/sickle). The reads that are less than 25nt in length after adapter trimming and base quality trimming were removed from analysis. The reads after the removal of adapter contamination and low quality bases were aligned to the draft genome v2.1 of Sorghum bicolor (ftp://ftp.jgi-psf.org/pub/compgen/phytozome/v9.0/early_release/Sbicolor_v2.1/) using TopHat v2.0.10 (Trapnell et al. 2012), with default parameters**.** Cufflinks v2.1.1 (Trapnell et al. 2012) with

default parameters was employed to assemble novel transcripts using the BAM files generated by Tophat as input. The newly assembled transcripts by cufflinks were then merged together with the original annotation of Sorghum genome. This augmented annotation gtf file was then used to generate updated mapping and the expression levels were calculated based on this updated mapping results. Based on the alignment information, HTSeq-count (Anders, 2015) was used to generate the raw-counts-table, applying "intersection_nonempty" mechanism to identify a read that belongs to a gene, which requires a read to have non-empty segment that aligns to the corresponding gene uniquely. All reads that have non-unique multiple alignment were ignored. All reads that could have been assigned to more than one gene were excluded as well.

The library size of samples varies significantly based on the total number of reads that have been assigned to any gene. This large difference among library sizes can not be handled properly by edgeR package, which will be used for the differential expression analysis later. Therefore, the raw counts were subjected to normalization process using binomial sampling so that the library size of each sample is similar.

Differential gene expression

For each plant condition (HT_HY, HT_LY, LT_HY, LT_LY), the two genotypes displaying it were considered biological replicates. The normalized counts were used as input into edgeR (Robinson et al, 2010) for differential expression analysis. There are four factors that potentially have effects on the gene expression pattern of a sample in the current experiment, which are: a) Experimental Condition (Control, Drought-stressed, Salt-stressed), b) Leaf-Temperature (High, Low), c) Yield (High, Low), and d) Tissue-Type (Leaf, Root). Because of this complex design, a four-factor ANOVA model was built, including all interaction effects between factors. Generalized linear model (McCarthy et al, 2012) implemented in edgeR was applied to estimate dispersion and test for differentially expressed genes. Differentially expressed genes using FDR (False Discovery Rate) <0.1, $|\log2 \text{ ratio}| \geq 1$, and P -value < 0.01 were collected into individual files for each comparison.

We identified; 1) transcriptomic responses under the specific plant conditions (HT_HY, HT_LY, LT_HY and LT_LY) in response to salt and/or drought stresses in leaf and root tissues, 2) differential gene expression between high grain yielding (HT_HY & LT_HY) and low grain yielding genotypes (HT_LY & LT_LY) under salt and drought stresses, 3) differential gene expression between high leaf temperature maintaining (HT_HY & HT_LY) and low leaf temperature maintaining (LT_HY & LT_LY) genotypes under salt and drought stresses, and 4) differential gene expression between genotypes maintaining different leaf temperature and yield under control no-stress conditions.

Gene ontology and enrichment analysis

Sorghum bicolor gene ontology (GO) term association information was obtained from http://www.phytozome.net. Using the above gene association file and the GO ID to term index file, the GO annotation file for sorghum was generated by a custom R script. Genes without GO terms were removed from the analysis. GO terms of all Differentially Expressed Genes (DEGs) were functionally classified into three major GO categories; molecular function (MF), biological process (BP) and cellular component (CC). Venny tool (http://bioinfogp.cnb.csic.es/tools/venny/index.html) was used to generate venn diagrams

showing common and differentially expressed genes between different stress/tissue/temperature and yield conditions. GO enrichment of different DGE profiles was performed with BiNGO (Maere et al., 2005), a plugin for Cytoscape 3.2.0 using best Arabidopsis ortholog of each Sorghum transcript from JGI. (http://phytozome.jgi.doe.gov). To identify significantly enriched or overrepresented GO terms among DEGs, a hypergeometric test was performed at P-value cut-off of ≤0.05 after applying Benjamini-Hochberg False Discovery Rate (FDR) correction. A custom annotation (gene_association.tair) and ontology (gene_ontology.obo) file was used for analysis, downloaded from (www.geneontology.org).

Pathway enrichment analysis

Statistical enrichment of DGEs in KEGG pathways was tested using KOBAS 2.0 (Xie et al, 2011) (http://kobas.cbi.pku.edu.cn/) (**K**EGG **O**rthology **B**ased **A**nnotation **S**ystem). Using g:GOST (http://biit.cs.ut.ee/gprofiler/index.cgi), functional profiling of sorghum genes list was done to identify UniprotKB/TrEMBL accessions for input to KOBAS 2.0. KEGG Pathways with P-values ≤0.05 were defined as significantly enriched in DEGs.

Identification of Sorghum stress responsive transcription factors

The gene annotation from JGI (v2.1) is used to identify transcription factors (TFs) of *Sorghum bicolor*. According to PlantTFDB v3.0 (Plant Transcription Factor Database), 2198 TFs (1826 loci) were identified in sorghum and classified into 56 families. Transcription factor list of *Sorghum bicolor* was downloaded from
http://planttfdb.cbi.pku.edu.cn/download/gene_model_family/Sbi

Results

A total of 1.31 billion reads were generated from the 48 samples. After quality filtering, about 1.27 billion high quality reads (ranging from about 9 to 61 million reads for each samples were obtained. The average Phred quality score was at least 25 at each base position for filtered reads, indicating high quality of filtered reads. The total length of all the reads was over 20 gigabases (Gb), representing about a 30 fold coverage of the sorghum genome. Approximately, 960 million reads from all 48 libraries after quality control were mapped to the transcriptome of the draft genome v2.1 of *Sorghum bicolor*. Reads that have non-unique multiple alignment and all reads that could have been assigned to more than one gene (5.6 % on average) were excluded from further analysis. In total, 31998 transcripts were identified from the alignment. The transcripts identified accounted for 82.9 % of the total annotated genes in Sorghum.

5585 unique genes were found to be differentially expressing at least in one tissue/genotype/stress and those with a GO annotation (3373) were further analyzed. (Table 2). Overall, the number of DEGs was greater in the leaves than those in the roots indicating more transcriptomic changes in leaf tissue compared to root under salt and drought stresses. Salt stress resulted in more differential gene expression in leaf tissue than drought stress, while in root tissue, drought stress resulted in more DEGs. The response to salt stress and drought stress was primarily down regulation in leaf, while in root, the primary response was upregulation under both stresses. We also identified the commonly up and downregulated DEGs between; 1) salt and drought stress in leaf/root tissue, 2) leaf and root tissues under salt/drought stress.

Table 4-32. Statistics of the number of reads from sequencer (Nraw), the number of reads after adapter and quality trimming (Nqc), the number of reads that were mapped to transcriptome (Nmapped), and the number of reads after down sampling (Ndownsample).

Sl. No	Library (barcode)	Library No	Plant Condition	Genotype	Sample Sl. No and stress	N_{raw}	N_{qc}	N_{mapped}	$N_{downsample}$	Quality of reads
1	AAGCA	6	HT_HY	PI576364	S1-leaf-control	34007579	33366960	26880213	6949288	98.12%
2	AATAG	5	HT_HY	PI576364	S1-root-control	28323338	27753848	20902385	6945717	97.99%
3	CAAGG	24	HT_HY	PI576364	S1-leaf-drought-36h	28301965	27761904	18413442	6945302	98.09%
4	CCAAT	23	HT_HY	PI576364	S1-root-drought-36h	25715005	24740724	17078903	6945769	96.21%
5	CAACT	26	HT_HY	PI576364	S1-leaf-salt-36h	31646586	30880591	22022891	6946623	97.58%
6	AGACG	25	HT_HY	PI576364	S1-root-salt-36h	26932907	26299763	19094322	6943136	97.65%
7	AATCT	8	HT_HY	PI533964	S2-leaf-control	30063219	29348842	23368692	6947274	97.62%
8	AAGGT	7	HT_HY	PI533964	S2-root-control	29158250	28710452	23046564	6947750	98.46%
9	CCTGA	28	HT_HY	PI533964	S2-leaf-drought-36h	20528765	19816916	13570497	6946051	96.53%
10	CTTAC	27	HT_HY	PI533964	S2-root-drought-36h	25227646	24356205	17639938	6943629	96.55%
11	TCATA	30	HT_HY	PI533964	S2-leaf-salt-36h	25360569	24543541	16677119	6944585	96.78%
12	CGTCC	29	HT_HY	PI533964	S2-root-salt-36h	10077006	9539750	6945971	6945971	94.67%
13	AACGA	3	LT_HY	PI533962	S3-leaf-control	29055511	28462511	22646384	6942387	97.96%
14	ACTCG	9	LT_HY	PI533962	S3-root-control	25641081	24830064	17877925	6946267	96.84%
15	GAATA	32	LT_HY	PI533962	S3-leaf-drought-36h	28303772	27572583	22734235	6945217	97.42%
16	CGCAT	31	LT_HY	PI533962	S3-root-drought-36h	15722330	14920604	11025109	6945383	94.90%
17	CTGCC	34	LT_HY	PI533962	S3-leaf-salt-36h	23902681	23326457	17963987	6949893	97.59%
18	CGGCT	33	LT_HY	PI533962	S3-root-salt-36h	15786292	14942088	11383656	6945604	94.65%
19	AGAAC	4	LT_HY	PI533794	S4-leaf-control	31341600	30635013	24879308	6944094	97.75%
20	ATCGT	10	LT_HY	PI533794	S4-root-control	28067739	27417669	20179030	6942142	97.68%
21	CATCG	36	LT_HY	PI533794	S4-leaf-drought-36h	26553538	25789127	19001864	6947686	97.12%
22	CCTAG	35	LT_HY	PI533794	S4-root-drought-36h	22956939	22110132	16827128	6946729	96.31%
23	CGGAC	38	LT_HY	PI533794	S4-leaf-salt-36h	21043844	20082534	14533924	6947831	95.43%
24	CTGAG	37	LT_HY	PI533794	S4-root-salt-36h	26455414	25651152	18418829	6949379	96.96%
25	TTGAC	2	LT_LY	PI656092	S5-leaf-control	21734555	21300962	14023322	6944900	98.01%
26	ACCAT	13	LT_LY	PI656092	S5-root-control	26523289	25693482	19139368	6945140	96.87%
27	CTAGT	40	LT_LY	PI656092	S5-leaf-drought-36h	27789890	27031026	15782726	6945432	97.27%
28	CCGCA	39	LT_LY	PI656092	S5-root-drought-36h	22148521	21519900	13713961	6946055	97.16%
29	GCTGC	42	LT_LY	PI656092	S5-leaf-salt-36h	21719033	21189197	13237630	6948740	97.56%
30	CTTCA	41	LT_LY	PI656092	S5-root-salt-36h	24518697	23237383	14878797	6946538	94.77%
31	AACTC	12	LT_LY	PI534124	S6-leaf-control	25820591	25184190	19097062	6945573	97.54%
32	TTGCA	1	LT_LY	PI534124	S6-root-control	62347861	61168545	48520793	6949409	98.11%
33	GCCTT	44	LT_LY	PI534124	S6-leaf-drought-36h	28641864	27755183	18880817	6947485	96.90%
34	CTGGA	43	LT_LY	PI534124	S6-root-drought-36h	27119084	26357356	16526262	6948198	97.19%
35	GCTAT	46	LT_LY	PI534124	S6-leaf-salt-36h	32625035	31855164	24602052	6943939	97.64%
36	GAGGC	45	LT_LY	PI534124	S6-root-salt-36h	20460557	19772231	13413442	6949606	96.64%
37	ATTGA	14	HT_LY	PI597960	S7-leaf-control	30710887	30027056	22087243	6947867	97.77%
38	AGATA	16	HT_LY	PI597960	S7-root-control	34463072	33666907	25602139	6945858	97.69%
39	GCGAA	48	HT_LY	PI597960	S7-leaf-drought-36h	28953091	28315515	21553162	6947611	97.80%
40	GGCTC	47	HT_LY	PI597960	S7-root-drought-36h	27507882	26940256	20453163	6942036	97.94%
41	GCAAG	50	HT_LY	PI597960	S7-leaf-salt-36h	34741148	34039901	25817442	6950230	97.98%
42	GGAGC	49	HT_LY	PI597960	S7-root-salt-36h	27977865	27449334	21184659	6948846	98.11%
43	ACGCT	15	HT_LY	PI656081	S8-leaf-control	31166832	30438386	22710745	6944965	97.66%
44	AGCGC	11	HT_LY	PI656081	S8-root-control	22731813	22159828	15978622	6946638	97.48%
45	TAGAG	52	HT_LY	PI656081	S8-leaf-drought-36h	33203931	32376171	22479406	6941730	97.51%
46	GATAC	51	HT_LY	PI656081	S8-root-drought-36h	33656587	32814648	23145901	6945134	97.50%
47	GTAGG	54	HT_LY	PI656081	S8-leaf-salt-36h	31164252	30618134	22100658	6942461	98.25%
48	GTTCG	53	HT_LY	PI656081	S8-root-salt-36h	23731126	23170795	15389817	6944768	97.64%

Table 4-33. Differential gene expression profiling Gene Ontology (GO) annotations and enrichment of differentially expressed transcripts

Sl. No.	DEG profile number	Plant condition	Genotypes Sl. No. and GRIN No.	Tissue	Stress	Down regulated genes	Up regulated genes	Total DEGs
1	1	HT_HY	S1-PI576364 S2-PI533964	Leaf	Drought	106	30	136
2	2	HT_HY	S1-PI576364 S2-PI533964	Leaf	Salt	584	236	820
3	3	HT_HY	S1-PI576364 S2-PI533964	Root	Drought	81	151	232
4	4	HT_HY	S1-PI576364 S2-PI533964	Root	Salt	47	28	75
5	5	LT_HY	S3-PI533962 S4-PI533794	Leaf	Drought	297	98	395
6	6	LT_HY	S3-PI533962 S4-PI533794	Leaf	Salt	606	401	1007
7	7	LT_HY	S3-PI533962 S4-PI533794	Root	Drought	29	128	157
8	8	LT_HY	S3-PI533962 S4-PI533794	Root	Salt	111	101	212
9	9	LT_LY	S5-PI656092 S6-PI534124	Leaf	Drought	735	519	1254
10	10	LT_LY	S5-PI656092 S6-PI534124	Leaf	Salt	958	521	1479
11	11	LT_LY	S5-PI656092 S6-PI534124	Root	Drought	134	71	205
12	12	LT_LY	S5-PI656092 S6-PI534124	Root	Salt	74	53	127
13	13	HT_LY	S7-PI597960 S8-PI656081	Leaf	Drought	630	203	833
14	14	HT_LY	S7-PI597960 S8-PI656081	Leaf	Salt	1251	568	1819
15	15	HT_LY	S7-PI597960 S8-PI656081	Root	Drought	15	166	181
16	16	HT_LY	S7-PI597960 S8-PI656081	Root	Salt	37	97	134

The successfully annotated DEGs from DEG profiles 1-16 were functionally classified into three major GO categories; molecular function (MF), biological process (BP) and cellular component (CC). To gain further insights to the functional significance of these DEGs, BINGO tool was used to identify the over-represented/enriched GO terms. Among the upregulated DEGs, 233 were found to be classified under MF, while 854 and 80 classified under BP and CC respectively. Among the downregulated DEGs, 342 were found to be classified under MF, while 1187 and 110 classified under BP and CC respectively. The enriched GO terms were also compared between DEGs across plant conditions, tissue and stress. The comparisons revealed; 1) enriched GO terms that were present across all plant conditions, tissue and stress in both up and downregulated DEGs, 2) enriched GO terms specific to up/ downregulated DEGs under salt/drought stress in leaf/root, 3) enriched GO terms specific to up/downregulated DEGs in leaf/root, and 4) enriched GO terms specific to leaf or root tissue.

Functional enrichment of significant genes

The potential involvement of the *Sorghum bicolor* DEGs in response to salt and drought stress in metabolic pathways was investigated using the KEGG annotation system. Similar to the GO enrichment analysis, the KEGG enrichment analysis depicted common, plant condition/tissue and stress specific patterns of overrepresentation.

Global transcriptome changes in leaf and root tissues of Sorghum genotypes differing in leaf temperature and grain yield in response to salt and drought stress

The genotypes grouped under different plant conditions showed marked differences in the number of DEGs in response to salt and/or drought stress. Among the four plant conditions studied, HT_LY genotypes showed the maximum number of total DEGs followed by LT_LY, LT_HY and HT_HY respectively.

Table 4-34. Plant condition specific DEGs

Sl. No	DEGs	HT_HY	LT_HY	LT_LY	HT_LY
1	Total DEGs in response to salt and/or drought in leaf and/or root (total DEGs)	1063	1406	2140	2183
2	Total DEGs in response to salt and/or drought in leaf (total DEGs in leaf)	847	1163	1930	2011
3	Total DEGs in response to salt and/or drought in root (total DEGs in root)	267	334	280	272
4	Total number of DEGs in response to drought in leaf and/or root (total DEGs in drought)	364	548	1428	995
5	Total number of DEGs in response to salt in leaf and/or root (total DEGs in salt)	876	1159	1578	1899
6	Total number of DEGs upregulated in response to drought in leaf and/or root (total DEGs upregulated in drought)	181	224	578	359
7	Total number of DEGs downregulated in response to drought in leaf and/or root (total DEGs downregulated in drought)	185	326	863	643
8	Total number of DEGs upregulated in response to salt in leaf and/or root (total DEGs upregulated in salt)	256	470	559	622
9	Total number of DEGs downregulated in response to salt in leaf and/or root (total DEGs downregulated in salt)	623	706	1024	1280
10	Total DEGs in leaf in response to drought	136	395	1254	833
11	Total DEGs in root in response to drought	232	157	205	181
12	DEGs upregulated in leaf in response to drought	30	98	519	203
13	DEGs downregulated in leaf in response to drought	106	297	735	630
14	DEGs downregulated in root in response to drought	81	29	134	15
15	Total DEGs in leaf in response to salt	820	1007	1479	1819
16	Total DEGs in root in response to salt	75	212	127	134
17	DEGs upregulated in leaf in response to salt	236	401	521	568
18	DEGs downregulated in leaf in response to salt	584	606	958	1251
19	DEGs upregulated in root in response to salt	28	101	53	97
20	DEGs downregulated in root in response to salt	47	111	74	37

We further analyzed the common and differential up and down regulation of DEGs in each plant conditions in leaf and root tissues.

No gene was commonly found upregulated under both stresses in both tissues in HT_HY, LT_HY and LT_LY, while 4 DEGs were common in HT_LY. The results show the common and differential gene expression in leaf and root tissues under salt and drought stresses for the four plant conditions.

Table 4-35. Comparison of differential gene expression in different plant conditions

Sl. No.	DEGs	HT_HY	HT_LY	LT_HY	LT_LY	HT_HY	LT_HY	LT_LY	HT_LY
1	Commonly upregulated genes under drought in leaf	26		74		22		138	
2	Differently upregulated genes under drought in leaf	4	177	24	445	8	76	381	65
3	Commonly upregulated genes under drought in root	86		35		61		44	
4	Differently upregulated genes under drought in root	65	80	93	36	90	67	27	122
5	Commonly downregulated genes under drought in leaf	69		186		81		338	
6	Differently downregulated genes under drought in leaf	37	561	111	549	25	216	397	292
7	Commonly downregulated genes under drought in root	9		14		16		6	
8	Differently downregulated genes under drought in root	72	6	15	120	65	13	128	9
9	Commonly upregulated genes under salt in leaf	184		238		171		324	
10	Differently upregulated genes under salt in leaf	52	384	163	283	65	230	197	244
11	Commonly upregulated genes under salt in root	15		16		16		19	
12	Differently upregulated genes under salt in root	13	82	85	37	12	85	34	78
13	Commonly downregulated genes under salt in leaf	436		382		392		734	
14	Differently downregulated genes under salt in leaf	148	815	224	576	192	214	224	517
16	Commonly downregulated genes under salt in root	25		45		39		25	
15	Differently downregulated genes under salt in root	22	12	66	29	8	72	49	12

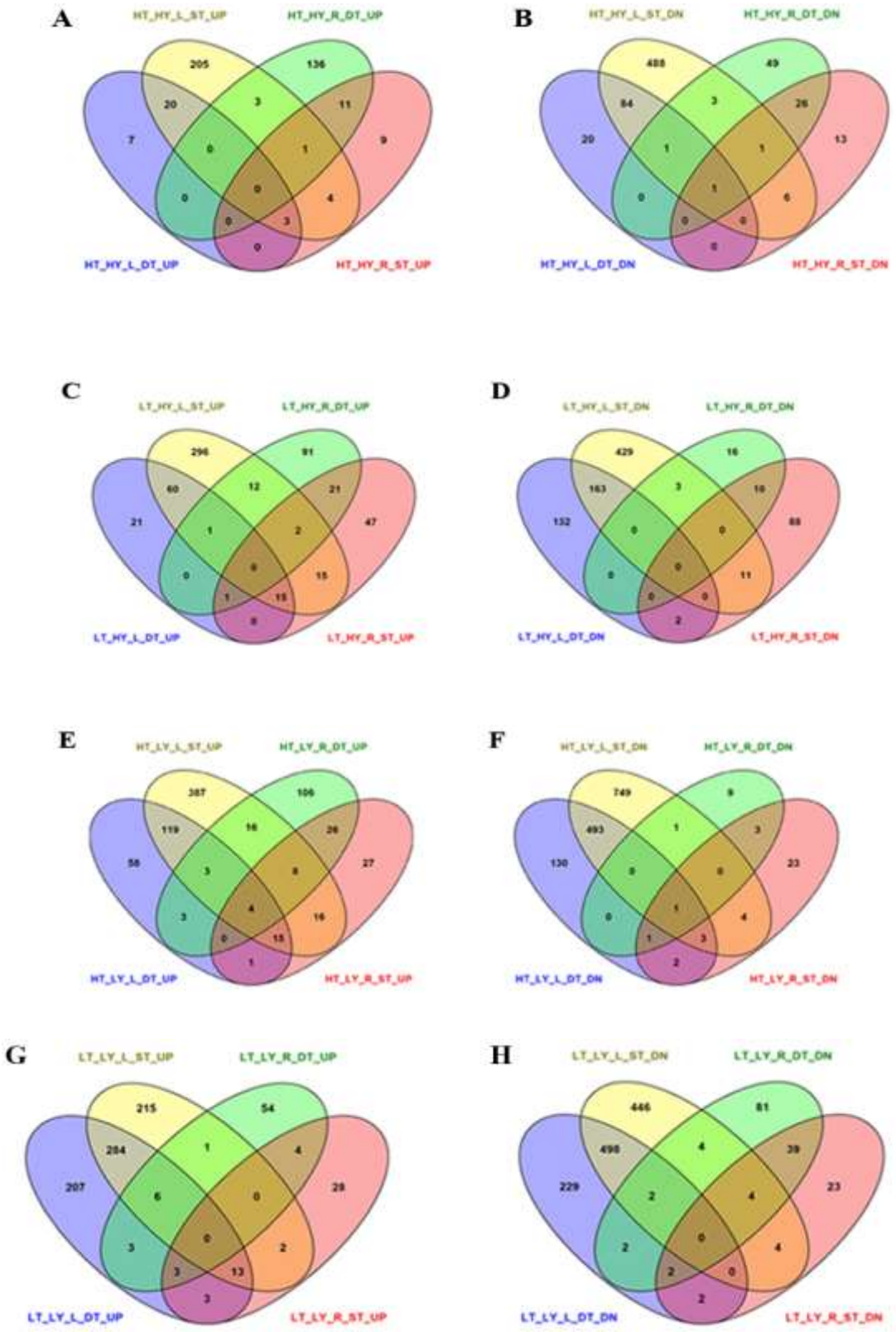


Figure 4-19. Common and differential up and down regulation of DEGs in each plant conditions in leaf and root tissues

We compared the differential gene expression under stress conditions between plant conditions that have either leaf temperature or grain yield in common (HT_HY & HT_LY, HT_HY & LT_HY, LT_HY & LT_LY, LT_LY & HT_LY) (Table 4). Considerable differences in differential gene expression were observed between genotypes differing in grain yield/leaf temperature. The results indicates that between different Sorghum genotypes, the responses to drought or salt stress varies vastly, and this has to be taken into consideration before recommending any given gene as potential candidate for improvement of stress tolerance in this species. This also points out the importance of carrying out breeding as well as transgenic efforts for crop improvement in a genotype specific manner. The commonly regulated genes across different conditions could be potential candidate genes for crop improvement in a species, while differently regulated genes could be candidate genes for crop improvement in a genotype specific manner.

4.4 Chickpea breeding lines with improved drought adaptation

Improving drought adaptation in chickpea through marker-assisted breeding

Two popular cultivars (JAKI 9218 and JG 16) of chickpea were selected to introgress root trait QTLs for enhancing the drought adaptation (Table 1). Eighty-six BC_1F_1 seeds harvested from the first cross (JAKI 9218 x ICC 4958) during March to September 2013, were planted in the field during the month of October, 2013. DNA was extracted from all these plants, and five plants were selected based on foreground marker analysis. These selected BC_1F_1 plants were backcrossed with the cultivar (JAKI 9218) using the cultivar as female parent. By end of February 2014, 206 BC_2F_1 seeds were harvested from the first cross. Of which 150 BC_2F_1 seeds were planted in the glasshouse during April 2014 and selected 36 heterozygous plants carrying foreground markers. The selected plants were backcrossed with JAKI 9218. After three backcross generations, 60 BC3F1 seeds were planted in glasshouse (Aug-Oct 2014) and harvested 16 plants carrying heterozygous foreground markers. During October 2014, 444 BC3F2 seeds were sown and 42 single plants selected carrying homozygous alleles for foreground markers. These plants will be harvested during February 2015. These selected single plants will be further grown as progenies from April to July 2015.

In the second cross (JG 16 x ICC 4958), 3 F_1 plants were identified based on foreground analysis and back crossed with the cultivar (JG 16) using the cultivar as female parent. 90 BC_1F_1 plants were raised during 2013 (Oct-Feb) and 14 plants were selected based on foreground analysis. These selected plants were backcrossed with JG 16, by using JG 16 as female parent and 144 BC_2F_1 seeds were harvested by end of February. 120 BC_2F_1 plants grown in glasshouse and selected 36 heterozygous plants for backcrossing with JG 16 during April-July 2014. 84 seeds of BC3F1 were planted and selected 21 heterozygous plants in glasshouse (Aug-Oct 2014). 629 seeds of BC3F2 were planted in the field in Nov 2014 and selected 68 single plants carrying all homozygous alleles for the foreground markers. These selected plants will be advanced as single plant progenies.

Table 4-36. Progress in MABC under Water4Crops Project		
Activity	**JAKI 9218 x ICC 4958**	**JG 16 x ICC 4958**
Oct 12 – Feb 13		
Identification of parental polymorphism for the markers linked to QTL-hotspot	CaM 1903 (274, 278), ICCM0249 (161, 191), NCPGR127 (218, 216), NCPGR21 (148, 151), TAA170 (264, 242)	CaM 1903 (274, 278), ICCM0249 (182, 191), CPGR21 (153, 151), TA130 (232, 221), TAA170 (254, 242)
Number of F1 seeds obtained	18	10
Mar 13 – Sep 13		
Number of F1 plants raised	15	8
Number of F1 plants confirmed	13	3
Markers used for confirmation of hybridity	ICCM0249, TAA170	TA11
Number of F1 plants used in backcrossing	5	3
No of BC1F1 seeds obtained	86	90
Activity	**JAKI 9218 x ICC 4958**	**JG 16 x ICC 4958**
Oct 13 – Feb 14		
No. of BC1F1 plants raised	86	90
No. of BC1F1 plants selected based on foreground selection	5	14
Markers used in foreground selection	ICCM0249, TAA170, NCPGR21	ICCM0249, TAA170, NCPGR21
No. of BC1F1 plants used in backcrossing	5	14
No of BC2F1 seeds obtained	206	144
Mar 14 – May 14		
No. of BC2F1 plants raised	150	120
No. of BC2F1 plants selected based on foreground selection	36	36
Markers used in foreground selection	NCPGR21, ICCM249, NCPGR21	NCPGR21, ICCM249, NCPGR21
No. of BC2F1 plants used in backcrossing	36	36
No of BC3F1 seeds obtained	60	84
Jun 14 – Sep 14		
No. of BC3F1 plants raised	16	21
No. of BC3F1 plants selected for selfing based on foreground selection	16	21
Markers used in foreground selection	NCPGR21, ICCM249, NCPGR21	NCPGR21, ICCM249, NCPGR21
No of BC3F2 seeds obtained	444	629
Oct 14 – Feb 15		
No. of BC3F2 plants raised	444	629
No of BC3F2 plants homozygous for foreground markers selected and selfed	42	68
Markers used in foreground selection	NCPGR21, ICCM249, NCPGR21	NCPGR21, ICCM249, NCPGR21
No of BC3F3 progenies established	35	55
Feb 15 – Sep 15		
Seed multiplication of BC3F3 progenies	35	35
Oct 15 – Feb 16		
Replicated yield trial		

Improving drought adaptation in chickpea through trait based selection
Evaluation of 300 chickpea breeding lines under rainfed and irrigated conditions.

Experimental details*:* Three hundred elite breeding lines, including desi and kabuli types, were evaluated under irrigated and rainfed conditions in the post-rainy season, for two years (2012/13 and 2013/14) at ICRISAT, Patancheru. Experiment was conducted in alpha-lattice design with three replications, 20 blocks per replication and 15 genotypes per block. Each genotype was grown on 3 m row length plot with 0.6 m inter row distance. Yield related observations were calculated on 1.0 m row length in both the conditions. Agronomic 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. Other derived parameters like harvest index, duration of growth before the start of 50% flowering °Cd (D_v), duration of growth after the start of 50% flowering °Cd (D_r); crop growth rate (C) and rate of partitioning coefficient (P) as given by standard formulas were also calculated.

Experimental findings:

Correlation coefficient:

Relationship among morphological traits and indices with rate of partitioning coefficient (P) indicating that, all the traits are positively correlated with seed yield (except D_r) under irrigated and rainfed conditions during 2013-14. Similarly Partitioning efficiency and crop growth rate (C) are also positively correlated with other traits, except for duration of growth after the start of 50% flowering °Cd (D_r). D_r showed a significant negative correlation with duration of growth before the start of 50% flowering °Cd (D_v), P and plant height in both growing conditions. D_v has a negative relationship with 100 seed weight and positive relationship with biological yield in both growing conditions. Biological yield showed a negative relationship with 100 seed weight and positively with plant height. In summary, P is highly depending on the number of days taken before the 50% flowering, and the genotypes those took more time to flowering showed high partitioning efficiency, and thus higher seed yield. Similarly crop growth rate has not shown any effect on partitioning coefficient either in rainfed or irrigated conditions.

Table 4-37. Phenotypic correlation coefficient, based on pooled data, among five metric traits and four components of partitioning coefficient, under irrigated conditions

Traits and Indices	HSW	HI	BY	D_v	D_r	C	P	SY
Plant height (cm)	0.22**	–0.35**	0.42**	0.35**	–0.23**	0.39**	0.01	0.21**
100 seed weight (g)		–0.14**	–0.28**	–0.38**	–0.03	0.15**	0.33**	0.35**
Harvest index (%)			–0.25**	–0.24**	0.24**	–0.25**	0.56**	0.33**
Biological yield (g)				0.48**	–0.14**	0.97**	0.13*	0.82**
D_v					–0.69**	0.34**	0.63**	0.33**
D_r						–0.18**	–0.55**	0.01
C							0.06	0.79**
P								0.45**

Table 4-38. Phenotypic correlation coefficient, based on pooled data, among five metric traits and four components of partitioning coefficient, under rainfed conditions

Traits and Indices	**HSW**	**HI**	**BY**	**D_v**	**D_r**	**C**	**P**	**SY**
Plant height (cm)	0.20**	–0.18**	0.19**	0.24**	–0.11*	0.14**	–0.03	0.04
100 seed weight (g)		–0.13*	–0.28**	–0.28**	0.08	0.21**	0.24**	0.30**
Harvest index (%)			–0.06	–0.08	–0.05	-0.02	0.78**	0.58**
Biological yield (g)				0.23**	–0.06	0.97**	0.06	0.77**
D_v					–0.69**	0.05	0.52**	0.14**
D_r						–0.07	–0.58**	–0.09
C							0.03	0.76**
P								0.55**

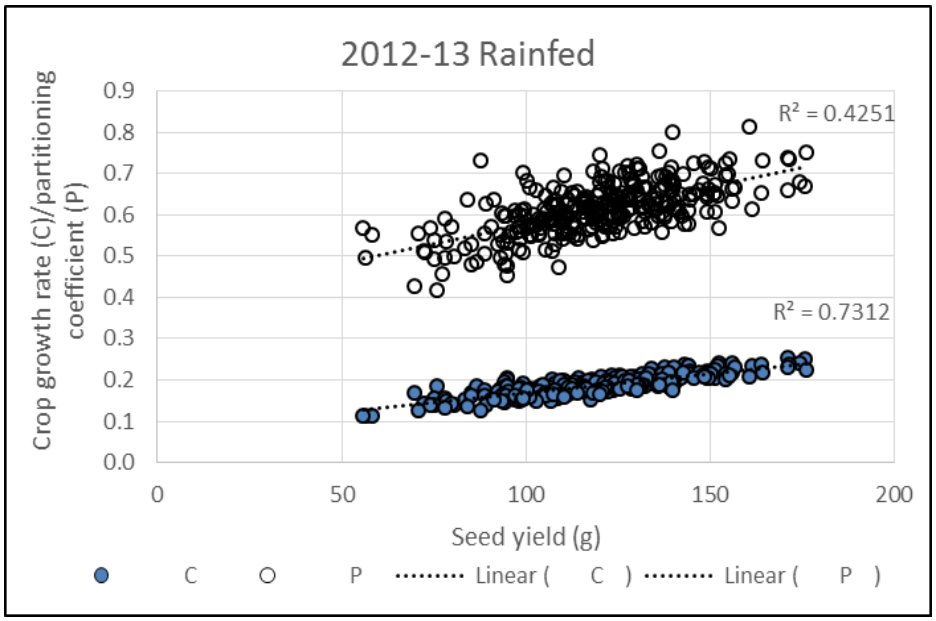
PH= Plant height (cm); HSW= 100 Seed Weight (g); HI=Harvest Index (%); BY= Biological yield (g); D_v=Duration of growth before the start of 50% flowering °Cd; D_r=Duration of growth after 50% flowering °Cd; C= Crop growth rate; P= Rate of partitioning coefficient and SY= Seed Yield/m plot (g). *, **= Significant at 5% and 1% probability level (table value of correlation with 298 degree of freedom at 5% 0.11 and 1% 0.14).

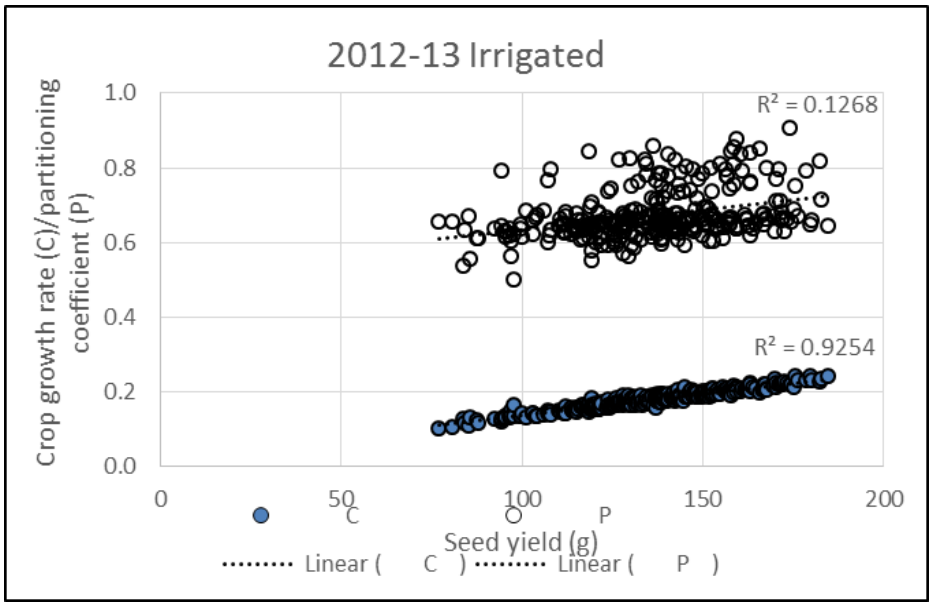
Selection of superior lines:

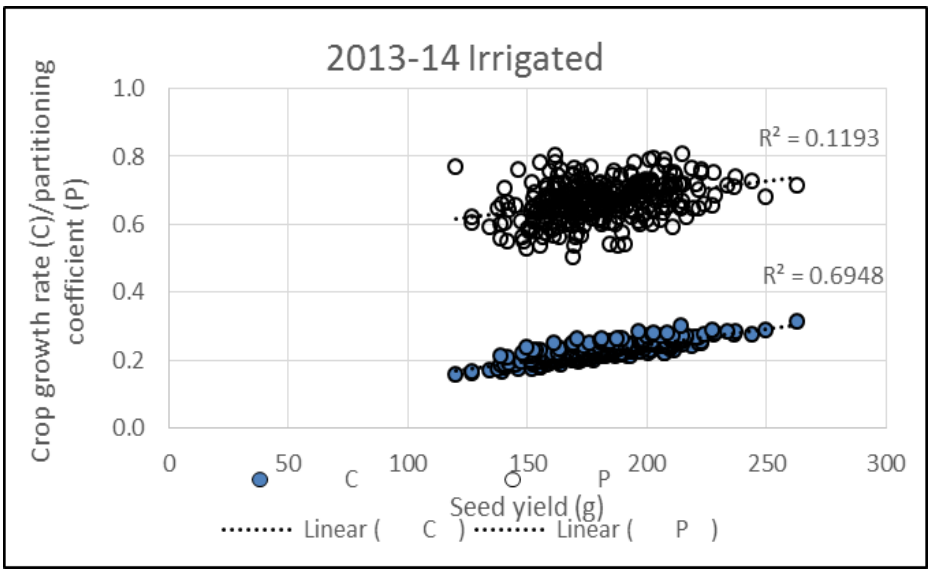
To improve the drought adaptation of chickpea, two derived indices C (crop growth rate) and P (partitioning coefficient) were used along with seed yield for identifying promising genotypes. Top 10 genotypes identified under rainfed and irrigated conditions based on seed yield, C and P (Table 3). The superior lines identified based on seed yield are common with the top lines identified based on C in each growing conditions and in both years. Contrary, none of the superior lines selected for seed yield and C showed up in the top list identified based on P. Two breeding lines ICCV 00108 and ICCV 03110 showed higher seed yield consistently in two years under irrigated conditions, and ICCV 03103 performed well under both rainfed and irrigated conditions during 2012-13. There was no trend observed for P either in two growing conditions in a year or across years. The relationship of seed yield was evaluated with C and P. C explained 70-93% of variation in seed yield across growing conditions and seasons. However P showed significant relationship with seed yield in one season (2012 rainfed) only. This indicates that crop growth rate index can be used as an indirect measure of seed yield for identifying promising lines under drought and irrigated conditions.

Table 4-39. Top 10 chickpea genotypes identified based on seed yield, crop growth rate and partitioning efficiency under rainfed and irrigated conditions during 2012-13 and 2013-14. (Underlined are common genotypes with in a growing condition; common genotypes across growing conditions are in bold)

Rank	Genotype	Seed yield (g)	Genotype	Crop growth rate (C)	Genotype	Partitioning coefficient (P)
2012-13-Irrigated						
1	ICCV 10105	185	ICCV 04104	0.240	ICCV 03205	0.905
2	**ICCV 03103**	183	ICCV 10105	0.240	ICCV 97103	0.877
3	ICCV 97126	183	ICCV 03110	0.239	**ICCV 97110**	0.859
4	**ICCV 00108**	180	ICCV 03103	0.233	ICCV 01101	0.852
5	**ICCV 03110**	180	ICCV 09107	0.232	ICCV 97114	0.850
6	ICCV 07103	179	ICCV 04304	0.229	ICCV 03207	0.844
7	ICCV 04304	176	ICCV 07103	0.228	ICCV 97106	0.841
8	ICCV 04104	176	ICCV 00108	0.228	ICCV 03210	0.839
9	ICCV 04110	175	ICCV 10111	0.227	ICCV 97109	0.837
10	ICCV 07118	175	ICCV 97126	0.226	ICCV 97127	0.834
2012-13-Rainfed						
1	ICCV 07101	176	ICCV 00402	0.253	ICCV 03213	0.811
2	**ICCV 03103**	176	ICCV 03103	0.248	ICCV 03211	0.800
3	ICCV 08111	175	ICCV 04106	0.239	ICCV 03201	0.752
4	ICCV 09101	172	ICCV 08111	0.239	ICCV 07101	0.751
5	ICCV 03202	171	ICCV 06305	0.239	ICCV 10316	0.742
6	ICCV 00402	171	ICCV 09113	0.237	ICCV 03202	0.736
7	ICCV 03207	164	ICCV 09101	0.236	ICCV 03210	0.734
8	ICCV 09102	164	ICCV 09102	0.235	ICCV 09101	0.732
9	ICCV 10103	162	ICCV 01302	0.233	ICCV 03207	0.730
10	ICCV 03213	161	ICCV 03107	0.233	ICCV 09302	0.729
2013-14-Irrigated						
1	**ICCV 00108**	263	ICCV 00108	0.314	JG 11	0.805
2	ICCV 05116	250	ICCV 03105	0.299	ICCV 00101	0.803
3	ICCV 09104	244	ICCV 03106	0.287	ICCV 07118	0.794
4	ICCV 00102	237	ICCV 05116	0.287	ICCV 05102	0.791
5	ICCV 09107	237	JAKI 9218	0.284	ICCV 97115	0.788
6	JAKI 9218	234	ICCV 00102	0.283	ICCV 04309	0.782
7	ICCV 04305	229	ICCV 01101	0.282	**ICCV 97110**	0.779
8	**ICCV 03110**	228	ICCV 05308	0.280	ICCV 09113	0.779
9	ICCV 03106	228	ICCV 01304	0.280	ICCV 01103	0.774
10	ICCV 10102	224	ICCV 04305	0.279	ICCV 97108	0.770
2013-14-Rainfed						
1	ICCV 07118	168	ICCV 07118	0.214	ICCV 05109	1.040
2	ICCV 10115	167	ICCV 09102	0.211	ICCV 09101	1.018
3	ICCV 10112	150	ICCV 10112	0.200	ICCV 04104	0.988
4	ICCV 07114	149	ICCV 10115	0.200	ICCV 03209	0.975
5	ICCV 10114	149	ICCV 09309	0.197	**ICCV 97110**	0.971
6	ICCV 03409	147	ICCV 01303	0.196	ICCV 04109	0.964
7	ICCV 09106	146	ICCV 09112	0.195	ICCV 03205	0.958
8	ICCV 03211	145	ICCV 03409	0.195	ICCV 07107	0.951
9	ICCV 09309	145	ICCV 95333	0.195	ICCV 03206	0.943
10	ICCV 97127	144	ICCV 07114	0.191	ICCV 09114	0.943


2012-13 Rainfed
Crop growth rate (C)/partitioning coefficient (P)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
R² = 0.4251
R² = 0.7312
0
50
100
150
200
Seed yield (g)
C
P
Linear (C)
Linear (P)


2012-13 Irrigated
Crop growth rate (C)/partitioning coefficient (P)
1.0
0.8
0.6
0.4
0.2
0.0
R² = 0.1268
R² = 0.9254
0
50
100
150
200
C
P
Seed yield (g)
Linear (C)
Linear (P)


2013-14 Irrigated
Crop growth rate (C)/partitioning coefficient (P)
1.0
0.8
0.6
0.4
0.2
0.0
R² = 0.1193
R² = 0.6948
0
50
100
150
200
250
300
C
P
Seed yield (g)
Linear (C)
Linear (P)

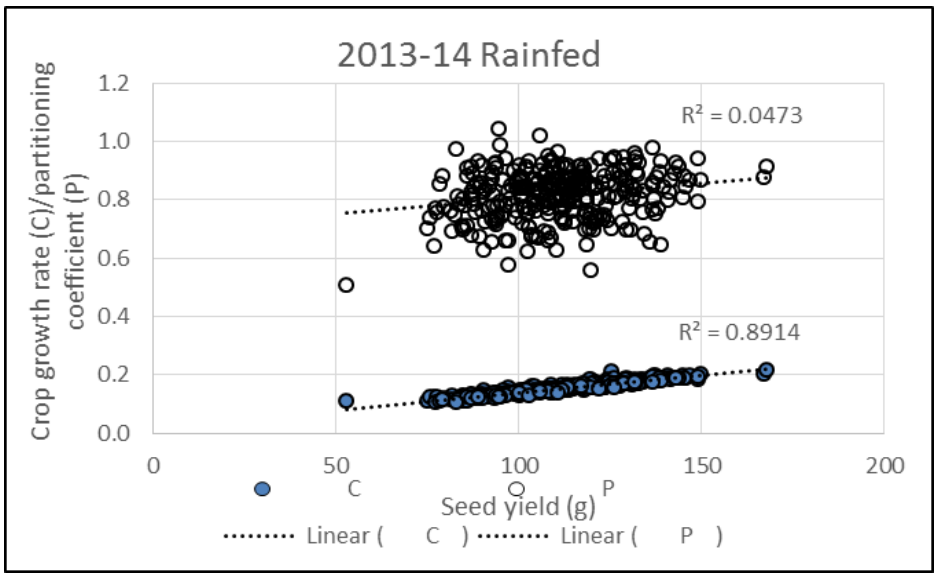


Figure 4-20.

Relationship of seed yield with crop growth rate (C) and partitioning coefficient (P) evaluated under rainfed and irrigated conditions during two years.

Evaluation of 1136 chickpea MAGIC lines under rainfed and irrigated conditions.

A total of 1136 homozygous MAGIC (multi-parent advance generation inter-cross) lines were developed using 8 diverse chickpea genotypes. Parents were crossed in all possible combinations by 2-way. 4-way and 8 way fashion. From seven 8-way crosses inbred lines were developed through single seed descent method. Experiment was conducted in augmented design in vertisols with 1136 MAGIC lines where 8 parental lines replicated 8 times used as checks under rainfed and irrigated conditions during 2013-14. Observations were recorded on phenology and yield related traits. Preliminary evaluation showed a large variation among MAGIC lines for flowering time (34-68 d), maturity (101-120), plant height (31.5-65 cm), number of pods per plant (14-150), number of seeds per plant (14-186), seed yield (255-4555 kg/ha), harvest index (0.10-0.88) and seed size (11.2-39.3). Different lines showed best performance under two different growing conditions. These MAGIC lines provide a useful germplasm source with diverse allelic combinations to be exploited by global chickpea community.

Table 4-40 List of top genotypes selected based on yield and SSI.

Rank	Genotype	Irrigated Yield (kg/ha)	Genotype	Rainfed Yield (kg/ha)
1	ICCML11026	4555	**ICCML10489**	4400
2	ICCML10758	4389	**ICCML10027**	3655
3	ICCML11077	4332	**ICCML10176**	3489
4	ICCML10148	4183	ICCML10383	3311
5	ICCML10491	4158	**ICCML10733**	3222
6	ICCML10239	4153	ICCML10833	2989
7	ICCML10570	4150	ICCML11160	2911
8	ICCML11094	4071	ICCML10215	2900
9	ICCML10993	4065	ICCML11107	2889
10	ICCML10562	4044	ICCML10387	2833

*Lines in bold have very less SSI

Large number of recombinations occurred by crossing eight different parents which led to accumulation of favorable alleles in common backgrounds of superior lines. Preliminary results indicated that top performing 49 lines showed a significant yield advantage of >23% over best check variety (ICCV 10, 2040 kg ha^{-1}) under rainfed condition (Figure 2). Similarly, ten lines under irrigated condition have produced 22-38% higher yield than best check variety (ICCV 10, 3298 kg ha^{-1}). Yield performance of these top lines need to be confirmed again in the next year and the stable lines will be shared with national partners for evaluation at respective locations.

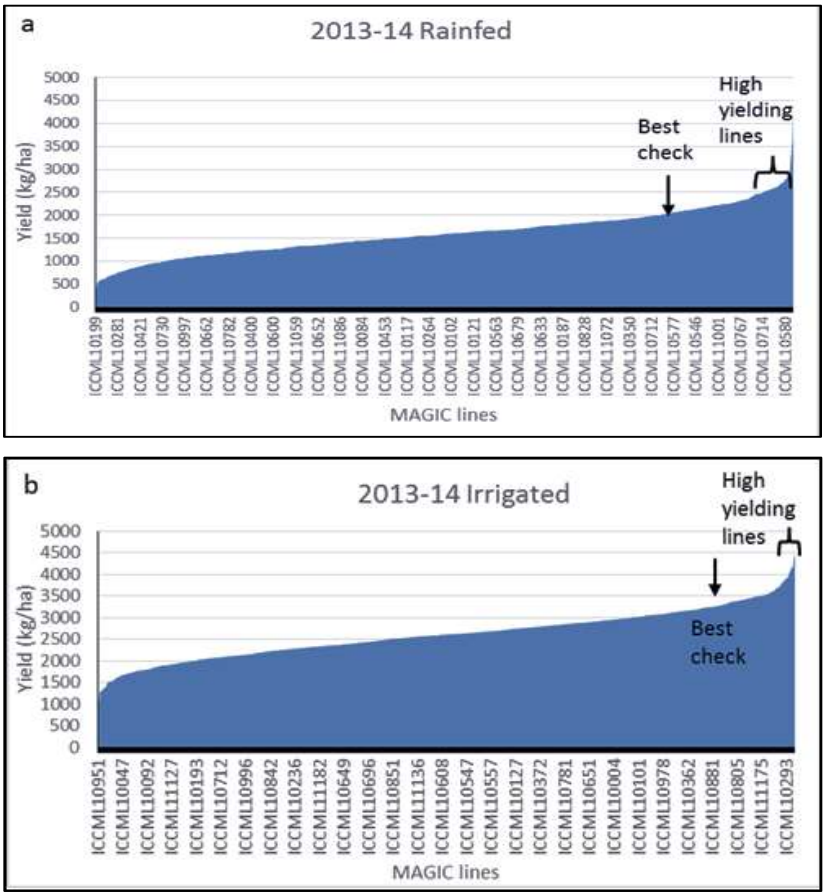


Figure 4-21. Range of yield variability observed in MAGIC lines evaluated under rainfed (a) and irrigated (b) conditions

Stress susceptibility index (SSI) proposed by Fisher & Maurer (1978) was calculated to identify drought tolerant and high yielding lines. SSI = 1 – (Ys / Yp) / SI, while SI = 1 – (Ŷs / Ŷp); whereas SI is stress intensity and Ŷs and Ŷp are the means of all lines under stress and well water conditions, respectively. The calculated values of SSI and observed yield under stress conditions (rainfed) gave a good fit to a negative linear relationship (R^2=0.60; Figure 3a). In other words, higher the negative index, lesser the drought stress sensitivity in the genotype. However, SSI also showed a significant positive relationship (R^2=0.32) with yield under irrigated condition (Figure 3b). As the SSI showed a very high significant relationship with yield, it can be utilized in identification of drought tolerant genotypes in chickpea.

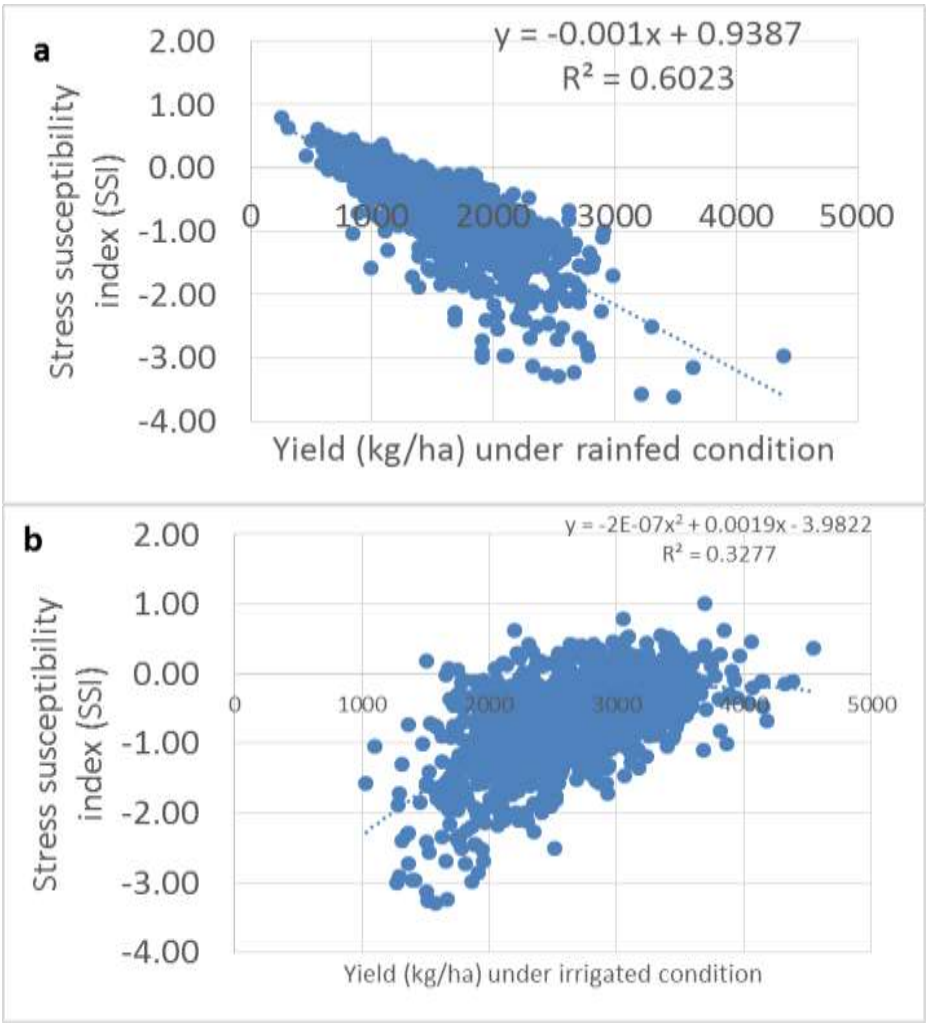


Figure 4-22. Relationship of stress susceptibility index (SSI) with yield under rainfed (a) and irrigated (b) conditions in MAGIC lines

4.5 Trained human resources in research on drought adaptation of crops and integrated breeding for drought adaptation

- A training course on "Pre-breeding and crop improvement in grain legumes" was organized at ICRISAT-Patancheru from 9-20 December 2013. The training course was aimed at updating knowledge of legume breeders working in the developing countries on recent developments in integrated breeding of chickpea, pigeonpea and chickpea. There were 26 participants representing 15 countries, including 3 participants from India. There were lectures, demonstrations and visits of field and laboratory experiments. The research areas covered in the training course included utilization of genetic resources, wide hybridization, and integrated breeding
    - The Research Associate working in the project at ICRISAT-Patancheru was provided training on marker-assisted breeding in chickpea.
    - Two PhD (BP Mallikarjuna and Pronob Paul) and one MSc student (Prity Sundaram) carried out research work on chickpea at ICRISAT. The research topics included molecular mapping of early flowering genes (BP Mallikarjuna), molecular mapping of heat tolerance genes (Pronob Paul) and effects of earliness on seed size and seed yield in chickpea (Prity Sundaram).

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

5.1 Database of stakeholders

EIRC and WP5 EU partners ALTERRA, STEP and GIZ had an internal discussion during the 1st EU-India Joint meeting which was held from 3rd to 5th Dec in Bari, Italy. The meeting focused on developing common strategies to bring together the research and industry players of their respective consortiums in order to mobilize the transnational knowledge and technology transfers between the partners from India and Europe.

Establishment of Innovation Platform

EIRC identified the key stakeholders and practitioners from knowledge (intrinsic and explicit) sector which included technology developers, researchers and industry experts. The profiles of the external experts were sent to the project Coordinator for feedback. Regular skype calls were made to discuss about organizing the Indian INNOVA meeting and the final list of experts were invited for the Meeting.

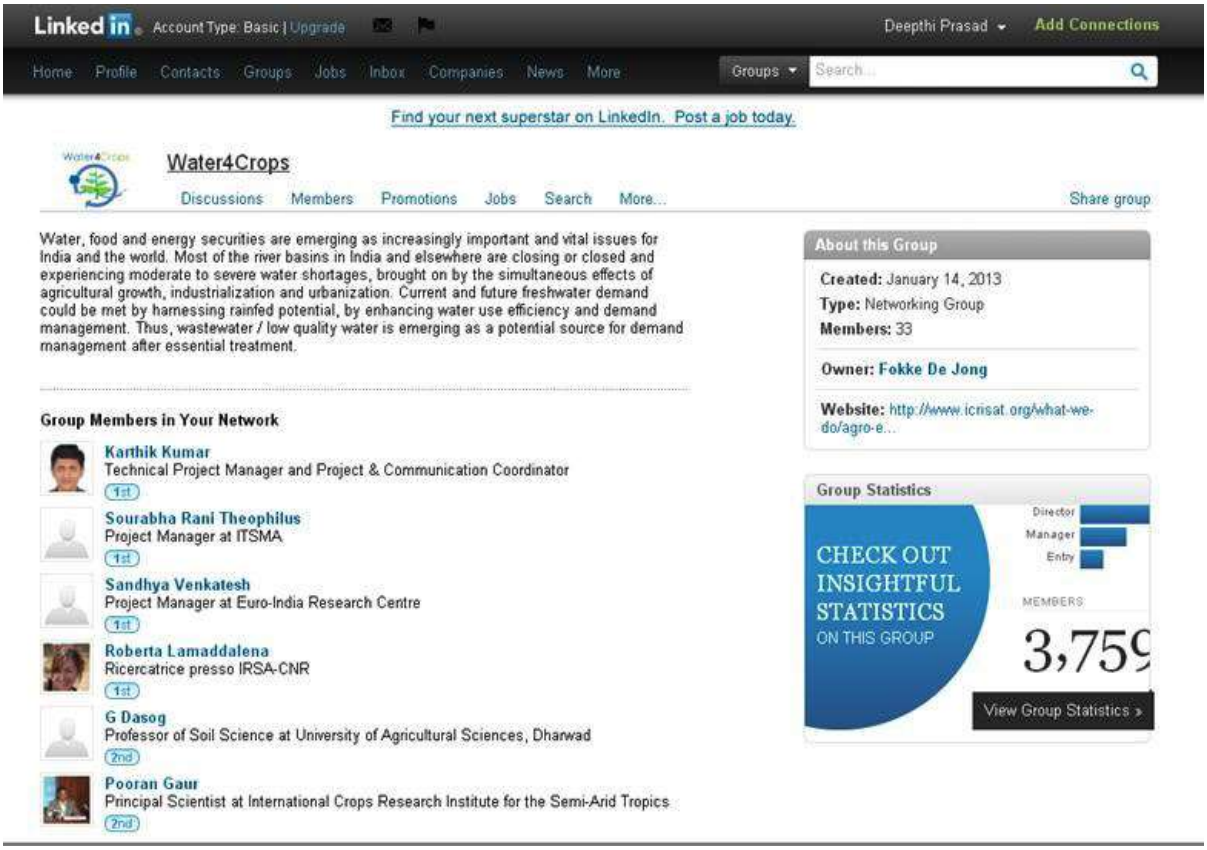
On 28th May 2014, EIRC organized the 1st Indian INNOVA Meeting at the Capital Hotel in Bengaluru, India. The meeting brought together the Industry experts from CII (Confederation of Indian Industry), EBTC (European Business Technology Center), Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) Germany and EnviroTech Water Management Pvt. Ltd. in the field of wastewater treatment and water use efficiency. These experts were challenged to explore business opportunities for the new technologies in the domains of wastewater reuse and valorization, and water use efficiency that are being developed in Water4Crops project. The meeting facilitated lively discussions between researchers and experts in which the relevance of technology for target users, economic viability and other issues related to applicability and market uptake were addressed.

Creation of Digiinnova Platform - LinkedIn Group

This is a common platform for both the EU and the Indian consortium to exchange and share their experiences about project activities they are undertaking. On the platform upcoming events, meetings, synthesis of specific newsletters and reports are being posted. It is also designed to host discussion on upcoming factsheets especially on the topics like legislation and cost-benefits of waste water treatment and reuse technologies. This discussion will provide inputs to the innovation process in WP5. The external stakeholders from Innova Platforms were also invited to the group. The task ahead for EIRC will be to enhance the group and encourage Indian partners and external experts to actively

participate in the discussions that have begun. EIRC will continue pursuing the Indian consortium partners to make best use of this forum to exchange ideas and share knowledge.

The Water4Crops discussion portal via Linked-in group is available under the link: http://www.linkedin.com/groups/Water4Crops-4799081
Database of stakeholders: Completed and submitted the deliverable to DBT.



Future trends and boundary conditions

EIRC and EU WP5 partner ALTERRA discussed and identified 5 topics on boundary conditions and trends to waste water treatment and reuse in India and EU which include – Legislation; Resource use and boundaries; Health and perceptions; Cost and benefits; and future food production. ALTERRA and EIRC together developed Factsheet Templates and were sent to all the partners to collect the "facts" and "figures", key trends and present scenario existing in both the regions in relation to waste water treatment and reuse in EU and India focused on these 5 topics. The factsheets are almost prepared and the final editing and fine tuning is under process.

Co-creation process of identifying innovation potentials to enable green economy

In order to prepare for the Innova platform meetings, a questionnaire was jointly prepared by the EU and Indian WP5 leaders namely Alterra and EIRC. Based on the questionnaire inputs, relevant experts (from within the consortium and also stakeholders from outside) were selected and invited for the 1st Indian INNOVA platform meeting. The technologies and issues that have been mapped through the questionnaires were discussed during the 1st Indian INNOVA platform meeting.

Synthesis of results and initiation of an implementation process:
Initial "list of technical innovations" were 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. Further, these innovations will be discussed with the stakeholders like SMEs, farmers, local investors to define a roadmap for implementation. This activity is under progress.

5.2 Report of agribusiness opportunities

This activity is under progress. The 1st Indian INNOVA meeting inputs are recorded and analysed. Business opportunities are being identified by both the consortiums. The final short list of business opportunities will be reported in the following months.

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

6.1 Internal report on customer / entrepreneur demands and technological offer

EIRC and EU WP 6 Partners STEP, ALTERRA, IRSA and GIZ had an internal meeting on 28th May 2014 at Bangalore, India. The dissemination and communication strategies and future plans were discussed. The objective for the year 2 was to ensure effective dissemination of the project results and defining means and actions 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.

Exchange of experiences and results within the Innovation Platforms (IPs) (EIRC)
At the INNOVA platform meetings, all the project partners were given a platform to share their experiences and research results to the external stakeholders. The industry experts were challenged to explore business opportunities for the new technologies in the domains of waste water reuse and valorization, and water use efficiency that are being developed in Water4Crops project.

LinkedIn Forum also acts as a common platform for both the EU and the Indian consortiums to exchange and share their experiences about project activities they are undertaking. On the platform upcoming events, meetings, synthesis of specific newsletters and reports are being posted. It is also designed to host discussion on upcoming W4C factsheets. This discussion will provide input to the innovation process in WP5. The external stakeholders from Innova Platforms were also invited to the group. The

task ahead for EIRC will be to enhance the group and encourage Indian partners and external experts to actively participate in the discussions that have begun.

Organization of special entrepreneur and SME knowledge brokerage event (establishment of the Science Practice interface (EIRC)

A special session called "Water4Crops – SME Brokerage Discussion" was organized in the framework of IFAT India 2014 on 9th & 10th October at the Bombay Exhibition Centre, Mumbai, India. IFAT India is the country's leading trade fair for water, waste, sewage, and recycling. The SME's, entrepreneurs, technology producers and industry experts were invited for the event. The event focused on treatment of industrial wastewater, its reuse and valorisation to support Green Economy in Europe and India. High-profiled researchers from India and Europe presented promising ideas and technologies, which are under development for treatment of wastewater and irrigation technologies. Besides, the event provided an opportunity to the SME's to interact and network with Indian and International experts representing premier Indian and European research organizations. The programme agenda, presentations and pictures are available under the link below:
http://www.water4crops.org/promising-research-results-water4crops-presented-ifat-india-2014-mumbai-october-9-10-2014/

6.2 Webpage and Public Dissemination material

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

The EU-India Joint water4crops website is the main dissemination tool to showcase significant results and outcomes and project events. The website is regularly updated with information from both EU and Indian side. Apart from project activities, the news, events and related articles are also posted in the website. This conveys to outsiders that W4Cs is a joint project between India & EU and both sides are working together. Moreover, it enables effective linkages between both projects partners. Every partner in this way is updated on the developments and progress on activities on both sides.

In order to measure the dissemination impact, EIRC regularly monitors the Website statistics including number of visits, duration of visits, number of downloads, download items, etc. EIRC has enabled the Awstats and Google Analytics tools to monitor the activity of the project web site and measure the progress and impact. The snapshots of the web statistics is provided at the end of the document.

Project Store: The "Project Document Store" or the "Intranet" has been developed by EIRC in the time frame of the 1st reporting period. 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. Two separate accounts have been created for both EU and Indian partners and the credentials are shared with them. 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.

Project Poster and roll ups: A common poster design was designed and developed after reviews and suggestions from both project partners. This poster was presented during the Water4Crops-India 1st Project Review and Planning Meeting which was held from 27th –

29th May 2014 at Bengaluru, India**.** The roll ups was specifically designed and developed for the 1st Indian INNOVA meeting. The snapshots of both the poster and roll up is attached in this document.

Technical posters for IFAT India 2014: Both EU and India consortium partners decided to develop technical posters to present at the IFAT India 2014 event at Mumbai. An earlier discussion was made with all the partners to know there interest and availability to develop the technical poster. A draft template was prepared and sent to all the partners for their inputs. Later EIRC and STEP collated all the information and developed a total of 9 posters for the event. The snapshots of the posters are attached at the end of the document.
Water4Crops Booklet: EIRC and EU partner STEP together developed a booklet for the IFAT India 2014 event. This booklet consist of all the technical Innovations which were developed under water4crops. EIRC designed the booklet and it was distributed to all the SMEs and entrepreneurs who attend the SME brokerage session at IFAT India 2014 on 9th & 10th October at Mumbai.

Elaboration of Annual Newsletters for the wider public
EIRC and EU partner STEP together developed the common W4C Newsletter. Two annual newsletter have been published till date. The 1st newsletter contains the information on the project progress, research findings and observations made especially at its Indian case study sites. The next issue, was focused mainly on the European case studies. The 1st NL was presented and distributed to the experts at the Water4Crops-India 1st Project Review and Planning Meeting and the 2nd NL was presented at the IFAT India 2014 event. The newsletter is also widely distributed to all the stakeholders. The newsletters can be downloaded from the link below:

- Water4Crops Newsletter Issue #2
- Water4Crops Newsletter Issue #1

Mass media and press releases, information to social media with project progress statements (EIRC, ICRISAT and SAB Miller India, All Partners)
This activity will be undertaken not only by EIRC, but also by other partners, especially ICRISAT, SAB Miller India, and other partners in their respective regions. The next press release is yet to be decided.

YouTube Channel for Water4Crops
EIRC created a YouTube channel for Water4Crops project. All project related videos are uploaded to the Water4Crops website and is also disseminated through this social media network: http://www.youtube.com/watch?v=tOCC7z2fUdQ

Twitter account for Water4Crops
EIRC has also created a Twitter account for the project and all the project activities, news and events are tweeted regularly. This way the subscribed followers are informed about current activities of the project and Importantly, it lets followers communicate with the project too.
Twitter account: @water4crops

Input to existing information hubs:
EBTC (European Business Technology Center) was identified as one of the important initiators to promote water4crops activities and results through their EBTC web portal. As per earlier discussions, it was decided that Water4Crops will be promoted and involved in several Water Initiatives and Channels that EBTC is connected to, and EBTC expert will be invited and involved in Water4Crops events and Innova Platform meetings. EIRC was in regular contact with EBTC officials and as promised EBTC created a separate web page for the water4crops project within their website and all the latest news and project outcomes are shared in this webpage. To view the webpage, please follow the link below:
http://ebtc.eu/index.php/sector/environment/water4crops
An article about "Water4Crops" was also published in the "EU Parliament Magazine" (pg 51, Issue 391, 26 May 2014). To read the article visit: http://www.water4crops.org/article-water4crops-eu-parliament-magazine/

6.3 Report on training course including online curricula

The demand on training and the priorities of the trainable topics are discussed and evaluated at the 1st INNOVA meeting at Bari. Using the results of this knowledge brokerage event as basis STEP and EIRC will develop a catalogue on Trainable Tools of W4Cs making it available via W4Cs internet portal. The first W4Cs tool, **SALTMED 2013**- An integrated management tool for Water, Crop, Soil and N-Fertilizers, tool is already available in the Water4crops website (http://www.water4crops.org/saltmed-2013-integrated-management-tool-water-crop-soil-n-fertilizers/). Dr. R. Ragab has developed a very useful tool which helps in agriculture resources management as well as in predicting the impact of future climate change on food production and on the environment.

The second tool called "**IHMS-Integrated Hydrological Modelling System** by Dr. R. Ragab, CEH, UK" is also available on the W4C website (http://www.water4crops.org/ihms-integrated-hydrological-modelling-system-dr-r-ragab-ceh-uk/)

STEP and EIRC also organized a one day **Workshop on SALTMED Management Tool** on 29th May 2014 at Bengaluru, India. The SALTMED Workshop was successfully carried out with 22 participants. Dr. Ragab Ragab, CEH Wellington who is the developer of the SALTMED model, gave an overview about his model and instructions for installing the SALTMED software program on the laptops of the participants. In the first part of the workshop Dr. Ragab explained the theoretical background of the SALTMED model indicating the range of its application area and its limitations. A special part of the workshop was dedicated to working with the SALTMED model in the afternoon session. The workshop flyer can be found under the link :
http://www.water4crops.org/wp-content/uploads/2014/05/SALTMED-Workshop-flyer-29th-May-2014.pdf

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

7.1 Workshop to workout common protocols to be adopted by the partners in the project

7.2 First year annual report to DBT

7.3 Second year annual report to DBT

Table 7-1. List of Staff recruited for consortium partners

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						

*** 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**

Partners (India side)

About ICRISAT

ICRISAT Science with a human face

International Crops Research Institute for the Semi-Arid Tropics

CGIAR

ICRISAT is a member of the CGIAR Consortium

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.

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Addis Ababa, Ethiopia

ICRISAT-Bamako (Regional hub WCA)
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ICRISAT-Bulawayo
Matopos Research Station
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ICRISAT- Kano
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Tarauni, Kano, Nigeria

ICRISAT-Lilongwe
Chitedze Agricultural
Research Station
PO Box 1096, Lilongwe, Malawi

ICRISAT-Maputo
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Caixa Postal 1906
Maputo, Mozambique

ICRISAT-Nairobi (Regional hub ESA)
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ICRISAT-Niamey
BP 12404, Niamey
Niger (Via Paris)

About ICRISAT: **www.icrisat.org**

ICRISAT's scientific information: **http://EXPLOREit.icrisat.org**