Model for economical and sustainable bioenergy production under greywater irrigation trial

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The present study focuses on sustainable utilization of greywater in short-rotation energy plantation – *Eucalyptus hybrid*, *Populus deltoides*, *Salix alba* and *Melia azedarach*. The dry matter produced by wastewater plots was 143%, 54%, 274% and 321% higher for *Eucalyptus hybrid*, *Populus deltoides*, *Salix alba* and *Melia azedarach* respectively, than the same plants in control plot. The calorific value of samples ranged from 4037 to 5190 Kcal kg^{-1} in greywater plots, and 3460 to 4469 Kcal kg^{-1} in control plots. The carbondioxide mitigation potential was 19, 13, 11 and 29 t ha^{-1} higher for *E. hybrid*, *P. deltoides*, *S. alba* and *M. azedarach* trees respectively, under wastewater irrigation.

Keywords: Bioenergy production, calorific value, carbon dioxide mitigation potential, greywater irrigation.

ACCORDING to the 2011 census, India's population has reached 1210 million. Around 833 million people reside in rural areas constituting 68.8% of the population. Approximately 44% of this rural population has no access to electricity and 85% still depends on firewood as a primary source of fuel. In India, bioenergy is the primary sources of energy for cooking, heating water, etc. About 76.3% of rural and 17.5% of urban population still depend on firewood for cooking purposes[1]. India is well bestowed with 450–500 million tonnes of biomass per year, which has a potential to generate 18,000–50,000 MW of power[2].

However, due to increasing population pressure, the consumption of firewood has by far exceeded its supply, thereby causing deforestation. Another problem faced is freshwater scarcity and increasing loads of wastewater. Grey fraction of domestic wastewater represents the largest potential source of water conservation[3].

The present study demonstrates a commercial model for plantation of short rotation bioenergy trees under the greywater irrigation trial and its impact on firewood and economic returns.

The present study was conducted in a 1 ha fallow land at the G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India (Figure 1). The soil of the experimental site is sandy loam in texture with a slightly acidic pH ranging from 6.3 to 6.5 and electrical conductivity of 140–165 μS cm^{-1}. It is a Tarai region with organic matter (OM) ranging from 1.42% to 1.49%, and bulk density and infiltration rate of 1.45 g cm^{-3} and 0.32 cm h^{-1} respectively.

The experiment was initiated in November 2008; greywater used for the experiment was exclusively from the bathrooms and kitchens of two residential colonies of the University, having a population of approximately 5000 inhabitants. The control plot was irrigated with water from a shallow borewell which was installed in the experiment field.

Two equal sub-plots of dimensions 60 m × 80 m were prepared for treatment and control trials leaving a buffer zone of 20 m width between the two plots to prevent the mixing of wastewater and control water. Each plot was further subdivided into three replicated subplots of dimension 30 m × 20 m having a buffer of 10 m between each sub-plot, in which four short-rotation energy species of *Eucalyptus hybrid*, *Populus deltoides*, *Salix alba* and *Melia azedarach* were planted. The chosen species are well adapted to the climatic conditions and have high commercial demand in the local market. The plant-to-plant and row-to-row spacing was kept 2 m × 2 m with plant density of 2500 plants/ha. To verify the statistical significance of the results, Student's *t* test was performed at 5%, 1% and 0.1% confidence level. Correlation between biomass, calorific value and nitrogen content was also estimated statistically using Pearson correlation coefficient. The economic benefits incurred by the study were estimated for five years of plant rotation for 1 ha plot with 2500 plants.

The trial will run for 5–7 years of rotation, but for the present initial study tree samples were harvested after 2 years in 2011. Biomass was estimated using the destructive method. The representative samples were harvested from each replicate plot using statistical distribution. The greywater and control water used in the trial were regularly monitored during the study period. Table 1 shows the physico-chemical characteristics of the water used in the trial. The harvested samples were divided into five components, i.e. stem, root, twig, branch and foliage. Fresh weight of all components was taken in the field for each sample tree using a heavy-weight spring balance. The samples (250–500 g) were then taken to the laboratory and oven-dried at 65°C till constant weight was attained to determine the moisture content. The calorific value and specific gravity were measured by bomb calorimeter and maximum moisture content method respectively[4,5]. Ash and carbon content were estimated by muffle furnace method at 600°C for 4–5 h to obtain ash. Carbon was assumed to constitute 50% of ash free dry-mass[6]. Carbon dioxide (CO_2) mitigation potential was estimated by multiplying the values of carbon stock by a factor of 3.66.

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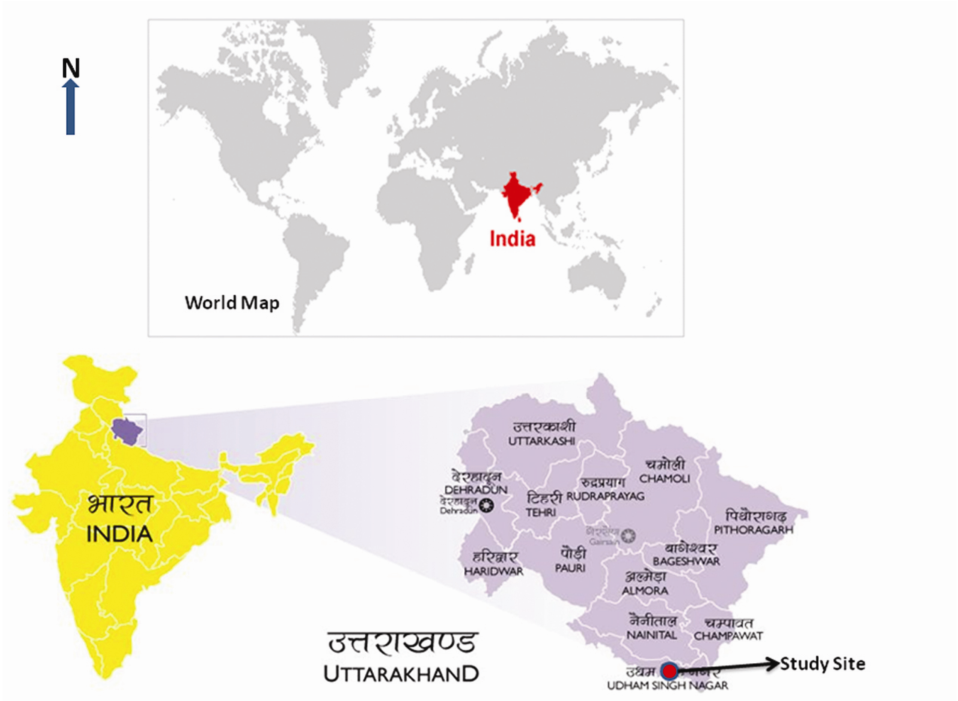

Coordinates	29°01'469"N and 79°29'393"E
Altitude	243.8 meter above msl
Annual average temperature	5.5°C – 41°C
Average annual rainfall	1350mm

Meteorological parameters	Minimum	Maximum
Daily maximum temperature (°C)	13	40
Daily minimum temperature (°C)	4	27
Monthly precipitation (mm)	0.1	325
Relative humidity (%)	37	90
Wind speed (km h^{-1})	1.8	11

Figure 1. Geographic location and meteorological condition of the study site in Pantnagar, Uttarakhand, India.

The monetary estimations were done by calculating the total expenditure and expected returns from the trial after five years. All estimations for expenditure and benefits were done for 2500 plants planted in 1 ha land. Intangible benefits to soil, environment and wastewater treatment efficiency were not accounted. Freshwater pricing was neglected deliberately as the Government provides irrigation water free of cost in the country, but this can be a major head in the near future. The cost of harvestable biomass was estimated based on the average cost mentioned by Forest Corporation of Uttarakhand, India (2008–2009) and Haryana Agro Forestry Company (2010)[7].

The performance of species was regularly monitored every month during the study period. The height, diameter at breast height (DBH), chlorophyll content and nutrient content were measured regularly for the samples in both treatment and control plots. In the present communication, we will only restrict the discussion to harvestable biomass, nitrogen content, energy parameters and carbon mitigation potential to confine to the objectives of our research.

The total dry biomass (DM) yield (t/ha) for the study period was recorded to be 18.2, 22.07, 9.45 and 21.25 for *E. hybrid*, *P. deltoides*, *S. alba* and *M. azedarach* respectively, in wastewater plot and 7.5, 14.3, 2.5 and 5.0 respectively, for control plot (Table 2). Though the highest dry biomass was obtained with *Populus* species, the difference in biomass of treatment and control plots was recorded highest for *Melia* species. The wastewater irrigation showed an initial positive impact on plant growth, which envisaged higher dry biomass in the treatment than control plots. The DM produced by wastewater plots was 143%, 54%, 274% and 321% higher for *E. hybrid, P. deltoides*, *S. alba* and *M. azedarach* respectively. The positive impact on the growth of plants irrigated with wastewater could be probably due to optimum loads of nutrients and organic matter present in it.

Student's *t* test showed that the difference in total DM of plants in treatment and control plots for all the four species *E. hybrid*, *P. deltoides*, *S. alba* and *M. azedarach* was statistically significant at $P < 0.001$ (0.1% confidence level), which revealed the positive impacts of

Table 1. Physico-chemical characteristics of irrigation water (freshwater and greywater) used in the study

Parameters	Greywater	Control water
pH	7.6–8.1 ± 0.01	7.6–7.8 ± 0.01
Electrical conductivity (µS cm^{-1})	508–551 ± 0.02	215–252 ± 0.01
Dissolved oxygen (mg l^{-1})	1.3–1.6 ± 0.2	2.9–3.8 ± 0.2
Nitrogen (mg l^{-1})	26–37 ± 0.2	1.5–1.7 ± 0.4
Phosphorus (mg l^{-1})	7.3–10.3 ± 0.01	0.9–1.2 ± 0.02
Potassium (mg l^{-1})	2.2–5.3 ± 0.01	0.67–0.86 ± 0.01
Sodium (mg l^{-1})	3–11.5 ± 0.01	3.8–4.9 ± 0.01
Biochemical oxygen demand (mg l^{-1})	50–58 ± 0.5	2.9–3.5 ± 0.4
Chemical oxygen demand (mg l^{-1})	192–292 ± 0.2	5.3–6.2 ± 0.4
Total dissolved solids (TDS; mg l^{-1})	323–352 ± 1.0	150–171 ± 1.0
Total suspended solids (TSS; mg l^{-1})	241–278 ± 2.1	115–124 ± 2.0
Total solids (TS; mg l^{-1})	564–617 ± 2.2	264–285 ± 1.5

Table 2. Total average dry biomass production from the study site after two years

Tree species	Total dry biomass in treatment plot (t ha^{-1})	Total dry biomass in control plot (t ha^{-1})	Yield increase (t ha^{-1})	Percentage increase in treatment plot
Eucalyptus hybrid	18.198	7.504	+10.7	143
Populus deltoides	22.068	14.332	+7.75	54
Salix alba	9.456	2.527	+6.93	274
Melia azedarach	21.240	5.042	+16.2	321

wastewater irrigation on tree yield due to the presence of nutrients required for the growth of plants.

Similar results of feasible productivity up to 20 t DM ha^{-1} year^{-1} for *E. globulus* have been reported when irrigated with domestic wastewater[8]. Mitchell *et al.*[9] reported an average production ranging from 2.2 to 13.5 t DM ha^{-1} year^{-1} for *P. deltoides* and willow depending on the site[9], while an average value of 10 t DM ha^{-1} year^{-1} was reported by Heller *et al.*[10]. Few studies on *M. azedarach* documented higher biomass yields with treated sewage[11].

The moisture content ranged from 9.5% to 14% in wastewater-irrigated wood (stem and branch) and 11%–14.5% under control conditions. The ash content was 0.38%–0.94% and 0.40%–1.62% in greywater and control water respectively. It was higher in branch samples than stem samples.

The specific gravity of wood samples irrigated with greywater was higher than those irrigated with control water. The difference was highest, viz. 0.29 g cm^{-3} for *M. azedarach* followed by *E. hybrid* which had 0.1 g cm^{-3} higher specific gravity in greywater-irrigated samples. The specific gravity of *M. azedarach* wood samples irrigated with greywater and control water was 0.67 and 0.38 g cm^{-3} respectively. The specific gravity of wood samples in greywater-irrigated plot water was 0.65, 0.81 and 0.40 g cm^{-3} for *E. hybrid*, *P. deltoides* and *S. alba* respectively. However, the values were 0.55, 0.72 and 0.32 g cm^{-3} for *E. hybrid*, *P. deltoides* and *S. alba* respectively, in control plot. The difference in the specific gravity of samples from the treatment and control plots revealed least variation in *P. deltoides* and *S. alba* (Table 3).

The calorific value of stem samples ranged from 4037 to 5190 K cal kg^{-1} in greywater plot, and 3460 to 4469 K cal kg^{-1} in the control plots. However, the branch samples revealed calorific value ranging from 3748 to 4902 K cal kg^{-1} in wastewater plot and 3172 to 3892 K cal kg^{-1} in control plot. Highest calorific values were observed in samples of *M. azedarach* under greywater irrigation, followed by *Salix*, *Poplar* and *Eucalyptus*. Low calorific value of *E. hybrid* samples could be probably due to higher ash content of the samples. *P. deltoides* showed calorific value of 4325 and 3460 K cal kg^{-1} in wastewater and control plot respectively (Table 3).

The moisture in branch samples was more than stem samples due to green and tender appearance. However, moisture content of wood is not an intrinsic property and can show variation with climate and time of harvesting[12]. Higher moisture decreases the net calorific value due to more heat requirement of moisture vaporization[13]. Similar results of increase in specific gravity of wood samples irrigated with wastewater have been reported[14,15]. The results show that the increase in specific gravity of samples due to higher nutrients can increase the heating value.

The higher calorific value of wood can be due to increase in its lignin and cellulose contents[16]. Ramaden *et al.*[11] reported 45% higher cellulose and 31% higher lignin content in *M. azedarach* under wastewater irrigation. The calorific value of wood samples was reported to increase with age; thus one can get significant results with wastewater irrigation after 5–6 years[16]. Presently, the Student's *t* test was not found to be statistically significant for calorific value, but is expected to show significant results after 5–6 years of continuous greywater irrigation.

Table 3. Energy value parameters of tree samples in wastewater and control water plots under study

Parameters	Moisture content (%)		Ash content (%)		Specific gravity (g cm^{-3})		Calorific value (K cal kg^{-1})		Nitrogen (%)	
Samples	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
Branch samples										
E. hybrid	13.5 ± 1.2	14.0 ± 1.6	0.94 ± 1.5	1.62 ± 1.9	–	–	3748 ± 2.12	3172 ± 0.89	0.83 ± 0.21	0.28 ± 0.23
P. deltoids	13.0 ± 1.5	12.5 ± 3.2	0.78 ± 1.2	0.80 ± 1.01	–	–	4037 ± 2.13	3172 ± 1.54	0.35 ± 0.05	0.29 ± 0.01
S. alba	12.0 ± 2.2	12.0 ± 2.2	0.71 ± 1.1	0.73 ± 1.5	–	–	3748 ± 1.25	3748 ± 0.67	0.46 ± 0.05	0.35 ± 0.05
M. azedarach	14.0 ± 2.3	14.5 ± 3.2	0.79 ± 1.4	0.84 ± 2.2	–	–	4902 ± 0.55	3892 ± 0.12	0.68 ± 0.04	0.26 ± 0.02
Stem samples										
E. hybrid	10.8 ± 2.5	12.3 ± 1.1	0.44 ± 1.2	0.40 ± 0.29	0.65 ± 1.3	0.55 ± 1.3	4037 ± 0.55	3748 ± 0.44	0.51 ± 0.22	0.22 ± 0.21
P. deltoids	9.50 ± 1.2	11.0 ± 1.0	0.47 ± 1.2	0.48 ± 3.2	0.81 ± 1.1	0.72 ± 1.7	4325 ± 0.54	3460 ± 0.54	0.26 ± 0.25	0.19 ± 0.31
S. alba	13.0 ± 1.5	12.5 ± 1.1	0.49 ± 1.5	0.50 ± 1.8	0.40 ± 1.7	0.32 ± 1.7	4613 ± 0.67	4325 ± 1.55	0.19 ± 0.01	0.15 ± 0.05
M. azedarach	11.5 ± 1.8	13.0 ± 1.6	0.38 ± 1.25	0.42 ± 1.3	0.67 ± 1.1	0.38 ± 1.5	5190 ± 0.89	4469 ± 1.15	0.44 ± 0.05	0.24 ± 0.02

Higher calorific value of wood samples was further correlated with nitrogen content in them. Nitrogen content was found to be the highest, viz. 0.83% and 0.51% in branch and stem wood samples of *E. hybrid* under greywater irrigations; however, the values were 0.28% and 0.22% respectively, in control samples. *M. azedarach* also envisaged higher nitrogen content, i.e. 0.44% and 0.68% in branch and stem wood samples respectively under grey water irrigation and ~0.25% in the respective control samples. The difference in nitrogen content in wood samples of *P. deltoids* and *S. alba* was comparatively less.

Pearson correlation of nitrogen with dry biomass (Figure 2) and nitrogen with calorific value (Figure 3) was found to be strongly positive ($r = 0.6$ and 0.5) in samples under wastewater irrigated plots. The results foresee the probability that high nitrogen uptake by plants can enhance biomass as well as calorific value of wood. However, in control plot samples, all the correlations were found to be either weakly negative or negligible. The results can be strongly interpreted after 5–7 years of continuous greywater irrigation. Similar results of high nutrient accumulation are reported in the literature under wastewater irrigation trials[17,18].

The total CO_2 mitigation potential of trees under wastewater irrigation was ~38, 37, 32 and 15 t ha^{-1} for *P. deltoides*, *M. azedarach*, *E. hybrid* and *S. alba* respectively, whereas the values were 24, 8.7, 13 and 4.4 t ha^{-1} for the same species respectively, in control plot. The highest difference of wastewater irrigation on CO_2 mitigation was noticed in *M. azedarach* followed by *E. hybrid*, *P. deltoides* and *S. alba* (Table 4). The difference in mitigation potential between wastewater and control plots was found to be statistically significant ($P < 0.05$). The high carbon stock and carbon mitigation potential in these plant species is attributed due to higher biomass production. The reason for the higher carbon stock and thus higher mitigation potential has been verified in the literature due to higher biomass accumulation in nutrient-rich environment. It has also been reported that the biomass allocation is found to be higher in above-ground parts compared with below-ground accumulation[19,20]. Carbon storage in the plantation also depends on factors such as age and structure of the system[21,22].

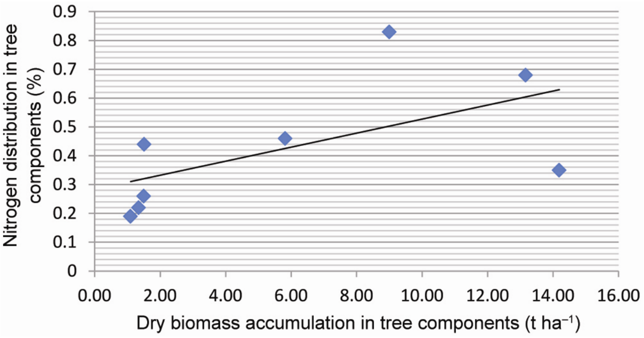

Figure 2. Statistical correlation between nitrogen and dry biomass accumulation of wood samples in the study.

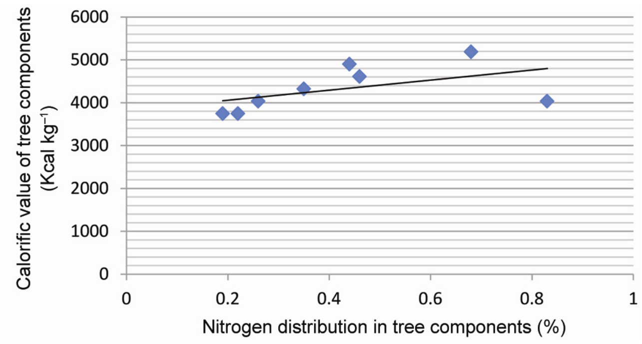

Figure 3. Statistical correlation between nitrogen accumulation and calorific value of wood samples in the study.

Two scenarios, one with the benefits incurred by a hectare plot planted with short-rotation trees under greywater and the other with the respective freshwater irrigation, are compared in the study. The price of freshwater, electricity, etc. is not accounted as they were provided by the GBPUAT for research.

The secondary data shows the price of plant varies from Rs 2100/tree to Rs 3600/tree (average price is Rs 2500/tree) depending on the growth and biomass of tree. The price of a tree under greywater is taken to be Rs 3000 due to higher biomass. However, the cost of carbon mitigation potential is estimated based on the average rate of certified emission reduction (CER), which varies from Rs 800 to 1600 (average Rs 1200) per tonne of carbon reduction under CDM projects. Cost of fertilizers was estimated from the literature[7,23].

It was observed that various site preparation activities, tree plantation and maintenance marked an expenditure of approximately Rs 8.5–9.0 lakhs, which was the same for both plots. However, the benefits incurred varied in the two scenarios due to good growth of trees under greywater irrigation, and thus high biomass and sequestration benefits. The freshwater-irrigated plot is expected to give returns of Rs 54 lakhs (about 90,000 USD; 1 USD = 60 INR) by the end of the experiment after five years; however, the greywater-irrigated plot can give returns of Rs 68 lakhs (about 113,000 USD) after five years (Table 5). The returns can be further enhanced if freshwater pricing and wastewater treatment cost are considered in the trial.

In the present experiment, significant difference was noticed among the trees planted under greywater and freshwater. The harvested biomass obtained after two years was 321% higher in *M. azedarach* and 274% and 143% higher for *Salix* and *Eucalyptus* respectively, under greywater irrigation trial. The calorific value for stem samples was found to be 288–721 K cal kg^{-1} higher in greywater-irrigated samples than the respective freshwater irrigated samples. The carbon mitigation potential was

Table 4. Total carbon mitigation potential (t ha^{-1}) in different tree components of treatment and control plots under study

Tree components	Stem		Branch		Twigs		Root		Foliage	
Species	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
E. hybrid	16.1	6.2	2.3	1.0	1.7	0.7	7.6	2.5	4.2	2.5
P. deltoides	25.0	16.8	2.6	2.2	0.8	0.3	1.2	1.6	8.4	4.1
S. alba	9.2	2.6	1.9	0.4	0.4	0.2	2.1	0.3	1.8	1.0
M. azedarach	23.6	4.7	2.6	0.5	1.6	0.5	6.8	0.9	2.7	2.1

Table 5. Estimated monetary expenditure and returns (in Rupees) after the five-year study period

Expenditure	Year 1	Year 2	Year 3	Year 4	Year 5	Total
Land lease	25,000	25,000	0	0	0	50,000
Site preparation (land ploughing and levelling)	4,000	0	0	0	0	4,000
Electricity charge (Rs 5000/year)*						
Average plant sapling cost (Rs 15/sapling)	37,500	0	0	0	0	37,500
Labour cost for field maintenance (@ 150/day – 2 labourers)	109,500	109,500	109,500	109,500	146,000	584,000
extra labour for channel, bund preparation, plantation (six additional labourers for one month @ Rs 150/day)	27,000	0	0	0	0	27,000
Irrigation check dam	15,000	0	0	0	0	15,000
Shallow bore-well installation	25,000	0	0	0	0	25,000
Miscellaneous cost @ Rs 20,000/year	20,000	20,000	20,000	20,000	20,000	100,000
Investment in wastewater irrigated plot						842,500
Fertilizer cost (approx. Rs 5/plant)*	12,500	12,500	12,500	12,500	12,500	62,500
Freshwater rate (Rs 60/100 litre)*						
Investment in freshwater irrigated plot						905,000
Returns by freshwater irrigated plot						
Harvestable biomass rate approx Rs 2500/tree	0	0	0	0	0	6,250,000
Carbon sequestration by trees (average CER rate is Rs 1200)	0	0	0	0	0	61,200
						6,311,200
Returns by wastewater irrigated plot						
Harvestable biomass rate approx Rs 3000 per tree	0	0	0	0	0	7,500,000
Higher carbon sequestration in wastewater (average CER rate is Rs 1200)	0	0	0	0	0	147,600
						7,647,600
Gross benefit in freshwater irrigated plot						5,406,200
Gross benefit in wastewater irrigated plot						6,805,100

*Parameters whose monetary value is not accounted in the present study.

also noticed to be 10–28 t ha^{-1} higher in trees under wastewater irrigation and thus can provide carbon-neutral source of energy.

The comparative monetary benefits were found to be around Rs 14 lakhs (24,000 USD) higher in greywater-irrigated plot due to higher biomass and carbon sequestration benefit after five years of rotation. The trial offers initial positive results and recommends further research. Such projects can make India energy self-sufficient and to cope with climate change.

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Prevalence and multiple antibiotic resistance of *Vibrio coralliilyticus*, along the southwest coast of India

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Samples from two different estuaries (Cochin and Kumarakom) and a shrimp farm located along the southwest coast of India were analysed for the presence of *Vibrio* species. *V. coralliilyticus*, a global marine pathogen had high prevalence in all the three sources. The incidence of *V. coralliilyticus* was very high in the Cochin estuary (40%) when compared to the shrimp pond (20%) and Kumarakom estuary (19%). The susceptibility of *V. coralliilyticus* strains to 20 different antibiotics and their plasmid profiles were also checked. All the tested strains exhibited multiple antibiotic resistance, showing resistance towards 5–9 antibiotics tested. Resistance was shown towards amoxycillin, ampicillin, carbenicillin, oxytetracycline, trimethoprim, nitrofurantoin, furazolidone, sulphamethoxasole, erythromycin, while all the strains were sensitive to streptomycin, gentamicin, amikacin, netillin, tetracycline, chloramphenicol, cotrimoxasole, nalidixic acid, norfloxacin and ciprofloxacin. Multiple antibiotic resistance index varied from 0.25 to 0.55. Forty-three per cent of the isolates harboured 1–3 plasmids, with size ranging from 0.5 to 33 kb. Thus the present study demonstrates the high incidence, multiple antibiotic resistance and plasmid profiling of *V. coralliilyticus* from the southwest coast of India.

Keywords: Antibiotic resistance, estuaries, plasmid profiling, shrimp pond, *Vibrio coralliilyticus*.

VIBRIO, a Gram-negative halophile, is found naturally in shallow coastal waters to the deepest parts of the ocean. It is highly abundant in aquatic environments, including estuaries, marine coastal waters and sediments, and aquaculture settings worldwide and consists of more than 74 species[1]. Many *Vibrio* species are pathogenic to humans and animals. Hence, their prevalence and distribution in aquatic environments is of utmost public health importance. *Vibrio coralliilyticus* is a global marine pathogen that has been associated with coral disease from geographically distinct global regions. First isolated from diseased and bleaching corals off the coast of Zanzibar[2,3], this species has also been implicated in white syndrome disease outbreaks in the Indo-Pacific[4]. It causes fatal

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