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Research Article

Effect of brewery wastewater on growth and physiological changes in maize, sunflower and sesame crops

K. Senthilraja*, P. Jothimani and G. Rajannan
Department of Environmental Sciences, Tamil Nadu Agricultural University,
Coimbatore, Tamil Nadu - 641 003, India.

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Abstract

Beer is the fifth most consumed beverage in the world. The brewing industry is one of the largest users of water. Even though substantial technological improvements have been made in the past, it has been documented that approximately 3 to 10 litres of wastewater is generated per litre of beer. The crops tested for management of brewery wastewater were maize, sunflower and sesame which had the characteristics of fast growth, high salinity tolerance; potential for producing commercial products such as food grains along with the remediation capability without affecting the food grade. The brewery wastewater was colourless and odourless with neutral in reaction (7.05) having an EC of 1.96 dS m^{-1}. Therefore, the potential of brewery wastewater as an irrigation source for crop production was assessed through pot culture and field experiments. In the pot culture studies, the growth parameters like plant height, stem girth, number of leaves, leaf area, total chlorophyll content, proline, soluble protein and biomass production of maize, sunflower and sesame performed better at higher concentration of brewery wastewater irrigation. The soil enzyme activities showed that increasing the concentration of wastewater lead to increase in enzyme activities of brewery wastewater irrigated soil.

Keywords : Brewery wastewater, impact, crops and enzyme activity.

Introduction

Globally there is a great political and social pressure to reduce the pollution arising from industrial activities. The brewing industry is one such industry that generates relatively large amounts of by-products and wastes such as spent grain, spent hops and yeast. However, as most of these are agricultural products, they can be readily recycled and reused. Thus, compared to other industries, the brewing industry tends to be more environmental friendly (Ishiwaki *et al*., 2000). A brewery is a dedicated building for the making of beer, though beer can be made at home, and has been for much of beer's history. The brewing industry is one of the largest users of water. Even though substantial technological improvements have been made in the past, it has been documented that approximately 3 to 10 litres of wastewater are generated per litre of beer production (Genner, 1988). In India, there are 64 brewing industries situated throughout the country. The common disposal route for brewery wastewater sludge has been through landfill. Due to increasing environmental concerns and regulations, attempts were made to utilize the brewery wastes in an eco-friendly manner. Reuse of wastewater requires careful management to prevent the potential problems such as secondary salinisation. Qadir *et al.* (2003) indicated the reuse through a plant-based system, which reduced the amount of waste-water requiring further management or disposal. The brewery effluent carrying abundance of organic

Corresponding Author : K. Senthilraja, Email : ksentha@gmail.com

carbon and plant nutrients were found to alter the soil chemical properties (Orhue *et al.,* 2005). Mohammad and Khan (1984) observed a significant increase in the available potassium, organic matter, ammonia-nitrogen and phosphorus of the soil, when 100 per cent brewery wastewater was used for irrigation. On the other hand, pH of the soil decreased gradually with increasing concentration of the effluent irrigation. The crops selected for management of brewery wastewater were maize, sunflower and sesame which had the characteristics *viz*., fast growth, high salinity tolerance potential with the remediation capability without affecting the food grade and soil quality. Hence, the present investigation was carried out to study the waste-water utilization pattern for the growth of crop plants based on the pot culture experimental studies.

Materials and Methods

The brewery wastewater samples were collected from the United Breweries Limited, Kanjikode, Palakkad, Kerala and analyzed for their physicochemical and biological properties as per the standard methods (APHA, 1980). In order to assess the efficiency of brewery wastewater for crop growth, a pot culture experiment was carried out using different dilutions of the brewery waste-water. Certified seeds of *Zea mays* (Co H (M) 5), *Helianthus annus* (TCSH 1) and *Sesamum indicum* (TMV (Sv) 7) were surface sterilized in 0.1 per cent $HgCl_2$ for 5 minutes, with continuous shaking followed by repeated washing with sterile water, so that all traces of $HgCl_2$ were removed from the seeds. The maize, sunflower and sesame seeds were sown at a depth of 1 cm in plastic pots (20.5 × 24.0 cm) filled with 2 kg of garden soil. After complete emergence of the seedlings, it was thinned out to 2 plants per pot. Plants were irrigated regularly with the brewery wastewater based on the 60 per cent water holding capacity of the soil (173 ml / irrigation). Growth was observed both in the control and treated plots.

The treatment details are given below,

T_1 : Control

T_2 : 25 percent brewery wastewater

T_3 : 50 percent brewery wastewater

T_4 : 75 percent brewery wastewater

T_5 : Brewery wastewater alone

The observations such as leaf area, stem girth, plant height, leaf number, total chlorophyll, dry matter production (g) and Na^+/K^+ ratio of seedlings were taken. The observation and analysis of the above parameters were carried out as per standard methods. Chlorophyll meter (model 502 of Minolta, Japan) was used to measure SPAD (Soil Plant Analysis Division) values. The most recently matured leaf was measured on the each side of the midrib at the point three - fourths of the way from base to leaf tip in plants for each replication and the mean value was worked out. Ethanol soluble protein content was determined by the procedure described by Lowry *et al*. (1951) and expressed as mg g^{-1} fresh weight. Proline accumulation in the leaf was estimated by the method of Bates *et al*. (1973). The third fully expanded leaf was used for proline estimation. The proline content was expressed in mg g^{-1} fresh weight. The experimental results were statistically scrutinized as suggested by Panse and Sukhatme (1985). The critical difference was worked out at 5 per cent (0.05) probability levels.

Results and Discussion

The treated brewery wastewater is colorless and odorless. This is in line with the findings of Orhue *et al*. (2005). The pH of treated brewery wastewater was neutral (7.05). The electrical conductivity of the effluent was 1.96 dSm^1 (Table - 1). This might be due to the presence of chemical substances like caustic soda, calcium sulphate, potassium meta bisulfate, ortho phosphoric acid, lactic acid and chlorine used during the beer manufacturing process. Similar results were reported by Luc Fillaudeau *et al*. (2006). In general, the vital quality parameters *viz.,* pH, Electrical Conductivity (EC), Total Dissolved

Table – 1 Characteristics of treated brewery industrial wastewater

Parameters	Unit	Values
Physical properties		
Colour	Pt. Co	Colourless
Odour	-	Odourless
Total suspended solids	mg L^{-1}	1.20
Total dissolved solids	mg L^{-1}	1320
Total solids	mg L^{-1}	1321.20
Chemical properties		
pH	-	7.05
Electrical conductivity	dS m^{-1}	1.96
Dissolved oxygen	mg L^{-1}	2.20
Biochemical oxygen demand	mg L^{-1}	32
Chemical oxygen demand	mg L^{-1}	168
Total Nitrogen	mg L^{-1}	52
Total Phosphorus	mg L^{-1}	4
Calcium	mg L^{-1}	75
Magnesium	mg L^{-1}	39
Sodium	mg L^{-1}	212
Total Potassium	mg L^{-1}	28
Chloride	mg L^{-1}	335
Sulphate	mg L^{-1}	48
Biological properties		
Bacteria (CFU mL^{-1})	10^6	10
Fungi (CFU mL^{-1})	10^4	6
Actinomycetes (CFU mL^{-1})	10^3	3
Total coli form count	MPN 100 mL^{-1}	BDL
Bio - assay test (survival of fish after 96 h in 100 per cenfluent)	per cent (%)	94

Solids (TDS), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), chloride (Cl), sulphate (SO_4) and sodium (Na) of treated brewery wastewater were within the permissible limits. It had considerable amounts of essential plant nutrients *viz.*, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg). This is in accordance with Ohio *et al.* (2005) who reported that the brewery wastewater had a BOD of 1000 - 1500 mg L^{-1}, suspended solids of 10 - 60 mg L^{-1}, COD of 800 - 3000 mg L^{-1}, plant nutrients of N and P of 30 - 100 and 10 - 30 mg L^{-1}, respectively and Na content of 212 - 258 mg L^{-1}. The bioassay test indicated the survival rate of fish up to 94 per cent in brewery wastewater. The total coli forms count was nil (Table – 1).

The results of growth attribute, plant height and stem girth (Table – 2), it is evident that the highest plant height (76.8 cm) in maize crop was recorded in the treatment that received the mixture of brewery wastewater and water at 1:3 ratio (T_2). In sunflower, the highest plant height (49.9 cm) was recorded in the treatment T_3 (irrigation with a mixture of brewery wastewater and water at 1:1 ratio) but in the sesame crop the highest plant height (38.1 cm) was recorded in the control (T_1). The statistical analysis of maize and sunflower plant height showed non-significant in all the stages. But the sesame crop showed a significant result at 30 and 45 Days after Sowing (DAS). In both the stages, the control recorded the highest plant height of 16.5 and 38.1 cm, respectively.

Table – 2 Effect of treated brewery wastewater on plant height and stem girth

Treatment	Plant height (cm)									Stem girth (cm)								
	Maize			Sunflower			Sesame			Maize			Sunflower			Sesame		
	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS
T_1	23.5	48.8	71.9	12.3	26.8	49.0	6.4	16.5	38.1	1.5	2.9	3.4	1.0	1.4	1.7	0.2	1.4	1.6
T_2	26.4	56.0	76.8	11.7	27.0	44.4	6.4	16.4	32.6	1.7	3.0	3.2	1.1	1.5	1.9	0.2	1.3	1.4
T_3	22.0	41.6	73.5	12.4	27.3	49.9	6.4	15.2	34.0	1.6	3.0	3.3	1.0	1.6	1.9	0.2	1.3	1.4
T_4	21.4	42.7	69.9	11.6	27.5	49.1	7.1	15.9	30.3	1.8	3.0	3.4	1.1	1.6	2.0	0.2	1.6	1.6
T_5	21.4	40.5	70.8	12.4	27.9	48.2	6.6	13.9	30.7	1.7	2.9	3.4	1.0	1.5	1.9	0.2	1.3	1.4
Mean	**23.0**	**45.9**	**72.6**	**12.1**	**27.3**	**48.1**	**6.6**	**15.6**	**33.1**	**1.7**	**3**	**3.3**	**1.0**	**1.5**	**1.9**	**0.2**	**1.4**	**1.5**
S.ED	**2.4**	**9.89**	**4.69**	**0.75**	**0.92**	**1.99**	**0.50**	**0.63**	**2.17**	**0.09**	**0.08**	**0.13**	**0.07**	**0.05**	**0.12**	**0.01**	**0.10**	**0.12**
CD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**1.32**	**4.53**	**0.18**	**NS**	**NS**	**NS**	**0.11**	**NS**	**NS**	**NS**	**NS**

Table – 3 Effect of treated brewery wastewater on number of leaves and leaf area

Treatment	Number of leaves									Leaf Area (cm^2)								
	Maize			Sunflower			Sesame			Maize			Sunflower			Sesame		
	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS
T_1	4.0	7.4	8.8	4.0	6.8	15.4	4.0	5.6	13.0	30.6	189.5	350.8	10.1	30.0	44.5	0.4	17.8	32.9
T_2	4.0	7.8	8.0	4.0	7.4	15.4	4.0	6.0	12.0	33.8	144.4	272.2	09.3	33.9	47.6	0.8	17.3	20.2
T_3	4.0	7.0	8.8	4.0	7.6	17.0	4.0	6.0	13.2	36.7	122.7	308.1	08.8	33.1	49.5	0.6	15.8	23.4
T_4	4.2	7.6	9.2	4.0	7.0	16.8	4.0	6.0	13.2	29.8	158.9	290.3	11.0	38.1	49.8	0.7	12.5	21.3
T_5	3.6	7.6	8.2	3.8	7.2	16.0	4.0	4.8	14.6	24.7	152.5	263.0	09.7	33.2	43.7	0.9	10.1	21.4
Mean	**4.0**	**7.5**	**8.6**	**3.96**	**7.2**	**16.1**	**4.0**	**5.7**	**13.6**	**31.1**	**153.6**	**296.9**	**09.8**	**33.7**	**47.0**	**0.7**	**14.7**	**23.8**
S.ED	**0.20**	**0.30**	**0.51**	**0.13**	**0.45**	**0.62**	**0.00**	**0.40**	**0.99**	**7.12**	**27.76**	**33.41**	**1.06**	**2.24**	**3.26**	**0.05**	**1.95**	**2.77**
CD (0.05)	**NS**	**NS**	**NS**	**NS**	**NS**	**1.29**	**NS**	**0.83**	**2.66**	**NS**	**NS**	**NS**	**NS**	**4.68**	**NS**	**0.11**	**4.06**	**5.77**

(DAS : Days after Sowing)

Thus the present study revealed that the crop growth with brewery wastewater alone improved the performance of crop up to vegetative stage. Similar observations were reported by Orhue *et al*. (2005). Regarding stem girth, maize, sunflower and sesame crops recorded 3.4 cm (T_1, T_4 and T_5) 2.0 cm (T_4) and 1.6 (T_1 (control) and T_4 (irrigation with a mixture of brewery wastewater and water at 3:1 ratio)) at 45 DAS, respectively.

The application of brewery wastewater had a marked difference in the number of leaves of sesame, but in maize and sunflower it showed non-significant result. The number of leaves recorded in maize (8 - 9 leaves); sunflower (15 - 17 leaves) and sesame (12-15 leaves) were maximum number at 45 DAS (Table – 3). All the treatments registered the maximum number of leaves. The brewery wastewater application had marked effect on leaf area. It varied from 24.7 to 36.7, 122.7 to 189.5 and 263.0 to 350.8 cm^2 in maize, 8.8 to 11.0, 30.0 to 38.1 and 43.7 to 49.8 cm^2 in sunflower and 0.4 to 0.9, 10.1 to 17.8 and 20.2 to 32.9 cm^2 in sesame at 15, 30 and 45 DAS respectively. The leaf area was relatively more at 45 DAS. In maize (350.8 cm^2), sunflower (49.8 cm^2) and sesame (32.9 cm^2), the maximum leaf area was observed in T_1 (control) and T_4 (irrigation with a mixture of brewery wastewater and water at 3:1 ratio). The least leaf area was recorded in maize (263.0 cm^2) and sunflower (43.7 cm^2), which received brewery wastewater alone (T_5).

The highest value of total chlorophyll content (Table – 4) in maize leaves (2.7, 3.8 and 3.1 mg g^{-1}) were recorded in the treatments that received irrigation with a mixture of brewery wastewater and water at 1:3 ratio (T_2) and irrigation with a mixture of brewery wastewater and water at 3:1 ratio (T_4) at 15, 30 and 45 DAS, respectively. But in sunflower, the application of brewery wastewater showed a significant difference in increasing the brewery wastewater application. In all the stages (15, 30 and 45 DAS) the brewery wastewater (T_5) application alone recorded the highest total chlorophyll content (2.2, 3.2 and 3.5 mg g^{-1}), Whereas control recorded the lowest total chlorophyll content in 2.0, 2.4 and 2.4 mg g^{-1} at 15, 30 and 45 DAS. A similar trend was also observed in the sesame crop in all the stages of crop growth. But the lower total chlorophyll content was observed in the treatment T_3 which received irrigation with mixture of brewery wastewater and water at 1:1 ratio (2.0 and 2.8 mg g^{-1}) at 15 and 30 DAS, respectively and, T_1 and T_2 (irrigation with a mixture of brewery wastewater and water at 1:3 ratio) recorded the lowest value of 3.1 mg g^{-1} at 45 DAS. The maize crop showed an increase in total chlorophyll content up to 30 DAS and decreased thereafter. Similar observations on chlorophyll content were reported by Orhue *et al*. (2005).

The different treatments significantly influenced the proline content (Table – 4). The proline content in the brewery wastewater alone (T_5) application was the highest (98.4, 123.1 and 79.3 mg g^{-1}) in maize, sunflower and sesame, respectively. The lowest proline content of 88.3, 100.1 and 72.4 mg g^{-1} was recorded in the control (T_1) in maize, sunflower and sesame crops, respectively at 30 DAS.

The soluble protein content of the maize, sunflower and sesame leaves was significantly influenced by the brewery wastewater irrigation. The highest value of soluble protein content in leaves (5.6, 6.3 and 5.7 mg g^{-1}) was recorded in the treatment which received irrigation with a mixture of brewery wastewater and water at 1:3 ratio (T_2) in maize and sunflower at 30 DAS. In maize and sunflower, the control (T_1 and T_4) recorded the lowest soluble protein content of 4.8 and 5.9 mg g^{-1}, respectively. The dry matter (shoot and root) produced by various crops in the pot experiment ranged from 2.5 to 3.0 and 1.7 to 1.9 g in maize, 2.3 to 2.4 and 0.7 to 0.8 g in sunflower and 1.2 to 1.4 and 0.4 to 0.6 g in sesame (Table – 5).

Table – 4 Effect of treated brewery wastewater on total chlorophyll, proline and soluble protein content

Treatments	Total Chlorophyll content (mg g^{-1})									30 DAS (mg g^{-1})					
	Maize			Sunflower			Sesame			Maize		Sunflower		Sesame	
	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	15 DAS	30 DAS	45 DAS	Proline	Soluble protein	Proline	Soluble protein	Proline	Soluble protein
T_1	2.1	3.7	3.0	2.0	2.4	2.4	2.1	2.9	3.1	88	4.8	100	6	72.4	5.7
T_2	2.7	3.8	2.9	2.0	2.5	2.6	2.2	2.9	3.1	91	5.6	104	6.3	73.9	5.6
T_3	2.4	3.6	2.7	2.1	2.8	3	2	2.8	3.2	97	5.4	118	6.1	75.6	5.5
T_4	2.4	3.5	3.1	2.1	3	3.2	2.3	3	3.2	95	5.3	116	5.9	77	5.2
T_5	2.4	3.5	2.7	2.2	3.2	3.5	2.5	3.1	3.3	98	5.2	123	6	79.3	5
Mean	**2.4**	**3.6**	**2.9**	**2.1**	**2.8**	**2.9**	**2.2**	**3**	**3.2**	**94**	**5.3**	**112**	**6.1**	**75.6**	**5.4**
S.ED	**0.1**	**0.07**	**0.06**	**0**	**0.06**	**0.1**	**0.1**	**0.06**	**0.1**	**1.9**	**0.11**	**2.3**	**0.12**	**1.51**	**0.11**
CD (0.05)	**0.1**	**0.15**	**0.12**	**0.1**	**0.11**	**0.1**	**0.1**	**0.12**	**0.1**	**3.9**	**0.22**	**4.7**	**NS**	**3.16**	**0.23**

DAS : Days after sowing

Table – 5 Effect of treated brewery wastewater on shoot, root biomass and Na^+ / K^+ Ratio

Treatments	45 DAS								
	Shoot biomass (g plant^{-1})			Root biomass (g plant^{-1})			Na^+ / K^+ Ratio		
	Maize	Sunflower	Sesame	Maize	Sunflower	Sesame	Maize	Sunflower	Sesame
T_1	2.7	2.3	1.2	1.9	0.7	0.4	0.9	1.3	1.2
T_2	2.5	2.4	1.4	1.8	0.7	0.5	1.1	1.4	1.3
T_3	2.8	2.3	1.2	1.7	0.8	0.5	1.1	1.8	1.4
T_4	2.9	2.4	1.3	1.9	0.7	0.6	1.2	1.8	1.5
T_5	3.0	2.4	1.2	1.9	0.8	0.6	1.2	1.8	1.6
Mean	**2.8**	**2.3**	**1.3**	**1.8**	**0.7**	**0.5**	**1.1**	**1.6**	**1.4**
SEd	**0.06**	**0.05**	**0.03**	**0.04**	**0.02**	**0.01**	**0.02**	**0.03**	**0.03**
CD (0.05)	**0.11**	**NS**	**0.05**	**0.08**	**0.03**	**0.02**	**0.05**	**0.07**	**0.06**

DAS : Days after sowing

The brewery wastewater application produced significantly more dry matter than the other treatments in all the crops. The higher concentration of brewery wastewater irrigation significantly increases the dry matter production. Such effect was more pronounced in maize.

In irrigation of brewery wastewater to the seedlings, Na^+/K^+ ratios (Table – 5) varied from 0.9 to 1.2 in maize, 1.3 to 1.8 in sunflower and 1.2 to 1.6 in sesame. The increasing concentration of Na from 25 to 100 per cent brewery wastewater gradually increased the Na^+/K^+ ratios of seedlings. Among the treatments, T_1 and T_2 (control and irrigation with a mixture of brewery wastewater and water at 1:3 ratio) registered significantly lower Na^+/K^+ ratios of 0.9 and 1.1, 1.3 and 1.4, 1.2 and 1.3, respectively in maize, sunflower and sesame. It is accepted that competition exists between Na^+ and K^+ that leads to reduce the level of internal K^+ at a higher external NaCl concentration (Botella *et al*., 1997). Na^+ and K^+ contents of maize and sunflower seedlings increased with the increase in irrigation of brewery wastewater concentration. Gaxiola *et al*. (2001) also reported that Na^+ and K^+ contents were markedly increased in transgenic lines of *Arabidopsis thaliana* than the wild type plants at 100 - 300 mM NaCl stress.

Conclusion

Field crops take up large amounts of water and minerals, serving as effective biological filters that reduce accumulation of minerals in soil and groundwater. An experiment was conducted to assess the efficiency of the brewery wastewater for crop growth. The growth parameters like plant height, stem girth, number of leaves, leaf area, total chlorophyll content, proline, soluble protein and biomass production of maize, sunflower and sesame performed better by increasing the concentration of brewery wastewater irrigation. The enzyme activities showed that increasing the concentration of wastewater lead to increase in plant enzyme activities. The relative increase in biomass was also recorded in the treatments that received brewery wastewater irrigation. The ions N and K uptake by the plant was more in the brewery wastewater irrigation.

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