Impact of Sugar Mill Effluent on Growth and Biochemical Characters of Acacia ferruginea DC.

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ABSTRACT

The present study deals with the effect of sugar mill effluent on seedling growth of Acacia ferruginea DC. The sugar mill effluent was collected near Sakthi Sugars and Chemicals Limited, Padamathur, Sivaganga district, Tamil Nadu. The effect on various growth and biochemical parameters have been estimated at different concentrations of sugar mill effluent (10%, 20%, 30%, 40% and 50%). The results showed that the application of different concentrations of sugar mill effluent on the 45th day treatment resulted in a significant decrease in growth characteristics of Acacia ferruginea DC compared with the control. The physico-chemical parameters of sugar mill effluent indicated that some parameters, such as pH, EC, acidity, TDS, TS, BOD, COD, sulphate, magnesium, nitrogen, zinc, iron, copper, lead, manganese and oil and grease exceeded the permissible limit compared to Tamil Nadu Pollution Control Board (TNPCB) and then germination and growth parameters increased in lower (10%) concentration of sugar mill effluent and this morphological parameters gradually decreased with increasing effluent concentration. But the sugar mill effluent affected at 45th day the plants of Acacia ferruginea DC seedling growth of shoot length, root length, fresh weight, dry weight, vigor index and biochemical contents viz., pigment content carbohydrates, and protein were significantly decreased compared with control. The sugar mill effluent leads to decrease the various growth parameters such as root and shoot of the seedlings. The reduction in all growth characteristics and biomass accumulation was parallel to decrease in carbohydrate, protein and nucleic acid, peroxidase, catalase and leaf nitrate activity of the leaf and an increase in the anthocyanin and L-proline. Finally, it was observed that the effluent can be used for irrigation purposes after appropriate dilution with ground water.

Keywords: Sugar Mill Effluent, Acacia ferruginea DC, Growth and Biochemical Parameters

1. INTRODUCTION

Environmental pollution poses a multifaceted threat with local, regional, and global impacts, significantly endangering the health of humans, animals and plants (Irshad, et al., (1997). Water, renowned for its universal solvent properties, is extensively utilized across various industrial applications. Nonetheless, the ongoing evolution of human activities, particularly with industrialization, has resulted in the generation of significant volumes of wastewater laden with toxic substances, notably heavy metals. The proliferation of these metals within water ecosystems is ascribed to diverse processes,

including industrial activities such as metal plating, electroplating, mining and battery manufacturing, tannery industry and sugar industry operations. Among the factories polluting the soil and groundwater, sugar mills certainly have a larger share in form of their discharges of the large account of wastewater as effluent. Increasing use of chemical fertilizers and pesticides in raising the sugar production has also resulted in the degradation of soil and groundwater quality. Fifteen groundwater samples were analysed for various parameters like pH, EC, TDS, Na, K, Ca^{2+}, Mg^{2+}, Cl, HCO_3, SO_4^{2-}, PO_4^{3-}, NO_3 and total hardness during the period of operation of sugar mill. The results revealed that there was significant variation in some parameters. The high values of Ca, Na, chloride, sulphates, nitrates and hardness in the samples which are located downstream suggest that sugar mill effluent is the source of soil and groundwater pollution. During this study, it was found that the samples flowing to the downward land direction located in the close proximity of stream of effluent are not suitable for either drinking or irrigation purposes. The investigation suggests that water quality management, an important issue for the sustenance of human civilization must become a major priority (Deshmukh, 2014).

Acacia ferruginea DC is one of important sources of medicinal active ingredients and widely used in Ayurveda system of medicine, in recent years, ethnobotanical and traditional uses of natural substances from plant origin have more attention and generally believed to be safe for human use. This plant has been reported for anti-inflammatory, analgesic, anti-diabetic, antibacterial, anti-fungal, anti–haemorrhoidal, antiulcer, hepatoprotective, wound healing and anti-protozoal activities. The data recorded can be used as a source for discovering modern medicine, due to its valuable medicinal characters, there is enormous scope for future research on Acacia ferruginea. DC and further clinical or pharmacological investigation should be carried out to investigate unexploited properties of this plant. Taking this view into amount, the present study aimed at conducting a detailed investigation of the impact of sugar mill effluent on the growth and biochemical characters of Acacia ferruginea DC and ground water quality in the vicinity of sugar mills in Padamathur.

2. MATERIALS AND METHODS

Collection of Sugar Mill Effluent

The wastewater used in this treatment process was collected from Sakthi Sugars and Chemical Limited, Padamathur, Sivagangai, Sivagangi District. The physico- chemical qualities of the effluent were examined based on the standard procedures (APHA, 2024)

Sample Preservation

The collected samples were preserved as per the standard preservation technique (APHA, 2024). The effluent samples were always kept in a suitable container in a refrigerator at $15 - 20^0$C. The effluent samples were taken out from the refrigerator only at the time of analyses.

Measurement of Physico-chemical parameters of Sugar Mill Effluent

The methods of chemical analyses and the measurement procedure for all Water Quality parameters were similar to the standards recommended by Bureau of Indian Standards (BIS). The Physicochemical parameters such as temperature, pH, electrical conductivity, total solids, total dissolved solids, total suspended solids, total hardness, alkalinity, sodium, calcium, magnesium, sulphate, carbonate, bicarbonate, phosphate, chloride, nitrite(NO2), nitrate (NO3), dissolved oxygen, biological oxygen demand, chemical oxygen demand were determined and Water Quality Index (WQI) were calculated.

Ground water was used as Control.

Water Quality Index (WQI)

The WQI is a single number that expresses a certain level of water quality by aggregating the measurements of water quality parameters (Lumb et al., 2011). However, the WQI is bound to depend on the indented use of the water. The standard values for drinking water recommended by Bureau of Indian Standard (BIS) for 10 parameters were chosen for the analysis along with the assigned weights (Punmia, 1977) are shown in the Table 1.

Table 1: Standards for Drinking Water (Trivedi, 1988)

Sl. No.	Parameters*	Standard (Si)	Weight (Wi)	Unit Weight (Uwi)
1	pH	7 to 8.5	1	0.04
2	Electrical conductivity	400	2	0.09
3	Total hardness	300	1	0.04
4	Total dissolved solids	500	2	0.09
5	Calcium	75	3	0.13
6	Magnesium	30	1	0.04
7	Chloride	250	4	0.18
8	Sulphate	150	3	0.13
9	Sodium	20	4	0.18
10	Potassium	20	2	0.09
			Total	0.99~1.00

*All the values are expressed in mg/L except pH and Electrical conductivity.

WQI calculation was carried out as per the Harton (1965) as modified by Tiwari and Mishra (1985). The Weights (wi) were assigned to various water parameters as indicated in Table 1, which ranged from 1-4. According to the role of various parameters on the overall quality of water, the rating scales were fixed. For example, Sodium, Chloride and Sulphate were the important parameters in the effluent and hence 4, 4 and 3 were assigned respectively. The weights for other parameters were assigned according to their importance and incidence in drinking water. Even if they were present, they might not be ruling factor. Hence they were assigned low weights. The weight (wi) for the ith parameter (i = 1, 2,…….10 in our case) was calculated from the following relation.

$$W_i = \frac{w}{10} \quad \Sigma w_i = 1 \quad \text{.............. (1)}$$

This ensure that

$$10 \, w_i = 1, \quad i = 1 \quad \text{……………… (2)}$$

The unit weights calculated from the relation shown are indicated in the Table 1. The rating scales for the 10-water quality parameters considered here are given in the following Table 2. Each

parameter has been divided into 5 intervals according to the ranges. The quality index (qi) corresponding to various value ranges, in descriptive terms, are also given in the following table.

Qi - 100 – Ideal Limit (BIS)

0 - Severe value (BIS).

Other ratings, namely qi - 25, 50 and 75 are intermediate scales between Ideal and Sever values of BIS for drinking water.

The WQI is the aggregate of the multiplication of qi and wi of the ith parameters.

$$\text{i.e., WQI} = \sum_{i=1}^{10} p_i w_i$$

Based on WQI value, the water quality status is assigned, i.e., if WQI is >100, the parameters are in Ideal limit as shown in the Table 2.

Table 2: Range of Values for the Extent of Pollution

Sl. NO.	Parameters*	EXTENT OF POLLUTION				
		Ideal	Slight	Moderate	Extreme	Severe
1	pH	7 - 8.5	8.6 - 8.7	8.8 - 8.9	9.0 - 9.2	> 9.2
2	EC	0 - 700	701- 900	901 - 1200	1201 - 1500	> 1500
3	Total hardness	50 - 100	101 – 150	151 - 200	201 - 250	> 250
4	TDS	0 - 700	701 – 800	801 - 900	901 - 1000	> 1000
5	Calcium	6 - 12	13 – 20	21 - 26	27 – 32	> 32
6	Magnesium	5 - 10	11 – 15	16 - 18	19 – 25	> 25
7	Chloride	35 - 70	71 – 100	101 - 150	151 - 250	> 250
8	Sulphate	10 - 15	16 – 20	21 - 25	26 – 30	> 30
9	Sodium	25 - 50	51 – 75	76 - 100	101 - 125	> 125
10	Potassium	5 – 8	9 – 10	11 - 12	13 – 18	> 18
	Rating (qi)	>101	76 – 100	51 – 75	26 – 50	0 - 25

*All the values are expressed in mg/L except pH and EC.

Seed Source

Healthy and viable seeds of Acacia ferruginea DC. were procured from Oddukam Seed Centre, Nallampatty, Dindigul, Tamil Nadu and regular treatment methods were followed.

Seedling Treatment

Healthy seeds of Acacia ferruginea DC. were surface sterilized with 0.1% mercuric chloride for one minute and washed with running tap water, followed by distilled water.

Various concentrations of the sugar mill effluent, such as 10%, 20%, 30%, 40% and 50% were prepared. The seeds used for Control were soaked overnight in ground water and seeds of experimental

plants were soaked overnight in their respective concentration of the effluent. Both the control and experimental seeds were allowed to grow in pots containing uniformly mixed red, black and sandy soil in 1:1:1 ratio. All pots were kept in diffused sunlight at room temperature.

The experimental pots were watered everyday with their respective concentrations of effluent with 200mL. The control pots were irrigated with the ground water. Both experimental pots and control pots were maintained in triplicates. On the 21st day, the seedlings were carefully plucked out without any damage and analyzed for the growth parameters like Germination percentage (Carley and Watson, 1968), Root length (Burris et al., 1969), Shoot length (Arts and Marks, 1971), Fresh weight (Burris et al., 1969), Dry weight (Burris et al., 1969) and Vigour index (Abdul Baki and Anderson, 1973). Further the pigments content such as Chlorophyll-a, Chlorophyll-b, Total Chlorophyll and Caroteniods (Arnon, 1949), and the biochemical parameters like total sugar by Jeyaraman, (1981), total protein by Lowry et al., (1951),   L- proline by Bates et al., (1973), free aminoacids by Moore and Stain, (1948), leaf nitrate by Cataldo et al., (1978), nitrate reductase activity by Jaworski, (1971), peroxidase and catalase by Addy and Goodman (1978) were quantitatively estimated.

Statistical Analysis

The morphometric parameters were determined with randomly selected 10 independent seedlings and pigments, biochemical and enzyme assays were carried out with randomly selected 5 independent seedlings from both control and experimental pots. The collected data were subjected to statistical analysis and reported as Mean ± SE. (Zar, 1984).

3. RESULTS AND DISCUSSION

Physico-chemical parameters of sugar mill effluent are given in Table 3. The evaluation of sugar mill effluent confirmed that it is acidic in nature and dull white in colour with decaying molasses smell. Colour place a vital role in an aquatic ecosystem and it affects photosynthesis. Colouration reduce some other parameters such as temperature, DO and BOD, etc., and it also reduce the decomposition of substances by microbes (Ingaramo et al., 2009; Buvaneswari et al., 2013; Saurabh and Shailja, 2014). Decomposition of organic matter under anaerobic condition produced various sulphides, particularly ferrous sulphide which is cause for the colour of the effluent. This is the most common gas and it simply soluble in water, colourless and inflammable, however, highly toxic. Some other gases, such as carbon dioxide, nitrogen, etc., from decomposition of organic compounds are responsible for odour of the effluent. This is in conformity with the earlier finding of Vijayaragavan et al. (2011) and Rathore et al. (2000).

In the present investigation, the pH value of the sugar mill effluent was 6.2, which is acidic in nature. It is a very important factor in an ecosystem that serves as an index for pollution. It is an indicator for the sustainability for the aquatic organisms. Various ions are present in the effluent is directly related with pH of the effluent. The reaction among effluent flowing from open drainage system with the soil has direct relevance to pH of the effluent. The pH was acidic in nature because of the use of phosphoric acid and sulphuric acid during the clarification of sugar juice (Ayyasamy 2008).

TABLE 3: PHYSICO-CHEMICAL PARAMETERS OF SUGAR MILL EFFLUENT

Sl. NO.	PARAMETERS	VALUES *
1	pH	6.2
2	Colour	Dark brown
3	Temperature	30°C
4	Electrical Conductivity	13,275
5	Total Solids	12.423
6	Total Dissolved Solids	12,001
7	Total Suspended Solids	422
8	Total Hardness	35.2
9	Alkalinity	2.1
10	Sodium	750
11	Potassium	18
12	Calcium	52
13	Magnesium	4.6
14	Sulphate	5.6
15	Chloride	2247
16	Carbonate	23
17	Nitrogen	10
18	Phosphate	15.2
19	Salinity	23
20	Sodium Absorption ratio	24.67
21	Soluble sodium percentage	32.26
22	Dissolved O_2	2.6
23	Dissolved CO_2	32.24
24	BOD	57.34
25	COD	422.23
26	Cadmium	0.03
27	Lead	0.04
28	Copper	0.05
29	Nickel	0.09
30	Zinc	0.01
31	Manganese	0.07
32	Water Quality Index	30

*All the values are expressed in mg/L, except Temperature, pH and EC (micromhos/cm)

The sugar mill effluent contains high level of EC (13.275 mM/cm) which has a harmful effect on living organisms in the ecosystem. The temperature plays a major role in aquatic environment which is very high in sugar mill effluent (30^0 C) that has a lethal effect on the diversity of the aquatic environment. Normally, the organisms present in aquatic conditions rapidly grow at a temperature in the range of 20 – 27ºC (Ezhilvannan et al., 2011). The increased temperature may accelerate the rate of chemical reaction and chemical changes in the aquatic condition (Shiva Kumar and Srikantaswamy 2015). In agriculture, irrigation has agreeable limits of temperature of 25ºC. So, this sugar mill effluent has suitable temperature for irrigation. High suspended particles present in the water bodies affect the light intensity of aquatic environment and impact the turbidity and transparency of water bodies. The TDS and TS of sugar mill effluent were 12,001 and 422.0 mg/ l respectively. The findings were also in accordance to Borole and Patil (2004), Vinod and Chopra (2014a, b). Dissolved solids in the form of colloidal substances are also present in the effluent. Dissolved and non-dissolved substances called as total solids and it composed of carbonates, bicarbonates, chlorides, sulphates, nitrates, Ca, Mg, Mn, organic matter, silts and other particles which cause pollution of water bodies. It affects the intensity of light and living organisms (Poddar and Sahu 2015).

BOD is an important parameter that indicates the magnitude of water pollution, by the oxidizable organic matter and the oxygen used to oxidize inorganic material such sulphides and ferrous ions. Present investigation showed that the effluent has high value of BOD (57.34 mg/l) and COD (422.23 mg/l). The chemical kinetic factors like temperature, pressure can affect the BOD reaction. BOD indicates of water pollution caused by oxidation of organic substances. It is one of the valuable parameters of the water quality. It is clear from the data that COD of effluent exceed the TNPCB limit. The high COD value is because of the presence of excessive amount of organic wastes. Saranraj and Stella (2012) analysed various parameters of sugar mill effluent and they have recorded the high COD value.

The COD test determines the required amount of oxygen for oxidation of organic substances by chemical oxidant. The strong oxidizing agents should oxidized completely all organic substances except some other substances of effluent in acidic condition. The BOD and COD tests are used in indication of toxic conditions and the presence of biologically resistance substances in the sugar mill effluent (Malik et al. 2014; Poddar and Sahu 2015). High amount of magnesium, sulphate, chloride, nitrogen, oil and grease (g/l) and the toxic heavy metals such as zinc , iron, copper, lead and manganese were recorded in the collected sugar mill effluent sample. Besides the metals, oil and grease were used in sugar mill for various processes. It influenced the temperature, pH, BOD, COD, DS and TS of the effluent. It affected all living organisms of aquatic and terrestrial ecosystems. Similar findings were recorded and reported by Rathore et al., (2000), Lakshmi and Sundaramoorthy (2000), Borole and Patil (2004). Effluents from different industrial sources possess distinct chemical compositions, leading to disparate effects on plants. For instance, textile industry wastewater is reported to have variable pH, high electrical conductivity (EC), significant levels of cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) and anions (HCO_3^-, CO_3^{2-}, Cl^-), and often heavy metals like Zn, Cu, Cr, Pb, Mn, Ni, and Fe. Sugar mill effluents are typically characterized by high biological and chemical oxygen demand (BOD and COD) Joshi and Kumar, (2011), Matkar and Gangotri (2002), which can deplete aquatic oxygen and reduce biodiversity.

Elevated EC, indicative of high dissolved ion concentrations, is a common feature of sewage and industrial wastewater (Singh et al., 2019). The carbon content and the balance of organic and inorganic constituents also vary with the source and treatment level. This variability means that while wastewater can be a valuable source of irrigation and nutrients for farmers' fields, its specific composition must be the primary determinant of its use.

Water Quality Index (WQI)

The WQI of the sugar mill effluent was calculated and it is shown in Table 4. Out of the 10 parameters prescribed for drinking water by BIS, only 4 parameters i.e., EC, TDS, Chloride and Sodium were within the permissible limit. The cumulative WQI is 54, which showed that the pollution level of the sugar mill effluent was "Moderate" in the rating scale.

TABLE 4: WATER QUALITY INDEX OF SUGAR MILL EFFLUENT

Sl. No.	Parameters*	Values (BIS)	Values of Sugar Mill Effluent	Rating (pi)	Unit Weight (wi)	Product (pi X wi)
1	pH	7 to 8.5	6.2	0	0.00	0
2	Electrical Conductivity	400	13,275	100	0.09	09
3	Total Hardness	300	35.2	0	0.00	0
4	Total Dissolved Solids	500	12,001	100	0.09	09
5	Calcium	75	52	0	0.00	0
6	Magnesium	30	4.6	0	0.00	0
7	Chloride	250	2247	100	0.18	18
8	Sulphate	150	5.6	0	0.00	0
9	Sodium	20	750	100	0.18	18
10.	Potassium	20	18	0	0.00	0
				Total Water Quality Index		54

*All the values are expressed in mg/L except pH and Electrical Conductivity (micromhos/cm)

RESULTS OF IMPACT OF EFFLUENT ON MORPHOMETRIC PARAMETERS

In order to find out the impact of sugar mill effluent a study was undertaken to observe the effect of various concentrations on the growth characteristics of Acacia ferruginea DC Table 5. Seed germination and seedling growth are vital for continuation of plant life and they are extremely vulnerable to environmental stress. Since germination is the first physiological process, several growth parameters such as percentage of germination and ultimately growth and yield of the crops are taken as criteria to assess the degree of pollution (Mishra and Pandey 2002).

TABLE 5: IMPACT OF SUGAR MILL EFFLUENT ON GROWTH CHARACTERS OF ACACIA FERRUGINEA. DC

Sl. No.	Parameters*	Control	Concentration of sugar mill effluent				
			10%	**20%**	**30%**	**40%**	**50%**
1	Germination (%)	97.0±0.03	87±0.05	75.0±0.05	69.2±0.06	59.2±0.07	41.5±0.03
2	Shoot length (cm)	20.6±0.89	21.7±0.08	19.8±0.07	14.6±0.06	13.2±0.08	5.91±0.067
3	Root length (cm)	9.12±0.02	9.21±0.04	8.76±0.03	5.67±0.01	3.2±0.06	1.50±0.09
4	Fresh weight (g)	7.45±0.01	8.02±0.05	6.1±0.056	5.23±0.05	4.1±0.02	2.61±0.023
5	Dry weight(g)	0.81±0.04	0.99±0.076	0.53±0.05	0.32±0.04	0.22±0.06	0.10±0.02
6	Vigour Index (%)	814.0±0.3	746.04±0.9	676.8±0.3	406.9±0.1	202.6±0.1	47.4±0.078

*All the values are averages of five observations. Mean ± Standard error

The increase in germination percentage over control at lower concentration (10%) indicates the stimulation of physiologically inactive seeds by the effluent treatment as reported by Vinod and Chopra (2014a), Vaithiyanathan et al., (2014) and Suresh et al., (2014). The favourable amount of nutrients may be presented in lower concentration of sugar mill effluent. It created good environmental condition for seed germination. The lower concentration of effluent had many nutrients such as nitrogen, phosphorous, etc. which might have promoted the plant growth as suggested by Augusthy and Annsherin (2001). At the same time, the higher concentrations of sugar mill effluent inhibited the germination of Acacia ferruginea DC. Large amount of organic and inorganic substances present in higher concentration of sugar mill effluent adversely affect the seed germination because of the higher salt content which caused change of osmotic pressure outside of the seed. It decreased water absorption of the seed and then inhibited the seed germination (Adriano et al., 1973).

The shoot length, root length, fresh weight and dry weight were decreased in lower concentration (10%) of sugar mill effluent when compared to control and the lowest shoot length, root length, fresh weight and dry weight were observed in higher concentration (50%) of sugar mill effluent. The highest decrease of vigour index of seedlings were observed in 10% of effluent treated seedling and lowest vigour index were observed in 50% of effluent. Seedling growth and development are essential processes of life and propagation of plant species. They continuously depend on the external environment. Presence of various pollutants in lower concentrations of sugar mill effluent (at 10%) increased the growth and development. These observations are in conformity with Lakshmi and Sundaramoorthy (2000), Saxena and Madan (2012), Ali et al., (2012) Malik et al., (2014). Higher concentrations of sugar mill effluent inhibited the root and shoot length of seedlings. It contained large amount of DS and SS which interfered and decreased the absorption of some other nutrients. The interference of heavy metals decreased the root and shoot length of the plant might be due to the effect of physiological processes of plant and it also involved in inhibition of enzyme activities, affected the nutrition, water imbalance and alternation of hormonal status changed the membrane permeability (Sharma and Dubey 2005). The fresh and dry weight of seedlings increased at 10% of sugar mill effluent concentration while decreased at higher concentration of sugar mill effluent. Some amount of macro

elements and trace elements may be needed for seeds. These elements also present in the effluent and are important for plant growth.

The lower concentration of sugar mill effluent contained required amount of nutrients which helped the growth of seedlings as well as fresh and dry weight of the seedlings. The required amount of various chemicals present in the lower concentration of sugar mill effluent promoted the plant growth (Lakshmi and Sundaramoorthy 2000; Siva Santhi and Suja Pandian 2012). The higher concentrations of sugar mill effluent minimized the fresh weight and dry weight of seedlings. The decrease of seedlings weight may be due to the poor growth of seedlings under the higher concentrations of effluent irrigation. The presence of various chemicals in wastewater often detrimentally affect early plant development. Research demonstrates that the impact varies significantly both by effluent type and crop species. A linear decrease in germination percentage has been noted in vegetables like tomato, chilli, and onion when irrigated with effluent from petroleum refineries, paper mills, ordinance factories, and distilleries. Gulfraz et al., (2003) evaluated the suitability of different industrial effluents for wheat irrigation, finding seed germination was most severely affected by textile mill wastewater, followed by soap and detergent, oil refinery, hydrogenated oil, and rubber industry effluents. This study concluded that untreated wastewaters should not be discharged into agricultural systems and advocated for industrial treatment plants. Similarly, Karunyal et al., (1994) reported a decline in germination, chlorophyll, and protein content in Oryza sativa with increasing concentrations of tannery effluent. Conversely, studies also show the potential benefits of properly managed effluent. Kalaiselvi et al., (2009) found that distillery spent-wash at concentrations up to 10% improved seed germination and seedling growth in maize, suggesting its safe use as a fertilizer substitute after appropriate dilution. Singh et al., (2006) observed that fertilizer factory effluent at 25% concentration could increase root/shoot length and chlorophyll content in gram (Cicer arietinum) after 21 days, though higher concentrations were toxic. These studies collectively affirm that the effects on germination and early growth are critically dependent on effluent source, concentration, and crop tolerance.

RESULTS OF IMPACT OF EFFLUENT ON BIOCHEMICAL PARAMETERS

Effect of various concentrations of sugar mill effluent on biochemical parameters of Acacia ferruginea DC is revealed in the Table 6. Among the various dilution factors, 10% dilution showed good amount of Chlorophyll-a, Chlorophyll-b, Total Chlorophyll and Carotenoid contents than other dilutions. A similar trend was reported in Phaseolus aureus .An increase in chlorophyll a, b and total chlorophyll content at lower concentration of distillery effluent might be due to presence of optimum conditions of nutrient element such as Mg and K required for pigment biosynthesis. Reduction in the pigments content was observed when the concentration of the effluent was increased from 20% up to 50% (v/v). Reduction in chlorophyll induced by higher concentration of effluent may be associated with mineral ions. It may also due to the formation of enzymes chlorophyllase which is responsible for chlorophyll degradation (Lakshmi and Sundaramoorthy, 2000). The inhibition of chlorophyll synthesis may be due to the induced inhibition of electron transport system in Photosystem (PS) II. Increasing concentration of wastewater is inhibitory to synthesis of chlorophyll molecules. A decrease in carotenoid content at higher concentration of textile effluent was reported (Baskaran et al., 2009).

TABLE 6: IMPACT OF SUGAR MILL EFFLUENT ON BIOCHEMICAL CONTENT OF ACACIA FERRUGINEA. DC

Sl. No.	Parameters*	Control	Concentration of Sugar Mill Effluent				
			10%	20%	30%	40%	50%
1	**Chl-a** (mg/g LFW)	1.86±0.05	1.80±0.045	1.72±0.04	1.65±0.021	1.45±0.45	1.23±0.34
2	**Chl-b** (mg/g LFW)	0.965±0.7	0.92±0.052	0.85±0.056	0.79±0.023	0.54±0.023	0.32±0.097
3	**Total Chl.** (mg/g LFW)	2.82±0.56	2.73±0.045	2.57±0.045	2.44±0.089	1.97±0.32	1.56±0.045
4	**Carotenoids** (mg/g LFW)	0.453±0.34	0.42±0.34	0.38±0.045	0.30±0.67	0.25±0.23	0.12±0.78
5	**Anthocyanin** (mg/g LFW)	0.92±0.89	1.26±0.56	2.23±0.56	3.25±0.78	3.90±0.45	4.25±0.45
6	**Total sugar** (mg/g LFW)	52.2±0.5	50.45±0.55	48.2±0.34	45.2±0.43	40.0±0.53	32.5±0.45
7.	**Total Protein** (mg/g LFW)	65.4±0.04	62.2±0.45	58.2±0.90	53.2±0.45	48.78±0.34	42.2±0.45
8	**L- Proline** (μmole/g LFW)	10.5±0.3	12.6±0.45	14.5±0.45	18.5±0.87	20.5±0.45	24.3±0.45
9	**Free Aminoacids** (mg/g LFW)	24.5±0.3	28.5±0.67	30.5±0.34	32.5±0.45	35.5±0.34	38.5±0.56
10	**Nitrate reductase** (μmole/g LFW)	20.4±0.04	18.4±0.45	15.4±0.45	12.4±0.09	09.26±0.05	07.23±0.06
11	**Peroxidase** (μmole/g LFW)	4.5±0.06	4.0±0.045	3.85±0.056	3.2±0.067	3.0±0.045	2.5±0.067
12	**Catalase** (μmole/g LFW)	1.32±0.02	1.02±0.034	0.925±0.05	0.86±0.045	0.76±0.45	0.45±0.56
13	**Leaf nitrate** (mg/g LFW)	23.4±0.04	20.4±0.67	18.4±0.06	15.4±0.56	12.4±0.05	09.4±0.56
14	**DNA** (mg/g LFW)	2.4±0.04	2.1±0.045	2.01±0.05	1.98±0.067	1.54±0.078	1.23±0.045
15	**RNA** (mg/g LFW)	1.5±0.04	1.23±0.45	1.04±0.56	0.94±0.45	0.84±0.012	0.76±0.67

*All the values are averages of five observations. Mean ± Standard error

The percentage of reduction was observed in various biochemical characteristics of plant species. The effect of sugar mill effluent revealed significant reduction of pigments viz., Chlorophyll- a, chlorophyll – b, total chlorophyll and carotenoids content when compared to those of control plants. The soluble protein content was found to decrease with sugar mill effluent treated plant species. The reduction observed in the leaf protein level directly to the photosynthetic product, namely sugar. The

total sugar content was found to be reduced in all concentrations of the effluent. The reduction in protein content also correlated to increase in the accumulation of free amino acids. The liberated aminoacid L-proline accumulated more in polluted area plants. The in vivo nitrate reductase (NR) activity was found decreased. The decrease NR activity can be correlated to an increase in leaf nitrate content. Reduction in protein synthesis and altered NR activity observed in the present study may also related to decline in enzymatic activity. The activity of enzyme such as peroxidase and catalase was found to decrease in sugar mill effluent treated Acacia ferruginea DC

The present study showed that sugar mill effluent had an adverse effect on growth and biochemical content of Acacia ferruginea DC at higher concentration (50%). But in lower concentration (10%) when compared to control, there was an enhancement in its growth parameters which may be due to the presence of optimum level of nutrients in the effluent. Photosynthetic pigments are highly sensitive to pollution stress. Under effluent-induced stress, chlorophyll can undergo photochemical reactions like oxidation, reduction, and pheophytinization, altering plant physiology and biochemistry (Puckett, 2003). Studies consistently show pigment reduction under effluent stress. Akujobi et al., (2011) reported decreased chlorophyll and protein in eggplants due to diesel oil pollution. Nath et al., (2009) observed significant reductions in chlorophyll, pheophytin, and carotenoids in Phaseolus mungo with tannery effluent and Cr^2. Bamniya et al., (2010) found similar decreases in chlorophylls and carotenoids in Brassica oleracea and Spinacia oleracea irrigated with mixed industrial-municipal wastewater. Kakar et al., (2010) provided a detailed physiological analysis, showing that municipal wastewater reduced stomatal conductance, transpiration rate, and photosynthetic rate by up to 69% in canola. Chlorophyll-a and chlorophyll-b were reduced by 68 - 86%, with the higher-concentration effluent (100%) causing the most severe damage. This direct impairment of the photosynthetic apparatus is a primary mechanism behind the observed growth reductions. Lakshmi and Sundaramoorthy (2000)

4. Conclusion

This comprehensive review confirms that industrial wastewater reuse in agriculture presents a complex trade-off between significant benefits and serious risks. The body of research indicates that with proper dilution and pre-treatment, certain effluents can be a valuable source of water and nutrients, enhancing crop growth and yield. Conversely, untreated or concentrated wastewater leads to toxic effects including suppressed germination, reduced growth and photosynthetic efficiency, posing a direct threat to ecosystem and human health.

Therefore, a universal endorsement of wastewater irrigation is not feasible. Its suitability is entirely context-specific, depending on the effluent's chemical profile, the degree of dilution or treatment, the crop species, and soil characteristics. A thorough economic and environmental impact analysis is prerequisite for any large-scale implementation. Future research should prioritize: 1) Long-term field studies on soil-pollutant-plant transfer dynamics; 2) The development of cost-effective, industry-specific tertiary treatment technologies; and 3) The breeding and selection of crop varieties with greater tolerance to common wastewater pollutants. Ultimately, rigorous, science-based guidelines and continuous monitoring are essential to harness the potential of this resource while safeguarding agricultural sustainability and public health.

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