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Impact of sugar factory effluent on growth and biochemical characteristics of terrestrial and aquatic plants

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Abstract : The physico-chemical characteristics of the sugar industry effluent were measured and some of them found that above the permissible limits of Indian irrigation water standard. Pot study was first investigated to study the effect of different concentrations (20, 40, 60, 80 and 100%) of sugar factory effluent on seed germination, seedling growth and biochemical characteristics of green gram and maize. Similar study was also carried out using the aquatic plants water hyacinth and water lettuce. Higher concentration (above 60%) of the effluent affected the plant growth. Diluted effluent (up to 60%) favored seedling growth.

Key words : Sugar factory effluent, green gram, maize, water hyacinth, water lettuce.

The sugar industry is playing an important role in the economic development of the Indian sub continent, but effluents released by them produce a high degree of organic pollution in aquatic and terrestrial ecosystems. They also alter the physico-chemical characteristics of the receiving aquatic bodies and affect aquatic flora and fauna. The sugar factory effluent in the environment, poses serious health hazard for the rural and semi-urban population that uses the stream and river water for agriculture and domestic purposes. There have been reports of fish mortality in the stream and river, and also damage to the paddy crops in this area due to the entry of these wastewaters in to the agricultural land (Baruah et al. 1993). The sugar factory effluent has obnoxious odour and unpleasant colour, when it is released into the environment without proper treatment. Farmers have been using these effluents for irrigation and found that the growth, yield and soil health were reduced. The contaminants like chloride, sulphate, phosphate, magnesium and nitrate are discharged with the effluent by various industries, which create nuisance by the way of physical appearance, odour and taste. Such harmful water is injurious to plants, animals and human beings. The effects of various industrial effluents on seed germination, growth and yield of crop plants have captivated the attention of many workers (Ozoh and Oladimeji 1984; Rahman et al. 2002; Street et al. 2007). However, no detailed experiments have been performed on the germination and plant growth using sugar factory effluent. In the present investigation an attempt has been made to study the effects of sugar factory effluent on the seed germination, seedling growth, amino acids, proteins and chlorophyll content of green gram and maize and aquatic plants water hyacinth and water lettuce.

MATERIALS AND METHODS

The sugar factory effluent was collected in pre-cleaned, acid washed 50 litre carboy from a sugar industry located in Erode district of Tamil Nadu, India and stored at cold room until used. Physico-chemical parameters of temperature, colour, pH, electrical conductivity (EC), dissolved oxygen (DO), biological oxygen demand (BOD), total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), chloride, alkalinity, total hardness, calcium, magnesium, sulphate, phosphate and total iron were measured using the standard methods (APHA 1998).

The impact of sugar factory effluent on the growth and biochemical characteristics of the green gram (*Phaseolus aureus* CO-4) and maize (*Zea mays* CO-1) were first investigated using soil pots (30cm height x 30cm width). Red soil was collected without any contamination by sugar factory effluent and sieved (2mm mesh). About 4kg of soil was taken in separate pots. Five different concentrations (viz., 20, 40, 60, 80 and 100%) of effluent were prepared and poured into each pot. Control was also maintained and irrigated with tap water. Five seeds of green gram and maize presterilised with 0.1% mercuric chloride were sowed separately in each pot and allowed to germinate. Periodically, the pots were irrigated with 1 litre of effluent at 48h interval. The percentage of germination was assessed (Rahman et al. 2002). The shoot length of the plants was recorded every 48h for 20 days.

Fresh and total dry mass of green gram and maize were determined after 20 days of the experiment. The plants were uprooted and washed thoroughly with distilled water and lengths of the roots were measured. The plants were dried under natural conditions at the open roof top garden for 2h. The fresh weight was taken and the plants were then packed in paper envelopes and over dried for 36h at 70°C. The dry weight of each plant was also recorded. Biochemical parameters of total amino acid, total protein and total chlorophyll were analyzed for each experimental plant leaves on the 20th day (Sadasivam and Manickam 1996).

The study for aquatic system, healthy water hyacinth (*Eichornia crassipes*) and water lettuce (*Pistia stratiotes*) were collected from a pond with an average weight of 60 gram and then washed thoroughly with distilled water to remove particles adhering to the plants. Further, sugar factory effluent with five different concentrations (20, 40, 60, 80 and 100%) were prepared and transferred about 1.5 litres into rectangular plastic vessels (25 x 15 x 12 cm). The cleaned plants (60g each) were introduced into the vessels with the roots submerged in the effluent and were kept under sunlight for 20 days. The fresh weight of the plants was determined using physical balance every 24h after removing water by blotting. The fresh and dry mass of the plants, total amino acid, total protein and total chlorophyll were estimated at final stage of the study (20th day). The procedure applied was similar to that described earlier for the determination of physical and biochemical properties of the plants. All the experiments were conducted in triplicate unless otherwise stated. Data points in tables and figures represent means, with all error bars shown (± 1 standard error of mean). Both mean and standard deviation were performed where appropriate using the statistical package on Microsoft® Excel Version 2003.

RESULTS AND DISCUSSION

Physico-chemical parameters of the effluent were found to be above the permissible level according to Indian Standards (Table 1). pH was relatively low due to usage of phosphoric acid and sulfur dioxide during clarification of sugar cane juice (Manivasakam 1987). Palharyal et al. (1993) reported pH is an essential factor in the formation of algal blooms and makes the water unfit for irrigation and the soil over a large area becomes acidic resulting in poor crop growth and yield. Similarly the effluent had very high TDS. This observation is in good agreement with the report by Abdul Jameel and Sirajudeen (2006). They also found that TDS in sugar factory effluent was very high (3950 mg/L).

The seeds of green gram and maize were germinates 100% in the sugar factory effluent at the lower concentrations (20 to 80%), whereas in the undiluted effluent the germination was found that 73 and 80% (Table 2). Ajmal and Khan (1983) proved, the lower concentration of effluent (25%) supports 100% seed germination and osmotic pressure associated with higher concentration of sugar factory effluent affect the germination in kidney bean, *Phaseolus aureus* and millet, *Pennisetum typhoides*. Rodger et al. (1957) reported that high osmotic pressures of the germination solution makes imbibitions more difficult and retard germination, while the ability of seeds to germinate under high osmotic pressure differs with variety as well as species.

The maximum shoot length of green gram and maize was observed in control followed by 20, 40, 60, 80 and 100% concentrations of the effluent (Table 3). There was a direct relationship between shoot length and concentration of effluent. Kaushik et al. (2004) clearly reported the toxicity of sugar factory effluent on the growth, photo synthetic pigments and nutrient uptake in wheat seedlings in aqueous versus soil medium. The presence of calcium and magnesium cause higher osmotic pressure resulting in the wilting of seedlings (Gomathi and Oblisami 1992). Thus, in our study, the plant growth was highly affected due to the excess amount of chloride, alkalinity, hardness, calcium, magnesium, sulphate and phosphate in the sugar factory effluent. The root length was severely affected at the higher concentrations of the effluent (100%) in green gram (13.5cm) and maize (25cm) when compare to control. Similarly, green gram and maize showed maximum values of fresh and dry weight in the control and the minimum were in 100% effluent concentration (Table 4).

Bio-chemical parameters of total amino acid, protein and chlorophyll content were analyzed in plant leaves of green gram and maize. The amount of amino acid, protein and chlorophyll content gradually decreased with increasing concentrations of the effluent (Fig. 1a and b). Plants treated with higher concentration of the effluent (above 20%) showed lower amount of amino acid, protein and chlorophyll content due to the presence of higher concentration of magnesium and acidic pH in the effluent. Calcium and magnesium (20mg/L) influences the plant growth, biomass partitioning, fruit yield and creates a symptom of leaf chlorosis after 8 weeks in green house tomato (Hao and Athanasios 2004). Lasa et al. (2000) also reported four different concentrations (0.1, 0.8, 5 and 10mM) of magnesium affect the growth of sunflower plants grown with ammonium and nitrate and proved that the magnesium-fed plants had lower content of free amino acids and soluble protein in the leaves.

In the aquatic system, water hyacinth plant showed a gradual decrease in plant weight throughout the study with effluent concentrations 80 and 100% (Fig. 2a). Weight of control plant increased from 60 to 93.3g after 20 days. In 20, 40 and 60% concentrations the plant weight gradually increased up to 85.4, 84.2 and 77.6g after 20 days. After 10 days the weights remained more or less constant. There was a decrease in plant weight at 80% and 100% effluent concentrations. Similar results were observed in the aquatic plant, water lettuce (Fig. 2b). The maximum loss of weight was observed in 100% concentration, whereas in the control the growth increased from 60 to 73.1g after 20 days. This might be due to the presence of moderate amounts of micronutrients in the diluted effluent that stimulated the plant growth. However, the excessive level at higher concentrations could result in stunted growth.

Effect of sugar factory effluent at different concentrations on total amino acids, protein and chlorophyll content of the aquatic plants (water hyacinth and water lettuce) were observed and the results are given in Fig. 2c and d. The above parameters at different concentrations of the effluent were found to be very low when compared to the control. The amount of amino acid, protein and chlorophyll content in the aquatic plants decreased due to the increased concentrations of sugar factory effluent. Owing to the toxic nature of the effluent, the leaves of the plants were affected by decreased photosynthetic rate. As a result of higher BOD, the photosynthesis of aquatic system was also affected (Rao et al. 1993) and reduces the growth parameters of the plant. Dry weight of water hyacinth and water lettuce showed high value in the control that was 640 and 350mg respectively. Dry weight was significantly decreased when the effluent concentration was increased (Table 5).

The study concluded that that the physico chemical parameters like BOD, chloride, alkalinity, hardness, calcium, magnesium, sulphate and phosphate were found to be relatively higher in the sugar factory effluent and they severely affect the plant growth. There was a gradual decrease in the shoot length, content of free amino acid, protein and total chlorophyll in both terrestrial and aquatic plants irrigated with various concentrations of effluent when compared to the control. The untreated effluents possibly lead to soil pollution, deterioration and low productivity. Even the terrestrial and aquatic environments are affected. This could be averted by proper treatment of the effluents by suitable conventional methods.

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Figure Captions

Figure 1 Effect of different concentration of sugar factory effluent on the biochemical properties of terrestrial plant. a) Green gram b) Maize

Figure 2 Effect of different concentration of sugar factory effluent on the growth and biochemical properties of aquatic plant. a) Water hyacinth b) Water lettuce c) Water hyacinth d) Water lettuce

Table 1. Physico chemical parameters of sugar factory effluent

Parameters	Value	Permissible Limits (IS)	Parameters	Value	Permissible Limits (IS)
Colour	Pale white	-	Chloride	70	600
Temperature ^{o}C	29.1	-	Alkalinity	80	200
pH	4.42	6.5 – 8.5	Hardness	1100	600
EC $\mu S.cm^{-1}$	1.01	300	Calcium	480	200
DO	10.1	> 6	Magnesium	620	100
BOD	1010	100	Sulphate	400	400
TS	1344	1200	Phosphate	25	10
TSS	120	200	Iron	1.0	1
TDS	1224	1000			

All the values are expressed in mg/L except pH and EC; IS - Indian Standard

Table 2. Effect of sugar factory effluent on the percentage germination of green gram and maize

Concentration	Percentage of germination		Time of germination (hours)	
	Green gram	Maize	Green gram	Maize
Control	100	100	24	48
20%	100	100	24	48
40%	100	100	24	48
60%	100	100	24	48
80%	100	100	24	48
100%	73 ± 11.5	80 ± 12	48	72

Values are mean ± standard error

Table 3. Effect of sugar factory effluent on the shoot length of green gram and maize

Effluent Concentrations	Shoot length (cm) / Time (days)									
	2	4	6	8	10	12	14	16	18	20
Green gram										
Control	0.5 ± 0.11	2.3 ± 0.12	4.5 ± 0.18	5.5 ± 0.13	7.2 ± 0.19	8.5 ± 0.12	10.5 ± 0.17	11.8 ± 0.21	12 ± 0.29	12.1 ± 0.11
20%	0.5 ± 0.19	2.2 ± 0.22	6 ± 0.18	6.5 ± 0.12	7.5 ± 0.11	9 ± 0.15	10.3 ± 0.17	10.5 ± 0.21	10.8 ± 0.28	10.9 ± 0.29
40%	0.5 ± 0.12	3 ± 0.12	6 ± 0.11	7.1 ± 0.13	7.8 ± 0.13	9.5 ± 0.13	10 ± 0.18	10.3 ± 0.2	10.4 ± 0.27	10.6 ± 0.18
60%	0.3 ± 0.11	2.8 ± 0.13	5 ± 0.18	6.6 ± 0.13	8 ± 0.12	8.3 ± 0.12	8.9 ± 0.19	10.1 ± 0.21	10.3 ± 0.2	10.4 ± 0.21
80%	0.3 ± 0.07	2.5 ± 0.13	3.8 ± 0.28	5.4 ± 0.12	7.1 ± 0.12	7.3 ± 0.12	7.9 ± 0.16	8.1 ± 0.19	8.3 ± 0.29	8.3 ± 0.21
100%	0.3 ± 0.11	2.1 ± 0.11	3 ± 0.18	5 ± 0.13	7.2 ± 0.12	7.8 ± 0.12	8 ± 0.16	8.1 ± 0.19	8.2 ± 0.27	8.3 ± 0.28
Maize										
Control	0.2 ± 0.11	2 ± 0.12	7.5 ± 0.12	14 ± 0.12	21 ± 1.2	23.8 ± 0.12	26.1 ± 0.99	30 ± 0.49	31 ± 0.47	31.8 ± 0.51
20%	0.2 ± 0.12	1 ± 0.13	6 ± 0.15	12 ± 0.14	18 ± 0.79	21.5 ± 0.62	25.5 ± 0.69	28 ± 1.09	28.6 ± 0.87	29.1 ± 0.59
40%	0.2 ± 0.11	0.5 ± 0.12	5.5 ± 0.13	13 ± 0.29	14.5 ± 1.2	20.5 ± 0.52	21.9 ± 0.85	25 ± 0.91	25.9 ± 0.57	26.2 ± 0.69
60%	0.2 ± 0.11	0.5 ± 0.12	3.5 ± 0.17	10 ± 0.71	15.5 ± 0.2	19.5 ± 0.12	20 ± 1.01	21 ± 0.99	21.6 ± 0.49	21.9 ± 0.29
80%	0.2 ± 0.11	0.5 ± 0.12	3.3 ± 0.15	7 ± 0.19	9.5 ± 0.21	11 ± 0.24	11.7 ± 0.29	13 ± 0.89	13.7 ± 0.77	14 ± 0.49
100%	0.1 ± 0.11	0.3 ± 0.12	3 ± 0.12	5 ± 0.14	8.7 ± 0.19	9 ± 0.52	10.2 ± 0.22	11 ± 0.29	12 ± 0.27	12.5 ± 0.21

Values are centimeter and mean ± standard error

Table 4. Effect of sugar factory effluent on the root length, fresh weight and dry weight of green gram and maize seedling

Effluent Concen-tration	Green Gram			Maize		
	Root Length (cm)	Fresh Weight (mg)	Dry Weight (mg)	Root Length (cm)	Fresh Weight (mg)	Dry Weight (mg)
Control	20.1 ± 6.11	390 ± 3.08	128 ± 1.08	53 ± 3.28	1345 ± 4.2	230 ± 1.64
20%	19.7 ± 0.75	378 ± 2.12	120 ± 1.37	52 ± 2.16	1290 ± 1.2	210 ± 0.98
40%	15.4 ± 0.28	352 ± 1.86	120 ± 2.04	45 ± 3.64	1200 ± 4.11	200 ± 0.18
60%	14.8 ± 1.51	335 ± 1.21	100 ± 0.62	43 ± 2.05	1180 ± 3.08	180 ± 2.06
80%	14.1 ± 0.82	322 ± 0.97	90 ± 0.87	42 ± 3.02	600 ± 2.28	170 ± 1.92
100%	13.5 ± 0.56	310 ± 2.41	90 ± 1.03	25 ± 2.23	380 ± 3.29	120 ± 1.79

Values are mean ± standard error

Table 5. Effect of sugar factory effluent on dry weight of water hyacinth and water lettuce

Effluent Concentration	Water hyacinth (mg)	Water lettuce (mg)
Control	640 ± 3.563	350 ± 2.342
20%	550 ± 2.981	340 ± 1.896
40%	520 ± 2.812	325 ± 1.124
60%	500 ± 1.023	315 ± 1.465
80%	480 ± 2.103	288 ± 1.234
100%	420 ± 2.745	276 ± 1.197

Values are mean ± standard error

Fig. 1 (a)

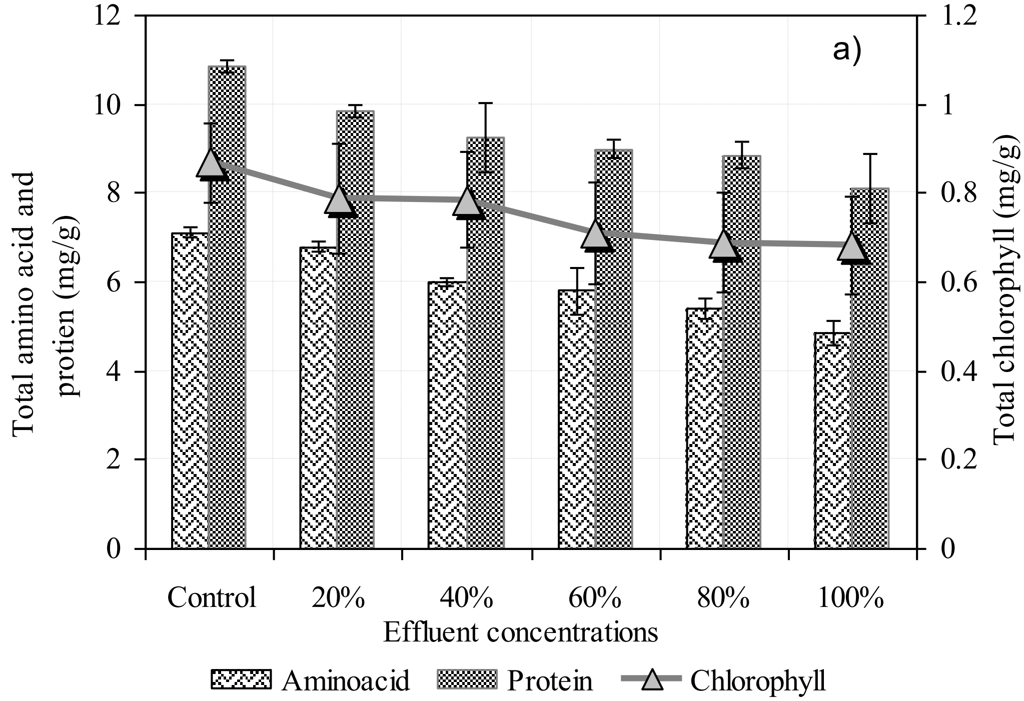

a)
Total amino acid and protien (mg/g)
Total chlorophyll (mg/g)
Control
20%
40%
60%
80%
100%
Effluent concentrations
Aminoacid
Protein
Chlorophyll

Fig. 1 (b)

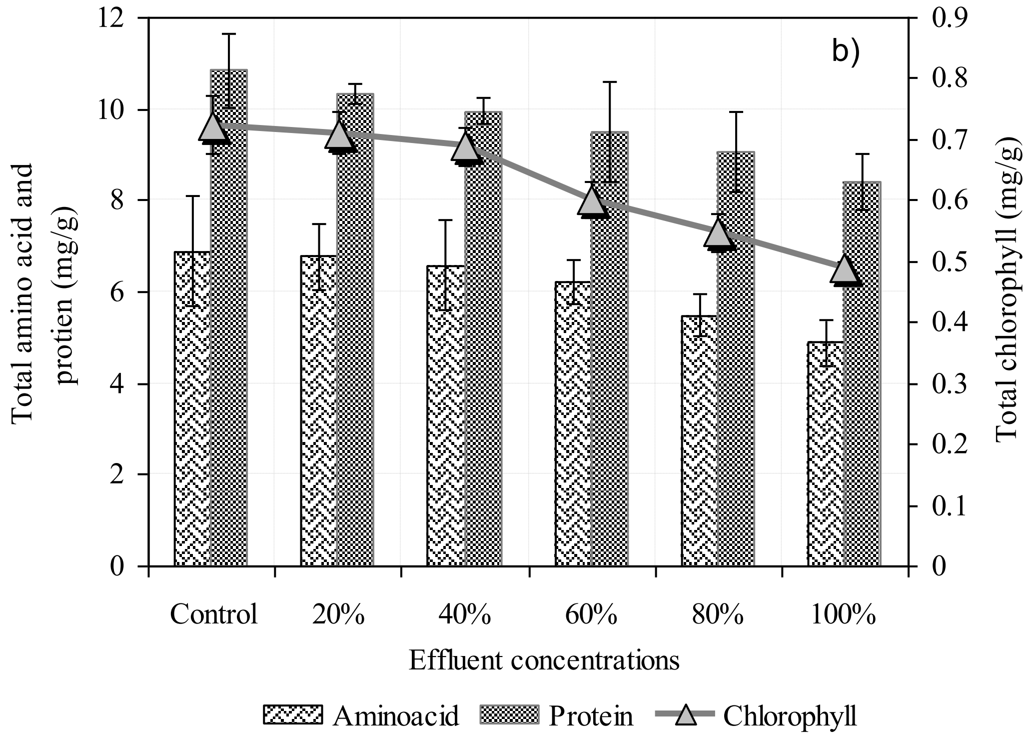
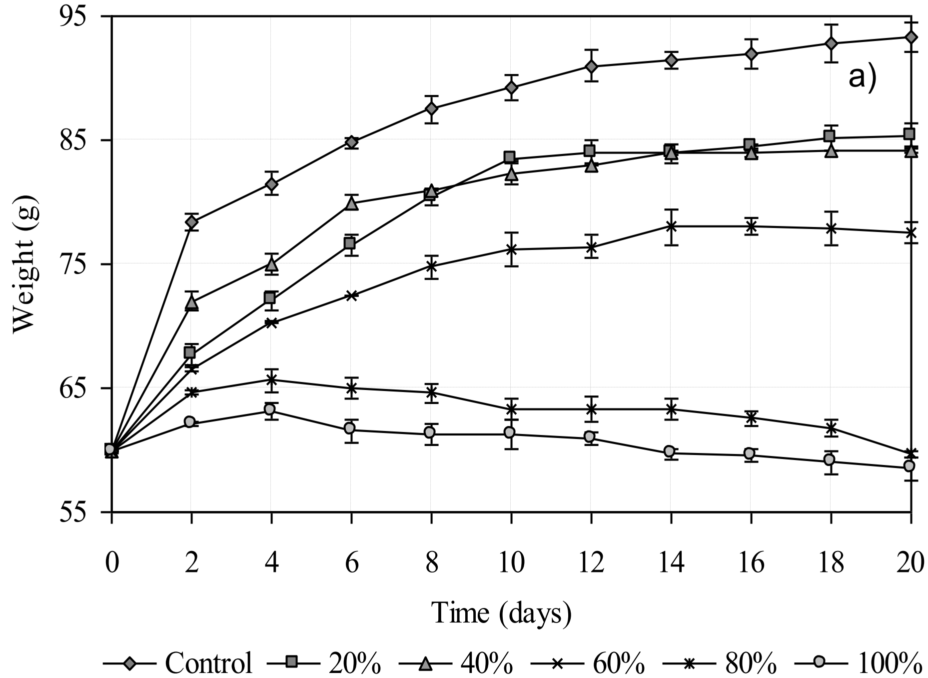

b)
Total amino acid and protien (mg/g)
Total chlorophyll (mg/g)
12
10
8
6
4
2
0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Control
20%
40%
60%
80%
100%
Effluent concentrations
Aminoacid
Protein
Chlorophyll

Fig. 2 (a)



a)
Weight (g)
95
85
75
65
55
0
2
4
6
8
10
12
14
16
18
20
Time (days)
Control
20%
40%
60%
80%
100%

Fig. 2 (b)

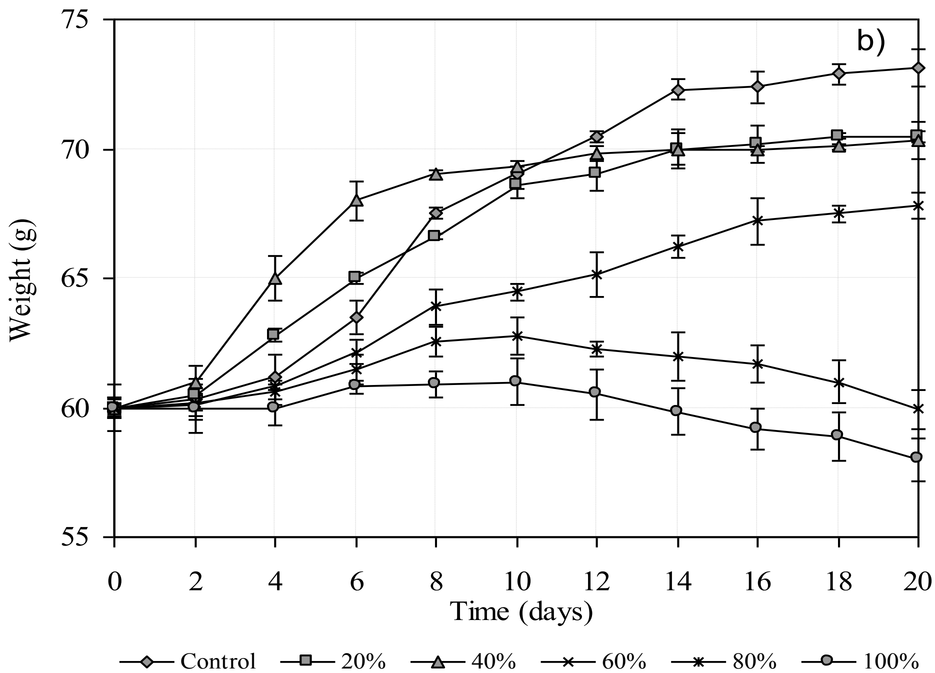

b)
75
70
65
60
55
Weight (g)
0
2
4
6
8
10
12
14
16
18
20
Time (days)
Control
20%
40%
60%
80%
100%

Fig. 2 (c)

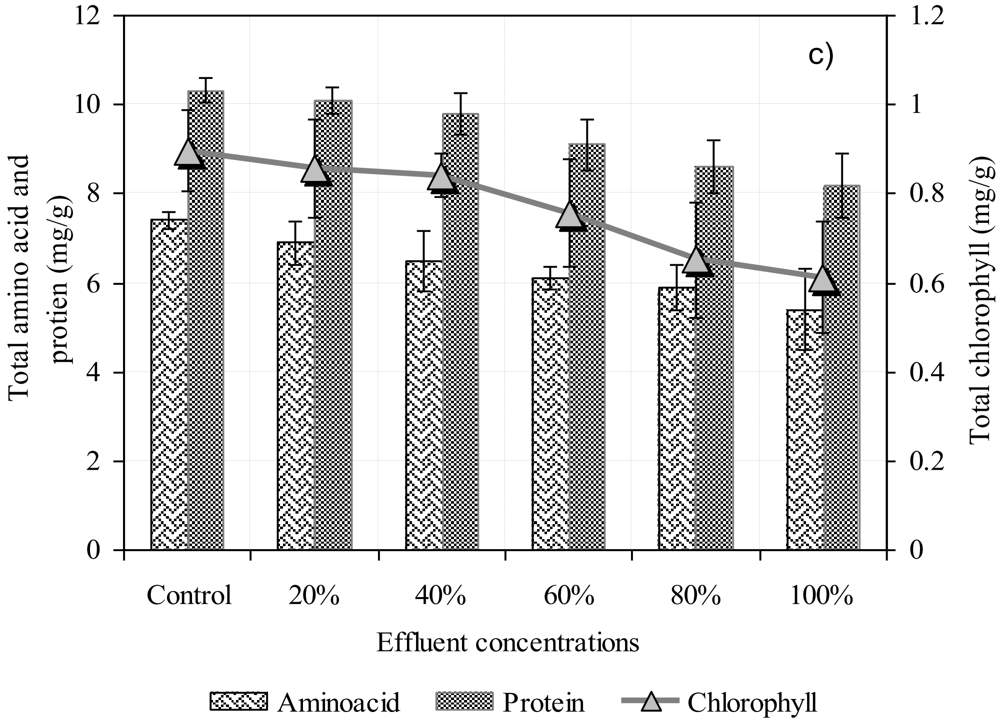
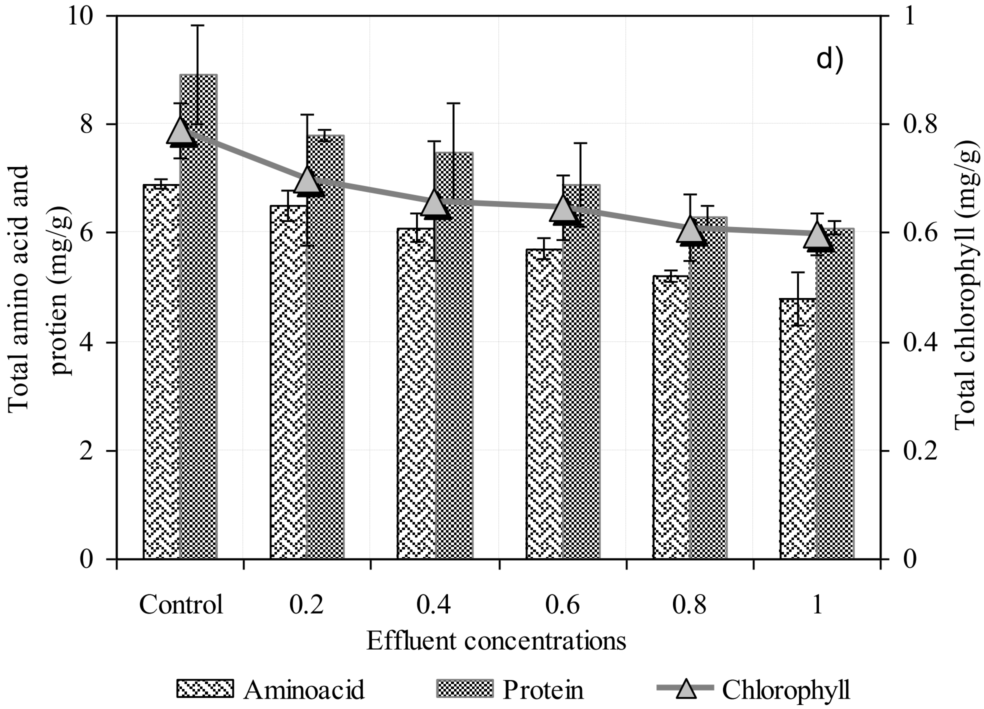

c)
Total amino acid and protien (mg/g)
Total chlorophyll (mg/g)
Control
20%
40%
60%
80%
100%
Effluent concentrations
Aminoacid
Protein
Chlorophyll

Fig. 2 (d)


d)
Total amino acid and protien (mg/g)
Total chlorophyll (mg/g)
Control
0.2
0.4
0.6
0.8
1
Effluent concentrations
Aminoacid
Protein
Chlorophyll

REFERENCES

Abdul Jameel A, Sirajudeen J (2006) Risk assessment of physico-chemical contaminants in ground water of Pettavaithalai area, Thiruchirappalli, Tamil Nadu, India. Environ Monitor Assess 123: 299-312.

Ajmal M, Khan AU (1983) Effects of sugar factory effluent on soil and crop plants. Environmental Pollution series A, Ecological and Biological 30: 135-141.

APHA (1998) Standard methods for the examination of water and wastewater. 20th edition, American Public Health Association, Water Pollution Control Federation. Washington, DC.

Baruah AK, Sharma RN, Borah GC (1993) Impact of sugar mill and distillery effluent on water quality of river Galabil, Assam. Indian J Environ Hlth 35: 288-293.

Gomathi V, Oblisamy G (1992) Effect of pulp and paper mill effluents on germination of the crops. Indian J Environ Hlth 34: 326-328.

Hao X, Athanasios P (2004) Effects of calcium and magnesium on plant growth, biomass partitioning, and fruit yield of winter greenhouse tomato. Hort Sci 39: 512-515.

Kaushik A, Kadyan, BR, Kaushik CP (2004) Sugar mill effluent effects on growth, photosynthetic pigments and nutrient uptake in wheat seedlings in aqueous vs. soil medium. Water, Air and soil Pollution 87: 39-46.

Lasa B, Frechilla S, Aleu M, Gonzalez-Moro B, Lamsfus C, Aparicio-Tejo PM (2000). Effects of low and high levels of magnesium on the response of sunflower plants grown with ammonium and nitrate. Plant and soil 225: 167-174.

Manivasakam N (1987) Industrial Effluents Origin, characteristics, effects, analysis and treatment. Sakthi publications, Kovai pudur, Coimbatore, India.

Ozoh PTE, Oladimeji AA (1984). Effects of Nigeria dye stuff effluent on germination latency, growth, and gross growth of *Zea mays*. Bull Environ Contam Toxicol 33: 215-219.

Palharyal JP, Siriah VK, Shobana M (1993) Environmental Impact of sewage and effluent disposal on the river systems. Ashish Publishing House pp. 325.

Rahman KSM, Banat IM, Rahman TJ, Thayumanavan T and Lakshmanaperumalsamy P (2002) Bioremediation of Gasoline contaminated soil by a bacterial consortium amended with poultry litter, coir pith and rhamnolipid biosurfactant, Biores Technol 81: 25-32.

Rao AV, Jain BL, Gupta IC (1993) Impact of textile industrial effluents on agricultural land – a case study. Indian J Environ Hlth 35: 132-138.

Rodger JBB, Williams GG, Davis RL (1957) A rapid method for determining winter hardiness of alfalfa. Agron J 49: 88-92.

Sadasivam S, Manickam A (1996) Biochemical methods, second edition. New age International Publishers, New Delhi, India.

Street RA, Kulkarni MG, Stirk WA, Southway C, Van Staden J (2007) Toxicity of metal elements on germination and seedling growth of widely used medicinal plants belonging to Hyacinthaceae. Bull Environ Contam Toxicol 79:371-376.