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

3
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Impact of sugar factory effluent on the growth and biochemical characteristics of terrestrial and aquatic plants

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).

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

11
12 Fresh and total dry mass of green gram and maize were determined after 20 days of the
13 experiment. The plants were uprooted and washed thoroughly with distilled water and
14 lengths of the roots were measured. The plants were dried under natural conditions at the
15 open roof top garden for 2h. The fresh weight was taken and the plants were then packed in
16 paper envelopes and over dried for 36h at 70°C. The dry weight of each plant was also
17 recorded. Biochemical parameters of total amino acid, total protein and total chlorophyll were
18 analyzed for each experimental plant leaves on the 20th day (Sadasivam and Manickam 1996).

19
20 The study for aquatic system, healthy water hyacinth (*Eichornia crassipes*) and water
21 lettuce (*Pistia stratiotes*) were collected from a pond with an average weight of 60 gram
22 and then washed thoroughly with distilled water to remove particles adhering to the plants.
23 Further, sugar factory effluent with five different concentrations (20, 40, 60, 80 and 100%)
24 were prepared and transferred about 1.5 litres into rectangular plastic vessels (25 x 15 x 12
25 cm). The cleaned plants (60g each) were introduced into the vessels with the roots
26 submerged in the effluent and were kept under sunlight for 20 days. The fresh weight of the
27 plants was determined using physical balance every 24h after removing water by blotting. The
28 fresh and dry mass of the plants, total amino acid, total protein and total chlorophyll were
29 estimated at final stage of the study (20th day). The procedure applied was similar to that
30 described earlier for the determination of physical and biochemical properties of the plants.
31 All the experiments were conducted in triplicate unless otherwise stated. Data points in tables
32 and figures represent means, with all error bars shown (± 1 standard error of mean). Both
33 mean and standard deviation were performed where appropriate using the statistical package
34 on Microsoft® Excel Version 2003.

35 36 **RESULTS AND DISCUSSION**

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

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

10
11 The maximum shoot length of green gram and maize was observed in control followed by
12 20, 40, 60, 80 and 100% concentrations of the effluent (Table 3). There was a direct
13 relationship between shoot length and concentration of effluent. Kaushik et al. (2004)
14 clearly reported the toxicity of sugar factory effluent on the growth, photo synthetic
15 pigments and nutrient uptake in wheat seedlings in aqueous versus soil medium. The
16 presence of calcium and magnesium cause higher osmotic pressure resulting in the wilting
17 of seedlings (Gomathi and Oblisami 1992). Thus, in our study, the plant growth was highly
18 affected due to the excess amount of chloride, alkalinity, hardness, calcium, magnesium,
19 sulphate and phosphate in the sugar factory effluent. The root length was severely affected
20 at the higher concentrations of the effluent (100%) in green gram (13.5cm) and maize
21 (25cm) when compare to control. Similarly, green gram and maize showed maximum
22 values of fresh and dry weight in the control and the minimum were in 100% effluent
23 concentration (Table 4).

24
25 Bio-chemical parameters of total amino acid, protein and chlorophyll content were
26 analyzed in plant leaves of green gram and maize. The amount of amino acid, protein and
27 chlorophyll content gradually decreased with increasing concentrations of the effluent (Fig. 1a
28 and b). Plants treated with higher concentration of the effluent (above 20%) showed lower
29 amount of amino acid, protein and chlorophyll content due to the presence of higher
30 concentration of magnesium and acidic pH in the effluent. Calcium and magnesium
31 (20mg/L) influences the plant growth, biomass partitioning, fruit yield and creates a
32 symptom of leaf chlorosis after 8 weeks in green house tomato (Hao and Athanasios 2004).
33 Lasa et al. (2000) also reported four different concentrations (0.1, 0.8, 5 and 10mM) of
34 magnesium affect the growth of sunflower plants grown with ammonium and nitrate and
35 proved that the magnesium-fed plants had lower content of free amino acids and soluble
36 protein in the leaves.

37
38 In the aquatic system, water hyacinth plant showed a gradual decrease in plant weight
39 throughout the study with effluent concentrations 80 and 100% (Fig. 2a). Weight of control
40 plant increased from 60 to 93.3g after 20 days. In 20, 40 and 60% concentrations the plant
41 weight gradually increased up to 85.4, 84.2 and 77.6g after 20 days. After 10 days the
42 weights remained more or less constant. There was a decrease in plant weight at 80% and
43 100% effluent concentrations. Similar results were observed in the aquatic plant, water
44 lettuce (Fig. 2b). The maximum loss of weight was observed in 100% concentration, whereas
45 in the control the growth increased from 60 to 73.1g after 20 days. This might be due to the

1 presence of moderate amounts of micronutrients in the diluted effluent that stimulated the
2 plant growth. However, the excessive level at higher concentrations could result in stunted
3 growth.

4
5 Effect of sugar factory effluent at different concentrations on total amino acids, protein and
6 chlorophyll content of the aquatic plants (water hyacinth and water lettuce) were observed
7 and the results are given in Fig. 2c and d. The above parameters at different concentrations
8 of the effluent were found to be very low when compared to the control. The amount of
9 amino acid, protein and chlorophyll content in the aquatic plants decreased due to the
10 increased concentrations of sugar factory effluent. Owing to the toxic nature of the effluent,
11 the leaves of the plants were affected by decreased photosynthetic rate. As a result of higher
12 BOD, the photosynthesis of aquatic system was also affected (Rao et al. 1993) and reduces
13 the growth parameters of the plant. Dry weight of water hyacinth and water lettuce showed
14 high value in the control that was 640 and 350mg respectively. Dry weight was
15 significantly decreased when the effluent concentration was increased (Table 5).

16
17 The study concluded that that the physico chemical parameters like BOD, chloride,
18 alkalinity, hardness, calcium, magnesium, sulphate and phosphate were found to be
19 relatively higher in the sugar factory effluent and they severely affect the plant growth.
20 There was a gradual decrease in the shoot length, content of free amino acid, protein and
21 total chlorophyll in both terrestrial and aquatic plants irrigated with various concentrations
22 of effluent when compared to the control. The untreated effluents possibly lead to soil
23 pollution, deterioration and low productivity. Even the terrestrial and aquatic environments are
24 affected. This could be averted by proper treatment of the effluents by suitable conventional
25 methods.

26
27
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32 researches.

33 34 35 **Figure Captions**

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

38
39 **Figure 2** Effect of different concentration of sugar factory effluent on the growth and
40 biochemical properties of aquatic plant. a) Water hyacinth b) Water lettuce c) Water
41 hyacinth d) Water lettuce

1
2

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 °C	29.1	-	Alkalinity	80	200
pH	4.42	6.5 – 8.5	Hardness	1100	600
EC $\mu\text{S.cm}^{-1}$	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 \pm 11.5	80 \pm 12	48	72

Values are mean \pm 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 Concentration	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

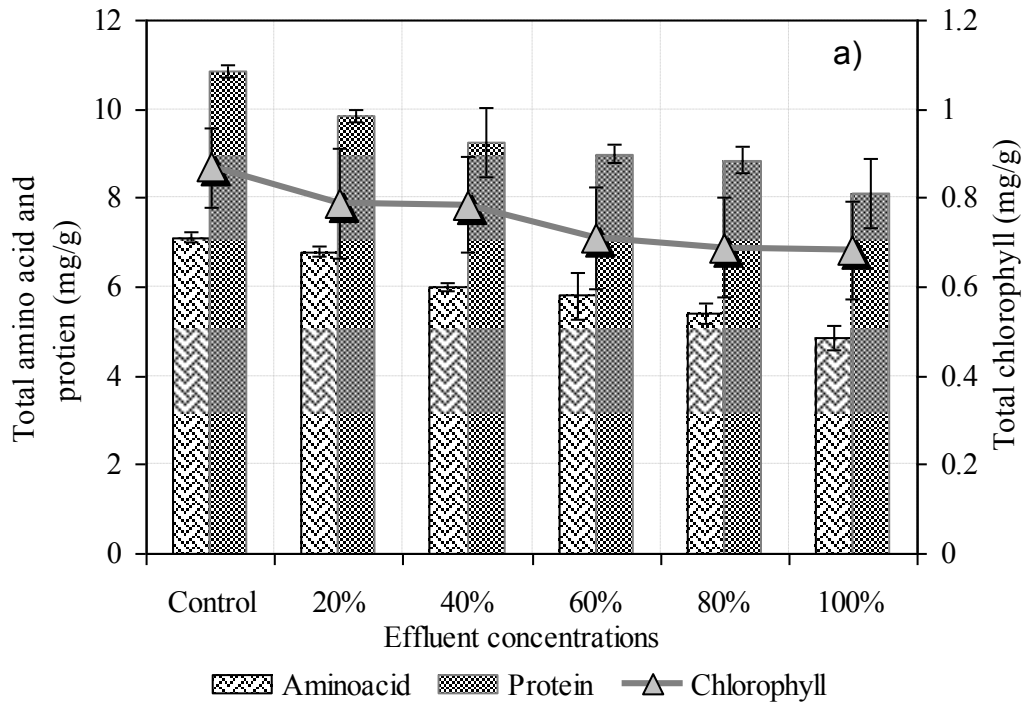
Values are mean ± standard error

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

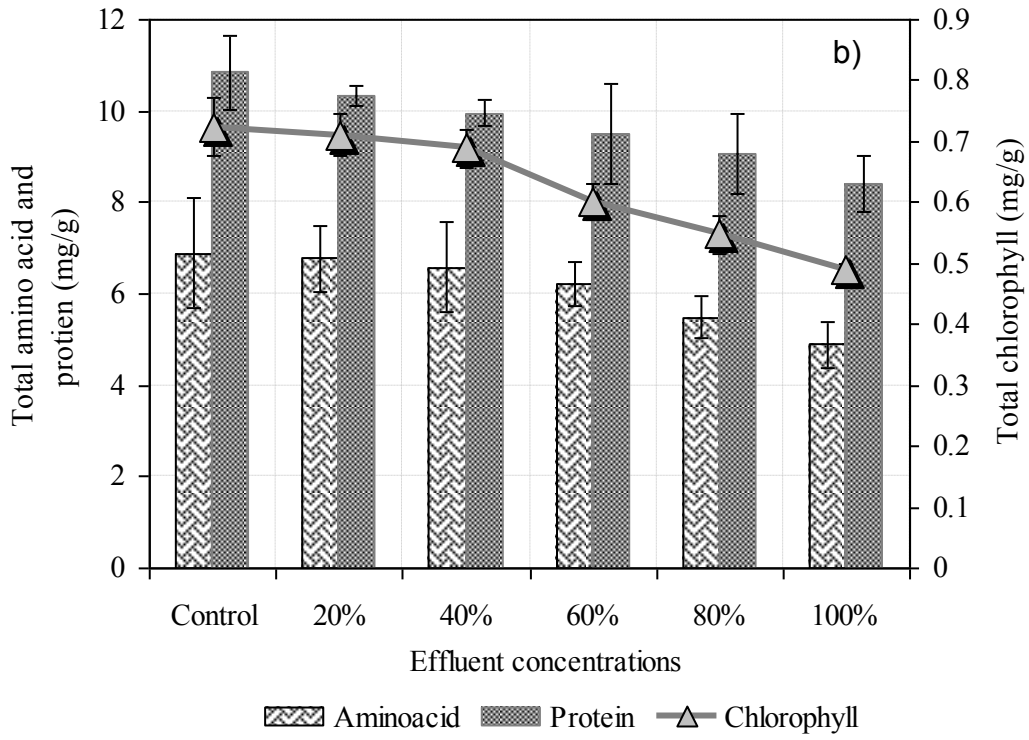
Effluent Concentration	Water hyacinth (mg)	Water lettuce (mg)
Control	640 ± 3.563	350 ± 2.342
20%	550 ± 2.981	340 ± 1.896
40%	520 ± 2.812	325 ± 1.124
60%	500 ± 1.023	315 ± 1.465
80%	480 ± 2.103	288 ± 1.234
100%	420 ± 2.745	276 ± 1.197

Values are mean ± standard error

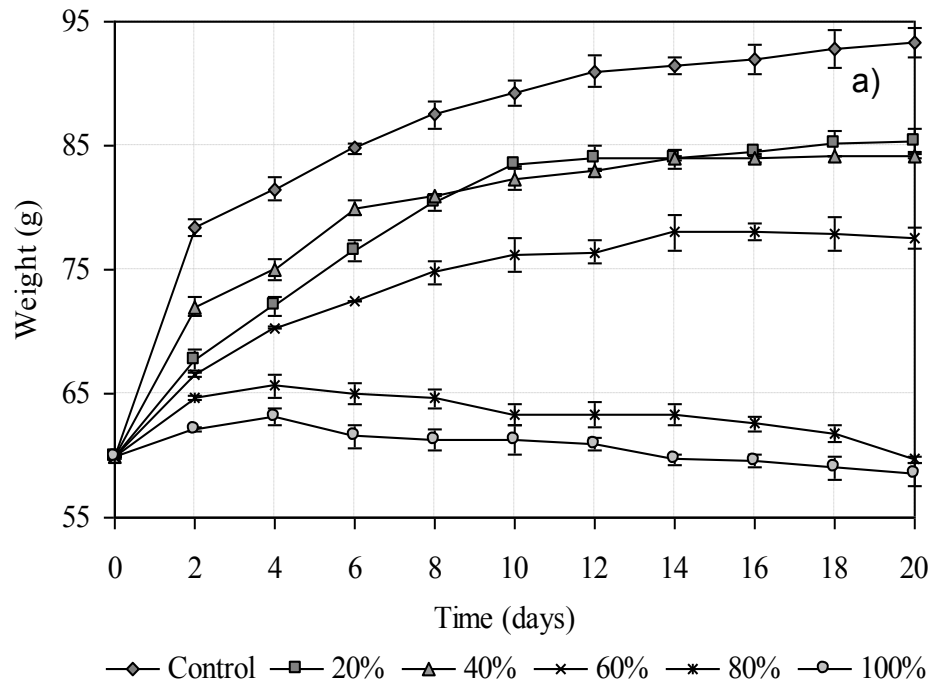
1 **Fig. 1 (a)**



1 **Fig. 1 (b)**



1 **Fig. 2 (a)**



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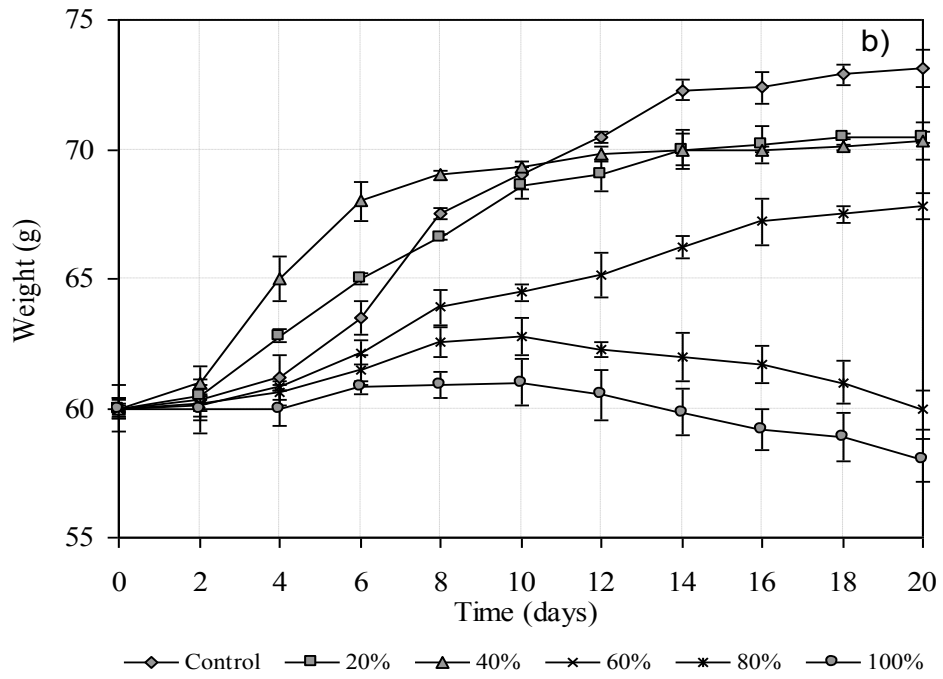
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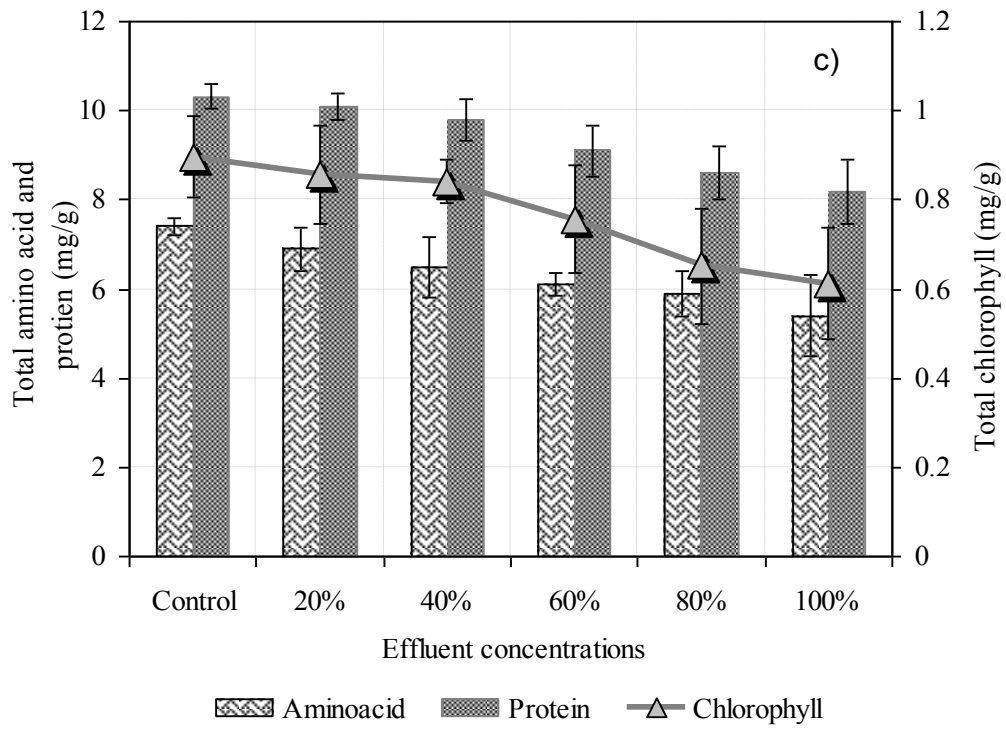
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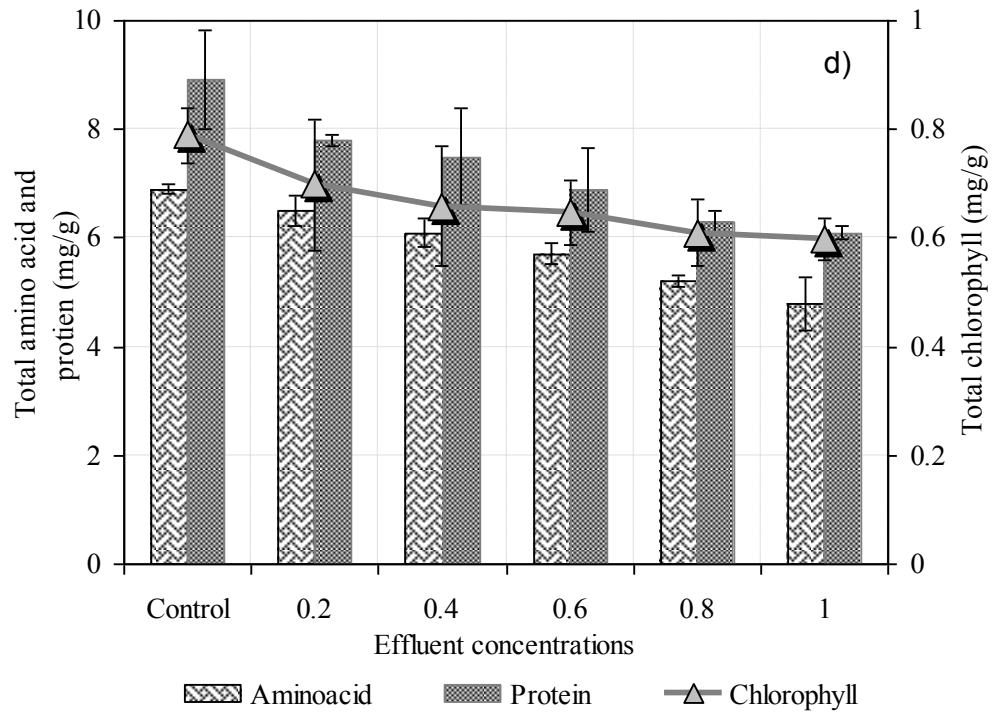
1 **Fig. 2 (b)**



1 **Fig. 2 (c)**



1 **Fig. 2 (d)**



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