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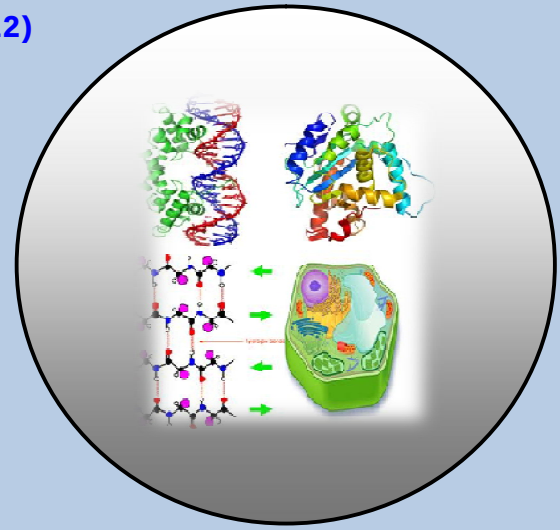

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Prof. P.K. Tandon Dr. Induja tripathi
http:// www.jbcr.in
jbiolchemres@gmail.com
info@jbcr.in

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Sugar Industry Effluent in Relation to Growth and Metabolism of Maize (*Zea mays* L.) Plants

P.K. Tandon and Induja Tripathi
Department of Botany, University of Lucknow, Lucknow 226007, U.P. India

ABSTRACT

Increasing doses of sugar industry effluents caused significant reduction in plant growth and germination percentage of Zea mays L. plants. Similarly metabolic parameters such as chlorophyll, sugar, protein and activities of enzymes catalase (CAT) and amylase were also found to have toxic effects of higher doses of sugar industry effluents. However activity of another iron enzyme viz. peroxidase (SOD) was found to stimulate at increasing doses of the effluent. Similar trend of increase was also observed at harvesting stage with respect to pH, percentages of $CaCO_3$ and organic carbon in the soil which were irrigated with sugar industry effluent.

Keywords: Sugar Industry, Zea mays L., Peroxidase (SOD), Catalase, Metabolism and Growth

INTRODUCTION

Industrial effluents are one of the major sources of water pollution in our country as most of the industries dump their effluent in the nearby river. This is true with sugar industry effluent also. Study reveals that pH of this effluent is slightly acidic in nature. Thus it creates lot of problems for flora and fauna present in the water bodies where it is released. This fact in view this study was carried out to investigate the harmful effects of this effluent on the growth and metabolism of maize plants.

MATERIAL AND METHODS

Experiment was carried out in earthen clay pots. The tap water washed soil was filled in medium sized earthen pots provided with a central drainage hole. The central drainage hole was covered by an inverted watch glass over a pad of glass wool. The soil pH was maintained repeatedly by flushing with distilled water. For each treatment including control, pots were taken in triplicates. The basal nutrient solution was prepared by method given by Hewitt (1966). Industry effluent was collected from a sugar industry.

Four concentrations (25%, 50%, 75% and 100%) of the effluent were used for the study. Plants were raised in basal nutrient solution except in controlled treatments, respective concentration of industry effluents were superimposed over basal nutrient solution. The treatments were supplied on alternate days. The controlled plants were watered with distilled water. 25 seeds were grown in each pot. The number of seeds germinated was noted and accordingly the germination percentage was calculated. Growth of the plants were measured in terms of shoot and root length in cms and total fresh weight and total dry weight in gms at maturity.

Soil pH was determined in 1:2 soil water ratio (Jackson, 1973). Calcium Carbonate in the soil was determined by the rapid titration method of Piper (1942), organic carbon in soil was determined by the method of Walkley and Black (1934).

Activities of enzymes catalase and peroxidase were assayed by the modified methods of Bisht (1972) and Luck (1963) respectively. Amylase activity in plant tissue was assayed by the method of Katsumi and Frekuhara (1969).

Chlorophyll, Protein and sugar concentration were measured by the method of Petering et al (1940), Lowry *et al* (1951) and *Dubias et al* (1956) respectively.

RESULTS AND DISCUSSION

(a) **Germination percentage:** It was found to be decreased at increasing concentration of effluent. Germination was found to be 94.67 in control and it decreased to 81.33, 72.00, 68.00 and 64.00% respectively, it was 14.09, 23.94, 28.17 and 32.40%decrease at 25, 50, 75 and 100% concentration of sugar industry effluent respectively as compared to control **(Table. 1.)**. According to Kirkby (1968) reduction in germination percentage at higher concentration of effluent may be due to the presence of excess amount of ammonia in effluent which may have caused depletion the acid from the tricarboxylic acid cycle which reduces the respiration rate and subsequently germination. Our findings are in conformity with above report.

(b) **Plant Growth:** The effects of different concentration of sugar industry effluent were observed on growth and biomass yield of maize plants. The results showed that the shoot and root lengths were significantly decreased at increasing concentration of effluent. Percentage of decrease in shoot length were of 15.36, 24.70, 39.76 and 43.07 while root length was decreased at the rate of 11.11, 19.44, 26.85 and 30.56 at 25, 50, 75 and 100% concentration respectively than the control **(Table. 1.)**. However fresh weight and dry weight was significantly decreased at increasing concentration of effluent (table).

Decrease in shoot and root length was due to the presence of toxic pollutants in the effluents. According to Singh et al (2005), a kind of pollutants mainly effects the respiration of roots. Respiration of roots and soil organism tends to reduce the oxygen and increase the CO_2 concentration. The soil becomes harder and closed and the closed pores of the soil cause less aeration which causes retardation in the growth of the plants. Jerome and Fergusan (1972) reported that the inhibition of growth might be due to fact that heavy metals present in the effluent bind with proteins.

(c) Metabolic Activities

(i) Chlorophyll: Different concentrations of effluent significantly reduced the level of photosynthetic pigments (Chlorophyll a, b and total and also carotenoids) **(Table. 2.)**. Chlorophyll reduction was found to be induced by waste water which could be associated with higher concentrations of mineral ions present in sugar industry effluent. It may also caused by inhibition of chlorophyll biosynthesis. Such inhibition of chlorophyll biosynthesis has been earlier reported for heavy metals such as Pb, Hg, Cd and Cr due to their effect on ∂-amino levulinic acid dehydrogenase (Prasad and Prasad, 1987, Padmaja et al, 1990, Vajpayee et al 2000). Reduction in pigments causes deficiency in light harvesting capacity and consequently decreased photosynthetic activity of the cells (Ouzounidou, 1996 and Srivastava et al 2005)

(ii) Sugars: Sugar concentration was significantly decreased at the increasing concentration of the effluents. It was found to be 5.06, 29.79, 57.58 and 63.64% decrease at 25, 50, 75 and 100% concentrations respectively **(Table. 3.)**. The various negative characters in the higher concentration of the effluents will act as a barriers for the natural process of the plant system and that may reduce leaf area and number of leaves and that may cause reduction in the surface area which receives light and thus rate of photosynthesis may be reduced. Manonmami *et al* (1992) reported that it may be due to the deranged sugar metabolism and poor translocation of starch and other metabolites to the growing axis and other possibility of reduction may be due to the heavy metal toxicity that may inhibit the membrane transport system mechanism which transport sugar to the phloem (Rauser, 1978) .

(iii) Protein: Protein concentration was also found to be significantly decreased at increasing concentration of effluent. It was 17.31, 28.84, 48.09 and 59.62% decrease at 25, 50, 75 and 100% concentration respectively than the control **(Table. 3)**. The decreased protein concentration in this study is in conformity with the study of Muthuswami and Jayabalan (2001) and Ayyasamy *et al* (2008). Reduction may be due to the breakdown of soluble protein or due to the increased activity of protease or other catabolic enzymes which might have activated and destroyed the protein (Singh *et al*, 2005)

(iv) Enzyme Activities: Catalase activity was non significantly decreased at increasing concentration of effluent. It was 0.66, 3.04, 9.24 and 21.62%decrease at 25, 50, 75 and100% concentration respectively than the control. **(Table. 4.)**

Peroxidase activity was however found to be increased. It was 52.80, 74.58, 91.78 and 101.65% increase at 25, 50, 75 and 100% concentration respectively than the control. Results indicated that the activity of amylase was significantly decreased at increasing concentration of effluent. The maximum decrease was observed at higher concentration of effluent and it was decreased 64.01% as compared to the control **(Table. 4.)**.

Decreased catalase activity with increasing concentration of effluents may be due to more generation of reactive oxygen species and H_2O_2 against this enzyme which may be unable to cope in the ROS generation. Decreased activity of this enzyme might also be due to in sufficient supply of iron for the synthesis of catalase. This finding is in agreement with some earlier findings (Kong xiang *et al*, 1999, Leon et al, 2002; Pandey and Sharma 2002; Tandon and Gupta, 2002). Increase in the activity of peroxidase enzyme may be due to the fact that effluents contain toxic metals which may cause enhancement in POD generation in plants. The other possibility of enhancement of peroxidase may be due to the effluents having large amount of various cations and anions (Behera and Misra, 1982). Heavy metals present in sugar industry effluent may cause a reduction in the hydrolysis products viz. α- amylase, phosphatase, RNAs and proteins. They interfere in the enzyme action by replacing metal ions from the metaloenzymes and inhibit different physiological processes of plants (Agarwal, 1999).

Table 1. Effect of different concentration of sugar industry effluent on germination percentage, growth and biomass yield of maize (*Zea mays L.)* plants.

S. No.	Effluent concentration (%)	Germination (%)	Shoot length (cm)	Root Length (cm)	Total Fresh weight (g)	Total Dry Weight (g)
1.	Control	94.66^{a} ±1.33	110.66^{a} ±3.84	36.00^{a} ±3.05	49.18^{a} ±8.42	14.18^{a} ±2.16
2.	25	81.33^{ab} ±1.33 (-14.09%)	93.66^{ab} ±1.20 (-15.36%)	32.00 ±1.15 (-11.11%)	30.29^{a} ±0.39 (-38.40%)	6.97^{a} ±0.004 (-50.86%)
3.	50	72.00^{abc} ±2.30 (-23.94%)	83.33^{abc} ±4.41 (-24.70%)	29.00^{a} ±2.08 (-19.44%)	23.58^{a} ±0.70 (-52.05%)	6.99^{a} ±1.03 (-50.68%)
4.	75	68.00^{ab} ±0.00 (-28.17%)	66.66^{abc} ±3.33 (-39.76%)	26.33^{a} ±0.88 (-26.85%)	20.41^{a} ±2.24 (-58.49%)	5.24^{a} ±0.02 (-63.03%)
5.	100	64.00^{ab} ±0.00 (-32.40%)	63.00^{abc} ±1.52 (-43.07%)	25.00^{a} ±0.57 (-30.56%)	18.84^{a} ±1.45 (-61.69%)	4.99^{a} ±0.98 (-64.79%)

(v) Soil chemical changes: pH value was found to be significantly increased at higher concentration of effluent. Percentage of calcium carbonate and organic carbon were also found to be increased at increasing concentration of effluent. Calcium carbonate had 20.05, 50.10, 80.07 and 90.04% increase while organic carbon showed 23.40, 19.15, 50.75 and 59.02% increase at 25, 50, 75 and 100% concentration respectively than the control **(Table. 5.)** this was

(vi) Already reported by many workers (Ajmal and Khan, 1983 and Orhue *et al* 2005b). The organic carbon in the soil irrigated with effluent was found to be higher than the soil irrigated with uncontaminated water. It may be due to the high organic nature of the effluent (Ale *et al*, 2008). The increased organic carbon is also a result of high total solid present in the effluent (Osaigbovo and Orhue, 2006). $CaCO_3$ was also found to be increased with increasing concentrations of effluents in the soil. Similar results of increased soil CaCO3 was reported by Ajmal and Khan, 1983.

Table 2. Effect of different concentration of sugar industry effluent on pigment content of maize (*Zea mays L.)* plants.

S. No.	Effluent concentration (%)	Chlorophyll a (mg/gFW)	Chlorophyll b (mg/gFW)	Total Chlorophyll (mg/gFW)	Carotenoids (mg/gFW)
1.	Control	2.15^{a} ±0.03	1.16^{a} ±0.01	3.32^{a} ±0.04	1.06^{a} ±0.009
2.	25	1.88^{ab} ±0.02 (-12.70%)	1.02^{ab} ±0.03 (-12.08%)	2.90^{ab} ±0.05 (-12.51%)	1.02^{b} ±0.02 (-3.49%)
3.	50	1.65^{abc} ±0.04 (-23.17%)	0.95^{abc} ±0.01 (-17.91%)	2.61^{abc} ±0.05 (-21.32%)	0.92^{abc} ±0.01 (-13.02%)
4.	75	1.46^{abcd} ±0.003 (-32.25%)	0.83^{abc} ±0.005 (-28.28%)	2.29^{abcd} ±0.008 (-30.89%)	0.84^{abc} ±0.006 (-20.47%)
5.	100	1.29^{abcd} ±0.004 (-40.08%)	0.81^{abc} ±0.01 (-30.33%)	2.10^{abcd} ±0.01 (-36.66%)	0.80^{abc} ±0.01 (-24.25%)

All values are means of triplicates ±SE. identical superscripts on values denote significant difference ($p<0.05$) between means of different treatments according to Duncan's multiple range test. The values given in the bracket shows the percent increase or decrease as compared to control.

Table 3. Effect of different concentration of sugar industry effluent on the concentrations of total sugar and protein of maize (*Zea mays L.)* plants.

S. No.	Effluent concentration (%)	Total Sugar Concentration (mg/g FW)	Protein Concentration (mg/g FW)
1.	Control	3.30^{a} ±0.02	2.06^{a} ±0.07
2.	25	3.13^{ab} ±0.33 (-5.06%)	1.70^{ab} ±0.10 (-17.31%)
3.	50	2.31^{abc} ±0.07 (-29.79%)	1.46^{ac} ±0.10 (-28.84%)
4.	75	1.40^{abcd} ±0.02 (-57.58%)	1.07^{abc} ±0.06 (-48.09%)
5.	100	1.20^{abcd} ±0.02 (-63.64%)	0.83^{abc} ±0.06 (-59.62%)

All values are means of triplicates ±SE. identical superscripts on values denote significant difference ($p<0.05$) between means of different treatments according to Duncan's multiple range test. The values given in the bracket shows the percent increase or decrease as compared to control.

Table 4. Effect of different concentration of sugar industry effluent on the activities of catalase, peroxidise and amylase in leaves of maize (*Zea mays L.*) plants.

S. No.	Effluent concentration (%)	Catalase activity (µ mole H_2O_2 decomposed /min/mg protein)	Peroxidase activity (ΔOD/mg protein)	Amylase activity (Starch hydrolyzed in mg/g FW)
1.	Control	30.73 ±1.40	2.05^{abcd} ±0.07	1.66^{a} ±0.06
2.	25	30.53^{NS} ±2.14 (-0.66%)	3.14^{d} ±0.19 (+52.80%)	1.33^{ab} ±0.17 (-20.04%)
3.	50	29.80^{NS} ±2.30 (-3.04%)	3.59^{c} ±0.25 (+74.58%)	0.73^{ab} ±0.06 (-56.03%)
4.	75	27.89^{NS} ±0.91 (-9.24%)	3.94^{b} ±0.25 (+91.78%)	0.66^{ab} ±0.06 (-59.99%)
5.	100	24.09^{NS} ±3.08 (-21.62%)	4.14^{a} ±0.33 (+101.65%)	0.60^{ab} ±0.00 (-64.01%)

All values are means of triplicates ±SE. identical superscripts on values denote significant difference ($p<0.05$) between means of different treatments according to Duncan's multiple range test. The values given in the bracket shows the percent increase or decrease as compared to control.

Table 5. Chemical properties of sugar industry effluent irrigated soils after harvesting of maize (Zea mays L.) plants.

S. No.	Effluent concentration (%)	pH (1:2 soil water)	Calcium carbonate (%)	Organi[c] Carbon (%)
1.	Control	7.13[abcd] ±0.03	0.83[abc] ±0.08	1.34[ab] ±0.05
2.	25	7.30[abcd] ±0.00 (+2.34%)	1.00[ab] ±0.14 (+20.05%)	1.65[ab] ±0.03 (+23.40%)
3.	50	7.43[ac] ±0.03 (+4.21%)	1.25[c] ±0.14 (+50.10%)	1.59[ab] ±0.12 (+19.15%)
4.	75	7.50[b] ±0.00 (+5.15%)	1.50[b] ±0.00 (+80.07%)	2.02[b] ±0.11 (+50.75%)
5.	100	7.60[a] ±0.05 (+6.55%)	1.58[a] ±0.08 (+90.04%)	2.13[a] ±0.06 (+59.02%)

All values are means of triplicates ±SE. identical superscripts on values denote significant difference ($p<0.05$) between means of different treatments according to Duncan's multiple range test. The values given in the bracket shows the percent increase or decrease as compared to control.

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Corresponding author: Professor Pramod Kumar Tandon, Department of Botany, University of Lucknow, Lucknow – 226 007, U. P., India
Email: tandonpramod@yahoo.in