SUGARMILL EFFLUENT EFFECTS ON GROWTH, PHOTOSYNTHETIC PIGMENTS AND NUTRIENT UPTAKE IN WHEAT SEEDLINGS IN AQUEOUS VS. SOIL MEDIUM

A. KAUSHIK,* BALA R. KADYAN and C.P. KAUSHIK
Department of Biosciences, M.D. University, Rohtak-124001, India

(Received 22 September, 1993; accepted in final form 12 October, 1994)

Abstract. Germination, seedling growth, concentrations of photosynthetic pigments and nutrient uptake in *Triticum vulgare* L. (Var. W-H-147) were studied in response to sugarmill effluent application (10% concentration) in aqueous Vs. soil medium. The effluent rich in various nutrients showed particularly high concentration of sodium. Germination was not affected by the effluent treatment. Seedling growth was reduced significantly by the effluent in aqueous medium, but not in soil. The effluent treatment increased the concentrations of various pigments, however, the pigment ratios got changed in the aqueous medium only. The uptake of nitrogen, magnesium and carbon by the seedlings decreased while that of calcium, sodium and phosphorus increased in effluent treated plants, the changes being more marked in aqueous medium except for phosphorus. In the effluent treated plants, uptake of potassium and chloride increased in aqueous medium, but decreased sharply in soil.

1. Introduction

Utility potential of industrial effluents for irrigation of crop-fields has been a controversial proposition due to the contradictory reports obtained on the effects of these effluents on crop plant responses (Sutton *et al.*, 1978; Ajmal *et al.*, 1984; Fayez and Shahin, 1987). The effects of effluents on plant responses have been investigated by several workers in pilot studies conducted in petri-dishes (Behera and Mishra, 1982; Ajmal and Khan, 1985) whereas the effect of the effluent on crop plants is expected to be different or modified, when applied actually to soil. In the present investigation, therefore, the effect of effluent from a sugarmill was studied on germination, seedling growth, photosynthetic pigments, biomass and nutrient uptake of wheat seedlings grown in aqueous effluent medium and in an effluent irrigated sandy loam soil with an objective to see if there are any differences in plant responses to the effluent under different experimental conditions.

2. Materials and Methods

2.1. Effluent Collection and Analysis

The effluent was obtained from a Co-operative Sugar Mill, Rohtak, India, which is double carbonation and double sulphitation plant. The liquid effluent, other than

* Present address: Department of Environmental Sciences, Kurukshetra University, Kurukshetra-132119, India

molasses, discharged from the mill are collected in an aerated lagoon, diluted ten times with canal water, and discharged directly into the soil of the crop fields just adjacent to the factory. This diluted effluent (10% concentration) used in the present study was chemically analysed using standard methods (Allen *et al.*, 1986; Michael, 1986).

2.2. Aqueous and Soil Culture of Wheat Seedlings

Grains of *Triticum vulgare* L. (var. W-H-147) a commonly grown cultivar in Haryana, India, were surface sterilized with 0.1% $HgCl_2$ solution.

For aqueous culture, a total of 12 petri-dishes were grouped into two sets of 6 each, receiving either 10 ml of distilled water or 10 ml of the 10% effluent. Twenty five grains were placed in each petri-dish lined with filter paper and kept at 25 ± 3 °C in a B.O.D. incubator.

For pot culture, a total of 12 pots (15 cm high, 13 cm diameter), each with 2 kg sandy loam soil were grouped into two sets of six pots each receiving on alternate days 250 ml either of distilled water or of the 10% effluent. Pre-soaked grains of *Triticum* were sown into soils prior to irrigation.

The soil was collected from agricultural fields which was old-alluvium with sandy loam texture, having on an average 0.8% organic carbon, 0.06% total nitrogen, 0.04% available phosphorus, 0.25% exchangeable calcium, 0.075% exchangeable magnesium, 0.24% exchangeable sodium, 0.06% exchangeable potassium, 0.07% chloride and 1.0 dsm^{-1} electrical conductivity.

Seed germination and seedling growth (upto 12 days) were studied in the two experiments.

2.3. Plant Analysis

Photosynthetic pigments and nutrient elements were determined in the leaves/shoots of the wheat seedlings grown in aqueous and soil culture, Chlorophyll a, b and carotenoids were extracted in 80% acetone and estimated spectrophotometrically (Arnon, 1949). Plant organic carbon was determined by rapid titration method (Walkley and Black, 1934). Total nitrogen was determined by conversion into ammoniacal form using modified Kjeldahl method (Lang, 1958) followed by colorimetric estimation using Nesseler's Reagent (Ballentine, 1957). For the analysis of phosphorus, calcium, magnesium, sodium and potassium, the plant materials were wet digested (Allen *et al.*, 1986). Phosphorus was analysed by molybdenum blue method, calcium and magnesium by EDTA method and exchangeable sodium and potassium were estimated by using flame photometer. Soluble chloride content in the soil, water and plant material was estimated after extraction by using standard $AgNO_3$ titration method (Allen *et al.*, 1986).

The data were subjected to t-test for testing the significance of differences in various parameters due to different treatments.

TABLE I

Chemical characterization of effluent water from sugar mill (10% concentration) used in the study

Characteristics	Parameter Value
pH	8.05 ± 0.022
Electrical Conductivity (ds m^{-1})	12.8 ± 0.0
Carbonate (meq/l)	1.5
Bicarbonate (meq/l)	8.5
Chloride (meq/l)	47.5
Sodium (mg/l)	3200
Potassium (mg/l)	40.48
Calcium (mg/l)	85.3 ± 0.27
Magnesium (mg/l)	78.1 ± 1.25
Lead	Nil
Sulphate (mg/l)	280
Total dissolved solids (mg/l)	2395
Total suspended solids (mg/l)	65
B.O.D (mg/l) (5 days at 20 °C)	74
C.O.D (mg/l)	142
Oil and Grease (mg/l)	7.5

3. Results and Discussion

3.1. Chemical Characterisation of the Effluent

Chemical analysis of the effluent (10% concentration) revealed slightly alkaline pH and high enrichment of the effluent with nutrients, as shown in Table I. Large amounts of the suspended, dissolved and volatile solids seem to be responsible for a high B.O.D and C.O.D of the effluent. The cationic concentration was quite high with exceptionally high concentration of sodium (3,200 ppm), which may be one of the prime pollutants in the sugarmill effluent. Amongst the anions, chloride and sulphate concentrations were high. Bicarbonate ions far exceeded the carbonate ions.

3.2. Germination and Seedling Growth

Effluent treatment did not have any adverse effect on percent seed germination of *Triticum vulgare*, but there was a reduction in seedling growth in both aqueous and soil medium (Table II). Effluent treatment caused 61 to 70% reduction in root and shoot length in aqueous medium, whereas the inhibitory effect was less in soil, showing a reduction of 21 to 24%, as compared to control. Likewise, the

TABLE II

Germination and seedling growth of *Triticum vulgare* (12-day old) in response to sugarmill effluent treatment

	Aqueous		Soil	
	Control	10% effluent	Control	10% effluent
Germination (%)	100±0.0[a]*	100±0.0[a]	100±0.0[a]	98±0.8[a]
Root length (cm)	10.5±1.1[a]	3.8±0.9[b]	9.8±0.9[a]	7.5±1.1[a]
Shoot length (cm)	10.8±0.7[a]	4.2±1.3[b]	12.6±0.8[a]	9.9±1.2[a]
Biomass** shoot (g)	0.14±0.02[a]	0.096±0.02[a]	0.16±0.025[a]	0.134±0.024[a]
Root (g)	0.08±0.01[a]	0.05±0.008[a]	0.12±0.15[a]	0.09±0.16[a]

* Values bearing the same alphabetic subscript for individual parameter do not differ significantly ($P > 0.05$) for t-values.
** Biomass represents the average dry weight of five plants.

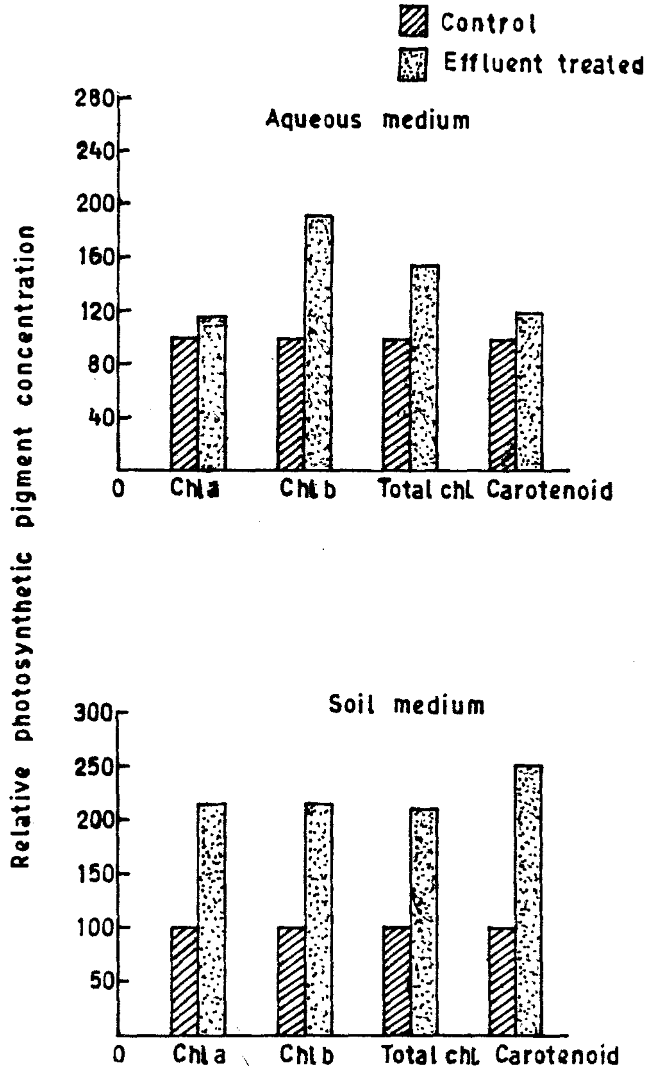
reduction in plant biomass due to effluent treatment was more (31 to 38%) in aqueous medium than that in soil (16 to 25%). The differences in the length of shoots and roots in control Vs. effluent treatment were statistically significant only in aqueous medium ($P < 0.05$) but not in soil. Biomass of shoots and roots were not affected significantly due to effluent as shown by t-test (Table II).

Excessive amounts of various ions in the effluent retarded seedling growth in wheat. However, the inhibitory action of the effluent was less when applied to soil which can be attributed to the buffering capacity of the soil. The organocolloids in soils are known to chelate many ions (Marshall, 1973) thereby binding many pollutants, which in the aqueous medium were in free soluble form and thus quite reactive.

3.3. Photosynthetic Pigments

Chlorophyll a, b and carotenoids studied in the leaves of 5, 7, 9 and 12-day old seedlings showed greater pigment concentrations in the leaves when grown in effluent. The relative concentrations of these pigments in 12-day old seedling of *Triticum* are shown in Figure 1. In aqueous medium, the effluent treatment stimulated chl, a, chl. b and carotenoid contents in the leaves by 17, 92 and 20%, respectively, whereas in the effluent treated soil there was about 2 to 2.5 times increase in pigment concentration of the leaves, as compared to control.

The relative increase in chl. a, chl. b and carotenoids in response to the effluent treatment varied in aqueous and soil medium resulting in different pigment ratios (Table III). In aqueous medium, the effluent caused greater increase in chl. b as



'ig. 1. Relative Photosynthetic Pigment concentrations in the leaves of wheat seedlings under ugar-mill effluent treatment (10% concentration) in aqueous and soil medium (pigment concentration n respective control taken as 100).

ompared to chl. a, thereby causing a drift in the pigment ratios in control vs. ffluent treated seedlings. However, the ratios were either identical or showed ninor variations in soils, indicating more uniform increase in all the pigment oncentrations in response to effluent irrigation. Similar increases in photosynthetic igments have been reported in response to fertilizer factory effluent (Sahai and ahai, 1988). The presence of some essential nutrients in abundance in the effluent eems to play a role in increasing the pigment concentrations. Various ions have

TABLE III

Pigment ratios in the leaves of 12 day old seedlings of *Triticum* in response to sugar mill effluent in aqueous and soil medium

Pigment ratio	Aqueous medium		Soil medium	
	Control	10% effluent	Control	10% effluent
Chl. a/chl. b	1.19	0.72	0.76	0.76
Chl. a/Total Chl.	0.54	0. 42	0.43	0.43
Chl. b/Total Chl.	0.46	0.58	0.57	0.57
Chl. a/carotenoids	138.05	132.6	111.8	108.6
Chl. b/carotenoids	116.1	185.1	147.1	140.8

TABLE IV

Average nutrient uptake by wheat seedlings grown in aqueous and soil culture (effluent concentration, 10%)

Nutrient	Uptake (mg/plant)			
	Aqueous culture		Soil culture	
	Control	Effluent	Control	Effluent
Organic carbon	19.47^{a*} $(100)^{**}$	13.9^{b} (71.4)	31.5^{c} (100)	27.4^{d} (86.9)
Available phosphorus	0.011^{a} (100)	0.014^{b} (127)	0.016^{b} (100)	0.023^{c} (143)
Chloride	0.44^{a} (100)	0.74^{b} (168)	1.28^{c} (100)	1.08^{d} (84)
Calcium	0.12^{a} (100)	0.22^{b} (183)	0.31^{c} (100)	0.42^{d} (135)
Magnesium	0.29^{a} (100)	0.27^{a} (93)	0.51^{b} (100)	0.50^{b} (98)
Sodium	0.70^{a} (100)	2.92^{b} (417)	1.17^{c} (100)	3.0^{b} (175)
Potassium	1.55^{a} (100)	1.67^{b} (107)	3.55^{c} (100)	1.87^{b} (53)
Nitrogen	0.32^{a} (100)	0.25^{b} (78)	0.54^{c} (100)	0.47^{d} (89)

* Values bearing the same alphabetic subscript for individual parameter do not differ significantly ($p < 0.05$), based on t-test.
** Figures in parentheses represent relative values (as % of control).

been reported to influence the bondage between pigment-protein-lipid complex in the chloroplast structure (Strogonov *et al.*, 1970) and the structure of chl. a is more labile than chl. b, and hence, differential effects are seen.

3.4. Nutrient Uptake

The effluent treatment caused considerable differences in the uptake of various nutrients by the seedlings in both aqueous and soil medium the statistical significance of which is shown in Table IV. Though differing in magnitude, the pattern of increase or decrease in the nutrients was mostly similar in both aqueous and soil medium. The decrease in carbon, nitrogen and magnesium uptake by the wheat seedlings due to the sugar-mill effluent treatment was more marked in aqueous medium as evident from the relative values of uptake (71, 78 and 93 in aqueous vs. 87, 89 and 89 in soil medium: Table IV). The uptake of calcium and sodium was very high in effluent treated seedlings both in aqueous medium (0.22 and 2.92 mg/plant) and in the soil (0.42 and 3.0 mg/plant), showing relatively greater uptake in aqueous effluent medium. The increase in phosphorus uptake due to the effluent was on the other hand, more marked when applied to soil. The pattern of uptake of potassium and chloride differed in aqueous and soil medium. While the effluent treated plants showed 7% increase in potassium and 68% increase in chloride uptake, their uptake was decreased by 47 and 16% respectively, in soil.

Overstreet and Dean (1973) reported increased availability of phosphates in the presence of soluble salts and organic matter which are found to be high in the sugar-mill effluent. Excessive uptake of sodium and calcium by the seedlings in the nutrient rich effluent medium is comparable to the behaviour of many plants exposed to high salt concentrations which is attributed usually to the osmoregulatory adaptive response of plants under high salinity (Kaushik, 1990) and that is obtained at the cost of growth (Sinha *et al.*, 1986). Reduced growth and biomass of the plants under salt stress due to extra expenditure of energy for active ion uptake and oxidation of the photosynthetic assimilates (Bernstein, 1975) explains as to why in spite of greater concentration of photosynthetic pigments in the seedling, the growth and dry matter production of the wheat seedlings were reduced under effluent treatment.

The soil used for experimentation showed a high concentration of exchangeable sodium and low potassium concentration, a situation met commonly in the soils of semiarid regions and the effluent used also had a higher concentration of sodium than potassium. Thus, the plants were exposed to high sodium stress in the medium. Reduced uptake of potassium in comparison to sodium seems to be due to competitive inhibition of K^+ by high Na^+ concentration in the medium.

In semi-arid regions like the present site at Rohtak, where economic use of water is warranted, diluted effluents of sugar-mills may be used for irrigation. The present study shows that the pollutants present in the effluent get buffered in the soil to an extent that no significant adverse effect on seedling growth is observed. However, sprinkling irrigation with effluent is not proposed since direct contact of the effluent with plant surfaces may be harmful to the seedlings.

References

Ajmal, M., Khan, M. A., and Nomani, A. A.: 1984, *Environ. Pollut. (Series A)* **33**, 97.
Ajmal, M., and Khan, A. U.: 1985, *Environ. Res.* **38**, 248.

Allen, S. E., Grimshaw, H. M., and Rowland, A. P.: 1986, in Moore, P. W., and Chapman, S. B. (eds.), *Chemical Analysis, in Methods in Plant Ecology*, Blackwell Scientific Publication, Oxford, pp. 285–344.
Arnon, D. I.: 1949, *Plant Physiol.* **24**, 1.
Ballentine, R.: 1957, *Methods Enzymol.* **3**, 984.
Behera, B. K., and Mishra, B. N.: 1982, *Environ. Res.* **28**, 10.
Bernstein, L.: 1975, *Annu. Rev. Phytopathol.* **13**, 295.
Fayez, M., and Shahin, R. R.: 1987, *Z. Pflanzenernaehr, Bodenkd.* **150**(4), 220.
Kaushik, A.: 1990, *Flora* **184**, 263.
Lang, C. A.: 1958, *Ann. Chem.* **30**, 1692.
Marshall, C. E.: 1973, in Truog E. (ed.), *Mineral Nutrition of Plants*, Oxford and IBH Publishing Co., N. Delhi, 57.
Michael, P.: 1986, *Ecological Methods for Field and Laboratory Investigation*, Tata McGraw Hill Publishing Co. Ltd., N. Delhi, pp. 95–130.
Overstreet, R., and Dean, L. A.: 1973, in Truog E. (ed.), *Mineral Nutrition of Plants*, Oxford and IBH Publishing Co., N. Delhi, 79.
Sahai, N., and Sahai, R.: 1988, *J. Environ. Biol.* **9**(1), 45.
Sinha, A., Gupta, S. R., and Rana, R. S.: 1986, *Int. J. Biometeor.* **28**, 147.
Strogonov, B. P., Kabanov, V. V., Shevyakova, N. I., Lapina, L. P., Kainizerko, E. M., Popov, B. A., Dostanova, R. K. H., and Prinkholko, L. S.: 1970, *Structure and Function of Plant Cells in Saline Habitats. New Trends in the Study of Salt Tolerance*, Israeli Program for Scientific Translations (English), Jerusalem.
Sutton, A. L., Nelson, D. W., Mayrose, V. B., and Nye, J. C.: 1978, *J. Environ. Qual* **7**(3), 325.
Walkley, A., and Black, I. A.: 1934, *Soil Sci.* **37**, 29.