Int. J. Chem. Sci.: 6(4), 2008, 2088-2098

PHYTOTOXIC STRESS OF TREATED DISTILLERY EFFLUENT ON SEEDLING GROWTH AND CHLOROPHYLL CONTENT OF *ORYZA SATIVA* L

M. A. ALI KHAN* and RUBY RANI

Environmental Science Lab., Department of Botany, Kisan P.G. College,
SIMBHAOLI – 245207, Ghaziabad (U. P.) INDIA

ABSTRACT

A study was carried out to observe the phytotoxic stress of distillery effluent on the seed germination of *Oryza sativa* L. The study revealed that 25 % concentration of effluent promoted seed germination and seedling growth of two cultivars of *Oryza sativa* cv. Pusa-44 and Saka-4. The higher concentration of effluents retarded not only seed germination but also seedling growth. Emergence index, vigour index, germination relative index, phytomass and chlorophyll content were found significantly higher in 25 % concentration than 50 % and 100 % of distillery effluent over control. It is recommended that 25 % distillery effluent may be recycled as ferti-irrigation for sustainable agriculture in Indian scenario for environmental conservation.

Key words: Distillery effluent, Phytotoxic stress, Ferti-irrigation, Sustainable agriculture, *Oryza sativa* L.

INTRODUCTION

After extraction of alcohol in molasses based distilleries, a tremendous amount of distillery effluent is discharged @ 15 litre/L of alcohol. Rapid industrial growth leads to considerable pollution problems. Distillery effluent discharged into fresh water bodies depleted dissolved oxygen of these water resources and thereby the aquatic life is lost[1] and caused **ecological time bomb** in river and ground water[2].

After the treatment of spent wash, with a proper dilution and systematic application it would not cause any harm to soil and in some cases, the nutrients which are exhausted by the crop would be brought back to soil[3]. The encouraging characteristic of distillery

* Author for correspondence Tel. : 0122-2306128, Mob. : 09837043647
E-mail : maalikhan@rediffmail.com

effluent is that its composition rate in soil is four times faster than farm yard manure (FYM) and it has exorbitant load of secondary nutrient like Ca, Mg and S, so it has ameliorate potential in the reclamation of sodic soil[4]. Soundarrajan et al.[5] studied the effect of distillery spent wash on seed germination, seedling growth and yield of Bhendi [*Abelmoschus esculentus* (L.)].

In recent years, use of distillery effluent for ferti-irrigation in agriculture after treatment is a favourable approach because effluent contains macro and micronutrients which are useful for growth of crops and as 'Complehumus'[6]. It serves as an additional source of organic fertilizer for agricultural use and is the safest and cheapest disposal for a safer environment along with superior quality of agricultural production[4].

Main objective of the present investigation is to determine the efficient management of treated distillery effluent in Indian scenario. Keeping this in central core, detailed laboratory experiments were conducted on environmental physiology of seedling growth.

EXPERIMENTAL

Materials and methods

Distillery effluent was collected from distillery division of S.S. Mills, Simbhaoli (Ghaziabad) U.P. Partially treated effluent was diluted with distilled water in 4 treatments in three replications as under :-

T_1 = 25 % distillery effluent + 75 % distilled water

T_2 = 50 % distillery effluent + 50 % distilled water

T_3 = 100 % distillery effluent

C = Distilled water

Seeds of *Oryza sativa* L. var. cv. Pusa-44 and Saka-4 were procured from Indian Agricultural Research Institute, New Delhi. Seeds were surface sterilized with 0.1 % mercuric chloride ($HgCl_2$) and 95 % alcohol for 1 and 2 minutes, respectively. Seed germination percentage and seedling growth were recorded. Chlorophyll contents were estimated following the method of Smith and Benitez[7].

Seed viability was tested with the help of tetrazolium chloride (TTC) test following Thakur et al.[8]. Seeds selected were uniformly healthy and viable for short term exposure experiment. Sterilized seeds were germinated in petriplates (10 cm in diameter) lined with Whatman No. 1 filter paper discs. 10 seeds were arranged equidistantly in concentric ring in petriplates.

10 mL of prepared solutions of each treatment were poured gently to petriplates twice weekly and emergence index, vigour index, phytotoxicity % and germination relative index (G.R.I.) were observed.

Germination relative index (G.R.I.) has been calculated as per the following equation :

$$\text{G.R.I.} = (S)\ Xn\ (K-n) \qquad \ldots(1)$$

Where Xn = No. of germinated seeds on n^{th} day

K = Total number of seeds

n = Number of days

Emergence index and vigour index were performed according to ISTA[9].

Emergence Index = dn/n

Where dn = Number of seeds germinated on a particular day

n = Days of emergence

Vigour index = Germination percent x shoot length.

Phytotoxicity percentage was determined following Chou and Muller[10] as per following :

Chlorophyll was estimated as per method given by Smith and Benitez[7].

Data were computed and statistically scrutinized for C.D. at 5 % level.

RESULTS AND DISCUSSION

Physico-chemical and biological characteristics of treated distillery effluent from distillery were analysed (Table 1).

Table 1: Physico-chemical characteristic of spent wash, post methanated effluent (PME) treated distillery effluent.

Parameters	Range values	PME
pH	3.9-4.3	7.8
EC (ds/m^{-1})	30.5-45.2	14
Biochemical Oxygen Demand (BOD)	46100-96000	5000 mg/L
Chemical Oxygen Demand (COD)	79000-87990	11200 mg/L
Total Dissolved Solid (TDS)	1660-4200	28000 mg/L
Total Solids (TSS)		2100 mg/L
Nitrogen (N_2)	1660-4200	2160
Phosphorus (P_2O_5)	225-3038	22-30
Potassium (K_2O)	9600-17475	4000-6000
Calcium	2050-7000	200
Magnesium	1715-2100	178
Sodium	492-670	40
Sulphate	3240-3425	255
Chloride	7238-42096	685
SAR	5.0-7.3	328
Zinc (ppm)	3.5-10.4	4.42
Copper (ppm)	0.4-2.1	0.52
Manganese (ppm)	4.6-5.1	4.38
Gibberellic Acid	3245-4943	0.63
Indole acetic acid	25-61	0.46

* All values are in mg/L.

Table 2 : Effect of different conc. of distillery effluents on seedling growth of Oryza sativa L. cv. Saka-4 and cv. Pusa-44.

Particulars	DAS	Cultivars	Control	Treated distillery effluent		
				25 %	50 %	100 %
Germination (%)	3	Pusa-44	10.00	30.00**	30.00**	0.00
		Saka-4	60.00	60.00	50.00	0.00
	5	Pusa-44	60.00	70.00**	30.00	10.00
		Saka-4	70.00	80.00	80.00**	20.00
	7	Pusa-44	70.00	80.00**	40.00	20.00
		Saka-4	80.00	90.00**	80.00	30.00
	10	Pusa-44	80.00	80.00	40.00	30.00
		Saka-4	80.00	90.00	90.00	50.00
Length of radicle plant^{-1} (cm)	5	Pusa-44	3.10	3.40	1.80	0.50
		Saka-4	3.20	4.20	2.40	0.80
	10	Pusa-44	3.90	5.30	3.20	1.80
		Saka-4	6.00	6.30*	4.20	3.10
	15	Pusa-44	6.20	7.30*	5.30	4.20
		Saka-4	7.40	7.80	6.80	5.00
Length of plumule plant^{-1}	5	Pusa-44	3.80	4.60	2.40	1.00
		Saka-4	4.50	6.30	3.20	1.50
	10	Pusa-44	5.40	7.20	5.50	2.50
		Saka-4	8.20	8.80	6.80	4.20
	15	Pusa-44	7.90	10.50**	7.80	5.40
		Saka-4	10.50	11.20	10.20	6.30
Phytomass of radicle plant^{-1}	15	Pusa-44	4.70	8.50**	4.00	2.30
		Saka-4	6.70	9.70	7.70	6.60

* CD at 5 % level; ** CD at 1 % level.

Cont…

Table 2 : Effect of different conc. of distillery effluents on seedling growth of Oryza sativa L. cv. Saka-4 and cv. Pusa-44.

Particulars	DAS	Cultivars	Control	Treated distillery effluent		
				25 %	50 %	100 %
Phytomass of plumule plant^{-1}	15	Pusa-44	5.30	6.00	2.70	2.40
		Saka-4	7.70	8.30**	6.80	3.10
Phytotoxicity % of root	5	Pusa-44	-	-9.67	41.93	83.87
		Saka-4	-	-31.25	25.00	75.00
	10	Pusa-44	-	-35.89	17.94	180.00
		Saka-4	-	-5.00	30.00	48.33
	15	Pusa-44	-	-17.74	14.51	32.25
		Saka-4	-	-5.40	8.10	32.43
Phytotoxicity % of shoot	5	Pusa-44	-	-21.05	36.84	73.68
		Saka-4	-	-40.00	28.89	66.67
	10	Pusa-44	-	-33.40	-1.85	53.70
		Saka-4	-	-7.31	17.07	48.78
	15	Pusa-44	-	-32.91	1.26	31.64
		Saka-4	-	-6.67	2.85	40.00
Emergence index	I	Pusa-44	3.00	1.00	1.00	0.00
		Saka-4	6.00	5.00	6.00	0.00
	II	Pusa-44	1.50	3.00	1.00	1.00
		Saka-4	1.50	2.50	1.00	2.00

* CD at 5 % level; ** CD at 1 % level.

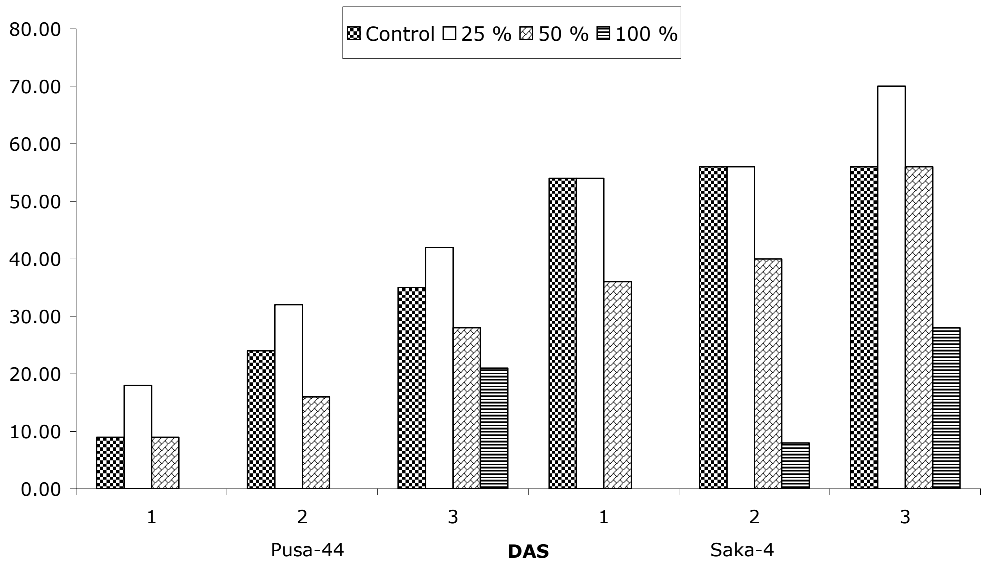

Fig. 1: Effect of distillery effluent on germination relative index of *Oryza sativa* L. cv. Saka-4 and cv. Pusa-44

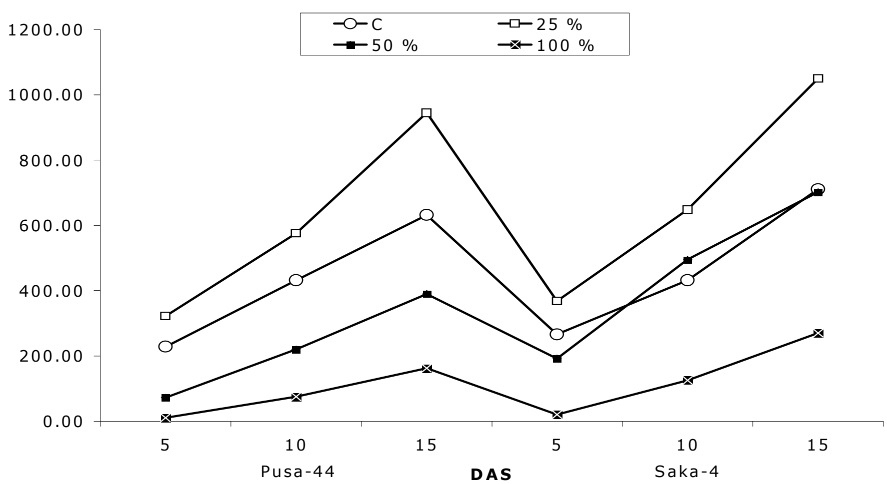

Fig. 2: Effect of distillery effluent on vigour index of *Oryza sativa* L. cv. Saka-4 and cv. Pusa-44.

The results of treated distillery effluent on germination percentage and seedling growth of two cultivars of *Oryza sativa* L. were observed. Germination % was higher at 25

% in cv. Pusa-44 (80 %) and Saka-4 (90 %) than 50 % in cv Pusa-44 (40 %) and Saka-4 (90 %), at 100 % in cv. Pusa-44 (30 %) and Saka-4 (50 %) over control cv. Pusa-44 (80 %) and Saka-4 (80 %). The germination relative index (Fig.1) and vigour index (Fig. 2) emergence index followed the same trends (Table 2). Similar observation were Observed by Pereira et al.[11] and Om et al.[12].

Experiment revealed that phytomass of radicle in cv. Pusa-44 increased at 25 % (8.50 mg), 50 % (4 mg), 100 % (2.30 mg) than control (4.7 mg) and it also increased in cv. Saka-4 at 25 % (9.7 mg) over 50 % (7.7 mg) and 100 % (6.6 mg) control (6.7 mg).

Phytomass of plumule cv. Pusa-44 increased at 25 % (6.0 mg) 50 % (2.7 mg), 100 % (2.4 mg) than control (5.3 mg) and Saka-4 increased at 25 % (8.3 mg), 50 % (6.8 mg), 100 % (3.11 mg) over control (7.7 mg) (Table 2).

Higher phytotoxicity % have been observed in 100 % effluent due to accumulation of salts like chloride from treated distillery effluent in solution and the seeds might have suffered salt (effluent) stress induced by high osmotic presence and corresponding low water potential. The seeds also might have suffered due to chloride ion toxicity. Among the treatments, 25 % diluted effluent recorded the highest plumule height (10.5 cm) followed by 50 % (7.8 cm), 100 % (5.4 cm) and control (7.90 cm). Higher concentration of distillery effluent decreased the height of *Oryza sativa* L. cv. Pusa-44. The major cause for inhibiting the growth was higher osmotic potential due to higher salt concentration in the rhizosphere but diluted effluent did not show stress. Results are in line with the findings of Farooqui[13].

The toxic effect of the distillery effluent is diluted at the lower concentrations reflected with increase in plumule length whereas the undiluted effluent though having abundant nutrient is quite toxic presenting the seedling growth and increasing trend of shoot and root growth have been marked from 3rd day to 10th days plant, which has negative effect as compared to that of control except 25 % distillery effluent. Our observations further support strongly to the findings of Naik et al.[14], Srikantha et al.[15] that concentration 25 % effluent significantly increased the plumule length as compared to higher concentrations (100 %), which has decreased significantly due to phytotoxic stress.

Experimental results revealed that chlorophyll content was recorded higher in 25 % than 50 % and 100 % concentration over control (Fig. 3). Higher concentrations of effluent decreased chlorophyll ‘a’ and ‘b’ contents. This was due to inhibition of enzyme concerned with chlorophyll biosynthesis. Similar results were reported by Mohite and Shingte[16], Devarajan et al.[3] and Bera and Kamta Bokaria[17].

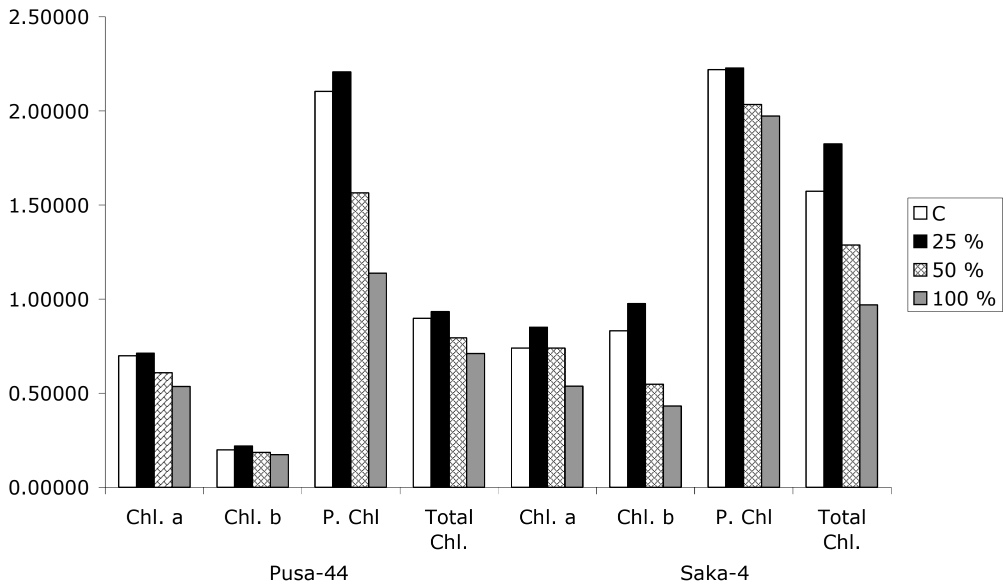

Fig. 3: Effect of distillery effluent on chlorophyll pigment content of *Oryza sativa* L. cv. Saka-4 and cv. Pusa-44.

From this study, it is suggested that direct use of distillery effluent could cause statistically a significant reduction on seed germination, growth and chlorophyll of rice crop. The undiluted effluent is not suitable or toxic for irrigation since low germination growth of seedling were noticed. Hence, 25 % concentration can be utilized as ferti-irrigation for sustainable agro-development in Indian scenario.

CONCLUSION

Thus, it is concluded that distillery effluent has phytotoxic stress on seedling growth and chlorophyll contents at higher concentrations but contains higher levels of soluble salts and rich in plant nutrients. The beneficial effect of distillery effluent on crop production was exerted only at lower rate of application. However, exceptionally high organic loading is likely to diminish surface and ground waters, destruction of aquatic life and pose serious threat to sustenance of soil and environmental health. Technologies based on scientific experimentation are needed for effectively utilizing this valuable resource in agriculture without any environmental hazards. Therefore, long term field experiments are needed to confirm these results with safer disposal cum utilization of distillery effluent for sustainable agriculture.

ACKNOWLEDGEMENT

We are grateful to Mr. I. S. Bhatia, G. M. of S. S. Mills, Simbhaoli (Ghaziabad) and members of Environmental Science Laboratory, Department of Botany, Kisan (P.G.) College, Simbhaoli and research scholars, who helped us in present investigation, without which it was not possible to gratinate it.

REFERENCES

1. Shukla, Pollution Control in the Sugar Industry, Treatment and Disposal of Factory and Distillery Effluent. Sugarcane Agro-Industrial Alternatives (1992).
2. M. A. Ali, Environmental Pollution due to Distillery Effluent Waste Management and Protection. Association for Advancement of Modeling and Stimulation Technique in Enterprise Periodical, Lune, France (1990).
3. L. Devarajan, G. Rajannan and G. Oblisami, Effect of Distillery Effluent Irrigation on Soil Properties, Yield and Quality of Oil Seed Crops. in National Seminar on Application of Treated Effluent for Irrigation, held at REC, Tiruchirappali, Mar. 23, (1988) p. 19.
4. M. A. Ali Khan, and P. Kaushik, Ferti-Irrigation Effects of Treated Spent Wash (Liquid Gold) on Saccharum Officinarum L. (Sugarcane) and Sustained Soil Health, Local Action for Restoration of Majestic Ganga river. (In press) (2007).
5. G. Soundarrajan, P. James and T. S. Kumar, Effect of Distillery Spent Wash on Seed Germination, Seedling Growth and Yield of Bhendi (Abelmoschus esculentus L.) Moench. Adv. Plant Science, **20 (I),** 141-143 (2007)
6. J. H. Pavgi and R. Paode, Production of Biofertilizer from Distillery Spent Wash. Report DSI, Pune (INDIA) (1986).
7. J. H. C. Smith and A. Benitez, Modern Methods of Plant Analysis (K. Peach and M. V. Iracey). Springer Heidelbergedi, **III,** (1955) pp. 142-192.
8. A. Thakur, R. Mehta and P. S. Thakur, Germination, Viability and Vigour of Fresh and Aged Seeds of Some Endangered Medicinal Plant Species of Western Himalayas, Indian J. Plant Physiol., **9(3),** 247-254 (2004).
9. ISTA International Rules for Seed Testing. Proc. Int. Seed Test Assco., **31,** 1-52 (1976).

10. C. H. Chou and C. H. Muller, Alleophathic Mechanism of Archtostaphylous Glandulosa var. Zazaensis, Am. Mid. Nat., **88,** 324-347 (1972).

11. J. P. Pereira, E. M. Alvarenga, J. R. P. Tortes and L. E. F. Fontes, Effect of Application of Different Rates of Vinasse a Dystrophic Raddish, Yellow Latosol on the Germination and Vigour of Maize Seeds. Revista Brasileva de Sementes, **14 (2),** 147-150 (1992).

12. H. Om, N. Singh and M. S. Aryo, Combined Effect of Waste of Distillery and Sugar Mill on Seed Germination, Seedling Growth and Biomass of Okra [Abelmoschus esculentus (L.)] Mocuch. J. Environ. Biol., **15,** 171-175 (1994).

13. A. Farooqui, Effect of Tannery Effluent on Growth Morphology and Yield of Lens cullinaris medic., Madras Agri. J., **(81) 1,** 26-28 (1994).

14. L. Naik, J. Hota, and I. P. Nanda, Effect of Dairy Effluent on Seed Germination and Seedling Growth of Doliohos bioflorus L. New Botanica, **31 (1-2),** 29-32 (1995).

15. K. Srikanta,, C. A. Srinivasamurthy and G. C. Sathis, Effect of Undiluted Dairy Effluent on Yield and Nutrient Composition of Two Vegetable Crops (French bean and Amaranthus). Indian J. Environ. Hlth., **40 (3),** 232-239 (1998).

16. A. V. Mohite and A. K. Shingte, Use of High Salt Water Dilution Method in Conjunction with Farm Yard Manure and Gypsum for Reclamation of Sodic Soils. J. Indian Soc. Sci., **29,** 512-517 (1981).

17. A. K. Bera and Kamta Bokaria Effect of Tannery Effluent on Seed Germination, Seedling Growth and Chlorophyll Pigment Content in Mungbean (Vigna Radiata L Wilczerk), Environ. Eco., **17 (4),** 958-961 (1990).

Accepted : 30.06.2008