Bull. Environ. Contam. Toxicol. (1989) 42:187–194

Bulletin of Environmental Contamination and Toxicology

Long–Term Effects of Land Application of Aqueous Oil Effluent on Photosynthetic Efficiency of Certain Varieties of *Oryza sativa* L.

K. Ilangovan and M. Vivekanandan

Department of Botany, Bharathidasan University, Tiruchirapalli 620 024, Tamilnadu, India

Effluents discharged from industries form the main sources of organic and inorganic pollutants in the environment. When untreated effluents are discharged into the environment, they disrupt the ecological niches of fauna and flora. Bharat Heavy Electricals Limited(BHEL), at Tiruchirapalli, Tamilnadu (one of the largest boiler plants in India) releases a huge volume of aqueous oil effluent through a drainage canal which empties into a huge reservoir 3 km east of the factory premises. From the reservoir, the effluent is directly used for cultivation of paddy in about 25 acres. The physico-chemical characteristics of the effluent have already been determined by Ilangovan and Vivekanandan (1987). The effluent composition is mainly of hydrocarbons, phenols and trace elements like Cd, Pb, Cu, Zn, Al and Cr. Since in many plants anions and cations at high concentration induce serious biochemical and physiological disorders (Behera and Misra 1983; Foy et al. 1978), an attempt is made in the present study to characterise the toxic effect of the aqueous oil effluent on photosynthetic efficiency of five varieties of *Oryza sativa* L. (CR-1009, ADT-36, IR-50, IR-20 and Ponni). In addition, all the rice varieties were screened for shoot and root tolerance indices to aqueous oil pollution.

MATERIALS AND METHODS

Aqueous oil effluent was collected in a 5 litre polyethylene container at the region of entry of the effluent into the rice field. Physico-chemical parameters were analysed using sensitive methods of APHA (1978). Total hydrocarbons were extracted from soil samples (0-5 cm depth) and the effluent using CCl_4 and quantification of hydrocarbons was done by Infrared Spectrophotometer (Perkin-Elmer) (CONCAWE 1972). The rice varieties, ADT-36, CR-1009, Ponni, IR-50 and IR-20 were used in the present experiment. The rice seedlings were raised in the field under natural photo-period, day temperature of 28-35°C and night temperature of 22-26°C, employing aqueous oil effluent.

For control, rice seedlings were cultivated using pollution free water. The rice seedlings at 4-leaf stage were transplanted in the field in hills (3-5 seedlings=1 hill) and the planting density was 29 hills/m^2. Photosynthetic studies in rice varieties were carried out in 40-day old plants. Root (dry weight of roots grown with effluent/ dry weight of roots grown without effluent) shoot (dry weight of shoot grown with effluent/dry weight of shoots grown without effluent) tolerance indices were calculated following the method of Taylor and Foy (1985). Five grams of leaf bits were homogenised in a prechilled pestle and mortar with 20 ml of isolation medium containing 330 mM sorbitol, 30 mM Mes, 2 mM ascorbate and 0.1% BSA adjusted to pH 6.5 with Tris. The brei was squeezed through double layer of cheese cloth and the filtrate was centrifuged at 1200 g for 1 min at 4°C. The pellet was washed twice with the same isolation medium. The final pellet was resuspended in 6 ml of 330 mM sorbitol, 30 mM Hepes and 0.2% BSA adjusted to pH 7.6 with Tris. O_2 evolution was measured polarographically with Hansatech O_2 electrodes. The basic assay medium contained 330 mM sorbitol, 2 mM EDTA, 1 mM $MnCl_2$, 1 mM $MgCl_2$,50 mM Hepes KOH, pH 7.6. The percentage of intact chloroplasts was determined by measuring O_2 evolution with FeCN before and after osmotic shock (Lilley et al. 1975). The rate of electron transport from water to MV was determined with intact chloroplasts (20 µg Chl/ml) plus 0.1 mM MV, 0.5 mM KCN, and 10 mM NH_4Cl. Electron transport from water to FeCN was measured with osmotically shocked chloroplasts (20 µg Chl/ml) plus 10 mM DL-glyceraldehyde, 1 mM FeCN, and 10 mM NH_4Cl. CO_2-dependent O_2 evolution was measured with intact chloroplasts (40 µg Chl/ml) plus 4 mM $NaHCO_3$, 0.3 mM Pi and 1000 units/ml catalase. Leaf photosynthesis, diffusive resistance and transpiration were analysed using portable Infrared Gas Analyser (IRGA) and steady state porometer (Licor Inc, USA).

RESULTS AND DISCUSSION

Physico-chemical properties of the aqueous oil effluent and soil are given in Table 1. The effluent was deficient in dissolved oxygen and contained large amounts of suspended solids which were responsible for the high BOD and COD. The effluent and soil contained significant concentrations of oil compounds and the results presented in Table 1 indicate the presence of trace elements in soil and water. The levels of trace elements in the soil and effluent are beyond the prescribed maximum contaminant level (US EPA 1973; Page 1974). Since most of the trace elements tend to accumulate in the soil, the levels of trace

elements in the receiving soil should be substantially elevated year after year by the long-term use of the

Table 1. Physico-chemical characteristics of aqueous oil effluent and soil (The results are means of three different experiments.)

Samples	pH	DO	BOD	COD	Total hydro-carbon	Pb	Cd	Cu	Zn	Cr	Al
	← mg/ℓ				→	← µg/ℓ					→
Pollution free water	7.2	8.4	16	31	ND	32	8.0	42	131	ND	ND
Aqueous oil effluent	6.2	2.1	128	278	760	254	274	2110	1860	140	170
					← mg/kg						→
Normal soil	7.4				ND	56.00	0.28	12.40	32.00	ND	4.7
Oil-polluted soil	6.5				1640	248.00	12.65	106.50	143.00	17.00	81.6

ND: Not detectable

aqueous oil effluent. Disposal of oily wastes to lands alters physico-chemical processes of soil; and may prevent water percolation (Collins 1983). The oil not only perculates into the soil and injures the rice plant by adhering to the root system, but also covers the surface of the rice field as a thin mat (Ilangovan and Vivekanandan, *unpublished*) and thereby prevents copious oxygen supply in the effluent and the soil.

Since the aqueous oil effluent is continuously used for irrigating *Oryza sativa* L., a preliminary study has been carried out to assess the most suitable tolerant variety by using shoot tolerance (STI) and root tolerance indices (RTI) (Table 2). Of all the rice varieties tested,

Table 2. Root and shoot tolerance indices of five varieties of *Oryza sativa* L. grown in aqueous oil effluent

Rice varieties	Mean STI ± SD	Mean RTI ± SD
ADT-36	0.97 ± 0.05	0.94 ± 0.02
CR-1009	0.91 ± 0.03	0.93 ± 0.06
Ponni	0.58 ± 0.03	0.61 ± 0.01
IR-50	0.38 ± 0.10	0.51 ± 0.01
IR-20	0.36 ± 0.06	0.39 ± 0.02

ADT-36 and CR-1009 showed highest STI and RTI. Tables 3 and 4 present the results of photosynthetic efficiency of five varieties of *Oryza*. The CO_2-dependent O_2 evolution in the isolated chloroplasts of 40-day old plants in the following rice varieties IR-50, IR-20 and Ponni decreased by 42-55% whereas in CR-1009 and ADT-36 the decrease was by 19-30% as compared to the control.

The rate of electron transport from $H_2O \rightarrow$ FeCN in chloroplast of IR-50, IR-20 and Ponni was reduced by 35-48% over the control but only marginal reduction was observed in CR-1009 and ADT-36 (18-20%). These results suggest that the aqueous oil effluent primarily inhibits the oxygen evolving capacity of isolated chloroplasts as well as electron transport system in IR-50, IR-20 and Ponni varieties significantly.

Leaf photosynthesis as measured by CO_2 uptake using IRGA

Table 3. Photosynthetic characteristics of leaves from five varieties of *Oryza sativa* L. grown in polluted and control soils (The data are mean values ± SD for five examples. All the analyses were carried out using portable IRGA and steady state porometer, Licor Inc, USA.)

	ADT-36		CR-1009		Ponni		IR-50		IR-20	
	Control	Polluted	Control	Polluted	Control	Polluted	Control	Polluted	Control	Polluted
Leaf photo-synthesis ($mg\ CO_2/m^2/s$)	0.265 ±0.07	0.198 ±0.04	0.276 ±0.08	0.212 ±0.03	0.219 ±0.03	0.117 ±0.07	0.238 ±0.04	0.148 ±0.06	0.219 ±0.03	0.149 ±0.01
Diffusive resistance (s/cm)	2.300 ±0.01	1.803 ±0.10	2.100 ±0.06	1.706 ±0.06	1.750 ±0.08	1.561 ±0.04	2.400 ±0.40	1.750 ±0.14	1.491 ±0.01	1.041 ±0.06
Transpiration ($\mu g/cm^2/s$)	14.51 ±1.01	18.40 ±0.91	13.10 ±0.31	18.40 ±0.46	17.40 ±0.04	16.80 ±0.37	14.60 ±0.14	19.05 ±0.14	17.92 ±0.16	23.40 ±0.08

Table 4. Effect of aqueous oil effluent on photosynthetic properties of chloroplasts isolated from five varieties of *Oryza sativa* L. (The rates of electron transport and CO_2-dependent O_2 evolution are expressed in μ mol O_2/mg chl/h .Values are means of three different experiments.)

Property	ADT-36		CR-1009		Ponni		IR-50		IR-20	
	Control	Polluted	Control	Polluted	Control	Polluted	Control	Polluted	Control	Polluted
Electron transport										
$H_2O \rightarrow$ FeCN	538	446	614	495	440	238	573	301	473	310
$H_2O \rightarrow$ MV	613	521	680	610	516	414	610	418	584	378
CO_2-dependent O_2 evolution	146	121	178	126	108	63	176	81	134	74

in ADT-36 and CR-1009 was reduced only marginally (24-26%) whereas in Ponni, IR-50, and IR-20 rice varieties 32-47% of reduction was observed. There was no significant difference in diffusive resistance and transpiration among all the rice varieties investigated. Therefore, actual rate of photosynthesis by the plant may be reduced by the cumulative effects of trace elements as well as oil in the effluent. The decrease in CO_2 uptake may be due to decreased rates of electron transport in rice varieties like IR-20, IR-50, and Ponni.

The results of the present study suggest that long-term application of aqueous oil effluents in the field enhanced not only the concentration of trace elements but also enriched the oil content making the soil unsuitable for cultivation. However, rice varieties like CR-1009 and ADT-36 are tolerant in such soils as growth characteristics and photosynthetic capacity are only least affected, whereas that of Ponni, IR-50 and IR-20 are drastically affected.

Acknowledgements. Financial assistance provided by the Council of Scientific and Industrial Research (CSIR), New Delhi, in the form of a senior research fellowship to the first author is gratefully acknowledged.

REFERENCES

APHA (1978) Standard methods for the examination of water and waste water. 15th edition, APHA, AWWA, WPCF

Behera BK, Misra BN (1983) Analysis of the effect of industrial effluent on pigments, proteins, nucleic acids, and the 2,6-dichlorophenol indophenol Hill reaction of rice seedlings. Environ Res 31:381-389

Collins CM (1983) Long-term active layer effects of crude oil spilled in interior Alaska. In Permafrost, Fourth International Conference Proceedings, National Academy Press, Washington, DC 175-179

CONCAWE (1972) Methods for the analysis of oil in water and soil. CONCAWE report 9/72; 1-8

Foy CD, Chaney RL, White MC (1978) The physiology of metal toxicity in plants. Ann Rev Plant Physiol 29:511-566

Ilangovan K, Vivekanandan M (1987) Chemical analysis of oil-polluted water and its suitability for agricultural purposes. In National Seminar on Estuarine Management Proceedings, State Committee on Science and Technology, Trivandrum, India (in press)

Lilley RMcC, Fitzgerald MP, Rienits KG, Walker DA (1975) Criteria of intactness and the photosynthetic activity of spinach chloroplast preparations. New Phytol 75:1-10

Page AL (1974) Fate and effects of trace elements in sewage sludge when applied to agricultural soils. A literature review study. EPA 670/2-74-005 USEPA, Cincinnati, Ohio
Taylor GJ, Foy CD (1985) Mechanisms of aluminium tolerance in *Triticum aestivum* L. (Wheat). II. Differential pH induced by spring cultivars in nutrient solutions. Amer J Bot 72(5):702-706
USEPA (1973) Water quality criteria. 1972 EPAR3.73.033. Ecological Research Series, EPA, Washington, DC
Received May 20, 1988; accepted August 19, 1988.