Asian Journal of Water, Environment and Pollution, Vol. 9, No. 2 (2012), pp. 61–71.

Effect of Rice Mill Wastewater on Soil Respiration and Enzyme Activities under Field and Pot Conditions

A. Padhan and S.K. Sahu*[1]

Environmental Engineering Division, Department of Chemistry, ITER
SOA University, Jagamora, Bhubaneswer – 751030, Orissa, India
[1]P.G. Department of Environmental Sciences (Auto.), Sambalpur University
Jyoti Vihar - 768019, Orissa, India
✉ sahusanjat@rediffmail.com

Received April 17, 2010; revised and accepted March 19, 2012

Abstract: The physico-chemical characteristics of rice mill wastewater was measured and the effect of rice mill wastewater on respiration and enzyme activities (amylase, invertase, protease and dehydrogenase) of rice cropped soil was investigated under field and pot conditions at 15 days interval for 90 days. For the pot experiment five different concentrations (0, 25, 50, 75 and 100%) of rice mill wastewater was used. The results of the field experiment revealed significant difference in the soil respiration and enzyme activities rates of control and experimental plots from 30 days onwards. After 90 days the soil respiration and enzyme activities were inhibited by about 25-34% in experimental plots, whereas the results of the pot experiment revealed a maximum increase of about 36% in soil respiration and 24-45% in enzyme activities (amylase, invertase, protease and dehydrogenase) in 50% wastewater irrigated soil and a maximum decrease of about 40% in soil respiration and 24-40% in enzyme activities in 100% wastewater irrigated soil. The adverse effects of rice mill wastewater (100%) on soil respiration and enzyme activities were attributed to alkaline pH (8.0) of wastewater with higher contents of phenols (35 $mg^{-1}l$), silica (58 mg^{-1} l) and sodium (235 $mg^{-1}l$). Significant increase in soil respiration and enzyme activities at lower concentrations (i.e. 50%) may be due to the fact that, the above parameters being in the diluted form could favour the microflora to boost their activities. On the basis of above findings, we suggest that the rice mill wastewater should be diluted up to 50% before use for agricultural purpose. However, further works on the effect of rice mill wastewater on different crops and soil animals are needed to corroborate the present findings.

Key words: Rice mill wastewater, soil respiration, amylase, dehydrogenase, invertase, protease, enzyme activities.

Introduction

A large and diverse group of microorganisms, such as bacteria, fungi, actinomycetes and yeast etc. are present in soil. These microorganisms dominate both in numbers and biomass in most of the terrestrial ecosystems of the world (Tiwari et al., 1989). They form the primary mediator of several basic ecological processes, such as biogeochemical cycling of element; in the formation of organic matters, by chemo and photosynthesis; in the mineralization of carbon, nitrogen, phosphorous, sulphur and other element necessary to maintain the fertility of soil, and in the decomposition plant and animal residues (Bernhart and Vestan, 1983). Assessment of microbial activity through soil respiration and enzyme activities are very often used to reveal the functional role of microbial component in relation to organic matter input, energy flow and rate of mineralization (Macfadyen, 1970; Kiss et al., 1975; Roberts and Chenu, 1992).

*Corresponding Author

Measurement of CO_2 evolution from soil is a common measure of soil respiration and this represents sum total of all soil metabolic activities (Lundergardh, 1927; Witkamp, 1966). Origin of enzymes in the soil is chiefly due to the vital activities of microorganisms living in it (Skujins, 1978; Burns, 1982). Amylase, invertase, protease and dehydrogenase are some of the important enzymes in soil, which are partially responsible for the rate and course of decomposition of plant and animal tissue (Pancholy and Rice, 1973; Makoi and Ndakidemi, 2008). Amylase and invertase belong to the glycoside-hydrolase group of enzymes and are chiefly supplied by microorganisms and plant roots respectively (Ross, 1976). Protease is chiefly produced by microorganisms and catalyses hydrolytic dissociation of protein to chemically simpler unit called amino acid, and therefore plays a leading role in regulating the dynamics of available form of nitrogen in soil (Speir and Ross, 1975; Mishra et al., 1979). Dehydrogenase, belongs to the class oxidoreductase, is an intracellular enzyme and mainly linked with microbial respiratory process and estimation of this enzyme gives an insight into the endogenous soil microbial activity (Ross, 1970; 1971). Now-a-days microbial activities of soil are largely disturbed due to the contamination of soil with varieties of solid and liquid pollutants (Mishra and Pradhan, 1987; Gong et al., 1997; Tam, 1998; Panda and Sahu, 2000; Pati and Sahu, 2004). Wastewater discharged from industries interact with soil microorganisms as soon as they enter the soil ecosystem and change metabolic activities and functions of soil microorganisms depending on the type of industry, nature of raw materials used and manufacturing processes involved (Hodges, 1973; Mishra and Sahoo, 1989).

Although a great deal of information has been accumulated on the effect of effluent of industries like tannery, olive mill, distillery, pulp and paper mill, sugar mill, petrochemical and galvanizing factory on soil microbial population and metabolic activities (Danana et al., 1985; Perez et al., 1992; Cox et al*.,* 1997; Panda, 2001; Dee et al., 2003, Hamida, 2005), little is available at present regarding wastewater of rice mill industries. Therefore, activities of soil enzymes along with soil respiration was undertaken in the present work to assess the effect of rice mill wastewater (or effluent) on soil metabolic activities under both field and pot conditions.

Material and Methods

For field and pot experiment the wastewater of a nearby rice mill having milling capacity 10 MT/day was used. The physicochemical characteristic of the wastewater was analyzed following the procedures recommended by APHA (1989) and values have been given in Table 1.

Experimental Design for Field Study

For field study an upland, uncontaminated field of 30 m × 30 m area was selected near the rice mill. Eight equal plots of 5 m × 5 m size were prepared and the distance between individual plots were kept 10 m apart to maintain hydrological isolation of plots and drains. Four plots were identified as control plots and other four plots as experimental plots. The control and experimental plots had initially the following soil characteristics: laterite type, sandy loam by texture, pH 6.79, organic matter 3.9 (g%), nitrogen 0.2 (g%) and C/N ratio 11.5. The control plots were irrigated with normal canal water of river, the Mahanadi, whereas the experimental plots were irrigated with wastewater from the rice mill. Twenty days old Jaya T_{90} variety of rice saplings having cropped during 120 to 130 days was collected from a nearby Agricultural Research Station at Chakuli and cropped in the present study. The saplings were planted during IV week of January immediately after preparation of the plots. Three saplings were planted in a single hill, at 15 cm gap in between both rows and columns. Normal canal water and rice mill wastewater were made available to the control and experimental plots respectively throughout the cropping period except planting and pre-harvesting period (Grist, 1953). Harvesting was done during the II week of May. Soil respiration and enzyme activities (amylase, invertase, protease and dehydro-genase) measurements from control and experimental plots were conducted at 15 days interval up to 90 days i.e. during the period between IV week of January and IV week of April. Four random soil samples of 100 g each were collected from 0-10 cm depth during each sampling occasion for measurement of enzyme activities, whereas for measurement of soil respiration four glass jars were laid down randomly in the control and experimental plots. The CO_2 evolution was recorded in situ following the method of Witkamp (1966).

Experimental Design for Pot Study

Twenty cemented pots of 40 cm (b) × 40 (l) × 80 (h) cm were used for the pot experiment. Pots were filled with 10 kg of soil each, collected from the upland non-irrigated paddy field, where field experiment was initiated. Different concentrations (0, 25, 50, 75 and 100%) of rice mill wastewater were prepared in dilution with water supplied from river, the Mahanadi, and irrigated to the pots before plantation of rice saplings. For each concentration four replicates were kept. Twenty days old

Table 1: Physico-chemical characteristics of the effluent of a rice mill at Sambalpur, Orissa with their maximum permissible limits as recommended by Indian Standard Institution

Parameters	*Range*	*Mean ± SD*	*ISI limit for discharge of industrial effluents*	
			On land for irrigation (ISI, 1977)	*Into inland surface waters (ISI, 1974)*
Colour		Brown	-	-
Odour		Unpleasant	-	-
Temperature (°C)	35.0-48.0	38.0±5.09	-	40
Conductivity (m mho cm^{-1})	0.46-0.86	0.66± 0.15	-	-
pH	7.2 - 8.8	8.0±0.54	5.5-9.0	5.5-9
Total solids (mg l^{-1})	998.1-1459.1	1200.0±189.48	-	-
TSS (mg l^{-1})	432.5-576.0	530.0±53.00	100	100
TDS (mg l^{-1})	522.1-883.1	670.0±149.2	2100	2100
Dissolved oxygen (mg l^{-1})	0.2-1.6	0.9±0.52	-	-
BOD at 20°C (mg l^{-1})	312.1-540.1	450.0±76. 61	100	30
COD (mg l^{-1})	400.2-892.1	630.0±183.03	-	-
Total alkalinity (mg l^{-1})	180.7-340.1	272.0±58.29	-	-
Total hardness (mg l^{-1})	98.3-256.4	182.0±59.84	-	-
Ca hardness (mg l^{-1})	38.4-98.3	78.0±22.22	-	-
Mg hardness (mg l^{-1})	14.1-24.3	21.0±3.68	-	-
Chloride (mg l^{-1})	95.1-170.3	140.0±28.06	600	1000
Sulphate (mg l^{-1})	28.4-70.1	40.0±15.66	1000	1000
Phosphate (mg l^{-1})	10.1-35.2	21.0±11.11	-	-
Nitrate (mg l^{-1})	0.3-0.8	0.5±0.15	-	-
Sodium (mg l^{-1})	213.4-263.7	235.0±20.34	60%	-
Potassium (mg l^{-1})	14.1-32.1	20.0±7.12	-	-
Phenols (mg l^{-1})	13.3-50.4	35.0±13.98	-	1.0
SiO_2 (mg l^{-1})	35.4-75.1	58.0±15.5	-	-

Jaya T_{90} variety of rice saplings was also planted in the pots with three saplings in each pot. Different concentrations of rice mill effluent were maintained throughout the cropping period except planting and pre-harvesting like the field experiment. The experiment was carried out during the month of winter (December-February) when prevailing atmospheric temperature was between 10-30°C. Soil respiration and enzyme (protease, dehydrogenase, amylase and invertase) activities were measured from each replicate at an interval of 15 days up to 90 days.

Measurement of Soil Respiration

Soil respiration was measured by alkali absorption technique (Witkamp, 1966) using glass jars, each of 15 cm (length) × 15 cm (width) × 25 cm (height) in size. The soil was exposed to 20 ml of 0.1N KOH in airtight glass jars for 2hours. After incubation the KOH solution was removed, precipitated with saturated solution of $BaCl_2$ to form barium carbonate ($BaCO_3$) and the unspent KOH was titrated with an equivalent strength of HCl using phenolphthalein indicator. A jar without soil, containing the same amount of KOH was also run simultaneously as control. The evolved CO_2 was expressed in mg CO_2 g $soil^{-1}$ h^{-1}.

Measurement of Enzyme Activities

Soil protease activity was determined following the method of Speir and Ross (1975). Two gram of fresh soil was taken and to it 0.2 ml of toluene and 10 ml of Tris-HCl buffer (0.1M, pH 8.1) containing 1% sodium caseinate was added. Then the whole mixture was incubated for 2 hrs. After incubation 4 ml of aqueous solution of trichloroacetic acid (17.5% wv^{-1}) was added to the mixture and centrifuged. The supernatant (2 ml) was taken and treated with 1.4 M sodium carbonate solution (3 ml) followed by 1 ml Folin-ciocalteu reagent (33.3% wv^{-1}). The blue colour developed was read after 30 minutes at 700 nm in a spectrophotometer (Systronics-106). A blank was run simultaneously taking Tris-HCl buffer without sodium caseinate. Tyrosine was used as standard. The activity was expressed in mg tyrosine g $soil^{-1}$ h^{-1}.

Dehydrogenase activity was determined by tri phenyl formazan method (Casida et al., 1964). Two gram of fresh soil was taken and to it 2 ml of 1% solution of 2,3,

5-triphenyl tetrazolium chloride (TTC) and 0.5 ml of 1% (wv^{-1}) glucose was added. The mixture was incubated at 37°C for 24 hours. The triphenyl formazan (TPF) formed from the reaction mixture was extracted with methanol and the resulting pink colour was measured at 485 nm in a spectrophotometer. Dehydrogenase activity was expressed in mg formazan g $soil^{-1}$ h^{-1}.

Invertase and amylase activity was determined by using 3,5-dinitrosalicyclic acid (Mishra et al., 1979). Soil samples were incubated with substrate (sucrose for invertase and starch for amylase) and Sorensen's buffer at 35°C for 24 hours and then centrifuged. A suitable aliquot of the supernatant was heated with 3, 5-dinitrosalicylic acid and the colour was measured at 540 nm in a spectrophotometer. Both the enzyme activities were recorded in mg glucose g $soil^{-1}$ h^{-1}.

One-way analysis of variance (ANOVA) was used to determine significant differences in the data. Least significance difference (LSD) test was used for multiple comparisons when differences were found by one-way ANOVA. Two-way ANOVA was also used to determine significant differences between the days and concentrations (Snedecor and Cochran, 1967).

Results

Physico-chemical Characteristics of Rice Mill Wastewater

In the present investigation the wastewater of rice mill industry showed an alkaline pH (8.0) with low concentration of DO (0.9 mgl^{-1}), nitrate (0.5 mgl^{-1}), phosphate (21 mgl^{-1}) and sulphate (40 mgl^{-1}); and moderate concentration of COD (630 mgl^{-1}), chloride (140 mgl^{-1}) and TDS (670 mgl^{-1}). The total suspended solids (530 mgl^{-1}) and BOD (450 mgl^{-1}) were much higher than the recommended standard set by ISI (1974, 1977) for the discharge of industrial effluent into inland surface waters as well as on land for irrigation, which indicate the presence of high amount of organic matter in the effluent (Table 1). Moreover, the wastewater was rich in sodium (235 mgl^{-1}), total phenols (35 mgl^{-1}) as well as silica (58 mgl^{-1}). The higher concentration of sodium in the wastewater may be due to the ingress of domestic sewage of the workers into the discharge outlet of the rice mill wastewater. The higher values of phenolic compounds and silica in the wastewater is perhaps because of boiling and cleaning operations involved during the processing of raw paddy. A detailed description of process details and effluent characteristics of rice mill has been given in the earlier report of Padhan and Sahu (2004).

Field Experiment

The rate of respiration (mg CO_2 g $soil^{-1}$ h^{-1} ± SD) of the control and experimental plots prior to irrigation was 7.12 ± 0.512. In control soil the rate of respiration went on increasing, showing a peak value of 21.73 ± 1.98 after 60 days and thereafter it remained more or less constant. In experimental plots, the respiration rate was also accelerated up to 60 days but it followed a steady decrease thereafter. Significant difference in the rate of respiration of control and experimental plots was, however, noticed after 30 days ($t \geq 3.58$, $P < 0.05$) and it continued up to the end of the experiment. After 90 days the rate of respiration was found to be inhibited by 34.28% in experimental plots as compared to control plots (Table 2).

The protease (mg tyrosine g $soil^{-1}$ h^{-1} ± SD), dehydrogenease (µg formazan g $soil^{-1}$ h^{-1} ± SD) and amylase and invertase (mg glucose g $soil^{-1}$ h^{-1} ± SD) activities of the soil was initially 16.68±1.73, 15.98 ± 1.56, 32.15±3.15 and 41.74±3.23 respectively. Like soil respiration, all the enzyme activities increased up to 60 days in both experimental and control plots. The peak values of four enzymes also remained more or less constant thereafter in control plots, whereas in experimental plots the values of enzymes were found to be declined. Comparison of protease, dehydrogenase, amylase and invertase activity between control and experimental plots revealed significant inhibition of enzyme activities in experimental plots throughout the study period (Table 2). After 90 days the enzymes activities were found to be inhibited by about the 25-33%.

Pot Experiment

The rate of respiration (mg CO_2 g $soil^{-1}$ h^{-1} ± SD) of soil was initially 6.01± 0.553. An increase in soil respiration was noticed up to 60 days in control soil. The same trend was also observed in 25, 50, 75 and 100% rice mill effluent treated soil (Table 3). But when comparison was made over control after 90 days, the soil respiration was found to be enhanced by 14.95 and 35.84% in 25 and 50% effluent irrigated soil and decreased by 23.97 and 40.41% in 75 and 100% effluent irrigated soil respectively (Figure 1). Two-way ANOVA indicated significant difference in soil respiration rate with respect to different days as well as concentrations ($F_1 = 19.14$, $F_2 = 22.56$, $P < 0.05$). Segmental analysis of soil respiration by one- way ANOVA also showed significant difference with respect to concentration from 15 days onwards ($F \geq 6.66$, $P < 0.05$). During comparison of LSD (Least Significant Difference) between respiration rates

Table 2: Soil respiration and enzyme activities of soil irrigated with rice mill wastewater under field conditions

Days	*Soil respiration (mg CO_2 g $soil^{-1}$ h^{-1})*			*Soil enzyme activities*											
				Protease (mg tyrosine g $soil^{-1}$ h^{-1})			*Dehydrogenase (µg formazan g $soil^{-1}$ h^{-1})*			*Amylase (mg glucose g $soil^{-1}$ h^{-1})*			*Invertase (mg glucose g $soil^{-1}$ h^{-1})*		
	C	*E*	*'t'*	*C*	*E*	*'t'*	*C*	*E*	*'t'*	*C*	*E*	*'t'*	*C*	*E*	*'t'*
0	7.12 ±0.512	7.12 ±0.512	-	16.68 ±1.73	16.63 ±1.73	-	15.98 ±1.56	15.98 ±1.56	-	32.15 ±3.15	32.15 ±3.15	-	41.74 ±3.23	41.74 ±3.23	-
15	8.98 ±0.768	8.65 ±0.576 (3.67)	0.972 NS	24.59 ±1.99	22.32 ±1.98 (9.23)	2.28 NS	25.21 ±2.35	22.75 ±2.76 (9.75)	1.91 NS	52.78 ±4.93	49.34 ±3.99 (6.51)	1.62 NS	60.23 ±4.93	5723 ±3.75 (4.98)	1.37 NS
30	14.38 ±1.12	12.36 ± 1.132 (14.04)	3.58*	33.54 ±2.95	30.12 ±2.76 (10.19)	2.39 NS	30.04 ±2.95	26.13 ±2.65 (13.01)	2.78*	87.27 ±6.52	75.68 ±5.93 (13.28)	3.71*	90.27 ±8.77	77.54 ±6.99 (14.1)	3.21*
45	18.44 ± 1.34	15.29 ±1.21 (17.08)	4.93*	45.79 ±3.76	37.23 ±2.39 (18.69)	5.43*	40.83 ±3.75	32.97 ±2.62 (19.25)	4.85*	98.68 ±7.77	83.32 ± 5.99 (15.56)	4.42*	112.38 ±10.83	86.55 ±7.68 (22.98)	5.5*
60	21.73 ±1.98	17.33 ±1.33 (20.24)	5.21*	48.13 ±4.12	39.59 ±3.33 (17.74)	4.55*	48.68 ±3.25	36.44 ±2.12 (25.14)	8.92*	106.73 ±9.23	79.33 ±6.25 (25.67)	6.95*	122.42 ±11.63	95.25 ±7.76	5.49* (22.19)
75	20.66 ±1.44	15.53 ±1.22 (24.83)	7.68*	45.53 ±3.67	35.39 ±2.34 (22.27)	6.58*	47.93 ±3.75	33.73 ±2.22 (29.62)	9.21*	92.23 ±8.73	66.55 ±5.23 (27.84)	7.13*	112.73 ±10.55	86.59 ±6.95 (23.18)	5.85*
90	19.98 ±1.33	13.13 ±1.25 (34.28)	10.61*	45.23 ±3.99	33.79 ±2.59 (25.29)	6.8*	45.93 ±3.11	30.88 ±2.98 (32.76)	9.88*	90.33 ±8.13	64.79 ±5.23 (28.27)	7.47*	111.53 ±8.43	77.27 ±5.35 (30.71)	9.7*

*$P < 0.05$, NS = Not significant, C = Control, E = Experimental

Values in parentheses denote percentage decrease over respective day's control.

Table 3: Effect of different concentrations of rice mill wastewater on soil respiration (mg CO_2 g $soil^{-1}$ h^{-1}±SD) under pot conditions

Days	*Control*	*Effluent treatment in soil*				*One-way ANOVA (F)*	*LSD ($P < 0.05$)*	*Two-way ANOVA*
		25%	*50%*	*75%*	*100%*			
0	6.01 ±0.553	6.01 ±0.553	6.01 ±0.553	6.01 ±0.553	6.01 ±0.553			
15	7.16^{acd} ±0.592	7.47^{abc} ±0.591	8.25^{b} ±0.653	6.85^{cd} ±0.469	6.35^{d} ±0.411	6.66*	0.825	
30	10.98^{a} ±0.573	11.99^{b} ±0.573	13.69^{c} ±0.777	9.58^{d} ±0.677	8.78^{d} ±0.46	39.22*	0.94	F_1= 19.14*
45	14.34^{a} ±0.486	15.98^{b} ±0.621	18.35^{c} ±0.683	12.18^{d} ±0.403	10.53^{e} ±0.392	134.82*	0.797	
60	18.99^{a} ±0.469	22.15^{b} ±1.32	25.87^{c} ±1.269	15.17^{d} ±0.496	11.65^{e} ±0.469	155.21*	1.35	F_2= 22.56*
75	17.25^{a} ±0.558	19.25^{b} ±0.901	23.78^{c} ±1.185	13.38^{d} ±0.583	10.49^{e} ±0.566	172.7*	1.2	
90	16.85^{a} ±0.648	19.37^{b} ±0.732	22.89^{c} ±0.959	12.81^{d} ±0.794	10.04^{e} ±0.472	191.22*	1.11	

* $P < 0.05$, F = Value of one-way ANOVA between concentrations, F_1 = Value of two-way ANOVA between days, F_2 = Value of two-way ANOVA between concentrations. Values in the same row with different alphabets were significantly different by LSD ($P < 0.05$).

in different days intervals, it was observed that after 30 days soil respiration significantly increased in 25 and 50% effluent treated soil and decreased significantly in 75 and 100% effluent treated soil.

The soil protease activity (mg tyrosine g $soil^{-1}$ h^{-1} ± SD) was initially 15.05 ±1.72. At the end of the experiment the protease activity was increased by 124.38, 171.49, 224.71, 90.89 and 38.73% in control, 25, 50, 75 and 100% effluent treated soil over 0 day (Table 4). But when comparison was made after 90 days over control, the protease activity was found to be increased by 20.99 and 44.71% in 25 and 50% effluent treated soil and decreased by 14.92 and 38.16% in 75 and 100% effluent treated soil (Figure 2). Statistical analysis by two-way ANOVA showed significant difference (F_1 = 31.6, F_2 = 13.88, $P < 0.05$) with respect to days and concentrations. One-way ANOVA test as well as LSD test showed significant difference ($P < 0.05$) with respect to concentrations from 30 days onwards till the end of the experiment.

The dehydrogenase activity at the start of the experiment was 14.98 ± 0.98 (µg formazan g $soil^{-1}$ h^{-1} ± SD). The dehydrogenase activity was maximum after 60 days and thereafter it declined in control as well as in

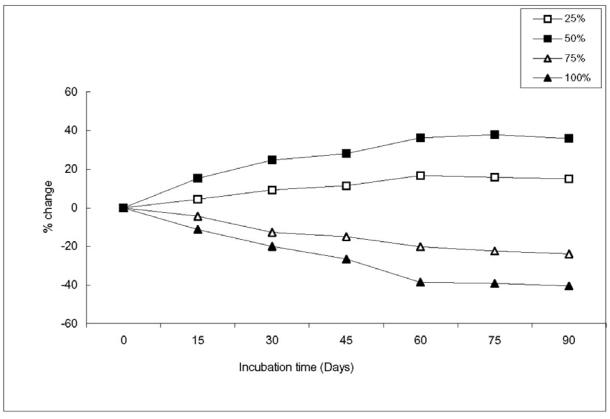

Figure 1: **Percentage change of soil respiration over control in different concentrations of rice mill wastewater treated soil.**

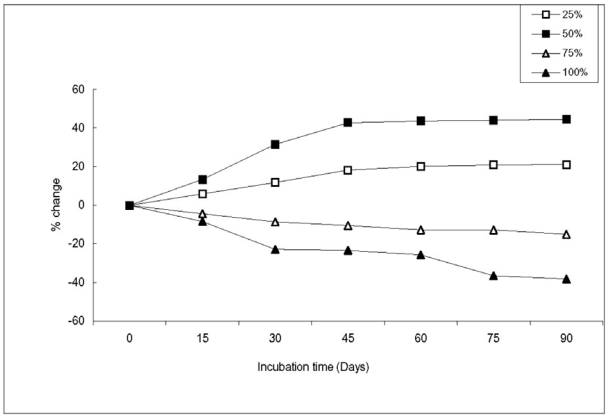

Figure 2: **Percentage change of protease activity over control in different concentrations of rice mill wastewater treated soil.**

Table 4: Effect of different concentrations of rice mill wastewater on protease activity (mg tyrosine g soil^{-1} h^{-1}±SD) under pot conditions

Days	*Control*	*Effluent treatment in soil*				*One-way ANOVA (F)*	*LSD (P < 0.05)*	*Two-way ANOVA*
		25%	*50%*	*75%*	*100%*			
0	15.05 ±1.722	15.05 ±1.722	15.05 ±1.722	15.05 ±1.722	15.05 ±1.722			
15	22.32^{ab} ±1.773	23.64^{ab} ±1.424	25.29^{a} ±2.546	21.34^{b} ±1.79	20.44^{c} ±1.624	1.43		F_1= 31.60*
30	31.54^{ac} ±2.25	35.23^{a} ±3.161	41.5^{b} ±3.367	28.82^{c} ±2.61	24.35^{d} ±1.493	23.84*	4.01	F_2= 13.88*
45	35.23^{ad} ±2.621	41.68^{b} ±3.221	50.3^{c} ±3.903	31.56^{d} ±2.398	26.99^{e} ±1.631	40.53*	4.307	
60	36.77^{a} ±2.391	44.18^{b} ±3.103	52.86^{c} ±3.575	32.12^{d} ±2.435	27.35^{e} ±1.748	54.74*	4.102	
75	34.25^{a} ±2.92	41.38^{b} ±2.79	49.35^{c} ±3.53	29.88^{d} ±2.04	21.73^{e} ±1.607	63.29*	4.01	
90	33.77^{a} ±2.061	40.86^{b} ±2.671	48.87^{c} ±3.075	28.73^{d} ±1.639	20.88^{e} ±1.873	86.2*	3.503	

* $P < 0.05$, F = Value of one-way ANOVA between concentrations, F_1 = Value of two-way ANOVA between days, F_2 = Value of two-way ANOVA between concentrations. Values in the same row with different alphabets were significantly different by LSD ($P < 0.05$).

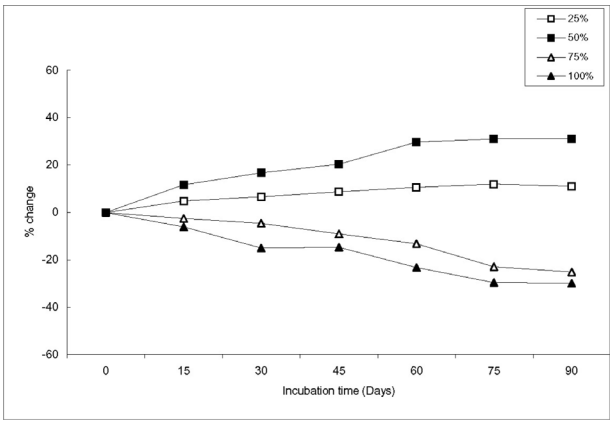

Figure 3: **Percentage change of dehydrogenase activity over control in different concentrations of rice mill wastewater treated soil.**

effluent treated soils. But when comparison was made with control, 11.0-31.0% increases in 25-50% effluent treated soil and 25-30% decreases in 75-100% effluent treated soil was noticed after 90 days (Figure 3). Two-way ANOVA test showed significant difference with respect to days and concentrations (F_1 = 18.52, F_2 = 21.39, $P < 0.05$). One-way ANOVA and LSD test revealed that the dehydrogenase activity was significantly higher from 30 days onwards in 50% effluent treated soil and significantly lower from 30 days onwards in 100% effluent treated soil (Table 5).

Amylase activity (mg glucose g soil^{-1} h^{-1} ± SD) was also recorded at 15 days interval up to three months. At the end of 90 days the amylase activity was enhanced by 220.59% over 0 day in control pot. During the same period increases in amylase activities were also recorded from 139.12 to 298.47% in 25 to 100% effluent irrigated soil (Table 6). But when comparison was made over control after 90 days the amylase activity was found to be enhanced by 7.98, 24.29% in 25 and 50% effluent irrigated soil and decreased by 11.72 and 25.41% in 75 and 100% effluent irrigated soil (Figure 4). Statistical analysis of data by one-way and two-way ANOVA showed significant difference in amylase activity with respect to different days and treatments from 30 days onwards (Table 6).

Invertase activity was increased by 100.76 to 231.54% in control and effluent treated soil at the end of 90 days. But when comparison was made with control soil the activity was increased by 6.97 and 25.37% in 25 and 50% effluent treated soil and decreased by 10.86 and 24.08% in 75 and 100% effluent treated soil after 90 days (Figure 5). Statistical analysis by two-way ANOVA test revealed significant difference with respect to days and concentrations (F_1 = 40.86, F_2 = 62.11, $P < 0.05$), whereas in one-way ANOVA test significant difference was noticed from 15 days onwards ($F \geq 9.47$, $P < 0.05$). LSD value showed significant difference from 30 days in all the concentrations (Table 7).

Table 5: Effect of different concentrations of rice mill wastewater on dehydrogenase activity (μg formazan g soil^{-1} h^{-1}±SD) under pot conditions

Days	*Control*	*Effluent treatment in soil*				*One-way ANOVA (F)*	*LSD (P < 0.05)*	*Two-way ANOVA*
		25%	*50%*	*75%*	*100%*			
0	14.98 ±0.982	14.98 ±0.982	14.98 ±0.982	14.98 ±0.982	14.98 ±0.982			
15	23.21^{abc} ±1.59	24.32^{ab} ±2.07	25.91^{a} ±2.047	22.57^{bc} ±1.694	21.77^{c} ±1.646	3.13*	2.744	F_1= 18.52*
30	30.04^{ac} ±2.76	32.01^{abc} ±2.879	35.06^{b} ±2.682	28.63^{cd} ±1.82	25.53^{d} ±1.536	8.89*	3.614	F_2= 21.39*
45	38.18^{ac} ±2.54	41.46^{a} ±3.218	45.94^{b} ±3.025	34.69^{cd} ±2.418	32.53^{d} ±1.584	16.65*	3.947	
60	44.68^{a} ±2.44	49.42^{b} ±3.178	57.92^{c} ±3.067	38.73^{d} ±2.45	34.29^{e} ±2.37	45.88*	4.102	
75	41.79^{a} ±2.848	46.75^{b} ±2.6	54.74^{c} ±3.452	32.19^{d} ±1.92	29.41^{d} ±1.839	64.13*	3.918	
90	40.99^{a} ±2.656	45.49^{b} ±2.978	53.72^{c} ±3.727	30.65^{d} ±2.388	28.73^{d} ±1.534	57.25*	4.146	

* $P < 0.05$, F = Value of one-way ANOVA between concentrations, F_1 = Value of two-way ANOVA between days, F_2 = Value of two-way ANOVA between concentrations. Values in the same row with different alphabets were significantly different by LSD ($P < 0.05$).

Table 6: Effect of different concentrations of rice mill wastewater on amylase activity (mg glucose g soil^{-1} h^{-1}±SD) under pot conditions

Days	*Control*	*Effluent treatment in soil*				*One-way ANOVA (F)*	*LSD (P < 0.05)*	*Two-way ANOVA*
		25%	*50%*	*75%*	*100%*			
0	30.21 ±2.24	30.21 ±2.24	30.21 ±2.24	30.21 ±2.24	30.21 ±2.24			
15	51.43^{ab} ±2.6	52.6^{a} ±2.70	54.23^{a} ±2.677	47.89^{bc} ±2.547	46.08^{c} ±2.12	7.05*	3.82	F_1= 30.85*
30	83.27^{a} ±3.77	86.71^{a} ±3.85	93.57^{b} ±4.578	76.44^{c} ±4.158	65.68^{d} ±3.045	29.45*	5.89	F_2= 48.66*
45	99.73^{a} ±4.489	106.44^{a} ±4.493	120.13^{b} ±5.166	89.99^{c} ±4.182	77.67^{d} ±4.161	50.97*	6.79	
60	103.28^{a} ±5.359	111.84^{b} ±5.613	129.19^{c} ±6.761	91.96^{d} ±3.954	79.16^{e} ±4.776	50.36*	8.09	
75	97.46^{a} ±3.781	105.54^{b} ±5.563	121.66^{c} ±5.559	85.62^{d} ±3.954	73.13^{e} ±3.664	65.58*	6.9	
90	96.85^{a} ±4.662	104.58^{b} ±5.23	120.38^{c} ±5.461	85.49^{d} ±4.213	72.24^{e} ±3.275	62.26*	6.98	

* $P < 0.05$, F = Value of one-way ANOVA between concentrations, F_1 = Value of two-way ANOVA between days, F_2 = Value of two-way ANOVA between concentrations. Values in the same row with different alphabets were significantly different by LSD ($P < 0.05$).

Discussion

Assessment of the effect of the pollutant on soil microorganism involves two approaches: (i) a direct study of the soil microbial population and biomass and (ii) a more efficient, indirect study of the soil metabolism through soil respiration and enzyme activities (Tam, 1998; Dee et al., 2003, Makoi and Ndakidemi, 2008). In the present study the assessment of the effect of rice mill effluent on soil microorganisms was done indirectly by studying the soil metabolism through soil respiration and enzyme activities. Our results showed a maximum increase of about 36.0% in soil respiration and 24-45% in enzyme activities (amylase, invertase, protease and

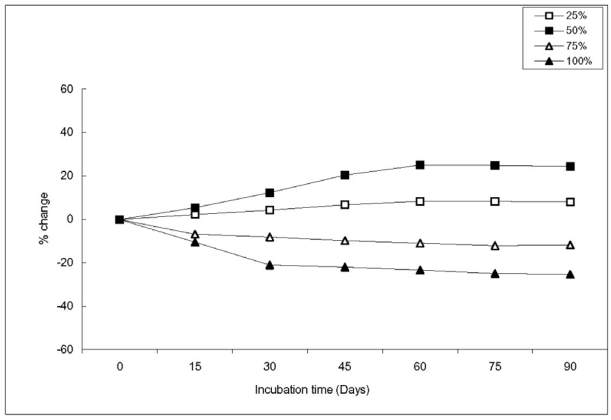

Figure 4: **Percentage change of amylase activity over control in different concentrations of rice mill wastewater treated soil.**

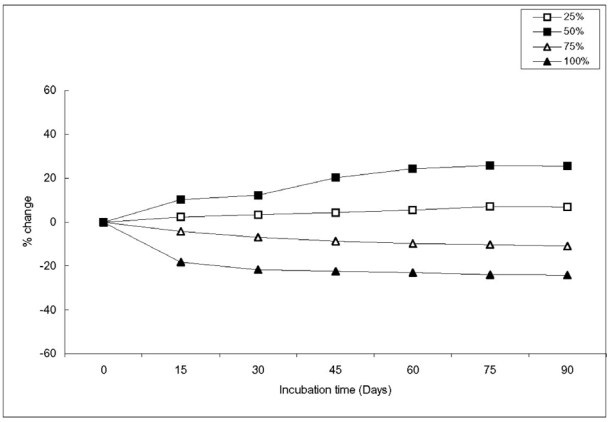

Figure 5: **Percentage change of invertase activity over control in different concentrations of rice mill wastewater treated soil.**

Table 7: Effect of different concentrations of rice mill wastewater on invertase activity (mg glucose g soil^{-1} h^{-1}±SD) under pot conditions

Days	*Control*	*Effluent treatment in soil*				*One-way ANOVA (F)*	*LSD (P < 0.05)*	*Two-way ANOVA*
		25%	*50%*	*75%*	*100%*			
0	40.64 ±3.41	40.64 ±3.41	40.64 ±3.41	40.64 ±3.41	40.64 ±3.41			
15	58.92^{ab} ±3.824	60.25^{ab} ±4.5	64.94^{a} ±4.351	56.46^{b} ±3.451	48.15^{c} ±3.811	9.47*	6.05	F_1= 40.86*
30	90.27^{a} ±3.998	93.34^{a} ±4.782	101.32^{b} ±4.565	83.96^{c} ±4.039	70.68^{d} ±3.898	28.91*	6.432	F_2= 62.11*
45	108.42^{a} ±5.583	113.2^{a} ±5.383	130.32^{b} ±8.687	98.98^{c} ±5.419	84.18^{d} ±4.429	31.67*	9.151	
60	116.35^{a} ±6.724	122.77^{a} ±6.429	144.63^{b} ±8.138	104.99^{c} ±6.417	89.54^{d} ±5.093	38.34*	9.98	
75	108.55^{a} ±6.737	116.31^{a} ±6.935	136.41^{b} ±6.163	97.34^{c} ±5.36	82.51^{d} ±3.633	47.28*	8.86	
90	107.47^{a} ±5.616	114.97^{a} ±5.014	134.74^{b} ±6.01	95.79^{c} ±4.966	81.59^{d} ±4.456	58.41*	7.89	

* $P < 0.05$, F = Value of one-way ANOVA between concentrations, F_1 = Value of two-way ANOVA between days, F_2 = Value of two-way ANOVA between concentrations. Values in the same row with different alphabets were significantly different by LSD ($P < 0.05$).

dehydrogenase) in 50% effluent irrigated soil and a maximum decrease of about 40.0% in soil respiration and 24-40% in enzyme activities in 100% effluent irrigated soil after 90 days.

The feasibility of industrial wastewater for irrigation depends on the several parameters like pH, EC, total solids (both suspended and dissolved) and total phenols (Dee et al., 2003). In light soil the parameters like organic solids increase the water holding capacity, silt and clay content, CEC and organic matter content. In heavy soil, organic solids clog capillary pores mainly in the upper soil layer and bring about a decrease in the rate of infiltration (Mishra and Sahoo, 1989). Organic solids also clog capillaries deeper in the soil profile, where under anaerobic condition decomposition of organic matter proceeds at a very low rate and makes the soil unfit for crop production (Gong et al., 1997). Further, the enrichment of phenols, sodium and silica may cause disruption in the population of microflora, leading to inhibition of soil microbial activities (Perez et al., 1992; Hamida, 2005). In the present study rice mill wastewater (100%) had alkaline pH (8.0) with higher contents of phenols (35 mg^{-1}l), silica (58 mg^{-1} l) and sodium (235 mg^{-1}l). This might be the reason for inhibitory activities

of microorganisms in 100% effluent irrigated soil. When the effluent was diluted to 50%, all the activities were found to be increased. It seems that effluent characteristics at 50% dilution are favourable to boost the microbial activities. This might be due to hormesis effect which can be explained as "stimulation of the effect when organisms are exposed to the lower concentrations of toxic substances, besides inhibiting the same at higher concentrations" (Calabrese and Blain, 2005). On the basis of above field and pot experiments we suggest that the effluent of rice mill should be diluted up to 50% before being used for irrigation in croplands. However, further works on the effect of rice mill wastewater on different crops and soil animals are needed to substantiate the present findings.

Acknowledgements

The authors are grateful to the Head, Department of Environmental Sciences, and Sambalpur University for providing the laboratory facilities and DST, Govt. of India for a FIST grant. Ms. Abanti Padhan is thankful to the UGC, New Delhi for financial assistance in the form of a research fellowship and ITER, SOA University for a placement.

References

APHA (1989). Standard methods for the examination of water and wastewater. 18th ed. American Public Health Association, Washington, D.C.

Bernhart, C.L.H. and R. Vestal (1983). Effects of environmental toxicants on metabolic activity of natural microbial communities. *Applied Environmental Microbiology*, **46:** 970-977.

Burns, R.G. (1982). Enzyme activity in soil: Location and possible role in microbial ecology. *Soil Biology and Biochemistry*, **14:** 423-427.

Calabrese, E. and R. Blain (2005). The occurrence of hormetic dose responses in the toxicological literature, the hormesis database: An overview. *Toxicology and Applied Pharmacology*, **202:** 289-301.

Casida, L.E. Jr., Klein, D.A. and T. Santoro (1964). Soil dehydrogenase activity. *Soil Science*, **98:** 371-376.

Cox, L., Celis, R., Hermosin, M.C., Becker, A. and J. Cornejo (1997). Porosity and herbicide leaching in soils amended with olive mill wastewater. *Agriculture Ecosystem and Environment*, **65:** 151-161.

Danana, G.H., Parekh, L.J. and C.V. Ramakrishnan (1985). Bacterial profile of petrochemical industry effluents. *Environmental Pollution (A)*, **39:** 27-37.

Dee, B.M., Haynes, R.J. and M.H. Graham (2003). Changes in soil acidity and the size and activity of the microbial biomass in response to the addition of sugar mill wastes. *Biology and Fertility of Soils*, **37:** 47-54.

Gong, P., Heng Sun, T., Beudert, G. and H. Hahn (1997). Ecological effects of combined organic or inorganic pollution on soil microbial activities. *Water, Air and Soil Pollution*, **96:** 133-143.

Grist, D.H. (1953). Rice. Longmans Green and Co., London.

Hamida, S.K. (2005). Fungal and Bacterial Populations in Cement-Incorporated Soil. *International Journal of Agriculture and Biology*, **7(2):** 158-161.

Hodges, L. (1973). Environmental Pollution. Holt, Rinehort and Winson Inc., New York.

ISI (Indian Standards Institution) (1974). Tolerance limit for industrial effluents discharged into inland surface waters. No. 2490, New Delhi.

ISI (Indian Standards Institution) (1977). Tolerance limit for discharge of industrial effluents on land for irrigation. No. 3307, New Delhi.

Kiss, S., Dragan-Bularda, M. and D. Radulescu (1975). Biological significance of enzymes in soil. *Advances in Agronomy*, **27:** 25-87.

Lundegardh, H. (1927). Carbon dioxide evolution of soil and crop growth. *Soil Science*, **23:** 417-453.

Macfadyen, A. (1970). Soil metabolism in relation to ecosystem, energy flow and to primary and secondary production. *In:* Method of study in soil ecology. J. Phillipson (ed.). IBP/UNESCO, Paris.

Makoi, J.H.J.R. and A. Ndakidemi (2008). Selected soil enzymes: Examples of their potential roles in the ecosystem. *African Journal of Biotechnology*, **7(3):** 181-191.

Mishra, P.C. and S.C. Pradhan (1987). Seasonal variation in amylase, invertase, Cellulose activity and carbon dioxide evolution in tropical grassland of Orissa, India, sprayed with carbaryl insecticide. *Environmental Pollution*, **43:** 291-300.

Mishra, P.C. and S. Sahoo (1989). Agropotentiality of paper mill wastewater. *In:* Soil Pollution and Soil Organisms. P.C. Mishra (ed.). Ashis Publ. House, New Delhi.

Mishra, P.C., Mohanty, R.K. and M.C. Dash (1979). Enzyme activity in subtropical surface soils under pasture. *Indian Journal of Agricultural Chemistry*, **12:** 19-24.

Padhan, A. and S.K. Sahu (2004). Process details and effluent characteristics of a rice mill in the Sambalpur district of Orissa. *Jr. of Industrial Pollution and Control*, **20(1):** 111-124.

Pancholy, S.K. and E.L. Rice (1973). Soil enzymes in relation to old-field succession: amylase, cellulase, invertase, dehydrogenase and urease. *Soil Science Society of America Proceedings*, **37:** 47-50.

Panda, R. (2001). Ecotoxicology of the effluent of a galvanizing factory on soil, *Oriya sativa*. *L.* (Rice plant) and *Drawida willsi Michaelsen* (Oligochaeta). Ph.D thesis, Sambalpur University, Orissa, India.

Panda, S. and S.K. Sahu (2000). Respiration and enzyme activities of soil following application of an organo-phosphorous insecticide. *International Journal of Ecology and Environmental Sciences*, **26:** 75-82.

Pati, S.S. and S.K. Sahu (2004). CO_2 evolution and enzyme activities (dehydrogenase, protease and amylase) of fly ash amended soil in the presence and absence of earthworms (*Drawida willsi* Michaelsen) under laboratory conditions. *Geoderma*, **118:** 289-301.

Perez, J., De la Rubia, T., Moreno, J. and J. Martizez (1992). Phenolic content and antibacterial activity of olive mill waste waters. *Environmental Toxicology & Chemistry*, **11:** 489-495.

Robert, M. and C. Chenu (1992). Interactions between soil minerals and microorganisms. *Soil Biochemestry*, **7:** 370-379.

Ross, D.J. (1970). Effect of storage on dehydrogenase activities in soils. *Soil Biology & Biochemistry*, **2:** 55-61.

Ross, D.J. (1971). Some factors influencing the estimation of dehydrogenease activities of some soil under pasture. *Soil Biology and Biochemistry*, **3:** 97-110.

Ross, D.J. (1976). Invertase and Amylase activities in rye grass and white clover plants and their relationship, with activities in soil under pasture. *Soil Biology and Biochemistry*, **8:** 351-356.

Skujins, J. (1978). History of abiotic soil enzymes. *In:* Soil Enzyme. R.G. Burns (ed.). Academic Press, London.

Snedecor, G.W. and W.G. Cochran (1967). Statistical methods. Iowa State University Press, USA.

Speir, T.W. and D.J. Ross (1975). Effect of storage on the activities of protease, urease, phosphatase and sulphatase in three soils under pasture. *New Zealand Journal of Science*, **18:** 231-237.

Tam, N.F.Y. (1998). Effects of waste water discharge on microbial population and enzyme activities in mangrove soils. *Environmental Pollution*, **102:** 233-242.

Tiwari, S.C., Tiwari, B.K. and R.R. Mishra (1989). Microbial community enzyme activity and carbon dioxide evolution in pineapple orchard soil. *Tropical Ecology*, **30(2):** 265-273.

Witkamp, M. (1966). Rate of carbon dioxide evolution from the forest floor. *Ecology*, **47:** 492-494.

Asian Journal of Water, Environment and Pollution

Volume 9 Number 1 **January – March, 2012**

Contents