EFFECT OF RICE MILL WASTEWATER ON GROWTH, REPRODUCTION AND METABOLISM OF *DRAWIDA WILLSI* (OLIGOCHAETA) UNDER LABORATORY CONDITIONS

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ABSTRACT

The effect of rice mill wastewater on the growth, reproduction and metabolism of *Drawida willsi*, Michaelsen earthworm was studied under laboratory conditions. Prior to the experiment, the physicochemical characteristics of the rice mill wastewater were measured. The wastewater revealed an alkaline pH (8.0) with low concentration of DO (0.9 mg l^{-1}), nitrate (0.5 mg l^{-1}), phosphate (21 mg l^{-1}) and sulphate (40 mg l^{-1}), moderate concentration and COD (630 mg l^{-1}), chloride (140 mg l^{-1}) and TDS (670 mg l^{-1}), and high concentration of total suspended solids (530 mg l^{-1}) and BOD (450 mg l^{-1}). Moreover, the wastewater was rich in sodium (235 mg l^{-1}), total phenols (35 mg l^{-1}) as well as silica (58 mg l^{-1}). *Drawida willsi* is a dominant earthworm (>80% both in number and biomass) in the crop fields of India and therefore was used as the test specimen. A 96-hour toxicity test of juvenile immature and adult age groups of *D. willsi* was conducted in different concentrations of rice mill wastewater, both by suspension and artificial soil methods. The toxicity test revealed no mortality of any age groups of *D. willsi* even at 100% wastewater. Experiments on growth, reproduction and metabolism were, therefore conducted up to 100% wastewater (0, 25, 50, 75 and 100%) for a period of 105 days to assess the suitability of rice mill wastewater for agricultural purpose. Growth of *D. willsi* earthworm showed significant increase in 50% wastewater treated soil and it was significantly reduced, when the concentration of wastewater irrigation exceeded a level of 75%. Like growth, the peak as well as average rate of reproduction of *D. willsi* was stimulated up to 50% wastewater treated soil. Both the rates were significantly dropped at or above 75% wastewater treated soil. In addition to this, *D. willsi* exhibited a delay in reproductive peak by 15 days in 75 and 100 % wastewater treated soil. Unlike growth and reproduction, the respiratory and excretory activities of *D. willsi* did not show any significant change up to 50 % rice mill wastewater irrigated soil. A remarkable increase in metabolic activities was observed from 75% wastewater treated soil and the increase was maximal at the highest concentration tested (100%). However, in control as well as wastewater treated soil, the rate of respiration of *D. willsi* went on decreasing, as the earthworm grew older up to 105 days. The adverse effect of rice mill wastewater on growth, reproduction and metabolism at higher concentrations may be due to alkaline pH (8.0). Besides pH, other parameters like higher contents of polyphenols, silica and sodium in wastewater were also responsible to impede the growth, reproduction and metabolism of *D. willsi* in soil. However, in diluted concentrations of rice mill wastewater (i.e. up to 50%), significant increase in growth and reproduction with little or no change in metabolism was noticed. This may be due to the fact that at lower concentrations, the toxic substances being in diluted form stimulating the growth and reproduction of *D. willsi* in soil without causing any adverse effect to metabolism. So on the basis of our findings, we suggest that rice mill wastewater should be either diluted to at least 50% or treated to make it suitable for agricultural purpose.

Keywords: *Earthworm, Rice mill, Wastewater, Reproduction, Respiration, Excretion*

1. INTRODUCTION

The use of industrial effluents for irrigation has emerged in the recent past as an alternative source of water in most of the arid and semi arid regions of India (Hedge & Patil 1983). But the ultimate effect of these effluents on soil, flora and fauna directly depends on their nature, type and characteristics (Somashekar et al. 1984). The nature of effluents and the degree of pollution caused by them varies from industry to industry, depending on the nature of raw materials used, the processes involved there in and the type of equipment used in the processes (Manivasakam 1987). Therefore, it has become essential to study the ecotoxicity of the effluent of every industry before it is used or recommended for agriculture. Among the soil organisms, earthworms constitute a major component of the invertebrate biomass in most of the agro-ecosystems of the world (Lee 1985). They contribute to the decomposition of organic matter and nitrogen mineralization directly through consumption, digestion and respiration and excretion (Mishra & Dash 1980; Engelstad 1991) and indirectly (i) by affecting the growth rates of other populations of soil

organisms through grazing (Winding et al. 1997; Binet et al. 1998) (ii) by influencing soil structure (Gilot 1997) and (iii) by fragmenting and redistribution of plant materials by excreting nutrient rich faeces (Peres et al. 1998; Tomati & Galli 1995). They ingest large quantities of soil during their normal life cycle processes and form important link in natural food chains (Granval & Aliaga 1988). For these reason, earthworms are used as an indicator species to both diagnose and prognose the impact of environmental pollutant on soil ecosystems (Kuhle 1983; Callahan 1988).
Earthworms have been shown to be affected by wide variety of anthropogenic activities. They have been studied in soils affected by mining (Ireland 1976), smelting (Wright & Stringer 1980; Spurgeon et al. 1994), industrial emissions and discharge (Mishra & Sahoo 1997) and land disposal of animal waste and sludge (Rhee 1975). By contrast, no report is available on the effect of rice mill wastewater on soil organisms in general and earthworm in particular, although rice mill industries are much old and history of rice mill industries is associated with history of human civilization.
There are four varieties of earthworms available in agricultural fields of India. They are *Drawida willsi*, Michaelsen, *Ocnerodrillus occidentalis*, Eisen, *Lampito mauritii*, Kinberg and *Glyphidrilus tuberosus,* Stephenson (Pani 1987). Of these *D.willsi* is dominant (> 80%) both in number and biomass. In the present investigation an attempt was made to assess the suitability of rice mill wastewater for agricultural purpose using *D.willsi* as test species and the growth, reproduction and metabolism as test parameters.

2. MATERIALS AND METHODS

For laboratory experiment the rice mill wastewater was collected from a rice mill located near Sambalpur University Campus, Orissa having milling capacity 10MT day^{-1}. The physicochemical characteristics of the effluent were analyzed following the procedures recommended by APHA (1989) and values have been given in Table 1.
Soil and *D.willsi* earthworms were collected from upland, uncontaminated field. The soil had the following 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.

2.1. Worm Culture

Earthworms sampled from the field were cultured in their habitat soil and acclimated for 1 month with adequate provision of food (10% organic matter, cow dung+ leaf litter), moisture (20±2 g%) and temperature 25 ± 2^0 C as standardized by Panda & Sahu (1999). Prior to inoculation, juvenile earthworms were removed from culture pots and half-immersed in glass Petriplates containing 30 ml of dechlorinated water at 25 ± 2^0 C for 24 h to purge their gut contents (Dash & Patra 1977).

2.2. Survivility Test

Survivility test for juvenile, immature and adult age groups of *D. willsi* earthworms was conducted in different concentrations of rice mill wastewater (0, 25, 50, 75 and 100%) both by suspension and artificial soil methods. Petriplates were used for suspension experiments, where 10 gut-evacuated worms of respective age groups in five replicates kept in half immersed condition. Suspension experiment was continued for 96 hours and the solutions were changed at every six hours interval. Artificial soil test for different age groups was also conducted for 96 hours with soil treated with respective concentrations of wastewater to maintain 20±2 g% soil moisture. Earthworms deaths, if any, were recorded and percentage of survival was calculated.

2.3. Growth, Reproduction and Metabolism

The growth, reproduction and metabolism of *D.willsi* was studied in the laboratory at 25 ± 2^0 C of soil temperature for 105 days. The experiment was maintained at 20+2g% of soil moisture with 0,25,50,75 and 100% rice mill wastewater. Five sets of plastic culture pots, each with, twenty-eight replicates, filled with 2 kg of soils were kept. To each replicate five gut evacuated pre-weighed juveniles *D.willsi* earthworms were inoculated. Out of twenty-eight replicate of each set, four replicates were taken at 15 days interval up to 105 days. Worms and cocoons were collected by hand-sorting and wet-sieving method (Sahu et al. 1988) and were grouped into their respective age classes (juveniles, immatures and adults). The earthworms were gut-evacuated and weighed alive. The dry mass was determined directly after drying the earthworms at 85 ^{o}C for 48 hours. Growth was calculated in terms of change in weight as a percent of starting weight. The rate of reproduction was calculated as the total number of cocoons produced per adult worm (Sahu & Senapati 1988). Before drying the worms at each sampling intervals, respiratory and excretory activities of the gut-evacuated worms were also determined. Rate of respiration was measured by Winkler's gaseous displacement method (Welch 1948) and expressed in mg O_2 consumed g dry tissue^{-1} h^{-1}. The rate of excretion (mg rejecta g dry tissue^{-1}h^{-1}) of ammonia and urea were measured by indophenol blue and diacetyl monoxime method respectively (Kaplan 1965).

Statistical analysis of the data was made according to Snedecor & Cochran (1967). One-way, two-way analysis of variance (ANOVA) was used to determine the significant difference among the treatments and days. The least significant difference (LSD) test was also used for multiple comparisons when significant difference was found by one-way ANOVA.

3. RESULTS

3.1. Physico-chemical Analysis of Wastewater

The wastewater of rice mill showed an alkaline pH (8.0) with low concentration of DO (0.9 mg l^{-1}), nitrate (0.5 mg l^{-1}), phosphate (21 mg l^{-1}) and sulphate (40 mg l^{-1}); and moderate concentration of COD (630 mg l^{-1}), chloride (140 mg l^{-1}) and TDS (670 mg l^{-1}). The total suspended solids (530 mg l^{-1}) and BOD (450 mg l^{-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 wastewater (Table 1). Moreover, the wastewater was rich in sodium (235 mg l^{-1}), total phenols (35 mg l^{-1}) as well as silica (58 mg l^{-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 in the processing of raw paddy.

Table 1. Physico-chemical characteristics of the effluent of a rice mill at Sambalpur, Orissa with their maximum permissible limits as recommended by Indian Standards 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 (^{0}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})	998.1-459.1	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 ^{0}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	-	-

3.2. Survivility Test

In survivility test (both suspension and artificial soil methods) all the three age groups (juveniles, immatures and adults) of *D. willsi* earthworms were thrived up to 96 hours without any mortality. Even 100 % wastewater was not lethal to any age groups of *D. willsi*. Therefore, further experiments on growth, reproduction and metabolism (respiration and excretion) were conducted up to 100 % rice mill wastewater.

3.3. Growth

The average biomass (mg dry wt ± SD) of *D.willsi* worms (juveniles) was initially 4.5±0.258. Growth of *D.willsi* worms in control soil after 15 days was found to be 84.44% whereas in 25, 50.75 and 100% wastewater treated soil, it was about 120.0, 171.11, 62.22 and 40.0% respectively (Table 2). This indicates 19 - 47 % accelerated growth in 25 and 50% and 12 -24 % retardation in 75 and 100% effluent treated soil. This trend of increase and decrease in growth was more or less continued up to the end of the experiment (Figure 1).

Table 2. Growth of D.willsi earthworms (mg dry wt worm^{-1}±SD) in different concentrations of rice mill wastewater treated soil under laboratory conditions.

Days	Control	Effluent treated soil				One-way ANOVA (F)	LSD (p<0.05)	Two-way ANOVA
		25%	50%	75%	100%			
0	4.5 ±0.258	4.5 ±0.258	4.5 ±0.258	4.5 ±0.258	4.5 ±0.258			
15	8.3[a] ±0.663	9.9[b] ±0.6	12.2[c] ±0.73	7.3[d] ±0.657	6.3[e] ±0.553	53.3*	0.952	F1= 66.33*
30	13.8[a] ±0.858	16.4[b] ±0.952	18.9[c] ±1.054	12.2[d] ±0.793	10.4[e] ±0.9	54.12*	1.38	F2=222.97*
45	20.1[a] ±1.655	22.4[ab] ±1.734	23.9[b] ±1.527	17.3[c] ±1.416	15.0[c] ±1.242	22.76*	2.3	
60	23.5[a] ±1.571	24.6[ab] ±1.39	26.0[b] ±1.918	21.1[c] ±1.477	18.5[d] ±1.424	14.36*	2.37	
75	24.0[ac] ±1.628	24.7[ab] ±1.71	26.6[b] ±1.54	22.2[cd] ±1.526	20.4[d] ±1.58	8.83*	2.4	
90	23.5[a] ±1.688	24.3[ab] ±1.574	25.8[a] ±1.523	22.0[bc] ±1.51	21.0[c] ±1.419	5.97*	2.32	
105	22.3[acd] ±1.442	22.3[acd] ±1.531	24.9[b] ±1.638	21.0[cd] ±1.419	20.2[d] ±1.376	5.78*	2.23	

*P<0.05, F = Value of one-way ANOVA between concentration, F1= Value of two-way ANOVA between days, F2 = Value of two-way ANOVA between concentrations, Values in the same row with different alphabets were significantly different by LSD (P<0.05)

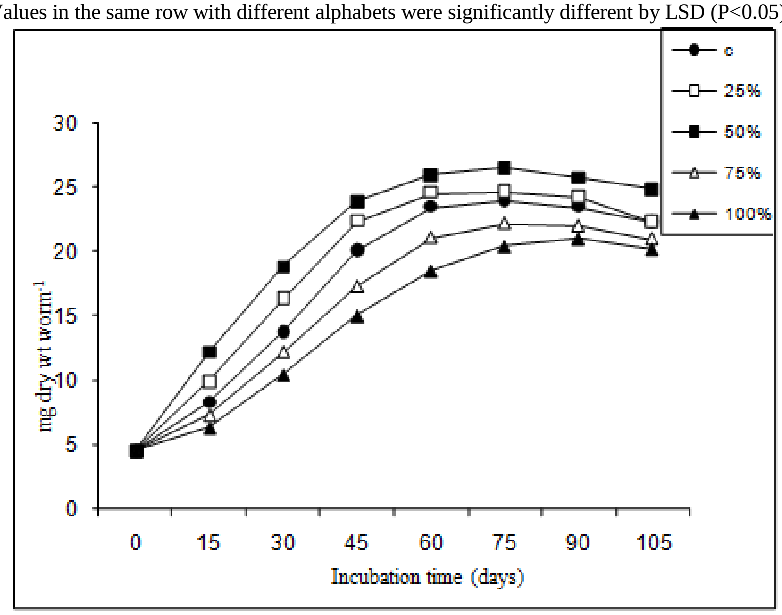

Figure 1. Growth of D. willsi (mg dry wt worm $^{-1}$) worms in different concentrations of rice mill wastewater treated soil.

The difference in the growth of *D.willsi* earthworms with respect to different percentage of effluent treatments was significant from 15 days onwards (F≥5.78, P< 0.05). Two-way ANOVA test also showed significant difference between treatments and also between days (F_1=66.33, F_2 =222.97, P< 0.05). The LSD test, however, revealed that the average growth Figure of *D.willsi* in different days intervals were significantly higher from control in 50% effluent treated soil and significantly lower from control at or above 75% effluent treated soil.

3.4. Reproduction

The rate of reproduction (C A^{-1}) of *D. willsi* earthworms showed a peak value of 1.8, 2.2, and 2.4 after 75 days in control, 25 and 50 % effluent treated pots, whereas in case of higher percentage of effluent treated pots i.e. (75 and100%) it showed a peak value of 1.33 and 1.16 respectively after 90 days (Table 3). This indicated that the peak rate of reproduction was increased by 22.22 and 33.33 % in 25 and 50 % and decreased by 26.11 and 35.55 % in 75 and 100 % effluent treated pots respectively. The average rate of reproduction was increased to a maximum Figure of 24.05% and decreased to a maximum Figure of 34.17 % in 50 and 100% rice mill wastewater treated pots respectively.

Table-3. Effect of rice mill effluent on the rate of reproduction (C/A) of D.willsi earthworms under laboratory conditions

Days	Control			Effluent treated soil											
				25%			50%			75%			100%		
	C	A	C/A	C	A	C/A	C	A	C/A	C	A	C/A	C	A	C/A
0	--	5(NA)		--	5(NA)		--	5(NA)		--	5(NA)		--	5(NA)	
15	--	5(NA)		--	5(NA)		--	5(NA)		--	5(NA)		--	5(NA)	
30	--	5		--	5		--	5		--	5		--	5	
45	8	5	1.60	8	5	1.60	10	5	2.00	5	5	1.00	4	5	0.80
60	8.5	5	1.70	9	5	1.80	10	5	2.00	6	5	1.20	5	5	1.00
75	9.0	5	1.80	11.0	5	2.20	12	5	2.40	6	5	1.20	5.5	5	1.10
90	10.0	7	1.43	11.0	7	1.57	14	8	1.75	7	6	1.33	7.0	6	1.16
105	11	8	1.37	12	8	1.5	15	9	1.66	8	7	1.14	8	7	1.14

C – Cocoon; A – Adult; NA – Non-adult

3.5 Respiration

The rate of respiration (mgO_2 g dry tissue^{-1}h^{-1} ±SD) of *D.willsi* earthworms was initially1.626 ±0.067. The rate of respiration went on decreasing, as the earthworms grew older up to 105 days in control as well as effluent treated pots (Table 4). But when comparison was made with control, the rate of respiration of earthworms was found to be increased by 0.735-1.61%, 1.68-3.16%, 5.57-8.87% and 6.01-13.61% in 25, 50, 75, and 100% of effluent treated pots respectively, with maximum increase in 100% effluent treated pots (Figure 2).

Table 4. Rate of respiration (mg O_2 g dry tissue^{-1}h^{-1} ± SD) of D. willsi earthworm in different concentrations of rice mill wastewater treated soil under laboratory conditions

Days	Control	Effluent treated soil				One-way ANOVA (F)	LSD (P<0.05)	Two-way ANOVA
		25 %	50 %	75%	100%			
0	1.626 ±0.067	1.626 ±0.067	1.626 ±0.067	1.626 ±0.067	1.626 ±0.067			
15	1.507 ±0.058	1.527 ±0.063	1.542 ±0.059	1.591 ±0.066	1.599 ±0.070	1.61		F1=101.28*
30	1.422^{a} ±0.059	1.445^{a} ±0.059	1.467^{ab} ±0.059	1.544^{bc} ±0.061	1.578^{c} ±0.063	4.93*	0.09	F2=237.12*
45	1.364^{a} ±0.058	1.376^{a} ±0.06	1.388^{a} ±0.065	1.485^{b} ±0.064	1.523^{b} ±0.068	5.2*	0.095	
60	1.307^{a} ±0.059	1.32^{a} ±0.06	1.334^{a} ±0.061	1.423^{b} ±0.062	1.485^{b} ±0.065	6.22*	0.092	
75	1.278^{a} ±0.055	1.294^{a} ±0.06	1.307^{a} ±0.061	1.36^{ab} ±0.061	1.41^{b} ±0.063	3.27*	0.09	
90	1.249^{a} ±0.049	1.262^{a} ±0.053	1.27^{ab} ±0.054	1.336^{bc} ±0.053	1.36^{c} ±0.057	3.41*	0.08	
105	1.224^{a} ±0.049	1.233^{ab} ±0.052	1.252^{ab} ±0.055	1.307^{bc} ±0.053	1.33^{c} ±0.053	3.16*	0.079	

*P<0.05, F= Value of one-way ANOVA between concentration, F1= Value of two-way ANOVA between days , F2= Value of two-way ANOVA between concentrations, Values in the same row with different alphabets were significantly different by LSD (P<0.05)

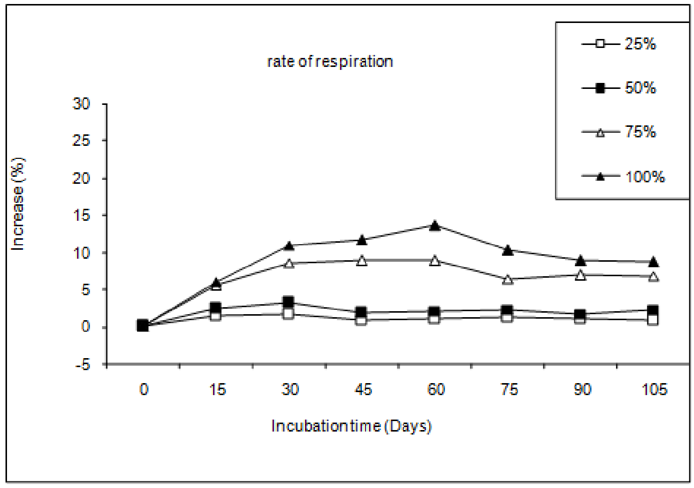

Figure 2. *Percentage increase in the rate of respiration of D. willsi earthworms over control in different concentrations of rice mill waste water treated soil.*

Two-way ANOVA test showed significant difference in the rate of respiration with respect to different days as well as concentrations ($F1 = 101.28$, $F2 = 237.12$, $P<0.05$). One-way ANOVA test also showed significant difference from 30 days onwards ($F \geq 3.16$, $P<0.05$). LSD test, however, did not show any significant difference in the respiration rates of control and 25 or 50 % effluent treated worms up to the end of the experiment, but significant difference was noticed between the respiration rates of control and 75 or 100% effluent treated worms from 30 days onwards.

3.6. Ammonia Excretion

The rate of ammonia excretion (mg g dry tissue^{-1}h^{-1} ± SD) of *D.willsi* worms before application of wastewater was 0.217±0.02. After 15 days, the rate of ammonia excretion of the worms was decreased by about 16% in control pots whereas it was decreased by about 14, 12, 9 and 4% in 25, 50,75 and 100% effluent treated pots. This trend of decrease in ammonia excretion was noticed in both control as well as experimental pots till the end of the experiment (Table 5) although, the worms in effluent treated pots showed higher rate of ammonia excretion as compared to the control (Table 5). An increase of 2.54, 7.62,16.94 and 20.33% in ammonia excretion was noticed in 25, 50, 75 and 100% effluent treated pots respectively after 105 days (Figure 3).

Table 5. Rate of excretion of ammonia (mg g dry tissue^{-1} h^{-1} ± SD) by D.willsi earthworm in different concentrations of rice mill wastewater treated soil under laboratory conditions.

Days	Control	Effluent treated soil				One-way ANOVA (F)	LSD (p<0.05)	Two-way ANOVA
		25%	50%	75%	100%			
0	0.217 ±0.0202	0.217 ±0.0202	0.217 ±0.0202	0.217 ±0.0202	0.217 ±0.0202			
15	0.183 ±0.012	1.187 ±0.011	0.191 ±0.017	0.198 ±0.0171	0.209 ±0.0133	1.98		F1=233.60*
30	0.161 ±0.0142	0.165 ±0.0138	0.171 ±0.013	0.179 ±0.0157	0.19 ±0.0159	2.53		F2=936.68*
45	0.136^{a} ±0.0106	0.142^{ab} ±0.0117	0.146^{ab} ±0.012	0.159^{bc} ±0.0117	0.171^{c} ±0.0137	5.5*	0.017	
60	0.123^{a} ±0.012	0.127^{ab} ±0.01	0.132^{abc} ±0.0115	0.141^{bc} ±0.0116	0.148^{c} ±0.0115	3.15*	0.017	
75	0.12^{a} ±0.0107	0.121^{a} ±0.0113	0.125^{a} ±0.0109	0.135^{ab} ±0.0117	0.143^{b} ±0.0048	3.75*	0.017	
90	0.117^{a} ±0.0102	0.118^{a} ±0.0108	0.124^{ab} ±0.0101	0.136^{ab} ±0.0116	0.141^{b} ±0.0116	3.92*	0.017	
105	0.118^{a} ±0.0106	0.121^{ab} ±0.0115	0.127^{abc} ±0.0112	0.138^{bc} ±0.0123	0.142^{c} ±0.0112	3.38*	0.017	

*P<0.05, F= Value of one-way ANOVA between concentration, F1= Value of two-way ANOVA between days, F2= Value of two-way ANOVA between concentrations, Values in the same row with different alphabets were significantly different by LSD (P<0.05)

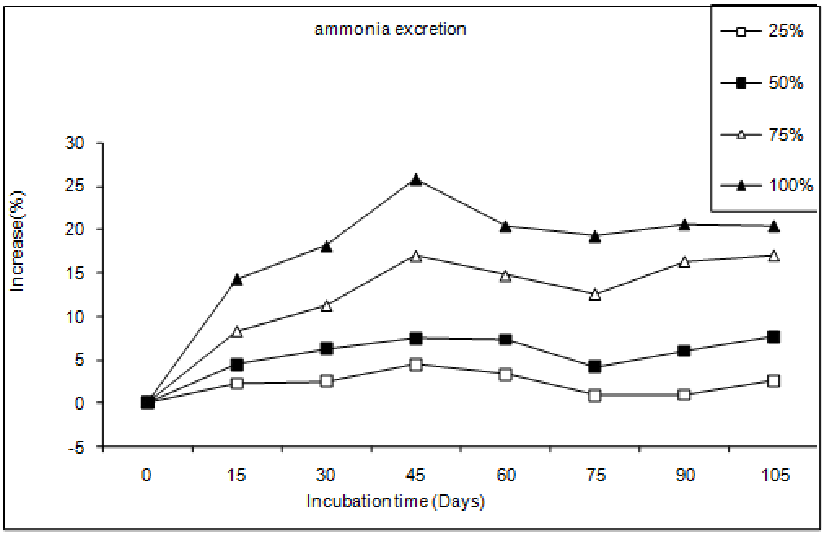

Figure 3. *Percentage increase in the rate of ammonia excretion of D. willsi earthworms over control in different concentrations of rice mill wastewater treated soil.*

3.7. Urea Excretion

At the start of the experiment the rate of urea excretion (mg g dry tissue^{-1} h^{-1} ± SD) of *D.willsi* earthworms was 1.181±0.075. A decrease in the rate of urea excretion was noticed up to end of the experiment in all the control and experimental pots and after 105 days the rate of urea excretion was decreased by 32.51, 30.56, 29.8, 25.74, and 22.52% in control, 25, 50, 75 and 100% effluent treated pots respectively (Table 6). When comparison was made with control worms of respective days, the rate of urea excretion was more in all the effluent treated pots. An increase of 2.88, 4.01, 10.03, and 14.80 % in urea excretion was noticed after 105 days in 25,50,75 and 100% effluent irrigated pots respectively (Figure 4).

Table-6. Rate of Excretion of urea (mg g dry tissue^{-1}h^{-1} ± SD) by D.willsi earthworm in different concentrations of rice mill wastewater treated soil under laboratory conditions.

Days	Control	Effluent treated soil				One-way ANOVA (F)	LSD (P<0.05)	Two-way ANOVA
		25%	50%	75%	100%			
0	1.181 ±0.075	1.181 ±0.075	1.181 ±0.075	1.181 ±0.075	1.181 ±0.075			
15	1.067 ±0.052	1.087 ±0.058	1.106 ±0.064	1.142 ±0.069	1.168 ±0.07	1.7		
30	0.98 ±0.066	1.015 ±0.068	1.029 ±0.063	1.08 ±0.071	1.107 ±0.07	2.27		F1=175.54*
45	0.892[a] ±0.06	0.897[a] ±0.063	0.926[ab] ±0.066	1.004[bc] ±0.076	1.038[c] ±0.077	3.65*	0.104	F2=484.28*
60	0.844[a] ±0.053	0.854[a] ±0.061	0.868[a] ±0.061	0.918[ab] ±0.064	0.975[b] ±0.063	3.22*	0.091	
75	0.803[a] ±0.058	0.828[a] ±0.062	0.844[a] ±0.06	0.897[ab] ±0.062	0.955[b] ±0.064	3.96*	0.092	
90	0.791[a] ±0.055	0.817[a] ±0.055	0.829[a] ±0.567	0.872[ab] ±0.056	0.93[b] ±0.06	3.72*	0.085	
105	0.797[a] ±0.045	0.82[a] ±0.056	0.829[a] ±0.054	0.877[ab] ±0.055	0.915[b] ±0.059	3.11*	0.0815	

*P<0.05, F= Value of one-way ANOVA between concentration, F1= Value of two-way ANOVA between days, F2= Value of two-way ANOVA between concentrations, Values in the same row with different alphabets were significantly different by LSD (P<0.05)

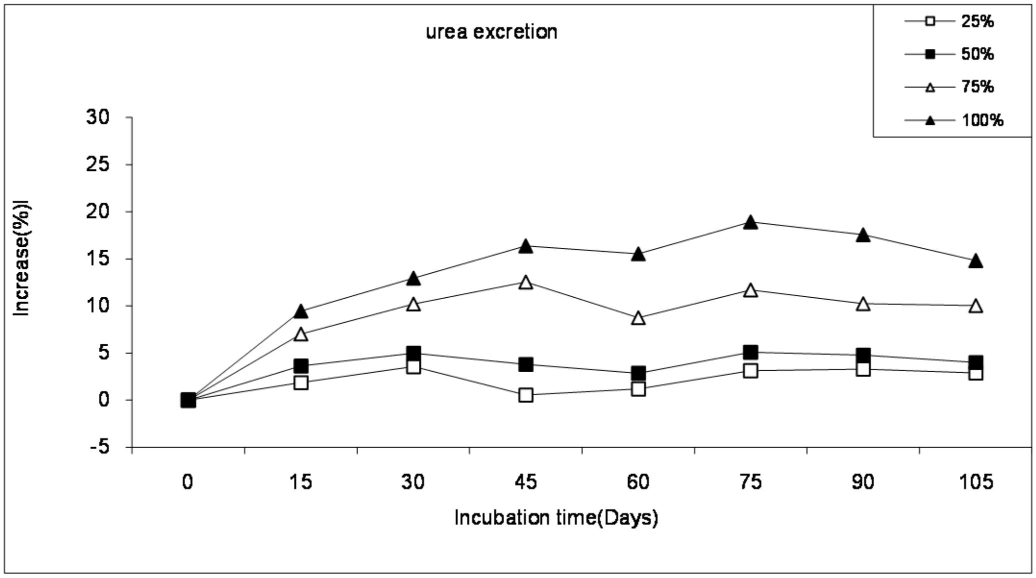

Figure 4. *Percentage increase in the rate of urea excretion of D. willsi earthworms over control in different concentrations of rice mill waste water treated soil.*

Statistical analysis by two-way ANOVA test revealed significant difference in the rate of urea excretion of *D.willsi* with respect to both days and treatments (F_1=175.54, F_2=484.28, P<0.05). One-way ANOVA test showed significant difference in urea excretion between control and effluent treated pots after 45 days and it was continued till end of the experiment ($F \geq 3.11$, $P<0.05$). LSD test, however, showed significant difference in the average rate of urea excretion of *D.willsi* only in 100% effluent where the difference was significantly increased over control after 45 days onwards (Table 6).

4. DISCUSSION

In the present study growth and reproduction of *D. willsi* earthworm was found to be influenced by rice mill wastewater. Growth of *D. willsi* showed significant increase in 50% effluent treated soil. It was significantly reduced when the concentration of effluent irrigated exceeded a level of 75%. Like growth, the peak as well as average rate of reproduction of *D. willsi* was stimulated up to 50% wastewater treated soil. Both the rates were significantly dropped at or above 75% effluent treated soil. In addition to this, *D. willsi* exhibited a delay in reproductive peak by 15 days in 75 and 100% effluent treated soil. Since growth is the first criteria for organisms to attain maturity and enter into reproductive stage, changes in growth and reproduction may alter the life cycle of an individual (Lofs-Holmin 1980; Okkermen et al. 1991) and influence the size and vitality of earthworm population in long run (Lofs-Holmin 1980).

Unlike growth and reproduction, the respiratory and excretory activities of *D. willsi* did not show any significant change up to 50% rice mill wastewater-treated soil. A remarkable increase in metabolic activities was observed in 75 and 100% wastewater treated soil, and the increase was maximum at the higher test concentration (100%). However, in control as well as wastewater treated soil, the rate of respiration of *D. willsi* went on decreasing, as the earthworm grew older up to 105 days.

Physiological functions of living organisms are modified by a great variety of factors, both internal and external. The internal factors are size, age, nutritional state, composition of diet and degree of activity etc, whereas external factors includes temperature, relative humidity, concentration of respiratory gases, nature of availability of food and exposure to different toxic substances etc. (Hoar 1991). Of these, one of the most important factors, which may influence the body physiology, is body size or age. Smaller organisms have a greater ratio of surface area to body weight and thus physiologically more active than larger organisms (Byzova 1965). This supports the present findings of enhanced respiratory and excretory activities in the case of juvenile worms. Like wise, organisms are exposed to chemicals in a variety of ways. Depending on the concentration and site of action of the toxic substance, the physiological process may result increase or decrease in the rate. Alteration in the metabolic rate due to exposure of anthropogenic substance has also seen reported for a wide variety of organisms and including earthworms (Greig-Smith et al. 1992).

In the present study, the effect of rice mill wastewater on growth, reproduction and metabolism at higher concentration may be due to alkaline pH (8.0). Earthworms are found in fairly large quantity in the pH range of 6.2 to 7.4 (Edwards & Lofty 1977). They are generally absent in very alkaline soils (pH > 8.5) and are scare in soils with pH ≥8.0. Further decrease in earthworm number in soils amended with alkaline coal fired ash (Pati & Sahu 2004) and irrigated with paper mill effluent (Mishra & Sahoo 1997) has been well reported. These soils had a fairly high level of pH. Besides pH, phenols, silica and sodium in wastewater can have serious impact on the growth, reproduction and metabolism of earthworm.

5. CONCLUSION

In diluted concentrations of rice mill wastewater (i.e. up to 50%), significant increase in growth and reproduction with little or no change in metabolism was noticed. This may be due to fact that at lower concentrations, the toxic substance being in diluted form, stimulating the growth and reproduction of *D. willsi* in soil, without causing any adverse effect to metabolism. On the basis of our findings, we suggest that the rice mill effluent should be diluted at least up to 50% before using for agricultural purpose. However, further works on the effect of rice mill effluent on soil and crop system is needed to reach at a concrete conclusion.

6. ACKNOWLEDGEMENT

The authors are grateful to the Head, Department of Environmental Sciences, Sambalpur University for providing the laboratory facilities. Ms. Abanti Padhan is thankful to the UGC, New Delhi for financial assistance in the form of a Research Fellowship and also thankful to the authority of Siksha O Anusandhan University and HOD chemistry ITER for their constant inspiration for publishing the paper.

7. REFERENCES

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

Binet, F., Fayolle, L., Pussard, M., 1998. Significance of earthworms in stimulating soil microbial activity. Biology and Fertility of Soils 27, 79-84.

Byzova, J.A., 1965. Comparative rate of respiration in some earthworms (Lumbricides; Oligochaeta). Revue d'Ecologie et Biologie du Sol 2, 207-216.

Callahan, C.A., 1988. Earthworms an ecotoxicological assessment tools. In: Edwards C.A., Neuhauser E.F. (eds) Earthworms in waste and Environmental management, SBP Academic publication, The Hague, The Netherlands, pp. 295-301.

Dash, M.C., Patra, U.C., 1977. Density, biomass and energy budget of a tropical earthworm population from a grassland site in Orissa, India, Revue d'Ecologie et Biologie du Sol 14, 461-471.

Edwards, C.A. Lofty, J.R., 1977. Biology of Earthworms. Chapman and Hall, London.

Engelstad, F., 1991. Impact of earthworms on decomposition of garden refuse. Biology and Fertility of Soils 12, 137-140.

Gilot, C., 1997. Effects of a tropical geophagous earthworms *Millsonia anomala* (Megascolecidae), on soil characteristics and production of a Yarn crop in ivory coast, Soil Biology and Biochemistry 29, 353-359.

Granval, P.H., Aliaga, R., 1988. Analyse critique des connaissances surles predature de lumbriciens. Gibier et faune Saurage 5, 71-94.

Greig Smith, P.W., Becker, H., Edwards, P.J. Heimbach, F. ,1992. Ecotoxicology of earthworms, Intercept, Andover.

Hedge, B.A., Patil, T. M., 1983. Utilization of industrial wastewater in agriculture. Pollution Research 2, 37-39.

Hoar, W.S., 1991. General and comparative physiology, Prentice Hall of India Pvt. Ltd., New Delhi, India.

Ireland, M.P., 1976. Excretion of lead, zinc and calcium by the earthworms *Dendrobaena rubida* living in soil contaminated with Zinc and Lead. Soil Biology and Biochemistry 8, 347-350.

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

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

Kaplan, A., 1965. The determination of urea, ammonia, and urease. In: Glick, D. (ed) Methods in Biochemical Analysis. Vol. 17, John Wiley and Sons, New York, pp. 311-321.

Kuhle, J.C., 1983. Ecotoxicological model studies to test the suitability of earthworms as bio-indicators. Verh. Dtsch. Zool Ges 147-151.

Lee, K. E., 1985. Earthworms, their ecology and relationship with soil and land use, Academic press, New York.

Lofs-Holmin, A., 1980. Measuring growth of earthworms in a method of testing sublethal toxicity to pesticides, Swedish Journal of Agricultural Research 10, 25-33.

Manivasakam, N., 1987. Industrial effluents: origin, characteristics, effects, analysis and treatment. Sakthi Publications, Coimbatore, India.

Mishra, P.C., Dash, M.C., 1980. Digestive enzymes of earthworms. Experientia, 36, 1155-1157.

Mishra, P.C., Sahoo, S., 1997. Production and energetics of earthworm population (*Lampito mauritii*, Kinberg) and metabolism in soil under paper mill waste water irrigation, Ecology and Environment Conservation 3(1), 49-61.

Okkerman, P.C., Plassche, E.J.V.D., Sloot, W., Leeuwen, C.J. Van, Canton, J.H., 1991. Ecotoxicological effects assessments: A comparison of several extrapolation procedures. Ecotoxicology and Environmental Safety 21, 182-193.

Panda, R., Sahu, S.K., 1999. Respiratory and excretory activity of *Drawida willsi* (Oligochaeta) in zinc applied Soil. Enviornment and Ecology 17(2), 383-387.

Pani, S.C., 1987. Aspects of ecological studies on tropical earthworms in irrigated agricultural systems of Orissa. India. Ph. D. thesis, Sambalpur University. Orissa, India.

Pati, S.S., Sahu, S.K., 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(3-4), 289-301.

Peres, G., Cluzeau, D., Curmi, P., Hallaire, V., 1998. Earthworm activity and soil structure changes due to organic enrichments in vine yard systems. Biology and Fertility of Soils 27, 417-424.

Rhee, J.A.Van., 1975. Copper contamination effects on earthworms by disposal of pig waste in pasture. In: Vanek, J. (ed) Progress in Zoology. B.V. Publishers, The Hague, pp. 451-457.

Sahu, S.K., Senapati, B.K., 1988. Alternative proposals for quantification of reproduction in a tropical earthworm, Tropical Ecology 29, 6-14.

Sahu, S.K., Mishra, S.K.. Senapati, B.K., 1988. Population biology and reproductive strategy of *Dichogaster bolaui* (Oligochaeta: Octochaetidae) in two tropical agro-ecosystems, Proceedings of Indian Academy of Science (Animal Science) 79(3), 239-250.

Singaram, P., 1996. Characterization of tannery effluent and its effect on soil physical properties, Madras Agricultural Journal 83(3), 205-206.

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

Somasekhar, R.I., Gowda, M.T.G., Shettigar, S.L.N. Srinath, K.P., 1984. Effect of industrial effluents on crop plants. Indian Journal of Environmental Health 26, 136-146.

Spurgeon, D.J., Hopkin, S.P., Jones, D.T., 1994. Effects of Cadmium, Copper, Lead and Zinc on growth, reproduction and survival at the earthworms *Eisenia foetida* (Savigny): assessing the environmental impact of point source metal contamination in terrestrial ecosystems, Environmental Pollution 84, 123-130.

Tomati, U., Galli, E., 1995. Earthworms, soil fertility and plant productivity, Acta Zoologica Fennica, 196, 11-14.

Welch, P.S., 1948. Limnological methods, Mc Graw Hill publication, New York.

Windings, A., Ronn, R., Hendriksen, N.B., 1997. Bacteria and Protozoa in soil microhabitats as affected by earthworms. Biology and Fertility of Soils 24, 133-140.

Wright, M.A., Stringer, A., 1980. Lead, Zinc and Cadmium content of earthworms from pasture in the vicinity of an industrial smelting complex. Environmental Pollution 23, 313-321.