Nature Environment and Pollution Technology © Technoscience Publications	Vol. 6	No.1	pp. 63-74	2007

ASSESSMENT OF ENVIRONMENTAL CONTAMINATION POTENTIAL OF DISTILLERY EFFLUENT USING PLANT AND ANIMAL BIOASSAYS

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ABSTRACT

Toxic effects of spent wash and crop field soil irrigated with it (hereafter referred to as distillery soil) were examined on 6 species of plants (2 cereals and 4 aquatic macrophytes) and 3 animals (cladoceran, fish and albino mice) by short-term bioassays using end point growth responses and mortality respectively. Seedling emergence was found nil (rice) to low (wheat = 30-40%) in distillery soil, but increased (48-00%) proportionately alongwith their vigor after mixing garden soil. In contrast to distillery soil, toxic effects of even cent percent soil leachate were nil on seed germination while meager (10-15%) on seedling vigor in both the cereals. Spent wash, however, decreased germination and seedling vigor in both rice (germination = 5-100%; vigor = 2-100%) and wheat (germination = 0-64%; vigor = nil-93%) with an increase in its concentration, though vigor was greater (43%) than control in wheat at 10%. Among aquatic macrophytes, free-floating species (*Lemna aequinoctialis* and *Spirodela polyrrhiza*) were found to be more sensitive than the submerged (*Ceratophyllum demersum* and *Hydrilla verticillata*) for spent wash; *Lemna* being the most sensitive species.

During a detailed study, sensitivity of different test organisms to spent wash (in terms of their EC/LC_{50} values) was in the order of: *Daphnia* (EC_{50} = 0.68%) > *Gambusia affinis* (LC_{50} = 1.31%) > *Lemna* (EC_{50} = 0.8-6.97%) indicating water flea to be the most sensitive test organism. The toxicity of soil leachate was, however, very low to these test organisms (*Gambusia*: LC_{50} = 12.1%; *Lemna*: EC_{50} = 46.5-278%) as also noted for cereals. The spent wash exposure not only decreased RBC counts (58-69%) in fish but also altered their morphology (77-97%).

Both food (7-14%) and water (27-45%) intake of albino mice (male) declined in soil leachate treatments (5-20%) alongwith their body (8-17%) and reproductive organ weights (4-63%), except that of prostrate gland weight increasing between 12% and 37%. The sperm counts (78-89%) and their motility (14-23%) also decreased in leachate treatments. Higher toxicity of spent wash may be ascribed to its greater conductivity (32.7mS), COD (54,900ppm) and chlorides (62,00ppm) than the soil leachate (EC = 3.56mS; COD = 2290ppm; chlorides = 220ppm).

INTRODUCTION

Indian distilleries utilizing molasses as a raw material are likely to play a major role in the near future by providing clean fuel (gasohol). The increased demand of alcohol will force expansion of existing industries, along with installation of new ones that is likely to aggravate both water and air pollution caused by discharge of dark brown, odorous effluent having very high values of COD, BOD and TDS, including plant nutrients (biogenic salts) such as N, P and K (Pathade 1999, Joshi 2004). Its economic, but safe disposal is a major challenge to distilleries. In view of its richness in biogenic salts, land treatment or agro recycling has been advocated as a convenient and most economic process of its safe disposal, though at times, it is impractical due to non-availability of sufficient land area or dilution water (Joshi 2004). The long-term unscientific application of spent wash also had adverse effects on physico-chemical properties of the soil including its microflora (Bhaduri 1980, Kumar et al. 2003).

During surveys, it was noted that application of spent wash in the crop fields in the town Behror, Alwar district, Rajasthan (India) has turned them barren. The villagers complain menace of house-flies and foul odour throughout the year that intensifies after rains. Such application also contaminates groundwater through leaching (Somawanshi & Yadav 1990) while its direct discharge pollutes the water bodies (Kumar et al. 2003). There are large number of studies describing toxic effects of spent wash on crops (Singh 2003, Suthar et al. 2005) and fish (Haniffa & Sundaravadanam 1983, Maruthi & Rao 2000), but effects on aquatic plants have not been detailed. In case of fishes, lethal dose (LC_{50}) of spent wash and soil leachate are still not known, including their effects on RBCs playing vital role in animal physiology. In areas having greater possibility of groundwater contamination, an assessment of soil leachate toxicity to mammals becomes imperative.

Since molasses based distilleries are contributing significantly to the economy in the developing countries including India, a detailed investigation of their impact on the environment becomes essential as waste generated in them is highly polluting in nature. With this backdrop, we explored toxicity of spent wash, distillery soil (effluent irrigated crop field soil) and its aqueous leachate on six species of plants (2 cereals and 4 hydrophytes) and three animals (water flea, fish and albino mice). The important findings are reported in this communication.

MATERIALS AND METHODS

The soil sample (about 50kg), collected from a spent wash irrigated crop field, was air-dried for 15 days in sunlight, ground and passed through a 2mm sieve. The solar dried garden soil containing compost (5:1) was mixed with distillery soil in different ratios (1:1, 1:2, 1:3 and 1:4). The physico-chemical characteristics of garden (G) and distillery soil (D) were analyzed according to Misra (1968) and Jackson (1973). 2/3rd part of plastic jars (volume = 300mL), filled in separately with these soils, were watered (tap water) to field capacity in the greenhouse. Ten seeds of rice (***Oryza sativa*** L)/wheat (***Triticum aestivum*** L) were sown in a jar during April (temperature (mean): minimum = 21.3°C; maximum = 35.4°C) and December (temperature (mean): minimum = 11.9°C; maximum = 25.2°C) respectively. About 5mL tap water was sprinkled on alternate days to compensate evaporative loss. The seedlings in jars were counted and their root and shoot length including dry weights (at 80°C in hot air oven) were recorded on 10th day (also on 15th day in wheat).

For preparing soil leachate, grounded distillery soil mixed (weight/volume) in distilled water (1:1) was stirred for almost 24h over a magnetic stirrer. After settlement of suspended soil, clear supernatant was pumped out and filtered through Whatmann filter paper 41. Soil leachate thus prepared was considered 100%. Its physico-chemical characteristics were assessed according to APHA (1989).

Toxic effects of soil leachate on germination and seedling growth of wheat and rice were studied in petri dishes maintained under sterilized conditions for 8 days in a growth chamber (25±2°C). The light source (duration: 24 h; intensity: 2100 lux) was fluorescent tubes (40W × 3) and bulbs (100W × 2). Ten seeds were placed on filter paper moistened below by cotton soaked in either distilled water (control) or soil leachate of varying concentrations made with distilled water. Observations were made daily. The emergence of radicle was considered as germination. Seedling lengths (root + shoot) were measured and their vigor was calculated as follows.

Seedling Vigor = % Germination × Seedling length

Lemna aequinoctialis Welwitsch, a test organism to examine toxicity, was collected from Rajasthan University Botanical Garden, Jaipur. Two control types; one in the tap water and the other in Hoagland medium (Volume = 200mL) were set in 300mL plastic jars. Different dilutions (200mL) of soil leachate (made in tap water) were also filled in similarly. 10pairs (20 fronds) of *Lemna* plants were added in a jar for observing changes in number of fronds; while 20pairs (40 fronds) for dry weight and chlorophyll contents were measured on 4th, 7th and 10th day of study. All data are based on the mean values of 5 replicates in each set. During initial trials, *Lemna* growth was better in continuous (24h) light exposure, in comparison to 12h exposure. Hence, these plants were kept under continuous light, about 8h, in presence of both fluorescent tubes and bulbs (light intensity = 2090 ± 35 lux) and remaining 16h in presence of fluorescent tubes (1000 ± 22 lux). The chlorophyll content in plants was estimated as per the method described elsewhere (Sharma 1985).

For fish bioassay, healthy mature fish *Gambusia affinis* (Baird & Gerard); length: 2.28 ± 0.10cm; width: 4.1± 0.20mm) caught from a tank (6m×3.7m×2m) in the University botanical garden were acclimated for 15 days in plastic troughs (40L) containing a good plankton population, to serve as food, and *Ceratophyllum* (submerged hydrophyte) to oxygenate water. Thereafter, fish starved for 24h in dechlorinated tap water, were transferred to tap water (control) and 6 different concentrations of soil leachate (3L) filled in plastic jars (volume = 5L) for determining acute toxicity (96h) as described in APHA (1989). Dead fish, found any, were removed immediately.

The toxicity of spent wash was also monitored, which was stored at 4°C after transport from a molasses based distillery. Its physico-chemical characteristics were assessed following standard methods (APHA 1989). The effects on germination and seedling growth of rice and wheat were monitored in petridishes, similar to soil leachate. In another experiment, emergence and growth of rice seedlings was assessed in river sand filled in plastic jars (300mL), as described for distillery soil. Both control and distillery treatments, initially irrigated respectively with 20mL of Hoagland solution and diluted effluent (by tap water), were later watered (5mL/jar) on alternate days to compensate evapotranspiration loss. The seedlings, harvested finally on 10th day of the study, were processed for growth measurements as described earlier.

Mortality of 2 species each of submerged *Ceratophyllum demersum* Linn. and *Hydrilla verticillata* (L. f) Royle and free-floating *Lemna aequinoctialis* and *Spirodela polyrrhiza* (L) Schleid macrophytes was also screened at 8 different concentrations (1, 3, 5, 25, 40, 60, 80 and 100%) of spent wash, prepared by diluting with tap water. 5 cm long shoots (explants) of *Ceratophyllum* and *Hydrilla* and 10 plants (20 fronds) each of *Lemna* and *Spirodela* were added separately in plastic jars (300mL) filled in with diluted effluent (distillery treatments) and tap water (control). Adding tap water, water level in the jars was kept constant. Plant health was recorded daily for 10 days in the greenhouse. In another set of study, *Lemna* growth in spent wash was detailed similar to distillery soil leachate for 10 days.

Water fleas (*Daphnia similis* Claus), collected from a lake (about 30km from Jaipur City) in February, 06, were cultured according to APHA (1989) in a BOD incubator at 20°C. Ten young neonates (24-48h old) were added in a flask (100 mL) containing spent wash/boiled and filtered lake water (control). A concentration gradient series from respective stock solution was prepared. Final concentrations were based on the results of preliminary acute static bioassay. Test concentrations were selected on a logarithmic scale as outlined in APHA (1989). Standard method described in APHA (1989) was followed for assessing acute toxicity.

Besides monitoring fish mortality as described earlier, morphology and counts of RBC in live fish of both control and effluent treatments were also studied, as detailed elsewhere (Saxena et al. 2003). LC_{50} (fish) and EC_{50} (*Daphnia* and *Lemna*) values were calculated by BASIC version 1.13.

Healthy Swiss albino male mice (*Mus musculus* L; age: 45-50 days; weight: 25-27g) acclimated for one week prior to the experiment in a well ventilated animal house, as per guidelines of the Institutional Ethical Committee (Temperature = 25 ± 3°C, Humidity = 40-60%, 12 hour light/dark cycle, Standard feed: Hindustan Lever Limited, India and unpolluted tap water *ad libitum*), were divided equally (according to their drink) into five categories (each having ten animals) viz: Control (tap water from a bore well in the campus), 5%, 10% and 20% soil leachate treatments. Both control and treated animals were weighed and sacrificed (5 mice of each category) after 15 days in all treatments, and also after 30days in 5% treatment. Their reproductive organs (testes, epididymides, seminal vesicles and prostate glands) were removed carefully and washed in saline solution (0.9%), blotted dry and weighed. Epididymal spermatozoa, separated as per the method of Brooks (1976), were counted using haemocytometer, and their motility was observed according to Prasad et al. (1972).

RESULTS AND DISCUSSION

Physico-chemical characteristics

Soil: pH values of garden (G) and distillery (D) soils differing little (G = 7.9; D = 8.4), were alkaline. Whereas, values of EC (G = 0.34mS; D = 3.53mS), chlorides (G = 20ppm; D = 220ppm), organic matter (G = 0.25%; D = 0.60%), total kjeldahl nitrogen (G = 0.021%; D = 0.078%) and total phosphorus (G = 16.5mg/100g; D = 23.0 mg/100g) were, however, higher in distillery soil as compared to garden soil.

Soil leachate: The dark brown soil leachate was alkaline (pH = 8.4). Its EC, chloride and COD values were 3.56mS, 220ppm and 2290ppm respectively.

Spent wash: The dark brown odorous effluent was almost neutral (pH = 7.8). It had very high values of EC (32.7mS), total dissolved solids (62.6g/L), chlorides (6200ppm), total hardness (8400ppm), chemical oxygen demand (54,890ppm), volatile fatty acids (27,720ppm) and total phosphorus (260ppm). Thus, in comparison to effluent, soil leachate was highly diluted.

Toxicity to Plants

1. Cereals

Distillery soil: The seedling emergence (%) decreased significantly in distillery soil treatments, being very low (30-40% in wheat) to nil (rice) in pure distillery soil (Figs. 1a, 2 a). The reduction in vigor and dry weights of wheat seedlings was inversely proportional to the quantity of garden soil in the mixture, but no definite trend was recorded for rice (Figs. 1 b, c; 2b, c). Thus, seedling emergence was found to be the most sensitive stage in rice, while their vigor and dry weight in wheat though magnitude of toxic effects decreased with seedling age.

Soil leachate: Percentage germination of rice and wheat seeds was almost similar to control in soil leachate (Figs. 3a, 4a). Seedling vigor was found to be greater (10-25%) than control in 25-75% leachate, which, however, decreased a little at 100% concentration (Figs. 3b, 4b). Seedling dry weights decreased (7-36%) with an increase in leachate concentration in rice (Fig. 3c), while they were greater (15-47%) than control plants in wheat, even in 100% leachate (Fig. 4c).

Spent wash: In comparison to control, percentage germination and seedling vigor of rice and wheat decreased significantly with an increase in spent wash concentration, becoming nil at 30% (Figs. 5a, 6a). Almost similar trend was found for emergence of rice seedlings growing in river sand irrigated with spent wash, though it was nil even at a higher dilution (15%, Fig. 7a). Seedling vigor and their

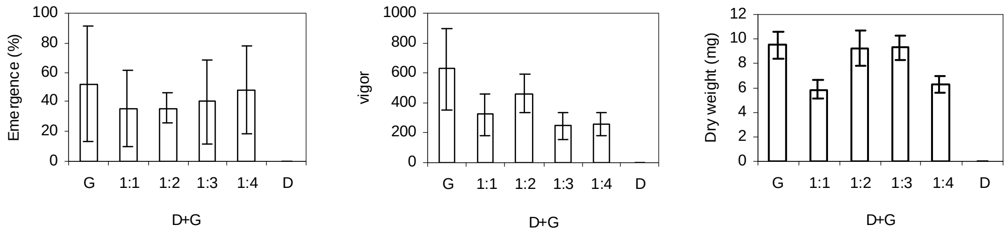

Fig. 1: Percent emergence of rice seedlings (a), their vigor (b) and dry weight (c) after 7 days growth in garden soil (G: Control), distillery soil (D) and distillery + garden soil mixed in different ratios (Jar study).

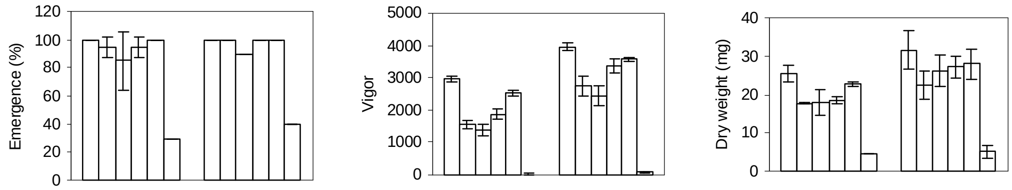

Fig. 2: Percent emergence of wheat seedlings (a), their vigor (b) and dry weight (c) after 10 and 15 days growth in garden soil (G: control), distillery soil (D) and distillery + garden soil mixed in different ratios (Jar study).

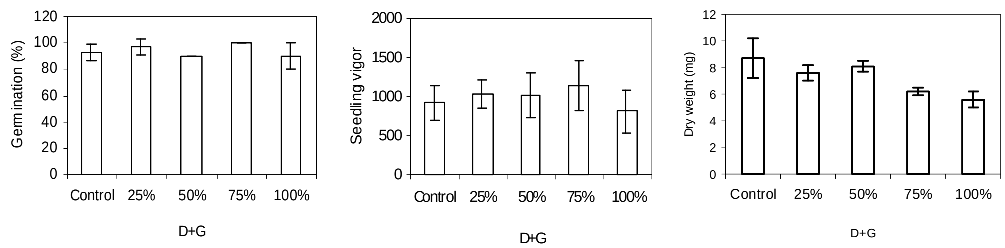

Fig. 3: Percent germination of rice (a), its seedling vigor (b) and dry weight (c) after 7 days growth in distillery soil leachate (Petridish study).

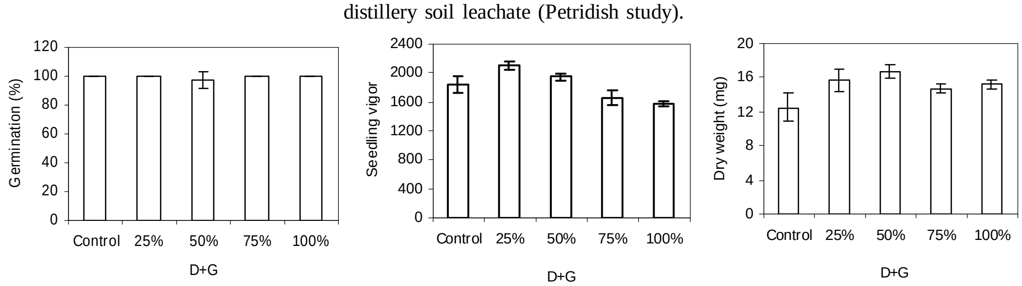

Fig. 4: Percent germination of wheat (a), its seedling vigor (b) and dry weight (c) after 7 days growth in distillery soil leachate (Petridish study).

dry weights in control were greater than spent wash treatments, with an exception at lower concentrations (5% in rice and 10% in wheat), indicating suitability of only very diluted spent wash for fertigation (Figs. 5b, 6b & c, 7b).

The toxic effects of distillery soil, its aqueous leachate and spent wash were comparatively higher in rice than wheat, establishing the former to be a more sensitive crop. Wang & Keturi (1990) also reported greater toxicity of heavy metals (in effluent) to rice as compared to wheat.

Dissolved solids and phenols are the two major pollutants in spent wash casting adverse effects on

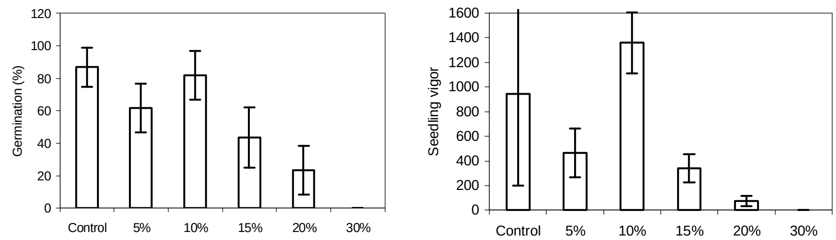

Fig. 5: Percent germination (a) of wheat and its seedling vigor (b) after 7 days growth in spent wash (Petridish study).

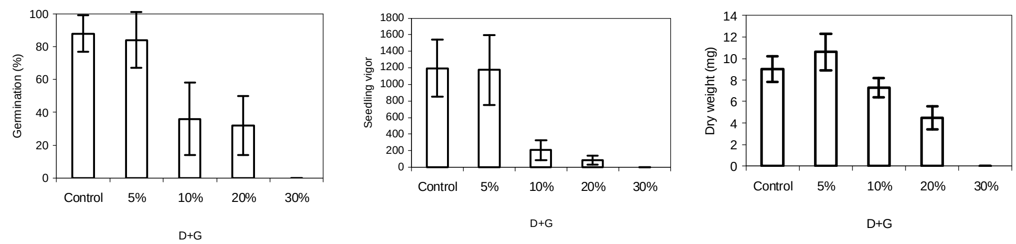

Fig. 6: Percent germination (a) of rice, its seedling vigor (b) and dry weight (c) after 7 days growth in spent wash (Petridish study).

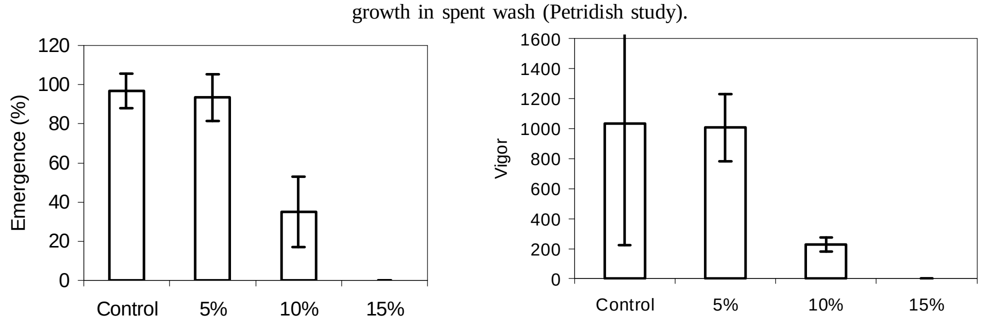

Fig. 7: Percent emergence of rice seedlings (a), and their vigor (b) after 7 days growth in the river sand irrigated with spent wash (Jar study). Significant at 5%* and !%** probability.

seed germination and seedling growth (Pathade 1999, Borja et al. 1993). Besides, higher concentration of biodegradable organics possibly increased soil (microbial) respiration that might have led to anoxic condition detrimental to germination (hence seedling emergence) and seedling growth. The lower toxicity of soil leachate (in petridish study) in comparison to distillery soil (filled in plastic jars) may partly be ascribed to increased oxygen availability to seeds in petridish, on account of their direct exposure to atmospheric oxygen. Another possible reason is degradation of toxic pollutants of spent wash by soil microflora.

2. *Hydrophytes*

Submerged (*Ceratophyllum* and *Hydrilla*) and free-floating (*Lemna* and *Spirodela*) macrophytes died within 24h of exposure between 25-100% spent wash since they lost their buoyancy and decayed afterwards. Their death was delayed between 3-10%, occurring after 2 days exposure in free-floating species, but after 7 days in submerged, suggesting former to be more sensitive. All the four species however, grew better, similar to control at 1%. Among free floating, detailed toxicity of distillery waste on *Lemna* (duckweed bioassay) was made, since it facilitates comparison with other related studies mostly made on it (Blinova 2000).

Duckweed bioassay: Slime formed over the surface of effluent and soil leachate was the most conspicuous feature. Its magnitude was concentration dependent. Initially, slime adhered at roots and lower surface of fronds due to which plants partially sank in the wastewater. Afterwards, it also covered upper surface of fronds. The slime layer was removed daily and also all plants were freed by agitating spent wash/leachate with a brush both in morning and evening. However, those exposed to concentrated waste (>3%) finally sank due to larger size of attached slime. Chemically, slime is a polysaccharide produced by aerobic microbes using carbohydrate of distillery waste as a substrate, to survive under stressed anaerobic condition developed due to higher BOD of wastewater (Wolfaardt & Plessis 2004).

***A. Spent wash*:** Control plants were green and buoyant, having thick spongy-paired fronds. Whereas, those growing in spent wash (1.0-7.5%) had relatively thin etiolated fronds whose size decreased (30-50%) between 2.0-7.5%. Roots were present only at lower concentration (0.5%). All plants died within 4 and 7 days of exposure respectively at 7.5% and 5%.

EC_{50} values of spent wash decreased with exposure period, being minimum for chlorophyll pigments, followed by dry weight and frond number (Table 1).

B. Soil leachate: The visible symptoms of leachate toxicity were similar to effluent, though plants growing at higher dilutions (>15%) were healthy, similar to control.

EC_{50} values decreased with an increase in exposure period and were markedly lower for frond number in comparison to chlorophyll pigments, suggesting severe toxic effects on vegetative

Table 1: EC_{50} values (%) of spent wash for *Lemna* in comparison to control plants growing in tap water.

Days	Regression Equation	EC_{50} (%)	Confidence level Upper	Lower	Chi-square
Frond Number					
4	Y=2.53 + 4.21 X	3.86	4.95	3.01	0.16591 7
Y=2.71 + 6.09 X	2.37	2.51	2.24	0.74935	10 Y=3.14
+ 6.04 X	2.03	6.67	0.62	4.75937*	Dry Weight
4	Y=4.73 + 0.32 X	6.97	NC	NC	30.18611*
7	Y=4.82 + 0.91 X	1.58	NC	NC	9.16833*10
Y=1.96 + 7.79 X	2.45	2.60	2.31	1.07733	Chlorophyll-*a*
4	Y=4.13 + 1.49 X	3.84	5.00	2.95	1.1077
7	Y=0.55 + 10.06 X	2.77	2.91	2.63	0.7495
10	Y=4.46 + 2.34 X	1.70	18.44	0.16	104.3054*
Chlorophyll-*b*					
4	Y=4.41 + 1.08 X	3.49	NC	NC	37.9489*7
Y=1.89 + 7.24 X	2.69	2.88	2.51	2.5850	
10	Y=5.13 + 1.32 X	0.80	5.42	0.12	20.86*
Chlorophyll-Total					
4	Y=4.34 + 1.23 X	3.45	4.56	2.61	4.03994 7
Y=1.17 + 8.75 X	2.74	2.90	2.59	1.4032	
10	Y=4.80 + 1.37 X	1.40	7.18	0.27	20.2038*

*- Chi-square highly significant; NC- Not calculable

Table 2: EC_{50} values (%) of distillery soil leachate for ***Lemna*** in comparison to control plants growing in nutrient poor medium (Tap water).

Days	Regression Equation	EC_{50} (%)	Confidence level Upper	Lower	Chi-square
Frond Number					
4	Y= 1.33 + 1.93X	79.5	94.8	66.7	1.31
7	Y=1.46 + 2.07 X	55.1	58.1	45.0	4.18
10	Y=1.21 + 2.27 X	46.5	52.6	41.1	1.0
Chlorophyll-*a*					
4	Not Calculable				
7	Y=-2.23 + 3.26 X	164.0	259.9	103.4	2.01
10	Y=-4.21 + 4.55 X	106.0	127.5	88.2	0.44
Chlorophyll-*b*					
4	Y=2.80 + 0.90 X	277.7	890.3	86.6	2.8
7	Y=-1.11 + 2.99 X	110.1	147.9	81.9	2.51
10	Y=-6.75 + 6.16 X	81.0	86.1	76.2	2.61E-03
Chlorophyll-Total					
4	Not Calculable				
7	Y=-5.06 + 4.91X	111.2	135.4	91.4	9.32E-02
10	Y=-5.27 + 5.26 X	90.2	99.5	81.8	0.13

Table 3: EC_{50} values (%) of distillery soil leachate for ***Lemna*** in comparison to control plants growing in nutrient rich medium (Hoagland solution).

Days	Regression Equation	EC_{50} (%)	Confidence level Upper	Lower	Chi-square
Frond Number					
4	Y=2.63 + 1.38 X	50.9	61.4	42.2	1.03
7	Y=2.51 + 1.62 X	35.1	43.7	28.1	2.9
10	Y=3.62 + 1.41 X	17.1	88.9	3.6	11.0*
Chlorophyll-*a*					
4	Not Calculable				
7	Y=-0.61+ 2.42 X	205.6	426.8	99.1	4.1
10	Y= -1.35+ 2.96X	139.5	188.2	103.4	1.3
Chlorophyll-*b*					
4	Y=3.51 + 0.64 X	207.6	747.8	57.6	1.68
7	Y=-0.97 + 3.08X	86.4	99.1	75.4	2.71
10	Y=0.85 + 2.37 X	56.98	63.7	50.96	5.02
Chlorophyll-Total					
4	Not Calculable				
7	Y=-1.54 + 3.20X	110.2	136.6	88.9	7.12E-02
10	Y=0.58 + 2.28 X	86.0	135.1	54.7	8.64*

* Chi-square highly significant

multiplication of plants. Their values decreased further when percent reduction in values of these two parameters were calculated with control values in Hoagland medium, indicating greater toxic effects of leachate in eutrophic water bodies, in comparison to oligotrophic ones having higher dilution factor for pollutants (Tables 2 &3).

EC_{50} values of soil leachate (44.5-277.7%) were 40-55 folds greater than that for effluent (0.8-6.97%), while COD value of the latter (Effluent: COD = 54,890ppm; Leachate: COD = 2290ppm) was only 24 times higher. Thus, in comparison to COD, reduction in leachate toxicity was higher, possibly on account of microbial degradation of toxic pollutants by soil microflora.

Toxicity to Animals

Daphnia: Water flea survived less than 12h in 1.5% spent wash. Whereas dose dependent mortality was observed between 0.25-1.0%; being nil at 0.1%. EC50 value for spent wash was 0.68%. Slime formation was meager in all spent wash treatments.

Fish: The slime formed in both effluent and soil leachate treatments increased their turbidity due to aeration in the study period (96h). Fish turned brown and moved faster than control fish. They were also slimy due to copious secretion of mucus; a protective mechanism to avoid direct contact with the pollutants. The dying fish lost their body balance. The gills of dead fish had deposition of slime and dark blackish brown material which interfered in respiration and was perhaps one of the major factors of their mortality. Fish mortality was dose dependent, being 100% within 24h exposure respectively in 2% and 30% effluent and leachate. The LC_{50} value of spent wash (1.31%) was significantly lower than that of soil leachate (12.1%), indicating higher toxicity of the former as also noted earlier in plant bioassays.

A comparison of LC_{50} value of spent wash with other industrial wastewaters revealed it to be little less toxic than tannery waste (0.66%; Thorat & Waugh 2001), but higher in comparison to dairy (25%; Amudha & Mahalingam 1999) and paper and pulp wastewaters (16.5-30%; Kumar et al. 1991, Varadraj & Subramanian 1991). The higher toxicity of tannery waste may be attributed to rich chromium content (100-250ppm) as its waste strength in terms of COD values (2500- 8000ppm) was much lower than spent wash (Rao et al. 1999).

In comparison to control, RBCs in spent wash treatments stained darker (hyperchromic) and their counts decreased markedly (58-69%). Percentage abnormalities in their shape (poikilocytosis) were

Table 4: Food and water intake of control and distillery soil leachate treated animals, along with their body and organ weights.

Parameters	Control	Exposure period (15 days)			30days
		5%	10%	20%	5%
Food intake (g)	5.61± 0.15	5.05 ± 0.14 (-10)	5.9± 1.57 (+5)	5.2± 1.39 (-7)	4.84± 0.16* (-14)
Water intake (mL)	13.9± 0.53	9.12 ± 0.34** (34)	10.2± 0.37** (-27)	7.6 ± 0.27*** (-45)	9.06 ± 1.63* (-35)
Body weight	30.3 ± 1.45	27.3 ± 3.7 (-10)	30±1.15 (-1)	28 ± 2.3 (-8)	25.3± 2.7 (-17)
Testes	0.096 ± 0.014	0.073 ± 0.01 (-24)	0.091± 0.002 (-5)	0.064± 0.03 (-33)	0.072± 0.014 (-25)
Epididymis	0.057 ± 0.012	0.051± 0.01 (-11)	0.052 ± 0.008 (-9)	0.043± 0.02 (-25)	0.043± 0.007 (-25)
Prostate	0.059 ± 0.012	0.078 ± 0.02 (+32)	0.081± 0.006 (+37)	0.037± 0.01 (-37)	0.066± 0.016 (+12)
Seminal Vesicle	0.080 ± 0.004	0.059 ± 0.01 (-26)	0.063 ± 0.004* (-24)	0.077± 0.02 (-4)	0.030 ± 0.011* (-63)

Significant at *5 %, **1% and ***0.1% probability; Data in parentheses indicate percentage change in comparison to control comparatively lower (about 20%) in control fish than those in spent wash treatments (77-82%), becoming almost cent percent (97%) at 1.5% concentration.

The permeability of erythrocytic membrane to pollutants not only destroys RBCs (Moss & Hathway 1964), but also alter their shape and size by changing structure and function of cell membrane (Udden 2000, Suwalky et al. 2004). This explains reduction in RBC counts and their poikilocytosis in the present study. We made similar observations in *Gambusia affinis* and *Poecilia reticulata* exposed respectively to detergents (Saxena et al. 2003) and an azo dye methyl red (Sharma et al. 2006). Kumar & Gopal (2001), however, reported increase in RBC counts in spent wash exposed *Channa punctatus*, attributing it to acceleration in erythropoiesis to cope anaemia. Such responses seem to be species specific.

A comparison of data on mortality and RBC (counts and poikilocytosis) revealed greater adverse effects on RBC, more particularly on their shapes, explaining greater physiological stress in fish. In view of these findings, we recommend examination of mortality and RBC (count and poikilocytosis) simultaneously, for deciphering wastewater toxicity more accurately.

Mammals: The oral administration of soil leachate (5, 10 and 20%) with drinking water decreased food (7-14%) and water (27-45%) intake in mice in comparison to control (Table 4). As a result, their body (8-17%) and reproductive organ weights (Testes = 5-33%; Epididymis = 9-25%; Seminal vesicle = 4-63%) decreased whereas that of prostrate increased (12-37%) (Table 4). Other workers also reported similar findings in albino mice exposed to phosphamidon (Sivasamy & Akabarsha 2000) and textile dye wastewaters (Suryavathi et al. 2005). Malnutrition of wastewater exposed animals may be related to their appetite loss caused by wide spread histopathological lesions in intestine, liver and kidney that affected their physiology (Pathak 2002, Mathur et al. 2003). Further, reduction in organ weight of testes and epididymis indicated damage respectively to organs of spermatogenesis and sperm maturation that decreased sperm counts (78-89%) and their motility (14-23%), which agrees with the findings of Suryavathi et al. (2005) made in mice and rats exposed to textile dye wastewaters. Thus, soil leachate exposure adversely affected health and reproduction capacity in male albino mice.

EC_{50} value of spent wash for fish (1.31%) was almost two-folds higher than that for *Daphnia* (0.68%) indicating greater sensitivity of the latter. In case of *Lemna*, minimum EC_{50} values of spent wash were recorded on 10^{th} day, which were almost 2-4 folds greater than LC_{50} value for fish for parameters such as frond number (3 folds), dry weight (3.6 folds) and chlorophyll contents (chl. *a*: 2.5 folds; total chl.: 2.1 folds). The only exception was EC_{50} value for chlorophyll *b*, being at par with *Daphnia* but lower than that for fish. In a similar comparative study on heavy metals, Wang (1991) also reported higher sensitivity of *Lemna* to some heavy metals (Cd, Cr, Pb, Ni and Se) than fish. Thus, duckweed bioassay is equally effective in monitoring industrial wastewater toxicity.

Although, cereals were found to be less sensitive to distillery wastes in comparison to duckweed, but they established toxic effects of land application of concentrated waste in crop ecosystems, as also noted during field surveys.

Present study has, thus, revealed toxic effects of current disposal practices of distillery effluent to both producers and consumers in terrestrial and aquatic ecosystems. Adverse effects on health and reproductive capacity of albino mice suggest that even human beings and domestic animals inhabiting nearby distilleries may be at a potential risk due to groundwater contamination. In view of these findings, the concerned regulatory agency is advised to monitor distillery waste disposal strictly.

ACKNOWLEDGEMENTS

We are thankful to the Department of Biotechnology, New Delhi for grants to K.P. Sharma and Subhasini, and Council of Scientific & Industrial Research, New Delhi for awarding Research Associateship to Shweta Sharma.

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