International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(1): 2236-2239

Received: 11-11-2018
Accepted: 14-12-2018

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Effect of long-term irrigation of treated distillery waste water on microbial activity and heavy metals content in a *Vertosol* under sugarcane cropping system

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Abstract
A field experiment was conducted to evaluate the effect of long-term application of spentwash on heavy metals content in soil and sugarcane crop in Northern Dry Zone of Karnataka. The distillery effluent was characterized by neutral pH (7.37), high EC (17.32 dS m^{-1}), high biological oxygen demand (7200 mg L^{-1}), and chemical oxygen demand (18032 mg L^{-1}). The application of spentwash significantly increased the microbial activity in the soil; among different periods of spentwash irrigation dehydrogenase activity ranged between 51.77 to 43.77 µg TPF g^{-1} of soil day^{-1}, phosphates activity 105.22 to 88.24 µg PNP g^{-1} of soil hr^{-1} and urease activity 141.7 to 107.5 µg NH_4- N g^{-1}soil hr^{-1}. Distillery effluent application for 5-10 years recorded the higher dehydrogenase (51.77 µg TPF g^{-1} of soil day^{-1}) and urease activity (141.7 µg NH_4- N g^{-1}soil hr^{-1}) while higher phosphates activity (105.22 µg PNP g^{-1} of soil hr^{-1}) in 10-15 years spentwash irrigated treatment. The distillery effluent application appeared to have promoted the enzymatic activities in soil as lower microbial activity was observed in the control treatment. The present study has shown that sugarcane distillery effluent had slightly elevated levels of toxic heavy metals like cadmium (Cd), arsenic (Ar), and chromium (Cr) therefore seemed not fit for long-term irrigation or disposal without applying proper remedial measures.

Keywords: Treated distillery wastewater, heavy metals, micro-organisms and sugarcane

Introduction
The distillery wastewater (spentwash) is a dark brown coloured liquid with an unpleasant odour of burnt sugar. The raw spentwash is acidic in nature with the pH ranging from 3.8- 4.2, while the primary treated spentwash otherwise known as post methanated distillery spentwash (PMDSW) is near neutral in reaction (pH of 7.2). The dark brown colour of raw spentwash is due to the presence of melanoidin of cane molasses which is not decomposed effectively by yeast and methane bacteria in its activated sludge process. In comparison to raw spentwash, the PMDSW contains lower BOD and COD values. The annual treated distillery spentwash obtained in India can supply 16,800 tonnes of N, 6,300 tonnes of P and 1.26 lakh tonnes of K and by this it is estimated that Indian distilleries could save about 10,000 million rupees annually as cost of fertilizers is increasing (Chandraju, 2005) [4].
Wastewater characteristics and levels of pollutants vary significantly from industry to industry. The use of industrial waste as soil amendment has generated interest in recent time (Devarajan and Oblisamy 1995). The wastewater produced continuously could cater the needs of irrigated crops. In the initial years, use of wastewater to the crop may benefit the crop due to its nutritive and growth promoting effect. However, continuous use of spentwash not only pollutes the environment but also results in accumulation of salts in the root zone adversely affecting the crop. In recent years, expansion of distilleries in the sugarcane growing areas and indiscriminate disposal of spentwash in sugarcane cultivating lands adjacent to industries has affected soil health through salinity and heavy metal toxicity.

Materials and Methods
The soil type of the experimental site was medium calcareous Vertisol. The soil was alkaline in nature with pH ranging from 7.84 to 8.11. The electrical conductivity was higher in spentwash irrigated plots (4.46 dS m^{-1}) compared to control treatment (0.45 dS m^{-1}).

The soil organic carbon in the surface layer ranged from 6.0 to 16.7 g kg^{-1}. The available N, P, K and micronutrients were higher in the spentwash applied treatments. The experiment consisted of five treatments laid out in randomized complete block design with five replications. The experiment was laid-out in 2-factor RCBD. The bio-methanated spentwash (dilution 1:10) was used as irrigation source to sugarcane @ 100 m^3 ha^{-1} $year^{-1}$. The treatments included bio-methanated spentwash irrigation for varied periods of time (factor-1); P0: Control, P1: 5-10 years, P2: 10-15 years, P3:15-20 years and P4: >20 years at different soil depths (factor-2); D1: 0-20 cm, D2: 20-40 cm, D3: 40-60 cm, D4: 60-80 cm and D5: > 80 cm. The experiment was conducted at the Ugar Sugar Factory premises, Ugar-Khurd, Athani taluk, Belagavi district, to know the effect of different periods of biomethanated spentwash application on sugarcane (Co-86032) crop. The physicochemical characteristics of the bio-methanated spentwash is given the Table-1.

Table 1: Characteristics of distillery bio-methanated spentwash (Ugar Sugar Factory)

Parameter	Value
Colour	Light brown
Odour	Tolerable
pH	7.37
Electrical conductivity (dS m^{-1})	17.32
Biological oxygen demand (mg L^{-1})	7,200
Chemical oxygen demand (mg L^{-1})	18,032
Bicarbonates (mg L^{-1})	3.9
Carbonates (mg L^{-1})	Trace
Chlorides (mg L^{-1})	3266
Sodium (mg L^{-1})	234
Potassium (mg L^{-1})	6213.12
Calcium (mg L^{-1})	521
Magnesium(mg L^{-1})	233
Total nitrogen (mg L^{-1})	748
Total phosphates (mg L^{-1})	112.35
Zinc (mg L^{-1})	8.34
Iron (mg L^{-1})	21.25
Manganese (mg L^{-1})	4.35
Copper(mg L^{-1})	6.55

(Source: Personal communication, Ugar Sugar Factory, Belgaum, Karnataka**)**

Enzymatic Activity

Dehydrogenase: Soil sample (5 g) was taken in a boiling tube. To this 1 ml of 3% 2, 3, 5-Tri phenyl tetrazolium chloride was added. Then 1 ml of 1% glucose and 2.5 ml of distilled water was added and incubated for 24 hrs. After that 10 ml of methanol were added and incubated for another 5 hrs. The content was filtered through Whatman No.1 filter paper. The samples were washed thoroughly with methanol. The red colour developed was read at 485 nm. The concentration of dehydrogenase in the sample was obtained from the standard graph using triphenyl farmazane (Casida, 1964) [3].

Phosphatase: Soil sample (5 g) taken in a boiling tube. To this 10 ml of distilled water, 0.25 ml of toluene and 1ml of 10 mM p- nitrophenyl phosphate (PNPP) were added and incubated at room temperature for 1hr. Then 5 ml of 0.5 M $CaCl_2$ and 20 ml of 0.5 M NaOH were added. The content was filtered using Whatman No.42 and volume made up to 50 ml with distilled water. The colour intensity was read at 420 nm. The concentration of phosphatase was obtained from a standard graph (Halstead 1964) [5].

Urease: Soil sample (10 g) was taken in a 100 ml volumetric flask. To this 1.5 ml of toluene was added, mixed well and incubated for 15 min. Then 10 ml of 10% urea solution and 20 ml of citrate buffer were added, mixed thoroughly, stoppered and incubated for 3 hrs at 37°C in an incubator. Then the volume was made up to 100 ml with distilled water, mixed by shaking immediately. The contents were filtered through Whatman No.1 filter paper and 1ml of filtrate was pippetted out into 50 ml volumetric flak. To this 9 ml of distilled water, 4 ml of phenate and 3 ml of NaOCl were added, mixed well and allowed to stand fo 20 min. The volume was made up to 50 ml and mixed well. The bluish green colour developed was read at 630 nm. Simultaneously a blank was also prepared (without urea solution). The concentration of urease in the sample was obtained from the standard graph using diammonium sulphate (Tabatabai and Bremner 1972) [11].

The population of bacteria, fungi and actinomycetes was assessed by serial dilution and plate count method as described by Bunt and Rovira (1955) [2], Martin (1950) [9] and Kuster and Williams (1964) [8], respectively.

The total metal ions can be extracted from the soil by using the solusion called Aqua-regia. It is prepared with the help concentrated nitric and hydrochloric acids at the ratio of 1:3. It is commonly used to remove noble metals ions particularly in micro fabrications and exchange complex of soil. Heavy metals (Cd, Cr, Co, Ar, Hg and Pb) were determined in clear digests by using atomic absorption spectrophotometer (AAS) and expressed as mg/kg.

Results and Discussion

Distillery spentwash is the residual liquid waste generated during alcohol production and pollution caused by it is one of the most critical environmental issues. Despite standards imposed on effluent quality, untreated or partially treated effluent very often finds access to water courses. The distillery wastewater with its characteristic unpleasant odor poses a serious threat to the water quality in several regions around the globe. The ever increasing generation of distillery spent wash on the one hand and stringent legislative regulations of its disposal on the other has stimulated the need for developing new technologies to process this effluent efficiently and economically including plant growth and yield (Sarayu *et al.* 2009) [10].

The dehydrogenase activity of sugarcane at harvest was significantly influenced by long-term spentwash application (Table 2). At harvest, the higher dehydrogenase activity (51.77 µg TPF g^{-1} of soil day^{-1}) was recorded in the treatment that was applied with spentwash for 5 to 10 years and lower was observed in control (29.55 µg TPF g^{-1} of soil day^{-1}). With respect to phosphatase activity the higher was seen in treatment that was applied with spentwash for 10 to 15 years. The higher activity (105.22 µg PNP g^{-1} of soil hr^{-1}) was higher in the spentwash irrigated fields compared to control. This was attributed to higher organic load present in the spentwash, which might serve as source of energy for the growth and multiplication of micro-organisms and also for various enzyme activities in soil.

Urease activity in sugarcane at harvest was significantly influenced by application of spentwash over different periods of time (Table 2) and it ranged from 141.7 to 75.22 µg NH_4-N g^{-1} soil hr^{-1}. Joshi (2002) [6] observed an increase of 10 to 20 per cent in microbial population over the control due to the application of distillery spentwash. He reported the dominance of *Bacillus sp*., *Pseudomonas sp*., *Citrobacter sp*.,

Arthrobacter sp., *Streptomycetes etc*., in the spentwash amended soil. Kalaiselvi and Mahimairaja (2009) [7] observed the highest enzyme activities due to the application of spentwash at the rate of 120 m^3 ha^{-1} plus NP fertilizers. The continuous application in split doses was found better than one time application of spentwash for promoting the enzyme activities throughout the crop growth, mainly by providing steady supply of nutrients and organic matter. The positive significant correlations observed between enzyme activities and nutrients mineralization clearly demonstrated the important role of these enzymes on the nutrients dynamics and the availability in soil.

The effect of long-term application of spentwash was found significant with respect to fungi, bacteria and actinomycetes population. The highest fungi population (24.01 cfu $x10^4$) was recorded in the treatment that was irrigated with spentwash for 15 to 20 years and it remained on par with (23.18 cfu $x10^4$) P_1 (10 to 15 years spentwash application). The lowest fungi population (11.66 cfu $x10^4$) was observed in control. Application of spentwash significantly increased the population of bacteria in the soil over control. Bacterial population in surface soil was significantly influenced by different periods of spentwash irrigation. However, spentwash irrigation for > 20 years significantly reduced bacterial population. The highest bacterial population (24.01 cfu $x10^6$) was noticed in treatment that received spentwash for 5 to 10 years. The data on population of actinomycetes showed that there was significant influence of long-term spentwash application on actinomycetes population. The P_1 (5 to 10 years of spentwash application) recorded higher actinomycetes population (29.88 cfu $x10^3$) and it remained on par with P_2 (29.10 cfu $x10^3$) (15 to 20 years of spentwash application). Further in P_4 (more than 20 years spentwash application) there was marginal reduction in the population of bacteria, fungi and actinomycetes which might be due to buildup of salinity and chloride. The results were in agreement with results reported by Altman and Lawlor (2008) [1].

Spentwash is purely of plant origin obtained during fermentation of molasses to produce alcohol using specific strains of yeast. It is a dark reddish brown coloured containing residual nutrients from sugarcane and yeast cells with rotten jaggery smell. It contains very small quantity of some heavy metals and it is highly polluting liquid because of its high BOD and COD. The data obtained after analyzing the surface soil samples (0-20 cm) for heavy metals like arsenic cadmium chromium cobalt lead mercury are presented in Table 4. The long-term application of spentwash found non-significant with respect to lead and mercury and there is slight increased some of the heavy metals like arsenic, chromium and cadmium content in surface soil.

Table 2: Effect of different periods of spentwash application on soil enzymatic activities in sugarcane field at surface (0-20 cm) of soil

Treatment	Dehydrogenase activity (µg TPF g^{-1} of soil day^{-1})	Phosphatase activity (µg PNP g^{-1} of soil hr^{-1})	Urease activity (µg NH_4-N g^{-1} soil hr^{-1})
P_0	29.55	61.82	75.22
P_1	51.77	103.44	141.7
P_2	45.39	105.22	134.1
P_3	47.75	93.02	113.9
P_4	43.77	88.24	107.5
S.Em.±	1.74	6.72	5.73
CD (P=0.05)	5.23	20.17	17.18

Table 3: Effect of different periods of spentwash application on soil microorganisms in sugarcane field at surface (0-20 cm) of soil

Treatment	Bacteria (CFU × 10^7 g^{-1} soil)	Fungi (CFU × 10^4 g^{-1} soil)	Actinomycetes CFU × 10^5 g^{-1} soil)
P_0	8.01	11.66	18.28
P_1	13.43	24.01	29.88
P_2	14.57	23.18	29.10
P_3	11.91	22.76	28.30
P_4	10.03	17.27	22.46
S.Em.±	0.61	1.01	1.52
CD (P=0.05)	1.82	3.05	4.56

P_0- Control. P_1, P_2 and P_3- correspond to periods of spentwash application; P_1-5 to 10 years, P_2-10 to 15 years and P_3- 15 to 20 years

Table 4: Effect of different periods of spentwash application on total heavy metals (mg/kg) in sugarcane field at surface (0-20 cm) of soil

Treatment	Arsenic	Cadmium	Chromium	Cobalt	Lead	Mercury
P_0	3.49	5.31	37.71	22.39	1.67	0.62
P_1	3.45	5.33	37.59	24.64	1.31	0.58
P_2	3.51	5.56	39.41	23.42	1.40	0.54
P_3	4.01	5.49	42.12	25.48	2.04	0.65
P_4	3.89	6.07	40.61	25.59	1.93	0.56
S.Em.±	0.12	0.23	1.85	1.23	0.10	0.05
CD (P=0.05)	0.35	0.71	5.59	3.63	NS	NS

P_0: Control. P_1, P_2, P_3 and P_4- correspond to periods of spentwash application; P_1:5 to 10 years, P_2:10 to 15 years, P_3: 15 to 20 years and P_4:>20years

D_1, D_2, D_3, D_4 and D_5- correspond to soil depth; D_1: 0-20 cm, D_2: 20-40 cm, D_3: 40-60 cm, D_4: 60-80 cm and D_5: >80 cm.

Conclusion

- Soil microbial activity was significantly influenced by spentwash application. Organic matter addition through spentwash had increased the microbial population and the highest bacteria, fungi and actinomycetes population were recorded in the treatment that received spentwash.
- The Environmental contamination by trace or heavy metals through industrial wastes is one of the main health concerns in industrial countries. The long-term use of sugar industrial effluents for irrigation may contribute to elevated levels of toxic heavy metals like Cd, Cr and Ar in surface soil.

Acknowledgements

The authors greatly acknowledge the financial assistance by the DBT funded EU-India "Water for Crops" Project and also thankful to Ugar Sugars Works Ltd. Ugar Dist: Belagavi for their help in executing the study.

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