Original Research Article
DYNAMICS OF SOIL MICROBIAL POPULATION AND ENZYMES ACTIVITIES UNDER DISTILLERY SPENTWASH IRRIGATION

ABSTRACT

Spentwash is a rich source of organic matter and nutrients like nitrogen, phosphorus, potassium, calcium and sulphur. The effect of different levels and methods of spentwash application on soil enzymatic activity was examined through a field experiment. The field experiment was conducted using Sesame VRI (Sv) 2 as a test crop at Research and Development Farm, The Sakthi Sugars pvt. Ltd., Appakkudal, Erode District. The experiment was formulated with six treatments with four replications, laid out in Randomised Block design. As per the treatment schedule the calculated quantity of biomethanated distillery spentwash for pre-sown application *was* uniformly applied to the plots before sowing viz., 25, 50, 75,100% along with recommended dose of NP for four treatments viz., 100%, 75%, 50%, 25% and Recommended dose NPK was treated as one treatment (control).The soil samples were collected at 30 days intervals and analysed for the changes in soil microbial population and enzyme activities. The results of the study showed that the microbial population and enzymatic activities of the soil were substantially increased throughout the crop growth period due to biomethanated distillery spentwash application.

Keywords: *Post methanated distillery spentwash, microbial population, enzyme activities*

1.INTRODUCTION

The spentwash is acidic (pH 3.94 - 4.30), dark brown liquid with high BOD (45,000 – 1,00,000 mg l^{-1}) and COD (90,000 – 2,10,000 mg l^{-1}), and produce obnoxious odour. Although it does not contain toxic substances, its discharge without any treatment brings about immediate discolouration and depletion of dissolved oxygen in the receiving water streams, in turn posing serious threat to the aquatic flora and fauna (Mane *et a*l., 2006). Distillery waste is rich in organic matter and nutrients especially nitrogen and potassium and also can be utilized as a source of irrigation water in water scarcity areas. However, they are also characterized by high soluble salts coupled with high BOD and COD. Hence while aiming for better crop production; their utilization has to be optimized for sustaining the environment. On an average, distillery effluent release 80 million kg of nitrogen and 520 million kg of potassium annually. Thus the availability of nutrients in distillery effluents and the possibility of substituting these for inorganic fertilizer in agriculture have a great promise (Joshi and Singh, 2010).The addition of organic matter through the BDS may be favorable for microorganisms and enzymes in soils. Batch *et al.* (1993) observed that the spentwash at 250 m^3 ha^{-1} rate stimulated the soil microorganisms and increased the dehydrogenase activity in soil. The spentwash addition increased the phosphatase, dehydrogenase and urease enzymes in dry land black and red soils especially at levels of 125 m^3 ha^{-1} (Murugaragavan, 2002).

2.MATERIALS AND METHODS

A field experiment was conducted at Research and Development Farm, The Sakthi Sugars pvt. Ltd., Appakkudal, Erode District, Tamil Nadu in randomized block design with three replications using sesame (*Sesamum indicum*) var .VRI (Sv)2 as a test crop. The experimental field was laid out and the calculated quantity of BDS (Table 1) was uniformly applied in each plot as per the treatment details given below. Then, the soil was ploughed at 10 days interval for providing better soil aeration and consequent reduction of BOD level in the soil-water system.

Treatment Details:

T_1 : Absolute control.

T_2 : Control – 100% recommended dose of NPK.

T_3 : 25 % N through DSW and 75 % N through inorganic source based on crop requirement.

T_4 : 50 % N through DSW and 50 % N through inorganic source based on crop requirement.

T_5 : 75 % N through DSW and 25 % N through inorganic source based on crop requirement.

T_6 : 100 % N through DSW.

While applying P, the available P in DSW and inorganic P will be taken together to meet the P requirement of crop. Potassium will be skipped in DSW applied treatments.

2.1 Collection and analysis of soil samples: Soil samples were collected at 30 DAS (R1), 60 DAS (R2), 90 DAS (R3), and 120 DAS (R4), and the number of fungi ,bacteria and actinomycetes colonies were assessed by plating dilution techniques (Waksman and Fred ,1922). The activities of urease, phosphatase and dehydrogenase enzymes were assayed as per the standard procedures (Tabatabai and Bremner, 1972).

Table 1.Characteristics of Post methanated distillery spentwash (PMDSW)

Characters	Unit	Values
Physical properties		
Colour	-	Greenish brown
Odour	-	Unpleasant
Moisture	%	82
Total suspended solids	mg L^{-1}	6850
Total dissolved solids	mg L^{-1}	45,120
Total solids	mg L^{-1}	51,970
Specific gravity	g cc^{-1}	1.12
Physico-chemical properties		

pH	-	7.75
EC	dS m^{-1}	37.8
Biological oxygen demand	mg L^{-1}	8,740
Chemical oxygen demand	mg L^{-1}	37,476
Organic carbon	mg L^{-1}	26,110
Total Nitrogen	mg L^{-1}	1,700
Total Phosphorus	mg L^{-1}	450
Total Potassium	mg L^{-1}	11,550
Total Sodium	mg L^{-1}	845
Total Calcium	mg L^{-1}	2,272
Total Magnesium	mg L^{-1}	1,580
Water soluble cations		
Calcium	m.e. L^{-1}	52.89
Magnesium	m.e. L^{-1}	61.25
Sodium	m.e. L^{-1}	32.87
Potassium	m.e. L^{-1}	227.35
Water soluble anions		
Carbonate	m.e. L^{-1}	Absent
Bicarbonate	m.e. L^{-1}	54.12
Chloride	m.e. L^{-1}	240.82
Sulphate	meq L^{-1}	75.70
SAR		4.56
RSC	meq L^{-1}	-61.20
SSP	Per cent	9.87
Potential salinity	meq L^{-1}	258.24
Biological properties		
Bacteria	× 10^6 CFU ml^{-1}	23.6
Fungi	× 10^4 CFU ml^{-1}	11.2
Actinomycetes	× 10^2 CFU ml^{-1}	7.2

3.RESULTS AND DISCUSSION

3.1 Soil microflora: In field experiment conducted with sesame under rainfed condition, the highest population of bacteria, fungi and actinomycetes (36.70 x 10^6 CFU g^{-1}, 15. 27 x 10^4 CFU g^{-1} and 6.87 x 10^2 CFU g^{-1} of soil) were observed in the treatment that received 100 per cent N through DSW at rainfed condition

respectively (Table.2). This is in line with Mattiazo and Ada Gloria (1985) who reported the increase in soil microbial activity due to oxidation of organic matter in treated spentwash applied soil. Devarajan *et al*. (1993) studied the population dynamics of bacteria, fungi and actinomycetes in field soil grown with turmeric, paddy, gingelly, cotton, and groundnut and the populations were increased with spentwash irrigations at different doses. Patil *et al.* (1982) stated that the spentwash contained 42.7 per cent polysaccharides which served as a source of carbon and sulphate for microbial proliferation. Such effect of spentwash on the population of bacteria, fungi and actinomycetes in the soil was also reported by Tauk *et al*. (1990); Goyal *et al*. (1995); Rajukkannu *et al.* (1996); Valliappan (1998). Similar results were reported in spentwash applied soils by Gopal *et al*. (2001) and Latha (2008). The above findings add strength to the present investigation.

The treatment of distillery spentwash with addition of inorganic fertilizer significantly influenced the soil bacterial population at different stages of crop growth.Among the treatments, T_6 (100 per cent N through distillery spentwash) recorded the highest soil bacterial population of 36.70 x 10^6 CFU g^{-1} of soil, followed by T_5 (75 per cent N through distillery spentwash + 25 per cent N through inorganic source) of 34.07 x 10^6 CFU g^{-1} of soil while the lowest bacterial population of 18.55 x 10^6 CFU g^{-1} of soil was recorded in T_1 (Control). The interaction effect on stages and different doses of DSW was positive only at S I (30DAS) and S II (60 DAS) stages. With respect to the stages of sampling, the highest soil bacterial population of 29.28 x 10^6 CFU g^{-1} of soil was recorded at S_1 (vegetative stage) and the lowest soil bacterial population of 26.25 x 10^6 CFU g^{-1} of soil) was recorded at S_4 (at harvest stage). The interaction effects of treatments and various stages were significant.

3.2 Fungal population

The different treatments significantly influenced the soil fungal population at four different stages of observation. Among the treatments, T_6 recorded the highest soil fungal population of 15.27 x 10^3 CFU g^{-1} of soil, which was on par with T_5 of 14.23 x 10^3 CFU g^{-1} of soil, while the lowest soil fungal population of 8.15 x 10^3 CFU g^{-1} of soil and 9.35 x 10^3 CFU g^{-1} of soil was recorded in T_1 (control) and T_2 (100 per cent RD of NPK) and were on par with each other. With respect to the stages of sampling, the highest soil fungal population of 13.15 x 10^3 CFU g^{-1} of soil was recorded at S_1 (vegetative stage) and the lowest soil fungal population of 10.45 x 10^3 CFU g^{-1} of soil was recorded at S_4 (at harvest stage). The interaction effects of treatments and various stages were non- significant.

3.3 Actinomycetes population

Application of distillery spentwash significantly influenced the soil actinomycetes population at different stages of observation. Among the treatments, T_6 (100 per cent distillery spentwash) recorded the highest soil actinomycetes population of 6.87 x 10^2 CFU g^{-1} of soil, followed by T_5 (75 per cent N through distillery spentwash + 25 per cent N through inorganic source) of 6.15 x 10^2 CFU g^{-1} of soil, while the lowest soil actinomycetes population of 4.77 x 10^2 CFU g^{-1} of soil was recorded in T_1 (Control). With respect to the stages of sampling, the highest soil actinomycetes population of 6.11 x 10^2 CFU g^{-1} of soil was

recorded at (vegetative stage) and the lowest value of 4.91 x 10^2 CFU g^{-1} of soil was recorded at S_4 (at harvest stage). The interaction effects of treatments and various stages were significant.

3.4 Dehydrogenase activity: The DSW application increased the activities of dehydrogenase, phosphatase and urease with different doses of DSW viz., 100 per cent N through DSW in the field experiments with sesame at rainfed condition, respectively (Fig.1, Fig.2 & Fig.3).The dehydrogenase activity of the soil was also influenced by other doses of DSW application. Significantly higher dehydrogenase activity of 31.62 and 28.15 µg of TPF g^{-1} of soil was recorded in T_6 and T_5, which were on par with each other. The lowest enzyme activity of 12.42 µg of TPF g^{-1} of soil was recorded in T_1 (Control). The soil dehydrogenase activity significantly differed at all stages of crop growth. The dehydrogenase enzyme activity was lowest at S_4 (at harvest stage) of 19.72 µg of TPF g^{-1} of soil and highest at S_1 (vegetative stage) of 23.40 µg of TPF g^{-1} of soil.

3.5 Phosphatase activity: The phosphatase activity of the soil was highly influenced by different doses of DSW application. Significantly higher phosphatase activity of 16.32 µg of PNPP g^{-1} of soil was recorded in T_6 (100 per cent N through distillery spentwash) followed by the treatments T_5, T_4 and T_3. The lowest enzyme activity of 9.85 µg of PNPP g^{-1} of soil was recorded in T_1 (Control). The soil phosphatase activity significantly differed at all stages of sesame crop growth. The enzyme activity was lowest at S_4 (harvest stage) of 11.60 µg of PNPP g^{-1} of soil and the highest at S_1 (Vegetative stage) of 14.21 µg of PNPP g^{-1} of soil.

3.6 Urease activity Urease activity of the soil was measured in distillery spentwash applied field. Significantly higher urease activity of 15.25 µg of ammonia released $g^{-1}$of soil h^{-1} was recorded in T_6 (100 per cent N through distillery spentwash), which was on par with T_5 (75 per cent N through distillery spentwash + 25 per cent N through inorganic source) of 14.00 µg of ammonia released g^{-1} of soil h^{-1}. The lowest enzyme activity of 8.87 µg of ammonia released g^{-1} of soil h^{-1} was recorded with T_1 (Control). The soil urease activity significantly differed at all stages of crop growth. The enzyme activity was the lowest at S_4 (at harvest stage) of 10.41 µg of ammonia released $g^{-1}$of soil $h^{-1}$and the highest at S_1 (vegetative stage) of 13.66 µg of ammonia released $g^{-1}$of soil h^{-1}. This might be due to tremendous increase in the microbial population, availability of most of the essential nutrients and organic carbon content of the soil applied with different levels of DSW. This is in close agreement with the findings of Kamalakumari and Singaram (1995) who observed a strong positive relationship among the available NPK and organic carbon for enzyme activities of the soil. The work of Goyal *et al.* (1995) and Rajannan *et al.* (1998), Murugaragavan (2002) lend support for the increased activities of soil enzymes owing to the addition of spentwash. Similar results were obtained by Sivashankari (2009) and Nandha Kumar (2009).

137 **Table 2. Effect of Distillery spentwash on soil microbial population at various stages of sesame**
138 **under rainfed condition**

Treatment / stages	Bacteria (x 10^6 CFU g^{-1} of soil)					Fungi (x 10^3CFU g^{-1} of soil)					Actinomycetes (x 10^2 CFU g^{-1} of soil)				
	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean	S_1	S_2	S_3	S_4	Mean
T_1	19.80	19.00	18.40	17.00	**18.55**	8.70	7.70	8.00	8.20	**8.15**	5.60	5.20	4.60	3.70	**4.77**
T_2	23.00	22.30	21.50	20.50	**21.82**	10.20	8.40	9.20	9.60	**9.35**	5.40	5.00	4.40	4.10	**4.75**
T_3	26.70	24.20	23.00	22.40	**24.07**	10.70	9.40	8.50	8.40	**9.25**	5.60	5.30	5.10	5.00	**5.25**
T_4	31.00	30.40	29.60	28.80	**29.95**	14.80	12.20	10.70	10.10	**11.94**	5.80	5.50	5.20	4.60	**5.27**
T_5	35.80	34.20	33.70	32.60	**34.07**	16.70	14.30	13.10	12.90	**14.23**	6.90	6.30	5.60	5.80	**6.15**
T_6	39.40	35.50	35.10	36.80	**36.70**	17.90	15.60	14.00	13.60	**15.27**	7.40	7.00	6.80	6.30	**6.87**
Mean	**29.28**	**27.60**	**26.88**	**26.35**	**27.52**	**13.15**	**11.27**	**10.58**	**10.45**	**11.36**	**6.11**	**5.71**	**5.28**	**4.91**	**5.50**
	SEd		**CD (0.05)**			**SEd**		**CD (0.05)**			**SEd**		**CD (0.05)**		
T	**1.03**		**2.06**			**0.56**		**1.12**			**0.15**		**0.31**		
S	**0.84**		**1.68**			**0.46**		**0.92**			**0.12**		**0.25**		
TxS	**2.06**		**4.12**			**1.13**		**2.26**			**0.31**		**0.62**		

Treatments:

T_1- Absolute control ,**T_2** -Control – 100% recommended dose of NPK ,**T_3 -** 25 % N through DSW and 75 % N through inorganic source ,**T_4 -**50 % N through DSW and 50 % N through inorganic source, **T_5 -** 75 % N through DSW and 25 % N through inorganic source ,**T_6 -** 100 % N through DSW

Sampling periods: S_1 : 30 DAS, S_2: 60 DAS, S_3: 90 DAS, S_4: At the time of harvest

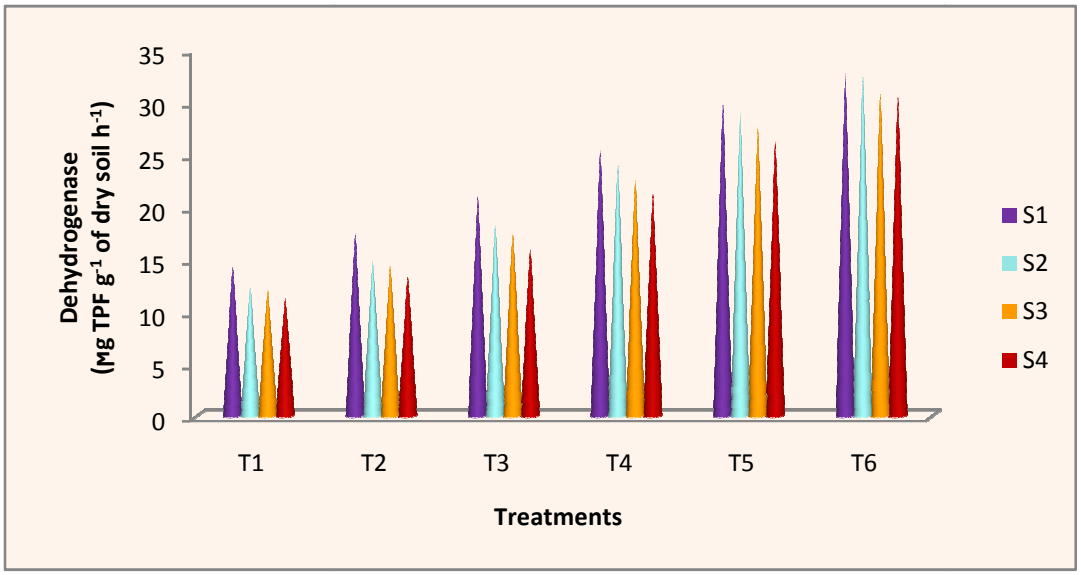

Fig.1. Effect of distillery spentwash application on soil dehydrogenase activity in rainfed sesame crop

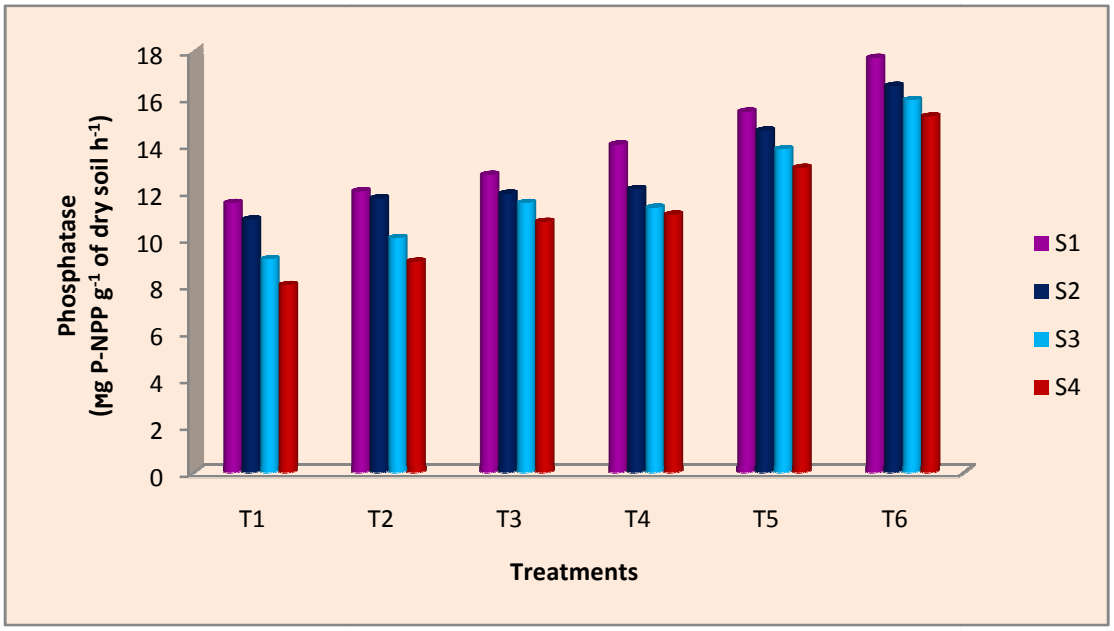

Fig.2. Effect of distillery spentwash application on soil phosphatase activity in rainfed sesame crop

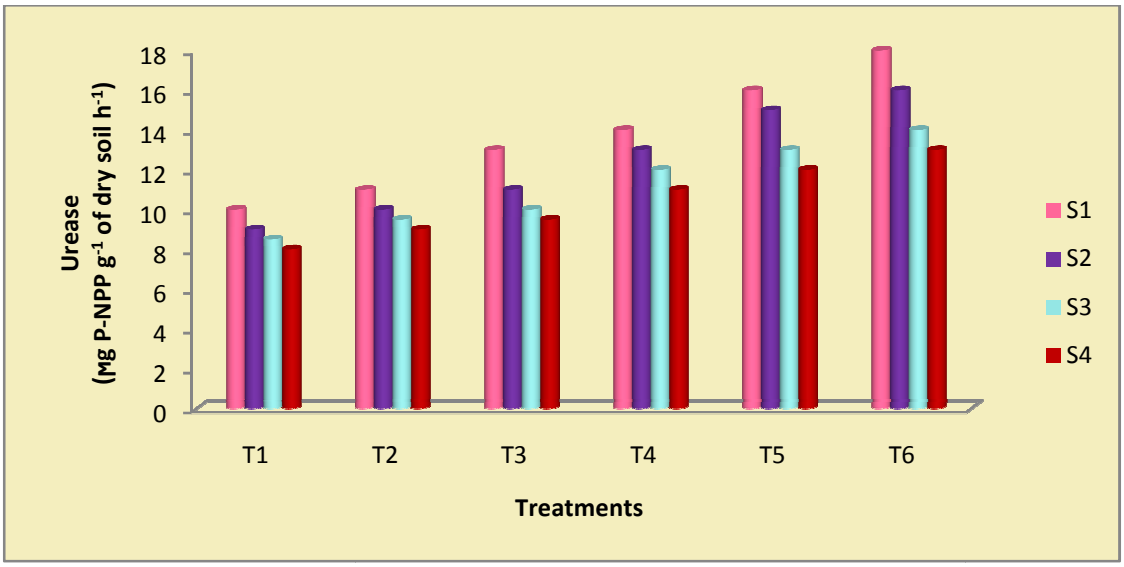

Fig.3. Effect of distillery spentwash application on soil urease activity in rainfed sesame crop

T_1- **Absolute control ,**T_2 **-Control – 100% recommended dose of NPK ,**T_3 - **25 % N through DSW and 75 % N through inorganic source ,**T_4 -**50 % N through DSW and 50 % N through inorganic source,** T_5 - **75 % N through DSW and 25 % N through inorganic source ,**T_6 - **100 % N through DSW**

CONCLUSION

Results of the present study indicated that application of post methanated distillery spentwash increased the microflora and enzyme activities of the soil throughout the crop growth period of sesame. Application of distillery spentwash in treatment T_6 (100 per cent N through distillery spentwash) recorded the highest bacterial, fungal and actinomycetes population at all four stages of crop growth compared to other treatments in rainfed condition. The increase in soil microbial activity due to oxidation of organic matter in treated spentwash applied soil .The enzyme activities *viz*., phosphatase, dehydregenase and urease recorded the highest value of 16.32 µg p-nitrophenol g^{-1} soil h^{-1}, 31.62 µg TPF g^{-1} soil h^{-1} and 15.25 µg NH_4-N g^{-1} soil h^{-1} respectively, in the treatment T_6 (100 per cent N through distillery spentwash) respectively under rainfed condition. Enhancement of the activities of phosphatase, dehydrogenase and urease enzyme was observed in the soil that received different doses of Post methanated Distillery SpentWash and maintained stable microbial population and enzyme activities till the harvest stage of the crop.

REFERENCES

Batch T, Martinez A, Mustelier-L-del A, Villegas D.R and Ponce-de- Leon D.Agricultural use of vinasse mixed with effluent from brown sugar production. **Quimica Fiscay Biologia de Suelos.1993. 1:** 229-232.

Gopal H., C. Kayalvizhi, M. Baskar, M. Sheik Dawood, M. Subash Chadra Bose and K. Rajukkannu. 2001. Effect of distillery effluent on soil properties microbial population dynamics, yield and quality of sugarcane. **In:** National seminar on use of poor quality water and sugar industrial effluents in agriculture. ADAC &RI, TNAU,Tiruchirapalli, Feb 5, p- 75.

Goyal S, Chander K and Kapoor K.K. Effect of distillery wastewater application on soil microbial properties and plant growth. **Environ. Eco.1995.13 (1):** 89-93.

Joshi H.C and Singh G. Use of distillery effluent in agriculture. **J. Socio Envl. Res. Org.2010.1(3):** 21-24.

Kamalakumari K and Singaram P.Relationship among soil chemical biochemical properties and enzyme activities. **Madras Agric. J., 1995. 82:** 69-70.

Latha K. 2008. Impact of repeated application of biomethanated distillery spent wash on the productivity of fodder and on soil health and ground water quality. 2008. **M.Sc**. **(Env. Sci.) Thesis**, Tamil Nadu Agric. Univ., Coimbatore.

Mane J.D, Modi S, Nagawade S, Phadnis S.P and Bhandari V.M.Treatment of spentwash using modified bagasse and colour removal studies, **Bioresource Technol.2006.53:** 80-89.

Mattiazo M.E and Ada Gloria N. Effect of vinasses on soil activity. **STAB (Portuguese)**.**1985.4(2):** 38-40.

Murugaragavan R. Distillery spentwash on crop production in dryland soils **M.Sc. (Ag.) Thesis.2002.**Tamil Nadu Agric. Univ., Coimbatore.

Nandakumar N.B.Eco-friendly utilization of post biomethanated distillery spentwash for enhanced crop productivity**. Ph.D. (Env.Sci.), Thesis**.**2009**. Tamil Nadu Agric Univ., Coimbatore.

Patil J.D, Arbatti S.V and Hapase D.G.Humification of spentwash in soil. **J. Maharasthra Agric. Univ.1982 7(1):** 80-83.

Rajannan G, Helkiah J, Parwin Banu K.S and Ramasami P.P.Preparation of quality compost from pressmud and distillery effluent. **In:** Proc. of National seminar on use of distillery and sugar industry wastes in agriculture, held at ADAC & RI, Trichirapalli, **Oc**t.**28 and 29.1998. pp.**149-151**.**

Rajukkannu K, Manickam T.S, Shanmugam K, Chandrasekharan A and Gladis R. Distillery Spentwash-Development of technology for using it as an amendment for reclamation of sodic soils. **In:** Proc. National Symp. Use of distillery & sugar industry wastes in agriculture, ADAC & RI, Tamil Nadu Agric. Univ., Tiruchirapalli.**1996**.pp. 30-39.

Sivasankari J**.**Studies on impact of post methanated distillery spentwash on the physiology and productivity of crop plants. **M.Sc. (Env. Sci.) Thesis. 2009**. Tamil Nadu Agric. Univ., Coimbatore.

Tabatabai M.A and. Bremner J.M.Use of p-nitrophenyl phosphate for the assay of soil phosphatase activity. **Soil Biol. Biochem.1969.1:** 301-307.

199 Tauk S.M, Schoenlein Crusius I. H and Petenate A. Ecological model of nutrients, microorganisms and
200 enzymatic activity of soil in a cerrado are treated with vinasse, corumbati. **Reviste de**
201 **Microbiologia.1990. 1:** 99 - 108.

202 Valliappan K. Recycling of distillery spent wash- An eco-friendly effective reclamation technology for sodic
203 soils. **Ph.D. Thesis**.**1998.** Tamil Nadu Agric. Univ., Coimbatore, India.

204 Veeresh H, Muthuraja R, Rehman A.H.M, Jayanthi S, Bhaskar and Srinivasamurthy C.A.Characterization
205 of distillery spentwash from Karnataka. In: The abstracts of National Conference on " Ecofriendly
206 Utilization of recyclable organic resources from the sugar and Distillery Industries for Sustainable
207 Agriculture" held at ADAC &RI.,Tiruchirappalli-9,TNAU**.2007**.March 6- 7th, 3p.

208 Waksman S.A and Fred E.B.A tentative outline of the plate method for determining the number of
209 microorganisms in soil. **Soil Sci.1922.14:** 27-28.