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Studies on nitrogen dynamics of spentwash applied soil in a closed incubation experiment

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Abstract
Distillery spentwash contains all nutrients and organic matter and used in agriculture as a source of plant nutrients. Besides all the nutrients, spentwash contains appreciable amount of nitrogen (N) also. The effect of different levels of spentwash application on soil nitrogen dynamics was examined through a laboratory closed incubation experiment. Application of different levels of spentwash had significant influence on soil N. Both the NH_4-N and NO_3-N fractions markedly increased with increase in the levels of spentwash (140 m^3). The spentwash not only adds mineral N (NH_4-N and NO_3-N) to soil, but also promotes the mineralization of soil organic N.

Keywords: Spentwash, levels of spentwash, mineralization, nitrogen fractions

Introduction
The spentwash, being loaded with organic and inorganic compounds could bring remarkable changes on the physical, chemical and biological properties of soils and thus influences the fertility of soil significantly. There is a significant increase in the available N, P, K, Ca and Mg contents of the soils due to spentwash application [1]. Similarly application of effluent @ 125 m^3/ ha enhanced the nutrient contents to 325, 60 and 640 kg/ ha N, P and K, respectively than the initial N, P and K status of the experimental soil [2]. The present study was, therefore, undertaken to study the N dynamics in soil under spentwash application and its impact on soil and environmental quality.

Materials and Methods
Collection and Characterization of distillery spentwash
The biomethanated distillery spentwash was characterized for its nutritive value and pollution potential by following standard methods [3] (Table 1). The biomethanated distillery spentwash sample was collected from the Salem Co-operative Sugar Mills Ltd, Mohanur, Namakkal District, Tamil Nadu, India.

Characterization of experimental soil
The soil samples used in the laboratory experiment were collected from a farmer's field in Kidaram village of Namakkal district. The soil belongs to Alfisol (red soil) – *Typic Rhodustalfs* in USDA classification. The soil samples were processed and analysed for important physical and chemical characteristics (Table 2).

Laboratory incubation experiment
The effect of different levels of spentwash on soil nitrogen dynamics was examined through a laboratory closed incubation experiment. Five hundred grams of air-dried soil (2 mm sieved) were weighed in plastic containers. The biomethanated spentwash was added at the rate equivalent to 0, 20, 40, 60, 80, 100, 120, 140 m^3/ ha and thoroughly mixed with soil. Wherever necessary required quantity of distilled water was added to achieve a final moisture content equivalent to 60% of field capacity. After adding the spentwash, the plastic containers were covered with polyethylene bags containing small pin-sized holes to permit aeration. Four replicates of each treatment were prepared, randomly placed and incubated in the laboratory at 25±2°C for 90 days. Based on the weight loss distilled water was added to the container to maintain the moisture content throughout the incubation experiment. At the end of 30, 60 and 90 days samples (≈100 g) were removed from all the treatments and used for chemical analyses. Moisture factor was computed and applied to express the results on oven dry basis.

The mineralization of N was determined by measuring mineral-N (NH_4-N and NO_3-N) in soil, at 0, 30, 60 and 90 days.

Estimation of soil nitrogen fractions

The fractionation of soil nitrogen was carried out by the procedure given by [4] and [5]. The individual fractions were estimated according to the procedure given by [6].

- **Preparation of Equilibrium extract:** A quantity of 10 g of soil (0.2 mm) treated with 100 ml of 2 M KCl solution was shaken for one hour and filtered through Whatman No.1 filterpaper.
- **Ammonical nitrogen (NH_4-N):** An aliquot of 20 ml of the above filterate was distilled with freshly ignited MgO in distillation apparatus and the distillate collected in 2% boric acid containing mixed indicator was titrated with standard H_2SO_4.
- **Nitrate nitrogen (NO_3-N):** On removal of NH_4 from the sample, added a pinch of devarda's alloy and continued the steam distillation. The amount of NO_3-N was determined as described for NH_4-N.

Table 1: Characteristics of biomethanated distillery spentwash

S. No	Characters	Biomethanated spentwash*
1.	Colour	Dark brown
2.	Odour	Unpleasant burnt sugar
3.	pH	7.1
4.	EC (dS/ m)	38
5.	Total dissolved solids	50000
6.	Total suspended solids	3300
7.	Total solids	53300
8.	Biological oxygen demand	12800
9.	Chemical oxygen demand	35000
10.	Carbon (g/ L)	24
11.	Nitrogen	420
12.	Phosphorus	40
13.	Potassium	9097
14.	Total sugars (%)	3.49
15.	Reducing sugars (%)	1.77
16.	Total phenols	84
17.	Zinc	7.20
18.	Iron	78
19.	Manganese	5.3
20.	Copper	5.5
21.	Bacteria (x 10^6 CFU/ mL of effluent)	12
22.	Fungi (x 10^4 CFU/ mL of effluent)	19
23.	Actinomycetes (x 10^3 CFU/ mL of effluent)	Nil

* Mean of triplicate samples (Values are in mg/ L unless otherwise stated)

Table 2: Characteristics of soil used in the incubation experiment

S. No	Characters	Values
a). Mechanical properties		
	Textural class	Sandy loam
	Sand (%)	79.5
	Silt (%)	14.8
	Clay (%)	10.3
	USDA classification	*Typic Rhodustalfs*
b). Physico-chemical properties		
1.	pH (1: 2.5 soil water suspension)	5.40
2.	EC (dS/ m) (1: 2.5 soil water suspension)	0.043
3.	Organic carbon (g/ kg)	5.20
4.	NH_4- N (mg/ kg)	52
5.	NO_3- N (mg/ kg)	55
6.	NaOH - P_i (mg/ kg)	194
7.	NaOH - P_o (mg/ kg)	488
8.	HCl - P (mg/ kg)	42
9.	NH_4OAc-K (mg/ kg)	80
9.	Sodium (cmol (p^+)/ kg)	0.26
10.	Calcium (cmol (p^+)/ kg)	0.69
11.	Magnesium (cmol (p^+)/ kg)	0.52
12.	Sulphate (mg/ kg)	26
13.	Chloride (mg/ kg)	17

Table 3: Effect of spentwash application on NH_4-N (mg/ kg) content of soil during incubation

Treatments	Incubation period (days)				
	0	**30**	**60**	**90**	**Mean**
T_1- Control	54	58	70	51	58
T_2- Soil + Spentwash @ 20 m^3/ ha	134	192	80	82	122
T_3- Soil + Spentwash @ 40 m^3/ ha	140	204	90	92	132
T_4- Soil + Spentwash @ 60 m^3/ ha	194	210	94	92	148
T_5- Soil + Spentwash @ 80 m^3/ ha	224	222	98	96	160
T_6- Soil + Spentwash @ 100 m^3/ ha	272	234	104	94	176
T_7- Soil + Spentwash @ 120 m^3/ ha	272	240	120	104	184
T_8- Soil + Spentwash @ 140 m^3/ ha	296	246	130	106	195
Mean	198	201	98	90	147
	SEd			CD (0.05)	
T	2.34			4.65	
D	1.66			3.29	
T x D	4.69			9.31	

Table 4: Effect of spentwash application on NO_3-N (mg/ kg) content of soil during incubation

Treatments	Incubation period (days)				
	0	**30**	**60**	**90**	**Mean**
T_1- Control	66	118	140	148	118
T_2- Soil + Spentwash @ 20 m^3/ ha	78	138	142	160	130
T_3- Soil + Spentwash @ 40 m^3/ ha	84	142	144	204	144
T_4- Soil + Spentwash @ 60 m^3/ ha	92	150	152	268	165
T_5- Soil + Spentwash @ 80 m^3/ ha	104	154	160	346	192
T_6- Soil + Spentwash @ 100 m^3/ ha	106	156	160	390	203
T_7- Soil + Spentwash @ 120 m^3/ ha	114	160	184	420	220
T_8- Soil + Spentwash @ 140 m^3/ ha	114	174	214	424	232
Mean	95	150	161	295	175
	SEd			CD (0.05)	
T	3.06			6.08	
D	2.17			4.30	
T x D	6.12			12.16	

Table 5: Effect of spentwash application on organic carbon (g/ kg) of soil during incubation

Treatments	Incubation period (days)				
	0	**30**	**60**	**90**	**Mean**
T_1- Control	5.2	5.0	5.7	3.3	4.8
T_2- Soil + Spentwash @ 20 m^3/ ha	8.0	6.8	6.1	4.5	6.4
T_3- Soil + Spentwash @ 40 m^3/ ha	8.8	7.6	6.3	5.6	7.1
T_4- Soil + Spentwash @ 60 m^3/ ha	9.7	8.2	6.8	6.8	7.9
T_5- Soil + Spentwash @ 80 m^3/ ha	10.7	8.3	7.8	7.4	8.5
T_6- Soil + Spentwash @ 100 m^3/ ha	11.6	8.5	8.2	8.0	9.1
T_7- Soil + Spentwash @ 120 m^3/ ha	12.2	9.8	8.3	8.0	9.6
T_8- Soil + Spentwash @ 140 m^3/ ha	13.4	10.5	9.5	9.3	10.7
Mean	10.0	8.1	7.3	6.6	8.0
	SEd			CD (0.05)	
T	0.20			0.50	
D	0.22			0.36	
T x D	0.51			1.13	

Result and Discussion

The effect of spentwash on N dynamics in soil is presented in Table 3. Application of different levels of spentwash had significant influence on soil N. Immediately after application of spentwash, the NH_4-N in soil markedly increased from 134 mg/ kg (control) to 296 mg/ kg. The highest concentration was recorded due to the application of 140 m^3/ ha (T_8). The spentwash application up to 60 m^3/ ha resulted significant increase in the NH_4- N at 30 days (T_1 to T_4) and thereafter, a decreasing trend was observed; whereas, in treatments T_5 (80 m^3/ ha) to T_8 (140 m^3/ ha), the concentration of NH_4-N in soil was progressively decreased during 90 days of incubation. The treatments and incubation period had significantly affected the NH_4-N content in soil. At all days of incubation, the concentration of NH_4-N was the lowest in control and the highest in treatment (T_8) that received spentwash at the rate equivalent to 140 m^3/ ha.

Similar to NH_4-N, the NO_3-N in soil under controlled condition was also changed significantly due to spentwash application. Initially the NO_3-N in soil ranged between 66 and 114 mg/ kg (Table 4). Increase in the rate of application had significantly increased the NO_3-N content of soil. During 90 days of incubation, the concentration of NO_3-N progressively increased at all treatments. At the end of incubation, the concentration varied between 148 and 424 mg/ kg. At all days of incubation, the NO_3-N content was the lowest in the control and the highest in treatment (T_8) which received the spentwash at a rate of 140 m^3/ ha. The effect of treatments, incubation period and their interaction had significant impact on NO_3-N in soil.

The N dynamics in soil was significantly influenced by the application of spentwash. The data indicated that the mineralization of N occurs continuously in soil during incubation up to 60 days and thereafter the mineralization rate decreased at 90 days due to possible net immobilization and microbial uptake. Increase in the levels of spentwash markedly increased the rate of mineralization of N during the incubation.

Both the NH_4-N and NO_3-N fractions markedly increased with increase in the levels of spentwash. This might be due to the inorganic N present in the distillery spentwash. This result corroborates with the findings of [7], who reported that under land-based treatment of effluent, NO_3-N and NH_4-N concentrations in soil may increase as a direct result of inorganic N present in the effluent and increased soil organic matter (SOM) mineralization. Similar results were observed by [8], who stated that effluent irrigation can significantly increase total N stored within a soil profile, according to effluent composition, loading rates and solution chemistry.

The increase in the NH_4-N faction was observed up to 30 days of incubation in spentwash applied soils. This was in line with the findings of [9] who reported that addition of pig slurry and cocomposted sewage sludge has resulted in an immediate but short term increases in inorganic N contents, particularly NH_4^+. After 30 days of incubation a decline in the NH_4-N fraction was observed, probably due to the N transformation process through which the NH_4-N is converted into NO_3-N (or) due to immobilization and microbial uptake. In an incubation experiment [10] found that various components of dairy compost the bulk mineralized ammonium was observed to have been nitrified to nitrate within 14 to 28 days. The reduction in NH_4-N could also be due to conversion of NH_4-N to NO_3-N (nitrification) and further the NO_3-N lost during incubation through biological denitrification, a microbial process through which NO_3 is reduced to nitrous oxide (N_2O) and molecular N (N_2) and lost from soil [11].

The NO_3-N fraction was found increased continuously until the end of the incubation experiment. Nitrification of NH_4-N added through spentwash, and mineralization and nitrification of soil organic N might have increased the NO_3-N in soil. Similar results were obtained in a study conducted with different organic amendments like sewage sludge, farmyard manure and municipal solid waste compost. The organic amendments are important source of soil nitrogen and the availability of total, mineral and organic nitrogen in the soil profile is greatly improved during mineralization [12, 13] and [14] reported that an increase in the rate of spentwash markedly increased the rate of both mineralization and nitrification in soil. However greater amount of NO_3 than NH_4-N was evident particularly at the later stage of incubation. The spentwash contains large amount of organic carbon. It increased the soil organic carbon content (Table 5) which in turn stimulated the soil microbial activity by providing a carbon substrate. The balance between N mineralization and immobilization is closely linked to C substrate availability and the C/N ratio of the available organic matter [15].

Conclusions

Both the NH_4-N and NO_3-N fractions markedly increased with increase in the levels of spentwash. This might be due to the inorganic N present in the distillery spentwash. The spentwash not only adds mineral N (NH_4-N and NO_3-N) to soil, but also promotes the mineralization of soil organic N.

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