Pak. J. Bot., 39(7): 2673-2680, 2007.

EFFECT OF BACTERICIDAL PLANTS TREATMENTS ON UREA HYDROLYSIS AND NITRIFICATION IN SOILS OF DIFFERENT CROPPING HISTORIES. PART-2. NITRIFICATION

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Abstract

Effect of some indigenous plants such as *Prosopis, Azadirachta, Sphaeranthus* and *Cassia* species, was studied on urea nitrogen transformations, especially nitrification. Soils selected from two different crop fields i.e. the wheat and kallar grass fields were used for incubation studies. Soil samples, up to depths of 0.5 meter were collected, to assess some important soil physico-chemical properties. Powdered plant material, @ 3% and 6%, was incorporated into the soils under study. The nitrogen released as NO_3^- –N was determined. The hydrogenase activity was determined to confirm the microbial growth index. Differential responses of indigenous plants on soil nitrification were observed in both the soils tested. The process of nitrification was slightly inhibited by the treatments of indigenous plants. *Prosopis* and *Cassia* treatments proved to be the most effective treatments for reducing N losses. Treatment effects of the wild indigenous plants will be useful in the maintenance of soil biological activities.

Introduction

The soils of Sindh are generally calcareous in nature, having less organic matter (<1%), alkaline pH range and their texture ranges from silty to clay loam. Nitrogen is the most essential nutrient for plant metabolism, as it is an integral part of many physiological systems of plants. The factors which make the nitrogen availability in the soil are rainfall, bacterial mineralization, organic matter decomposition and synthetic fertilizer application. Urea and its derivatives are the major sources (80%) of the nitrogenous fertilizers used in tropics. In case of rapid hydrolysis of urea, depending upon the soil and environmental conditions, accumulation of NH_4^+ may result in the phytotoxicity and volatilization losses. Ammonia reduces to produce nitrate, which is one of an ultimate source of available-N in the soils, but nitrates being mobile are subject to the leaching losses of nitrogen (Bouman *et al*., 1995). Therefore, it is essential to understand the nature of the catalytic activities associated with soil microbial flora, in particular the complete breakdown of urea into nitrate *via* reduction of ammonium through the process of nitrification. Microorganisms present in the soil bring about the various transformations. Various attempts have been made to prevent excessive N losses. In many intensive agricultural systems, synthetic inhibitors are attractive proportions to minimize losses of nitrogen (Keerthisinghe & Freney, 1994). But heavy cost of such compounds has become prohibitive for their frequent and economic use. The method which may prove to be the most promising, is the use of naturally occurring plant products and materials (Beyrouty *et al*., 1988 a-b; Baqir *et al*., 1985, 1987).

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To increase agricultural production agriculture sector would be making efforts for efficient use of organic materials, a key component of agricultural systems, its availability, composition and decomposition has an important affect on various soil nutrient transformations (Batjes, 1996). Nutrient cycling through straw incorporation is widely practiced in modern agriculture (Mahler & Hamamda, 1993; Becker *et al*., 1994). A number of plants with bactericidal activities have been reported (Chopra, 1958; Modan *et al.,* 1972; Lodhi, 1978, 1982; Lodhi & Killingback, 1980). These plants can slow down the hydrolytic process by suppressing microbial population thus increasing the efficiency of fertilizer (Felsot & Dzantor, 1995). Bremer & Kessel (1992) reported that temporal fluctuations in microbial biomass may have a considerable influence on the amount of plant available N; thus there is a possibility of controlling N losses by controlling microbial activity.

Hydrogenase is the enzyme presumed to function in the evolution or utilization of molecular hydrogen. The enzyme appears to be particulate in several aerobes and facultative anaerobes and soluble in various clostridia (Sumner, 1951). The enzyme, being integral part of a large number of microorganisms, is recommended as an indicator of microbial regulation (Casida *et al*., 1964; Felsot & Dzantor, 1995). This also provides an indication of the effectiveness of inhibitors and organic amendments on microbial population (Wilke & Brautigam, 1991; Patra *et al*., 1990).

Physico-chemical characteristics of the soil play a vital role in soil N transformations. Among the various factors involved, soil organic matter (Medina & Radel 1988), soil organic carbon (Batjes, 1996; Antill *et al*., 1993) and soil nitrogen (Hector & Westerman, 1989) have a special influence.

Keeping in view the facts that (i) Urea hydrolysis is mainly of microbial origin and (ii) the hydrogenase enzyme is an indicator of active microbial populations; the present study was carried out to see the influence of the residues of some indigenous plants, reported for having bacteriostatic potential, on release of NO_3^- -N in some soils of Sindh province. The use of dried and powdered, easily available plant material, may increase the fertilizer use efficiency of urea fertilizer.

Materials and Methods

Soil sampling: Efforts were made to select the sampling sites, which represented specific crop cultivation/rotation prevalent in the areas of survey. The samples for this purpose were collected at different sites from N.I.A experimental farm Tando Jam. The crop fields selected were wheat and kallar grass fields. Bulk of soil samples was collected at the top 0-15 cm layer. The soil sampling was done with the help of Iron Auger having 6" diameter. Four spots were selected for sampling. Soil collected from various spots was mixed together to make one homogenous sample. The polythene bags (12" x 8") containing soil samples were brought to the laboratory and samples were air dried for 48 hours in a glass house. Air-dried samples were ground to pass through a 2-m.m sieve. The samples were then stored in covered plastic jars at room temperature. All the samples collected from different spots were analyzed for some soil physico-chemical properties (Appendix-1) by using standardized methods. Texture was determined by Hydrometer method given by Bouyocos (1962), Electrical Conductivity (1:5 soil- water extract) on Conductivity meter, pH (1:5 soil- water extract) on pH meter, Organic matter were determined by Walkely-Black method (Walkely, 1947), Total Nitrogen was determined by Kjeldhal's method as described by Bremner (1970), Urea-N was determined by the method of Mulvaney & Bremner (1979) and Hydrogenase activities were determined by the method of Casida *et al*., (1964).

Collection of bactericidal plants: The plants reported by Baqir *et al.,* (1985, 1987) known to have bactericidal properties were collected from fields around Tando Jam (Table 1). The plant material was brought to the laboratory and washed several times with tap water. The material was then oven dried for 48 hours at 70^{o}C. The dried plant material was crushed and ground in a mechanical grinder to pass through 20-mesh sieve and stored in plastic screw capped bottles.

Assay of hydrogenase: A three-week incubation experiment was conducted comprising of three sets. A six gram of each of the two soils was taken in quadruplicates in screw capped glass bottles. For every set there were three treatments (0, 3% and 6%) of ground plant material (leaves and flowers) added to the respective soils. The tubes were incubated for four incubation periods at 24 hour intervals up to 96 hours.

For the 1st set one ml of 3%Triphenyl Tetrazolium Chloride (TTC) solution was added to each of the tubes and incubated at 37^{o}C for 24 to 96 hours at 24 hour intervals. After the completion of each incubation period the respective tubes were removed from the incubator and proceeded further as described for the assay of Hydrogenase activities. The method used for Hydrogenase activity was that of Casida *et al.,* (1964).

For the 2nd set the same procedure as described for set - 1 was followed up to the addition of plant material. Then instead of TTC solution 1 ml distilled water was added. The bottles were placed in the incubator for one week at 37^{o}C. After one week 1 ml of 3% TTC solution was added and mixed thoroughly and the samples were reincubated for 24 to 96 hours at 24-hour intervals. After the completion of each time of incubation only the tubes, reserved for that particular time were removed and proceeded further as described for the assay of Hydrogenase activity.

For the 3rd set the same procedure, as described for set –I, was followed up to the addition of plant material. Then instead of TTC solution 1 ml distilled water was added. The bottles were placed in the incubator for two weeks at 37^{0} C. After two weeks 1 ml of 3% TTC solution was added and mixed thoroughly and the samples were reincubated for 24 to 96 hours at 24-hour intervals. After the completion of each time of incubation only the tubes, reserved for that particular time were removed and proceeded further as described for the assay of Hydrogenase activity.

Study of nitrate nitrogen by incubation method: A three week incubation experiment was conducted, to see the effect of four indigenous plants (i.e. *P. glandulosa., A. indica., S. indicus* and *C. angustifolia*), incorporation in the soil on nitrate nitrogen release from urea fertilizer. Soils, from kallar grass fields and wheat fields were used for this experiment. Order of sampling was same as described previously. Soil samples were taken in quadruplicate for three treatments and four incubation periods i.e. 24 to 96 hours at 24 hour intervals. Powder form of Indigenous plants (leaves and flowers), was added to the soils at the concentrations of 0, 3% and 6%.

Assay of nitrate nitrogen: A three-week incubation experiment was conducted comprising of three sets. A 10 gram of each of the two soils was taken in quadruplicates in screw capped glass bottles. For every set there were three treatments (0, 3% and 6%) of ground plant material (leaves and flowers) added to the respective soils. The tubes were incubated for four incubation periods at 24 hour intervals up to 96 hours.

Table 1. List of bactericidal plants used in this study.

S. #	Local name	Botanical name	Family name	Abbreviations
1.	Devi	*Prosopis glandulosa*	Mimosaidae	*P. glandulosa* Torr
2.	Neem	*Azadirechta indica*	Meliaceae	*A. Indica* L.
3.	Mundi flower	*Sphaeranthus indicus*	Compositeae	*S. indicus* Vohl
4.	Sanna-i-makki	*Cassia angustifolia*	Leguminosae	*C. angustifolia* L.

Table 2. Cumulative effects of various indigenous plants incubation in kallar grass and wheat field soils, on nitrate nitrogen.

Plants used	Concentration	Kallar grass	Wheat field
P. glandulosa	0%	42.36 A	18.85 A
	3%	10.14 C	10.39 C
	6%	22.11 B	14.82 B
C. angustifolia	0%	42.36 A	18.85 A
	3%	5.9 C	6.93 C
	6%	10.31 B	11.36 B
S. indicus	0%	42.36 A	18.85 A
	3%	14.08 B	6.39 C
	6%	13.22 C	7.94 B
A. indica	0%	42.36 A	18.85 A
	3%	6.9 C	5.34 C
	6%	8.73 B	11.07 B

Table 3. Inhibitory effects of indigenous plants on nitrate released from ammonium N.

Indigenous plants treatment	Incubation weeks	Kallar grass field soil			Wheat field soil		
		0%	3%	6%	0%	3%	6%
P. glandulosa	0	73.21	7.33	22.81	24.35	8.08	2.11
	1	16.46	9.07	32.53	17.52	11.37	17.92
	2	37.42	14.01	10.99	14.67	11.71	24.41
C. angustifolia	0	73.21	5.00	11.35	24.35	4.62	10.39
	1	16.46	4.23	8.95	17.52	7.31	15.01
	2	37.42	8.47	10.63	14.67	8.76	8.66
S. indicus	0	73.21	26.37	21.94	24.35	4.93	3.44
	1	16.46	10.01	6.83	17.52	7.31	10.49
	2	37.42	5.87	10.87	14.67	6.93	10.04
A. indica	0	73.21	7.70	11.35	24.35	4.14	9.52
	1	16.46	6.45	6.54	17.52	4.57	9.91
	2	37.42	6.54	8.27	14.67	7.31	13.76

Table 4. Correlation between NH_4^+–N & NO_3^-—N. (T. values: 0.5%= 0.159., 1%= 0.208).

Treatments	Soil types			
	Kallar grass		Wheat	
	R-values	Slope	R-values	Slope
P. glandulosa	-0.999 **	-0.999	-1 **	-1
C. angustifolia	-0.995 **	-0.997	-1 **	-1
S. indicus	-0.98 **	-0.994	-0.996 **	-0.986
A. indica	-1 **	-1	1 **	-0.999

Note: ** Represents significance at 1% level.

For the first set 5 ml of 0.5% Urea solution and plant material, were simultaneously added. The amount of water, used for urea solution, was in accordance with water holding capacity of soil. Samples were then incubated at 32^{o}C for 24 to 96 hours at 24-

hour intervals. After completion of each incubation period, respective samples were removed from the incubator and proceeded further for obtaining the extract.

In case of second set, no urea was added but after mixing of the plant material, distilled water was added (5 ml) per bottle and incubated at 32°C for one week. After one week all bottles were removed from the incubator and to each bottle 5 ml of urea, solution (0.5% urea), were added and bottles were reincubated for 24 to 96 hours at 24-hour intervals. After the completion of each incubation time, the respective bottles were removed and proceeded further for obtaining the extract.

Same method was followed for the third set up to addition of plant material, after that 5.0 ml of distilled water per bottle was added and bottles were incubated for 2 weeks at 32°C. After which all these bottles were removed from the incubator and to each bottle 5 ml (0.5%) urea solution was added and bottles were reincubated at 32°C for 24 to 96 hours at 24-hour intervals. After the completion of each incubation period, respective bottles were removed from the incubator and to each bottle 50 ml of 2M KCl PMA solution was added and bottles were shaken on a mechanical shaker for one hour. After shaking the solution was filtered through Whatman No. 42 filter paper. The filtrate was made to 100 ml. This was the stock solution used for the determination of NO_3-N by the method of Barness & Folkard (1951).

Results and Discussion

The objective of the study was to understand the nature of the catalytic activities associated with soil microbial flora, particularly the complete break down of urea into nitrate *via* reduction of ammonium to nitrate, through the process of nitrification, being an ultimate source of nitrogen for the plants. The bactericidal/bacteriostatic action, of the water extract of the plants, such as *P. glandulosa., C. angustifolia., S. indicus* and *A. indica* (Table 1) was tested against a wide range of bacteria by Baqir *et al.,* (1985,1987) and Maryam *et al.,* (1972,1973). In the present studies, the powdered form (leaves and flowers) of these plants was tested in the soils. Generally an increase in microbial activity was observed in both types of soils tested (kallar grass and wheat field soils).

In spite of the enhancements in urea hydrolysis, (Author's un published data), the nitrification was significantly retarded by incorporation of the indigenous plants in both the soils tested. Results presented in Tables 2 and 3 and a negative correlation observed for NH_4^+—N and NO_3^-—N (Table 4) suggests the inhibition of nitrification at both the plant treatment concentrations.

In certain cases a temporary decrease in activity was observed only at lower concentration (3%) of *C. angustifolia.* However, significant depressive effect on microbial activities was observed in the kallar grass soil. Kallar grass soil was observed to be saline in nature (Appendix-1) may be less conducive to soil transformations. Hydrogenase activity, (an index of microbial growth) in such cases, was also observed to be suppressed. Nevertheless the effect of chemical substances resulted from the degradation of plant residues cannot be ruled out. These types of studies are reported by many authors (Fernando & Roberts, 1976; Rashid, 1977). However at lower treatment levels in comparison to the higher treatment levels inhibition was more apparent. Nevertheless slower rates of nitrification, observed at higher treatments, was due to enhanced heterotrophic microbial action, suppressing inhibitory effects of indigenous plants vulnerable to nitrifers only as indicated by Santhi *et al.,* 1986. Consistent to these

studies are the results presented by Minenko, (1987) and Terrirena *et al.*, (1977), who reported the selective effects of mineral fertilizer and straw on the biological activity of soil. Recent studies indicates that nitrification is some times inhibited by natural products of plants like polyphenols, condensed tannin, ellagic and gallic acids as reported by Lodhi (1978,1982) and Rice & Pancholy (1973). Use of nitrification inhibitors holds promise for improving the efficiency of nitrogenous fertilizers under the situations where losses due to leaching and denitrification are severe. Considerable literature on the use of nitrification inhibitors regarding their growing importance in fertilizer use efficiency is available (Basaraba, 1964; Batjes, 1996; Lodhi & Killingback 1980). The use of indigenous plants in this study, can be substituted with the use of synthetic inhibitors reported in the literature due to the low cost input rates and abundant availability of indigenous plants in the local areas may provide higher economic return to our developing country.

Appendix-1. Physico-chemical properties of the soils used in these studies.

Soil (fields)	Soil texture			Texture	pH	Tss mmoh	O.M %	N %	Urease ug/10g	Hydrogenase mg/kg
	Sand %	Silt %	Clay %							
Orchard	49.1	20.0	30.9	SCL	7.8	1.19	0.94	0.06	260	10.2
Wheat	33.2	33.4	33.4	CL	7.9	0.39	0.73	0.05	280	4.4
Cotton	31.60	30.0	33.4	CL	7.6	0.33	0.81	0.04	90	6.0
Rice	12.8	40.0	47.2	C	8.0	0.35	0.92	0.05	90	7.8

Note: ** = Cl= Clay loam, L= Loamy, C= Clay, SC= Silty clay, SCL= Silty clay loam.

Acknowledgement

The principal author is highly thankful to higher authorities of Pakistan Atomic Energy Commission for extending research work facilities for Ph.D.

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(Received for publication 14 February 2006)