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INFLUENCE OF UREA FERTILIZER ON SOME SOIL MICROBIOLOGICAL AND BIOCHEMICAL PARAMETERS

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ABSTRACT

A laboratory incubation study was conducted to estimate the influence of N-fertilizer (urea) on a paddy field soil health under controlled moisture (flooded soil) and temperature (25 °C) conditions. The fertilizer was applied @ 0, 25, 50, 100, 200, and 400 μg N g^{-1} soil. The fertilizer concentrations ranging from 0 to 100 μg N g^{-1} soil showed positive response on ETS activity. However, at concentrations of 200 and 400 μg N g^{-1}, the activity was reduced significantly. The higher rates of fertilizer caused an improvement in the soil phenol contents and it increased with increasing concentration of fertilizer. The fertilizer incorporation didn't produce any significant change in soil protein content while the response of biomass phospholipid content was nearly similar to ETS activity; it increased with lower rates of N-fertilizer but decreased at higher rates.

Keywords: Urea, ETS activity, phenol, protein, phospholipids, paddy soil

INTRODUCTION

There is considerable interest in study of soil microbiological, biochemical and particularly enzyme activity of soils (Burns, 1978) because such activities may reflect the potential capacity of a soil to perform certain biological transformations of importance to soil fertility. Most studies of soil enzymes have been confined to arable agricultural and forest soils. But a flooded rice soil is predominantly anaerobic and as a result differs from a nonflooded soil in several physical, chemical and biological characteristics. Despite extensive studies on the chemistry (Ponnamperuma, 1972) and microbiology (Yoshida, 1975) of flooded soil, our knowledge of their enzyme activities is limited, especially under different management/agronomic practices. One of the general criteria used to determine microbial activity and biomass in soil is dehydrogenase/ETS activity (Trevors *et al.*, 1982). Dehydrogenase activity (DHA) is an indicator for potential non-specific intracellular enzyme activity of the total microbial biomass. It has been used as an indicator for active microbial biomass (Ladd, 1978). Intracellular dehydrogenases belong to the oxidoreductases and catalyze the oxidation of organic compounds by separating two-H atoms. The overall dehydrogenase activity of a soil depends on the activities of various dehydrogenases, which are a fundamental part of the enzyme system of all microorganisms. Dehydrogenase activity thus serves as an indicator of the microbiological redoxsystems and may be considered a good measure of microbial oxidative activities in soils (Tabatabai, 1982).

Productivity of irrigated rice crops is declining over years of continuous, intensive cultivation and appears to be associated with a decreasing N supply capacity of the soil (Olk *et al.*, 1998), which in turn may be attributed due to the enrichment of phenolic compounds in these soils (Olk *et al.*, 1996). It has further been hypothesized that soil microbial biomass is turned over rapidly enough to be able to constitute a major source of N for rice crops (Inubushi and Watanabe, 1987). Hence, rice soil microbial biomass may be considered as key to the better understanding of a decreasing nutrient supply capacity.

Microbial activities important to effect on crop productivity and nutrient cycling can be altered by agricultural management practices (Doran *et al.*, 1987). Management practices have been used primarily in the past to improve productivity and in many cases it usually involves N management (Lovell *et al.*, 1995). The microbial biomass is a sensitive indicator of changes resulting from agronomic practices and other perturbations of the soil ecosystem (Gunapala and Scow, 1998). Soil microbial biomass and dehydrogenase activities increase with increasing microbial populations following amendments of soils with nutrients/fertilizer application (Ladd and Paul, 1973). Inorganic-N at concentrations ranging from 40-120 μg g^{-1} showed no inhibitory effect on ETS activity, however, at 160 and 200 μg g^{-1}, activity was reduced 13.8 and 10.3 per cent, respectively (Trevors, 1984). Kucharski *et al.* (1996) also reported that application of higher doses of N fertilizers was negatively correlated with the dehydrogenase activity. Free phenolic acids exist in the soil solution comprising <0.01% of the total soil organic matter (Vaughan and Ord, 1980) and both toxic or stimulatory effects on plant and microbial growth have been documented (Olk *et al.*, 1996). These phenolic acids can be readily utilized by soil microbes (Sparling *et al.*, 1981).

The present study was designed to investigate the effects of fertilizer (urea) applied at various concentrations on soil microbiological and biochemical properties in a paddy field soil.

MATERIALS AND METHODS

Soil

Silt loam soil was collected from surface top (0-15 cm) of an agricultural field (used for paddy cultivation) near Jinhua city, Zhejiang Province, P. R. China, after removal of surface water. The soil was dried to moist condition, passed through a 2 mm sieve and stored at 4 °C. Various characteristics of the soil were measured and are presented in Table 1.

Soil Incubation

The homogenized moist soil in portions equivalent to 150 g oven-dry weight was transferred to 250 ml glass beakers. The soil samples were first

adjusted to 40 per cent of the soil water-holding capacity (WHC) by adding distilled water and then pre-incubated at 25 °C for 7 days (conditioning period).

After conditioning, designated amounts of N in the form of 1.0 ml urea solution were added to obtain the N concentrations of 0 (control), 25, 50, 100, 200, and 400 μg N g^{-1} soil, and mixed thoroughly. The moisture contents in the treated (and control) soil samples were adjusted to flooded soil/waterlogged condition (the water level was 2 cm above the soil water interface) and incubated at 25 °C for 21 days in the dark. The temperature was maintained within ±1 °C of the desired temperature (25 °C) and the soil moisture was kept at the same level by adding distilled water at regular intervals throughout the incubation period. All results are the mean of three replicate determinations and are expressed on an oven-dry weight basis (105 °C, 24 h). At the end of the incubation period, soil samples were taken out and analyzed for electron transport system (ETS)/dehydrogenase activity, total protein concentration, total soluble phenol compounds and total phospholipids.

Table 1: Characteristics of the soil under study.

pH*	4.74	CEC, cmol kg^{-1}	7.33
WHC, g kg^{-1}**	510	Sand, g kg^{-1}	278
O.C., g kg^{-1}	15.25	Silt, g kg^{-1}	562
Av. N, μg g^{-1}	106.40	Clay, g kg^{-1}	160
Av. P, μg g^{-1}	13.34	Soil texture	Silt loam

* Soil pH in 1: 2.5 soil: water suspension

** The methods used for estimation of general soil properties are taken from Anderson and Ingram (1993).

Assay

Electron transport system (ETS)/dehydrogenase activity was measured using the reduction of 2-(p-iodophenyl-3- (p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) to iodonitrotetrazolium formazan (INT-formazan/INTF) (Benefield *et al.*, 1977). The absorbance values obtained photometrically were converted to nmoles INT-formazan min^{-1}. g^{-1} (dry soil) using a standard curve of INT-formazan (INTF). The total soluble phenol compounds of the soil organic matter were determined with Folin Ciocalteau's phenol reagent as described by Box (1983) and total protein also by reaction of Folin Ciocalteau reagent with amino acids/proteins containing phenolic hydroxyl groups (Lerch *et al.*, 1993). The total phospholipids (polar lipid fraction in lipids extracted from soil) were determined by means of its phosphate content. Inorganic O-phosphate is released by digestion of the lipid extract with potassium persulfate, and color is developed by reaction of phosphate with ammonium molybdate and malachite green (Frostegard *et al.*, 1991). Data were examined by analysis of variance using Statistix 4.1 software.

RESULTS

i. Effect of N-fertilizer on dehydrogenase/ETS activity

The lower rates of N i.e. 25, 50, 100 μg g^{-1} soil increased the activity by 2.3, 5.4, and 17.5 per cent, respectively, as compared with the control. The lowest level (25 μg N g^{-1} soil) failed to produce any significant increase, while N_{50} and N_{100} resulted in significant increase in ETS activity. However, higher N concentrations of 200 and 400 μg g^{-1} soil caused significant reductions of 4.5 and 8.7 per cent, respectively, as against the control. The maximum activity (942.1 nmol INTF g^{-1} soil min^{-1}.) was observed with N_{100}, while minimum (731.6 nmol INTF g^{-1} soil min^{-1}.) activity was recorded at N application rate of 400 μg g^{-1} soil. There appears to be no linear relationship between N concentrations and ETS activity, since higher rates reduced activity, and lower concentrations did not cause inhibition (Fig. 1 & Table 2).

Table 2: Effect of nitrogen fertilization on soil microbiological and biochemical properties in a paddy field soil

Nitrogen (mg g^{-1} soil)	ETS nmol INTF min^{-1} g^{-1} soil	Phenol mg phenol g^{-1} soil	Protein mg protein g^{-1} soil	Phospholipids nmol phosphates g^{-1} soil
N 0	801.5 C	106.58 B	2.261^{ns}	27.60 AB
N 25	819.6 BC	104.27 C	2.255	27.63 AB
N 50	844.5 B	103.14 C	2.250	27.66 AB
N 100	942.1 A	102.38 C	2.244	27.89 A
N 200	765.1 D	110.07 A	2.263	27.53 AB
N 400	731.6 D	112.25 A	2.269	27.44 B
LSD (0.01)	36.33	2.230	0.0232	0.3943
Corr. (r)	-0.3041	0.6047	0.3559	-0.2902

Mean values followed by the same letter (s) are not significantly different at P<0.01 level based on Duncan's multiple range test ns = non-significant; Corr.= correlation coefficient

ii. Effect of N fertilizer on soil phenol content

Nitrogen application as urea markedly affected the soil phenol contents (Fig. 2). The data revealed that the lower concentrations of N additions at rates 25 and 100 μg g^{-1} soil resulted in the reductions of phenol contents, while higher doses of 200 and 400 μg N g^{-1} soil produced significant enhancement in its concentration in the soil, as against the control. The results exhibited that the N application at 25 μg g^{-1} soil significantly reduced (2.2 per cent) the phenol contents, compared with the control. A further decline occurred at N concentration of 50 μg g^{-1} soil, where the phenol contents were decreased by 3.2 per cent of the control and this decline was enhanced further (3.9 per cent) by 100 μg N g^{-1} urea addition, compared to the control. The three lower levels of N addition (25, 50, and 100 μg g^{-1} soil) were found to be nonsignificant among them. The higher N levels of 200 and 400 μg g^{-1} soil caused marked increase in

phenol contents, by 3.3 and 5.3 per cent, respectively, as reference to the control and these two higher N levels were found at par between themselves. The minimum (102.38 µg phenol g^{-1} soil) phenol contents were observed in 100 µg N g^{-1} soil treatment and maximum (112.25 µg phenol g^{-1} soil) in the soil treated with 400 µg N g^{-1} soil. A relatively high and positive correlation ($r = 0.605$) was found between N levels and phenol concentration in the soil.

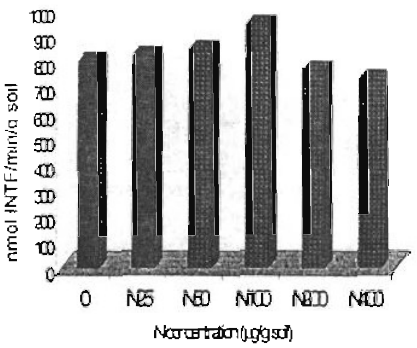

Fig. 1: Effect of N concentrations on ETS activity

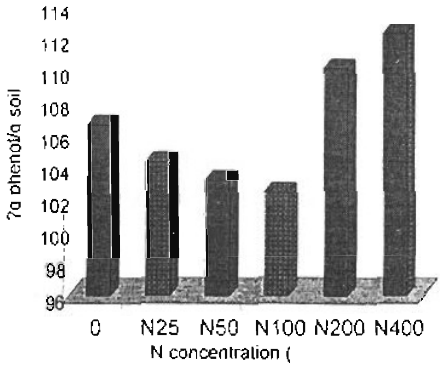

Fig:2 Effect of N concentrations on soil phenol content

iii. Effect of N fertilizer on soil protein content

The addition of N fertilizer at all levels didn't cause any marked change in soil protein contents (Fig. 3). However, the data exhibited that the lower levels of N additions (25, 50, and 100 µg g^{-1} soil) slightly but consistently reduced the soil protein contents with the increased N level, as against the control. However, the higher levels of N application (200, 400 µg g^{-1} soil) produced small increments in protein contents.

iv. Effect of N fertilizer on phospholipid content

The data revealed that the application of N has produced some changes in the phospholipid contents of the soil microbial biomass (Fig. 4). It was noticed that the addition of N at the lower rates of 25, 50, and 100 µg g^{-1} soil produced no significant stimulating effect in the phospholipid contents, as against the control. A slight but nonsignificant inhibition by 0.25 and 0.58 per cent relative to the control was occurred at N concentrations of 200 and 400 µg g^{-1} soil. Most of the N treatments were found to be nonsignificant among themselves or with the control, except the two treatments of 100 and 400 µg g^{-1} soil, which were found to have significant differences. The data further indicated that the maximum phospholipid contents (27.89 nmol PO_4^{-3} g^{-1} soil) were recorded in the 100 µg N

g^{-1} soil treatment and its minimum content (27.44 nmol PO_4^{-3} g^{-1} soil) was found in the soil treated with 400 µg N g^{-1} soil. The increase/decrease in phospholipid contents was found to be negligible (< 1.0 per cent), as against the control.

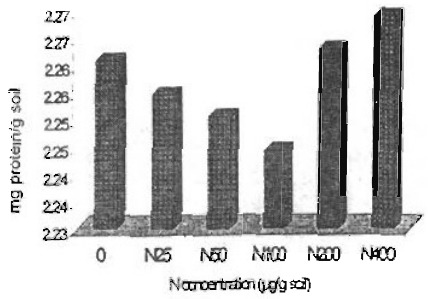

Fig. 3: Effect of N concentrations on soil protein content

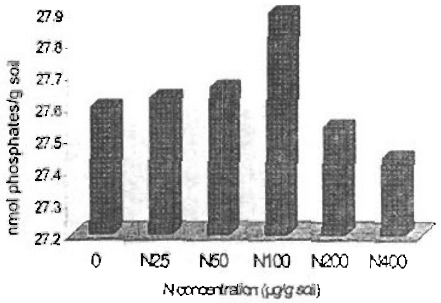

Fig. 4: Effect of N concentrations on phospholipid content

DISCUSSION

The results demonstrated that N fertilizer applied in the form of urea from 0 to 100 µg N g^{-1} soil showed a positive response on the dehydrogenase/ETS activity. However, higher concentrations of 200 and 400 µg N g^{-1} soil significantly reduced it. This was probably because nutrient supply has been reported as one of the controlling factors of the enzyme activities in upland soils (Sinsabaugh *et al.*, 1991). The activity of much of the soil biomass is severely limited by nutrient availability and many soil organisms have very low metabolic rates or spend most of their lifetime in dormant or resting phases (Gray, 1976). Similar results showing microbial biomass and dehydrogenase stimulation by glucose amendments have been earlier reported by Sparling *et al.* (1981) and Tsai *et al.* (1997). The increase in ETS activity of soil microorganisms up to a certain level of N application is might be due to the usage of nutrient as their energy source and thus resulted in increased their activity and enzyme production through their increased number. The lower rate might be not sufficient to activate the microorganisms and the higher doses might be excessive enough to produce inhibitory effects on soil microflora (Cooper and Warman, 1997). Sparling *et al.* (1981) also observed greater increases at the lower rates of glucose amendment than at the higher ones. Soderstrom *et al.* (1983) studied the effects of N fertilizations on microbial activity and found decreases in microbial activity, particularly at higher N rates, and they suggested several possible reasons for this decrease. It might be a direct inhibiting effect of nitrogenous compounds

making C less available and/or a partial sterilization effect from the raised osmotic potential of the soil solution due to excess fertilizer salts. Any of these effects could be causing the significant decrease in ETS in the higher doses of N treated soils. Chrost (1991) reported that nutrient availability also influences the enzyme activities, as various microorganisms control their enzyme production in response to nutrient availability. Ladd and Paul (1973) and Trevors (1984a) also reported the similar results.

The addition of urea fertilizer exhibited a considerably greater effect on soil phenol contents. An inverse relationship was observed ETS activity and soil phenol contents.

The decrease in phenol content by lower rates of N fertilizer and vice versa might be due to the increased microbial activity at lower rates of N application which resulted in greater decomposition of phenol content and opposite might be is true for higher doses of fertilizer (Stevenson, 1994). It is also established that soil microorganisms can both produce and decompose phenolic compounds (Stevenson, 1994). There are many soil fungi capable of utilizing phenolic acids as their sole source of carbon (Henderson and Farmer, 1955). Olk *et al.* (1996) also reported an accumulation of phenolic compounds in the soil organic matter, which he suggested that it is a characteristic of the anaerobic, or nearly anaerobic, soil conditions that exist at the initial stages of soil organic matter formation in submerged irrigated rice soils. Stowe and Osborn (1980) have shown that phenolic acid toxicity also depends on the nutrient status of the soil, particularly in respect to N and P. They suggested that such acids have their most marked toxic effects on soils of low fertility or during periods of the year of low fertility. Wingfield *et al.* (1977) has also give similar explanation. It was found that phenolic acids can be readily utilized by soil microbes (Sparling *et al.*, 1981) as they occur as the free acids in the soil solution and hence should be readily available for microbial degradation (Wang *et al.*, 1967).

The addition of fertilizer did not cause any marked change in the soil protein content and it was found nearly stable with N application. A slight (non-significant) difference in control and lower rates of N treatments might be due to comparatively more mineralization in N-treated soils than control soil. The N addition might have increased microbial population and activities as was obvious by increased ETS activity in the present study (Anderson and Domsch, 1978). However, a slight increase in protein contents was noted at higher N-additions, which might be due to decreased N-mineralization resulted from lower microbial population and activity (McCarty and Meisinger, 1997). It was reported elsewhere that N fertilization at rates in excess of crop requirements tended to decrease amounts of organic matter in soils and hence the soil microbial biomass and their activities (Green *et al.*, 1995).

Microbial biomass C, N, and P gradually decreased as the soils became more reduced (anaerobic). This was probably explained by the reduced energy captured by microorganisms using alternate electron acceptors. Consequently,

as the microbial biomass decreased there was a decrease in the amount of enzyme activity and decreased organic C, N, and P mineralization (McLarchey and Reddy, 1998).

The high resistance (stability) of organic N complexes in soil to microbial attack is of considerably significance to the N balance of the soil and several explanations are often given to explain this phenomenon. For instance, proteinaceous constituents (e.g., amino acids, peptides, proteins) are stabilized through their reaction with other organic constituents, such as lignins, tannins, quinones, and reducing sugars and biologically resistant complexes are formed in soil by chemical reactions involving NH_3 or NO_2^- with lignins or humic substances. The complexes thus formed have been shown to be highly resistant to mineralization by soil microorganisms (Stevenson, 1994). The increased phenol content with increasing pesticide concentrations in this study might be a strong reason for increased protein content (Olk *et al.*, 1996).

A slight (non-significant) increase in microbial phospholipid contents was observed at lower rates of fertilizer application and vice versa. The reasons might be the same as given by Green *et al.* (1995) and McCarty and Meisinger (1997) and are discussed above as the microbial biomass and phospholipid contents are directly related to each other. It has been reported that soil under aerobic condition contains larger amounts of phospholipids than under anaerobic (flooded) conditions (Reichardt *et al.*, 1997). Inubushi *et al.* (1991) also reported similar findings. Frostegard et al. (1991) evaluated the use of total lipid phosphate (L-PO_4) as a measure of microbial biomass in soils with different organic matter content and suggested that the soil samples typically contained ? 30-50 nmol L-PO_4 after persulfate digestion, a range that was suitable for the method used. Slightly lower values in present study might be due to anaerobic conditions as anoxic conditions caused reduction in (aerobic) microbial biomass (Inubushi et al., 1991). Also the lower quantities of phospholipids observed in agrochemical's treated soils than control soil, might be due to their toxicity as explained by Wingfield *et al.* (1977) and Soderstrom *et al.* (1983). Reichardt *et al.* (1997) also noticed a decline in phospholipids, due to flooding, in continuously cropped, irrigated rice fields. So, results of these studies are quite consistent with earlier findings.

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تأثير إستخدام سماد اليوريا على بعض قياسات التربة الميكروبيولوجية والبيوكيميائية.

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أجريت تجربة تحضين معملية لدراسة تأثير التسميد النيتروجيني (اليوريا) على جودة الأراضي المغمورة المنزرعة بالأرز مع تثبيت كل من ظروف الرطوبة (درجة التشبع) ودرجة الحرارة (٢٥ °م). وقد أضيف السماد بمعدل صفر، ٢٥، ٥٠، ١٠٠، ٢٠٠، ٤٠٠ ميكروجرام ن/جم تربة. وقد لوحظ أن معدل تركيز السماد المضاف من صفر - ١٠٠ ميكروجرام ن/جم تربة يشير إلى استجابة إيجابية على نشاط ETS. بينما التركيزات ٢٠٠، ٤٠٠ جرام ن/جم تربة لوحظ بها انخفاض معنوي في تأثيرها على ETS. ولوحظ أن المعدلات العالية من السماد تسبب زيادة في محتوى التربة من الفينول الذي يزيد مع زيادة السماد المضاف. والسماد المضاف لا يؤدي إلى تغير في محتوى التربة من البروتين بينما استجابة الفوسفوليبيدات تتشابه إلى حد ما مع نشاط ETS فهي تزداد مع المعدلات المنخفضة من السماد النيتروجيني وتنخفض مع المعدلات العالية من الإضافة.