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Effects of cotton ginning mill effluents on soil enzymatic activities and nitrogen mineralization in soil

G. Narasimha[a], A. Sridevi[b], G. Venkata Subba Reddy[c] and B. Rajasekhar Reddy[*c]

[a]*Department of Virology, Sri Venkateswara University, Tirupathi, India*
[b]*Department of Biotechnology, School of Engineering & Technology, Sri Padmavathi Mahila Viswavidyalayam, Tirupathi, India*
[c]*Department of Microbiology, Sri Krishnadevaraya University, Anantapur, India*

ABSTRACT

In view of importance of nitrogen mineralization in sustenance of soil for agricultural production, influence of effluents of cotton ginning mill on activities of enzymes- protease and urease and two processes in nitrogen mineralization- ammonification and nitrification in a black cotton soil was examined under laboratory conditions. The soil samples with effluent discharges exhibited higher activities of protease and urease than soil samples without effluent discharges. Unlike enzyme activities, rates of ammonification and nitrification occurred at lower pace in polluted soils than in control. On the 7th day incubation, 740 µg (microgram) of nitrogen in the form of ammonia was formed from peptone in polluted soil as against 1445 µg of NH^{+}_{4}-N in control soil. About 576 µg of nitrogen in the form of $N0^{-}_{3}$-N was recovered from polluted soil as against 1624 µg of $N0^{-}_{3}$-N in control soil. Increase in enzyme activities and reduction in ammonification and nitrification could be attributed to proliferation of fungi and decrease in population of bacteria in polluted soil.

Keywords: Cotton ginning mill effluents; Protease; Urease; Nitrogen mineralization; Ammonification; Nitrification.

INTRODUCTION

There is increasing pressure to provide basic needs such as food, fiber and shelter to the growing population, in particular, developing countries in the world. In order to meet basic needs, many agro-industries are being developed with least concern towards environment. Agro-industries include pulp, paper, sugar, ginning, textile, dairy, dyes, edible oil and fruit processing and

generate large volume of liquid/solid effluents and release them into the environment [1, 6]. Thus, advance in technology and industrialization bring with them unpleasant partners, pollution and degradation of the environment. The effects on the environment, connected with industrial activities are mainly related to the production of industrial wastes. Damage to the environment, in particular, soil a natural resource through industrial effluents, adversely affects agricultural production and may lead to food crisis. The main industrial activity of cotton ginning industry is ginning process that separates cotton fibers lint from cotton seeds. Residual lint left over on cotton seeds after ginning is removed with acid treatment in order to get clean seeds for raising crops in the next season. The acidic effluents generated in this fashion are released into surroundings including agricultural lands without treatment. Analysis of these soils with these effluents revealed occurrence of changes in physico-chemical and biological properties of soil due to discharge effluents from cotton ginning mill [25]. The present study is aimed at monitoring health status of soil under the influence of effluents of cotton ginning mill by examining the impact of effluents of cotton ginning mill on two soil enzymes and nitrogen mineralization as sensitive indicators of nitrogen cycle.

EXPERIMENTAL SECTION

Soil samples

Soil Samples of black cotton soil were collected from different locations where effluents had been discharged by M/s. Gajalakshmi ginning mill located at Nandyal, Kurnool district of Andhra Pradesh state and mixed together to make composite soil sample with effluent discharges (pH 5.52, organic matter 6.46 gkg $^{-1}$ and total nitrogen 0.204 gkg^{-1}). Samples of soil of the same type without effluent discharges collected from the farm of Regional Agricultural Research Station at Nandyal, located adjacent to the M/s. Gajalakshmi ginning mill. These two soil samples were air-dried and passed through < 2 mm (millimeter) sieve. The soil samples without effluent discharges served as control (pH 7.95, organic matter 1.21 g kg $^{-1}$and total nitrogen 0.0188 gkg^{-1}). Soil samples with/without effluent discharges were used in the present study and their physico-chemical and biological properties were reported elsewhere [25].

Different quantities of soil samples soils with/without effluent discharges- five grams for protease, one gram for urease and five grams of soil with 1mg of peptone per gram of soil for nitrogen mineralization were placed in test tubes (25 × 200 mm) for determining activity of two enzymes and nitrogen mineralization. Sterile water was added to these soils to adjust moisture to 60% water holding capacity (WHC). Moisture in soil samples incubated at room temperature 28 ± 4°C at the same level was maintained throughout the experiment by replacing water loss that occurred during incubation. Similar model was used earlier in the study on effect of insecticides on microbial activities in soil [10, 11]. Triplicate soil samples with/without effluent discharges were withdrawn after 0, 7, 14, 21 days of incubation to determine the soil enzyme activities and two processes in nitrogen mineralization.

Assay of Enzymes

Protease assay

Activity of protease in soil sample was determined according to the method of Speir and Ross [33]. At desired intervals one set of triplicate soil samples with/without effluent discharges received 10 ml (milliliter) of 0.1 M Tris (2-amino-2-hydroxy-methyl propane 1:3 diol, pH 7.5)

containing sodium caseinate (2%W/V) (weight volume^{-1}) whereas addition of 10ml of 0.2 M Tris buffer without caseinate was made to another set of triplicate soil samples. Both sets were incubated for 24 hrs at 30°C and four milliliter of (17.5%W/V) trichloro acetic acid was then added and the mixture was centrifuged. A suitable aliquot of the supernatant was treated with 3 ml of 1.4M Na_2CO_3 followed by the addition of Folin-Ciocalteau reagent (33.3% V/V) (volume volume^{-1}). The blue color was read after 30 minutes at 700 nm in a spectronic-20D spectrophotometer. Microgram of Tyrosine Equivalents (TE) formed in the supernatant was estimated by referring to tyrosine standard curve and protease activity is finally expressed in µg TE g $^{-1}$ 24 h^{-1}.

Urease assay

Urease activity in soil samples was estimated according to Phenol-hypochlorite method [7]. At desired intervals, withdrawn soil samples were split into two sets for determination of urease activity in soil samples with/without effluent discharges in the presence and absence of buffer. One set of soil sample received one ml of 0.1M phosphate buffer (pH-7.1), whereas another set of soil samples received one ml of distilled water. Soil samples of each set was further sub-grouped into two halves. To one half of soil samples of both sets, one ml of 30% urea was added. Another half of soil samples of both sets with receipt of distilled water in the place of urea served as control. After 30 minutes of incubation all soil samples were shaken at 37°C in a water bath shaker. The flasks were placed in ice until ammonia was extracted with 10 ml of 2M KCl. Five milliliters of phenol-sodium nitroprusside solution and 3ml of 0.02 M sodium hypochlorite were added to 4ml aliquot of KCl extracts. The mixture was shaken and incubated for 30 minutes in the dark room and the bluish color developed was measured at 630 nm in a spectronic-20D spectrophotometer.

Nitrogen Mineralization

Estimation of ammonia

Different forms of inorganic nitrogen- NH^+_4-N, $N0^-_2$-N and $N0^-_3$-N, formed in soil samples incubated with peptone were determined after extraction. At regular intervals one set of soil samples were extracted for NH^+_4-N with 2M KCl whereas another set of soil was used for extraction of $N0^-_2$-N and $N0^-_3$-N with distilled water in the same fashion as described earlier [11]. Ammonium (NH^+_4-N) extracted from peptone amended soil samples in 2M KCl extract was analyzed by Nesslerization [12]. To suitable aliquots of the soil extracts, 0.5 ml of Nessler's reagent was added and the volume was made up to 5ml. The yellow color developed was read at 495 nm in a spectronic-20D spectrophotometer. The amount of ammonium was calculated by referring to calibration curve prepared with standard solution of known ammonium concentration.

Estimation of nitrite

Nitrite, extracted from peptone incubated soil, in distilled water was estimated by diazotization following the method of Barnes and Folkard [3]. Suitable aliquots from the filtrate of soil extract were pipetted out into test tubes and 1ml of 1% sulphanilamide in 1N HCl was added and shaken thoroughly. Then 1ml of 0.12% (N-1-Naphthyl-ethylene diamine dihydrochloride) was added to the test tubes for the formation of colored diazocompound. After 30 min, the volume was made up to 10 ml with distilled water. The absorbance of the pink colored solution was read at 520 nm

in a spectronic-20D spectrophotometer. The amount of nitrite was calculated by referring to a calibration curve prepared with standard solution of nitrite.

Estimation of nitrate

Nitrate extracted from peptone-amended soil samples in distilled water was determined by the method of Ranney and Bartlett [28]. Three drops of brucine reagent (2g brucine in 50 ml of methanol) were added to suitable aliquots of the soil extracts followed by 2 ml of concentrated sulphuric acid. The solution was mixed by rotating and placed in the dark room for 30 minutes to ensure full color development after which the volume was made up to 15 ml with distilled water and the yellow color was read at 410 nm in a spectronic-20D spectrophotometer. The amount of nitrate in the filtrate was calculated by using calibration curve.

RESULTS AND DISCUSSION

Soil is a natural resource utilized for various activities to meet human needs including food production. Sustainability of soil for agricultural production rests on maintenance of soil fertility. Soil fertility is a nothing but a supply of nutrients for growth of plants from decomposition of organic matter mediated by life processes of microorganisms under congenial conditions. Exposure of soil microorganisms to effluents from industries including agro-industries may cause damage to soil health. Measurements of enzyme activities such as protease and urease and biochemical nitrogen transformations can be sensitive indicators of soil microbial activity which plays a major role in affecting soil quality [5, 24].

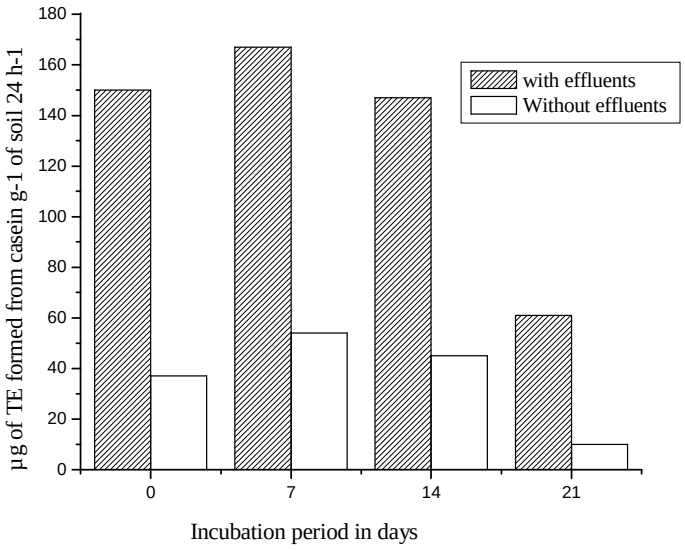

Figure 1. Protease activity in soils incubated with/without effluents.

Protease activity

Proteases in soils play a significant role in nitrogen mineralization [19], an important process in regulating the amount of plant available nitrogen for plant growth. In the present study the protease activity remained steady over a period of first 14 days and then onwards slightly declined and results are presented in Fig. 1.

Higher activity of protease was recorded in soil samples with effluent discharges than in control soil samples. For instance, at 7-day interval, soil samples with effluent discharges exhibited 170 µg TE g^{-1} 24 h^{-1} as against 55 µg TE g^{-1} 24 h^{-1} in respect of control soil sample. The protease activity shown by soil samples with effluent discharges increased in the range of 2 to 5 folds over control soil samples at all intervals.

The effect of industrial effluents on soil enzyme activities received less attention than the effect of agrochemical on soil enzyme activities [9, 26, 29]. The present study pertains to influence of effluent discharges from cotton ginning mill on soil protease activity and soil with effluents displayed higher protease activity than control soil. Similarly, the addition of sewage sludge to the soil had stimulatory effect on proteolytic activity [16, 37, 21, 8, 15]. It was also further observed that occurrence of initial rise followed by declining in proteolytic activity in sludge–amended soils resulted from the depletion of organic nitrogen substances applied to the soils in the form liquid dairy sludge [36]. In contrast, protease activity on soils polluted with nonagroindustry-cement dust reveals that the protease activity was higher in unpolluted soils than in the polluted soils [31]. The percent decrease in the protease activity in soils was correlated with degree of cement dust pollution which in turn decreased with increase in distance from the factory site. Soil protease activity was correlated with the number of soil bacteria [30]. Similarly, display of higher protease activity by effluents-amended soils in the present study, probably is due to increase in fungal flora reported in the same soil [25] because of availability of proteins in the form that stimulated both microbial growth and microbial synthesis of protease.

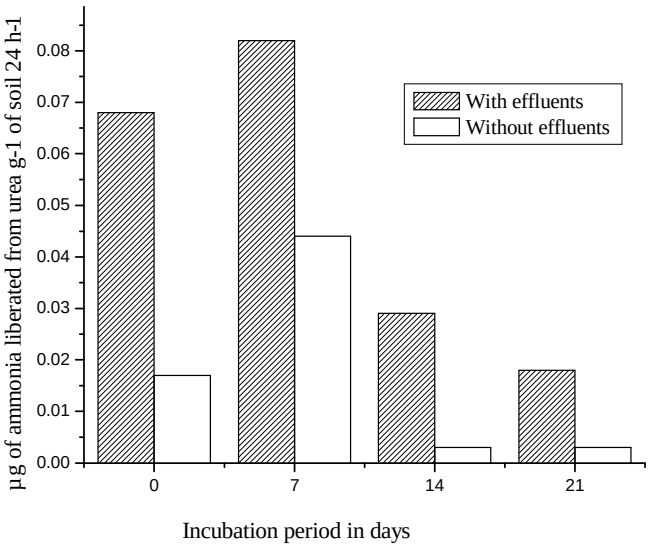

Figure 2. Urease activity in soils incubated with/without effluents in the absence of buffer.

Urease activity

Urea is an organic chemical complex used mainly as nitrogenous fertilizer in agriculture. Conversion of this nitrogen to inorganic nitrogen - ammonia and carbon dioxide takes place due to activity of urease enzyme, secreted by certain microorganisms and is responsible for supply of nitrogenous demand to growing crops. Assay of urease activity in soil samples involves

quantification of ammonia released upon hydrolysis of urea [22, 4]. Urease activity in soil samples with/without effluent discharges under nonbuffering conditions measured in this fashion is presented in Fig. 2.

Like soil protease enzyme, urease activity also increased in the first week and thereafter declined in both soil samples with/without effluent discharges under nonbuffering conditions. Polluted soil released 0.082 µg of ammonia from urea g^{-1} of soil as against 0.0436 µg in control soil sample at 7^{th} day interval under nonbuffering conditions. The similar trend was observed when urease activity was measured even in the presence of buffer in both soil samples with/without effluent discharge were presented in Fig. 3.

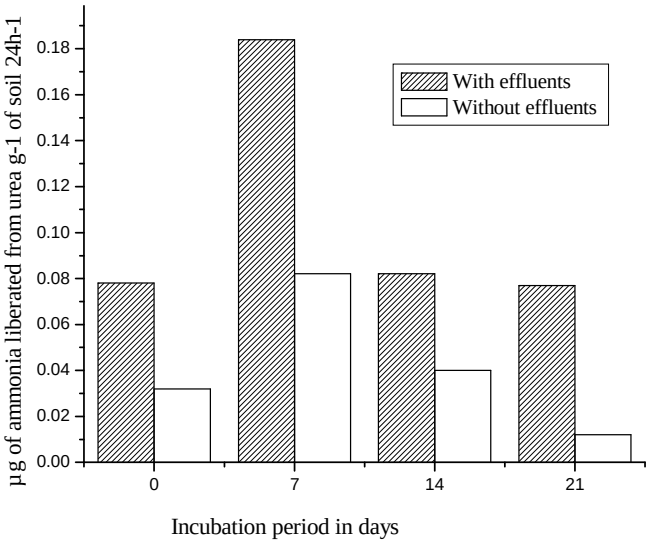

Figure 3. Urease activity in soils incubated with/without effluents in the presence of buffer.

Under both buffering and nonbuffering conditions, soil samples with effluent discharges exhibited about 2-10 fold higher urease activity over control. But inclusion of buffer in assay mixture enhanced urease activity in both soil samples with effluent discharges and control soil samples.

Presence of buffer in the assay medium increased urease activity in soil samples with/without effluent discharges in the present study. This observation is in agreement with recording of 2-fold enhancement of urease activity in agricultural soils upon addition of buffer solutions [34]. Discharge of effluents to soil elevated urease activity by many folds in soil in the present study. Similarly, dumping of sugar industry wastes not only brought changes in physico-chemical properties of soil but also enhanced both bacterial and fungal populations of soil and activity of enzyme such as cellulase in soils [23]. Addition of biomethanated spent wash increased the activity of enzyme including urease in soil [17]. There was a consistent and significant increase in the activity of urease upon addition of higher doses of sewage sludge to the soil [35, 2, 8, 18, 20]. In a most recent study [13], the administration of sewage sludge to soil resulted in enhanced ureolytic activity. In contrast, cement dust pollution caused significant decrease in urease activity in soil samples [31]. It appears that influence of industrial effluents on soil enzyme activities

were dependent on the nature and composition of chemicals in the effluents. Enhancement in fungal populations accompanied by reduction in bacterial population in soil of the same type used in the present study due to discharge of cotton ginning mill was earlier observed [25]. But, higher urease activity associated with elevated fungal flora in polluted soils with higher organic matter content and low pH in the present study, suggests participation of fungal populations in enzyme activities

Effect of effluents on soil nitrogen mineralization

Soil microorganisms are dynamically involved in many basic ecologic processes such as the biogeochemical cycling of elements, and the mineralization of carbon, nitrogen, phosphorous and sulfur. Among these ecological processes nitrogen mineralization play a vital role in conversion of organic nitrogen compounds to various inorganic forms such as NH^{+}_{4}-N, $N0^{-}_{2}$-N and $N0^{-}_{3}$-N through ammonification and nitrification. Direct discharge of effluents may affect microbial proliferation and enzymatic activities leading to decrease in the rate of bio-chemical processes in soil environment. In view of nitrogen as limiting factor for improving crop yield and importance of nitrogen mineralization in maintenance of soil fertility by providing useful forms of inorganic nitrogen, inorganic forms of nitrogen such as ammonium, nitrite and nitrate, converted from organic nitrogen, peptone added to soil samples with/without effluent discharges were quantified and are presented in the Table 1.

Table 1: Nitrogen mineralization in soil samples with/without effluent discharges

Soil incubation in days	Nitrogen mineralization in terms of liberation of µg of inorganic nitrogen g^{-1} of soil					
	Soil samples with effluent discharges			Soil samples without effluent discharges		
	NH_4^+-N	NO^-_2-N	NO^-_3-N	NH_4^+-N	NO^-_2-N	NO^-_3-N
0	434±22	19±3	2±0.1	470±8	27±0.5	5±0.5
7	700±20	32±3	7±0.6	1200±76	52±6	193±9
14	115±4	24±3	95±4	380±20	31±3	324±9
21	20±2	4±0	576±46	94±8	2±0.1	1624±113

- *Values represented in the table are means of triplicates ± S.D (Standard deviation)*

Analysis of different forms of inorganic nitrogen from organic nitrogen revealed that ammonical nitrogen recovered from organic peptone was increased at earlier intervals up to 7th day and then declined whereas oxidized forms of nitrogen ($N0^{-}_{2}$-N and $N0^{-}_{3}$-N) were increased with increase in the incubation period in both soil samples with/without effluent discharges. Drop in levels of ammonical nitrogen with concomitant rise in levels of $N0^{-}_{2}$-N and $N0^{-}_{3}$-N at later intervals due to oxidation of ammonia in nitrification was observed in both polluted as well as control soil samples. However, formation of NH^{+}_{4}-N, $N0^{-}_{2}$-N and $N0^{-}_{3}$-N at higher rate in control soil samples than in polluted soil samples occurred indicating that ammonification and nitrification

were affected by discharge of effluents into soil. For instance, 700, 32, and 7 µg of nitrogen g^{-1} of soil in the form of NH^{+}_{4}-N, $N0^{-}_{2}$-N and $N0^{-}_{3}$-N were recorded from peptone in polluted soil as against 1200, 52, 193 µg of nitrogen g^{-1} of soil in the form of NH^{+}_{4}-N, $N0^{-}_{2}$-N and $N0^{-}_{3}$-N in control soil samples at 7th day interval, respectively. Levels of ammonical nitrogen dropped down to 20 and 94 µg of nitrogen g^{-1} of soil in polluted soil and control soil samples with concomitant rise in $N0^{-}_{3}$-N level to 576 and 1624 µg of nitrogen g^{-1} of soil in polluted and control sample at 21st day interval, respectively.

Ammonification and nitrification are important processes responsible for mineralization of organic nitrogen (peptone) into different forms - NH_4^+, NO_2^- and NO_3^- in soil. Unlike soil enzymes, both these processes were inhibited in soil with effluent discharges in comparison to control in the present study. Rates of ammonification and nitrification in polluted soil on 7th day of incubation in the present study were reduced by about 2 and 6 folds respectively. Similar observations were made by Shanthi [31] in soil polluted with cement dust. A significant decrease in the ammonification and nitrification occurred in soils polluted with cement dust throughout incubation period in polluted soil.

According to Sharada Devi [32], mineralization of organic nitrogen in terms of NH^{+}_{4}-N, $N0^{-}_{2}$-N and $N0^{-}_{3}$-N and ammonification was not influenced by the presence of heavy metals even at 100 ppm level whereas nitrification was more sensitive to the presence of heavy metals. Premi and Confield [27] found some stimulatory but more usually inhibitory effects of trace elements (copper, manganese, zinc and chromium) on both processes in soils. These effects varied considerably depending on the level and type of cation added. On the other hand, N-mineralization was significantly improved in soils with incorporation of dairy sewage sludge as reflected by recovery of exchangeable NH^{+}_{4}-N and $N0^{-}_{3}$-N in larger amounts [14]. Further, the decrease in the concentration of exchangeable NH^{+}_{4}-N, generally coincided with an increase in $N0^{-}_{3}$- levels in dairy sewage sludge amended soils during later stage of incubation confirmed the occurrence of nitrification.

In the present study, both processes- ammonification and nitrification were inhibited in soil with effluents of cotton ginning mill. These two processes are generally mediated by microorganisms, in particular bacteria. Reduction in bacterial population in the same soil due to discharge of effluents from cotton ginning mill was also observed [25]. Decrease in the rate of ammonification and nitrification in polluted soil where reduction in bacterial population also took place suggests the involvement of bacteria rather than fungi in two important processes of nitrogen mineralization, ammonification and nitrification. This needs to be further examined.

CONCLUSION

In the present investigation the results clearly indicated that discharge of effluents from cotton ginning industry has stimulated activity of two enzymes protease, and urease in soils and exhibited maximum activity at 7th day interval followed by downward trend in their activities at lateral intervals of incubation in comparison to control soil. Increase in activities of enzymes in soil with effluent discharges over activity of corresponding enzyme in control soil varied from one individual enzyme to another enzyme within range of 2-25 folds at 7th day interval with

minimum and maximum limits. Unlike the soil enzymes- protease and urease, both ammonification and nitrification were inhibited in soil samples with effluent discharges.

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