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Toxic Effects of Distillery Sludge Amendment on Microbiological and Enzymatic Properties of Agricultural Soil in a Tropical City

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Abstract

Distillery sludge is an easy source of plant nutrient but its fertilizer value can vary considerably. In the present study anaerobically digested distillery sludge was applied to agricultural soils and its effect on soil biological and biochemical properties was evaluated. The sludge treatments were comprises of 0, 10, 50, 100 and 150 t ha^{-1} as single application in an agricultural field and tested for six months. Microbial respiration, microbial biomass carbon, FDA hydrolysis, phosphatase, urease and dehydrogenase activity were evaluated temporally throughout the incubation time for different amount of distillery sludge amendments. These parameters were sensitive enough to show the effect of distillery sludge application on soil microorganisms. The results revealed that sludge application at different rates initially increased the microbial activity, its highest activity was found between 30 and 60 days after application when sludge was applied at the rate of 150 t ha^{-1} but afterwards the microbial activities decreases gradually. Results show that at high dose soil microbial number increases but the diversity of soil microbial population decreases. Aerobic heterotrophic and symbiotic nitrogen fixing bacteria seems to be more sensitive to sludge addition and shows a marked decrease in population on higher doses. The results also shows that the sludge from distillery wastewater treatment plant may have potential as a beneficial soil amendment up to certain extent for improving biological properties of the soil but at higher doses its contamination can create harm for the beneficial soil inhabitant microbial population and their activities.

Keywords: Distillery sludge; Microbial biomass; Microbial respiration; Flourescein Diacetate (FDA); Hydrogenase; Urease; Phosphatase; Dehydrogenase

Introduction

Distillery sludge application in degraded soils is amongst the most important economical resources for the soil fertility amelioration through improvement in soil water-holding capacity, texture, structure, nutrients retention, roots penetration, and reduction in soil acidity [1-3]. Presently due to the ever increasing number of sugar mills and distillery units in the country, it is now become mandatory to use the distillery sludge as a fertilizer to enhance the fertility levels of the soil. However, its application in soil also results in environmental problems [4] because apart from organic content and nutrients, sludge also includes toxic compounds, heavy metals, coloured compounds i.e., melanoidin, caramel colorants, dissolved inorganic salts, chlorinated lignin, and phenolic derivatives [5]. Thus, soil microorganisms may be affected via variation in soil temperature, pH, nutrient status, heavy metals, oxygen level and which in turn can affect the ecological processes linked with nutrients cycling [6]. Soil microbial activity, often linked with fertility levels, has been found to be indicated by microbial biomass, microbial respiration, hydrolysis of fluorescein diacetate (FDA), and activity of phosphatase and urease [7-9]. Microbial biomass is the 1-5% active fraction of organic matter of the soil, related with the soil fertility and is highly susceptible to soil pollution than the bulk organic matter. It acts as a reservoir of nutrients and gradually releases it into soil with passage of time which influences soil fertility levels. Soil respiration determines the effect of sludge amendment on soil microbial activity terms of carbon dioxide evolution. In this study, we have chosen enzymatic activities representative of main steps of soil biogeochemical nutrient cycles, i.e. C (FDA hydrolytic activity), N (urease), P (phosphatases), and microbial biomass (Dehydrogenase and FDA hydrolytic activity). Fluorescein diacetate has been used to determine amounts active microorganism. Hydrolysation product of FDA by a number of soil enzymes, such as proteases, lipases, and esterases is fluorescein, which can be easily visualized to estimate microbial activity. Urease catalyzes the hydrolysis of urea and amides to carbon dioxide and ammonia and helps in nitrogen and carbon cycling. Phosphatase is involved in phosphorus mineralization in soils through attack on phosphomonoesterase bond presents in soil organic matter thus increasing the phosphorus availability to microorganisms and plants. Dehydrogenase is a good indicator of metabolic activity soil microorganisms as it is not active independently of the parent microbial cell as extracellular enzymes in soil (Quechan et al. 2002). Short term evaluation of distillery effluent application in crop fields showed positive effect on crops growth [6,10-12] but long-term in-depth understanding of effect of distillery sludge is lacking on soil microbiological properties responsible for sustainability of soil. The present study, carried out in Indian tropical agroecosystems, aims to evaluate the effect of varying amount of distillery sludge on soil microbiological characteristics including major soil microbial groups, microbial respiration, microbial biomass carbon and nitrogen, FDA hydrolysis, phosphate activity and some other soil fertility indices.

Material and Methods

Experimental design, sampling and analysis

The study was performed at the agricultural farm at the Narang

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distillery at Nawabganj (26°52' N, 82°08' E and 98 m above the mean sea level). Experiment was designed in randomized block design with four replicate plots of 5 m×5 m size for various doses of sludge amendment. Likewise four un-amended plots were also established as control. The sludge was collected from sludge bed of ETP of distillery, sun dried in the form of cakes and placed in clean sterile polythene bags. Before application, distillery sludge was dehydrated and applied as single dose at a rate of 10, 50, 100 and 150 t ha $^{-1}$ (on dry weight basis) in January 2009. Composite soil samples were collected from A-horizon (0–20 cm soil depth) of the plot without any crop at different time intervals from the experimental field. The experimental soil was an inceptisol with a pale brown colour, and sandy loam texture. The soil consisted of 9.5% clay, 10.5% silt, 16.5% coarse silt, 36.7% fine sand, 21.0% coarse sand, and 5.8% humic acids. Ten soil samples were collected and composited into one sample then packed in sterile polythene bags and were stored at 4°C in the dark and maintained at 50% water holding capacity then it was sieved through a 2mm-pore size sieve before use. The physico-chemical properties of sludge, amended and un-amended soil including organic matter, cation exchange capacity were estimated using standard methods [13]. Moisture content was determined by wet oxidation method. pH of sludge, un-amended and different sludge-amended soil (1:5 soil/sludge water suspensions) was determined by using digital pH meter. The electrical conductivity (EC) of was measured (1:5 soil/sludge water suspensions) with the help of conductivity meters. Samples were analyzed for total Nitrogen, Phosphorus and Potassium. The extractable heavy metal concentrations in soil samples were measured by atomic absorption spectrometry after extraction with aquaregia.

Estimation of microbial respiration

Basal respiration was determined by the carbon dioxide released in the process of microbial respiration during 10 days of incubation after sludge application. Homogenized samples of fresh moist soil of 500 g (oven-dry basis at 105°C, 24 h), each for control and soil-sludge mixtures, were placed into 1L capacity jars, sealed properly and further incubated for at 25°C. The incubation of moist soil of each treatment in sealed jars was conducted for 180 days in a growth chamber at constant temperature (25±2°C); the humidity was adjusted to 50-60% of water holding capacity (WHC) and maintained at a constant level throughout the experiment by distilled water filled vial. Two 20 ml beakers were placed on top of the soil. One beaker contained 10 ml 0.5 M NaOH to absorb CO_2 and the other contained 5 ml 2% H_3BO_3 to absorb NH_3. The NaOH solution was changed after every 15 days. The H_3BO_3 solution contained phenolphthalein diluted into 100 mL ethanol (60%, v/v) as an indicator and was titrated once at the end of the incubation. Cumulated microbial respiration of the soils and soil-sludge mixtures were estimated by measuring CO_2 evolution by alkali absorption of CO_2 evolved periodically after each 15 days followed by titrating the residual NaOH with a solution of 0.1 M HCl after precipitating the carbonate with 1 mL of 1.5 M $BaCl_2$ solution using phenolphthalein as indicator. The data were expressed as mg CO_2-C 100 per gm of soil [14,15].

Soil microbial biomass carbon

Chloroform fumigation-extraction method [16] for the estimation microbial biomass carbon in soil, is considered as a representative method to estimate the whole soil functional entity [17-19]. 25 g each of fresh moist soil and soil-sludge mixture were fumigated with alcohol-free $CHCl_3$ for 24 hr at 25°C in a closed vessel. The organic carbon content of fumigated and control soil and soil sludge mixture was extracted with 0.5 M K_2SO_4 and further estimated by treatment with a mixture of 0.5 M sodium dichromate and sulfuric acid (Sadzawka et al. 2004). An extraction efficiency coefficient of 0.45 was used to convert organic C to MBC [20]. Microbial biomass C was calculated as E_C/k_{EC}, where EC is organic C extracted from fumigated soils - organic C extracted from non-fumigated soils and k_{EC} 0.45 [21-23].

Number of culturable microorganisms

Microbial populations in both amended and unamended fresh soil samples of 1 g each (in triplicate) were enumerated following standard serial dilution plating technique on selective media using one-fourth strength Ringers solution [24] and expressed as CFU g^{-1} dry soil. Culturally viable bacteria, fungi and actinomycetes were counted on nutrient glucose agar, Martin's Rose Bengal Agar (amended with 30 mg/l streptomycin sulphate), and Kenknight's Agar (amended with 0.05 g/l cyclohexamide), by using 10^{-6}, 10^{-4} and 10^{-5} dilutions respectively [25]. Plates inoculated with 0.1 ml soil suspension cultured for 4, 7 and 10 days for heterotrophic bacteria, fungi and actinomycetes, respectively, at 28°C [26]. Nitrogen-fixing bacteria enumeration in soil was based on the most probable number technique, using a semi-solid nitrogen-free combined carbon medium [27]. The CFUs were calculated using Accu Count™ 1000 automated colony counter.

Enzyme activity

FDA activity: FDA activity (representing soil organic matter decomposition) was measured by incubating soil and soil-sludge mixtures (both 1.5 g) with 9.9 ml sodium phosphate buffer [9]. Hydrolysis, started at addition of 0.1 ml FDA solution (1 mg ml^{-1}), was stopped after 1 hour of incubation at 25°C by adding 10 ml of acetone and absorbance of supernatant filtrate (by Whatman No. 40 filter paper) was measured at 490 nm. The concentration of the released fluorescein was calculated by pre-drawn standard curve and expressed as µg FDA g^{-1} h^{-1}.

Urease: 0.5 mL toluene and 12 mL phosphate buffer mixed with 3g sieved soil and soil sludge mixtures and incubated for 15 min at 37 °C in a BOD incubator. After mixing with 3 ml of 10 % urea solution it was again incubated for 1 hr at 37°C and 15 mL of a KCl 2 M solution containing 5 mg phenyl mercury acetate were added. The sample was agitated for 5 min and then filtered through a Whatman no.1 filter paper. 0.2 g magnesium oxide previously heated at 500– 600 °C for 1 hr was added to 10 microlitres of the filtrate to remove carbonates. The filtrate was added to 5 mL boric acid solution, with methyl red and bromocresol green as indicators and then titrated with a H_2SO_4 0.001 M standard solutions. A blank was prepared for each treatment, and the same procedure utilized for the samples was adopted, with the only difference that the urea solution was added after the KCl and phenylmercury acetate solution. Urease activity was expressed as µg $N–NH_4^+ g^{-1}$ produced in 1 h of incubation [28].

Phosphatase: 5 g of soil sample, 0.25 ml of toluene, 1ml of 10 mm p-nitrophenyl phosphate and 10 ml of distilled water were mixed and after 1hr incubation 5 ml of 0.5 M $CaCl_2$ and 20 ml of 0.5 M NaOH were added. The content was filtered using Whatman No.42 filter paper and volume made up to 50 ml with distilled water. The colour intensity was read at 420 nm. The concentration of phosphatase was obtained from a standard graph and the enzyme activity was expressed as µmol PNF g^{-1} h^{-1} [29,30].

Dehydrogenase: A 20 g of soil sample was taken in a boiling tube. To this 1 ml of 3% 2, 3, 5-Tri phenyl tetrazolium chloride (TTC) was added. Then 1 ml of 1 % glucose and 2.5 ml of distilled water was added and incubated for 24 hrs at 37°C. After that 10 ml of methanol were added and incubated for another 5 hrs. The content was filtered and

the samples were washed thoroughly with methanol. The red colour developed was read at 485 nm. The concentration of dehydrogenase in the sample was obtained from the standard graph using triphenyl farmazane. Results were expressed in µg TPF g^{-1} day^{-1} [30,31].

Result

Soils and sludge characteristics

Physico-chemical analysis of sludge was done in triplicate sets of samples showed that all the collected samples have high pH, EC, BOD, COD, TSS, TDS, phenolics and metals (Cu, Cd ,Cr, Zn, Fe, Ni, Mn, Pb etc) as shown in Table 1. High concentrations of nitrogen, phenol, chloride and heavy metals were detected in distillery sludge versus the agricultural soil (Table 1). The values of pH (9.2) and EC (3.25 $mScm^{-1}$) were also higher in sludge than the soil where the pH and EC were 6.8 and 1.55 $mScm^{-1}$respectively. High values of pH and EC in the sludge may be due to the presence of high concentrations of soluble salt. High concentrations of heavy metals and salts in sludge are due to the condensation process, which takes place during sugar manufacturing and alcohol production. The effect of distillery sludge treatment on some soil chemical properties is also shown in the same table which shows that certain properties increase from the control soil after amendment.

Respiration

The cumulative CO_2 for control soil, sludge and for different soil-sludge after 180 days of different soil sludge amendments was calculated.

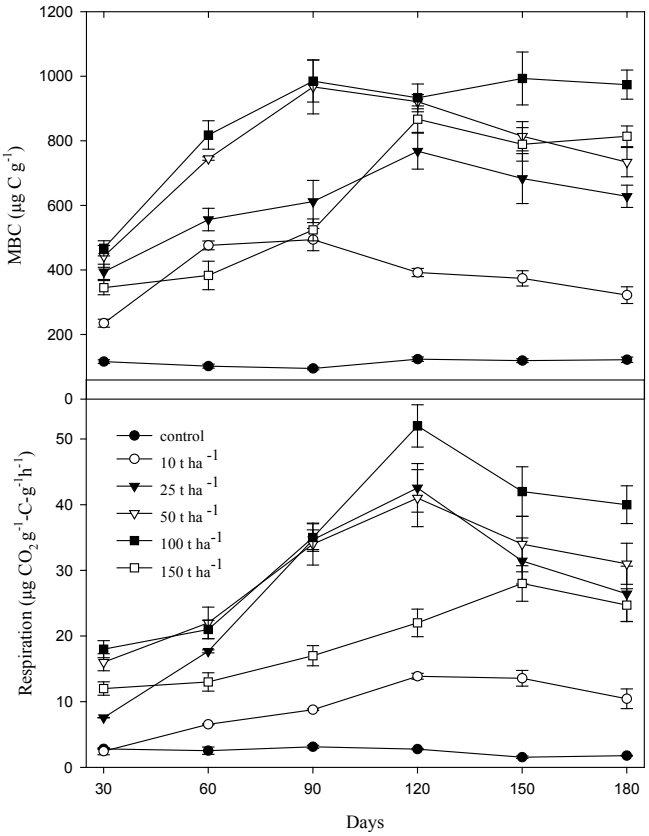

Figure 1: Changes in Microbial biomass carbon and Microbial basal respiration in distillery sludge amended soil at rates of 10, 25, 50, 100, 150 t ha^{-1} for varying time of incubation (the mean values are presented, n=3).

The microbial respiration of the sludge was many times higher than observed in soil. The addition of sludge increased the CO_2 production of soils initially, increasing almost ten times the respiration activity when 100t ha^{-1} was applied, in respect to the unamended soils and then declined (Figure 1).This increment is associated with large amount of easily available soluble organic matter [3], nitrogen, phosphorous and other nutrient sources of the sludge that would have triggered microbial activity causing high rate of microbial-mineralization in the soil-mix [32]. The moistening of soil through addition of sludge plays a major role in increase in respiration which reactivates the growth and activity of microorganisms. The decline in CO_2, evolution in the later stage is caused by the exhaustion of one or more essential nutrients and the accumulation of toxic metabolites and heavy metals during incubation at higher doses of sludge application [33].

Microbial biomass C

Soil microbial biomass and biological activities are the indicator for changes in soil resulting from different stresses in soil ecosystems [34]. It was found from the experiment that the microbial biomass carbon in soil increased with the increment of sludge application and higher level (mg kg^{-1}) was obtained after 90 days of incubation with the application of 150 t ha^{-1} of sludge (Figure 1). After 120 days of incubation, the MBC decreased in amended soil. However, the values of MBC in amended soils were always higher than that of unamended control soil. All the incubation experiment is carried in controlled condition so that effect of seasonal change and other environmental factor in microbial biomass can be removed.

Microbial population

Quantitative analysis of soil microbial populations show variations in the populations of different groups of microflora in the soils following amendment with distillery sludge (Figure 2) The results show a marked decrease in total culture numbers of the different microbial groups for the highly contaminated soil samples. Aerobic heterotrophic, nitrifying and denitrifying bacteria seem to be more sensitive to high dose of distillery sludge contamination than the other microbial groups under evaluation. A difference in the viable counts of fungal and actinomycetes were also significant; however, these two microbial groups seemed to be less sensitive to the presence to the amendment.

FDA activity

The FDA activity in soil increased with the increment of sludge application, and the higher level was obtained with the application of 150 t ha^{-1} of sludge, after 60 days of incubation (Figure 3). Results of FDA hydrolysis obtained with soil throughout the all incubation period (0-180 days), the FDA hydrolysis in sludge-amended soil was significantly different ($P<0.05$) compared to unamended. After 90 days of incubation, the FDA values decreased in both amended and unamended soil, however, the changes in values of FDA obtained in unamended soil was not very much significant. The initial increase of FDA hydrolysis indicates increment in the activity of the microorganisms due to organic matter contribution, macro and micronutrients, and decrease due to the toxic compound accumulation later.

Urease activity

The mean urease activity was highest at the 150 t/ha application of distillery sludge i.e. while control shows the lowest activity (Figure 3). Enzyme activity changes both according to the level of application and incubation period during the experiment it was also seen that

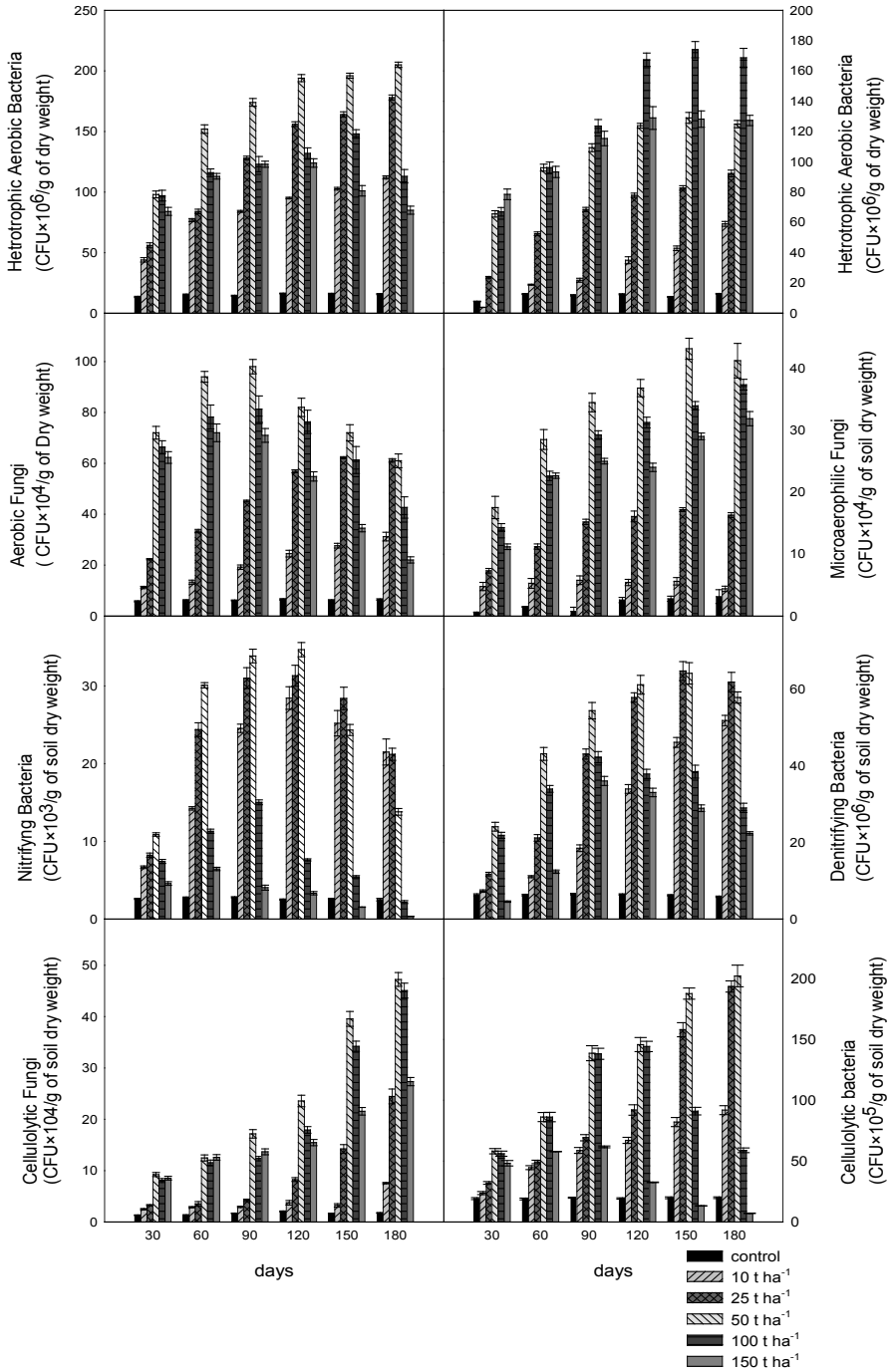

Figure 2: Changes in microbial population (CFU per gram of dry soil) sample following amendment with different amount of distillery sludge for varying length of time (all values are mean n=3, and SE bars are shown).

after application the soil show rapid increase in urease activity during incubation period when no additional amendment is applied. A slight decrease in urease activity is seen at 100 t/ha application of the sludge. Urease activity was expressed as mg $N–NH^{+}_{4}$ produced in 1 hr of incubation.

Dehydrogenase activity

Dehydrogenase activity increased significantly at 150 tha^{-1} amendment on the 10th day of incubation (Figure 3). The activity increased within the period of incubation as reflected in the mean values within the days of incubation respectively. Dehydrogenase is an important enzyme for the oxidative processes in the soil which increases gradually with the rate of sludge application and its activity is very much dependent on the organic matter supply through distillery sludge. Our results are in agreement with Tesařová (2000) and Kubát et al. (1999), who also considered the activity of dehydrogenase,

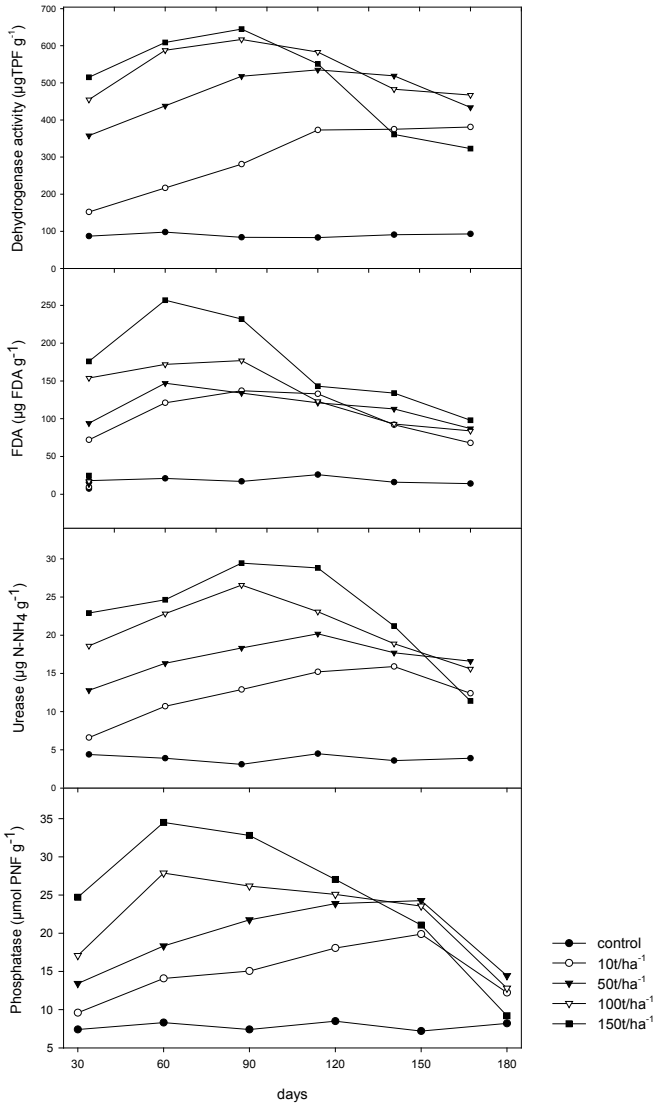

Figure 3: Changes in different enzymes activities in soil amended with varying amount of distillery sludge for varying days of incubation (the mean values are presented, n=3).

microbial biomass, soil respiration, and counts of N-fixing bacteria, etc. as sensitive bio-indicators of soil quality µgTPF/g.

Phosphatase activity

The phosphatase activity in distillery sludge treated soil is maximum when 150 t/ha sludge is applied and it further increases with the increase of the duration of incubation (Figure 3). It was maximum i.e. at the 180th day for the 150t/ha sludge application. Phosphatase recorded the maximum activity of 140.6 g kg hr that might have been favored by the copious quantity of phosphate present in the effluent. Sludge addition in experimental soil increases the plant-available phosphorus content and decreases acidity of the soils and consequently increases available P [1]. An increase of phosphorus availability in soil have been well correlated with the increase of phosphatase activity, being an adequate indicator of microbial activity and of modifications occurred in the soil due to sewage sludge application [8,35,36]. The increases the phosphatase activity is shown throughout incubation time (0-180 days), being significantly different ($P<0.05$) compared to unamended soils. The maximum activity was observed at 60 days of sludge application, and then the phosphatase activity decreased. In the experimental soil the levels of this enzymatic activity was 5 times higher after 60 days of incubation when 150 t ha^{-1} of sludge was applied, compared to unamended soil. The highest acid phosphatase activity observed in sludge-amended soils is similar to the results obtained for microbial biomass carbon and FDA hydrolysis, indicating that the sludge addition contributes to increase the overall microbial activity in this soil.

Discussion

It is observed that distillery sludge amendment influences physical, chemical and microbiological properties of the agricultural soil [9] which is in turn vital for the nutrient turnover and productivity [1,17,19]. Generally it is expected that the microbial activity in soil would always increase with the application of organic matter as distillery sludge, but, on higher doses by simultaneous enrichment of organic pollutants and heavy metal salts causes toxicity hence reduction in microbial biomass, activity and microbial populations of the soil. Experimental soils increased in organic matter, electrical conductivity, soluble heavy metals and toxic organic contaminants up to toxic level. The buffering capacity of the soil to degrade the pollutants is also not very effective at such a large quantity of toxic chemical addition [37]. The toxic effects of heavy metals and toxic organic substances present in distillery sludge on microbes are also determined by their solubility which is influenced its pH. This work showed that microbiological properties (MBC, microbial respiration, enzyme activity) increased as increasing rates of distillery sludge addition soils. Here the amount 50t ha^{-1} to100 t ha^{-1} exhibited increasing tendency in terms of biological properties from 60 days to 120 days after application, thereafter it became lower at 180 days.

The quantitative analysis soil samples collected at different time period after different amount of application of distillery sludge showed continuous increasing counts of bacteria, fungi, and actinomycetes initially which were according to soil organic C and nutrient status when the concentration of the pollutants in the soil are below the toxic level then the number decreases drastically. At low doses it showed microbial population of reduced diversity but of greater number, biomass and activity, but at higher doses it causes decrease in number of microorganism, corresponding enzymatic activities and other microbiological properties. At lower dosages the sludge addition supports the growth of only those microorganisms which have developed resistance against the toxic compounds and metals thus lowering the diversity but increasing the population of resistant microorganisms. But as the toxicity of the soil increases by further addition of the sludge, the resistance of these organisms fail. Aerobic heterotrophic and nitrogen fixing bacteria seem to be more sensitive to the toxic effects of distillery sludge than the other microbial groups under evaluation, undergoing a decrease in population size on higher doses. Differences in the viable count of fungal and actinomycetes were also significant; however, these two microbial groups seemed to be less sensitive to higher doses of sludge application in the soil.

The addition of sludge also increased the CO_2 production and microbial biomass carbon of soils initially, increasing almost ten times when 150 tha^{-1} distillery sludge was applied, in respect to the unamended soils and then declined during the incubation period. Highest level was obtained after 90 days for MBC and 120 days for respiration of incubation with the application of both 150 t ha^{-1} and 100 t ha^{-1} of sludge then they decreased.

Parameter	Sludge	Soil	10 t ha^{-1}	25 t ha^{-1}	50 t ha^{-1}	100 t ha^{-1}	150 t ha^{-1}
pH	9.2±0.34	6.8±0.27	7.3± 0.17	7.8± 0.38	8.47± 0.35	8.2± 0.31	8.2± 0.32
CEC	42.02± 1.56	22.64± 0.56	25.13± 0.82	28.35± 1.27	31.24± 2.69	38.67± 1.84	40.71± 1.56
Moisture	77± 2.12	70± 1.67	71± 1.62	72± 2.88	72± 3.43	74± 3.22	76± 3.71
Organic matter	25± 1.14	1.09± 0.03	3.09± 0.056	7.56± 0.31	13.26± 0.86	17.22± 0.75	19.09± 1.12
EC ($mScm^{-1}$)	3.25± 0.13	1.55± 0.024	2.85±0.12	2.87± 0.15	2.93± 0.083	3.02± 0.12	3.17± 0.17
Phosphate	313± 10.06	65± 2.88	76± 3.27	103± 4.82	183± 6.92	232± 8.43	264± 8.46
Sulphate	273.34± 8.45	55.68± 1.53	88.67± 1.82	120± 4.33	178 .50± 6.45	211.36± 8.77	234 .22± 9.39
Phenol	422.82± 7.21	3.09± 0.21	34.78± 1.47	89.12± 3.56	215 .02± 8.82	322.45± 11.23	378 .87± 12.56
Total Nitrogen	512± 11.56	86.76± 2.34	103.34± 2.66	126.56± 5.38	212.32± 7.33	371.56± 13.08	434.68± 13.33
Amm. Nitrogen	16.2± 0.68	19.5± 0.84	17.07± 0.63	17.32± 0.52	16.22± 0.73	17.34± 0.5	17.96± 0.72
Sodium	68.45± 3.56	23.67± 0.82	29 .14 ± 0.85	34.44± 1.72	48.45± 2.18	59.44± 2.07	62.34± 3.42
Chloride	245± 8.46	96.72± 1.51	109.28± 3.57	133.33± 4.07	168.45± 8.89	211.14± 9.19	221.34± 9.44
Magnesium	22.76±1.49	9.48± 0.32	9.88± 0.48	11.2 ± 0.43	14.42± 0.77	17.22± 0.63	18.86± 0.42
Calcium	76.22± 2.62	11.55± 0.21	19.33± 0.64	22.34± 0.83	45.3± 2.21	62.12± 3.46	69.25± 3.28
Aluminum	13.6± 0.62	3.94± 0.27	4.34± 0.47	5.12±0.13	8.12± 0.34	10.88± 0.64	11.2± 0.82
Potassium	18.22± 0.81	0.39± 0.017	2.82± 0.13	5.44±0.21	8.55± 0.41	13.33± 0.88	15.77± 0.75
Cadmium	1.89± 0.03	0.06± 0.0021	0.32± 0.016	0.74±0.017	1.02± 0.042	1.38± 0.04	1.56± 0.037
Chromium	2.25± 0.08	0.89± 0.014	2.88± 0.065	2.89±0.054	2.92± 0.12	3.08± 0.07	3.16± 0.076
Copper	165.02± 6.62	2.25± 0.043	18.22± 0.78	34.77±1.05	62.88± 3.27	88.17± 4.77	104.12± 5.72
Iron	97.08± 4.38	3.73± 0.069	19.12± 0.67	31.12±1.65	54.34± 2.11	73.74± 3.38	79.44± 4.41
Manganese	102.73± 2.28	1.86± 0.047	12.66± 0.48	17.44±1.02	58.55± 1.94	62.55± 2.7	81.26± 3.88
Nickel	34.18± 1.23	4.11± 0.021	13.66± 0.63	17.59±0.82	20.33± 0.87	25.6± 1.34	29.12± 1.23
Zinc	167.46± 6.18	12.18± 0.15	18.65± 0.86	21.34±1.67	46.55± 2.24	51.61± 2.45	68.12± 2.88
Lead	22.18± 0.83	4.31± 0.062	8.86± 0.32	14.77±0.73	23.72± 1.28	22.44± 1.55	22.32± 1.08

Table 1: Physicochemical properties of soil, distillery sludge, and sludge amended soil (values represent mean n=3 ±SE). All values presented in mg kg^{-1} except electrical conductivity ($mScm^{-1}$) and pH.

Enzyme activity changes both according to the level of application and incubation period during the experiment. Dehydrogenase activity increases up to 90th day of incubation. The activity increased many times within the period of incubation as reflected in the mean values. The FDA hydrolysis activity in soil increased with the increment of sludge application, and the higher level was obtained with the application of 150 t ha^{-1} of sludge, after 60 days of incubation. After 90 days of incubation, the FDA values decreased, however, the changes in values of FDA obtained in unamended soil was not very much significant. There was a rapid increase in urease activity initially in all the amendments and in the last phase of incubation there is a decrease in activity. The phosphatase activity in distillery sludge treated soil is maximum when 150 t/ha sludge is applied but it further decreases with the increase of the duration of incubation. It was maximum i.e. at the 60th day for the 150t/ha sludge application.

Conclusion

Distillery sludge amendment increased organic matter and electrical conductivity in the experimental soils and at high doses the amounts of soluble heavy metals and toxic organic contaminants were also higher up to toxic level. This work showed that microbiological properties (MBC, microbial respiration, enzyme activity) increased as increasing rates of distillery sludge addition in agricultural field soil in controlled conditions. Here the amount 50t ha^{-1} to 100 t ha^{-1} exhibited increasing tendency in terms of biological properties from 60 days to 120 days after application, thereafter it became lower at 180 days. 150 tha^{-1} distillery sludge shows negative effect on these properties comparatively very early. At the higher concentration of distillery sludge, decreasing tendency of soil fertility indicators observed becomes faster hence, we recommend the use of distillery sludge as improver of biological properties of soils if used in low concentration previously verifying its level of toxicity and stability to avoid potential hazardous effects over the soil microorganism. To minimize the shown environmental risks an adequate sludge management is proposed.

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