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NITRIFICATION AND UREA HYDROLYSIS IN ARID SOIL AMENDED WITH DIFFERENT LEVELS OF BIO-SOLID

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Abstract

A lab experiment was conducted to study the denitrification in soil mixed with different nitrogen sources (sludge, 10, 30, 50 ton. ha^{-1}). Ammonium sulphate, and urea at 250 kg. ha^{-1}. Mixed with soil and packed into leaching tubes (15 cm length and 5 cm diameter). Water was added up to 60% of the field capacity then tubes were incubated at 37 C^o for 10 weeks. Nitrogen ($N\text{-}NH_4$ and $N\text{-}NO_3$) was extracted by 0.01 M KCl solution biweekly to determine the concentration of NH_4, NO_3, Urease, and nitrification microorganisms' counts. Results showed that ammonium was decreased rapidly in the first period of incubation while nitrate was increased with the period of incubation. Sludge (50 kg. ha^{-1}) treatment has recorded the highest concentration of $N\text{-}NO_3$ on the tenth week at 78.973 $mgm.kg^{-1}$ in soil as compared to control treatment that gave 21.007 $mgm.kg^{-1}$ soil. The highest activity of urease was in sludge treatment at 274.30 µgm $N\text{-}NH_4$ .gm^{-1} soil per 2 hours and the least value was 34.39 µgm $N\text{-}NH_4$ .gm^{-1} soil per 2 hours in control treatment. The nitrification microorganisms' counts were the most in eighths week of incubation at $30.8*10^{-3}$ Cfu mg^{-1}. dry soil.

Keywords: Nitrification, Urease, Bio-Soild, Arid soils.

Introduction

Wastewater treatment has multiple benefits and is a pressing need in growing cities around the world. An additional benefit of wastewater treatment is the beneficial use of the resulting bio-solids in land application, which is becoming common practice in the world due to the potential recovery of nutrients and incorporation into degraded soils (Mata-Gonzalez *et al.*, 2004). Arid and semi-arid soil are ideal location for bio-solid land application because soils from these areas are usually low in nutrients and their typically high pH and low moisture tend to reduce the risk of trace element contamination (White *et al.*, 1997; Wester *et al.*, 2003). The benefits of municipal biosolid application to arid and sime-arid soils have been more than enough documented (Fresquez *et al.*, 1990; Benton and Wester, 1998; Jurado and Wester, 2001; Rostagno and Sosebee, 2001; Mata-Gonzalez *et al.*, 2002, 2004; Martinez *et al.*, 2003; Moffet *et al.*, 2005). Issues concerning the safety biosolid application, due to the potential leaching of nutrients or trace elements to deeper soil layers and groundwater (Keller *et al.*, 2002; Mclaren *et al.*, 2004; Pierzynski and Gehl, 2005). Early reports indicated that metal mobility through soil columns amended with biosolids was minimal and little risk of ground water contamination existed (Giordano and Mortvedt,1976; Emmerich *et al.*, 1982). Few direct measurements of the quality of soil solutions or drainage waters in soils treated with biosolids have been reported in intact columns (Keller *et al.*, 2002; McLaren *et al.*, 2004) and to our knowledge no reports exist on soils from arid or semi-arid regions. The objective of this study is to measure the concentration of nitrate (NO_3) in water leached through intact columns of arid soil amended with different levels of biosolid. Our goal is to evaluate the possibility of nitrate formation from bio-solid applied to arid land that potentially affecting the quality of subsurface waters.

Material and Methods

Soil samples were taken from surface (0-30 cm). Analyzed using standard methods as described by American Society of Soil Science (pH, EC, texture, NO_3, NH_4, CEC, $CaCO_3$, O.M). Nitrification rates of various nitrogenous compounds (ammonium sulfate, bio-solid and urea) were also evaluated. A 50-gm sample (oven dry basis) of air-dried soil mixed with an equal to weight of sand washed with 1N HCl and subsequently amended with ammonium sulfate, bio-solids, and urea. Quantity of urea and ammonia sulfate calculated to give an equivalent of 250 Kg N ha^{-1}, whereas bio-solid applied as 10, 30, and 50-ton ha^{-1}. The amended soil-sand mixture retained in a leaching tube (5 cm diameter and 15 cm length) by means of glass wool pad and a layer of washed fine sand. Water added to bring the soil moisture content to 60% of the water holding capacity. A thin glass wool pad placed over the soil to avoid dispersing the soil when solution poured over the treated soil. A control treatment consisted of a soil-sand mixture without the addition of the nitrogen sources. The most probable numbers (MPN) of nitrifying bacteria counted in the ten weeks of incubation at 35C using the method of Alef (1998). After 2 weeks of incubation at 35C, each leaching tube placed on a 250 mL suction flask and leached with 100 mL of 0.01M KCl in 4 to 5 increments. The leaching tube will place in the incubator at the temperature indicated. The leaching procedure repeated every 2weeks 1 of 10 weeks. The moisture contents of the columns incubated at 35C adjusted by adding distilled water. The leachate thus obtained will made to 100 mL with distilled water. A liquates taken for analysis of $N\text{-}NH_4$ and $N\text{-}NO_3$. Urease activity of 25gm soil amended with various amount and types of nitrogenous compounds evaluated (treatments as indicated above). The flasks stoppered and allowed to equilibrate at 30C and 60% water holding capacity for 15 days. The 15 days equilibration period chosen to allow ample time for the soil microflora to adapt to the soil environment. The soil moisture contents of the flasks adjusted by weighting the flasks every 3 days by adding distilled water and aerated for 20 min. After amendments soil samples (5gm oven dry basis) placed in 50 mL flasks and soil urease activity assayed. non-amended controls include to measure urease activity without the influence of nitrogenous compounds. All values reported an average of triplicate determinations

Table: 1 Some soil properties

Total Bacteria Count cfu g^{-1} soil $\times 10^{5}$	1.2
OM	0.7
$CaCo_3$	24.25
CEC C mol. Kg^{-1}	18.4
NH_4 $mgkg^{-1}$	13.41
No_3 $mgkg^{-1}$	8.12
%Sand	53.52
%Silt	36.14
%Clay	10.34
EC dsm^{-1}	1.8
pH	7.2

Results and Discussion

Results in table 2 showed that $N\text{-}NH_4$ concentration was constantly decreasing throughout 10 weeks of incubation period for all treatments. Where $N\text{-}NH_4$ concentration at the beginning of incubation period was 16.22 $mgm.kg^{-1}$ soil in sludge treatment (50 ton. ha^{-1}) while it became 2.278 $mgm.kg^{-1}$ soil at the end of incubation period (10 weeks). Urea treatment had the same behavior of $N\text{-}NH_4$ decrease, where it decreased from 7.621 $mgm.kg^{-1}$ soil at the beginning of the incubation and continued decreasing with time until the end of incubation where it wasn't able to determine (n.d). These results came parallel to what Alburguerane *et al.*, 2012, and Huang and Chen, 2009 that they referred to the decrease of $N\text{-}NH_4$ throughout incubation period is related to soil properties and applied biomass.

$N\text{-}NH_4$ behavior in soil treated with mineral nitrogen (Urea and ammonium sulphate that was similar to other treatments in spite of the low concentrations and the rapid decrease throughout incubation period. That could be explained by the matter of being controlled by different mechanisms that leads to transform it to more soluble form ($N\text{-}NO_3$) through nitrification process of nitrification bacteria or transforming it directly to ammonia NH_3 and volatilization (Saaud *et al.,* 2012). The reason of rapid process of nitrification is related to the availability of the appropriate conditions for such process to occur throughout the availability of $N\text{-}NH_4$ in soil, and the appropriate soil reaction, where the average range of nitrification pH is ranging from 6.6-8 (Colderon *et al.,* 2005; Huang and Chen 2009).

In the meanwhile, $N\text{-}NO_3$ concentration was found to have a different behavior throughout incubation period for all treatments. Concentration of $N\text{-}NO_3$ in sludge (50 ton. ha^{-1}) has increased from 22.350 to 78.973 $mgm.kg^{-1}$ soil as compared to control treatment with the increase of $N\text{-}NO_3$ from 13.125 to 21.007 $mgm.kg^{-1}$ soil. While in sludge treatments of 10 and 30 ton. ha^{-1}, the increase in $N\text{-}NO_3$ was 55.84 and 46.500 $mgm.kg^{-1}$ soil throughout the incubation period respectively. Urea and ammonium sulphate lead to an increase in $N\text{-}NH_4$ 31.251 and 23.107 $mgm.kg^{-1}$ soil at the end of incubation. These results confirm the occurrence of an opposite process of $N\text{-}NH_4$ and $N\text{-}NO_3$ concentrations throughout the obvious decrease in $N\text{-}NH_4$ and increase in $N\text{-}NO_3$ during the incubation period. That could be related to the activity of nitrification bacteria and nitrogen transformation by different mineralization mechanisms of microorganisms (Bar-Tel 2004). The increase of $N\text{-}NO_3$ in soil is one of unwanted processes that causes a big loss of useful forms of nitrogen ($N\text{-}NH_4$) and transform it to $N\text{-}NO_3$ by nitrification of nitrobacter. The increase of $N\text{-}NO_3$ ion in soil and leaching it to the ground water will cause a pollution of these waters and the environment.

Urease Activity

Results of table 3 showed that the activity of urease was varying in all treatments. Sludge treatment has revealed the highest values 274.30 µgm $N\text{-}NH_4.gm^{-1}$ soil per 2 hours and the lowest value were 34.39 µgm $N\text{-}NH_4.gm^{-1}$ soil per 2 hours of control treatment at the period two weeks of incubation. While activity of urease has decreased for all treatments at week eight because of the decrease in the material submitted to the enzyme therefore, the value of enzyme activity of sludge treatment (50 $ton.ha^{-1}$) was 98.59 µgm $N\text{-}NH_4$ $.gm^{-1}$ soil per 2 hours as compared to control treatment 10.22 µgm $N\text{-}NH_4$ $.gm^{-1}$ soil per 2 hours.

Maximum probable number (MPN) of nitrification bacteria

Results showed differences in nitrification bacteria due to different treatment, where sludge treatment of 50 $ton.ha^{-1}$ was superior to other treatments where it reached $25.8*10^3$ ml^{-1} bacteria in the eighth week of incubation, while it was $0.10*10^3$ in control treatment in spite of the number was ranging from $(0.12\text{-}0.14)*10^3$ ml^{-1} in all treatments. Where numbers of nitrification bacteria have increased throughout incubation period (Table 4) then they decreased in the last period of incubation for all treatments that could be related to the scarcity of energy resources of nutrients that could be consumed by bacteria in the first periods of incubation. At the beginning of incubation period, it was noticed that nitrification bacteria of urea treatment were low as compared to ammonium sulphate treatment then numbers were increasing with the incubation period increase and that could be related to the high solubility and oxidation of ammonium sulphate when compared to other sources of nitrogen. These differences in nitrification bacteria reflects the variation of activity an dynamic nature of nitrification societies in soil and the important role in soil as a source of nitrogen source oxidation as Belser and Schmidt 1978 referred to in studying nitrification bacteria role in ammonium oxidation.

The existence of nitrate in high concentrations in soil treated with different levels of sludge reflects the nitrogen transformation through ammonium oxidation to nitrate by nitrification bacteria and the increase of nitrate levels in soil where some of it will be leached to the ground water and pollute both ground water and environment. Where Alrashidi *et al.*, 2013 has mentioned that the long-term use and the surface application of processed waste water had an impact on the mechanism of nitrogen and carbon in the ecosystem.

Table 2 : Concentration of NH_4-N and NO_3-N (mg kg^{-1} soil) in soil with incubation period

Urea		Ammonium sulfate		Bio – solids						Control		Weeks
				50 tan ha-1		30 tan ha-1		10 tan ha-1				
N-NO_3	N-NH_4	N-NO_3	N-NH_4	N-NO_3	N-NH_4	N-NO_3	N-NH_4	N-NO_3	N-NH_4	N-NO_3	N-NH_4	
13.625	7.621	14.437	8.140	22.350	16.122	18.625	13.435	15.750	8.949	13.125	7.458	2
20.457	3.998	18.598	3.635	36.520	10.191	30.434	7.433	25.363	4.957	16.908	3.305	4
35.283	1.839	22.189	1.672	48.572	4.104	36.309	3.420	30.258	2.280	20.172	1.520	6
29.002	1.105	27.335	1.005	60.796	3.367	42.330	2.806	35.275	1.871	24.850	0.914	8
31.251	n.d	23.107	n.d	78.973	2.278	55.811	1.899	46.500	1.266	21.007	n.d	10

Table 3 Soil urease activity (mu g N-NH_4) g^{-1} soil 2 $hours^{-1}$

Urea	Ammonium sulfate	Bio-solid			Control	Weeks
		50 tan ha^{-1}	30 tan ha^{-1}	10 tan ha^{-1}		
72.30	59.84	274.30	155.89	123.42	34.39	2
18.10	15.72	98.59	74.11	50.32	10.22	8

Table: 4 The most probable numbers (MPN) of nitrifying microorganisms in the ten weeks

Weeks	× 10^3 ml^{-1}					
	Control	10 tan ha^{-1}	30 tan ha^{-1}	50 tan ha^{-1}	Ammonium sulfate	Urea
0	0.12	0.12	0.12	0.12	0.12	0.12
2	0.15	1.52	1.95	2.51	0.29	0.21
4	0.17	4.20	5.11	9.22	2.37	3.00
6	0.16	8.71	11.00	19.35	5.35	6.15
8	0.10	10.25	15.36	25.80	4.18	5.20
10	0.5	5.10	7.45	20.71	3.22	4.31

Conclusion

This study has focused on the increase of N-NO_3 in soil and decrease of N-NH_4 throughout ten weeks of incubation for all treatments especially sludge treatment of different application levels. Nitrogen is facing a unstable conditions in the soil system and transformations and loss from different nitrogen sources applied to soil. The environmental damage that occur because of that causes a pollution of ground water due to nitrate leaching and increasing its concentrations because of the oxidation of N-NH_4 by nitrification bacteria which is reflected through the harm effect on human beings and animal health because of the increase of nitrate ions in ground water, in addition to the increase of urease activity in the sludge treatment and the increase of nitrification bacteria numbers. expressed on a moisture-free basis. The experiment design in a completely randomized design.

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