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Impact of Animal Waste Application on Runoff Water Quality in Field Experimental Plots

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Abstract: Animal waste from dairy and poultry operations is an economical and commonly used fertilizer in the state of Louisiana. The application of animal waste to pasture lands not only is a source of fertilizer, but also allows for a convenient method of waste disposal. The disposal of animal wastes on land is a potential nonpoint source of water degradation. Water degradation and human health is a major concern when considering the disposal of large quantities of animal waste. The objective of this research was to determine the effect of animal waste application on biological (fecal coliform, Enterobacter spp. and Escherichia coli) and physical/chemical (temperature, pH, nitrate nitrogen, ammonia nitrogen, phosphate, copper, zinc, and sulfate) characteristics of runoff water in experimental plots. The effects of the application of animal waste have been evaluated by utilizing experimental plots and simulated rainfall events. Samples of runoff water were collected and analyzed for fecal coliforms. Fecal coliforms isolated from these samples were identified to the species level. Chemical analysis was performed following standard test protocols. An analysis of temperature, ammonia nitrogen, nitrate nitrogen, iron, copper, phosphate, potassium, sulfate, zinc and bacterial levels was performed following standard test protocols as presented in Standard Methods for the Examination of Water and Wastewater [1]. In the experimental plots, less time was required in the tilled broiler litter plots for the measured chemicals to decrease below the initial pre-treatment levels. A decrease of over 50% was noted between the first and second rainfall events for sulfate levels. This decrease was seen after only four simulated rainfall events in tilled broiler litter plots whereas broiler litter plots required eight simulated rainfall events to show this same type of reduction. A reverse trend was seen in the broiler litter plots and the tilled broiler plots for potassium. Bacteria numbers present after the simulated rainfall events were above 200/100 ml of sample water. It can be concluded that: 1) non-point source pollution has a significant effect on bacterial and nutrients levels in runoff water and in water resources; 2) land application of animal waste for soil fertilization makes a significant contribution to water pollution; 3) the use of tilling can significantly reduce the amount of nutrients available in runoff water.

Keywords: Non-point source pollution, fecal coliform, runoff water, land application

Introduction

Animal waste from dairy and poultry operations is an economical and commonly used fertilizer in the state of Louisiana. The application of animal waste to pasture lands not only is a source of fertilizer, but also allows for a convenient method of waste disposal. One type of animal waste product that is commonly used is dairy lagoon sediment or effluent. Most dairies in Louisiana have a one or two stage lagoon that collects liquid and semi-liquid manure from loafing barns and milking parlor areas. The solid waste settles in the lagoon where it is reduced by the process of anaerobic digestion. The liquid effluent is often pumped onto fields or is recycled for other uses. Periodically, the lagoon must be emptied of the sediment build up. The sediment is typically agitated in order to suspend it into a semi-liquid state and is pumped onto the fields. The disposal of animal wastes on land is a potential non point source of water degradation. Runoff and percolation could possibly

transport organic matter and nutrients to surface and ground water. Animal wastes applied to the land come from wastes that have been removed from feeding facilities, runoff from feeding areas, and waste from animals on pasture and rangeland. Proper application of animal wastes provides nutrients for crop production and also reduces surface runoff.

With the demand for fresh water increasing, agriculture is under increasing pressure to ensure that its practices do not contribute to the decline of water quality both nationally and in the state of Louisiana. According to the U.S. EPA Louisiana 1996 305(b) report, fecal coliform bacteria are the most common pollutants in rivers and streams. It has been noted that of the waterways in Louisiana that were surveyed, 37% of the river miles, 31% of the lakes, and 23% of the estuarine waters had some level of contamination. This shows a clear violation of fecal coliform and bacteria standards. Possible sources of elevated coliform counts include sewage discharges from municipal treatment plants and septic tanks, storm water overflows, and runoff from pastures and range lands [2].

The Louisiana Department of Agriculture has completed an inventory of beef cattle and swine feedlots for various basins in the state ("Inventory of Beef Cattle and Swine Feedlots," 1978). It was noted that loose grazing animals have a similar negative effect on water quality, but this effect is less concentrated than for feedlots [3].

The focus of this study was to determine the effect of animal waste application on the biological (fecal coliform, *Enterobacter spp.* and *Escherichia coli*) and physical/chemical (temperature, pH, nitrate nitrogen, ammonia nitrogen, phosphate, copper, zinc, and sulphate) characteristics of runoff water in experimental plots.

Materials and Methods

Materials

Six feet sections of 4 inch PVC pipe were purchased at Home Depot (Bossier City, LA.). The Tlaloc 3000 Rainfall Simulator was obtained from Joern's Inc. (West Lafayette, IN). Poultry litter and cattle sludge was supplied by Louisiana State University (LSU) AgCenter, Hill Farm Research Station, Mastitis Lab (Homer, LA). Landscape border and rain gauges were obtained from Wal-Mart (Minden, LA). The SMART 2 Colorimeter, 25 mm test vials, reagents and standards for ammonia nitrogen, copper, phosphate, potassium, sulphate and zinc analysis were purchased from A. Daigger and Company, Inc. (Vernon Hills, IL).

Method

A total of nine plots were used in this experiment. Each plot was 6 ft X 6 ft in size. This area was marked and defined by landscape border for each plot. The border was used on only three sides. The selected site for the plots was on the property of LSU Ag Center, Hill Farm Research Station, Mastitis Lab in Homer, LA. The suitable area chosen was a hay meadow that had a slope equal to 5% to facilitate runoff. Side four of the plots, which is the lowest position due to the slope, had PVC pipe cut to fit and positioned at the low end of the plot. A trench was dug at this position so that the PVC pipe would be level with the ground and would not extend above ground level. Metal flashing was positioned on the ground and fitted so that it would allow for the smooth runoff of the water into the PVC pipe. A 2 inch size hole was drilled into one end of the PVC pipe and a 100 ml plastic vial was positioned underneath to catch the sample runoff water.

The plots were divided into three groups to accommodate for three replicates of each test. Plots 1-3 received cattle sludge as the treatment. Plots 4-6 received broiler litter as the treatment. Plots 7-9 received broiler litter and were tilled. All plots treated received an amount equal to 1 inch of the appropriate type of animal waste when applied. Prior to the addition of the animal waste to the plots, simulated rainfall events were conducted on each plot in order to serve as the control in which no treatment was utilized. Runoff water samples were collected after each .5-inch rain event. A minimum of five simulated rainfall events was conducted for each plot. If the pre-treatment values were found to be lower than the measured values after five simulated rainfall events, additional simulated rainfall events were used. The first simulated rainfall event was conducted immediately prior to the application of animal waste and once weekly thereafter. These experiments were collected in late summer to avoid natural rain. An analysis of chemical and physical parameters was conducted on all samples by testing for the following: ammonia nitrogen, nitrate nitrogen, copper, phosphate, potassium, sulphate, and zinc. All tests conducted were referenced from the SMART 2 Colorimeter manual [4] as well as the 20th edition of the Standard Methods for the Examination of Water and Wastewater [1]. All blanks used were equivalent in volume to the samples. Samples that had a lot of soil mixed into the water were filtered prior to testing. The accuracy of all equipment was determined by using known standards.

Collection of Samples

All liquid samples were collected with a volume of not less than 100ml. A space of at least 2.5 cm was left in the bottle to facilitate mixing by shaking. The containers used were according to the 20th edition of Standard Methods for the Examination of Water and Wastewater. Samples were collected in non reactive glass or plastic bottles that had been cleansed and rinsed carefully, given a final rinse with distilled water, and sterilized. The samples were placed immediately on ice during a maximum transport time of 6h [1].

Temperature

Prior to placing the sample on ice, the temperature was taken by placing the probe of the portable pH/EC/TDS/Temperature meter into the collected sample.

pH

The pH was measured by using the conductivity/pH/TDS meter. The pH selection was chosen on the instrument and the probe was then inserted into the water sample. The appropriate pH reading was taken.

Fecal Coliform Count

Ten ml of water sample were vortexed and filtered onto a membrane filter using a sterile filtration unit. The approved technique used was from Clesceri et al. [1].

After filtration, forceps were used to place the membrane filter on an MFC agar plate. The plate was then incubated in an incubator at 45 °C for 24 hours. The plates were then checked for bacteria colony growth.

API 20 E System

The API 20E System was used in conjunction with the API Profile Recognition System (bioMerieux, Inc., Hazelwood, MO) so that members of the family *Enterobacteriaceae* and other Gram-negative bacteria could be accurately identified.

The API 20 E strip consists of 20microtubes containing dehydrated substrates. These tests were inoculated with the bacterial sample suspension. Each sample was incubated for 18-24 hrs. at 35-37ºC. This system is a standardized, miniaturized version of conventional procedures for the identification of *Enterobacteriaceae* and other Gram-negative bacteria.

Nitrate Nitrogen

Nitrate nitrogen was measured by using Waterworks Test Strips (Thomas Scientific Swedesboro, NJ). The test strip was placed into the water sample. The color change on the strip was compared to the chart provided with the strips.

Ammonia-Nitrogen - The Nesslerization Method

The colorimeter test vial was filled to the 10 ml line with the sample. The test vial was then placed into the colorimeter and scanned as a blank. The test vial was then removed and 8 drops of ammonia nitrogen reagent #1 was added and mixed. The mixture was allowed to sit for 1 minute. One ml of ammonia nitrogen reagent # 2 was added. After waiting 5 minutes, the test vial was inserted again and a reading was taken.

Phosphate-The Vanadomolybdophosphoric Acid Method

The test vial was filled with 10 ml of the sample water. The test vial was inserted into colorimeter and scanned as a blank. Two ml of VM Phosphate Reagent was added and mixed well. After waiting for a period of 5 minutes, a reading was obtained using the colorimeter.

Copper - Diethyldithiocarbamate Method

Ten ml of the sample water was added to the test vial and 5 drops of copper 1 reagent was added. The sample mixture was then mixed well. The solution turned yellow which indicated that copper is present. The test vial was then placed into the colorimeter and a reading was taken.

Zinc - The Zincon Method

Preparation of the dilute zinc indicator solution: Five ml zinc indicator solution was added to 17.8 ml of methyl alcohol and mixed well. Fill colorimeter test vial with water sample to the 10 ml line. The test vial was then inserted into the colorimeter and scanned as a blank. One tenth of a gram of sodium ascorbate powder was added along with 0.5 g zinc buffer powder. The mixture was capped and shaken vigorously for 1 minute. Three drops of sodium cyanide, 10% was added to the mixture and mixed well. One ml of dilute zinc indicator solution was then added and mixed. Four drops of formaldehyde solution, 37% was added and was mixed by inverting 15 times. The test vial was then inserted into the colorimeter and scanned for a reading.

Sulphate - The Barium Chloride Method

The colorimeter tube was filled to the 10 ml line with the sample water and was then inserted into colorimeter and scanned as a blank. One tenth of a gram of the sulfate reagent was added. The test vial was then capped and shaken until powder dissolved.

The mixture was allowed to sit for 5 minutes. After mixing the tube again, it was then inserted into the colorimeter and scanned for a reading.

Potassium - The Tetraphenylboron Method

One hundreth of 5 g of Tetraphenylboron Powder was added to the test vial containing 10 ml of the sample water. The vial was then capped and shaken vigorously until all of the powder had dissolved. The mixture was allowed to stand undisturbed for 5 minutes. The same procedure was followed to make a blank. Prior to taking the measurement, the vials were mixed again to suspend any settled precipitate. The vials were then inserted into the colorimeter and scanned for a reading.

Statistical Analysis

Descriptive statistics were applied to determine the mean values of all physical/chemical and bacteriological parameters evaluated. Standard deviations were computed as measures of variance. Statistical analysis was performed using GraphPad InStat program version 3.00 for Windows 95, GraphPad Software, San Diego, California.

The Student-Newman-Keuls Multiple Comparisons test was applied to determine significant differences in mean values among the experimental plots. The level of significance was considered a p-value ≤ 0.05.

Results

Prior to adding animal sludge to the experimental plots, an analysis on the pure sludge was conducted.

Table 1: Before treatment and After treatment levels (ppm) of total nitrate, phosphate, ammonium nitrogen, sulphate, potassium, copper and zinc in runoff from experimental plots.

Test Plots	*Total Nitrate*	*Phosphate*	*Ammonium Nitrogen*	*Sulphate*	*Potassium*	*Copper*	*Zinc*
Sludge BT	0.00	0.43	0.65	27.70	9.50	0.06	0.09
Sludge AT	0.50	1.80	1.16	28.00	4.67	0.02	0.04
Broiler Litter BT	0.50	0.40	0.99	30.00	8.13	0.07	0.09
Broiler Litter AT	0.26	14.43	0.55	24.67	4.50	0.14	0.33
Tilled Broiler Litter BT	0.00	0.50	1.75	31.00	7.67	0.09	0.37
Tilled Broiler Litter AT	0.16	6.00	0.20	7.00	15.67	0.00	0.02

Measurements of the levels of copper, ammonium nitrate, phosphate, sulphate, potassium and nitrate were found to be .05ppm, 4.68ppm, 5.07ppm, 10.31ppm, 27.49ppm and 1.21ppm, respectively. Table 1 shows that runoff water from experimental plots containing cattle sludge had final total nitrate levels of 0.50ppm, final potassium levels of 4.67ppm, final copper levels of 0.02 and final zinc levels of 0.04ppm. This shows a decrease in the nitrate, potassium, copper and zinc levels below the original pre-treatment levels of 0.50, 9.50, 0.06 and 0.09, respectively after 6 simulated rainfall events. The sulphate level for these same plots is at the pre-treatment level of 28ppm in that it was actually measured at 27.7ppm. Data for the first simulated rainfall event had a sulphate level of 121.33ppm while the second simulated rainfall event had a level of 57ppm. This shows a 50% reduction between the first and second trial for the sulphate levels. The final levels of phosphate at 1.80ppm and ammonium nitrogen at 1.16ppm were found to be above the pre-treatment level of 0.43ppm and 0.65ppm respectively even after a period of 6 trial simulated rainfall events. A comparison of the data collected from the simulated rainfall events to dates of known natural rainfall did not indicate a significant pattern that could be established.

Prior to spreading plots with broiler litter, measurements of the levels of ammonium nitrogen, phosphate, copper, sulphate, total nitrate, and zinc were found to be 7.54ppm, 17.60ppm, 0.40ppm, 49.62ppm, 4.29ppm and 0.03ppm, respectively. Differences can be seen in the data collected for the plots containing broiler litter and the plots containing broiler litter that had been tilled. Only the levels of total nitrate at 0.26ppm, ammonium nitrogen at 0.55ppm, and potassium at 4.50ppm are below the pre-treatment levels of 0.50, 0.99 and 8.13ppm, respectively in the plots containing broiler litter by the end of the experimental simulated rainfall events. In the plots containing tilled broiler litter, ammonium nitrogen (0.20ppm), sulphate (7.00ppm), copper (0.00ppm) and zinc (0.02ppm) are the only test parameters that were found to be below the pre-treatment levels of 1.75ppm, 31.00ppm, 0.09ppm and 0.37ppm, respectively. The ammonium nitrogen required less time to reach a point below the pre-treatment level in the plots containing broiler litter that was tilled than was noted in the plots containing broiler litter. This decrease was detected after four simulated rainfall events for the tilled broiler litter plots whereas it took eight simulated rainfall events for the plots containing broiler to exhibit this same type of reduction. This was also found to be the case for sulphate levels. A reverse trend was detected in the plots containing broiler litter for the levels of potassium. A decrease below the pre-treatment level after only two simulated rainfall events was found in the plots containing broiler litter whereas the plots containing tilled broiler litter had potassium levels that remained above the pre-treatment level even after a total of 6 simulated rainfall events.

For most of the chemical parameters that were tested, plots that were not tilled had a larger amount of nutrients present after the first rainfall event than those plots that were tilled. This was evidenced by the high amounts of nutrients present after the first rainfall event. A comparison of all plots indicated that the only significance among them was for the levels of sulphate and phosphate. Using nonparametric ANOVA, a p-value of 0.0294 was found for sulphate within the plots. This p- value is considered significant.

Figure 1 shows that the mean sulphate levels for the plots containing cattle sludge, broiler litter and tilled broiler litter were 56.33 ±33.90, 38.89±15.40, and 23.16 ± 11.40ppm, respectively. Based upon Dunn's Multiple Comparisons Test, the only significant difference, $p<0.05$, is between the plots containing cattle sludge and the plots containing tilled broiler litter.

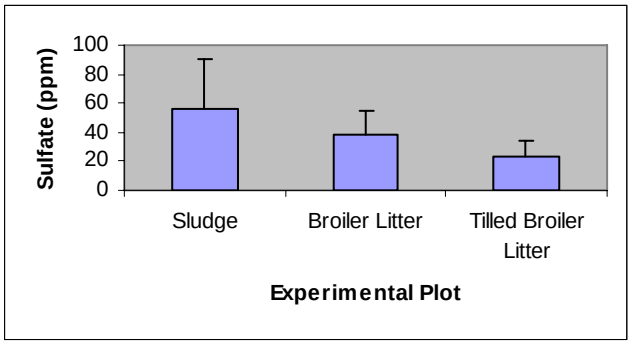

Figure 1: Sulfate levels (ppm) detected within the experimental plots. A significant difference ($p<0.05$) is detected between plots containing sludge and tilled broiler litter.

In the case of phosphate levels, the p- value is 0.0022. This p- value is considered highly significant. A comparison of the phosphate levels of plots containing broiler litter and plots containing cattle sludge, it was noted that the p value is less than 0.01. This indicates a highly significant difference in the mean levels of phosphate between these plots. Figure 2 shows that the mean levels of phosphate for the plots containing cattle sludge, broiler litter, and tilled broiler litter were 3.72 ± 1.50ppm, 27.87 ± 31.30 and 21.10 ± 16.70, respectively.

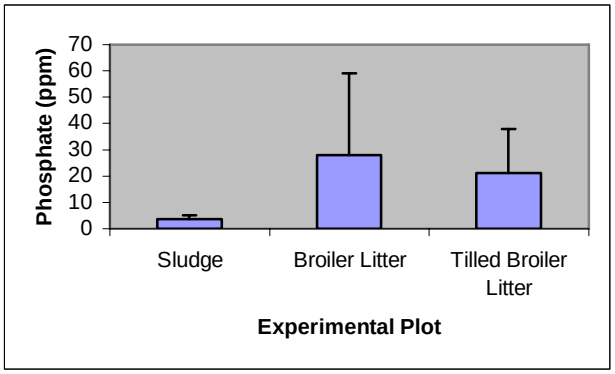

Figure 2: Phosphate levels (ppm) detected within the experimental plots.

Levels of the metals (copper and zinc) were measured within the experimental plots. Figure 3 shows the mean copper levels for the plots containing cattle sludge, broiler litter and broiler litter in plots that had been tilled to be 0.14 ± 0.07ppm, 0.39 ± 0.60ppm and 0.29 ± 0.50ppm, respectively. There appears to be no apparent significant difference (p>0.05) when comparing the mean copper levels among the experimental plots. The amount of time required to decrease below the pre-treatment levels was less than the time noted in the plots containing tilled broiler litter. The pre-treatment level of the plots containing broiler litter that had been tilled was 0.09ppm. It was noted that after only three simulated rainfall events, this level had decreased to 0.06ppm. It was further noted that after five simulated rainfall events, the copper levels were not detected at all. In the case of the plots containing cattle sludge and broiler litter, decreases below the pre-treatment level took a total of six simulated rainfall events.

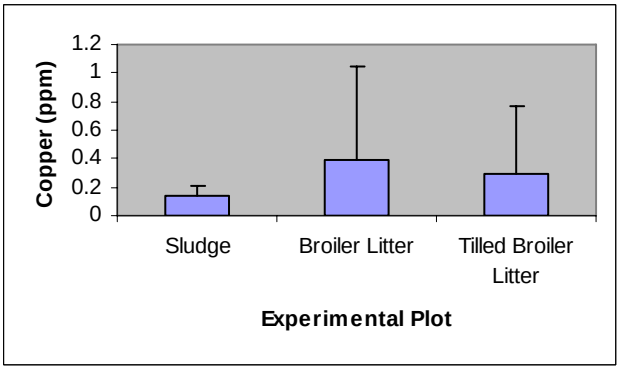

Figure 3: Mean copper levels (ppm) detected in the various experimental plots.

Figure 4 shows the mean zinc levels (ppm) for the experimental plots. These levels were 0.15 ± 0.10ppm for plots containing cattle sludge, 0.29 ± 0.30ppm for plots containing broiler litter, and 0.34± 0.35ppm for plots containing broiler litter that had been tilled. A comparison of the mean zinc levels between the plots containing broiler litter and plots containing broiler litter that had been tilled showed a faster rate of decline in the plots that had been tilled. The tilled plots required four simulated rainfall events to decrease to 0.20ppm below the pre-treatment level of 0.37ppm. In the case of broiler litter that had not been tilled, a decrease below the pre-treatment level of 0.09ppm was obtained after a total of nine simulated rainfall events. Plots containing sludge took only five simulated rainfall events to decrease below the pre-treatment level. The variations that were seen among the means of the zinc levels for all of the plots were not significant (p>0.05).

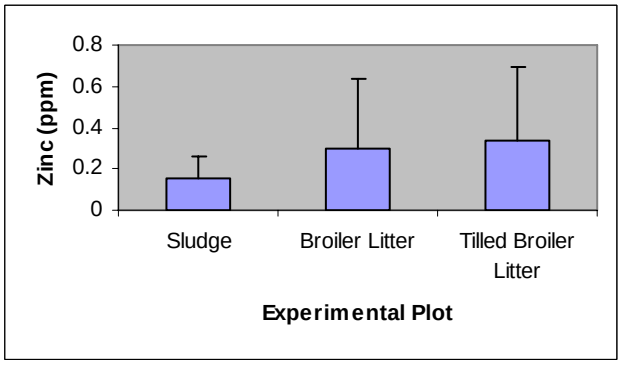

Figure 4: Mean zinc levels (ppm) detected in the various experimental plots.

Prior to adding any type of animal waste to the experimental plots, the pH was taken. The pH for all of the experimental plots before treatment was found to be 5.5. The mean pH for the plots containing cattle sludge was found to be 6.20 ± 0.38. The mean values of pH for the plots containing broiler litter and tilled broiler litter were found to be 6.57 ± 0.57 and 6.38 ± 0.37, respectively. There appears to be no apparent significant difference (p> 0.05) when comparing the mean pH levels among the experimental plots.

The temperature of all water samples was measured from the experimental plots immediately upon collection. The experimental plots containing cattle sludge were found to have a mean temperature of 26.60 ± 0.52°C. The mean temperatures of water samples collected from the experimental plots containing broiler litter and tilled broiler litter was found to be 26.0 ± 0.63°C and 26.02 ± 0.60°C, respectively. There appears to be no apparent significance difference (p > 0.05) when comparing the mean temperature among the experimental plots.

A comparative analysis was conducted in order to identify the predominant bacterial community found associated with the experimental plots. The most prevalent kind of bacteria found within the experimental plots containing the cattle sludge was *Escherichia coli.* The pre-treatment level of *E. coli* showed 300 CFUs/100 ml of sample runoff water collected. After the addition

of the cattle sludge, testing showed an increase to 15,500 CFUs/100ml after the first simulated rainfall event. Over the course of six simulated rainfall events, the number of *E coli* colonies decreased to 250 CFUs/100 ml. The mean number of bacterial colonies found among the experimental plots containing cattle sludge was 4352.67 ± 5801.00 CFUs/ 100 ml.

The plots containing broiler litter and tilled broiler litter showed a different predominant type of bacterial colony. *Enterobacter spp.* was noted as being the most prevalent type of bacteria. Two of the total of six simulated rainfall events revealed the presence of *E. coli* in experimental plots containing broiler litter. After simulated rainfall events, the number of colonies decreased from 21,000 CFUs/100ml of sample water tested to a low number of 4,700 CFUs/100 ml in these plots. Experimental plots containing tilled broiler litter revealed a very small number of *E. coli*, *Klebsiella spp.* and *Enterobacter sakazakii* colonies. The initial number of the *Enterobacter spp.* colonies found in the experimental plots containing broiler litter and tilled broiler litter was 1350 CFUs/100 ml and 1200 CFUs/100ml, respectively.

The total number of *Enterobacter spp* colonies increased after treatment to 30,500 CFUs/100 ml and later decreased to a level of 1600 CFUs/100 ml of sample water tested for those experimental plots containing broiler litter. A similar trend was seen in the tilled broiler litter plots with an increase after treatment to 15,000 CFUs/100 ml and a later decreased to a final level of 750 CFUs/100ml. The mean numbers of *Enterobacter spp.* colonies found among the experimental plots containing broiler litter and tilled broiler litter were 7530.00 ± 12,846.00 CFUs/100ml and 3970.00 ± 6177.50 CFUs/100ml respectively.

Discussion

A major concern of using animal waste as a source of fertilizer is the potential of degrading any nearby natural water sources. According to the Spring 1998 issue of Farm Santuary News [5], when manure is applied to crop land in amounts greater than it can be used or retained by the soil, nitrogen, phosphorous and other nutrients leach into surface and ground water. The plots that were constructed for the purpose of adding the different types of animal waste and utilizing simulated rainfall events were located in Homer, Louisiana. The type of soil in which the plots were built upon is described as being of Darley series. The Darley series consists of well drained, moderately slowly permeable soils. The soils were formed in iron-rich, clayey sediment of Tertiary age and are situated on ridge tops and side slopes on uplands. Although this type of soil has slopes that can range from 1 to 30 percent, the slope used was 5 percent. Soils of the Darley series are also kaolinitic, thermic Typic Hapludults.

The differences in the amount of time that was required for the measured nutrient and metal levels of each experimental plots to decrease below the pre-treatment levels was possibly due to not only plant uptake of the nutrients but also the penetration and leaching of the substances into the tilled soil. This is easier done when tilling has occurred because this process opens up the soil providing more pore space which can hold the nutrients for a longer period of time while allowing plants more time to slowly take up the material through their root systems. The Darley series soil type contains relatively stable organic compounds having very high cation-exchange capacities. This exchange capacity increases the ability of the soil to absorb and store nutrients. Soil tillage is a practice that is often utilized in the field of agriculture. The major purpose of soil tillage is seedbed preparation and weed control. Tillage implements stir the surface and in some cases, leave crop residue in place. This protects the soil from beating rains, thereby reducing runoff and increasing infiltration. Decomposition of crop residue and other materials within the soil serves as a major source of organic residue. The mixing of this formed organic matter into the soil by micro organisms enhances the structural development and increases the infiltration rate and available water capacity in soils [6].

Although a comparison of the data collected from the simulated rainfall events to the dates of known natural rainfall did not indicate any significant pattern, it is possible that the addition of the excess moisture during or near the time of simulated rainfall usage could make a difference in the amount of nutrients tested. Depending upon the relative amount of pollution within the atmosphere, it is possible for fallout to increase the nutrient amounts received in the surface runoff water. Comparisons of all plots in this experiment and the amount of time needed to reduce the nutrient and metal amounts indicate that when using animal waste for fertilizer, one must calculate the appropriate load amount so as not to impair water quality. Animal waste could have an impact on water quality based upon the elevation of some nutrients that are associated with the waste. It must be noted that some nutrients associated with animal waste did not have a negative impact on water quality.

The amount of runoff water, the length of time that the animal waste is in contact with the runoff water as well as the length of time between the spreading of animal waste, and the amount of animal waste used will be the determinant as to whether damage or degradation to the water source will occur. Increasing the amount of time between each application of animal waste spreading allows the nutrient amounts to leach into the soil or to be utilized by the plants in the area. Tilling seems to decrease the impact of the nutrients on water quality in that it has the ability to reduce the time in which the nutrients are at high levels. The levels of the heavy metals, copper and zinc, were tested because they are added to animal feed to prevent diseases and to improve digestion. It is possible that these metals could accumulate in soil when animal waste is used as a source of fertilizer [7]. The Louisiana Department of Environmental Quality sets the numerical criteria for acute limits of zinc and copper at 64 ppb and 10 ppb, respectively and the chronic limits for zinc and copper at 58 ppb and 7 ppb, respectively [8]. Only three readings of the experimental plots had values within the acute range for zinc. This level decreased far below the acute level after the next simulated rainfall event was conducted. Almost all of the plots had copper levels

within the acute range during the first three simulated rainfall events. This level decreased below the acute level after the fourth simulated rainfall event in the broiler litter that was tilled and after the sixth week in the plots containing cattle sludge and broiler litter. Tilling allowed the metal to penetrate and infiltrate into the soil thus, reducing the amount that would be found in the surface runoff water. Prior to the spreading of the animal waste, the pre-treatment levels of zinc for the plots containing cattle sludge and for the plots containing broiler litter were within the acute criteria range for freshwater of 0.06ppm [8]. The pre-treatment levels for copper and zinc were found to be 0.06ppm and 0.09ppm for cattle sludge, 0.07ppm and 0.09ppm for broiler litter and 0.09ppm and 0.37ppm for tilled broiler, respectively. Although these levels increased after the addition of the animal waste, it is highly unlikely that the animal waste is the only contributor of the metals copper and zinc to the soil in this region.

In the U.S., the pH of natural water usually ranges between 6.5 and 8.5. At extremely high or low pH values the natural water becomes unsuitable for most organisms. Very acidic waters have the ability to cause heavy metals such as copper to be released into the water [9]. The pH and temperature for all of the experimental plots was found to be very similar to one another. The pH for broiler was slightly higher at 6.57 than that of cattle sludge and tilled broiler litter. The pH for tilled broiler litter at 6.38 was higher than that of the cattle sludge at 6.20. The experimental plots containing cattle sludge had a higher measured temperature of 26.60 °C, tilled broiler litter experimental plots was found to have a higher temperature than broiler litter experimental plots with 26.02 °C and 26.00 °C, respectively.

The predominant type of bacteria found in the experimental plots containing cattle sludge was *Escherichia coli*. The predominant type of bacteria found in the broiler litter and tilled broiler litter experimental plots was *Enterobacter spp.* The tilled plots also had small colonies of *E. coli*, *Klebsiella* and *Enterobacter sakazakii*. The competition that existed among the many types of bacterial colonies found in the tilled plots could have contributed to the decreased levels of *Enterobacter spp.* that were found. Gagliardi and Karns found that because disturbed soil cores were well mixed and often more nutrients leached out of these soil cores, coliforms probably have to compete more with the soil microflora for available nutrients that were in short supply [10]. The bacteria levels in the broiler litter plots was found to be higher in beginning numbers and final numbers than was noted in the tilled broiler litter plots. The survival of *E. coli* organisms have been found to be greatest in soil cores containing rooted grass [11]. Thus this could explain the differences noted in the broiler litter and tilled broiler litter plots. All experimental plots experienced a decrease in the number of bacteria colonies after the simulated rainfall events. Periods of heavy rainfall can cause significant losses of *E. coli* by both leaching and runoff [12].

Conclusions

A major concern of using animal waste as a source of fertilizer is the potential of degrading any nearby natural water sources. According to the Spring 1998 issue of Farm Santuary News [5], when manure is applied to crop land in amounts greater than it can be used or retained by the soil, nitrogen, phosphorous and other nutrients leach into surface and ground water. To be specific in the identification of the actual source of water contamination, there is a clear need to incorporate the use of bacterial source tracking. Based upon the data analyzed from the simulated rainfall and experimental plots containing animal waste, it is evident that using animal waste as fertilizers has a significant effect on various nutrients that could impact surface runoff water quality and thus can have an effect on surrounding water sources. It must be noted that some of the nutrients associated with animal waste will not impact water quality in a negative way. It was shown that the effects could be decreased by the use of tilling in an effort to allow nutrients to leach into the soil, be taken up by the various plant roots and thus decrease the amount of nutrients available in surface runoff water.

It can therefore be concluded from the finding of this research that: 1) non-point source pollution has a significant effect on bacterial and nutrients levels in runoff water and in water resources; 2) land application of animal waste for soil fertilization can elevate some nutrients levels which could contribute to water pollution; 3) the use of tilling can significantly reduce the amount of nutrients available in runoff water.

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