RESEARCH ABSTRACT

PUBLIC HEALTH/WORKER SAFETY

Title: Influence of Setback Distance on Antibiotics and Antibiotic Resistance Genes in Soil and Runoff Following the Land Application of Swine Manure Slurry, **NPB # 16-072**

Investigator: Xu Li

Intuition: University of Nebraska – Lincoln

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Scientific Abstract

Setback distance is a commonly used measure to minimize the impacts manure land application on the quality of nearby surface water. The objective of this project was to determine how varying setback distances affect the concentrations of antibiotics and ARGs in runoff and soil following the land application of swine slurry. To achieve this objective, a portable rainfall simulator was assembled over a series of plots with varying lengths. Manure was applied by broadcasting at the top of each plot (manured region) and the rainfall simulator was placed so that it covered the entire length of the plot (manured region and setback region). At the end of each plot, a trough collected runoff and directed it to a flume where flow was measured and runoff sampled. The rainfall simulation with sampling of runoff was repeated once the following day. Five days after the second rainfall simulation, soil cores were sampled at various setback distances. Each soil core was divided into three depths. The runoff and soil samples were analyzed for both antibiotics and ARGs.

Out of the ten genes tested, *erm*(B), *erm*(C), *tet*(O), *tet*(Q), *tet*(X), *intI1*, and the 16S rRNA gene showed statistically significant decreases in their concentrations in runoff with increased setback distance. The log concentrations of these seven genes in runoff decreased linearly with increased setback distances, with *tet*(O), *tet*(Q), *erm*(B), and *erm*(C) having slopes ranging from -0.0800 to -0.0838, the 16S rRNA gene and *intI1* having slopes ranging from -0.0638 and -0.0641, and *tet*(X) having a slope of -0.0715. For the three antibiotics tested, chlortetracycline, lincomycin, and tiamulin, their all showed statistically significant decreases in their concentration in runoff with increased setback distance. The log concentrations of these antibiotics in runoff decreased linearly with increased setback distances, with the slopes for chlortetracycline, lincomycin and tiamulin being -0.0639, -0.0505, and -0.0722. By using the linear equations for ARGs and antibiotics, we calculated the setback distance needed to reduce the ARG and antibiotic concentrations in runoff to values similar to those in runoff from control plots where no manure was land applied. That setback distance was 50 meter.

Among the genes tested, the 16S rRNA gene, *bla*$_{TEM}$, *intI1*, and *tet*(D) were the only genes that were consistently detected in the surface soil within the setback region. Among them, *bla*$_{TEM}$ was the only gene that decreased significantly with increased setback distance. The other six ARGs tested (*erm*(B), *erm*(c), *erm*(F), *tet*(O), *tet*(Q), and *tet*(X)) were only detected in surface soil with the short setback distances (e.g., less than 9 meters). Because their concentrations in surface soil at most of the setback distances tested were below the method detection limits, ANOVA tests could not determine the statistical significances of setback distance on their concentrations. Nonetheless, these six genes, plus *bla*$_{TEM}$, are believed to decrease significantly in surface soil with in the setback region with increased setback distance. Similarly, the concentrations of chlortetracycline, lincomycin, and tiamulin decreased significantly in the surface soil with in the setback region with increased setback distance.

As revealed from the high-throughput qPCR analyses, the number of ARGs in runoff dropped slightly from 140 to 111 as the setback distance increased from 0.0 to 18.3 meter. However, the sum of the relative abundance, a measure that sums up the relative abundance of all ARGs tested, dropped from 1.2 to 0.2 ARG copy per the 16S rRNA gene copy as the setback distance increased from 0.0 to 18.3 meter. The percentages of resistance genes against aminoglycoside and MSLB decreased in runoff samples as the setback distance increased. The resistome of the soil within the setback region was not substantially affected by the land application of manure in the manured region, in terms of the number of ARGs detected, the sum of the relative abundance, or the composition of the resistome.