Increase in Antibiotic Resistance of Soil Microbes Found Near Hospital Sites

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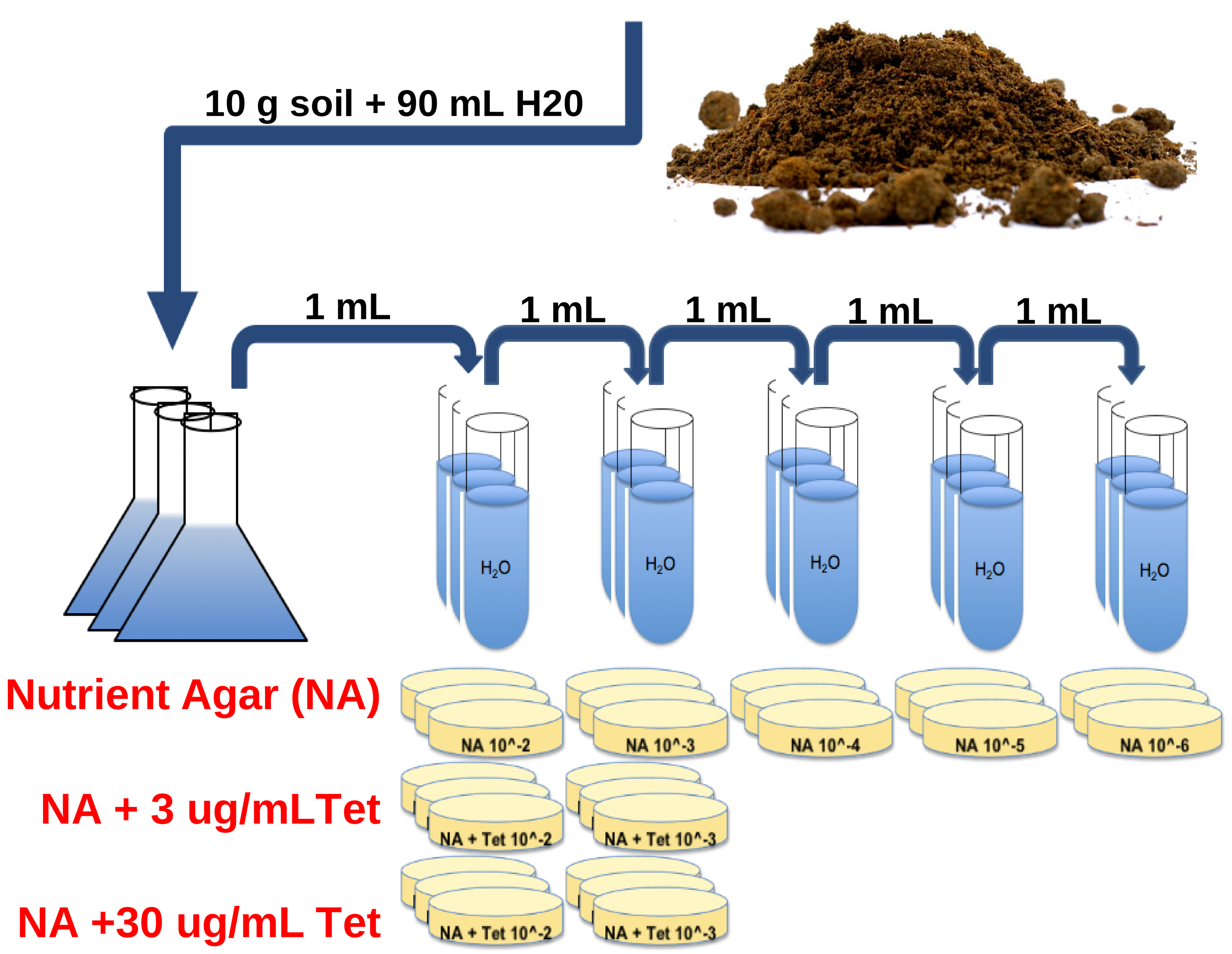
Research Question

Human activities such as industrialized animal farming, hospital usage, and improper antibiotic disposal by individuals are all potential sources of environmental antibiotic pollution. This pollution directly impacts the biodiversity of our soil by causing selective pressure for antibiotic-resistant soil microbes. **Our study asked whether the percentage of antibiotic-resistant soil microbes would differ between two soil environments: schools and hospitals. We hypothesized the use and disposal of antibiotics in hospitals would significantly increase the percentage of antibiotic-resistant microbes compared to schools.**

Methods

Ten grams of the soil sample were placed in an Erlenmeyer flask and mixed with 90 mL of sterile water to produce a 1/10 dilution. The sample was manually swirled for 10 minutes at room temperature. Using the 1/10 solution, two dilution series (SD1 and SD2) were prepared. For each series, five test tubes were filled with 9 mL of sterile water and labeled 10 -2 to 10-6. The serial dilutions were started by adding 1 mL of the 1/10 solution to the test tube labeled 10 -2 tube, followed by mixing. One mL from tube 10-2 was then added to 10-3 and mixed. This process was repeated for the remaining tubes. In the end, each step in the series diluted the previous solution by a factor of ten.



Results

Plates containing between 30-300 visible colonies were counted (see Fig. 1). The number of colony forming units (CFUs) per gram of soil was then calculated for the usable plates (NA, NA3, and NA30) by the formula: [CFUs]=counted CFUs x 5 x dilution factor. The relative percent of tetracycline resistant microbes at the different concentrations was then calculated by the formula: % of terR CFUs = (terRCFUs/g)/(control CFUs/g) x 100%. These formulas were used for both NA3 and NA30. Data analysis carried out using a t-tests, with Vassar stats, of the CFUs/g soil, % resistant at 3ug/ml, and % resistant at 30 ug/ml between the school and hospital sites.

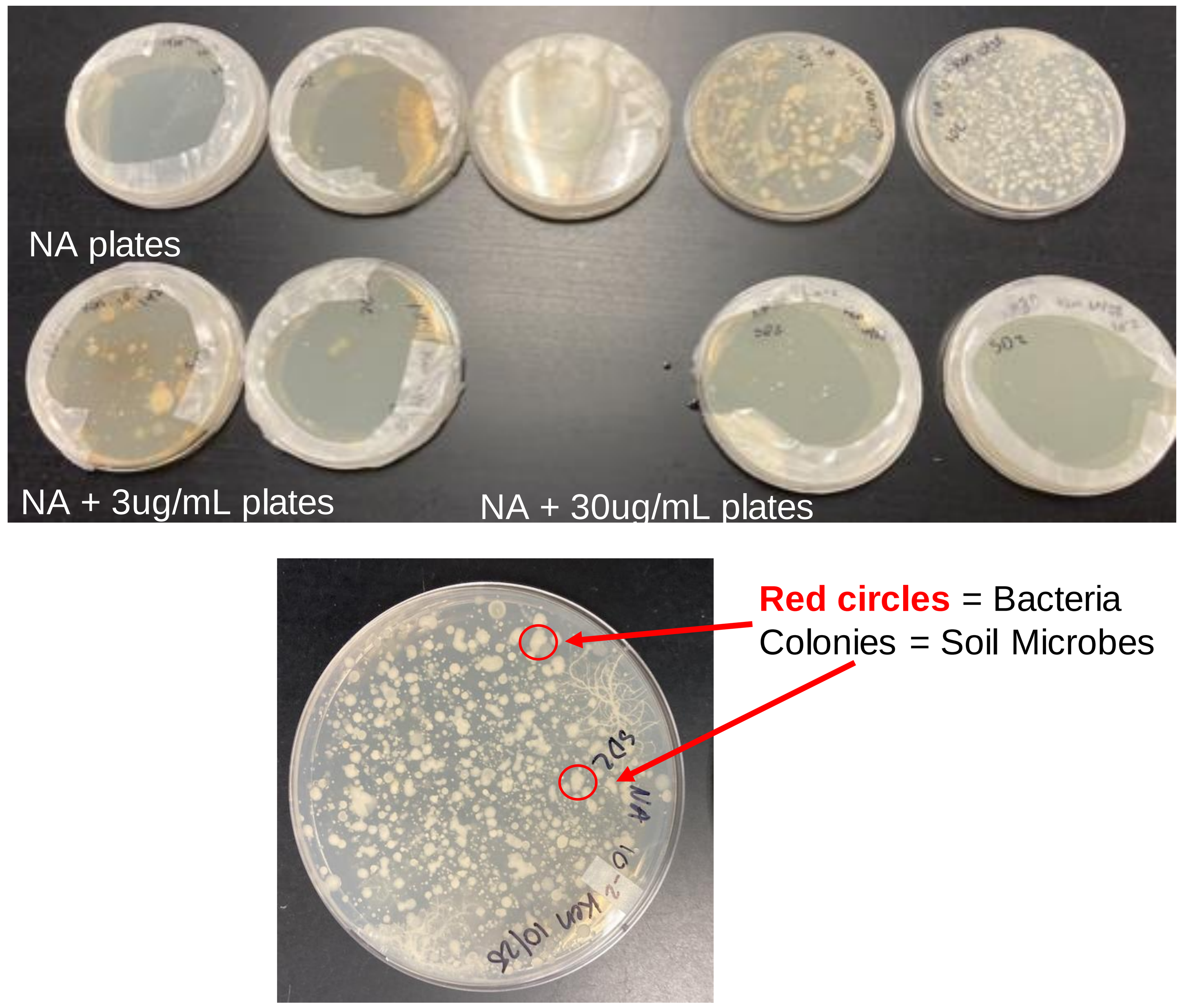


Fig. 1. Nutrient Agar (NA), NA + 3 ug/mL tetracycline, and NA + 30 ug/mL tetracycline plates inoculated with soil dilutions.

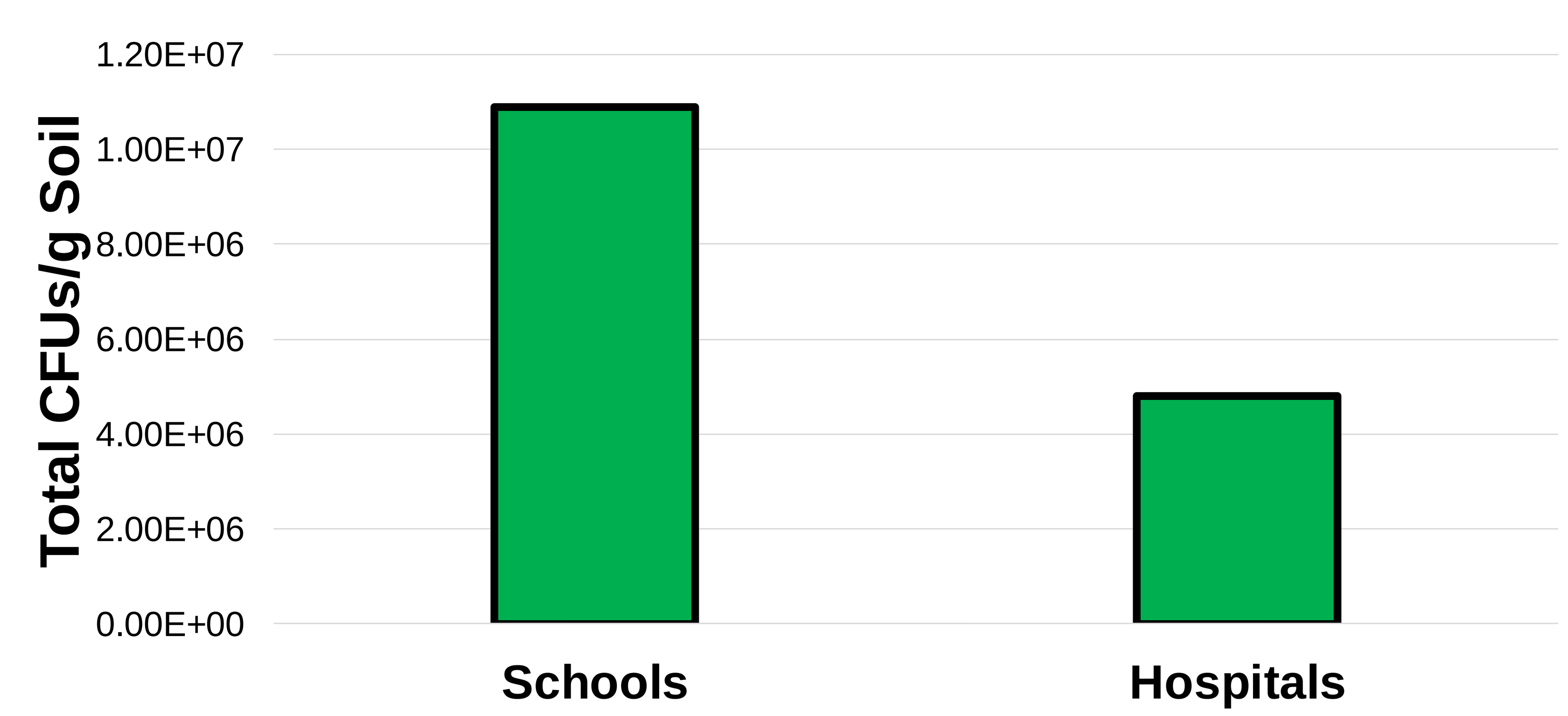


Fig 2. Average Colony Forming Units (CFUs) per gram soil at the soil sample sites.

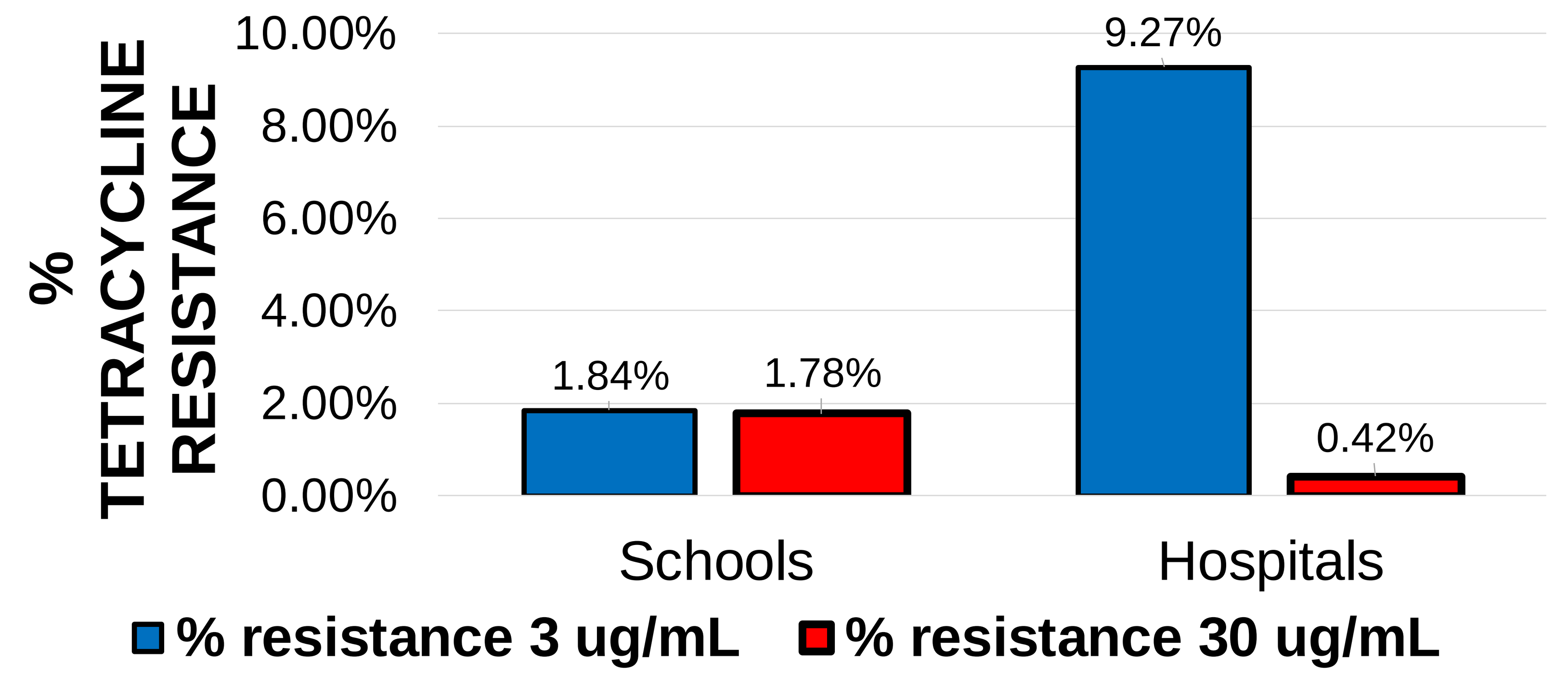


Fig 3. Percentage of tetracycline-resistant soil microbes at schools and hospitals.

Analysis and Interpretations

As seen in Fig. 2, the average CFUs/gram was highest at schools and less at hospitals. Both soil locations had a larger percentage of resistant microbes at 3ug/mL than at 30ug/mL, but the degree of difference varied (Fig. 3). A Student's t-test was only applied to the schools and hospitals. Average CFUs/grams at controls sites was 1.09E7 and at treatment site it was 4.82E6. There was no statistical difference between site type (t= 0.98, df=10, p= 0.3502). Average % tetR at 3 ug/mL was 1.84% at control sites and 9.27% at treatment sites. There was no statistical difference (t=-1.14, df=10, p= 0.1405). Average tetR at 30 ug/mL was 1.18% at control and 0.42% at treatment. There was no statistical difference (t=1.05, d=10, p= 0.1592)

Conclusion

We observed a trend toward more antibiotic resistance in soils near hospitals compared to schools, however, statistical analysis found no significant difference. Despite the study being limited by the number of soil samples collected at each location, the data supports the hypothesis of human activity contributing to antibiotic-resistant soil microbes. For future studies, we would like to collect more samples from each site category to determine the microbial biodiversity, percentage of tetR, and how the mechanisms of antibiotic resistance arose by using molecular analyses. Our data suggests that humans need to be more vigilant of their antibiotic use and disposal.

References

E.A. Genné-Bacon and C.A. Bascom-Slack. *The PARE Project: A Short Course-Based Research Project for National Surveillance of Antibiotic-Resistant Microbes in Environmental Samples*. J. Microbiol. Biol. Educ. Oct. 2018 vol. 19 no. 3.

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