



Tetracycline Resistance in Aerobic and Anaerobic Bacteria Recovered from Landfill Leachate.



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Introduction and Purpose



Solid waste landfills are inhabited by various microorganisms, including bacteria. Leachate accumulating within these landfills has the potential to contaminate surrounding groundwater and surface waters with potentially pathogenic bacteria, some of which may exhibit resistance to currently prescribed antibiotics. Tetracycline, an antibiotic which has found widespread use in a variety of applications, may be useful as a model substance for investigating development of bacterial resistance.

The purpose of this experiment was:

- To estimate the density of aerobic and anaerobic bacteria in landfill leachate and surrounding groundwater.
- To evaluate their resistance to tetracycline.

Methods



Groundwater sample (left); Leachate sample (right)



BBL GasPak Anaerobic System



Manifold filtration system

Leachate from a closed landfill and adjacent groundwater samples were obtained from a regional landfill site. All samples were extracted via a well/pump collection system integrated with the landfill and the surrounding ground. Samples were diluted 10^{-1} to 10^{-3} using phosphate buffered saline. Ten ml aliquots were vortexed and filtered through 0.22um membrane filters. Replicate filters were transferred onto pads with 2mL of Plate Count Broth. One-half of the plates contained 20ug mL^{-1} Tetracycline. The other half contained Plate Count Broth without Tetracycline. Of these plates, one-half were incubated aerobically, the other half in a BBL GasPak Anaerobic System for 48 hrs @35°C. Statistical analyses were performed. A Mann-Whitney test was used to determine the probability of significant differences between tetracycline resistant and tetracycline sensitive populations.

Results

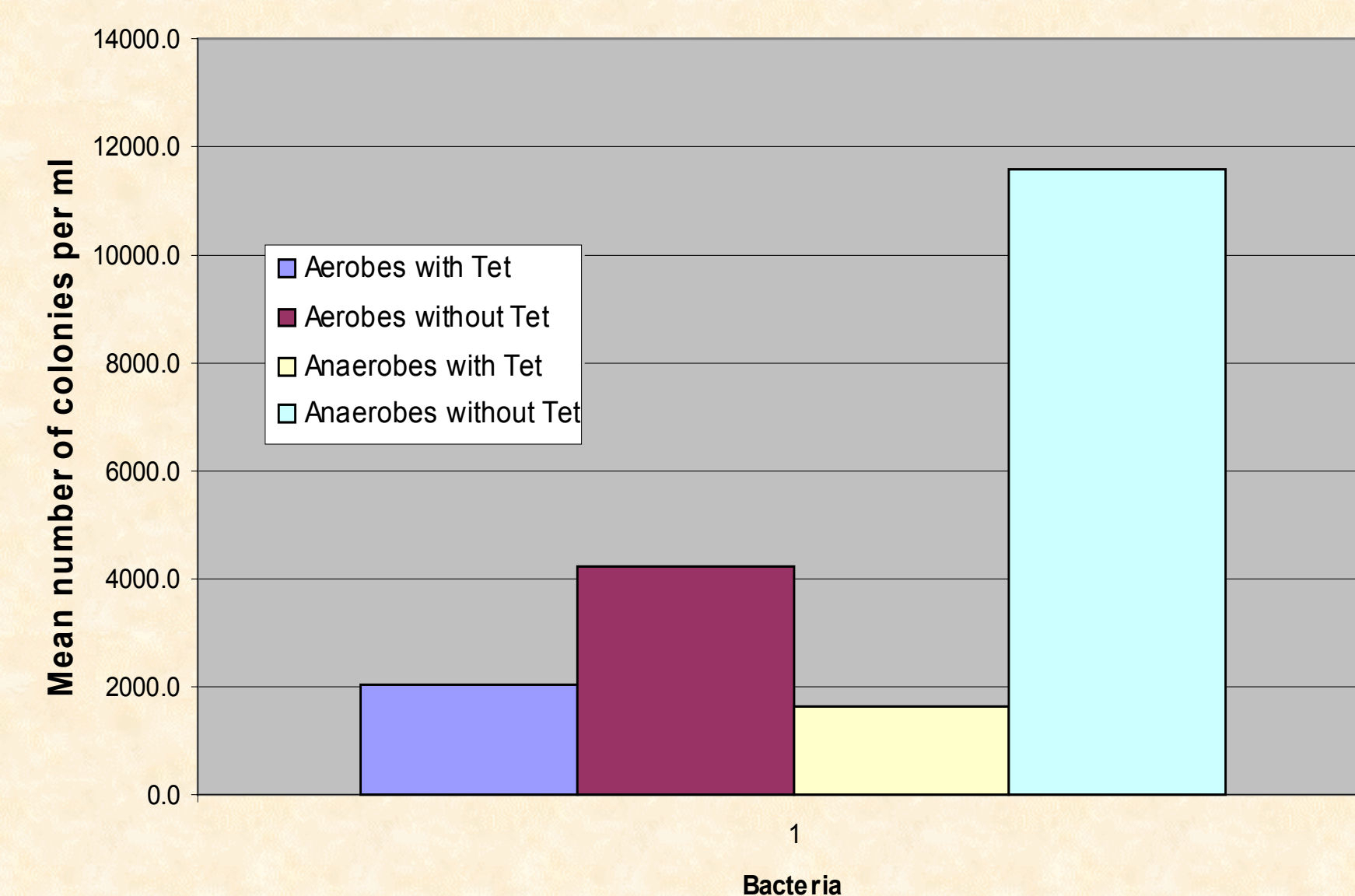


Fig. 1: Mean heterotrophic count of bacteria from groundwater

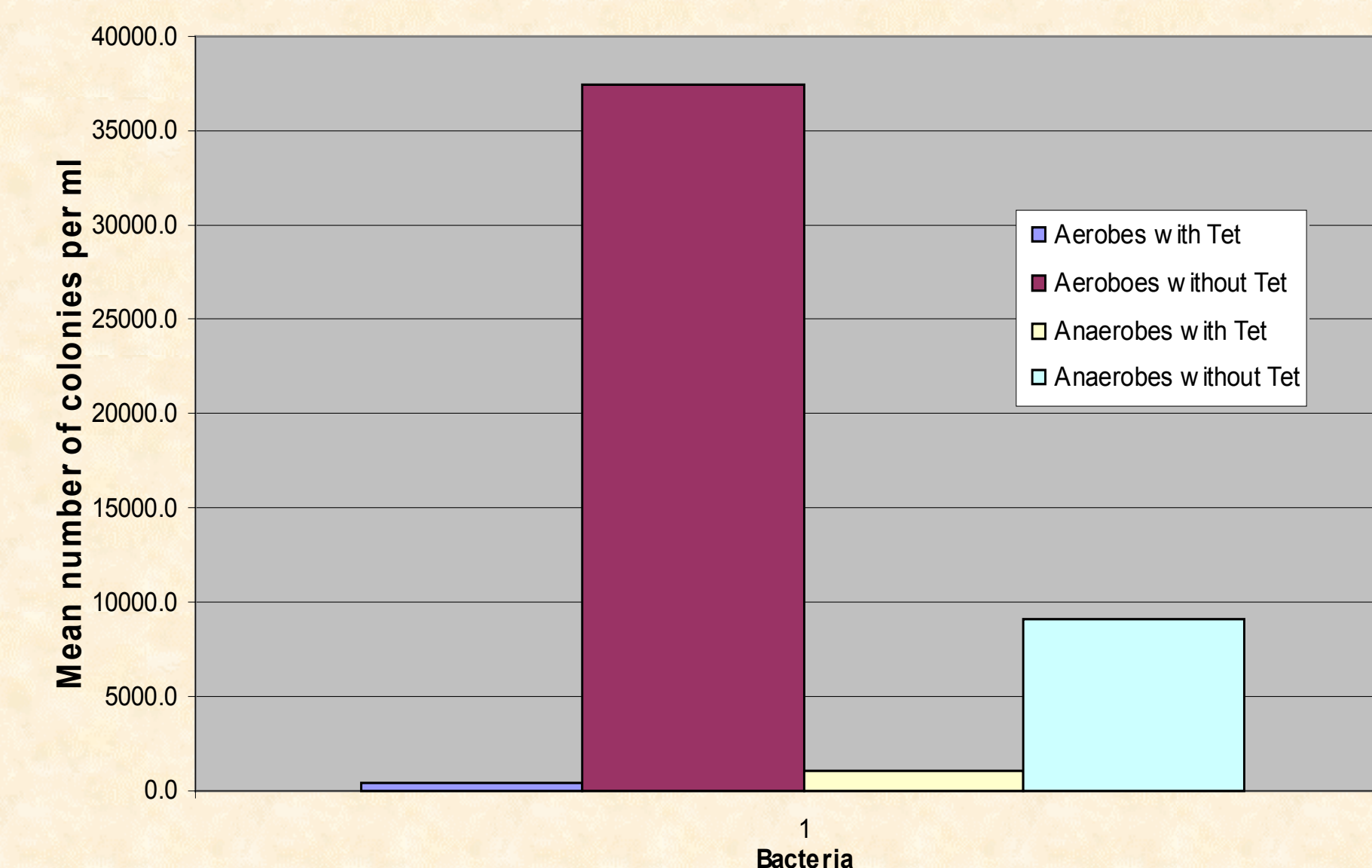


Fig. 2: Mean heterotrophic count of bacteria from leachate

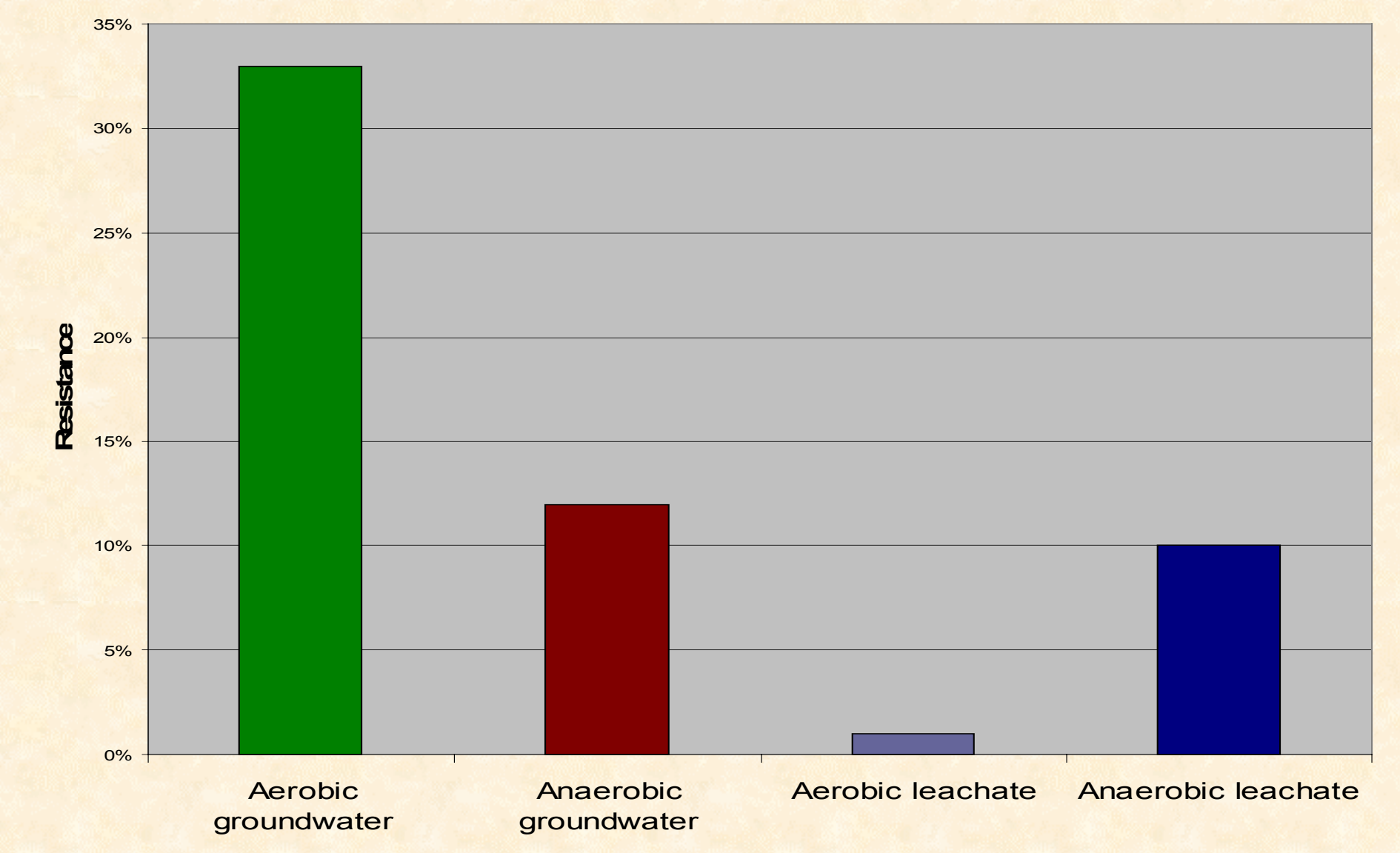


Fig. 3: Heterotrophic bacteria resistant to tetracycline.

Table 1: Mann-Whitney test results. Heterotrophic counts with and without tetracycline ($p < 0.05$).

	Groundwater		Leachate	
	Aerobic	Anaerobic	Aerobic	Anaerobic
Mann-Whitney results	54.5	38.5	0.0	7.0
Significant Difference ($p < 0.05$)	0.127	0.004	0.000	0.001

- Populations of aerobic heterotrophs from groundwater ranged from 2000 - 4000 mL^{-1} ; anaerobic populations ranged from 1000 - 1200 mL^{-1} .
- Populations of aerobic heterotrophs from leachate ranged from 400 – 38000 mL^{-1} ; anaerobic populations ranged from 1000 – 9100 mL^{-1} .
- 33% of aerobic and 12% of anaerobic populations from groundwater were resistant to tetracycline.
- 1% of aerobic and 10% of anaerobic populations from leachate were resistant to tetracycline.
- Mann-Whitney tests indicate a significant difference for tetracycline resistance among aerobic and anaerobic bacteria recovered from groundwater, and aerobic bacteria recovered from leachate.
- Mann-Whitney tests indicate no significant difference for tetracycline resistance among aerobic heterotrophic bacteria recovered from groundwater.

Summary

- Populations of aerobic and anaerobic bacteria recovered from landfill leachate and groundwater display a wide range of values.
- Resistance to tetracycline is highest among bacteria isolated from groundwater and lowest among bacteria isolated from leachate samples.
- Anaerobic bacteria seem to be more resistant to tetracycline than aerobic bacteria.
- Tetracycline appears to maintain its effectiveness in reducing populations of heterotrophic bacteria, with the exception of the aerobic groundwater populations.

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