**MADsludge – Material and Methods**

***Data analysis***

Statistical analysis was performed using R version 4.1.2 (R Core Team 2022 [1]). To study the significance of the difference between the ΔCt used to calculate ΔΔCt values, two methods were carried out to compare the results and select the best suited method for our data. The « DESeq2 » package was first used [2]. DESeq2 uses positive integer abundance values to calculate and compare log2FC values. Transformation of the ΔCt into relative abundance was first performed using R= 2−ΔCt. The result was then multiplied by 1,000,000 and rounded to eliminate floating values and keep only integers for the rest of the analysis. *DESeq* was then used to select genes with an adjusted p-value (padj) < 0.05 for each comparison of ΔCt before and after the condition studied (treatment, incubation, spreading on soil) ~~and each treatment (anaerobic digestion, lime, drying, compost, anaerobic digestion + compost)~~. A linear model taking into account the effect of treatment/incubation on the abundance of genes (unchanged ΔCt) in the samples was also used ~~(~~*~~gene~1+comparison~~*~~)~~. A post hoc Tukey test was performed to identify the genes with significantly different abundance before and after the condition studied (treatment, incubation, spreading on soil) depending on the sludge treatment [3].

To study the significance of the difference between ΔΔCt for each gene depending on the treatment, a Linear Mixed Model (LMM) was performed using the « lme4 » R package (*lmer* function [4]). Relative gene abundance (ΔΔCt) was modeled using the batch as fixed effect and the genes and the treatment as random effects *~~(ΔΔCt ~ batch + (1|gene) + (1|treatment))~~*. Likelyhood Ratio Test (LRT) was then performed using the « lmtest » package (*lrtest*) to identify the factors with a significant effect on the data (pval < 0.05) [5].

**REFERENCES**

1. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing. 2022.

2. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 2014;15:550.

3. Vandeweyer D, Milanović V, Garofalo C, Osimani A, Clementi F, Van Campenhout L, et al. Real-time PCR detection and quantification of selected transferable antibiotic resistance genes in fresh edible insects from Belgium and the Netherlands. International Journal of Food Microbiology. 2019;290:288–95.

4. Zeileis A, Hothorn T. Diagnostic Checking in Regression Relationships. R News 2. 2002;3:7–10.

5. Karkman A, Johnson TA, Lyra C, Stedtfeld RD, Tamminen M, Tiedje JM, et al. High-throughput quantification of antibiotic resistance genes from an urban wastewater treatment plant. FEMS Microbiology Ecology. 2016;92:fiw014.