

# Biological risk associated to bio-treatments: monitoring and modeling bacterial dispersion into the atmosphere in a soil bioremediation plant and in a wastewater treatment plant

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## INTRODUCTION

Wastewater is a mixture of domestic, municipal and industrial wastes dissolved in water. Treatment of polluted water and soil takes place at bioremediation plants, which contain a big amount of microorganisms. Such microorganisms can become aerosolized in the atmosphere during different steps of treatment. This process may cause different infections in plant workers and affect the population living in surrounding areas. Some bacterial species as *E.coli*, *C. perfringens*, *Enterococcus* spp. are very pathogenic. Their concentration in the drinking water, soil or air is an ideal indicator to test environmental samples for fecal contamination.

### The main objective of this project

- to quantify and characterize the dynamics and pathogenicity of bacteria in the air, wastewater and contaminated soil present at the municipal wastewater treatment and soil bioremediation plants during different steps of the bioremediation processes.
- to estimate biological risk associated with working or living in an area close to bioremediation plant.

## MATERIAL AND METHODS

The study was performed at soil bioremediation plant "Sistemi ambientali S.r.l.", situated in Calcinante from Apr. 2014 till Jul. 2014 during different bioremediation steps. Biodiversity of bacteria in the air and soil was analyzed quantitatively and qualitatively.

- The **structure** of microbial communities was described by **massively parallel Illumina sequencing** technology. A hypervariable regions of 16S rRNA gene, the most common housekeeping genetic marker used to study bacterial phylogeny and taxonomy was analyzed.
- Libraries of **16S rRNA hypervariable region** with attached specific sequence, consisting on adaptor, particular barcode and forward or reverse primers sequence were prepared. Illumina sequencing method gave as result **relative abundance** of bacterial orders in the samples. Relative abundance of *E.coli*, *C. perfringens*, *Enterococcus* spp. was translated in the actual number of bacterial copies in the samples.
- Total quantification** of bacteria in the samples was performed by using the **Real-Time quantitative PCR** technique.

## RESULTS

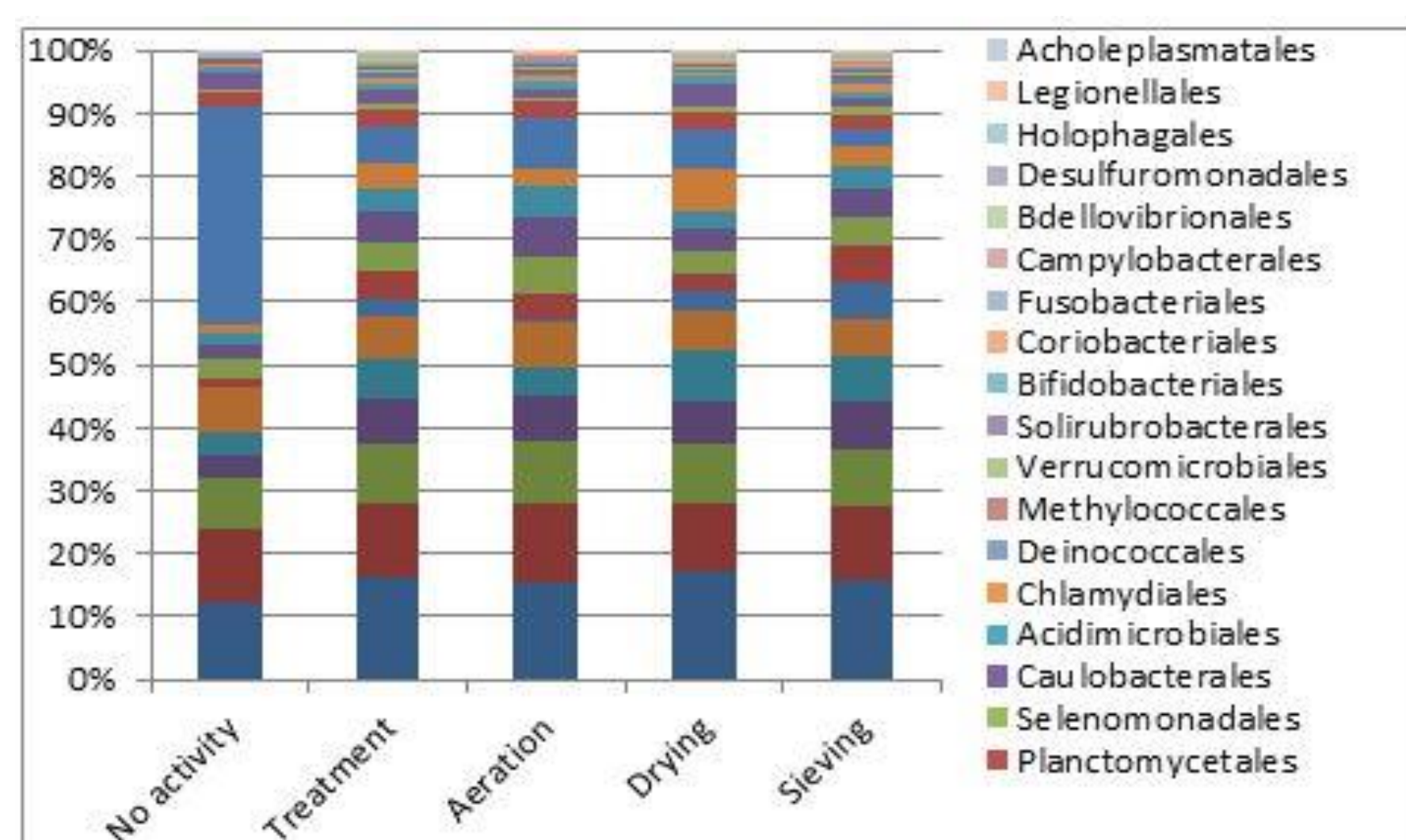


Fig.1. Bacterial community structures of the air filter samples during no activity, treatment, aeration, drying and sieving processes at the Calcinante plant.

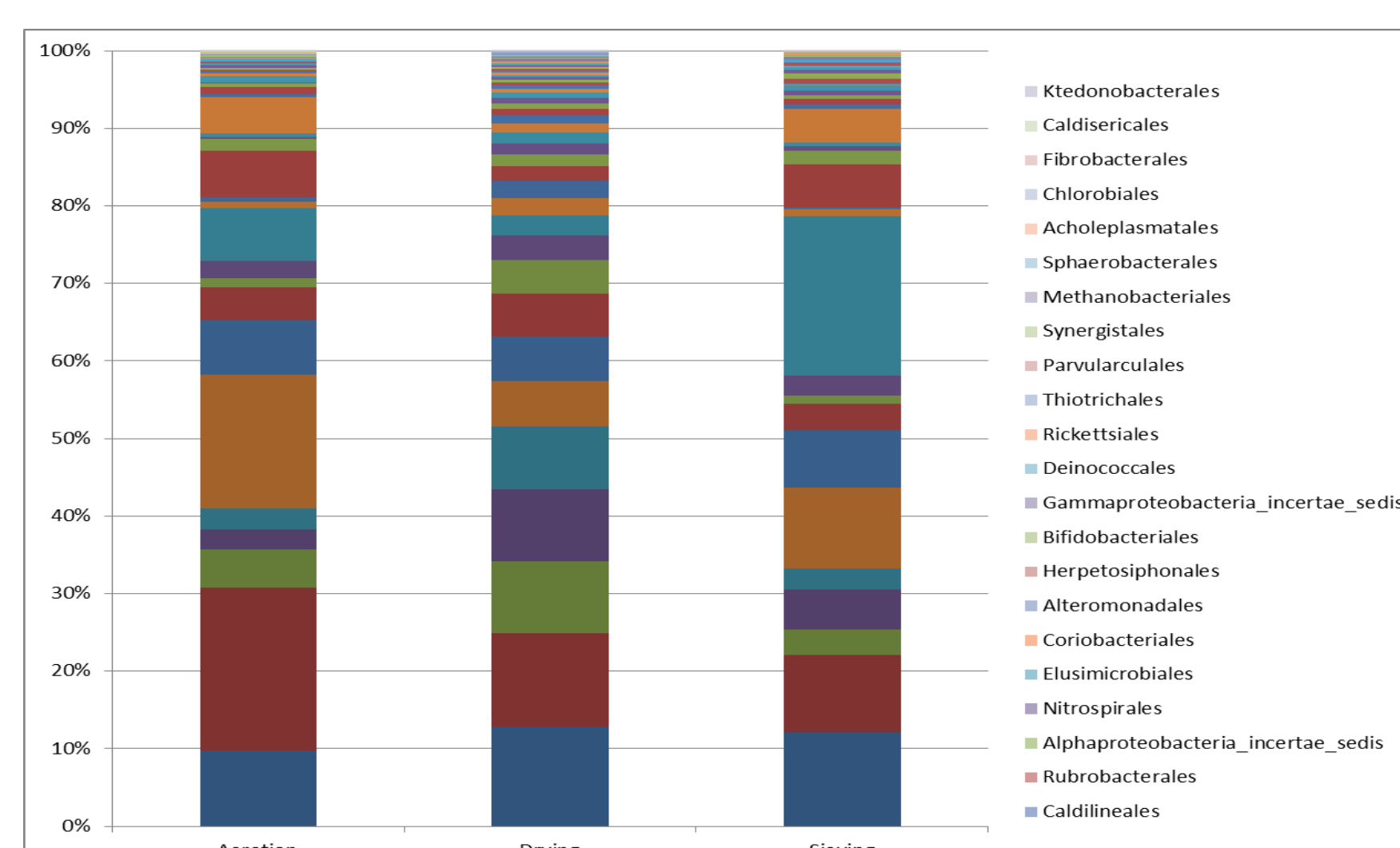


Fig.2. Bacterial community structures of the soil samples during aeration, drying and sieving processes at the Calcinante plant.

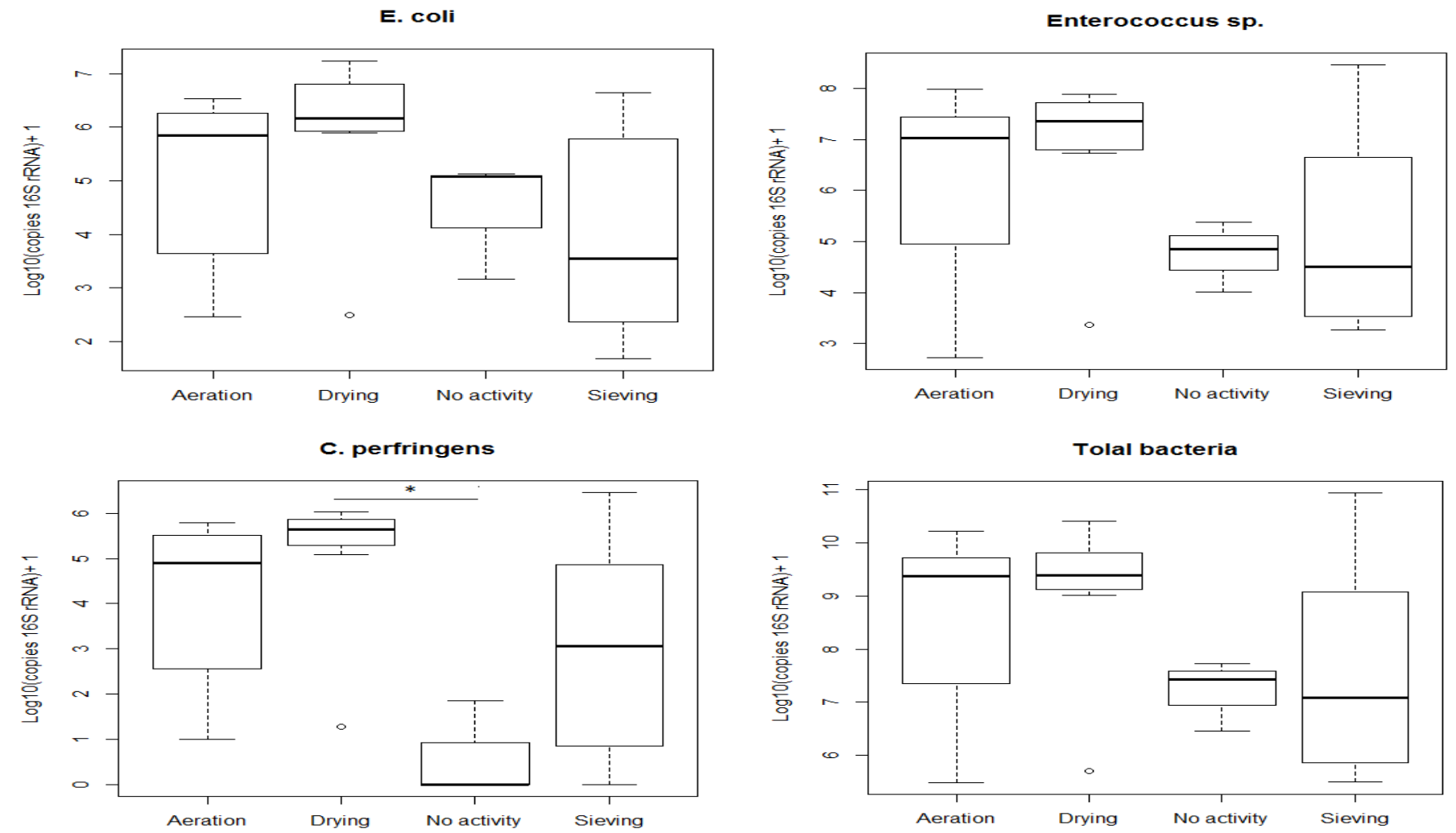


Fig.3. Comparison of means for bacterial species *E.coli*, *Enterococcus* spp., *C. perfringens* in the air filter samples at the Calcinante plant.

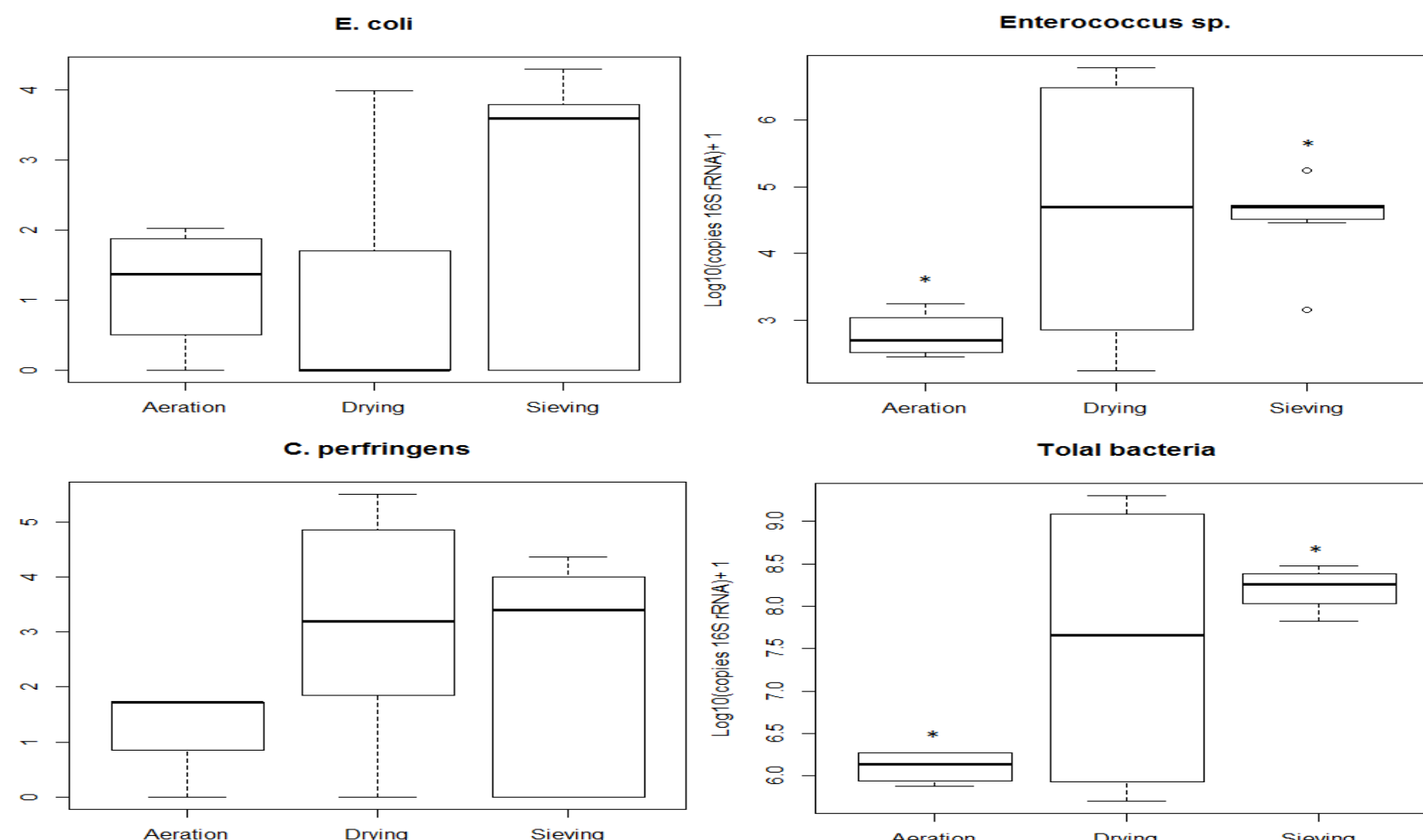


Fig.4. Comparison of means for bacterial species *E.coli*, *Enterococcus* spp., *C. perfringens* in the soil samples at the Calcinante plant.

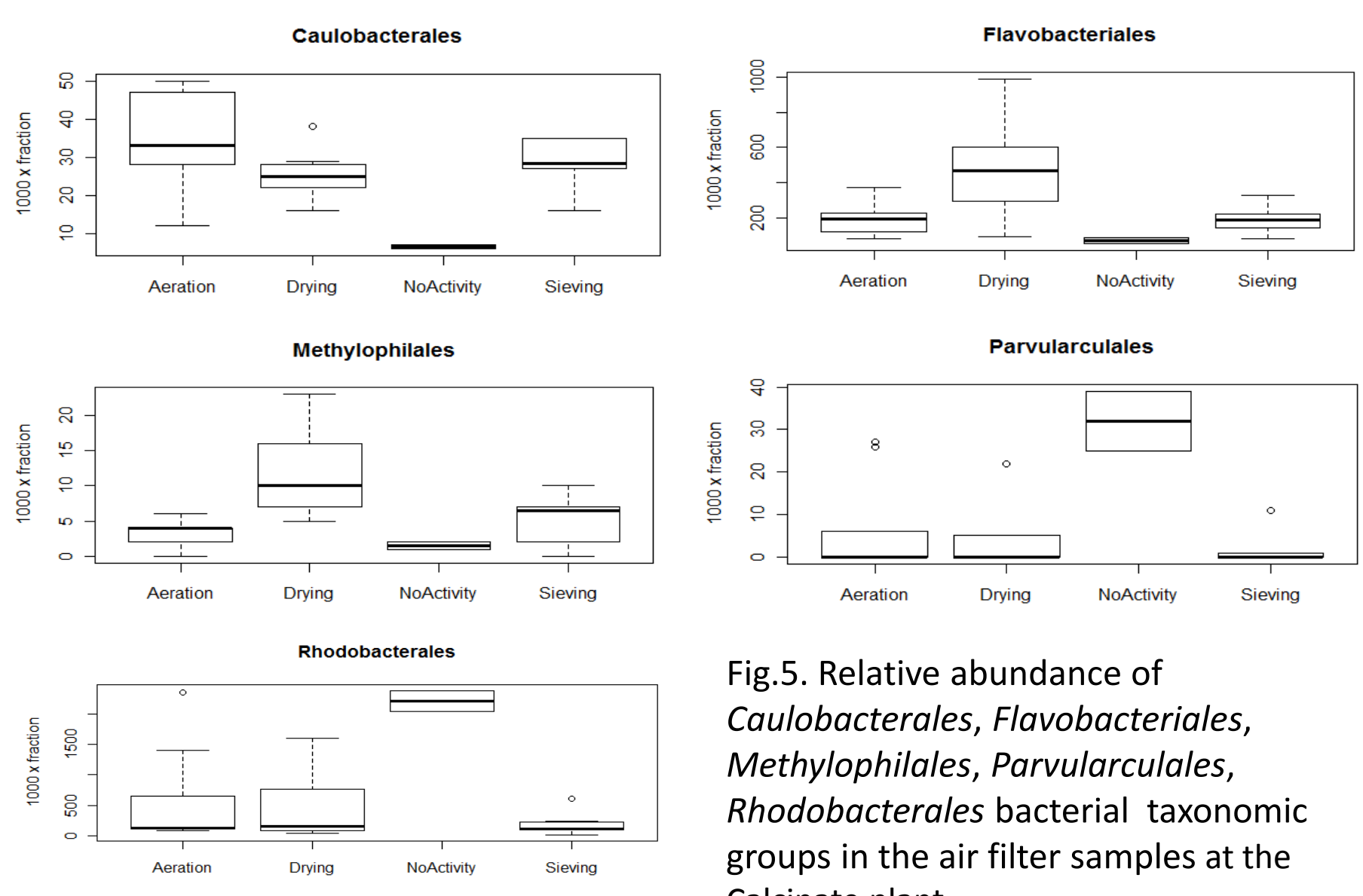


Fig.5. Relative abundance of *Caulobacteriales*, *Flavobacteriales*, *Methylophilales*, *Parvularculales*, *Rhodobacteriales* bacterial taxonomic groups in the air filter samples at the Calcinante plant.

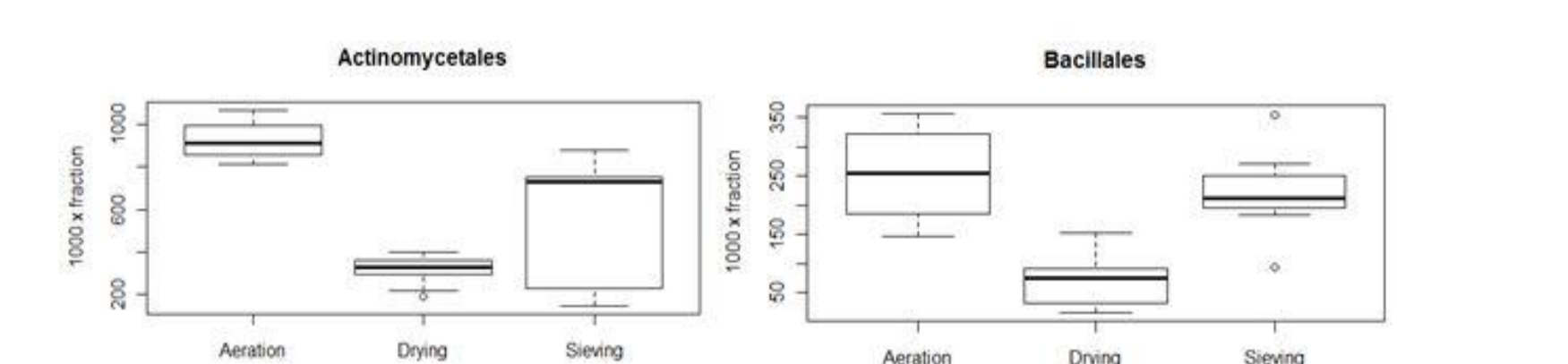


Fig.6. Relative abundance of *Actinomycetales* and *Bacillales* bacterial taxonomic groups in the soil samples at the Calcinante plant.

## CONCLUSIONS

Total bacterial quantification in the **air filter** samples of Calcinante plant was up to **10<sup>4</sup>-10<sup>8</sup> #/m<sup>3</sup>** (# - number of ribosomal operons). The highest bacterial concentrations were found during the step of **drying and aeration** (about 10<sup>7</sup> and 10<sup>8</sup> #/m<sup>3</sup>), while the lowest were found in the no activity (control) samples (about 10<sup>5</sup> #/m<sup>3</sup>). Total bacterial concentration in the **soil** samples was very high during steps of **drying and sieving** (about **10<sup>12</sup> #/g**) and lowers at the step of aeration. Occupational Exposure Limits are established by the government on the threshold limit values of 1.0×10<sup>5</sup>, 2.0×10<sup>4</sup>, and 5.0×10<sup>4</sup> CFU/m<sup>3</sup> for mesophilic bacteria, Gram-negative bacteria and fungi, respectively.

It can be conclude that:

- the soil bioremediation plant gives significant **increase** of bacteria in the air.
- aeration** and **drying** steps contribute the most to increasing of bacteria in the **air**.
- drying** and **sieving** are steps that contribute mostly to the increase of the bacteria in the **soil** samples.
- sieving does not contribute much to the bacterial move into the air because the soil is dry

Hypothetically, bacteria move from soil into the air

- when water is **evaporates** from the soil.
- during mechanical **mixing**.
- due to **salinity** shift and inappropriate conditions for bacterial growth.

Most abundant bacteria in the analyzed samples belong to **Alphaproteobacteria** (marine bacteria) or **Betaproteobacteria** (fresh water bacteria). An important factor for bacterial grow is the **oxygen** supply during the aeration step.

## REFERENCES

- G.Tortora, B.Funke, C.Case. Sewage (wastewater) treatment. Microbiology. 2010;16:1-6
- Gandolfi, V.Bertolini, R.Ambrosini, G.Bestetti, A.Franzetti. Unravelling the bacterial diversity in the atmosphere. Appl Microbiol Biotechnol. 2013;97:4727-36.
- E.Longhin, E.Pezzolato, P.Mantecca, J.A.Holme, A.Franzetti, M.Camatini. Season linked responses to fine and quasi-ultrafine Milan PM in cultured cells. Toxicology in Vitro. 2013;27:551-9.

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