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Ecotoxicological assessment of a copper-based nanopesticide towards *xenopus laevis* and its gut microbiota

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HAL Authorization

Introduction

Concerns regarding copper use in agriculture and accumulation in the environment have led to restrictions on its use

Copper-based nano formulations have been proposed as an alternative with improved efficiency compared to conventional analogues, supposing reduced application rates [1]

Environmental risk of such formulations remain poorly understood



Voluntary Release on Crops

Runoff



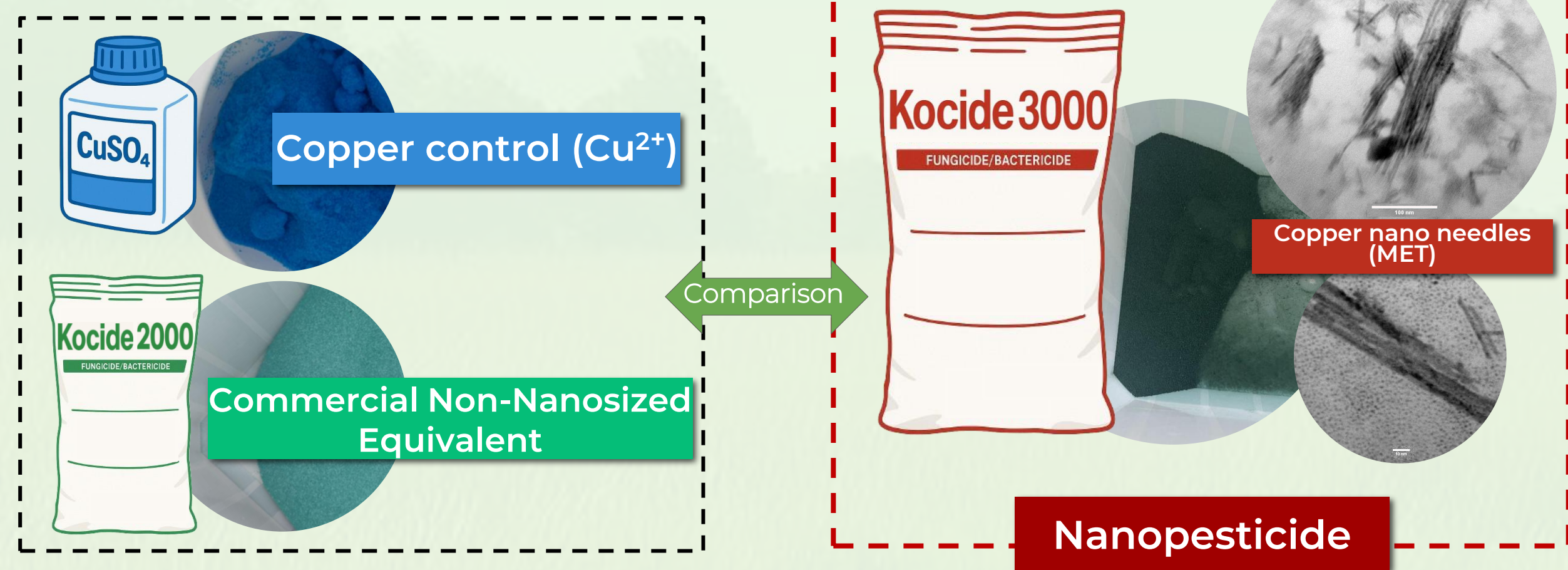
Aquatic Compartment as Final Receptacle



Xenopus laevis used as a sensitive bioindicator of aquatic toxicity

Objective: To assess both classical toxicity endpoints and gut microbiota alterations following exposure to a copper-based nanopesticide, such as Kocide® 3000

Material and Method



ISO Standardized exposure conditions ISO 21427-1

- 12 days semi-static exposure
- 15 larvae per condition

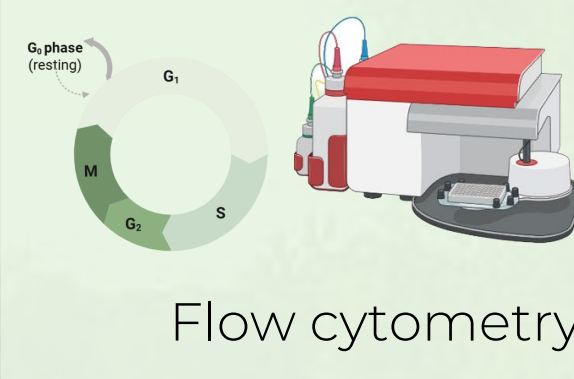


Xenopus laevis Larvae Stage 50 N&F [2]

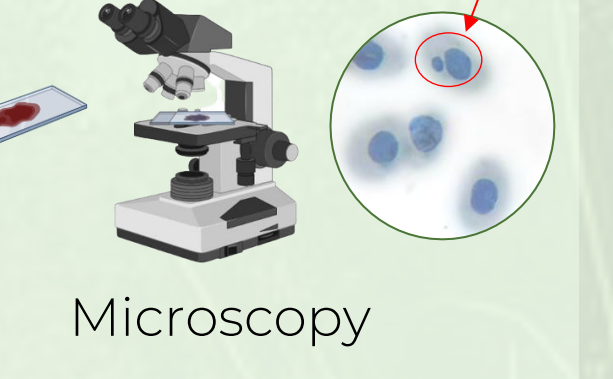
Environmentally relevant concentrations [3]

- C1 = 5 µg Cu/L
- C2 = 10 µg Cu/L
- C3 = 25 µg Cu/L
- C4 = 50 µg Cu/L

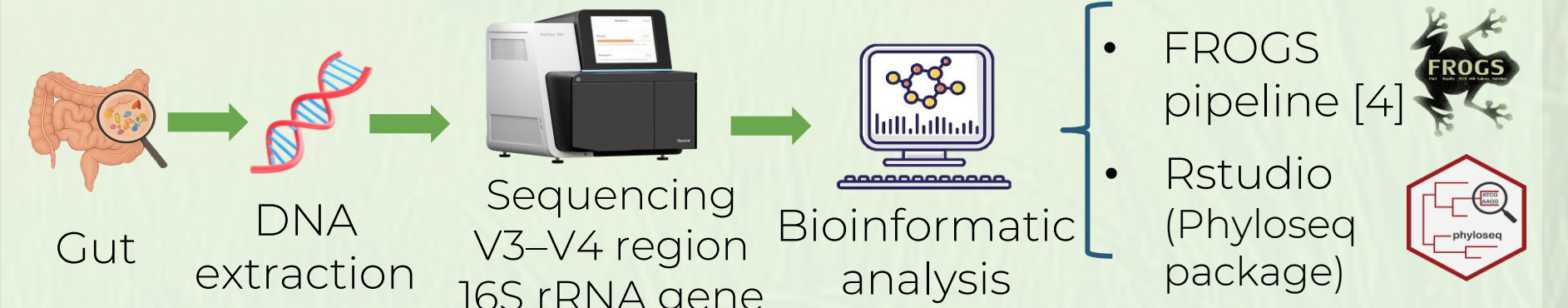
Cell Cycle Analysis



Micronucleus Assay



Microbiota Analysis



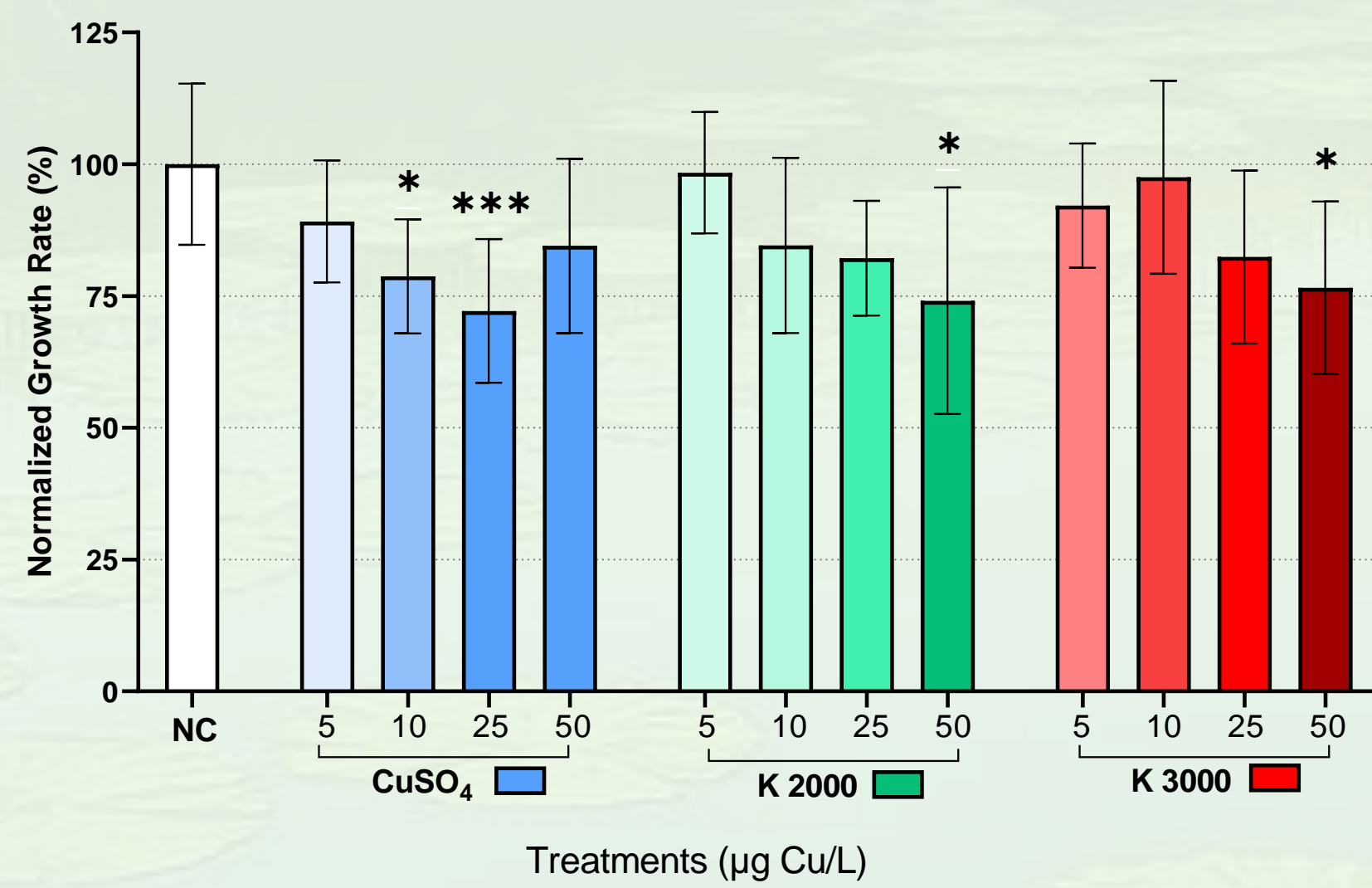
Toxicity Endpoints

Growth

Significant Growth Inhibition

Increased sensitivity to CuSO₄ (effects observed at 10 µg Cu/L)

No difference between Kocide 2000 and Kocide 3000 at equivalent concentrations

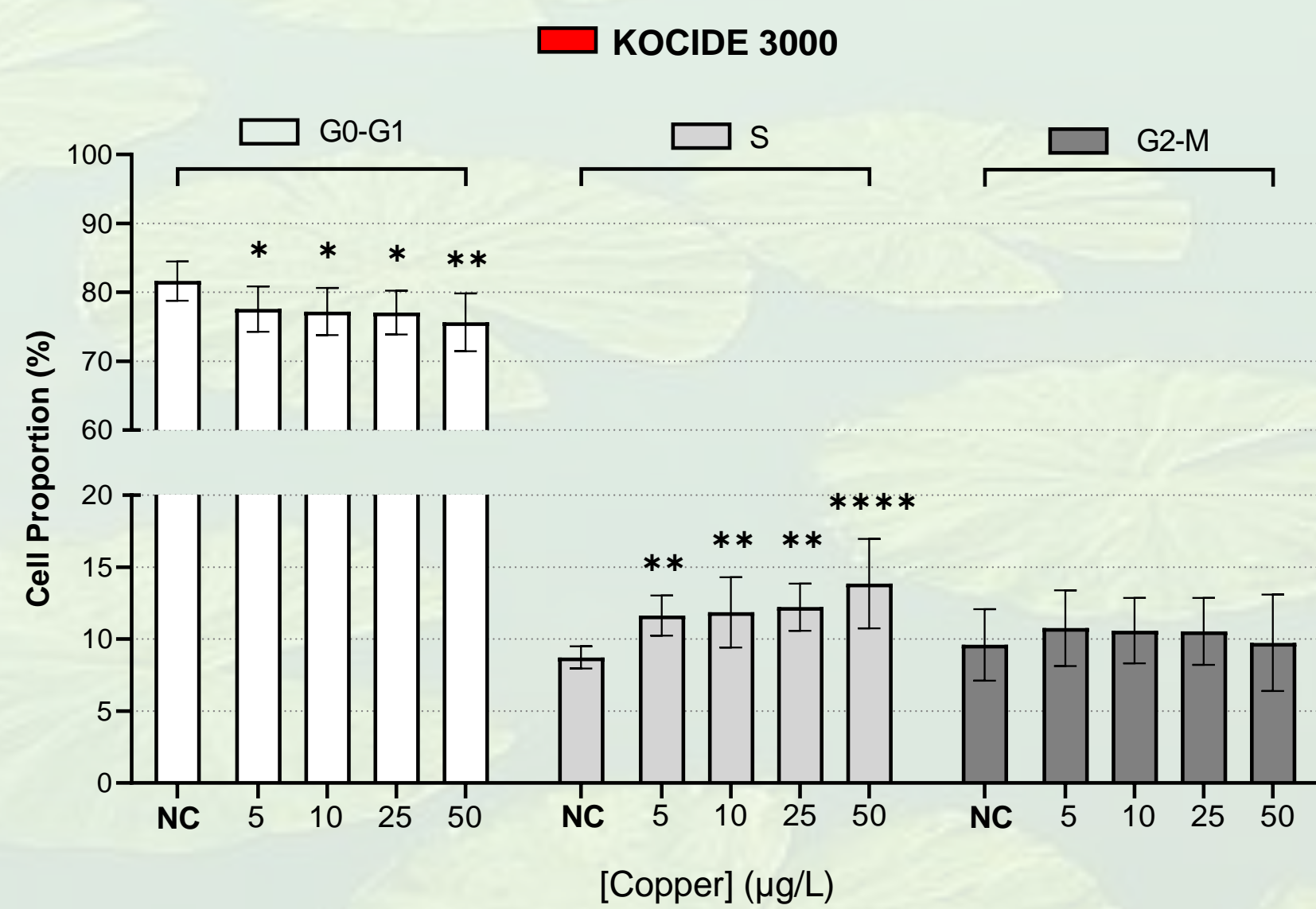


Cell Cycle Analysis

All concentrations induced an increase in the proportion of cells in the S-phase, with a concomitant decrease in G0/G1 percentage

Slowdown or partial Blockade of the S-phase: DNA replication stress or early genotoxic effects

All copper formulations showed the same response pattern



Micronucleus Assay

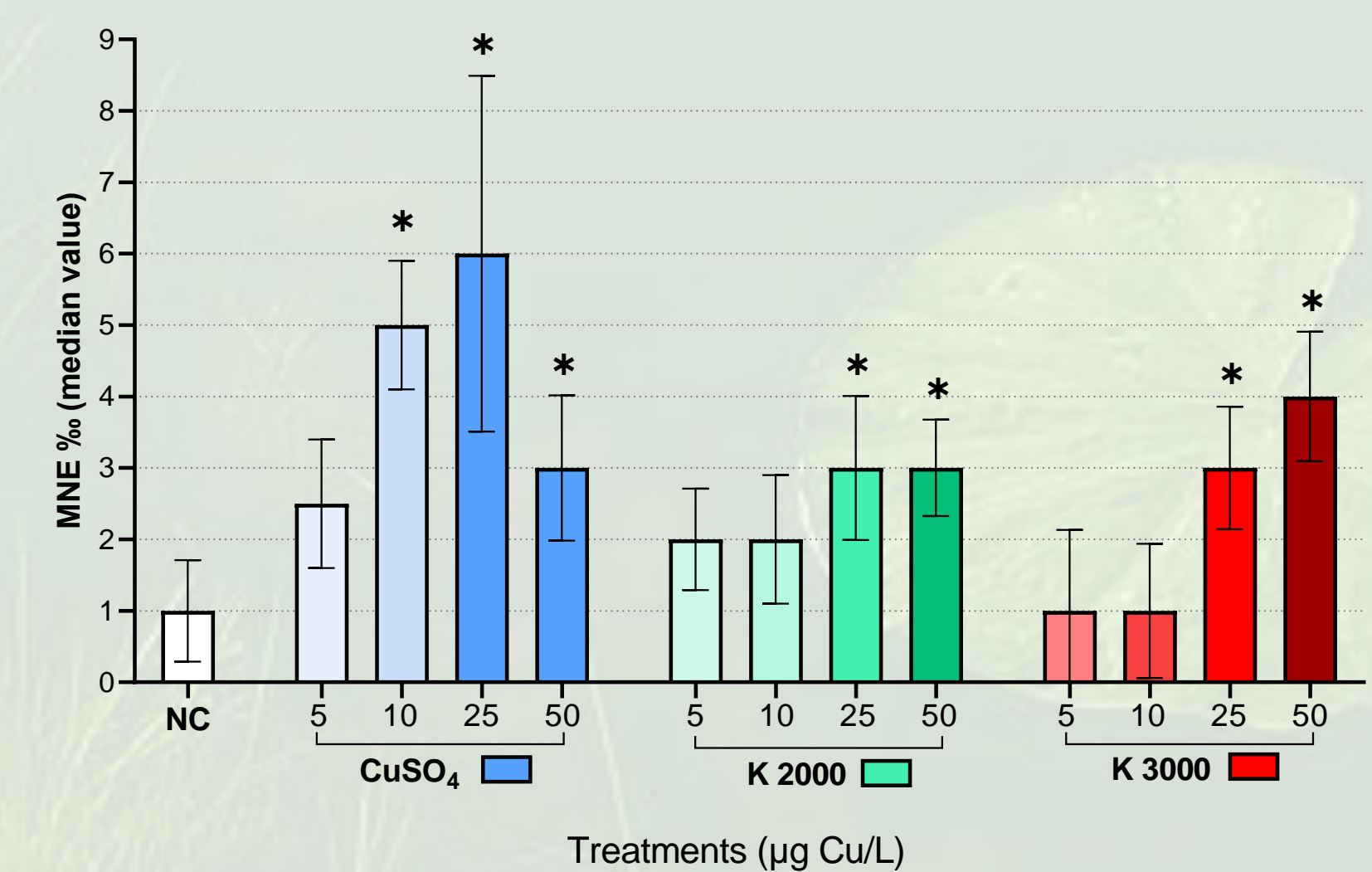
Significant induction of micronuclei

Similar results with copper exposure toward fish [5]

Increased sensitivity to CuSO₄ (effects at 10 µg Cu/L)

Genotoxic Compounds

No difference between Kocide 2000 and Kocide 3000 at equivalent concentrations

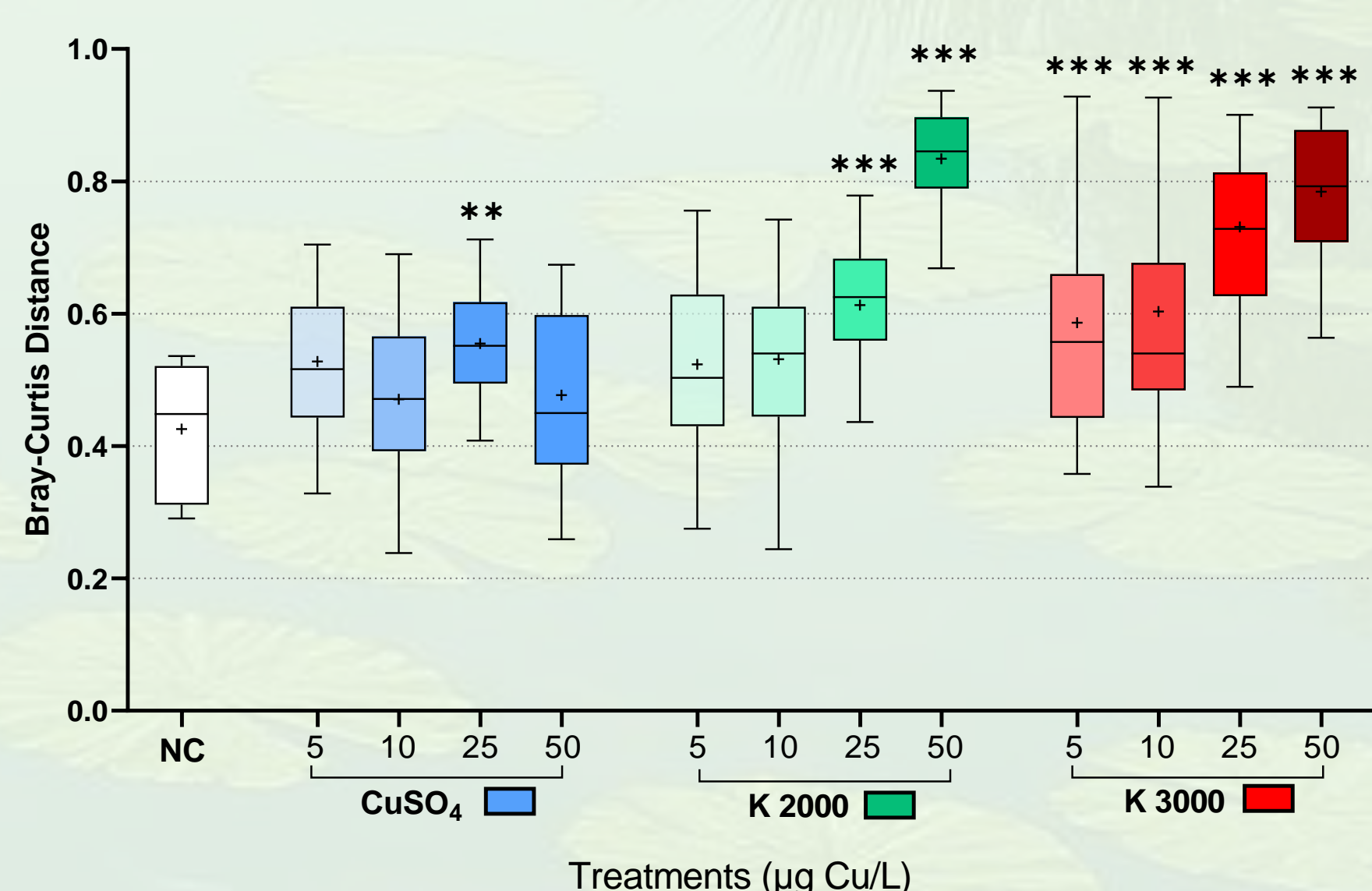


Gut Microbiota

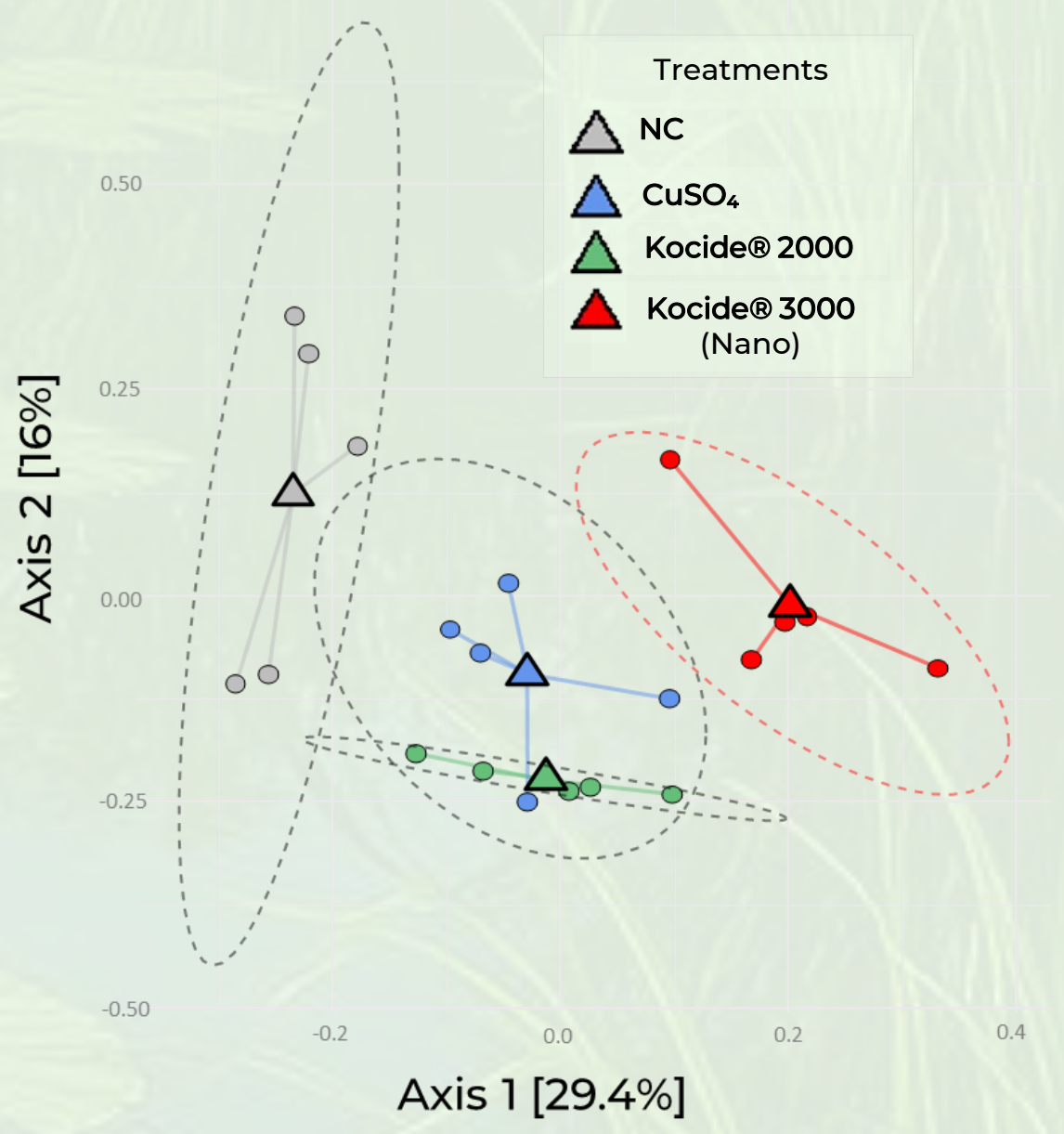
Alpha Diversity

No significant difference on alpha diversity indexes between negative control and exposed conditions (Chao1, Shannon, Simpson)

Beta Diversity



PCoA - Bray Curtis - 25 µg Cu/L

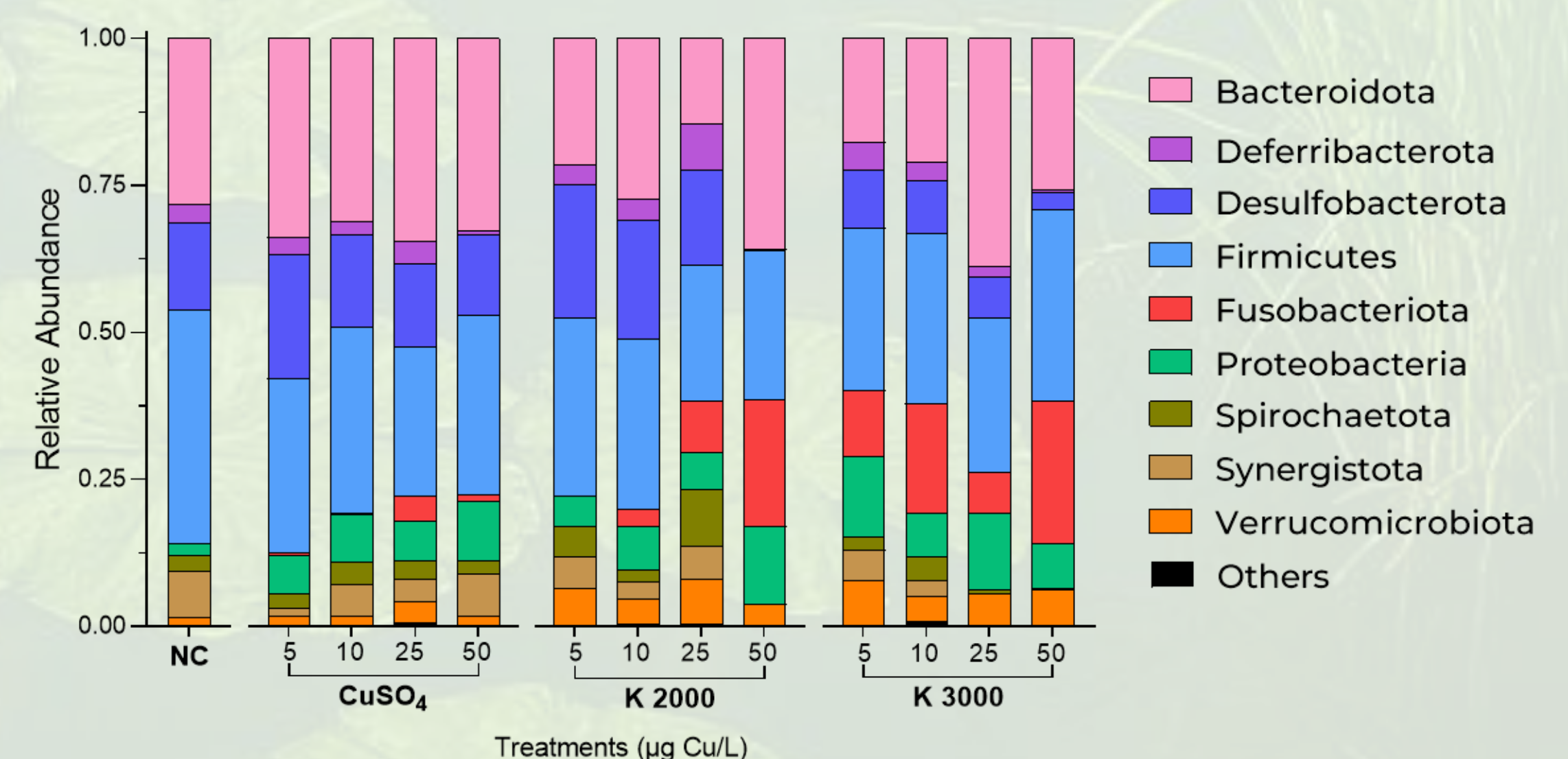


Bray-Curtis distances suggest changes in the structure of the gut microbial communities

Only Kocide 3000 (nano formulation) had a significant impact at all tested concentrations

PCoA revealed an exacerbated impact of Kocide 3000 compared to the non nanosized equivalent and copper control (Permanova, p < 0.05)

Microbiota Composition



Changes on the relative abundance at the phylum level occurs in exposed conditions compared to negative control. Dominant phyla are impacted by pollutants, benefiting to minor phyla [6]

An increase in Fusobacteriota is frequently observed during dysbiosis, notably in various aquatic organisms [7,8]

Conclusion

• Exposure to environmentally relevant concentrations highlights potential risks to aquatic ecosystems

• Standard toxicity endpoints revealed comparable effects of the commercial formulations. However, gut microbiota exhibited distinct responses between the nanopesticide (Kocide 3000) and its non-nanosized equivalent across all tested concentrations.

• Our findings emphasize the need for a holistic approach integrating host-microbiota interactions in ecotoxicological assessments