

1. The study asks how the arbuscular mycorrhizal fungus *Rhizophagus irregularis* affects mercury (Hg) accumulation, translocation, and tolerance in the plant *Medicago truncatula*. Because Hg and Zn share similar chemical properties, literature suggest that they may compete for the same uptake pathways. This lead the authors to investigate the ways in which *R. irregularis* can improve the plant's Zn nutrient status and regulate Zn transporter genes under Hg exposure.
2. Their hypothesis was not clearly expressed but they want to know if *R. irregularis* improves the tolerance of *M. truncatula* to Hg. They ask 3 main questions to answer this:
 - a. Does *R. irregularis* affect biomass and Hg accumulation of *M. truncatula* under Hg exposure?
 - b. How does Hg translocate across roots, stems, and leaves of *M. truncatula* associated with *R. irregularis*?
 - c. What effects does *R. irregularis* have on Zn nutrient uptake and Zn transporters under Hg exposure?
3. To test these hypotheses, the authors measured Hg and Zn concentrations in different plant parts (roots, stems, leaves), determined the Hg tolerance index of the plants, assessed the levels of Zn transporter gene expression (ZIP2, ZIP6), and evaluated mycorrhizal colonization in plant roots. Their experiment was a 2×3 complete factorial design, with 5 replications per treatment arranged in a randomized block design in a greenhouse with about 30 plants. The treatments were a single dose of 5 ml HgCl₂ at concentrations of 25 $\mu\text{g g}^{-1}$ or 50 $\mu\text{g g}^{-1}$ per plant. Observations lasted 5 weeks.
4. A mixed model two-way ANOVA to assess the main effects of Hg treatment and *R. irregularis* inoculation, as well as their interaction.
T-tests and one-way ANOVA with Tukey's *a priori* test for Zn and Hg content and concentration in plants.
Spearman's rho for correlations.
Their findings supported the hypotheses, showing that *R. irregularis* enhanced Hg tolerance, affected Hg partitioning, and upregulated Zn transporter genes under different Hg concentrations.
5. I believe that the the statistical tests were appropriate. The two-way ANOVA is accurate for examining the interactions between multiple factors (Hg treatment and AM inoculation). The t-tests allowed for specific comparisons between inoculated and non-inoculated plants at each Hg level, and the correlation analysis effectively examined relationships between Zn and Hg levels, further validating their conclusions. Some areas of concern for me are that their data was non-normal. They state having done Box-Cot transformation but that their figures represent the non-normal data... The experiment was also quite short and done on a quite small population.