Arsenic lab trial

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Abstract:

Arsenic is a metalloid widely distributed in the environment and recognized for its high toxicity and carcinogenic potential. In agricultural soils, arsenic contamination may compromise essential biological processes related to ecosystem functioning and crop productivity. This study aimed to evaluate the effects of realgar (As_2S_4) and arsenic trioxide (As_2O_3) on microbiological, biochemical, and ecological indicators of soil, as well as on plant growth–promoting microorganisms and soybean seed germination. Controlled experiments were conducted with increasing doses of arsenic applied to incubated soil, followed by the determination of microbial respiration, microbial biomass carbon, metabolic quotient, enzymatic activities, and bacterial diversity analyses through sequencing of the 16S rRNA gene. In addition, biological assays with beneficial bacteria and seed germination tests were performed. The results showed that arsenic increased microbial respiration and the metabolic quotient, accompanied by reductions in microbial biomass carbon and carbon use efficiency, indicating physiological stress in the soil microbiota. Enzymatic activities, particularly dehydrogenase and phosphatase, were significantly reduced, demonstrating a direct impact on biochemical processes associated with nutrient cycling. Experiments with microorganisms revealed high toxicity of As_2O_3, with *Bradyrhizobium japonicum* being more sensitive than *Azospirillum brasilense*, suggesting a potential reduction in the efficiency of microbial bioinoculants in contaminated soils. In germination assays, a progressive reduction in germination percentage and an increase in morphological abnormalities of soybean seedlings were observed with increasing arsenic doses. Furthermore, trivalent arsenic reduced bacterial diversity in the soil, highlighting the potential risk associated with the application of fertilizers contaminated with arsenic to soil microbial communities. Overall, the findings demonstrate that arsenic exerts multiple impacts on soil microbiota, affecting microbial and biochemical properties, microbial diversity, and the performance of beneficial microorganisms used as agricultural bio inputs.

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Introduction:

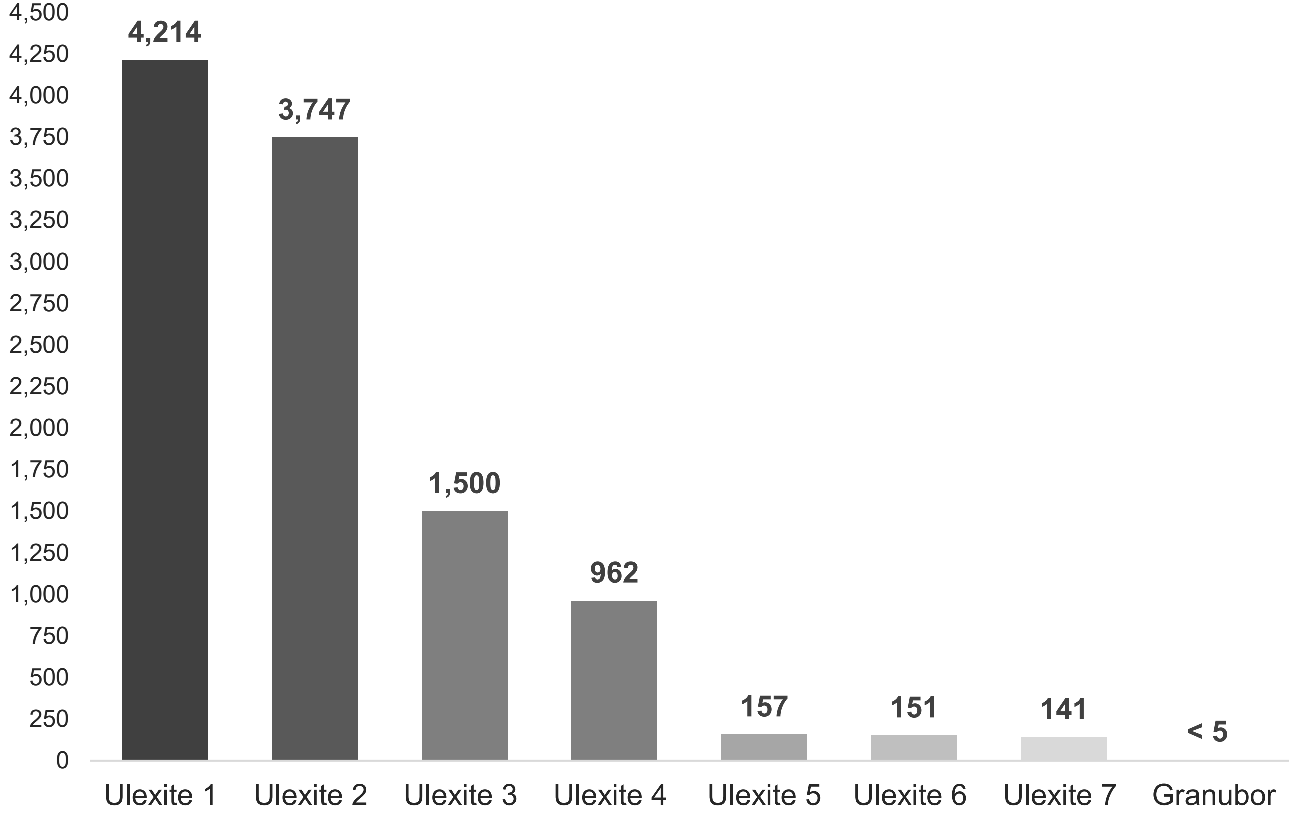
Arsenic is a metalloid widely distributed in the environment and recognized for its high toxicity and carcinogenic potential. The toxic effects of this metalloid on organisms vary according to its concentration, chemical form, and bioavailability. In natural environments, arsenic occurs predominantly in two redox states: the trivalent form (As^{3+}) and the pentavalent form (As^{5+}). Among these species, As^{3+} exhibits greater mobility and is considered more toxic to biological systems. Excessive and frequent exposure to As^{3+} may lead to its accumulation in cells, consequently promoting the denaturation of proteins and nucleic acids. Soil contamination by arsenic can trigger a series of direct negative impacts on soil functionality and the composition of microbial communities. These impacts may lead to the loss of soil quality and soil health, compromising the sustainability of agricultural systems and causing adverse effects in other ecosystems, including fauna such as insects and animals. In China, there is significant concern regarding soil contamination in irrigated rice cultivation areas due to evidence that arsenic can accumulate in rice grains and be transferred through different levels of the food chain, being associated with diseases in humans, including several types of cancer. In Brazil, the agricultural production chain uses various fertilizers that may contain significant concentrations of arsenic. The continuous application of these inputs over the years may increase the accumulation of this element in the soil, reaching levels that are toxic to microbial activity and microbial community diversity. Among fertilizers used as a source of boron, ulexite stands out and is widely applied in crops such as soybean, coffee, eucalyptus, and vegetables. However, ulexite may contain mineral impurities originating from the extraction process, including arsenic-bearing minerals such as realgar (As_2S_4). Despite this potential risk, reports on arsenic toxicity in these crops remain scarce, highlighting the need for studies that assess the risks of soil contamination and food chain transfer in agricultural systems.

Arsenic content in borate fertilizers

Boron (ulexite) + realgar (As_2S_4)

Realgar (As_2S_4)

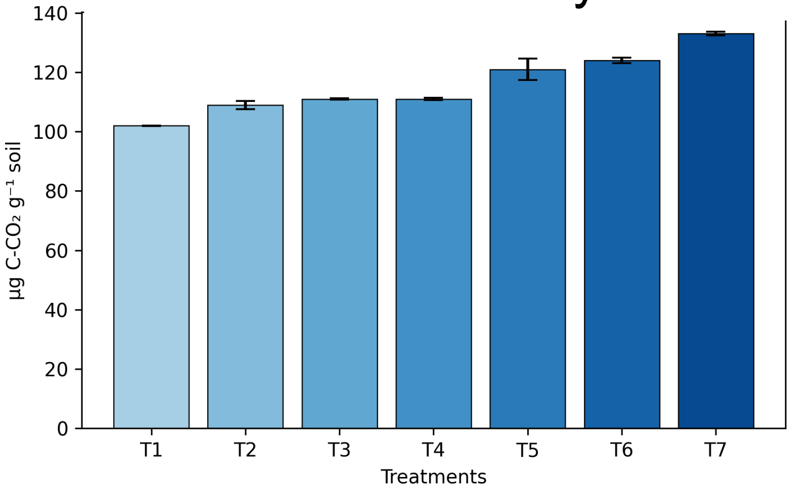
Arsenic content (ppm) of boron fertilizers



Source: Sample testing performed at the U.S. Borax Quality Labs in Boron, CA, and Denver, CO, USA; and Fundacão ABC Lab in Castro, PR, Brazil

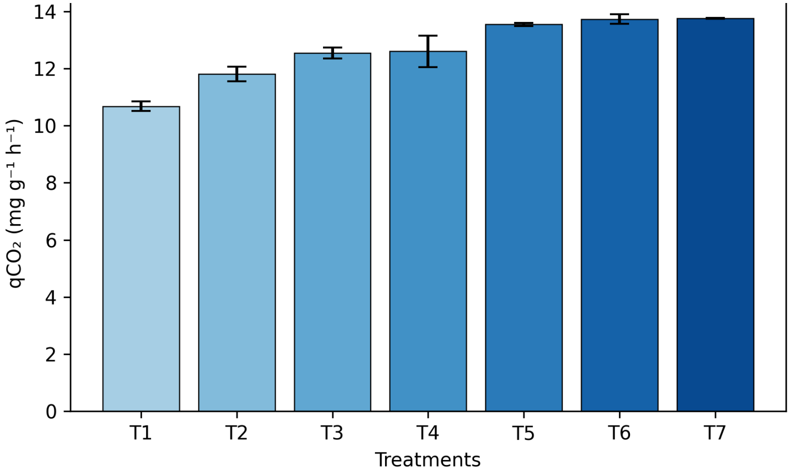
Effect of realgar (arsenic sulfide) on soil microbial indicators

Soil microbial activity



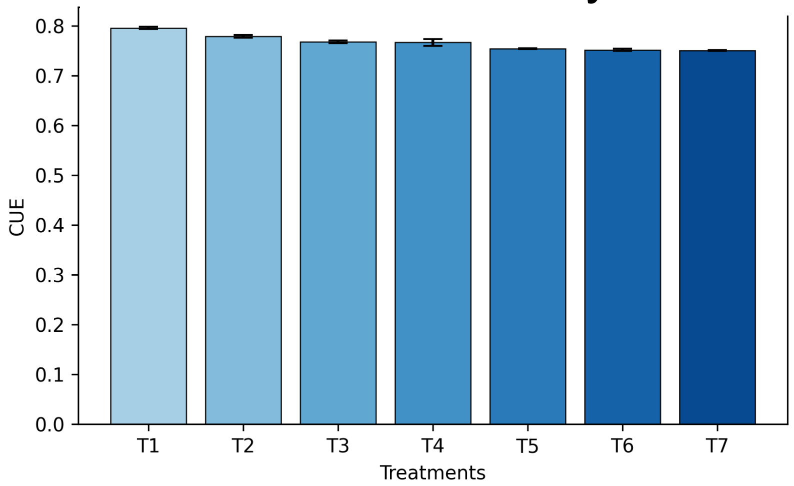
The addition of realgar in the soil increased soil respiration significantly demonstrating that As results in an increase in the soil respiration due to the toxic effect of the element on microorganisms.

Soil metabolic coefficient



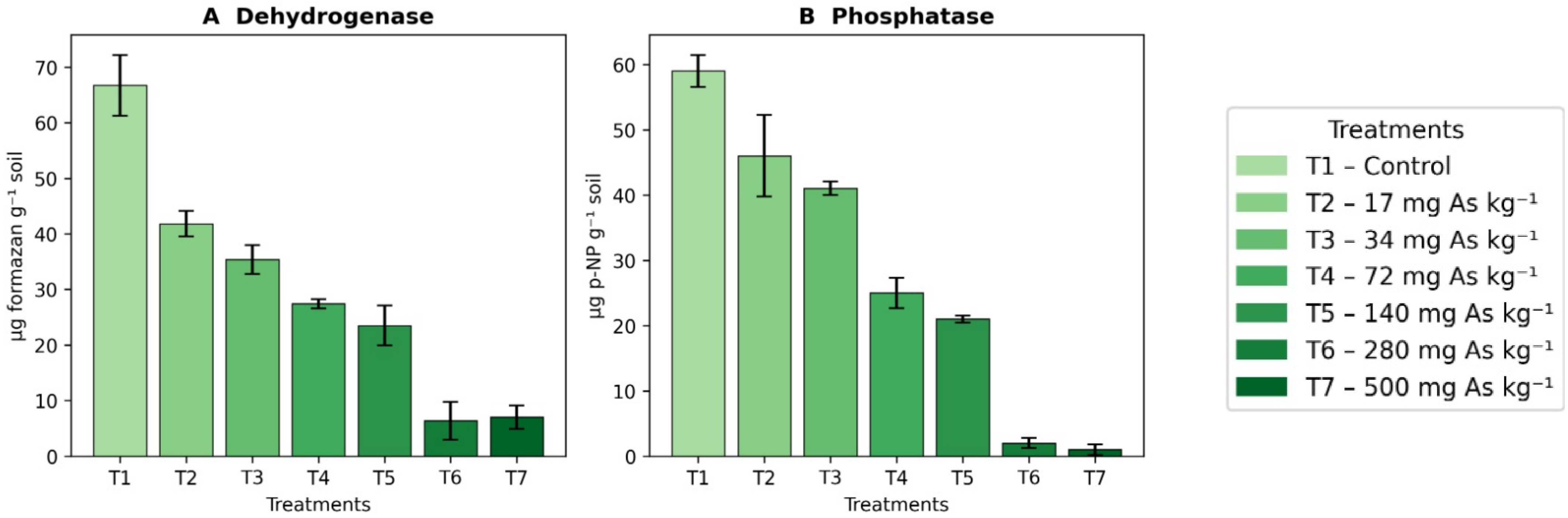
The results show that soil metabolic coefficient (qCO_2) increased significantly with realgar application, demonstrating metabolic stress.

Carbon use efficiency



The addition of realgar also has an effect on the soil carbon use efficiency by microorganisms. Soil carbon use efficiency is also associated with the formation of organic substances in the soil, such as humic acids.

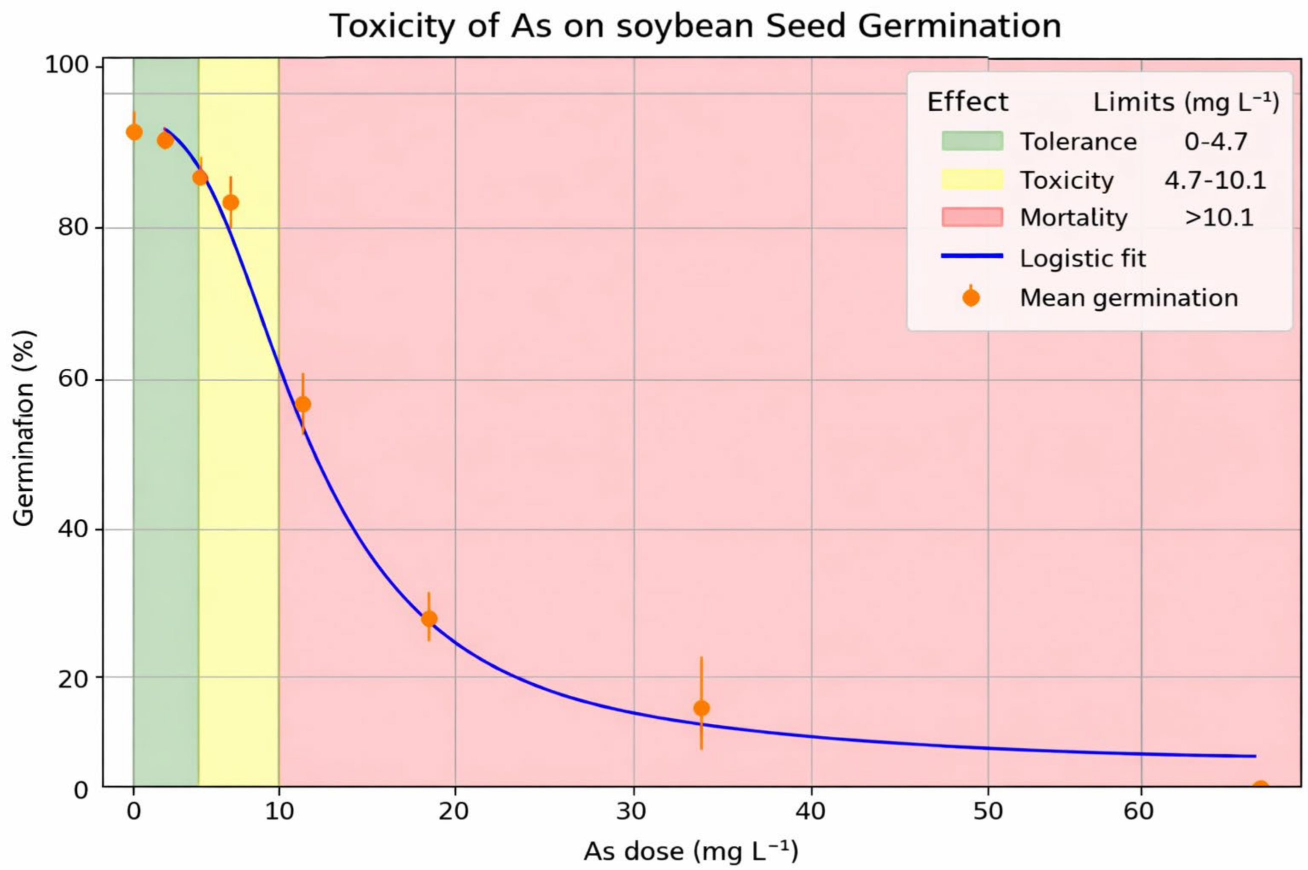
The impact of realgar on the biosynthesis of soil enzymes by microorganisms



The activities of dehydrogenase and phosphatase in the soil were evaluated at the end of a 21-day arsenic assay, using realgar as the contaminant source. These enzymes were selected because they are important biochemical indicators of carbon transformations and are directly associated with nutrient cycling in the soil.

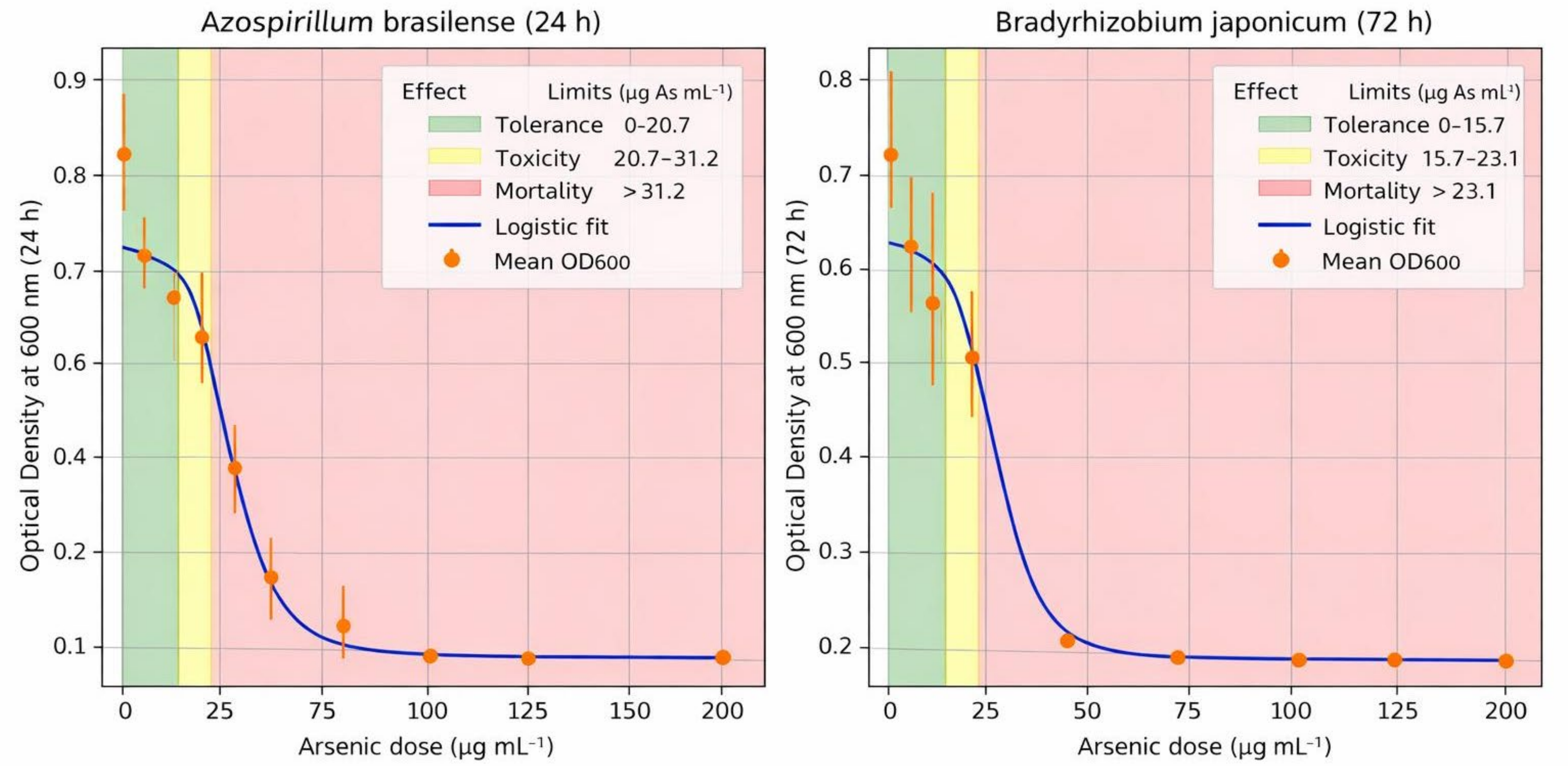
The results demonstrated that the addition of realgar reduced dehydrogenase activity in the soil (Figure A), with this reduction being proportional to the dose of arsenic applied. The estimated decrease ranged from 37 to 90% compared to the control treatment with soybean extract. This enzyme is an important metabolic indicator, associated with the oxidation and transformation of organic carbon in the soil, reflecting intracellular processes of the microbiota. Furthermore, dehydrogenase activity is widely used as an indicator of the metabolically active microbial fraction of the soil. The results show that the addition of arsenic, in the form of realgar, impacts microbial metabolism even at the lowest dose applied (17 µg As g^{-1} of soil). Phosphatase activity (Figure B) was also significantly affected by the application of realgar to the soil. This observed effect may represent a risk of functional loss in the mineralization and cycling processes of organic phosphorus in the soil.

Arsenic trioxide (As_2O_3) toxicity in soybean seed germination



The toxicity of As_2O_3 on germination was significant and showed a typical dose-dependent response curve, allowing the determination of tolerance, toxicity, and mortality ranges of the seeds. The tolerance limit to As_2O_3 was estimated at values below 4.7 mg L^{-1}. The toxicity range was between 4.7 and 10.1 mg As L^{-1} (ED50), while doses above 10.1 mg As L^{-1} resulted in a marked increase in seed mortality. In addition to the reduction in germination, the doses classified as toxic also affected seedling morphology—promoting significant abnormalities in root development and hypocotyl axis.

Arsenic trioxide (As_2O_3) toxicity in *Azospirillum brasilense* and *Bradyrhizobium japonicum*



The results demonstrated that As_2O_3 showed high toxicity to both bacteria evaluated, fitting the log-logistic model of toxicity analysis. The tolerance limits were 20.7 µg As mL^{-1} for *A. brasilense* and 8.2 µg As mL^{-1} for *B. japonicum*. The toxicity range was relatively narrow for both species, with functional mortality observed above 31.2 µg As mL^{-1} for *A. brasilense* and 17.6 µg As mL^{-1} for *B. japonicum*.