Copper-based nanopesticides: synthesis and application of more sustainable plant protection products

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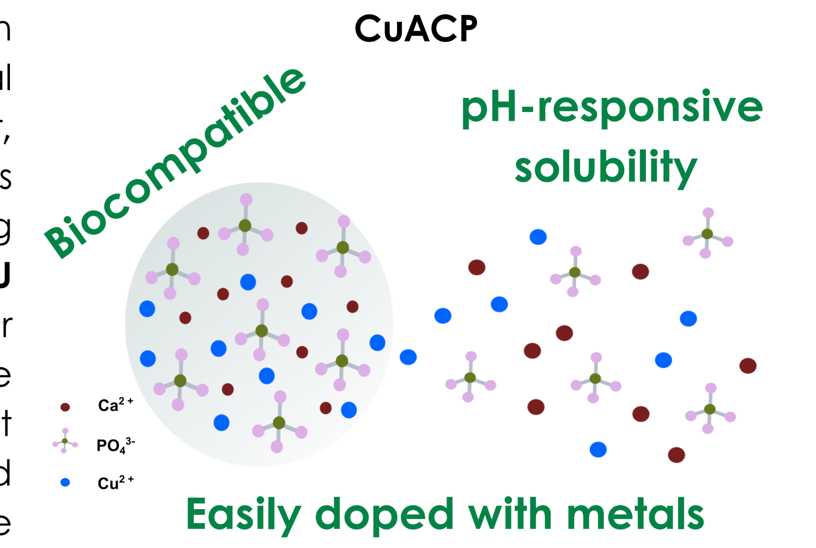
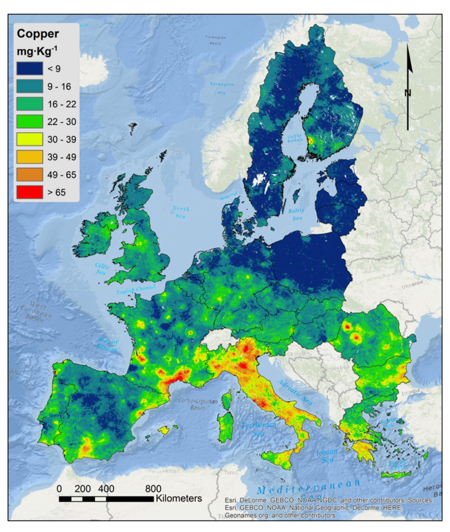
Bioinspired nanocarrier for copper delivery

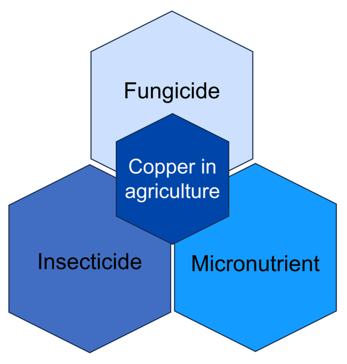
Copper-based compounds are widely applied in agriculture for their fungicidal and insecticidal properties, and as a micronutrient source. However, their **intensive use** and **high soil accumulation** of this metal have led to contamination in several cropping systems.[1,2] In light of these environmental concerns, **EU regulatory bodies** have progressively restricted copper applications in order to foster more sustainable agricultural practices.[3] Consequently, the development of innovative formulations with **enhanced efficacy** and **reduced environmental impact** can support the transition to **more sustainable agriculture**.



Soil accumulation



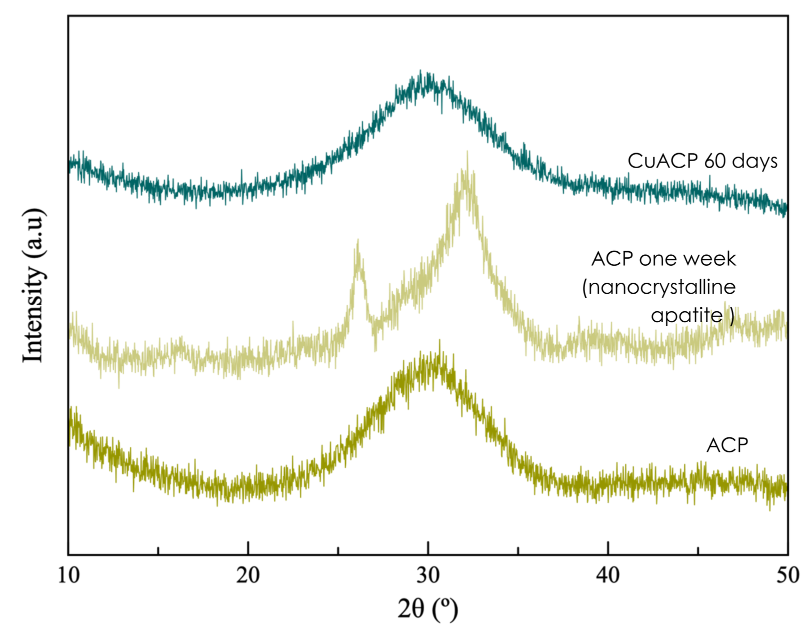
High solubility and poor leaf retention



Law Restrictions

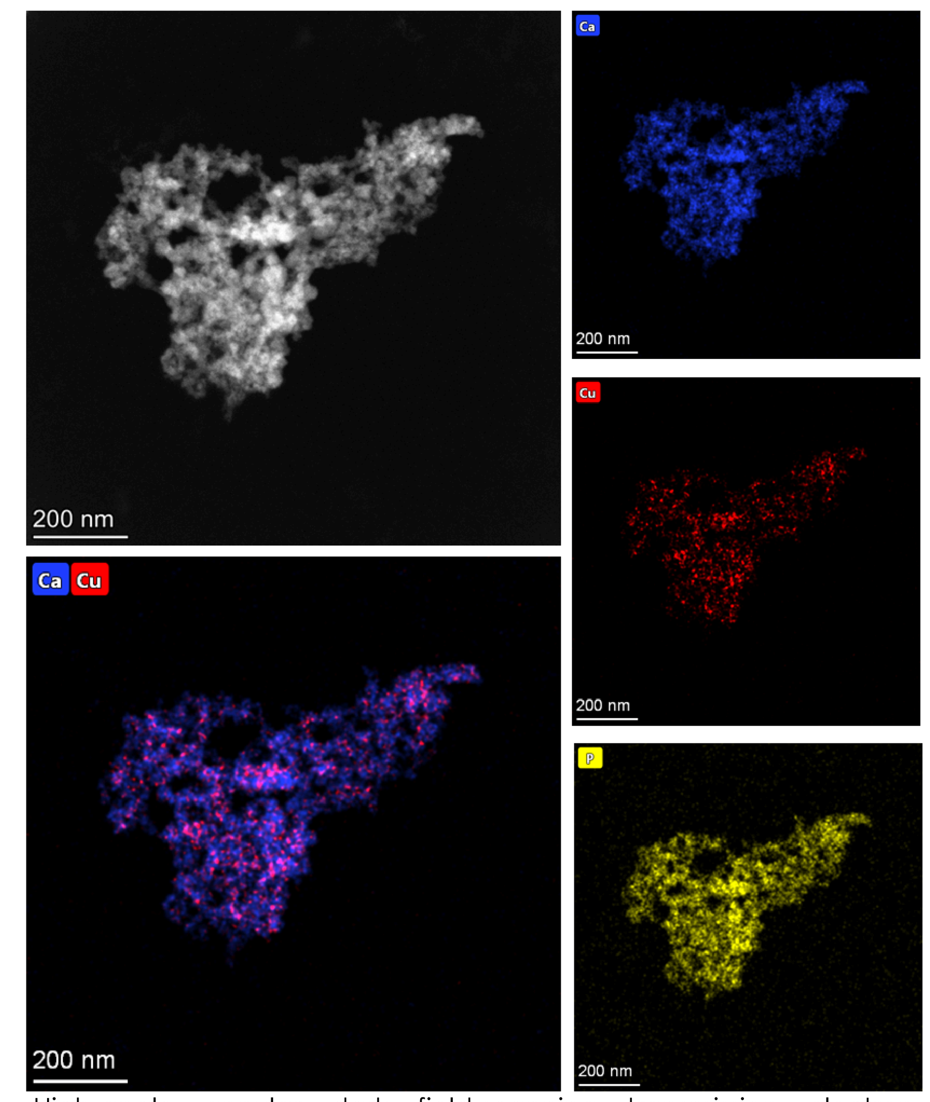
Amorphous calcium phosphate (ACP, $Ca_3(PO_4)_2 \cdot nH_2O$) is precursor in bone hard tissue formation. It presents desireable properties for adsorption and delivery of ions and molecules due to its structure and pH dependent properties.[4] ACP has shown promising results as a **nanocarrier in agriculture**, including applications such as **biostimulants** based on methyl jasmonate[5] or **biofortification** and **protection** through zinc delivery. With copper doping, the aim is to explore its effects in fungal control and nutrition.

Characterization



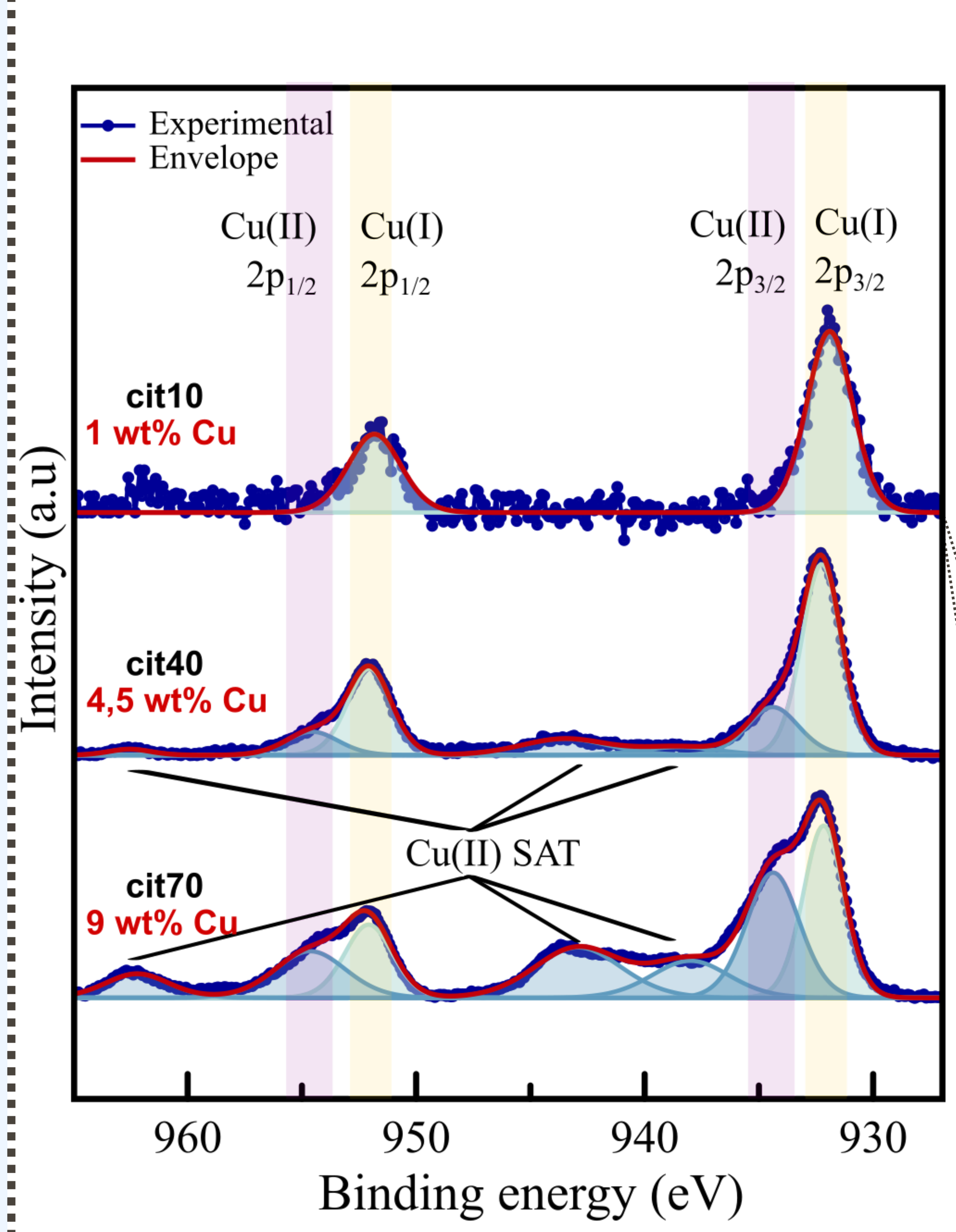
Different concentration of copper were used for the synthesis. **Powder X-ray diffraction** patterns of all CuACP confirmed the synthesis of the **amorphous** form. Moreover, copper stabilized the amorphous phase for more than 6 months, and no crystalline coprecipitation was observed.

TEM microrpahs and EDS elemental maps of CuACP showing homogeneous Cu (red) distribution within the nanoparticle, along with Ca (blue) and P (yellow) of ACP.



High-angle annular dark field-scanning transmission electron microscopy (HAADF-STEM) images and energy-dispersive X-ray (EDS) elemental maps

XPS analysis

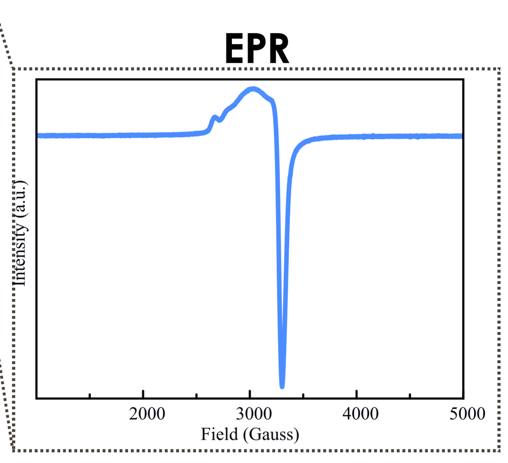


*paper submitted

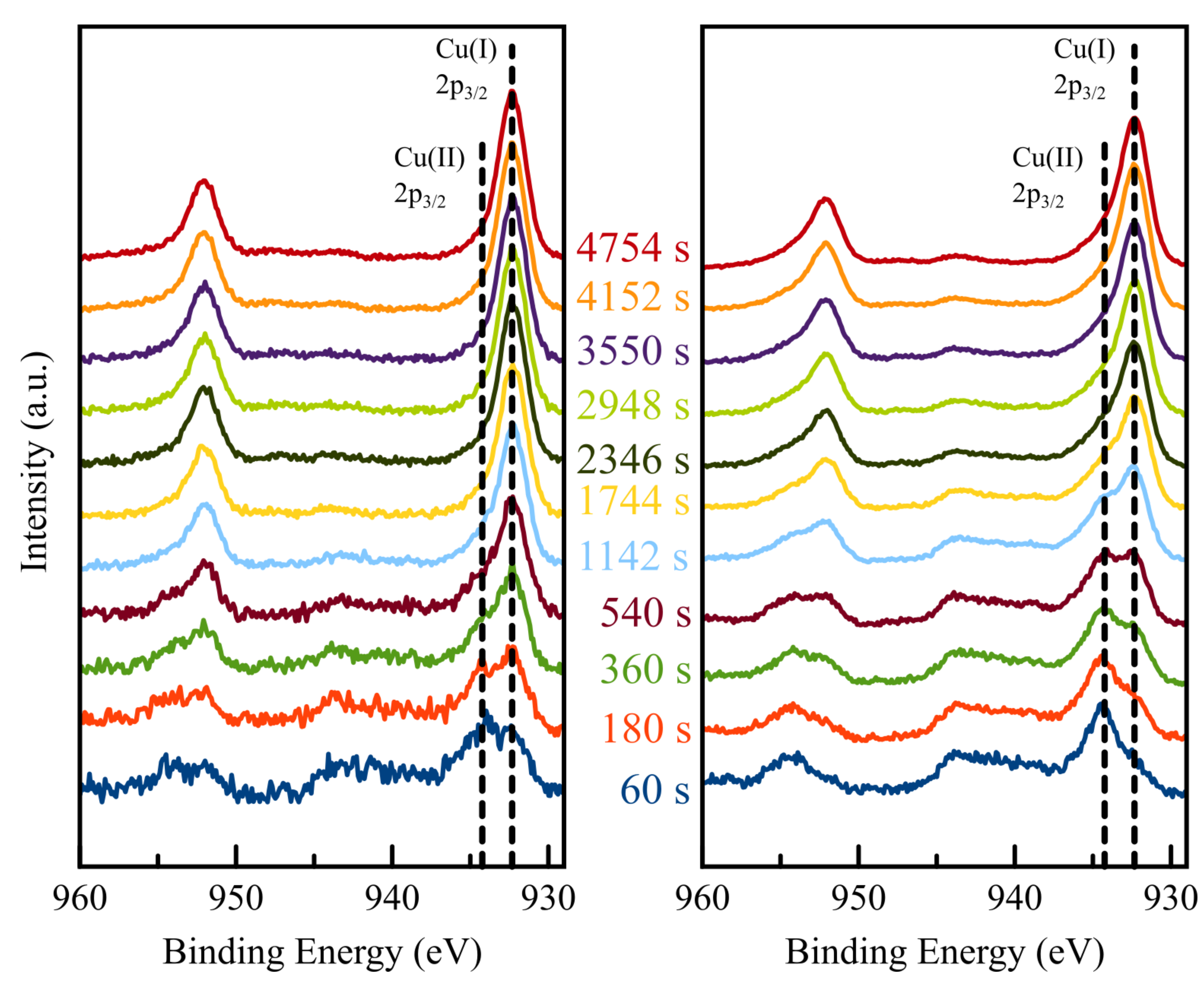
X-ray Photoelectron Spectroscopy (XPS) was employed to investigate the oxidation state of copper in the synthesized materials. In all samples copper a partial or even complete **conversion of Cu^{2+}** (used as reactant) **to Cu^{+}** was observed.

Several **hypotheses** were considered: i) reduction of copper by reactants, ii) precipitation of amorphous by-products, or iii) an artefact of the analysis (i.e., beam-induced surface reduction).

After eliminating potential reducing agents from the synthesis and still observing the same conversion, study of EPR evidenced the presence of Cu(II) in all samples. Thus, time-resolved XPS under controlled X-ray exposure was carried out to investigate **beam-induced reduction** hypotesis, confirming the progressive reduction of copper with increasing exposure.* Therefore, cit70 (9 % Cu) was selected for following experiments.



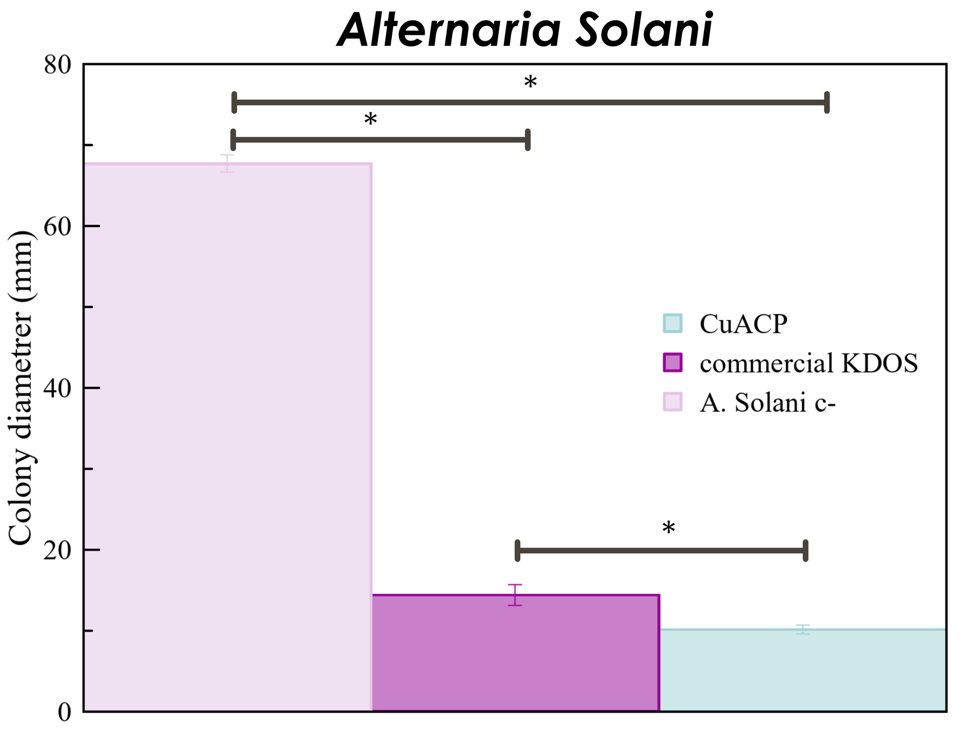
Electron Paramagnetic Resonance (EPR) of cit10. Since Cu(I) is EPR-silent, no signal was expected; however, a clear signal was detected, supporting the beam-induced reduction hypothesis.



Time resolved XPS under controlled exposure times. On the left **cit40** sample and **cit70** on the right. Notably, in the sample with the lowest copper amount, the conversion was immediate, preventing any time-dependent monitoring.

Antimicrobial tests

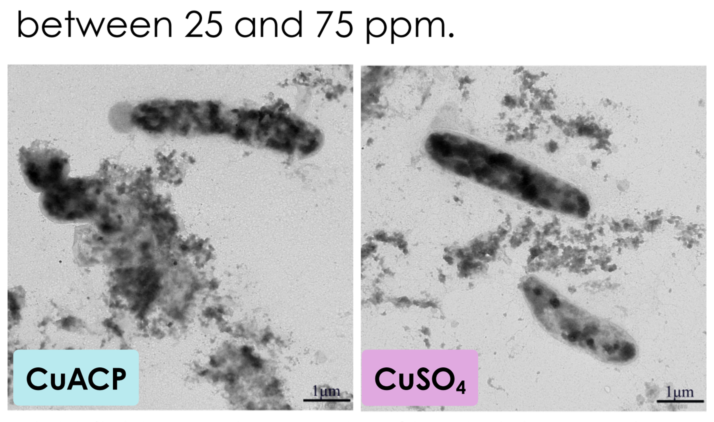
Alternaria Solani



Colony diameter after 8 days. Statistics: one-way ANOVA with Bonferroni post hoc test when $p < 0.05$.

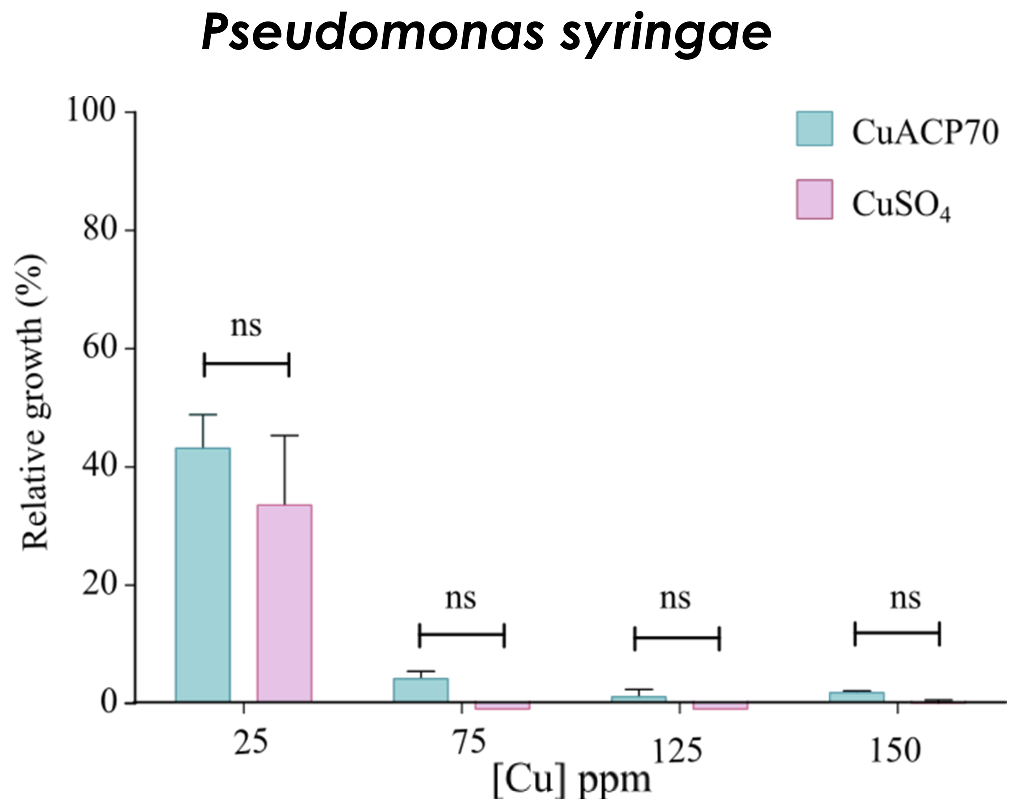
In vitro assays against fungus ***Alternaria solani*** demonstrated that, at 200 ppm of copper, CuACP provided a **stronger inhibition of mycelial growth** compared to the commercial copper hydroxide formulation (KDOS), which showed significantly different control of the pathogen.

In the case of bacterium ***Pseudomonas syringae***, the antibacterial activity was evaluated across increasing copper concentrations and compared with copper sulfate. Both products displayed **similar control of bacterial growth**, with IC_{50} values estimated between 25 and 75 ppm.



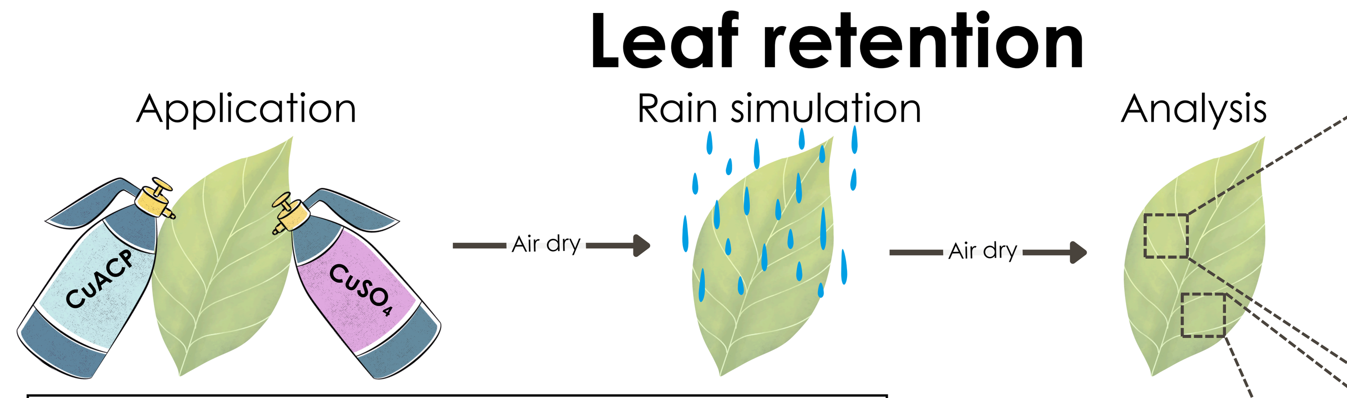
Bright-field TEM micrographs of bacterial suspension after 24 hours of contact with treatement.

Pseudomonas syringae

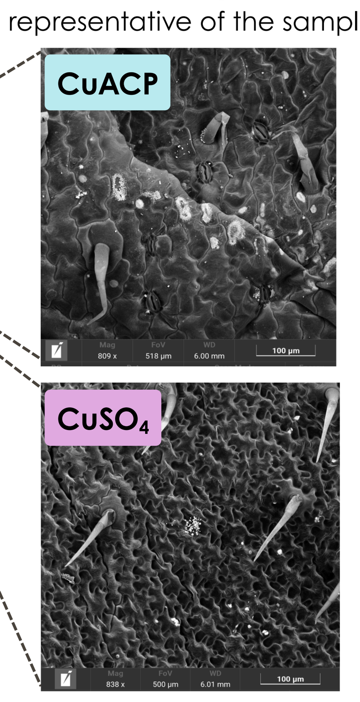


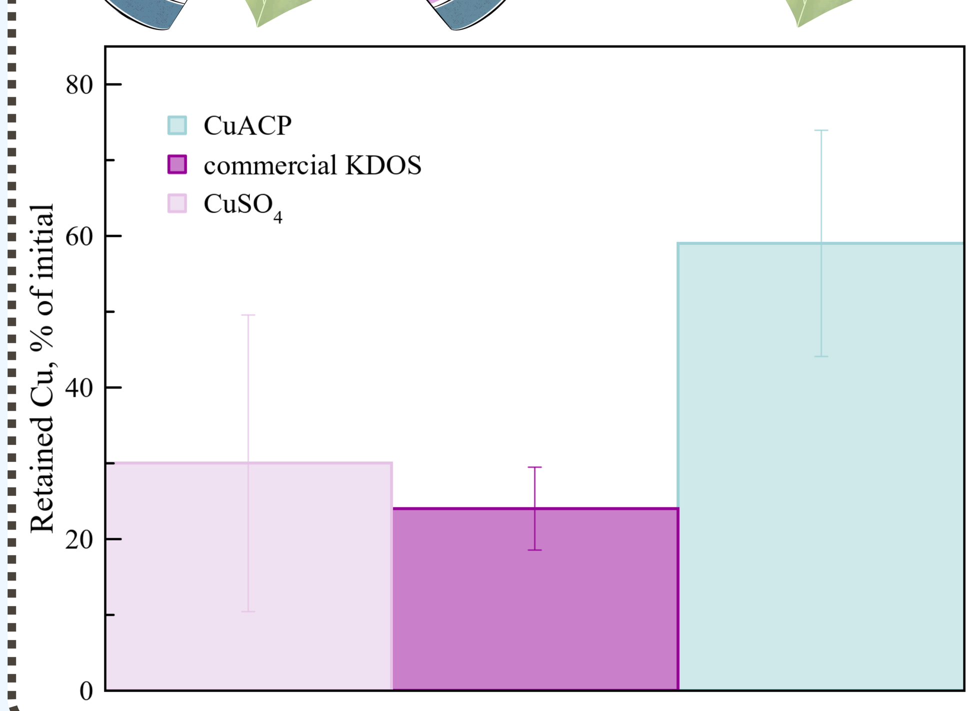
Antimicrobial activity was measured using MTS assay. Statistics: two-way ANOVA with Bonferroni post hoc test when $p < 0.05$.

Leaf retention



SEM micrographs representative of the sample





Leaf retention was tested on tomato leaves and assessed with **ICP-OES**. The results showed that, after rain simulation, leaves treated with CuACP, **retained 60% of the copper** initially present, compared to commercial $CuSO_4$ and KDOS, which retained 30% and 24%, respectively.

Conclusions

This work presents promising results for a new copper-based nanomaterial. Solid-state analysis confirmed the presence of 9% wt Cu(II) in the amorphous nanomaterial, while antimicrobial tests show increased in vitro efficacy of CuACP against *A. solani* and equivalent control of *P. syringae*, compared to conventional products. Planned **greenhouse experiments will** soon be conducted to **integrate in vitro and leaf retention results,** which so far suggest enhanced performance for CuACP. Finally, soil **ecotoxicity** studies will be carried out to assess the environmental impact of CuACP nanoparticles.

Acknowledgements

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Contact me!

I will be happy to hear your thoughts

References

(1) Ballabio, C. et al., 2018. Copper distribution in European topsoils: An assessment based on LUCAS soil survey. Science of the Total Environment, 636: 282-298 (2) Chengpeng S., 2024, Science of The Total Environment, 926, 171948. (3) Food Safety Authority, E. Review of the Existing Maximum Residue Levels for Copper Compounds According to Article 12 of Regulation (EC) No 396/2005. EFSA Journal 2018, 16 (3), e05212. (4) Ramírez-Rodríguez, G. B. et al. Engineering Biomimetic Calcium Phosphate Nanoparticles: A Green Synthesis of Slow-Release Multinutrient (NPK) Nanofertilizers. ACS Appl. Bio Mater. 3, 1344–1353 (2020). (5) Parra-Torrejón, B.; Ramírez-Rodríguez, G. B.; Giménez-Bañón, M. J.; Moreno-Olivares, J. D.; Paladines-Quezada, D. F.; Gil-Muñoz, R.; Delgado-López, J. M. Nanoelicitors with Prolonged Retention and Sustained Release to Produce Beneficial Compounds in Wines. Environ Sci Nano2021, 8 (12), 3524–3535.