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X-rays shed light on enhancing zinc uptake in pepper plants

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Zinc is an important trace element for plants and animals alike. Learn how nanoparticles could supply zinc to crops without having to add it to the soil.

The following was adapted from an ESRF News article.

Why is zinc important?

Zinc (Zn) is a vital element for animals, including humans. It is essential for growth and health and we get it from our diet. But where does the zinc in food come from? Plants also require zinc to grow, and since they don't eat, they must get it from the soil. In farming, zinc-deficient soil significantly impacts crop quality, causing yellowing of the leaves, stunted growth, and small or deformed leaves. This means that it is often necessary to use zinc fertilizers. However, conventional methods of adding zinc to the soil result in low zinc uptake by the plants, and increase the risk of soil and groundwater pollution.[1]

Figure 1. Maize plants with severe zinc deficiency in the foreground, with healthier plants (planted at the same time) in the background.
Image: Alandmanson/Wikimedia Commons, CC BY-SA 4.0

Could nanoparticles be part of the solution?

To address these issues, researchers are exploring the application of zinc-based nanoparticles directly to plant leaves (foliar application) for controlled, sustained zinc delivery without having to add zinc to the soil. This approach has potential, but the mechanisms underlying the uptake of zinc nanoparticles into the leaves (foliar uptake), their transport within the plant, and their transformation along the way are not fully understood. Which routes lead to the foliar uptake of these nanoparticles? In which compartment of the leaf do they accumulate and transform? Do they reach the vasculature and spread to the developing tissues that need zinc the most? In particular, the influence of nanoparticle surface modifications on these processes required further investigation to ensure the best possible uptake.[2]

How can we study this?

An international team of researchers studied the bioavailability of different zinc nanoparticles after they were applied to the leaves of bell pepper (capsicum) plants.[4] The pepper plants were exposed to two types of nanoparticles: bare zinc oxide nanoparticles and zinc oxide nanoparticles coated with a zinc-phosphate shell. They then explored how the nanoparticles interacted with the leaves, from their deposition on the cuticle to their uptake in the interior of the leaf (the epidermis), the mesophyll where most of the photosynthesis takes place, and the vasculature. They also tracked zinc transport from leaf to stem and fruits.

Figure 2. Varieties of *Capsicum annuum*, also called pepper plants (not to be confused with the spice pepper), produce bell peppers and chillies.
Image: Léna/Wikimedia Commons, CC BY 3.0

To track the fate of the zinc nanoparticles in the plants, leaf and stem samples were collected two hours and one week after leaf application. Small discs were punched out of the leaves (figure 4 b), then the samples were placed in a special resin and flash frozen in liquid nitrogen to preserve them (figure 4 c). Very thin cross-sections just 20 µm thick (finer than a human hair) were then cut using a precision cutting machine called a microtome.

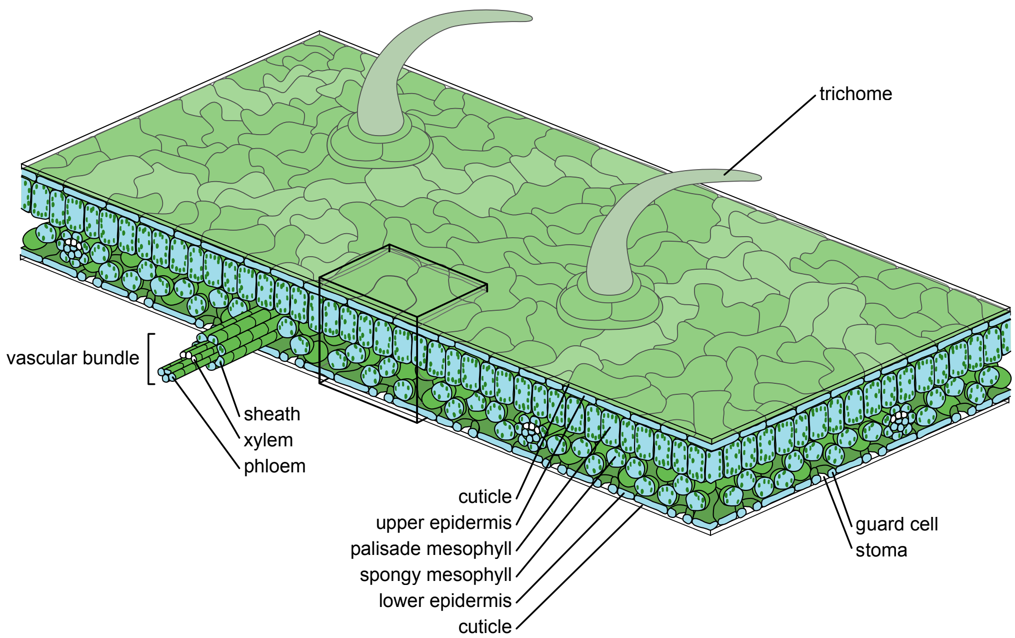


Figure 3. The structure of a leaf showing the major tissue regions: the upper and lower epithelia (and associated cuticles), the palisade and spongy mesophyll and the guard cells of the stoma. Vascular tissue (veins), made up of xylem, phloem, and sheath cells, and example trichromes are also shown. The green spots within the cells represent chloroplasts and indicate which tissues undergo photosynthesis.
Image: Zephyris/Wikimedia Commons, CC BY-SA 3.0

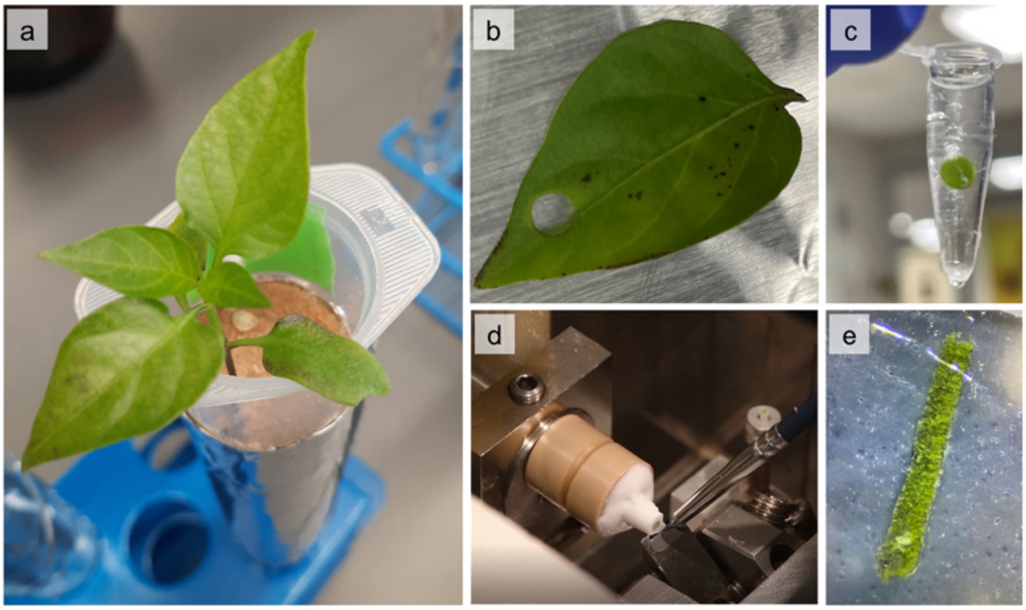


Figure 4: Experimental setup for leaf sampling and cross-sectioning. a) Pepper plant grown in sand matrix, b) punched leaf, c) flash-freezing of the punched sample in 'optimal cutting temperature' resin, d) cryo-sectioning with a microtome, and e) leaf cross-section.
Image from [ESRF News]

These thin samples were then analyzed at the European Synchrotron Radiation Facility (ESRF) in Grenoble to answer two questions: how does the zinc distribute in the plant tissues, and what chemical form does the zinc take (native nanoparticulate form or transformed into other zinc species)? The researchers used two special X-ray techniques: micro X-ray fluorescence (μ-XRF) and micro X-ray absorption near-edge structure (μ-XANES) spectroscopy. μ-XRF collects all the fluorescence signals of each pixel, enabling researchers to construct a map of the elements at each position in the sample. μ-XANES spectroscopy gives information on the atoms surrounding the zinc, which indicates its chemical form. The beamline at ESRF, at which this was done, allows these measurements to be performed with a very small beam size (ca. 500 nm in diameter), which is required to study these very small structures.

Does nanoparticle structure make a difference?

The results revealed clear differences in what happened to the zinc particles in pepper plants and where they ended up depending on how the nanoparticles were designed.

The bare zinc oxide nanoparticles resulted in high zinc accumulation in the lower epidermis cell walls and cytosol of leaves (figure 6), as well as in the stem epidermis one week after exposure. In contrast, plants treated with the nanoparticle that had a zinc phosphate shell showed more zinc accumulation in leaf and stem vasculature, suggesting that uptake in the vasculature was improved for this treatment. These findings indicate that the surface chemistry of the nanoparticles may cause differences in how zinc is distributed, stored, or transformed within the leaves of pepper plants, and offers ideas to make zinc delivery via leaves more efficient.

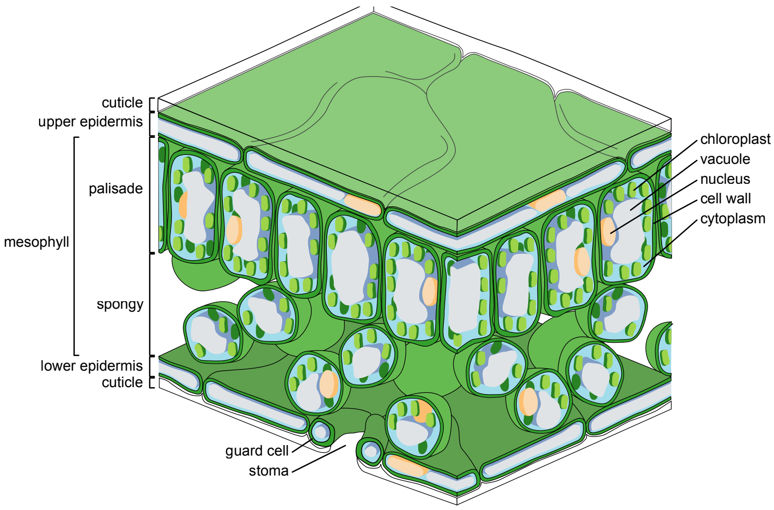


Figure 5. A magnified structure of a leaf showing the major tissues: the upper and lower epithelia (and associated cuticles), the palisade and spongy mesophyll, and the guard cells of the stoma. Vascular tissue (veins) not shown. Key plant cell organelles (the cell wall, nucleus, chloroplasts, vacuole and cytoplasm) are shown.
Image: Zephyris/[Wikimedia Commons], [CC BY-SA 3.0]

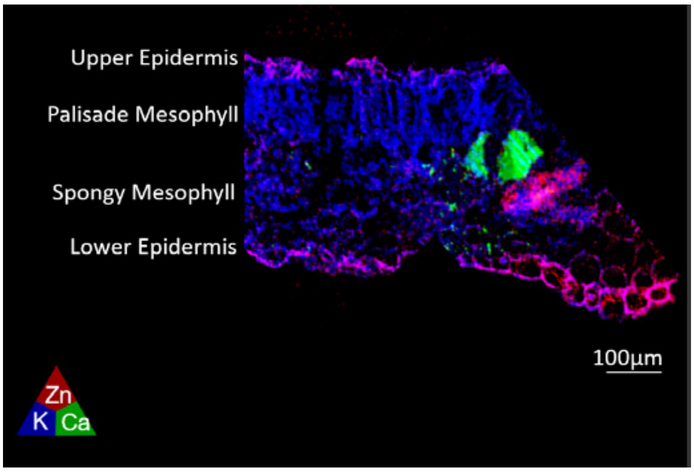


Figure 6. µ-XRF mapping of a pepper plant leaf after exposure to zinc oxide nanoparticles (1 week). This cross section of an exposed leaf shows the locations of potassium (blue), calcium (green), and zinc (pink).
Image adapted from Ref. [4]

The µ-XANES spectroscopy, which was done for different points of interest on each sample, revealed more details of how the plants was using zinc. Firstly, none of the zinc taken up inside the leaf was detected in nanoparticle form, even just 2 h after application, which indicates that the nanoparticles dissolved very fast upon uptake. Secondly, after 2 h of exposure to the uncoated zinc nanoparticles, the zinc was mainly associated with carboxyl and phosphate groups, which indicates that the zinc was mainly associated with cell walls (which are rich in peptides containing carboxyl groups) and/or precipitated inside the cytosol of the cells right after uptake. After treatment with the zinc phosphate coated nanoparticles, more points on the leaf showed zinc associated with thiol groups. This indicates it was binding to metalloproteins,[3] which are proteins that bind metal ions, often via thiol groups on the amino acid cysteine. The fact that the zinc from the zinc phosphate coated nanoparticles was taken up into these proteins suggests better incorporation into the plant compared with zinc from the bare zinc oxide nanoparticles.

Where do we go from here?

Altogether, these results indicate that zinc from zinc phosphate coated nanoparticles moved more easily within the plant than the zinc from the bare zinc oxide nanoparticles. The presence or absence of phosphate on the surface of the nanoparticles caused differences in both translocations inside the plant and cellular internalization of zinc after uptake. This change in mobility due to the phosphate shell correlated with the fact that zinc was only transported into the pepper fruits after treatment with the zinc phosphate coated nanoparticles. This is of interest since there are two possible goals to supplementing crops with zinc: 1) improving the health of the plants to improve crop yields and 2) increasing the amount of zinc in the part of the plant we eat to improve human nutrition. It's important to note that, at this stage, the zinc was no longer in the nanoparticle form but had been incorporated into the plant tissues, so the nanoparticles didn't end up in the fruit.

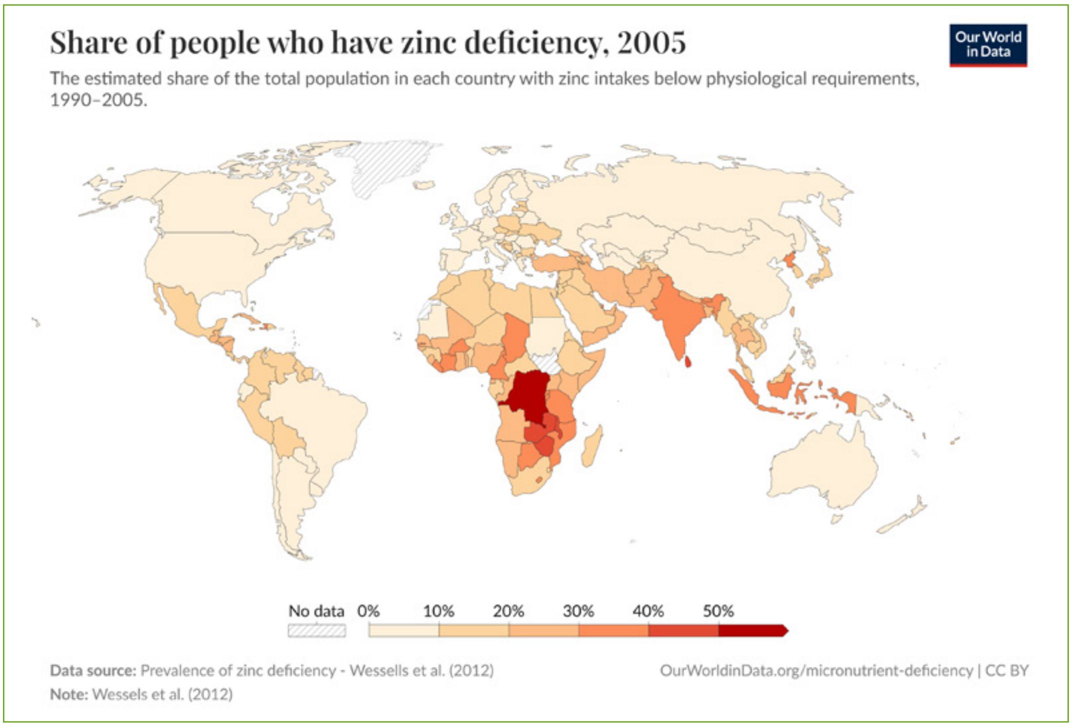


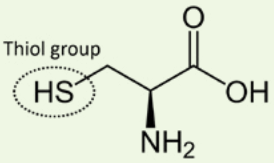
Figure 7. Prevalence of zinc deficiency [5]
Image: Wessels et al./Our World in Data, CC BY 4.0

Glossary

Bioavailability: how easily a nutrient or other molecule in a particular form can be absorbed and used by the target organism. For example, the heme iron found in animal products has good bioavailability for humans but pure iron metal doesn't; swallowing a lump of iron (not recommended!) won't help much with iron deficiency.[6]

Crops: plants grown for human use, often for food

Cysteine: an amino acid that contains a thiol group, which is used in proteins to build disulfide bridges or to bind to metal ions through coordination chemistry



Cysteine

Fertilizer: a substance (natural or synthetic) that applied to the soil to provide plants with the essential nutrients they need to grow. Many fertilizers can cause environmental problems if they leach into the environment.

Nanoparticles: really small particle less than 100 nm in diameter

Metalloproteins: proteins that bind to metal ions as part of their function. They have a wide range of important biological functions, including oxygen transport and enzyme catalysis.

Synchrotron: a circular particle accelerator. The synchrotron at ESRF is used to produce extremely bright X-ray beams (billions of times brighter than medical X-rays) for research.

Thiol group: a sulfur-containing functional group that is like the alcohol functional group (hydroxyl group) but with sulfur instead of oxygen

R–OH	R–SH
Alcohol	Thiol

Vasculature: in plants, the xylem and phloem vessels that transport water and nutrients around the plant

Zinc oxide: a zinc compound that is often used in zinc supplements (for humans) and zinc fertilizers. It is also used in so-called 'physical sunscreens'.

X-rays: very high-energy electromagnetic radiation with a shorter wavelength than visible or ultraviolet light.

Zinc: a transition metal that is an essential element for both plants and animals

Overall, the results showed that the zinc phosphate coating on zinc oxide nanoparticles seems to boost zinc uptake after application and accelerate phloem loading for distribution around the plant. This study highlights how small differences in the structure of a nanoparticle can have big effects on their fate in living things. This effect is well known in medicine, but other applications such as agriculture are sometimes less highlighted, although agricultural practices have a huge effect on both human and planetary health with links to sustainable development goals (SDGs) 2 (zero hunger), 3 (good health and well-being), 6 (clean water and sanitation), 12 (responsible consumption and production), 13 (climate action), 14 (life below water), and 15 (life on land). Research like this could be key for enhancing nutrient delivery in plants and bolstering crop resilience. Further research into the applications of phosphate-coated nanoparticles and their effects on plant nutrient uptake mechanisms are needed to harness their potential benefits in agriculture.

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The ESRF

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Resources

- Learn why zinc is an essential mineral for humans and how we get it from our diet.
- Explore global data on zinc deficiency on an interactive map.
- Learn why zinc is important for plants.
- Read about some of the problems of excess fertilizer use, especially nitrogen fertilizers.
- Learn why excess zinc in the environment can cause problems.
- Read about the vital importance of sustainable nutrient management.
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