X-ray study reveals distribution of the toxic metalloid in leaves

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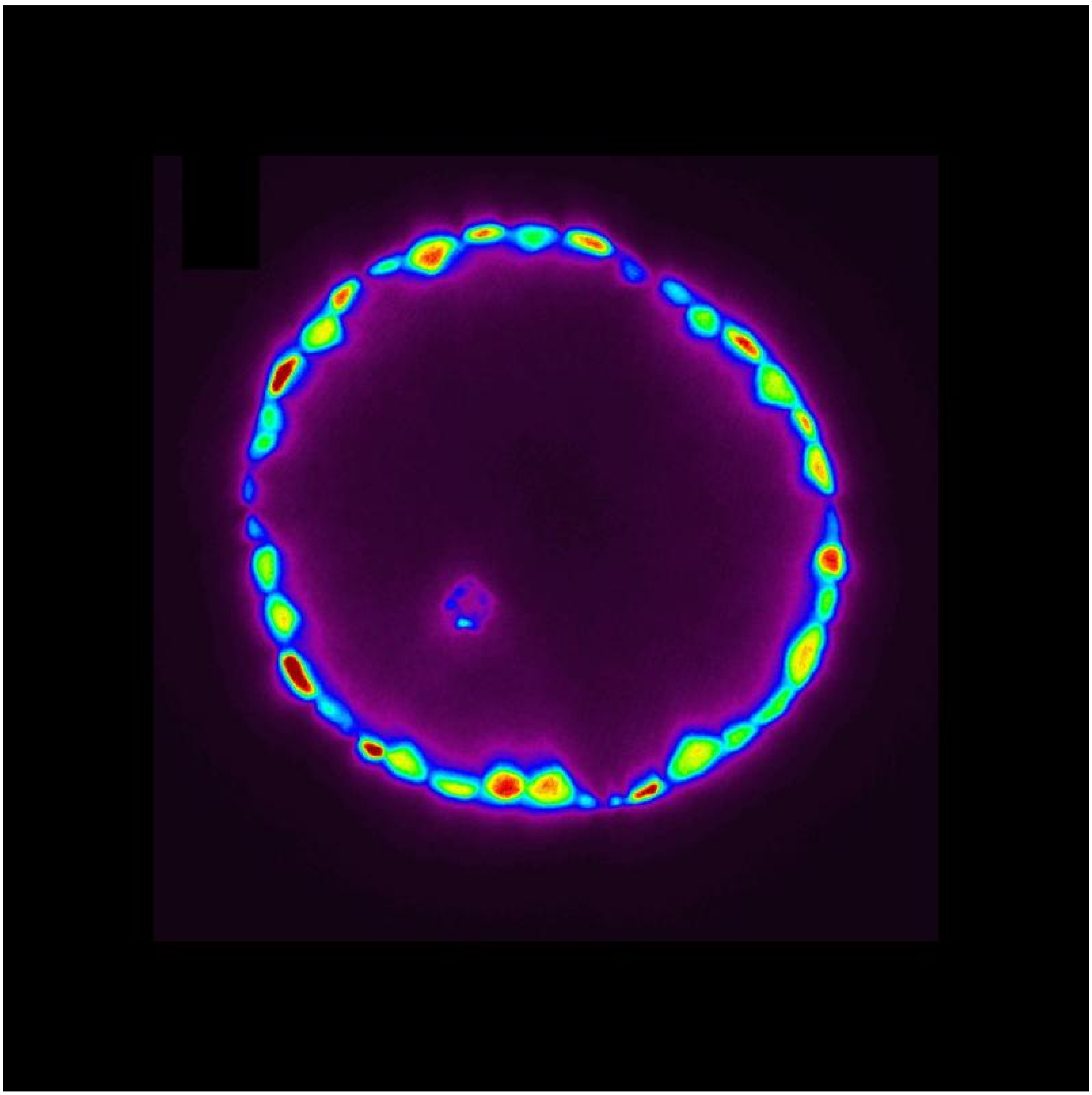


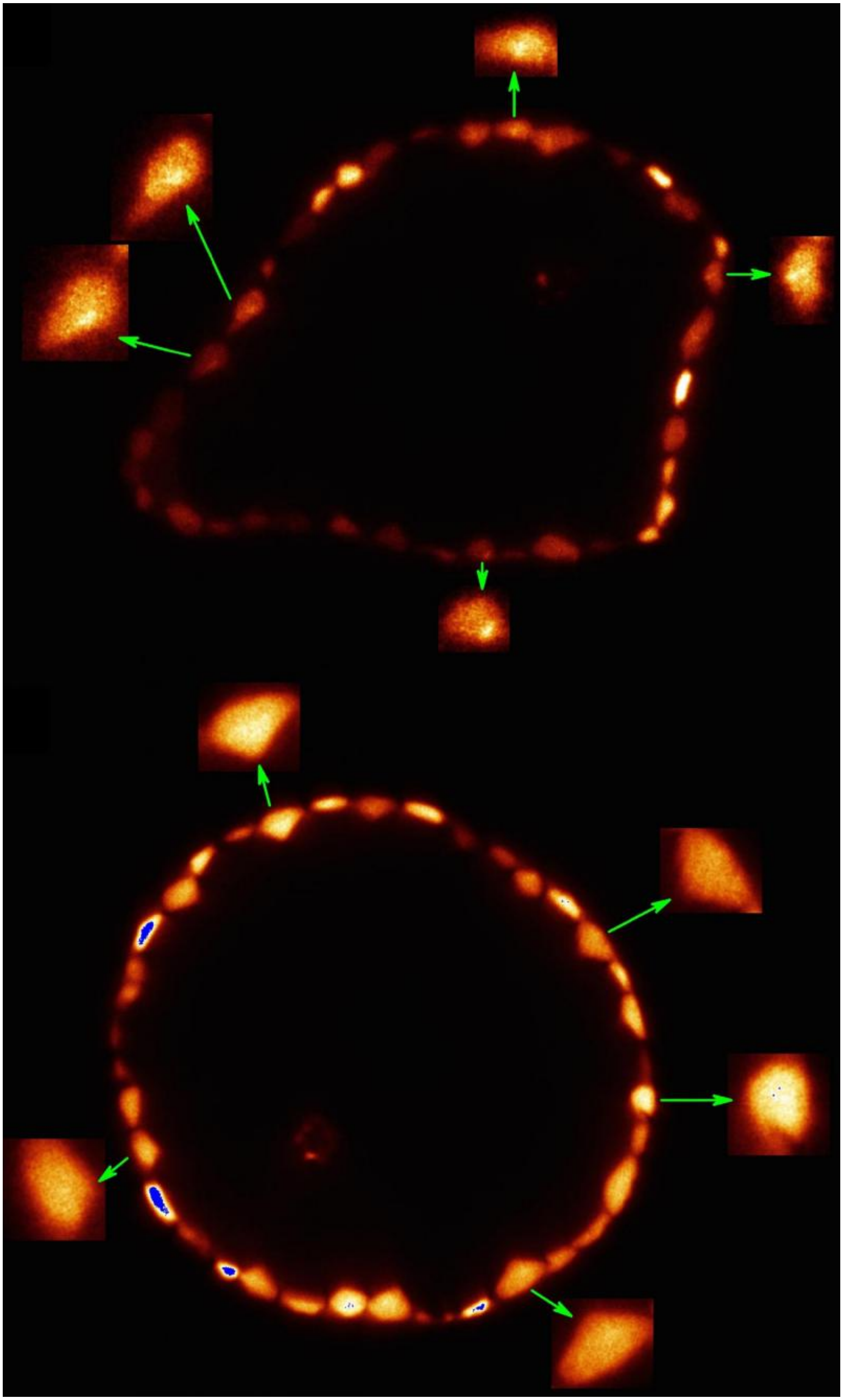
Distribution of arsenic within a mature leaf of rigid hornwort at a concentration of 5 micromols (375 micrograms) arsenic per litre water. Credit: Mishra et al., adapted from JExpBot [Source]

Toxic arsenic initially accumulates in the nuclei of plants' cells. This has been revealed by an X-ray examination of the aquatic plant rigid hornwort (Ceratophyllum demersum) using DESY's X-ray source PETRA III. Even at comparatively low concentrations, the arsenic also floods the vacuole, a liquid-filled cavity which takes up most of the cell. Researchers surrounding Hendrik Küpper of the Czech Academy of Sciences, who is a professor at the University of South Bohemia in Ceské Budejovice (Czech Republic), have made this discovery in the course of a project that was set up in Küpper's group by Seema Mishra (now at the National Botanical Research Institute in Lucknow, India). The scientists report their findings in the *Journal of Experimental Botany*.

Arsenic is highly toxic and poses a growing environmental and health problem all over the world. The concentration of arsenic in the soil is increasing as a result of human activities, and in many countries – especially on the Indian subcontinent – the concentration of arsenic in the groundwater has become a problem. Although arsenic is a natural component of soils there, well drilling and other human impacts on the ground have in recent decades mobilised it, leaching it into the drinking water. In humans, arsenic can cause cancer, necrosis, or acute renal and circulatory failure. The metalloid is also toxic to plants. It is taken up by the same transport mechanism as phosphorus, an element that is essential to plants, and even at levels far beneath the lethal concentration, it impairs plant growth and therefore reduces the size of crops.

"On top of this, humans eat plants, of course, and feed them to their livestock, so that arsenic accumulates in these and eventually ends up in human beings," explains Küpper. "By carrying out our analysis, we wanted to find out exactly how arsenic poisoning occurs in plants," adds co-author Gerald Falkenberg, who is in charge of the P06 beamline at DESY, where the experiments were performed. The results could help researchers to breed plants that absorb less arsenic.

According to Küpper, previous research on plants has usually been carried out with arsenic concentrations that were far too high. "Whereas concentrations of just one micromol, corresponding to 75 micrograms, per litre are already relevant in terms of plant physiology, it was not unusual to work with concentrations of up to 250 micromols per litre – concentrations at which completely different things are going on," explains the biologist. "We wanted to know what happens at ecologically and physiologically relevant concentrations." Up to 33 micromols arsenic per litre were reported in contaminated areas in irrigation water and soil solution.



Distribution of arsenic within leafs of rigid hornwort at concentrations of 1 micromol (75 micrograms, top) arsenic per litre water and 5 micromols (375 micrograms, bottom) arsenic per litre water. At low concentrations, arsenic accumulates predominantly in the nuclei of epidermal cells, while at high concentrations, it floods the whole cell. Credit: Mishra et al., adapted from JExpBot [Source] CC-BY-3.0 creativecommons.org/licenses/by/3.0/

According to the researchers, the rigid hornwort is a kind of indicator plant for metals, and experiments with this species can mostly be transferred to other species as well. The scientists exposed the plant under investigation to arsenic concentrations between one and five micromols per litre, and then shone a narrowly focused X-ray beam from PETRA III through the leaves. "Thanks to PETRA III we could peer into individual cells of the plant for the first time," reports Küpper. "This allowed us to localise the arsenic more precisely within the cell – after all, it makes a difference whether it is in the cell wall, for example, or in the vacuole."

A concentration of one micromol of arsenic per litre of water is still tolerated by the plant. The plant first deposits the toxin in its outer layer, the epidermis. "Surprisingly enough, we found that arsenic initially accumulates in the cell nuclei," Küpper reports. Only when the concentration rises to five micromols per litre, a level that the plants are unable to withstand for prolonged periods, is arsenic also found in the vacuole and thus more or less throughout the entire cell.

"This means that the capacity of the epidermis is exhausted and the plant can no longer get rid of the toxin, and that's when things start to get serious," says Küpper. The arsenic now spreads to the so-called mesophyll, which makes up most of the leaf. This is where photosynthesis takes place, in other words where the plant absorbs light and produces sugars. This shift in the distribution can be clearly observed in the X-ray tomogram of the leaves.

The investigation showed that arsenic damages the enzymes that are responsible for producing chlorophyll, the green pigment required for photosynthesis. Arsenic first inhibits photosynthesis, which is not due to the removal of chlorophyll but rather to the reduced production of the pigment, as Küpper points out.

In future studies, the scientists are hoping to find out what arsenic does inside the cell nucleus. "Presumably, it causes genetic damage," says Küpper. For example, arsenic might replace the phosphorus in the genes. Whereas the current X-ray examination shows that arsenic already accumulates in the cells' nuclei at low concentrations, the scientists are planning further experiments in which they will look at the chemical bonds that are formed by arsenic in the nucleus as compared with other parts of the cell.

More information: Analysis of sub-lethal arsenic toxicity to Ceratophyllum demersum: Subcellular distribution of arsenic and inhibition of chlorophyll biosynthesis; Seema Mishra, Matthias Alfeld, Roman Sobotka, Elisa Andresen, Gerald Falkenberg, Hendrik Küpper; *Journal of Experimental Botany*, 2016; DOI: 10.1093/jxb/erw238

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