Hendrik Küpper – at the service of plants and humans

Prof. Hendrik Küpper had his first experience as a scientist in 1992 when, together with his brother and a friend, he participated in the German government's "Jugend forscht" contest. The students came first in biology in the national competition and first in both the "Young Europeans' Environmental Research Contest" and the "European Community Contest for Young Scientists". Back then, Küpper was already interested in the interaction between heavy metals and plants. Nowadays, he is junior professor at the University of Constance where he is responsible for providing important insights into the increasing pollution of the soil with zinc, copper and other heavy metals.

Prof. Dr. Hendrik Küpper

European agriculture is one of the main sources of heavy metal pollution of the environment. Zinc and copper salts are still used as pesticides and water that drains off fields that have been treated with pesticides poses a deadly risk to aquatic plants. In addition, high concentrations of cadmium, nickel and iron can have a negative effect on plant growth and can, for example, have a negative effect on the production of phytoplankton, which is the nutritional staple of many animals that live on lake and ocean floors. Toxic heavy metal concentrations such as often observed in polluted waters and lakes, have an inhibitory effect on photosynthesis, as a result, amongst other things, of the exchange of the central chlorophyll ion that acts as an important target for many external attackers.

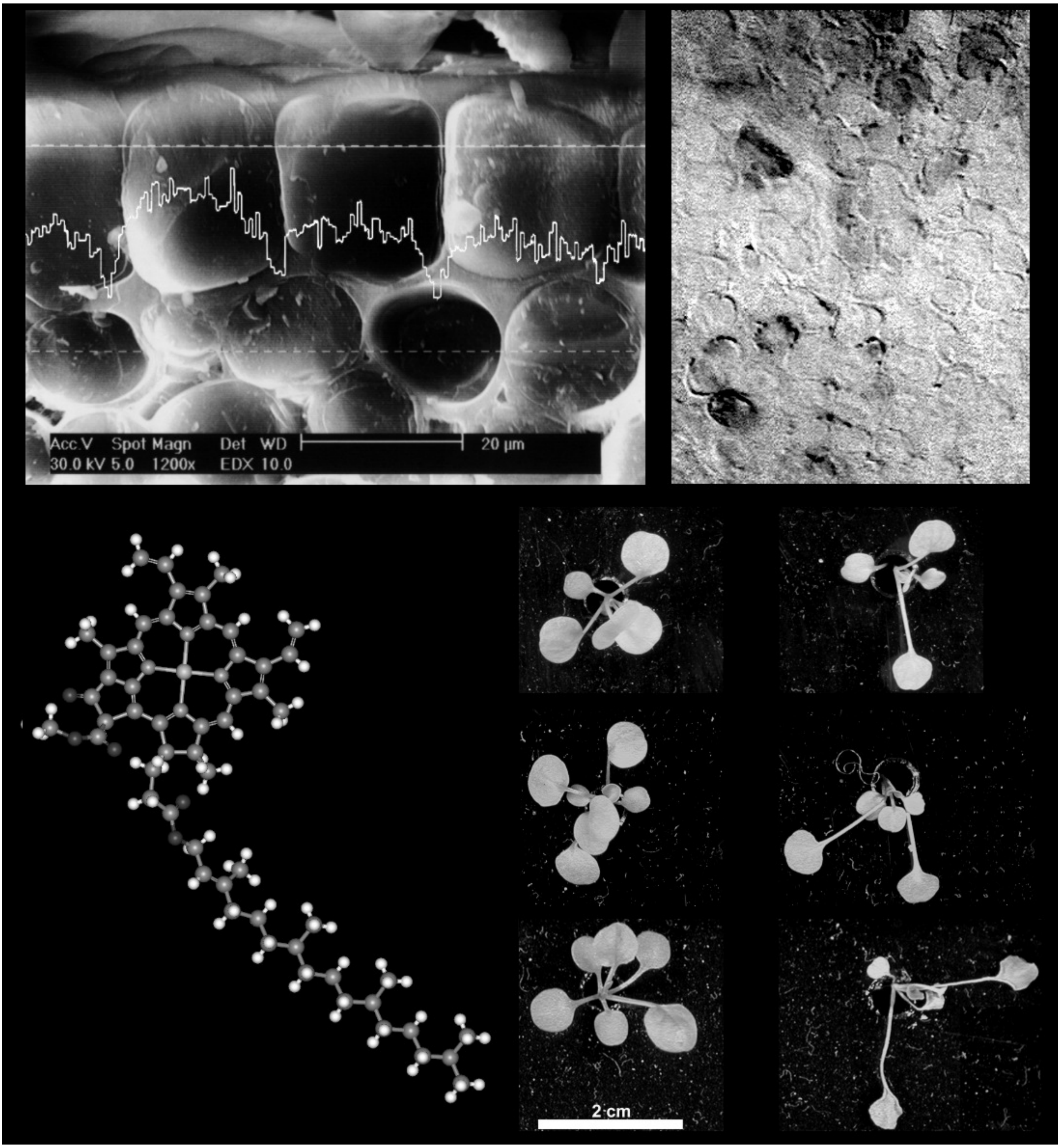
For Prof. Hendrik Küpper, who focuses mainly on the transport, toxicity and detoxification of heavy metals in plants, this interaction is of a very complex nature. "On the one hand, the majority of metals, including many heavy metals, are used by the human metabolism as trace metals. These enable the proper functioning of around one third of all enzymes. A total lack of copper, for example, is fatal. On the other hand, intoxication can result from the exposure of the body to large quantities of heavy metals," said the scientist from Constance. In general, the higher the concentration of heavy metals to which the body is exposed, the more unspecific the inhibition becomes," said the scientist. The inhibition is far more specific at low, but nevertheless toxic, concentrations of heavy metal. "In such cases, the location of the inhibition site depends on the kind of metal and its different chemical properties," said the plant physiologist. Environmental conditions such as pH and light intensity and cycle, play a particularly important role in this process.

Metal incorporation is the result of pump mechanisms

In the past, Prof. Hendrik Küpper's research contributed to the clarification of the mechanism of heavy metal-induced inhibition. He discovered that the exchange of magnesium in the chlorophyll for heavy metal ions weakens the process of photosynthesis, even at very low concentrations of copper, for example. The heavy metal ions enter the plants and chloroplasts by way of the normal transport pathways (those used for nutrition), but cannot be incorporated into enzymes or be detoxicated due to regulation overload.

Instead, the heavy metals react in a somewhat uncontrolled fashion with molecules to which they are able to bind strongly due to their chemical structure: these molecules are the chlorophylls in the chloroplasts. Due to the substitution of magnesium with heavy metal ions, the chlorophylls are no longer able to produce energy during photosynthesis as a result of their altered chemical and physical properties. They even inhibit photosynthetic energy production of those chlorophylls that still have magnesium ions in their active centre.

In plants, specific proteins transport the metals in the xylem of the roots and stem, from where they are pumped further on to their target cells, during which process they are bound to specific ligands. “A specific pump is required whenever the ions have to cross a membrane,” said Prof. Hendrik Küpper. When the metals have reached their final destination, they are integrated into the active centres of enzymes by way of specific proteins known as chaperones.



Top left: EDX-scan (Ni Kα line) of the nickel distribution in an Alyssum lesbiacum leave. Top right: photosynthesis heterogeneity in Thlaspi caerulescens (a Cd hyperaccumulator, which is, however, negatively affected in the initial growth phase on Cd-containing agar plates) under cadmium stress. Bottom left: copper chlorophyll (structural difference to Mg-chlorophyll: the central ion is only bound by way of the four nitrogens of the porphyrin ring, while Mg still has a ligand (protein or solvent) below and above. Bottom right: comparison of the growth of Cu-resistant (left) and Cu-sensitive (right) individuals of the Cad-/Zn hyperaccumulating plant Thlaspi caerulescens grown on media containing 10 µM copper.

Plants use smart strategies to defend themselves

Not all plant species die as a result of high concentrations of heavy metals. Many plants have protective counter mechanisms which lead to the exclusion of heavy metals from the plant metabolism, for example. This is achieved, for example, by sealing the roots (lignification) or by actively pumping the metals out of the plant. Strong ligands bind heavy metals in sensitive plant parts or cells, thereby preventing undesired reactions. "Another option is the accumulation of heavy metals in less sensitive plant tissues and cell compartments through active pumping processes," said Prof. Hendrik Küpper.

According to Küpper, far fewer than one percent of all plant species are not only resistant to heavy metals, but are also able to actively accumulate the metals in their dry mass (up to several percent). These plants are referred to hyperaccumulators. "It is worth noting that these plants accumulate huge quantities of heavy metals without intoxicating themselves," said the plant physiologist. Hendrik Küpper found out that these hyperaccumulators protect themselves very efficiently by accumulating the metals in the large storage cells of the leaf epidermis. The accumulation of large quantities of heavy metals (up to a concentration of one molar) in the storage cells is achieved by the elevated synthesis (expression) of specific transport proteins. Küpper's group of researchers is currently working on the biochemical characterisation of one of these proteins.

The hyperaccumulation of toxic heavy metals has the biological function of defending the plants against animals that feed on them and against pathogens. For humans, this process is a cheap way of counteracting minor to moderate heavy metal pollution (in particular Cd pollution) so that a particular area of cultivation can be reused for agricultural purposes a few years after previous use. In some regions (e.g., Africa and the USA), plants that hyperaccumulate nickel are also used for commercial ore extraction (phytomining).

Development and use of own methods

Prof. Hendrik Küpper and his team mainly use biophysical methods (e.g., different types of light and x-ray spectroscopy methods) to investigate the relationship between heavy metals and plants, particularly for investigations into microscopically small, living and undamaged cells and tissues. "We also use biochemical and molecular biology methods," said the plant physiologist explaining that this involves the use of a broad range of different devices, e.g., X-ray spectrometer (X-ray emission spectroscopy (EDX) using scanning electron microscopes, "beamline" for X-ray absorption spectroscopy (XAS) using the synchrotron) as well as UV/VIS spectrometers.

The biologist from Constance also uses a fluorescence-kinetics microscope which he has developed himself. This microscope enables the researcher to measure rapid temporal alterations (=kinetics) of fluorescence and/or absorption on living cells with spatial and spectral resolution. This can involve the measurement of the photosynthesis biophysics, an application for which the device was originally developed, but also the determination of metal accumulation or the formation of reactive oxygen species. Previously, the reliable simultaneous measurement of these processes on the microscopic level had not been possible without damaging the cells. These in vivo measurements can be carried out with much less light (between 10 to 10,000 times less depending on what kind of device this method is compared to) than with normal light microscopes. This is achieved by a specific illumination system and a specific measurement camera.

Other organisms can also benefit from these findings

Prof. Küpper's clarification of the general principles and mechanisms are also of medical relevance because humans also possess proteins involved in the transportation of heavy metals. Küpper's findings can also be used for the research and treatment of fatal diseases such as Menkes and Wilson's diseases which develop as a result of mutations occurring in copper-transporting enzymes.

Dr. Küpper’s future research projects will specifically focus on clarifying metal transport mechanisms and on comparing the relevance of difference toxicity mechanisms, a field where many questions still await clarification. Thanks to the biotechnological application of his findings on the improvement of phytoremediation and phytomining using hyperaccumulators, the researcher has received funding from a broad range of institutions and companies, including the Chemical Industry Fund (FCI). He also told us that he finds “exciting collaborations with industry highly interesting and most welcome.”

Background:
Prof. Hendrik Küpper studied biology at the University of Constance from 1994 to 1998. After several practical research periods at the Institute of Microbiology at the Czech Academy of Sciences in Třeboň (Czech Republic), where he did most of his degree thesis, and the IACR Rothamsted (Rothamsted Experimental Station) in the English town of Harpenden, he received his PhD from the University of Constance in 2001. He spent a postdoctoral period in the Department of Bioinorganic Chemistry at the University of Constance and two years (August 2002 - July 2004) in the Plant, Soil and Nutrition Laboratory at Cornell University, USA. He has been junior professor at the University of Constance since August 2004.

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