

Detection of Ni-Species and Co-Species in single living plant cells with high lateral resolution

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Nutrients in air, water and soil as well as ions and metals constitute the basic living conditions of all organisms. Environmental interferences like industrial pollution, mining, traffic, etc. affect both, the concentration (e.g. by accumulation) and the chemical state of individual heavy metals. Plants may take up these metals, some of them being essential for their metabolism. Adversely, they may act toxic as well, such affecting the metabolism of an organism. In the course of the food chain, thus accumulated heavy metals acquire in animals and finally in humans, with the consequence of similar toxic effects. The toxicity of heavy metals directly correlates with their chemical state (e.g. oxidation state, state of binding), so that further interpretations necessitate the analytical discrimination between the individual species.

Standard analytical techniques in element speciation with high sensitivity and high specificity focus on bulk materials, like cell cultures in biological research. A prerequisite for the interpretation of the underlying reaction mechanisms, however, is information on exchange reactions between the individual living cells – even individual cell compartments – and the surrounding fluid phase.

Analyzing heavy metal species in single cells needs high lateral resolution techniques combined with extreme sensitivity for individual species. The required standards may provide optical techniques, sensitive for fluorescence and autofluorescence like confocal laser scanning microscopy (CLSM) or scanning near-field optical microscopy (SNOM). Detection of fluorescent heavy metal species with CLSM resp. SNOM may yield a lateral resolution down to 100 nm respectively 50 nm. Fluorescent dyes with high specificity to individual heavy metal species may provide basic information, on the distribution of heavy metals in a single cell.

Standard fluorescent dyes are available for labelling heavy metals or even individual metal species, e.g. Newport Green DCF for Ni(II) and Co(II). Its fluorescence is increases with Ni(II)/Co(II)-binding and may be detected CLSM in fluorescence mode. In a pilot experiment plant cells were incubated with Ni-species and CoCl_2 (100 μM for 120 h). At 24 h intervals, one specimen was incubated with the fluorescent dye Newport Green DCF and then visualized by CLSM. Soluble Ni-species – NiCl_2 and NiSO_4 – tended to be taken up by the cell and were stored mainly in vacuoles and in the cytoplasm. After less than 24 h fractions of the initial Ni(II) additionally could be localized in the nucleus. Additionally, the insoluble species (Ni_2S_3) was acquired in the cytoplasm and the vacuole as well. In contrast to the soluble Ni-species the species in the nucleus fluoresces with 48 h retardation but much weaker. Under these conditions the CLSM technique can discriminate between soluble and insoluble Ni-species. In nutrient only solutions (without metal-species) for other 120 h the Ni(II) is depleted (decreasing fluorescence in the cytoplasm and the vacuole) from the cells after 120 h of incubation. Accordingly, in cells incubated with Ni_2S_3 the fluorescence in the nuclei almost completely is quenched. Similar to Ni_2S_3 , Co(II) ions are stored in the cytoplasm and in the vacuole. Extended heavy metal exposure, with respect to the nucleus results in more intensive fluorescence.