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## Mechanism of heavy-metal tolerance in shade plant *Aucuba japonica* via the possible function of root-endophytes

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**Purpose:** *Aucuba japonica* is an evergreen shrub and grows normally in the forest floor of our research site, which has been contaminated with heavy metals, such as copper, lead, and zinc. Recently, root-endophytes are reported to enhance heavy-metal tolerance of plants. Our purpose is to clarify the mechanism of heavy-metal tolerance of *A. japonica*, considering the interaction with root-endophytes.

**Methods:** *Aucuba japonica* is growing in forest floor of deciduous mixed forest, where the light condition and temperature are changing through a year, therefore, we collected plants both in July 2017 (summer) and January 2018 (winter). Leaves, branches and roots were analyzed for heavy-metal concentration by ICP-OES and identification of detoxicants, phenolics by HPLC/DAD, and organic acids-TMS by GC/MS. Distribution of zinc in roots was also observed by staining with zinpyr-1, which has Zn-selective fluorescence under confocal laser microscope.

Endophytic fungi were isolated from surface-sterilized root segments, and evaluated microbial ability to chelate zinc by the culture medium containing insoluble zinc.

**Results:** *Aucuba japonica* highly accumulated zinc in roots, and produced citric acid and aucubin as detoxicants both in summer and winter. Zinc was localized in cell walls of epidermis, cortex and stele, and also detected in fungal structures and hyphae in cortical cells.

Root-endophyte, *Pezizula* sp. was most frequently isolated, showing Zn-chelating abilities.

**Conclusions:** *Aucuba japonica* accumulated zinc in roots, suggesting zinc-tolerance by sequestering zinc in cell walls and producing detoxicants such as aucubin and citric acid through the year. *Pezizula* sp. might enhance the heavy-metal tolerance via producing Zn-detoxicants.