

Hyperspectral Bioindicators of Heavy Metal Exposure in Tall Fescue

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Expanded industrial globalization has resulted in the release of high concentrations of heavy metal ions into environmental water sources and soils. Phytoremediation has attracted increasing attention because it can safely remove environmental contaminants via plant uptake, accumulation, and harvesting. Tall fescue (*Festuca arundinacea* Shreb.) is the predominant temperate perennial grass in the United States and serves as soil erosion prevention and livestock feed, but also exhibits phytoremediation potential due to its endurance and high stress tolerances. Here, we discovered unique degradation bioindicators of photochemical and structural responses in tall fescue to heavy metals copper and chromium (VI) exposure. To quantify structural and pigment changes in tall fescue in response to copper and hexavalent chromium stresses, we performed brightfield and confocal fluorescence imaging. Fluorescence images were collected over a 9-day period, which confirmed decreasing total chlorophyll content in tall fescue cross-sections in response to Cr(VI) and Cu exposure. Brightfield fluorescence microscopy images presented multiple structural features of tall fescue: rib cross-section, vascular fiber bundle, adaxial fiber bundle, abaxial fiber bundle, metaxylem element 1, and metaxylem element 2. Observations from structural analysis indicated that the copper and chromium exposed plants became compromised by their respective stressor after 4 days of exposure resulting in varying plant survival responses. On day 9 copper and chromium treated plants displayed near-complete collapse of the plant vascular systems. To spectrally separate the closely related chlorophyll pigments (Chl-a, Chl-b, and Chl in Photosystem I) and visualize their relative localizations within the plant tissue, hyperspectral confocal fluorescence microscopy was also conducted with multivariate curve resolution (MCR) analysis of the data. Overall, our MCR analysis of hyperspectral confocal fluorescence images revealed unique localization patterns of photosynthetic pigments for Cu and Cr(VI) at different stages of stress. We observed significant changes in the spatial localization of the chlorophyll components compared to normal fescue tissue by day 9 post Cr(VI) or Cu exposure. Of particular interest, Chl-a became concentrated in large foci within the tissue, and the other chlorophyll components were decreased in abundance and dispersed diffusely. In contrast, these pigments were located within chloroplasts in healthy tall fescue tissue. These novel spatial-temporal bioindicators of heavy metal uptake in tall fescue can be used to ameliorate phytoremediation practices through remote and passive detection.

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