Metabolomic study of the effects of parabens and pharmaceuticals in recycled water on metabolic pathways of lettuce using NMR and GC-MS followed by chemometric techniques

Neda Feizi, Mahsa Seraj, Reyhane Shavandi, Nastaran Nikzad, Hadi Parastar*
Department of Chemistry, Sharif University of Technology, Tehran, Iran
E-mail address: h.parastar@sharif.edu

ABSTRACT

A great number of integrated water resource management schemes are being implemented with focus on utilization of non-conventional water resources, such as treated wastewater (TWW) [1]. TWW may contain undesirable chemicals such as contaminants of emerging concern (CECs). Plant metabolomics aims to study the plant system at the molecular level to provide a non-biased characterization of the metabolome of a plant's tissue in response to its environment [2]. In this study, a non-targeted metabolomic analysis was performed on lettuce exposed to nine CECs by irrigation and the subsequent changes have been traced comprehensively by nuclear magnetic resonance (NMR) and gas chromatography-mass spectrometry (GC-MS) and the huge amount of acquired data was analyzed by chemometric techniques. Lettuce seedling was planted in pots and watered with Hoagland solution. The plants were watered with mixture of CECs 30 days after the transplantation of seedlings for 33 days under controlled conditions. This dataset contains three doses groups consist of: low dose (10 µg L^{-1}), medium dose (50 µg L^{-1}) and high dose (100 µg L^{-1}) and a control sample. Each dose group consists of three replicates. Samples were collected three, four and five weeks after exposure to CECs. After sampling, the leaves were comminuted with liquid nitrogen and stored at $-20\circ$C until analysis. The extraction of metabolites was performed as reported elsewhere [2]. For NMR analysis, 550 µL of D$_2$O was added to the dry residue and the analysis was performed. For GC-MS analysis, 100 µL of 30 µL of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS) was added to the dry residue and the mixture was injected. The NMR and GC-MS data of control and exposed samples were arranged in a data matrix and then analyzed using principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA) using variable importance in projection (VIP) and ANOVA-simultaneous component analysis (ASCA). The metabolic response indicates that exposure to CECs at environmentally relevant concentrations causes large metabolic alterations in plants (carbohydrate metabolism, TCA cycle, pentose phosphate pathway and glutathione pathway) linked to changes in morphological parameters.

Keywords: "Metabolomics", "Chemometrics", "NMR", "GC-MS", "CEC", "ASCA"

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