

Evaluation of enzymatic-assisted extraction protocols for the analysis of total arsenic and arsenic species from individual leaves of terrestrial plants by means of IC/ICPMS and ICPAES

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Different enzymatic-assisted extraction protocols were evaluated regarding their suitability for the extraction of total arsenic and arsenic species from individual leaves of *Tropaeolum majus* plants contaminated by phenylarsenic compounds. Regarding the diminution of the sample size for total arsenic determination a high biological variability between different leaves and stalk segments was ascertained. To avoid a false estimation of extraction yields their calculation was based on the arsenic concentrations remaining in the extraction residues.

Using a two-step extraction comprising incubation of ground leaf material with cellulase followed by digestion with trypsin $7 \pm 3\%$ higher arsenic concentrations were extracted compared to non-enzymatic water extraction. For non-ground intact leaves an elevation of the extraction yield by means of enzymes was not achieved although a large fraction amounting to 50 up to 70% of the total foliar arsenic was dissolved from intact leaves in water within four hours without any further sample pretreatment. These results indicate a high mobility and solubility of phenylarsonic acid dominating in leaves independently on the application of amino- or nitro- and hydroxy-substituted phenylarsenic species. A cleavage of the functional groups from the phenyl ring was deduced from the arsenic species analysis using ion exchange chromatography coupled to inductively coupled plasma mass spectrometry. Some differences between the arsenic species distributions in aqueous and methanolic extracts and in enzymatic extracts were observed.

Due to the high water extractability of the arsenic species the improvement of the extraction yield using enzymatic-assisted extraction remained relatively low but the enzyme-extracted fraction of foliar arsenic can be assumed to be bound to biological macromolecules such as carbohydrates or proteins.