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Compound specific isotope analysis confirms biotransformation of 2,3-dichloroaniline in constructed wetlands

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Constructed wetlands can be a suitable and environment-friendly remediation approach for industrial contaminants. However, providing direct evidence for *in situ* transformation in wetlands using concentrations and biogeochemical parameters alone is often challenging. Compound specific isotope analysis (CSIA) is an established tool to assess the fate of legacy groundwater contaminants in the environment. In this work, we evaluated the potential of CSIA to identify and quantify *in situ* transformation of 2,3-dichloroaniline (2,3-DCA) which is a major industrial feedstock. To date, CSIA has never been applied to track the fate of 2,3-DCA *in situ*. First, we performed a controlled-laboratory aerobic experiment using a mixed enrichment culture derived from the site that transformed 2,3-DCA presumably via a dioxygenation pathway. Concentration and isotope signatures for carbon, hydrogen, and nitrogen of 2,3-DCA were measured over time to determine enrichment factors, i.e., values, using the Rayleigh model. We found negligible carbon and hydrogen isotope fractionation, yet a significant inverse nitrogen isotope effect. The corresponding Apparent Kinetic Isotope Effect for Nitrogen ($AKIE_N$) ranged from 0.9938±0.0003 to 0.9922±0.0004; whereas the bulk values ranged between +6.2±0.3 and +7.9±0.4‰. Next, the lab-derived results were applied to investigate the potential *in situ* 2,3-DCA biotransformation in pilot-scale constructed wetlands at a contaminated site. The treatment system consisted of an upflow gravel bed wetland followed by three planted marshes, all in series. Contaminated groundwater was supplied to the first wetland and samples were collected from the outlet of different units for concentration and isotope analysis. No significant changes in carbon isotope signature, i.e., ≤2‰ were observed despite the contaminant concentration decreasing by more than 99% between the inlet and outlet of the system. However, changes in nitrogen isotope signature were significant, i.e., >10‰ and inverse confirming *in situ* transformation. Using laboratory-derived , we confirmed 80% to 90% removal of 2,3-DCA in the planted marsh was due to biotransformation; whereas, sorption was identified as the dominant process in upflow gravel beds. This study proposes multi-element CSIA as a novel quantitative tool to track the fate of semi-volatile contaminants, such as 2,3-DCA in dynamic environmental conditions of wetlands, groundwater, and surface water.