

Assessment of biochar safety via its leachate characterization using physicochemical and biological assays

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The present study investigates the physicochemical composition of water aliquots derived from biochars produced from the pyrolysis of malt spent rootlets, in combination with the concomitant toxicological profile in each case. Specifically, physicochemical parameters and heavy metal ions were determined in aliquots of six (6) serial washes of biochar (1.5 g of solid was added in column and washed 6 times with 40 mL of distilled water per wash). The chemical analysis of each aliquot showed increased levels of PO₄-3, Cl-, NO₃-, SO₄-2, F- and Br- in the first wash aliquot, followed by a significant decrease over washes. Non-detectable concentrations were observed after 3 washes in almost all cases. Similarly, the increased levels of Zn, Be, Cs, Mn, V and Se determined in the first wash aliquot were eliminated followed successive washes. In parallel, the toxic potency of each wash aliquot was recorded by (a) a multi-well test plate bioassay, using instars II-III larvae of the fairy shrimp *Thamnocephalus platyurus*, hatched from cysts derived from Screening Toxicity test supplied by MicroBio Tests Inc. (Thamnotoxkit FTM) and (b) the Microtox bioassay, using bioluminescent bacteria *Vibrio fischeri*. According to the results, first and second wash aliquots were toxic for *T. platyurus* (LC₅₀ values of 22.12 and 68.28% v/v, respectively), followed by a significant elimination of toxicity after further washes in all cases. Similarly, the Microtox bioassay showed a significant inhibition of *Vibrio* luminescence after treatment for a period of 5-90 min (98-100% inhibition of luminescence) with the first wash aliquot (EC₅₀ ≤ 0.01 % v/v), with no toxicity to be observed after successive washes. According to the results, at least one wash of biochar is prerequisite for improving its safety for further use. Moreover, the removal of both inorganic and organic, such as metal ions, substances commonly washed by the biochar, could be a crucial step for its sustainable use and final application, thus avoiding the induction of adverse effects on biota.