Removal and Phytotransformation of Benzotriazole in Urban Stormwater with *Carex praegracilis* in Bench-Scale Constructed Subsurface Treatment Wetlands

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Background

Urban stormwater harvesting and treatment is imperative to augment water supply in drought-prone regions in the western United States. Constructed wetlands for stormwater treatment are an increasingly popular technology for decreasing pollutant concentrations before recharging ground and/or surface water.

Chemical contaminants of emerging concern in urban stormwater such as the xenobiotic anticorrosive agent benzotriazole (BT) that impair surface water quality are not removed by traditional drinking water or wastewater treatment technologies. BT is used in engine coolants, brake fluid, deicing fluid, and antifreeze, and thus ubiquitous in urban runoff.[1, 2, 3]

Treatment wetlands using combined subsurface and free water surface unit processes transform urban and suburban landscapes. For example, in Sun Valley, California, a former sand and gravel mine site will be converted into a multi-use park (Rory M. Shaw Wetlands Park) with constructed treatment wetland units to manage flooding and treat stormwater runoff. At this site, BT is a major contaminant of concern.

Natural filtration, bioaccumulation, and transformation of chemical contaminants such as BT with treatment wetlands is critical to preserving and conserving water supply. Vegetation can play a major role in pollutant removal. In a previous study at Stanford University, model *Arabidopsis* in hydroponic solution spiked with benzotriazole assimilated the contaminant from solution, and rapidly transformed BT into useful metabolites analogous in structure to natural plant compounds including auxin and tryptophan.[4]

In order to customize Rory M. Shaw Wetlands Park and other future stormwater treatment wetland projects in California, and with this background, we seek to discover if similar BT uptake and metabolization processes can occur in a simulated constructed wetland treatment environment populated with a California native plant, *Carex praegracilis*, in a laboratory setting.

Hypothesis

Subsurface treatment mesocosms planted with *Carex praegracilis* will remove a greater fraction of BT from stormwater than control mesocosms during and following simulated spiked storm events. In addition, BT and BT metabolites will be detected in *C. praegracilis* leaf and root tissue samples with LC-MS/MS.

Methodology and Materials

Nine mesocosms were constructed with irrigation tubing for vertical water infiltration and a two parts sand to one part compost mixture by volume over a layer of drainage rock and a perforated effluent PVC pipe covered with fine wire mesh. Six mesocosms were populated with *C. praegracilis* (Figure 1) and three served as controls (Figure 2). Over a four week period, weekly storm events (7 gallons per mesocosm at an average flow rate of 0.2 gallons per minute) occurred, weekly alternating spiked and unspiked conditions. During spiked storm events, three of the planted mesocosms received 1 mg/L BT and three 1 ug/L BT in synthetic stormwater. One control received 1 mg/L BT, one 1 ug/L BT, and one unspiked synthetic stormwater. After 5 gallons were flushed through each mesocosm, each system was closed and left saturated for one week during which daily 200 mL bulk water samples were harvested. Plant tissue samples were harvested twice a week. Bulk water was analyzed for BT and plant tissue was analyzed for BT and BT metabolites with LC-MS/MS. Bulk water subsamples were saved for future water quality testing with TOC-L, DA, and ICP-MS.

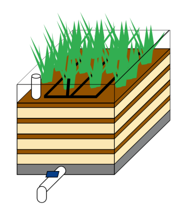


Figure 1.

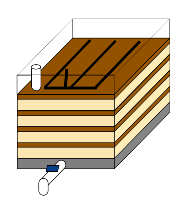
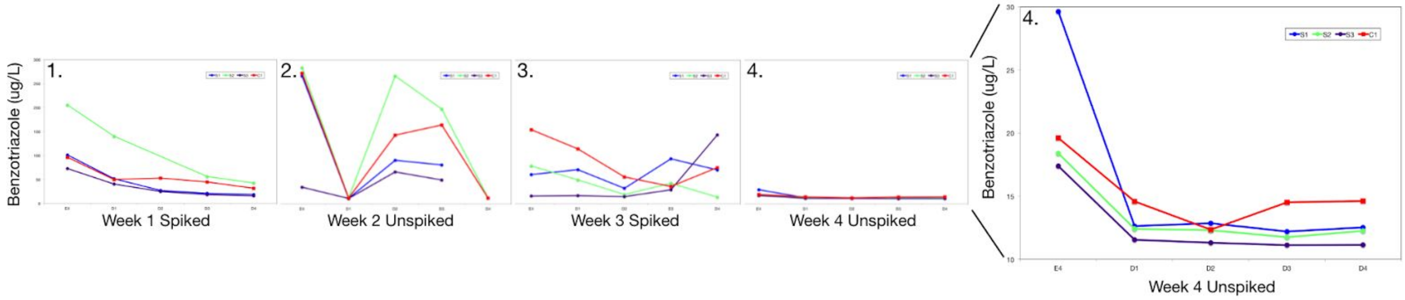


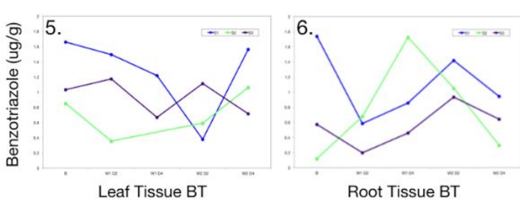
Figure 2.

Results and Discussion

For high BT (spiked with 1 ug/L) mesocosms, 79.4-92.7% of influent BT was removed from synthetic stormwater during the initial Week 1 storm event (E4, 1). After 4 days, all mesocosms experienced a further 66.9-79.1% reduction. Breakthrough of BT was observed in Weeks 2 and 3 (2, 3), suggesting the need for further investigation of retention time in the natural removal of benzotriazole in a saturated wetland system. By Week 4, following the last unspiked event, residual BT levels (11.1-14.6 ug/L) were established in each mesocosm (4). The control mesocosm exhibited a 2.7-17.9% higher BT residual than treated mesocosms.



A significant background level of BT was present in both leaf and root tissue samples from planted *C. praegracilis* (5,6). Major variations in BT concentration occurred over the first two weeks of the sampling period, and no clear trends related to the spiked and unspiked storm events are clear. However, the fluctuations in BT levels between the mesocosms could be explained by the phytotransformation of BT to benzotriazole alanine and glycosylated benzotriazole (GBT) (Figure 3), both BT metabolites detected in heightened levels. Increases in GBT levels were demonstrated in both leaf and root tissue relative to a blank BT internal standard (7, 8) with LC-MS/MS (9).



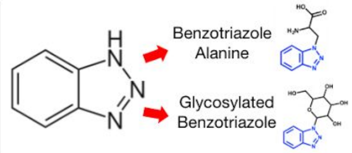
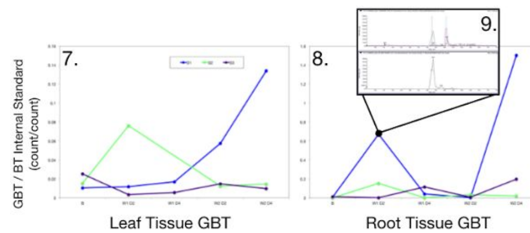


Figure 3.



Conclusions

No definite conclusions can be stated from this preliminary work. During the four week sampling period, BT was removed up to 79.4-92.7% in both vegetated and unvegetated mesocosms, demonstrating that model constructed wetlands can naturally remove a significant amount of BT from synthetic stormwater. However, concerns about breakthrough of BT suggest that future studies should focus on the effect of retention time on effluent BT concentration. In addition, it is imperative to measure and account for BT metabolites when sampling for BT in plant tissue. Simply accounting for BT is not enough because of its fast phytotransformation following assimilation. Future work must increase sampling frequency of *C. praegracilis*, target metabolite production, and potentially investigate the effect of metabolite synthesis on plant function.

References

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