

Objective

As reported in Deliverables 4.1 and 3.2, three eight-inch surrogate columns were constructed with the same bioretention soil materials and following the same installation and conditioning protocol used during the installation of the field bioretention columns. Following conditioning, influent and effluent water samples were tested on zebrafish embryos for any potential toxicity contributed from the combined gravel + bioretention soil media (BSM).



Figure 1. Surrogate columns for conditioned toxicity testing and destructive soil layer sampling.

Methods

Influent and effluent waters were stored at -20°C until the bioassays were performed. Zebrafish embryos (*Danio rerio*) ages 2-4 hours post fertilization (hpf) were exposed at 28.5°C to the influent, the effluent, or freshly made system water as a laboratory control (24 replicates for each treatment). After 48 h of exposure, embryos were checked for mortality and photographs were taken using a digital camera mounted on a Nikon SMZ 800 stereomicroscope. Embryo length, eye area, periventral and pericardial areas were measured using Image J (Figure 2), an open source image processing program (Rueden et al. 2017¹). ¹Rueden, C. T.; Schindelin, J. & Hiner, M. C. et al. 2017. ImageJ2: ImageJ for the next generation of scientific image data. BMC Bioinformatics 18:529. doi:10.1186/s12859-017-1934-z

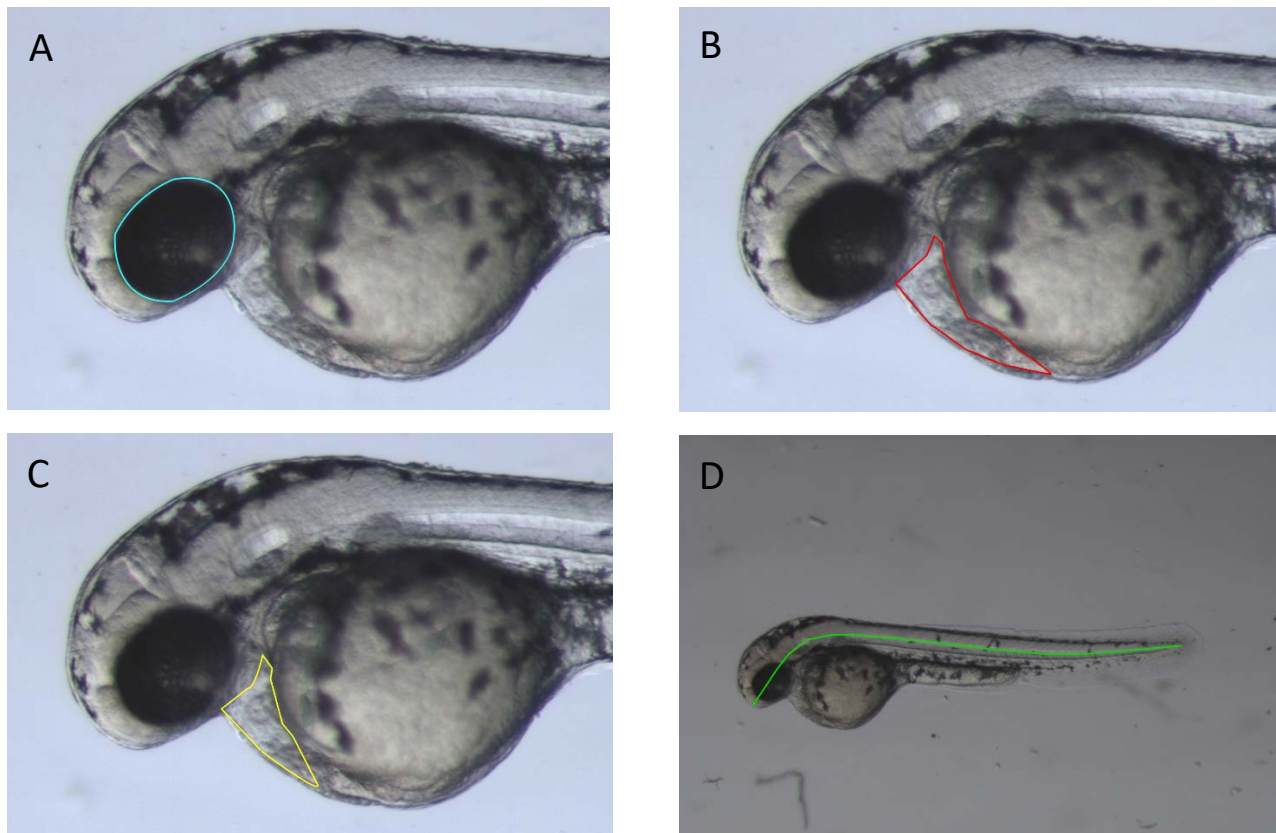


Figure 2. A: Eye area; B: Periventral area; C: Pericardial area; D: Embryo length.

Baseline toxicity

As expected, the clean water effluent from the bioretention columns was not acutely toxic to zebrafish embryos. The endpoints measured (survival, eye area, pericardial area, periventral area and length) after 48-h exposure to the effluent were not statically different from control and from the embryos exposed to the influent water (MANOVA, $p > 0.05$; Table 1).

Table 1: Summary of sublethal effects of runoff on zebrafish development at 48 hpf (hpf = hours post fertilization). Values presented are mean \pm s.e. from each endpoint.

Treatment	hpf	Hatched	survival	Pericardial area (mm ²)	Periventral area (mm ²)	Eye area (mm ²)	Length (mm)
Control	48	2	100%	0.023 \pm 0.001	0.018 \pm 0.001	0.043 \pm 0.001	2.839 \pm 0.011
Effluent	48	0	100%	0.023 \pm 0.001	0.019 \pm 0.001	0.042 \pm 0.002	2.852 \pm 0.025
Influent	48	0	100%	0.025 \pm 0.002	0.019 \pm 0.001	0.044 \pm 0.001	2.834 \pm 0.010