Mitigation of chemical flocculation toxicity with a proprietary mitigation agent

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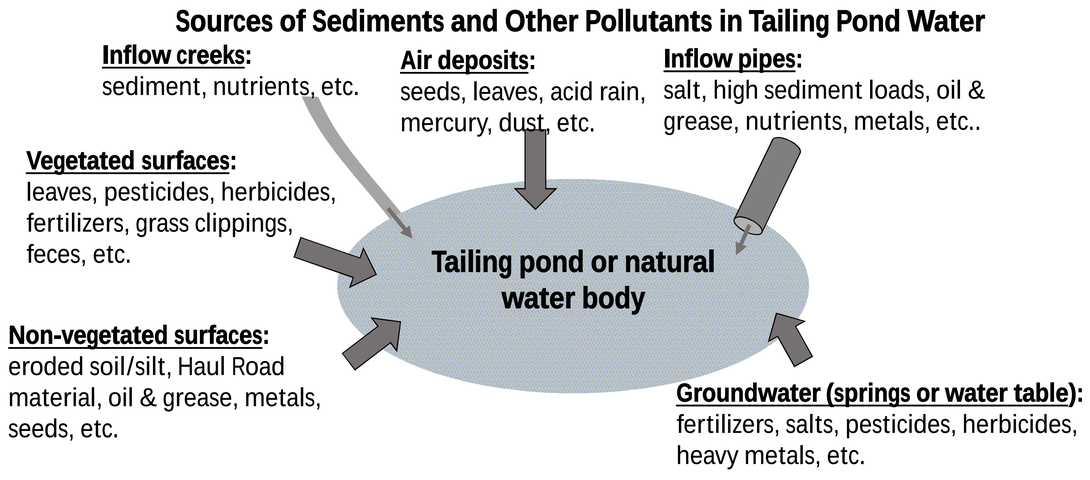
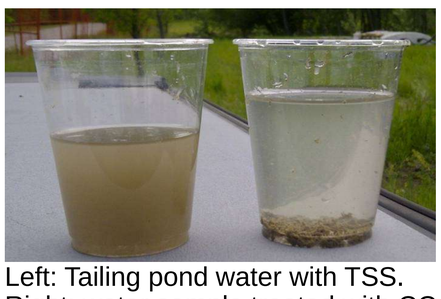
Background

Industrial operations such as mining, road building and aggregate washing result in high concentrations of **suspended particles (Total Suspended Solids; TSS)** in effluent waters.



Tailing/settling ponds promote sedimentation of TSS and prevent adverse effects of sediment deposition in aquatic environments prior to release into natural waters. However, small suspended particles do not settle rapidly and are easily transported over long distances in flowing water.

The use of **chemical coagulation (CC) and subsequent flocculation** is a common secondary treatment to improve the efficacy of TSS removal prior to discharge into the environment; however, these flocculating agents, when used in excess, have a high toxic potential at very low concentrations (0.3 - 0.5 mg L^{-1}) with noted effects including gill adherence and subsequent epithelial damage.



Left: Tailing pond water with TSS.
Right: water sample treated with CC

Novel treatment technologies exist to treat flocculated water with a **proprietary mitigation agent (MA)**, thus eliminating the toxicity of treated water. However, the mechanism of this mitigation has not been empirically demonstrated.

Given that flocculent exposure causes gill agglomeration/damage, we ***hypothesize*** that the mode of flocculent toxicity is *via* impairment of oxygen uptake.

Objective

To empirically determine the **mechanism** and **efficacy** of flocculent toxicity mitigation by **MA.**

Methods

96 h NOEC toxicity tests

- Rainbow trout (*Oncorhynchus mykiss*) exposed to varying ratios of **CC and MA** (**1:[0, 0.25, 0.5, 0.75, 1, 1.5])**
  - Determine mitigation efficacy
  - Determine effective NOEC using CC:MA ratio

Whole-mount gill morphology

- Rainbow trout exposed to CC (0.5 mg L^{-1}), CC/MA (0.5/0.75 mg L^{-1}), MA alone (0.75 mg L^{-1}) and Control water.
  - 4 h exposure, gill arch removed – whole-mount Nomarski interference contrast

Oxygen uptake studies

- Rainbow trout exposed to CC (0.5 mg L^{-1}), CC/MA (0.5/0.75 mg L^{-1}), MA alone (0.75 mg L^{-1}) and control water alone
  - Oxygen consumption (MO_2) measurements at 0, 12, 24, and 48 h

96 h NOEC toxicity tests

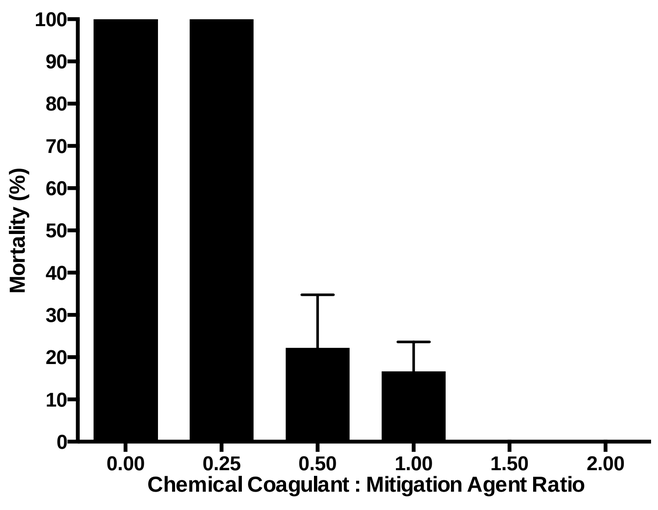


Figure 1. Rainbow trout mortality following exposure to varying ratios of CC:MA mixtures.
Fingerling (8-10 cm long) Rainbow trout (n=10) were exposed to mixtures of CC and MA in varying ratios while mortality was characterized following 96 h of exposure. Data presented as means + s.e.m. (16-24 replicates).

- **Full mitigation at CC:MA ≥ 1.5**

Gill morphology

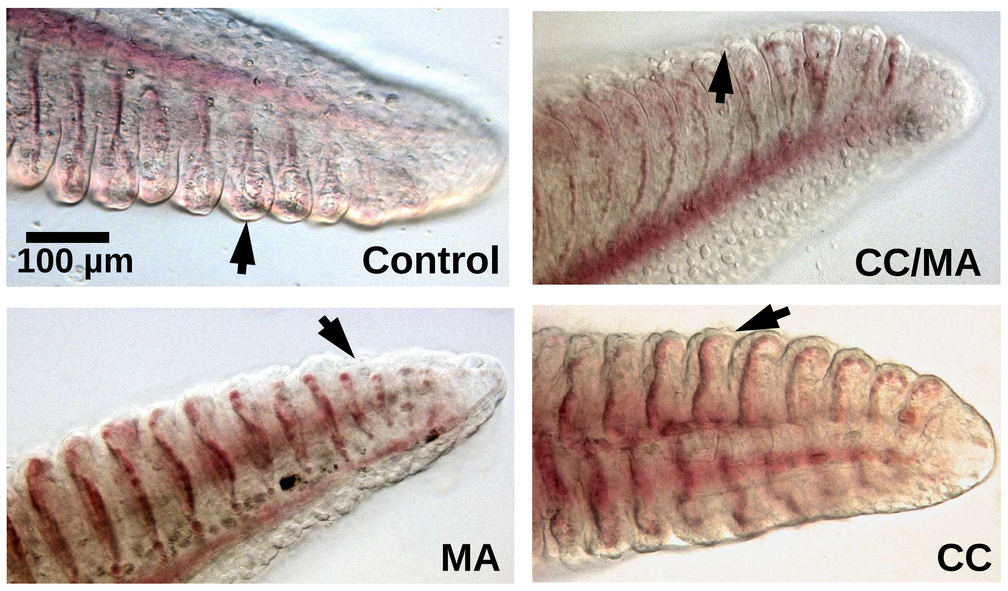


Figure 2. Effects of CC and MA exposure on gill morphology.
Whole mount of 2nd right arch gill filaments viewed using Nomarski interference contrast (Leica DMRXA light microscope; x20 objective). Fingerling Rainbow trout were exposed for 4 h to either CC (0.5 mg L^{-1}), MA (0.75 mg L^{-1}) or CC/MA (0.5 L^{-1}/0.75 mg L^{-1}). Control fish were held in freshwater only.

- **Control: Clear well-defined lamellar edges on filament (➨)**
- **CC/MA, MA: Small amounts of translucent globules loosely adhering to the epithelium (➨)**
- **CC: gill filament coated with translucent film obscuring internal structure of the gill (➨)**

Effects of CC and MA exposure oxygen uptake

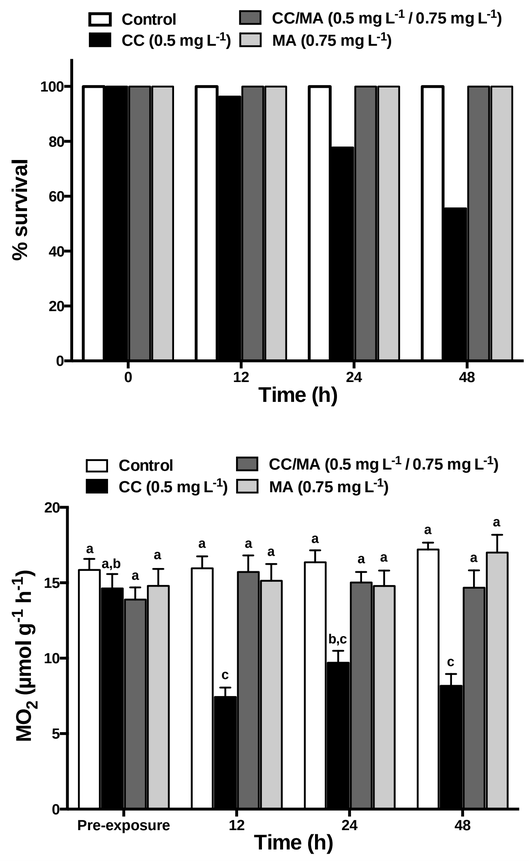


Figure 3. Effects of CC and MA exposure on oxygen consumption.
Metabolism (MO_2) in Rainbow trout prior to, and during exposure to either CC (0.5 mg L^{-1}; black bars), MA (0.75 mg L^{-1}; light grey bars) or CC:MA mixture (0.5 mg L^{-1}/0.75 mg L^{-1}; dark grey bars). Control animals (open bars) were held in freshwater containing neither CC nor MA. Trout (N=70) were acclimated overnight prior to determination of routine MO_2 (Pre-exposure), then separated into the aforementioned groups and exposed to CC/MA spiked freshwater for up to 48 h. At intermittent time-points, up to four (4) randomly selected trout from each exposure condition were selected and MO_2 was determined over a 1 h period in their respective conditions. Following sampling, trout were returned to exposure conditions. Data presented as means + s.e.m. *n* = 8. Bars not sharing the same letter are significantly different; $p<0.05$, two-way ANOVA with Tukey *post hoc* analysis.

- **Mortality only observed in CC**
- **Impairment of O_2 uptake only in CC**

Conclusions

- CC toxicity is fully mitigated by MA with a mixture ratio of ≥ 1:1.5 in rainbow trout.
- Whole-mount microscopy suggests that CC binds to gill epithelium
- Small amount of adhesion in CC/MA and MA , yet no apparent toxicity
- MO_2 studies suggest that the mechanism of toxicity of CC is *via* hypoxia
- No effects on MO_2 in MA or CC/MA conditions

Further studies

- Characterize fuel (tissue glycogen/glucose, ATP, phosphocreatine) and metabolite (tissue lactate) levels in brain, liver, muscle
- Quantitative PCR analysis on HIF1α in brain, heart, liver as hypoxia markers

Acknowledgements

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