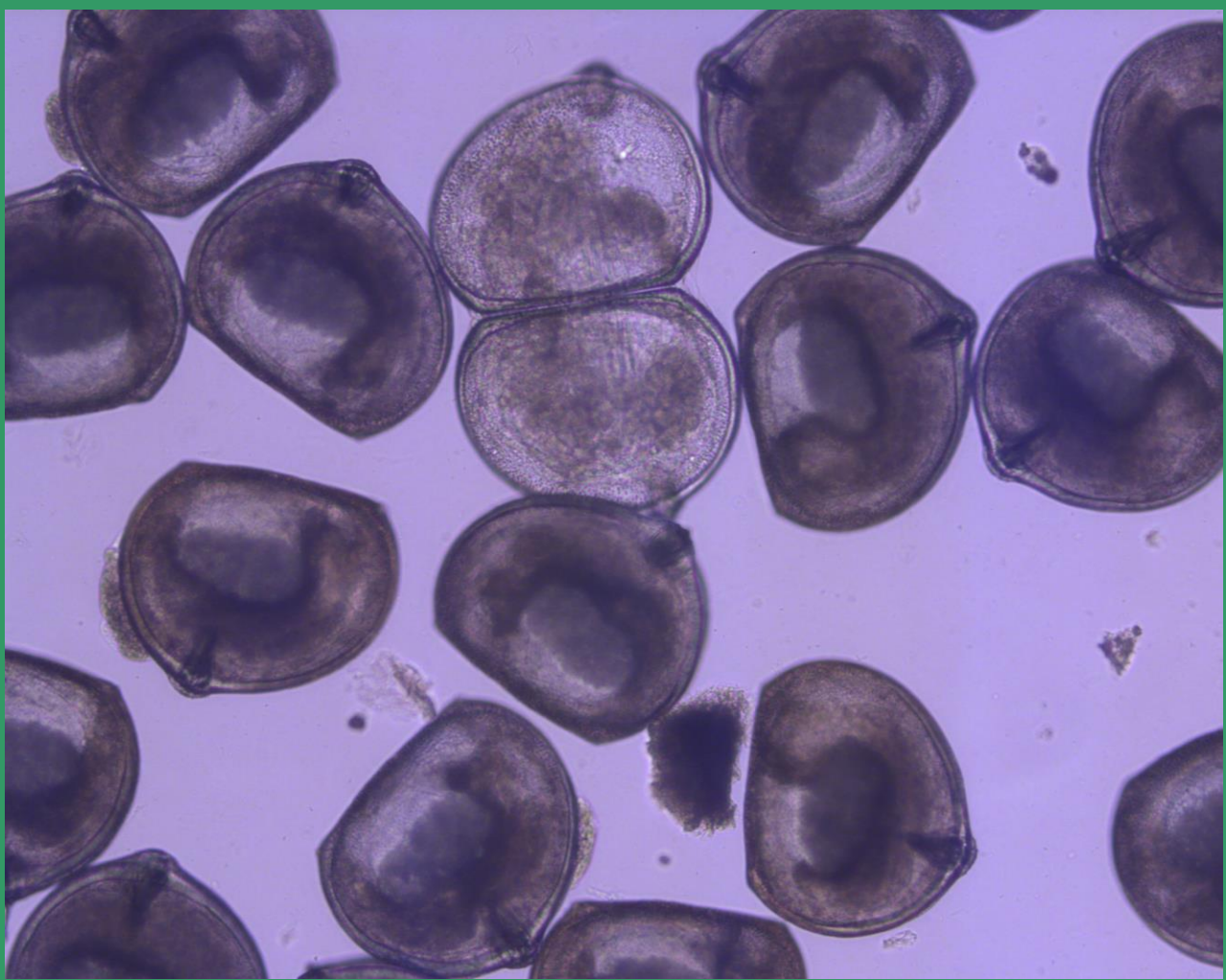


Chronic Copper–Zinc Toxicity to Freshwater Mussel Glochidia Is Strongly Modified by Plant-Derived Dissolved Organic Matter

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Background

- New Zealand's declining freshwater mussel species, *Echyridella menziesii*, also known as kākahi, kāeo or torewai in Te Reo Māori, are a native taonga (treasured) species.
- Kākahi are important freshwater ecosystem engineers, improving water quality through filter feeding.
- Copper (Cu) and zinc (Zn) are common freshwater contaminants whose toxicity is influenced by dissolved organic matter (DOM) via metal binding and bioavailability.
- In partnership with iwi, Ngāti Whātua Ōrākei, and ecotoxicology experts we investigate the potential for DOM derived from NZ native plants to modify Cu, Zn, and Cu:Zn mixture toxicity in freshwater. The native plants tested were Kānuka (*Kunzea robusta*), Kōwhai (*Sophora molloyi*), Mānuka (*Leptospermum scoparium*) and Pōhutukawa (*Metrosideros excelsa*).
- Chronic toxicity testing involving the sensitive early larval (glochidia) life stage of kākahi was used to assess if, and to what degree, native plant derived DOM can reduce Cu and Zn toxicity to this native taonga.

Methods

- Leachates were prepared by aerating 25 g/L dried leaves in aged UV-treated water for 7 days.
- Gravid female adult kākahi were collected and spawning induced to obtain mature glochidia (Fig. 1).
- Glochidia viability was assessed using an osmotic shock test.
- Approximately 200 pooled viable ($\geq 90\%$) glochidia per replicate were exposed to five concentrations of Cu, Zn and Cu:Zn mixtures (1:6 ratio) in the presence of plant-derived DOM.
- Tests were conducted at 20 °C and hardness of ~ 11 mg/L CaCO_3 in 35 mL volumes with an initial pH of 7.5 ± 0.4 , 5 control replicates and 3 treatment replicates per concentration at 5 and 10 mg/L dissolved organic carbon (DOC) (Fig. 1).
- Glochidia viability was assessed after a 48 h exposure. Physicochemical parameters (pH, dissolved oxygen, conductivity, and temperature) were measured at the start and end of the exposure.



Figure 1: Field collection (top left) of adult kākahi (top right) and microscope setup used for glochidia testing (bottom)

Results

- Glochidia survival was influenced by both DOM source, concentration and metal treatment (Cu, Zn and mixtures), highlighting complex interactions between DOM characteristics and metal toxicity (Fig. 2-4).
- Increasing DOC generally increased toxicity mitigation i.e. increased EC_{50} , however Kōwhai exhibited adverse effects (3.8% survival) at 10 mg/L DOC without added metals (Fig. 2-3).
- **5 mg/L DOC:** Pōhutukawa had the highest EC_{50} values (lowest toxicity) for Cu (88 $\mu\text{g/L}$) and Zn (490 $\mu\text{g/L}$). Kānuka showed an additive Cu:Zn interaction, indicating combined toxicity matched expected effects. Kōwhai, Mānuka and Pōhutukawa showed antagonistic interactions, where mixture toxicity was less than expected, with the strongest antagonism observed for Kōwhai (Fig. 3-4).
- **10 mg/L DOC:** Pōhutukawa had the highest EC_{50} values for Cu (276 $\mu\text{g/L}$) and Zn (772 $\mu\text{g/L}$). Kānuka and Mānuka showed antagonistic Cu:Zn interactions, indicating reduced metal toxicity relative to expectation. Pōhutukawa showed a synergistic interaction, where the Cu:Zn mixture was more toxic than expected (Fig. 3-4).

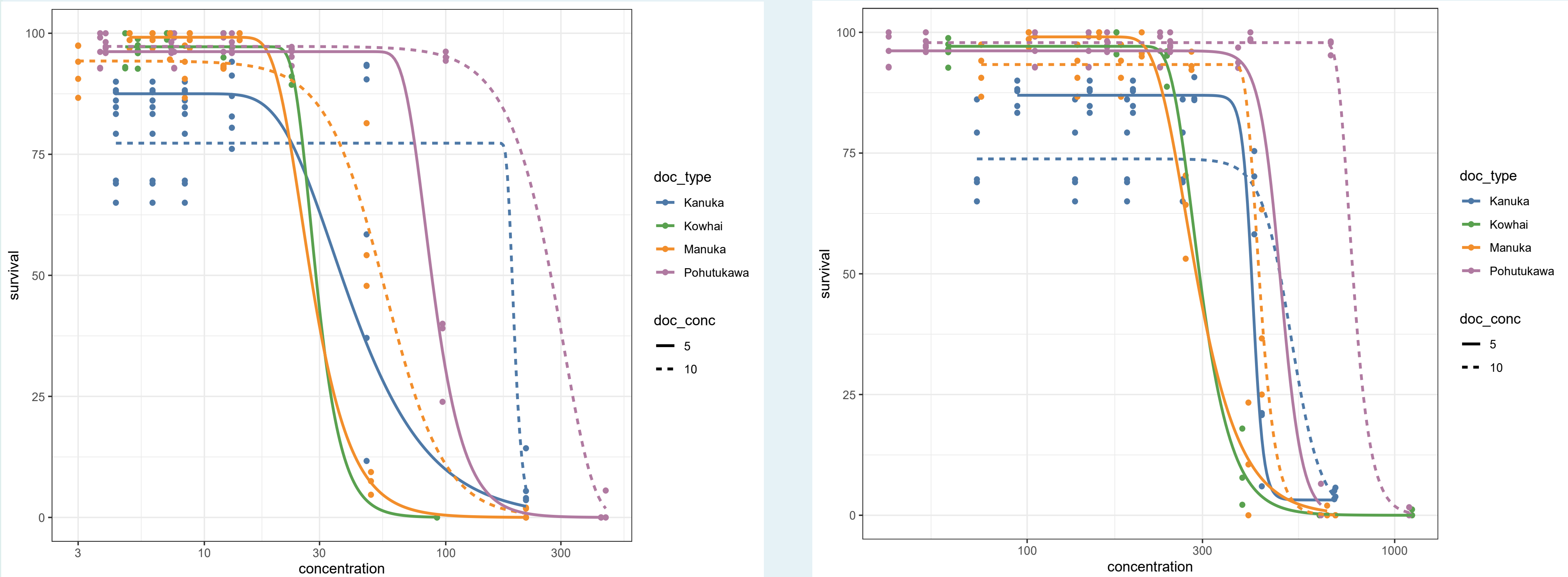


Figure 2: Concentration-response relationships for glochidia 48 hour survival percentage when exposed to $\mu\text{g/L}$ Cu (left) or Zn (right) in the presence of plant derived DOM.

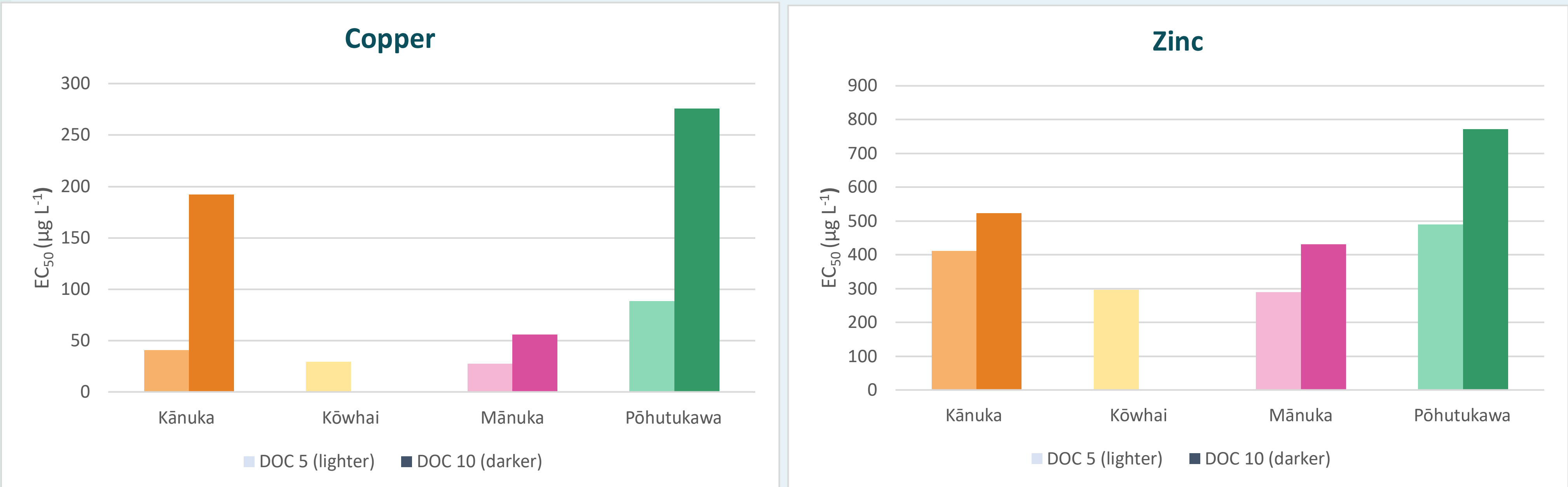


Figure 3: Glochidia 48 hr survival EC_{50} (50% effect concentration) values for Cu and Zn across DOC sources and concentrations. Note: Increasing EC_{50} = reduced toxicity.

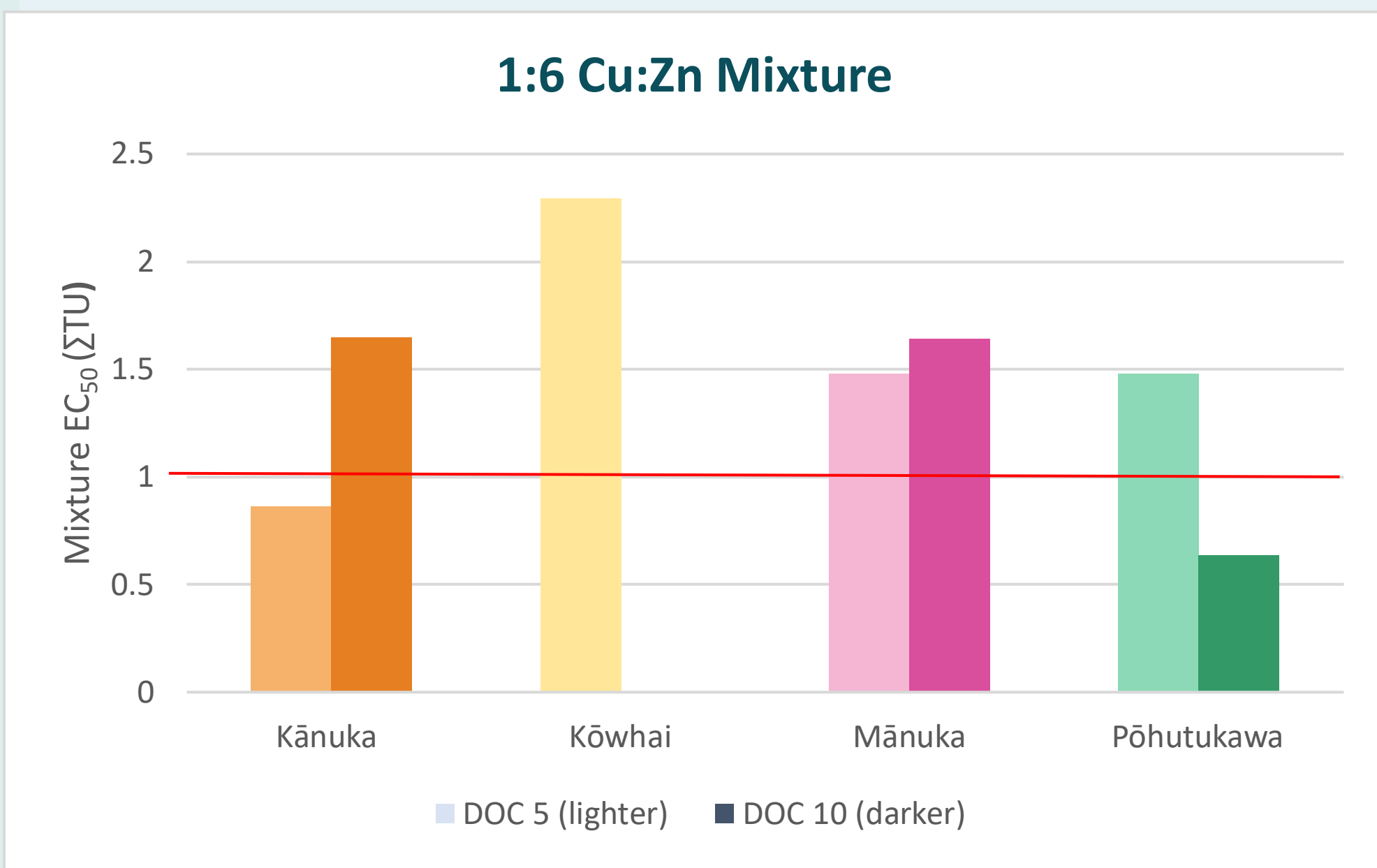


Figure 4: Mixture EC_{50} values (ΣTU) for glochidia exposed to a 1:6 Cu:Zn mixture with DOM from different plant sources at 5 and 10 mg/L DOC. The accompanying ΣTU table classifies interactions as synergistic ($\Sigma\text{TU} < 1$), additive ($\Sigma\text{TU} \approx 1$), or antagonistic ($\Sigma\text{TU} > 1$).

DOM Source	Interaction 5 mg	Interaction 10 mg
Kānuka	Additive ≈ 1	Antagonistic > 1
Kōwhai	Antagonistic > 1	No data
Mānuka	Antagonistic > 1	Antagonistic > 1
Pōhutukawa	Antagonistic > 1	Synergistic < 1

Summary and Next Steps

- Native plant-derived DOM show potential as a nature-based solution for mitigating Cu and Zn toxicity to *E. menziesii*. Protective effects varied among DOM sources, with some DOM sources also causing toxicity in the absence of metals.
- DOM influenced metal bioavailability and Cu:Zn mixture interactions, with additive, antagonistic and synergistic responses observed.
- Integrating these results with other toxicity testing and DOM characterisation data will support the development of predictive models for DOM-mediated metal toxicity and bioavailability.
- Combined research findings will inform practical design guidance for nature-based solutions, co-developed with engineering and iwi partners to support metal mitigation, freshwater health and species protection.

