The influence of bioavailable copper and zinc concentrations on metallothionein levels and DNA damage in the polychaete *Nereis (Alitta) virens*

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Introduction

- The King ragworm, *Nereis (Alitta) virens* (Fig. 1), is an ecologically and commercially important polychaete species of soft sediment inter-tidal communities throughout the northern hemisphere and is known to be impacted by various anthropogenic activities.
- Metals such as copper (Cu) and zinc (Zn) are naturally present in the ecosystem but can accumulate in the sediment at high levels due to industrial activities and therefore have an impact on polychaete species.

Aim : To establish (1) the resistance capacities by the induction of detoxification processes and (2) the level of DNA damage in a population of *N. virens* Exposed to environmentally relevant metal concentrations based on an integrated multi-biomarker approach.

Fig. 1 - *N. virens.*

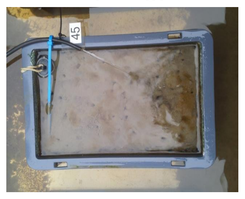
Experimental design

Duration : **9 months** with sampling every 3 months

Metal sediment **spiking approach** using target concentrations based on environmentally relevant metal concentrations from low (Poole Harbour), medium (Tamar Estuary) and high (Fal Estuary) contaminated UK sites.
Experimental concentrations are shown for month 3 (± SEM).

	Low		Medium		High	
(mg kg^{-1})	Target	Experimental	Target	Experimental	Target	Experimental
Cu	70	77 (± 7)	120	157 (± 11)	575	614 (± 109)
Zn	206	197 (± 42)	267	322 (± 35)	1160	615 (± 26)
Cu + Zn	70 + 206	62 (± 13) + 173 (± 34)	120 + 267	81 (± 6) + 188 (± 21)	575 + 1160	563 (± 25) + 607 (± 31)

- **10 treatments** including control
- **9 replicas** of each treatment
- 8 worms (1-3g) per box

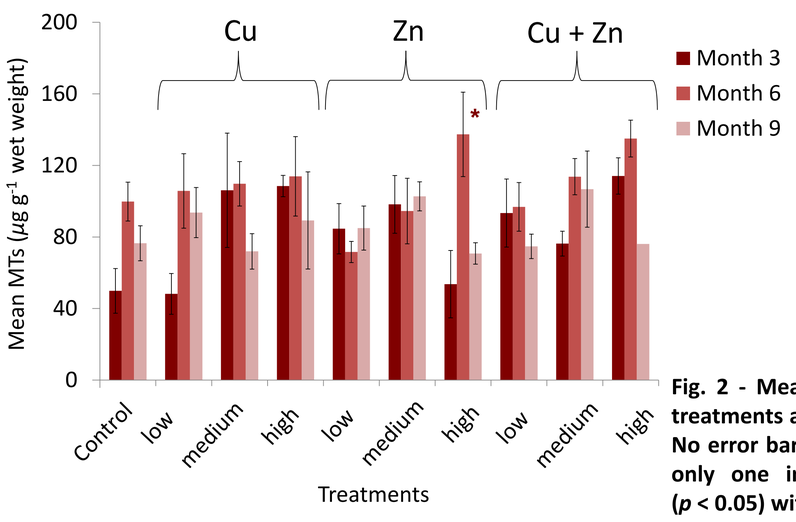


- Continuous seawater flow
- Temperature, pH, salinity, dissolved oxygen monitored weekly

Month 3 - 6 - 9	
Sampling	**Analysis**
Sediment (< 63µm)	Cu and Zn (BCR sequential extraction)
Pore water	Cu and Zn (Acid extraction)
Worms	Cu and Zn (Acid digestion)
Tissues	Metallothioneins (MTs) (Spectrophotometric method)
Coelomic fluid	DNA damage (Comet assay)

Results & discussion

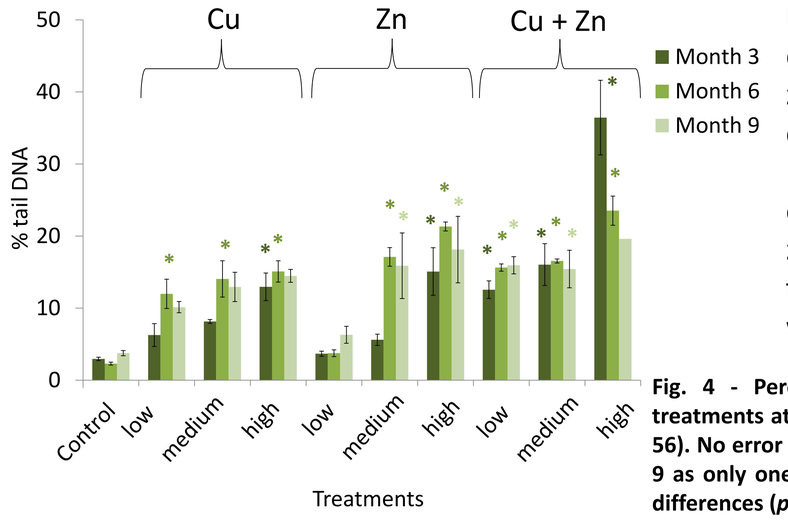
MTs



MT concentrations increased from month 3 to month 6 and decreased from month 6 to month 9 in all treatments except in low zinc and medium zinc (Fig. 2) revealing the induction of detoxification processes.

Fig. 2 - Mean MT concentrations in the worms for all treatments at month 3 (n = 30), 6 (n = 29) and 9 (n = 27). No error bar was shown for high Cu + Zn at month 9 as only one individual survived. Significant differences ($p < 0.05$) within the treatments are indicated (*) .

DNA damage



Data indicated high DNA damage in high copper, high zinc and high copper & zinc combined treatments. The maximum DNA damage was obtained in the high copper & zinc combined treatment after three months of exposure with 36.44 % (Fig. 4).

Fig. 4 - Percentage tail DNA in *N. virens* for all treatments at month 3 (n = 54), 6 (n = 103) and 9 (n = 56). No error bar was shown for high Cu + Zn at month 9 as only one individual survived. Monthly significant differences ($p < 0.05$) from control are indicated (*).

Influence of metal bioavailability on MTs

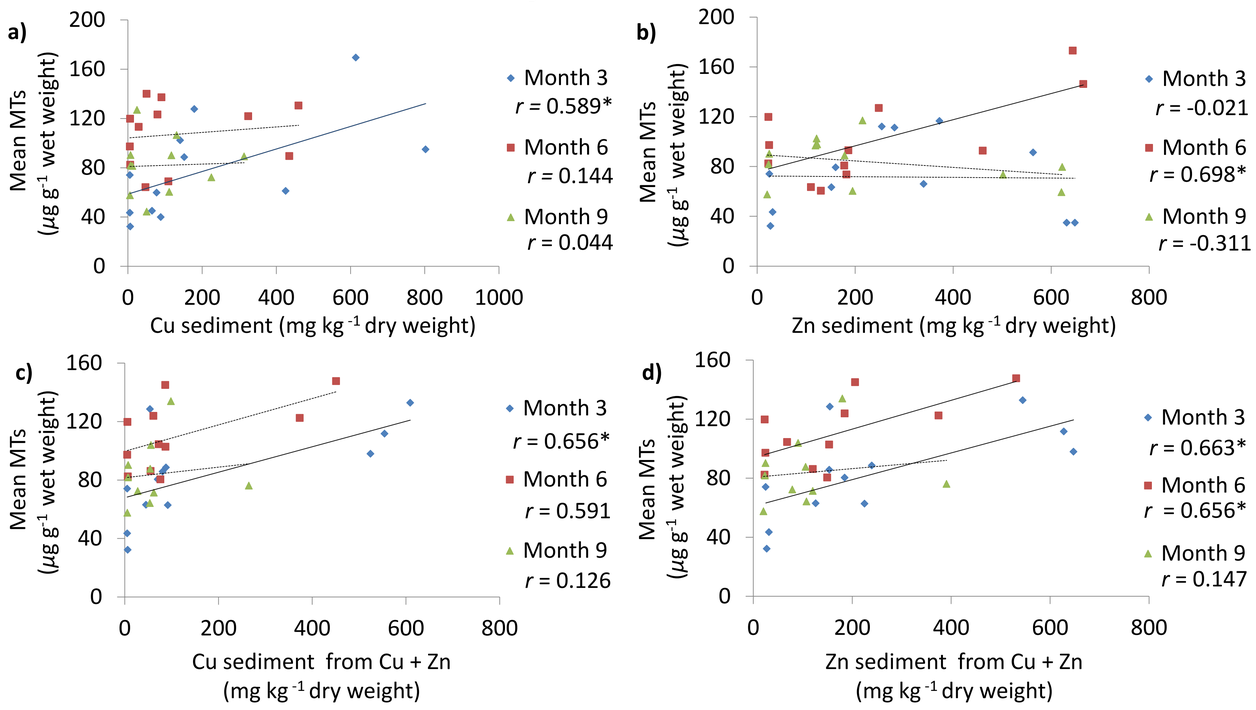


Fig. 3 - Relationships between MTs and sediment metal bioavailable concentrations for month 3, 6 and 9. Pearson's correlation coefficients (r) are reported on the graphs and the significant correlations ($p < 0.05$) are indicated (*). Non significant correlations are indicated by a dash line.

Influence of metal bioavailability on DNA damage

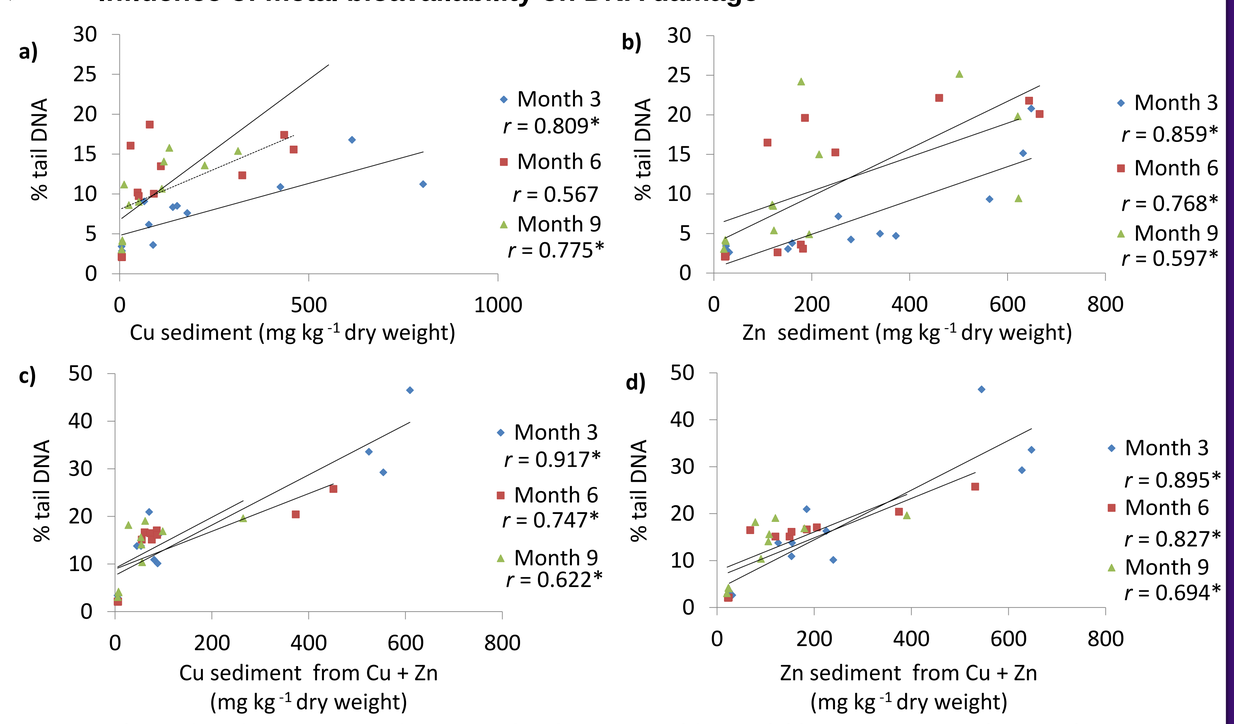


Fig. 5 - Relationships between DNA damage and sediment metal bioavailable concentrations for month 3, 6 and 9. Pearson's correlation coefficients (r) are reported on the graphs and the significant correlations ($p < 0.05$) are indicated (*). Non significant correlations are indicated by a dash line.

Conclusion

- MTs analysis revealed that *N. virens* were capable to induce metal defence mechanisms through the activation of detoxification processes.
- High copper & zinc combined treatments showed the greatest impact revealed by high DNA damage. Similar levels of DNA damage were found in *N. virens* inhabiting the Tamar Estuary[1].

=> Environmentally relevant concentrations of Cu and Zn can lead to high levels of DNA damage while inducing metal resistance capacities.

- Relationships between MTs or DNA damage with metal bioavailability varied over time.
- MTs and especially DNA damage prove to be sensitive endpoints when evaluating the impacts of metal contamination on marine invertebrates.

[1] Lewis, C. & Galloway, T. (2008), 'Genotoxic damage in polychaetes: A study of species and cell-type sensitivities', *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 654(1), 69–75.