

Clover Rhizobia

Clover Rhizobia population continues to decline in sewage sludge amended soils after 20 years

PAT COOPER¹, JEFFREY BACON¹, COLIN D. CAMPBELL^{1,2}, DUNCAN WHITE¹.

The James Hutton Institute, Craigiebuckler, Aberdeen, AB15 8QH, UK

Soil and Environment Department, Swedish Agricultural Sciences University, Uppsala, Sweden

Email: pat.cooper@hutton.ac.uk



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Introduction

Much debate surrounds which metals from sewage sludge are toxic to rhizobia, in this work we are able to show that Zn is the most toxic (Chaudri et al) and that effects can be seen in levels below those set in the guidelines.



Since 2003 the *Rhizobium leguminosarum* biovar *trifolii* (clover rhizobia) population has decrease to such an extent that in 2013 there were no Rhizobia present in any of the zinc amended plots including the long term build up plots which had reached 244 mg/kg zinc in 2013.



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Methods

Topsoil samples (0 – 25 cm) for soil analysis were taken usually late spring every 2 years from 1999. The soils were analysed for most probable numbers (MPNs) of clover Rhizobium (Vincent 1970; Blodgett 2010).

The most probable number (MPN) method was used to estimate the number of indigenous clover rhizobia in the soil from each plot, using a 10 fold dilution series and White clover (*Trifolium repens* cultivar *Huia*), as the trap host. This method of enumeration relies on the fact that a specific N-fixing *Rhizobium* (in this case *Rhizobium leguminosarum* biovar *trifolii*) has a symbiotic relationship with white clover.

Plants were scored for the presence/absence of nodules and the scores converted to most probable numbers (Blodgett, 2010).



Results

The first significant effect on the rhizobia population was seen in 1999 in the zinc 450mg kg⁻¹ rate and has become continuous since 2003. Also in the same year the same effect was seen in the zinc 350mg kg⁻¹ plots and the toxic effect has continued to the most recent sampling in 2013 without any recovery. Zinc toxicity has also had an effect on the zinc 250mg kg⁻¹ rate since 2005 and started to show an effect on the long term build up plots from 2009 (total zinc concentration 150mg kg⁻¹ in 2009) with complete decline to no rhizobia in 2013 (total zinc concentration 244mg kg⁻¹ in 2013).

Actual Zinc concentrations mg/kg	
Control	82
Blank Sludge	104
Zinc 150	164
Zinc 250	264
Zinc 350	343
Zinc 450	500
Zinc long term build up	244

Table 1. Total Zinc concentration mg/kg 2013

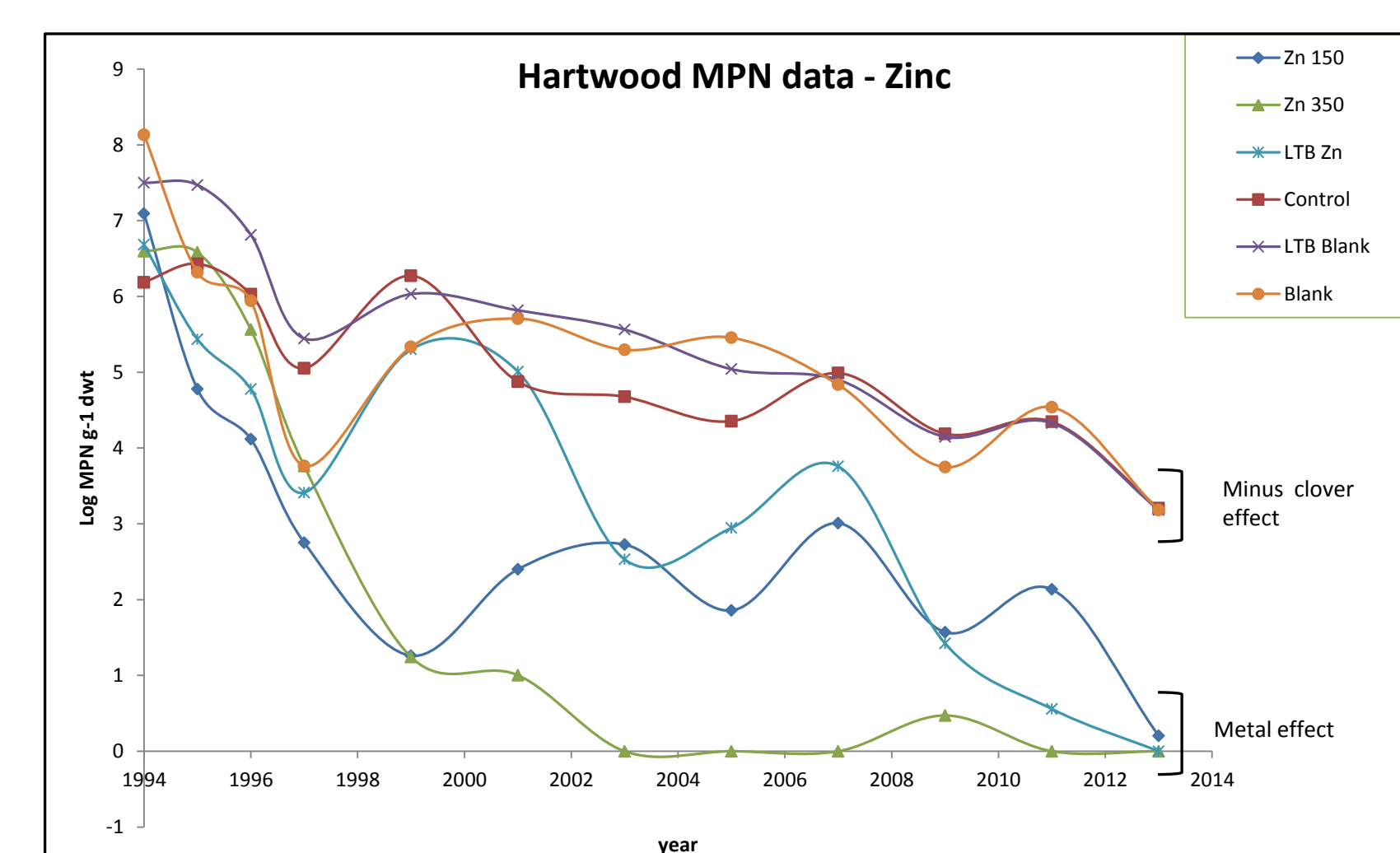


Fig 1. Numbers of indigenous clover in soil over time



Fig 2. Clover plants from Zinc treatments in 2005

Conclusion

Zinc is more toxic than other suspected metals and the effects seems to be long – term with little evidence of lasting recovery, it remains to be seen when the same effects are seen in the Copper and Cadmium treatments. It may be time to propose new zinc levels as guidelines.

- Future work -Testing remedial treatments
- Role of organic contaminants

Ref.: Chaudri, A., et al (1993) Enumeration of indigenous *Rhizobium Leguminosarum* biovar *trifolii* in soils previously treated with metal contaminated sewage sludge. *Soil Biology and Biochemistry*, 25, 301-309.