Review of impacts on soil biota caused by copper residues from fungicide application

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Abstract
Unlike most other agricultural chemicals, a significant weight of evidence exists that copper based fungicides have a long-term impact on a wide range of soil biota. Effects can occur at relatively low Cu concentrations and influence a number of soil processes including microbial activity, earthworm activity and bioturbation. In most soils, copper residues are likely to remain indefinitely, and will continue to influence the health of the soil. This manuscript reviews Australian and International data on the effects of copper based fungicides on soil health, and the implications for future land management.

Key words
Copper fungicide, earthworm avoidance, microbial activity.

Introduction

The use of pesticides has long been a feature of conventional agricultural practice and their use has made it possible to increase crop yields and food production (Lee 1985). However, many of these chemicals have toxic effects that are not confined to their target species, and their application may have impacts on organisms that benefit the wider agroecosystem.

There is significant uncertainty in the prediction of impacts of external stressors such as pesticide application on the agricultural ecosystem (Liess 2004). Questions have been raised over the significance of short and even long-term population changes of soil organisms, especially where no other indicator of harm is documented. To further challenge the evaluation of impacts, there are no standard tests for soil biota, such as those found in other fields of ecotoxicology. Soils contain an extremely diverse array of microorganisms including bacteria, fungi, yeasts; photosynthetic organisms including algae, and macroorganisms such as protozoa, nematodes, mites, springtails, spiders, insects and earthworms. The functions of this complex array of biota, often referred to as the 'soil food web,' are diverse, and include residue decomposition, nutrient storage and release, soil structure and stability, resistance against disease and degradation or immobilisation of pesticides and other pollutants. Soil biota are measured or studied in many different ways, including direct observation under microscopes and other direct counting mechanisms, analysis of intra- and extracellular enzymes, respiration and biomass carbon, fatty acid profiles, and DNA fingerprinting.

It was not until the 1880s that copper fungicides rapidly developed due to the 'accidental' discovery of the Bordeaux mixture. At this time, farmers in the Bordeaux region, France were applying a paste mixture of copper sulphate and lime to grapes that were bordering the highways to deter passer-by's. The French scientist, Millardet observed these grapes were also free of downy mildew (Copper Development Association 2003). By 1885, Millardet had completed experiments which confirmed this mixture controlled the mildew disease at very low cost. The Bordeaux mixture was the first fungicide to be used on a large scale world-wide (Schneiderhan 1933). Copper based fungicides have multi-site activity with a low risk of pathogens developing resistance.

In Australia, copper-containing sprays of various formulations (Table 1) have been used to control fungal diseases in pome and stone fruit orchards, vineyards and vegetable crops for well over 100 years (Merry *et al.* 1983). Over 7500 t yr^{-1} of Cu fungicides have been used, representing 13% of the global total (Lepp and Dickinson 1994). In stark comparison, England and Wales were estimated to use only 8 t of Cu fungicide between them in the year 2000 (Nicholson *et al.* 2003).

Table 1. Inorganic copper compounds used as fungicides in Australia.

Name	Chemical formula	Uses
Cupric sulfate	$CuSO_4.5H_2O$	Seed treatment and preparation of Bordeaux mixture
Copper dihydrazine sulfate	$CuSO_4(N_2H_5)_2SO_4$	Powdery mildew Black spot of roses
Copper oxychloride	$3Cu(OH)_2.CuCl_2$	Powdery mildews
Copper oxychloride sulfate	$3Cu(OH)_2.CuCl_2$ and $3Cu(OH)_2.CuSO_4$	Many fungal diseases
Copper zinc chromates	$15CuO\text{-}10ZnO.6CrO_3.25H_2O$	Diseases of potato, tomato, cucurbits, peanuts and citrus
Cuprous oxide	Cu_2O	Powdery mildews
Basic copper sulfate	$CuSO_4.Cu(OH)_2.H_2O$	Seed treatment and preparation of Bordeaux mixture
Cupric carbonate	$Cu(OH)_2.CuCO_3$	Many fungal diseases
Copper hydroxide	CuH_2O_2	Many fungal diseases

Source: Ware (1978)

Horticultural and viticultural operations with a long history of copper fungicide application have resulted in accumulations of copper in surface horizons (Gallagher *et al.* 2001; Chaignon *et al.* 2003). Prolonged use in Europe has lead to high levels in the soil (200-500 mg/kg in France, Brun *et al.* 1998), which has affected a large portion of agricultural land. An Australian study found up to 250mg/kg total copper in a 20-30 yr old vineyard soil, while 8-14 vineyards studied exceeded 60 mg/kg (Pietrzak and McPhail 2004). Similarly, avocado orchard soils in northern NSW were recently observed to have even greater soil Cu residues (280-340 mg/kg) (Merrington *et al.* 2002).

An example of an industry reliant upon copper fungicides in Australia is the avocado industry. Avocado orchards on the north coast of New South Wales have had copper oxychloride, cuprous oxide, copper hydroxide and copper ammonium acetate applied at up to 15 times per year mainly against anthracnose (*Colletotrichum gloeosporoides*) at recommended foliar application rates of 3-6 kg/ha (Van Zwieten *et al.* 2004). By contrast the use of copper-containing products in organic farming practices in Australia is restricted: Copper sulphate and hydrated lime mixtures, copper hydroxide and copper sulphates are permitted by certifying authorities but copper oxychloride is prohibited (Biological Famers of Australia 2003). Further, since 2002, the International Federation of Organic Agriculture Movements (IFOAM) has regulated total copper input on organic farms to a maximum of 8 kg/ha/yr. These restrictions applied by the organic farming industry acknowledge the potential for copper levels in orchard top-soils to accumulate with repeated application.

Impacts of copper on soil biota in an Australian study

Cu residues in avocado orchards have recently been shown to impact significantly on soil microorganisms (Merrington *et al.* 2002). Cu residues (280 and 340 mg/kg, respectively) in surface soils (0-2cm) of an established orchard were shown to be significantly greater than a nearby reference site under natural vegetation (13 mg/kg) (Table 2). The available fraction of Cu in these soils was also shown to be significantly greater (2.15 and 1.29 mg/kg, *c.* pCu^{2+} 8.64) than in the reference site (0.71 mg/kg, *c.* pCu^{2+} 9.2), as measured by ion-selective electrode in $CaCl_2$ extraction. Similar trends were observed for the 2-10 cm soil profile. Data (Table 3) suggested that the Cu residues were responsible for significant reductions in biomass carbon (C_{mic}) even though the orchard soils had similar or elevated levels of total organic carbon (C_{org}). The C_{mic}:C_{org} ratio was significantly lower in all of the Cu contaminated soils. Soil respiration was elevated (6.04 and 5.57 mg CO_2- C/kg/day) compared to the reference soil (3.04 mg CO_2-C/kg/day), and the metabolic quotient (qCO_2) was also significantly greater, indicating the microbial populations in these soils were stressed

Table 2. Free Cu as pCu^{2+}, and expressed as free Cu concentration and percentage of $CaCl_2$ extractable Cu in the orchard and reference soil.

Values are means ± standard error; n = 6. Significantly (*$P \leq 0.05$) different from respective soil layer in the reference soil

Sample site	'Total' Cu (mg/kg)	$CaCl_2$ extractable Cu (mg/kg)	pCu^{2+}	Free Cu^{2+} concentration (μg/L)	% Free Cu :$CaCl_2$ extractable Cu	Organic C (%)	pH (1:5 $CaCl_2$)
Block 1, 0-2 cm	280 ± 33*	2.15 ± 0.39*	8.64 ± 0.25*	0.363	0.017	8.82 ± 0.48*	6.01 ± 0.23*
Block 1, 2-10 cm	176 ± 48*	0.76 ± 0.12*	8.89 ±0.22*	0.206	0.027	4.88 ± 0.59	4.50 ± 0.47
Block 2, 0-2 cm	345 ± 78*	1.29 ± 0.21*	9.17 ± 0.16	0.108	0.008	8.43 ± 1.00*	5.80 ± 0.20*
Block 2, 2-10 cm	301± 112*	0.71 ± 0.15*	8.90 ± 0.21*	0.201	0.028	5.37 ± 0.14	5.34 ± 0.23
Reference, 0-2 cm	13 ± 1.8	0.71 ± 0.19	9.14 ± 0.05	0.115	0.016	6.74 ± 0.14	4.43 ± 0.05
Reference, 2-10 cm	14 ± 0.1	0.24 ± 0.05	9.36 ± 0.07	0.070	0.029	4.93 ± 0.30	4.51 ± 0.13

Source: Merrington *et al.* (2002)

Table 3. Microbial biomass C, soil respiration, metabolic quotient (qCO_2) in the orchard and reference soil.

Values are means ± standard error; n = 6. *($P \leq 0.05$) significantly different from respective soil layer in the reference soil

Sample site	Biomass-C (mg C/kg)	Biomass-C (% of Organic-C)	Soil respiration (mg CO_2-C/kg/ day)	qCO_2 (mg CO_2-C/g biomass C/ day)
Block 1, 0-2 cm	2167 ± 197*	2.48 ± 0.23*	6.04 ± 0.93*	2.85 ±0.40*
Block 1, 2-10cm	1021 ± 228*	2.01 ± 0.25*	1.11 ± 0.27	1.08 ± 0.21
Block 2, 0-2 cm	2023 ± 226*	2.48 ± 0.42*	5.57 ± 2.23*	2.85 ± 0.13*
Block 2, 2-10cm	1429 ± 170*	2.68 ± 0.47*	0.60 ± 0.13	0.48 ± 0.15
Reference, 0-2 cm	2617 ± 23	3.88 ± 0.05	3.04 ± 0.26	1.17 ± 0.01
Reference, 2-10cm	2424 ± 243	4.90 ± 0.20	3.00 ± 0.16	1.26 ± 0.13

Source: Merrington *et al.* (2002)

The avocado farmer from the site of the Merrington *et al.* 2002 study also observed that very few worms were located in the orchard. To study the impact of copper on earthworms, avoidance studies were undertaken using methods described by Yeardley *et al.* (1996). In these trials, worms were given the option to move between the target soil (ie, copper contaminated orchard soil), and a control soil. Various dilutions of the orchard soil were also made with the control. Results (Van Zwieten *et al.* 2004) showed the worms preferred non-contaminated control soils, sourced from adjacent to the orchard or an OECD standard uncontaminated soil, when Cu residues in the orchard soils reached 4-34 mg/kg. At levels of 553 mg/kg, 90% avoidance of orchard soil was observed (Figure 1).

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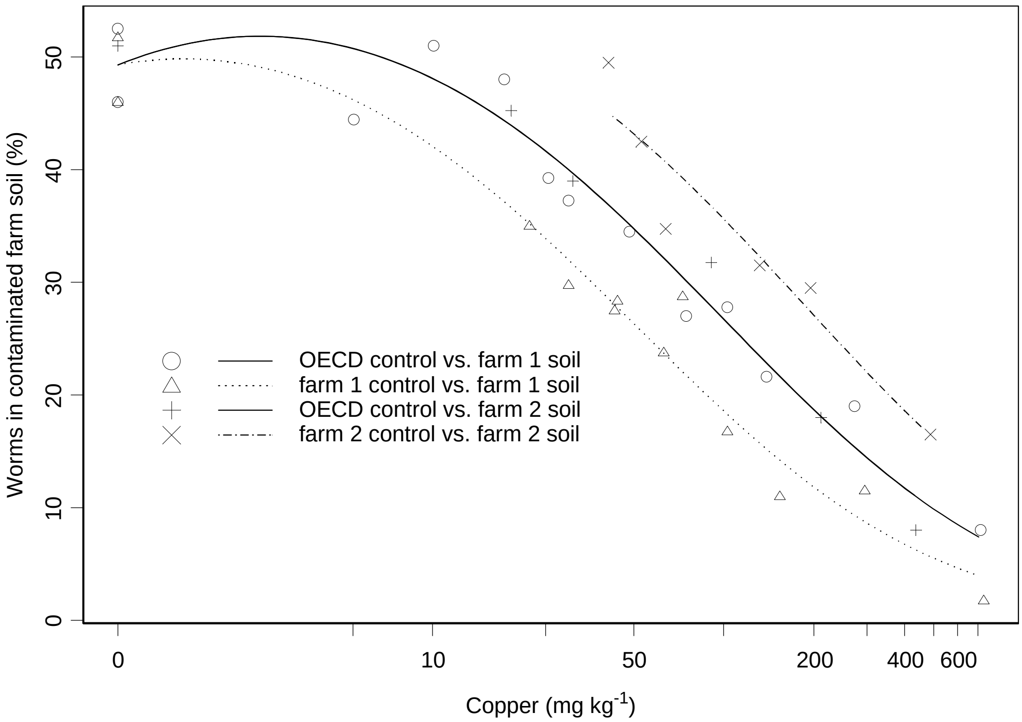

Figure 1. Observed and predicted response of worms to copper concentration in each test soil. Note that the curves for the OECD control vs farm 1 soil and OECD control vs farm 2 soil overlap. A 50% distribution of worms indicates 50% of the worms were found in the test soil and 50% were found in the control (uncontaminated) soil (ie no avoidance found).
Source: Van Zwieten *et al.* (2004)

The avoidance of soils contaminated by copper was reflected in a field study. The avocado orchard was sampled using a methodology described by Kingston and Temple-Smith (1988). Ten 20 cm square holes at distances of 10 m apart were dug to a depth of 30 cm. Soil was removed and placed on a plastic sheet where it was manually broken down. All worms found at each hole, whether whole or damaged, were placed directly into a jar containing 70% ethanol in water (by volume) and returned to the laboratory. The worms were removed from the jars, counted, rolled on tissues to remove excess fluid, counted and weighed.

Copper residues of between 180-338 mg/kg resulted in very low earthworm numbers and biomass (Table 4), when compared to a reference site directly adjacent to the contaminated orchard on similar soil type. Worms found under the canopy were small hence the low biomass found, while a large individual specimen found inter-row resulted in a high biomass/ worm number.

Table 4. Earthworm number and biomass in the orchard and reference soil.

Site location	Copper (mg kg^{-1})	Earthworm number m^{-2}	Earthworm biomass (g m^{-2})
inter-row	180	<0.1	0.8
under canopy	338	2.5	0.3
Control site	8	70.0	13.3

Source: Van Zwieten *et al.* (2004)

Discussion

Copper is an essential element and required by all organisms. However, elevated concentrations of copper in soils are toxic and may result in a range of effects including reduced biological activity and subsequent loss of fertility (Dumestre *et al.* 1999). It has been demonstrated here in Australian studies that copper residues in avocado orchards affect several soil health indicators; including microbial activity, earthworm populations and other processes such as bioturbation.

Earthworms have been suggested as useful indicators of soil health (de Bruyn 1997; Paoletti *et al.* 1998). Through their feeding and burrowing activity, earthworms aid in decomposition and incorporation of organic matter, increase the number of water soluble aggregates, improve water infiltration, aeration, drainage and root penetration, and increase microbial activity (Lee 1985). Earthworm casts and burrow walls exhibit higher concentrations of total and plant-available elements than surrounding soil and it has been recognised that surface feeding species horizontally and vertically disseminate micro-organisms, spores, pollen and seeds (Makeschin 1997) and can reduce plant pathogens through digestion of fungal spores (Hirst *et al.* 1955). Therefore practices that reduce earthworm populations in soil can lead to a reduction in soil health.

Within Australia, earthworms have been given serious consideration only in the context of temperate and Mediterranean pastures and cropping. Little relevant information had been gathered for sub-tropical agriculture. It has recently been shown (Dr. Tim Kingston, pers com) that a far greater incidence of native worms is present in sub-tropical agriculture than in many other regions in Australia. The role of native earthworms in sub-tropical agriculture is still under investigation.

Soils that contain significant copper residues have been observed to have few earthworms (Van Rhee 1967, Van Zwieten *et al.* 2004), reduced surface activity (fewer castings visible at the soil surface) and greater litter build-up (Ma 1984). At sites in a study on avocado orchards in northern NSW (Merrington *et al.* 2002) an absence of earthworms in areas of copper contamination was accompanied by a thick layer of organic matter (ca. 10-30 cm deep) that was clearly stratified on the soil surface, with little evidence of breakdown and incorporation into the sub-surface layers. This phenomenon is not seen in other sub-tropical horticultural systems where copper fungicides are not used.

A strong correlation has been observed between soil-copper concentration and the level of copper in earthworm tissues (Ma *et al.* 1983). It has been noted that earthworms exhibit chronic toxic responses at relatively low concentrations of copper (< 4-16 mg/kg) (Helling *et al.* 2000). The enchytraeid worm *Cognettia sphagnetorum* was shown to actively avoid copper contaminated soil (Salminen and Haimi 2001). Copper oxychloride has recently been shown to reduce populations of the earthworm *Aporrectodea caliginosa* in field trials six months following application of the fungicide (Maboeta *et al.* 2003).

Elevated Cu concentrations have been shown to reduce beneficial mycorrhizal associations (Liao *et al.* 2003) reduce microbial activity and function (Bogomolov *et al.* 1996) and impact a range of mesofauna (Böckl *et al.* 1998).

Thrupp (1991) found that copper arising primarily through application of Bordeaux tended to be associated with areas of high organic matter build-up in former banana plantations in Costa Rica. These soils were damaging to subsequent crops (i.e. phytotoxic). Residues ranging between 20 and 4000 mg/kg and were shown to have had a significant adverse effect on once fertile productive agricultural soils. It is likely that copper residues were toxic to soil organisms, restricting bioturbation of soil, hence resulting in accumulation of organic materials. This result is reflected in the findings of the current study where a thick layer of organic matter was found in the avocado orchard soils, however, phytotoxicity has not been evident. Phytotoxicity of copper depends on its bioavailability (Alva *et al.* 2000), which is controlled principally by pH, redox potential, oxide content, and clay mineral influences. However, there is limited unequivocal evidence suggesting the overriding importance of any one of these soil physico-chemical parameters. For example, Lock and Janssen (2001) noted that at relatively low organic matter content (1.5%) a soil pH increase from 4-7 reduced toxicity (14 day LC_{50}) in the worm *E. albdus* from 169 to 530 mg Cu/kg. Ma (1984) observed that a pH increase over a similar range had no effect on toxicity to the earthworm *Lubriculus rubellus.*

Other non-target species impacted by copper include mites (Michaud and Grant 2003), entomopathic fungus (McCoy *et al.* 1996) and nematodes (Jaworska and Gorczyca 2002). In some instances, these species have been stimulated by copper applications and have resulted in major outbreaks of a pest and subsequent losses in production. Thus, the environmental side effects of copper-based fungicides may be more wide spread than the impacts on soil health and soil biota.

A recent study in SE QLD assessed the effects of copper fungicide on anthracnose (a fungal disease) on avocados. The study showed the use of copper significantly reduced the numbers of bacteria, filamentous fungi and yeasts on leaf and fruit surfaces. Furthermore, there was a significantly higher disease occurrence on the copper-sprayed fruit (Stirling *et al.* 1999). The authors attributed this to the ability of the naturally occurring microorganisms to suppress pathogenic species, while the copper based fungicides eliminated the possibility of natural predation.

Conclusions

The evidence reviewed in this paper demonstrates that copper residues originating from fungicide application reduce soil microbial biomass, while stressing the microorganisms that are present. In addition, copper has resulted in an elimination of earthworms in the orchard where residues averaged between 180-338 mg/kg. This has influenced soil processes by reducing bioturbation, thus potentially influencing other soil physical and chemical processes. There is a need for a better evaluation of the potential impacts of copper contamination in agricultural land, and management strategies/remediation technologies need to be developed to reduce the bioavailability of existing residues. One of the strategies that could be implemented immediately is the reduction of further inputs, through the use of alternative fungicides, of either chemical or biological origin. A recent review of alternative fungal control agents has recent been published (Van Zwieten *et al.* 2004b), with products including: Selected biological control agents; Compost; Inoculated compost; Surfactants and biosurfactants; Antifungal compounds; Compost tea; pH modifiers and bicarbonates; Foliar calcium and silicone; Milk products and other organic amendments (eg. molasses); Essential oils (eg. tea tree) and Polymer coatings all showing promise as alternatives to copper for disease control.

References

Alva AK, Huang B, Paramasivam S (2000) Soil pH affects copper fractionation and phytotoxicity. *Soil Science Society of America Journal* **64**, 955-962.

Böckl M, Blay K, Fischer K, Mommertz S, Filser, J (1998) Colonisation of a copper-decontaminated soil by micro- and mesofauna. *Applied Soil Ecology* **9** (1-3 Special Issue SI), 489-494.

Bogomolov DM, Chen SK, Parmelee RW, Subler S, Edwards CA (1996) An ecosystem approach to soil toxicity testing: a study of copper contamination in laboratory soil microcosms. *Applied Soil Ecology* **4**, 95-105.

Brun LA, Maillet J, Richarte J, Herrmann P, Remy JC (1998) Relationships between Extractable Copper, Soil Properties and Copper Uptake by Wild Plants in Vineyard Soils. *Environmental Pollution* **102**, 151-161.

Chaignon V, Sanchez Neira I, Herrmann P, Jaillard B, Hinsinger P (2003) Copper bioavailability and extractability as related to chemical properties of contaminated soils from a vine-growing area. *Environmental Pollution* **123**, 229-238.

Copper Development Association (2003) Uses of copper compounds. www.copper.org/applications/compounds/copper_sulfate02.html.

de Bruyn LAR (1997) The status of soil macrofauna as indicators of soil health to monitor the sustainability of Australian agricultural soils. *Ecological Economics* **23**, 167-178.

Dumestre A, Sauve S, McBride M, Baveye P, Berthelin J (1999) Copper speciation and microbial activity in long-term contaminated soils. *Archives of Environmental Contamination and Toxicology* **36**, 124-131.

Gallagher DL, Johnston KM, Dietrich AM (2001) Fate and transport of copper-based crop protectants in plasticulture runoff and the impact of sedimentation as a best management practice. *Water Research* **35**, 2984-2994.

Helling B, Reinecke SA, Reinecke AJ (2000) Effects of the fungicide copper oxychloride on the growth and reproduction of *Eisenia fetida* (Oligochaeta). *Ecotoxicology and Environmental Safety* **46**, 108-116.

Hirst JM, Storey IF, Ward WC, Wilcox HG (1955) The origin of apple scab epidemics in the Wisbech area in 1953 and 1954. *Plant Pathology* **4**, 91-95.

Jaworska M, Gorczyca A (2002) The effect of metal ions on mortality, pathogenicity and reproduction of entomopathogenic nematodes *Steinernema feltiae Filipjev* (Rhabditida, Steinernematidae). *Polish Journal of Environmental Studies* **11**, 517-519.

Kingston TJ, Temple-Smith MG (1988) Earthworm populations under Tasmanian pastureland. In: Stahle PP, editor. *Proceedings of 5th Australasian Conference on Grassland Invertebrate Ecology*, August 15-19, University of Melbourne, Australia, 192-198.

Lee KE (1985) Earthworms Their Ecology and Relationships with Soils and Land Use. Academic Press: Australia.

Lepp NW, Dickinson NW (1994) Fungicide-derived Cu in tropical plantation crops. In: 'Toxic Metals in Soil-Plant Systems' (Eds S.M.Ross) 367-393. (Wiley and Sons: Chichester, UK)

Liao JP, Lin XG, Cao ZH, Shi YQ, Wong MH (2003) Interactions between arbuscular mycorrhizae and heavy metals under sand culture experiment. *Chemosphere* **50**, 847-853.

Liess M (2004) Enhancing realism and practicability in ecotoxicological risk assessment. *Proceedings of Interact 2004*, Gold Coast, Australia, 123.

Lock K, Janssen C (2001) Test designs to assess the influence of soil characteristics on the toxicity of Cu and Pb to the oligochaete *Enchytraeus albidus*. *Ecotoxicology* **10**, 137-144.

Ma W-C, Edelman T, Beersum IV, Jans T (1983) Uptake of cadmium, zinc, lead and copper by earthworms near a zinc smelting complex: Influence of pH and organic matter. *Bulletin of Environmental Contamination and Toxicology* **30**, 424-427.

Ma W-C (1984) Sublethal toxic effects of copper on growth, reproduction and litter breakdown activity in the earthworm *Lumbricus rubellus*, with observations on the influence of temperature and soil pH. *Environmental Pollution* **33**, 207-219.

Maboeta MS, Reinecke SA, Reinecke AJ (2003) Linking lysosomal biomarker and population responses in a field population of *Aporrectodea caliginosa* (Oligochaete) exposed to the fungicide copper oxychloride. *Ecotoxicology and Environmental Safety* **56**, 411-418.

Makeschin F (1997) Earthworms (Lumbricidae: Oligochaeta): Important promoters of soil development and soil fertility. In: Benckiser G, editor. *Fauna in Soil Ecosystems*. *Recycling Processes, Nutrient Fluxes and Agricultural Production*. Marcel Dekker Inc, New York, 172-223.

McCoy CW, Lye, BH (1996) Effect of copper sprays on the population dynamics of the citrus rust mite, *Phyllocoptruta oleivora* (Acari: Eriophyidae) and its fungal pathogen, *Hirsutella thompsonii*. *Proceedings of the Florida State Horticultural Society* 126-129.

Merrington G, Rogers SL, Van Zwieten L (2002) The potential impact of long-term copper fungicide usage on soil microbial biomass and microbial activity in an avocado orchard. *Australian Journal of Soil Research* **40**, 749-759.

Merry RH, Tiller KG, Alston AM (1983) Accumulation of copper, lead and arsenic in some Australian orchard soils. *Australian Journal of Soil Research* **21**, 549-561.

Michaud JP, Grant AK (2003) Sub-lethal effects of a copper sulfate fungicide on development and reproduction in three coccinellid species. *Journal of Insect Science* 3, 1-6.

Nicholson FA, Smith SR, Alloway BJ, Carlton-Smith C, Chambers BJ (2003) An inventory of heavy metals inputs to agricultural soils in England and Wales. *The Science of the Total Environment* **311**, 205-219.

Paoletti MG, Sommaggio D, Favretto MR, Petruzzelli G, Pezzarossa B, Barbafieri M (1998) Earthworms as useful bioindicators of agroecosystem sustainability in orchards and vineyards with different inputs. *Applied Soil Ecology* **10**, 137-150.

Pietrzak U, McPhail DC (2004) Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. *Geoderma* (in press).

Salminen J, Haimi J (2001) Life history and spatial distribution of the enchytraeid worm *Cognettia sphagnetorum* (Oligochaeta) in metal-polluted soil: Below-ground sink-source population dynamics? *Environmental Toxicology and Chemistry* **20**, 1993-1999.

Schneiderhan FJ (1933) The discorvery of Bordeaux mixture: Three papers: I. Treatment of mildew and rot./ II. Treatement of mildew with copper sulphate and lime mixture./ III. Concerning the history of the treatment of mildew with copper sulphate. By Perre Marie Alexis Millardet 1885. A Translation from the French by Felix John Schneiderhan. *American Phytopathological Society Phytopathological Classics* 3.

Stirling AM, Pegg KG, Hayward AC, Stirling GR (1999) Effect of copper fungicide on *Colletotrichum gloeosporioides* and other microorganisms on avocado leaves and fruit. *Australian Journal of Agricultural Research* **50**, 1459-1468.

Thrupp LA (1991) Long-term losses from accumulation of pesticides: A case of persistent copper toxicity in soils of Costa Rica. *Geoforum* **22**, 1-15.

Van Rhee JA (1967) Development of earthworm populations in orchard soils. In: Graff O, Satchell J, editors. *Progress in Soil Biology*. North Holland Publishing Company, Amsterdam, Netherlands, 360-371.

Van Zwieten L, Rust J, Kingston T, Merrington G, Morris S (2004) Influence of copper fungicide residues on occurrence of earthworms in avocado orchard soils. *The Science of the Total Environment* **329**, 29-41.

Van Zwieten M, Stovold G, Van Zwieten L (2004b) Literature Review and Inventory of Alternatives to Copper for Disease Control in the Australian Organic Industry. A report for the Rural Industries Research and Development Corporation. RIRDC Project DAN-208A. ISBN 0 7347 1590 0, 101p.

Ware GW (1978) 'The Pesticide Book.' (W.H. Freeman and Company: San Francisco).

Yeardley RB, Lazorchak JM, Gast LC (1996) The potential of an earthworm avoidance test for evaluation of hazardous waste sites. *Environmental Toxicology and Chemistry* **15**, 1532-1537.