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The impact of Cu, Zn and Cr salts on the relationship between insect and plant parasitic nematodes: A reduction in biocontrol efficacy

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ABSTRACT

Accumulation and potential health risks of toxic elements are associated with excessive fertilizer and manure applications in vegetable production systems. The goal of the present study was to explore the effects of potential toxic elements on the function of a cohort of beneficial organisms, specifically, the insect parasitic nematodes known as entomopathogenic nematodes. We assessed the impact of heavy metal accumulation on entomopathogenic nematodes (*Steinernema carpocapsae* and *Heterorhabditis bacteriophora*) and plant parasitic nematodes (*Meloidogyne incognita*). Genearlly, pretreatment with 1.5 mg Cu/L, 50 mg Zn/L or 7.5 mg Cr/L solution for 24 h caused direct mortality of infective juvenile *S. carpocapsae* and *H. bacteriophora* nematodes, and lowered virulence of *H. bacteriophora* against *Galleria mellonella*. Interestingly, we found that 24 h exposure to 4.5 mg Cu/L, 50 mg Zn/L or 7.5 mg Cr/L solution already caused higher mortality in *H. bacteriophora* than *M. incognita* in a laboratory assay. Moreover, in a potted plant experiment, pretreatment of *H. bacteriophora* with Cu, Zn or Cr salts resulted in increased penetration of *M. incognita* into cucumber roots compared with *H. bacteriophora* that were not treated (thus reducing biocontrol efficacy). Pretreatment of *S. carpocapsae* with Cu also caused increased penetration of *M. incognita* into cucumber roots; however, none of the Zn and Cr pretreatment concentrations exposed to *S. carpocapsae* resulted in increased *M. incognita* root penetration, and lower concentrations of these elements caused decreased penetration (enhancing biocontrol efficacy). Our results indicate that heavy metals can have a direct negative effect on entomopathogenic nematodes (via direct mortality) and also an indirect effect by disrupting biocontrol efficacy against plant parasitic nematodes, but in some cases low concentration may stimulate biocontrol activity. High levels of heavy metal accumulation in greenhouse vegetable production systems could substantially influence nematode community structure and impact other soil biota as well.

1. Introduction

Excessive fertilization is a significant environmental concern in agriculture (Teklic et al., 2008; Ren et al., 2010). One of the important negative outcomes is a buildup of heavy metals in the soil (Ondrasek et al., 2009; Li et al., 2009; Vogel et al., 2014). In our previous study, we found that soil total nitrogen increased up to 160% (Ruan et al. 2013). In the same study, concentrations of total phosphorous, Cu and Zn increased up to 2.95 g/kg, 20.3 mg/kg and 117.6 mg/kg when compared to 0.87 g/kg, 23.8 mg/kg, and 59.7 mg/kg in open fields, respectively (unpublished data). Yang et al. (2015) reported accumulation of Cu and Zn in greenhouse soil as compared to open-field soil. Additionally, soil available phosphorous accumulated in greenhouse soil (Kong et al., 2014). Vogel et al. (2014) predicted that the excessive application of phosphate fertilizer would likely lead to Cr buildup in soil, and

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higher levels or different fertilizer regimes might also lead to Cr buildup. Toxins derived from heavy metal buildup can enter the edible parts of vegetables (Huong et al., 2010; Sharma et al., 2007; Xue et al., 2012), which may pose human health risks (Chen et al., 2010). Additionally, heavy metal buildup can have a profound effect on soil biota (Stankovic et al., 2014).

Nematodes are the most numerous soil mesofauna, and occupy all consumer trophic levels in soil food webs. According to their feeding habits, soil nematodes were classified as plant feeders, bacteria feeders, fungal feeders, predators, omnivores, and insect parasites. Soil nematodes are beneficial in energy flow, nutrient cycling and even pathogen and pest insect control (Grewal et al., 2005; Ferris, 2010). Studies indicate that the soil nematode community is particularly sensitive to heavy metals and thus the nematode biota could be used as bioindicators of soil quality (Georgieva et al., 2002; Shao et al., 2008; Martin et al., 2014; Sharma et al., 2015). In the present study we focus on heavy metal effects on two important groups of soil nematodes, root knot nematodes (plant feeders) and entomopathogenic nematodes (EPNs) (insect parasites).

Root knot nematodes are probably the most notorious of the soil-dwelling plant parasitic nematodes, causing tremendous crop yield losses (Atkinson et al., 2012). Chemical nematicides e.g., oxamyl and fenamiphos and fumigants such as methyl bromide have been widely employed to kill *Meloidogyne* spp. and caused adverse effects on non-target soil biota (e.g, EPNs) and their functionality on an ecosystem level (Patel and Wright, 1996; Hägerbäumer et al., 2015).

There is an urgent need to exploit safe and effective biological control agents considering the negative side effects of chemical nematicides on humans and other non-target organisms. In nature, EPNs are obligate parasites of insects and thus have been primarily developed as commercial biocontrol agents for insect pest suppression (Grewal et al., 2005). EPNs can control a wide range of soil-dwelling insect pests as well as certain aboveground insect pests (Lacey and Georgis, 2012). Moreover, various studies also indicated the biocontrol potential of EPNs against *Meloidogyne* spp. and other plant parasitic nematode genera (e.g., Molina et al., 2007; Caccia et al., 2013; Bird and Bird, 1986; Lewis et al., 2001; Perez and Lewis 2002; Kepenekci et al., 2016). Thus, EPNs have been suggested as potential agents for integrated management of plant parasitic nematodes (Lewis and Grewal, 2005).

Soil parameters are associated with EPN survival and fitness (Campos-Herrera et al., 2016; Kanga et al., 2012). For example, increasing soil pH led to higher percentages of samples containing EPNs (Kanga et al., 2012). Soil moisture is a critical factor in modulating EPN populations (Campos-Herrera et al., 2016; Shapiro-Ilan et al., 2006). Soil types can have various effects on infectivity and persistence of EPNs (Koppenhofer and Fuzy, 2006). However, there is a dearth of knowledge on the impact of heavy metals on EPNs and their interaction with plant parasitic nematodes. A soil survey in La Rioja (Northern Spain) found that natural entomopathogenic population densities were negatively correlated with Zn and Cu concentrations (Campos-Herrera et al., 2008). Previous laboratory studies documented effects of certain metal ions on EPN activity (Jaworska et al., 1997; Jaworska et al., 1996) and reproductive capacity (Jaworska and Tomasik, 1999). The authors found that *Steinernema carpocapsae* was sensitive to Cu and Zn but *Heterorhabditis bacteriophora* was not affected; also different ions had variable effects with some having positive effects at low concentrations.

Plant parasitic nematodes and EPNs both occur in soil and are often found in close proximity and are also simultaneously exposed to heavy metal salts. However, the differences in the response to heavy metal exposure between plant parasitic nematodes and EPNs have remained largely unclear. We hypothesized that heavy metal exposure might affect the ability of EPNs to suppress plant parasitic nematodes. Based on expected toxicity to soil biota (Cervantes et al., 2001) and the results of a previous field survey (unpublished data), Cu (II), Zn (II) and Cr (III) salts were chosen to estimate heavy metal effects on two EPN isolates (*Heterorhabditis bacteriophora* and *Steinernema carpocapsae*) and one plant feeder (*Meloidogyne incognita*). Thus, the objectives of the present study were to 1) evaluate the impact of heavy metals on EPN virulence and viability, 2) estimate the impact of heavy metals on viability of *M. incognita*, and 3) assess the effects of heavy metals on the ability of EPNs to suppress the penetration of *M. incognita*. Understanding the effects of heavy metal salts on EPNs and plant feeders will provide new insights into the adverse effects of heavy metal salts on the function of biota in soil ecosystems.

2. Materials and methods

2.1. Heavy metal salts

Three heavy metals $CuSO_4{\cdot}5H_2O$, $ZnCl_2$, and $CrCl_3{\cdot}6H_2O$, were selected for this study. The three metals were chosen based on results of our previous field survey. The survey indicated that the soil concentrations of these elements increased in a 1-yr to $\geq$5 year course of greenhouse production, ranging from 28.7 to 40.3 mg/kg d.w. soil for Cu, 76.3–117.6 mg/kg d.w. soil for Zn, and 70.6–85.0 mg/kg d.w. soil for Cr (unpublished data), and reaching significant levels for Cu and Zn but not for Cr. $CrCl_3{\cdot}6H_2O$ was chosen mainly because, like the other two elements, it is known to be a potential toxin to soil biota (Cervantes et al., 2001). The three elements were purchased from Tianjin Reagent Corp, P.R. China. The compounds, all of analytical grade, were dissolved in sterilized double-distilled water with the final concentrations of 1.5, 4.5, 7.5, 9.0, 10.5 mg/L for Cu (as $CuSO_4{\cdot}5H_2O$); 50, 100, 200, 500, 1000 mg/L for Zn (as $ZnCl_2$); and 7.5, 17.5, 35, 75, 150 mg/L for Cr (as $CrCl_3{\cdot}6H_2O$). For brevity, the concentrations will hereafter be referred to by their element followed by mg used per liter, e.g., Cu1.5 indicates $CuSO_4{\cdot}5H_2O$ at 1.5 mg Cu/L. Sterilized double-distilled water was included as control.

We chose a wide range of concentrations so that the breadth of toxicity against the nematode subjects could be determined. These were the same concentrations as previously tested by Jaworska et al. (1997). Although our test concentrations in mg/L cannot directly be translate to soil concentrations in mg/kg, our study may still provide useful information on evaluating the exposure effect of buildup heavy metals on soil biota under field conditions.

2.2. Nematode preparation and quantification

2.2.1. Entomopathogenic nematodes (EPNs)

Two strains of EPNs *Heterorhabditis bacteriophora* TJ-1 (Hb) and *Steinernema carpocapsae* TJ-1 (Sc) used in this study were isolated from soil samples collected in Tianjin, China, and identified by sequencing ribosomal DNA from the entire internally transcribed spacer regions (Nguyen et al., 2001). EPNs were produced in wax moth larvae (*Galleria mellonella*) using the White trap method (White, 1927). Infective juvenile nematodes (IJs) were collected for use within 3 days after emergence.

2.2.2. Plant feeders

Meloidogyne incognita was reared on pepper plants (*Capsicum annuum* L.) under greenhouse condition. Egg masses were hand-picked from infested roots. Freshly hatched second stage juveniles (J2) were used in all experiments.