Effects of copper and the insecticide cypermethrin on a soil ciliate (Protozoa: Ciliophora) community

Hai T. T. Luu[a,1], Genoveva F. Esteban[a], Andrew A. Butt[a] and Iain D. Green[a]

[a] Bournemouth University, Faculty of Science and Technology, Department of Life and Environmental Sciences, Talbot Campus, Fern Barrow, Poole, BH12 5BB, UK.

[1] Present address - Tra Vinh University, No. 126, Nguyen Thien Thanh St., Ward 5, Tra Vinh Province, Vietnam.

Corresponding Author:

Iain D. Green
Bournemouth University,
Faculty of Science and Technology,
Department of Life and Environmental Sciences,
Talbot Campus,
Fern Barrow,
Poole,
BH12 5BB,
UK.
e-mail: igreen@bournemouth.ac.uk
Tel: +44(0)1202 849681

Highlights

- Total ciliate abundance was increased by Cu and cypermethrin treatment
- Sensitive species were lost from the community at high Cu and cypermethrin levels
- Tolerant species seemed to increase in abundance in the absence of sensitive species
- The ciliate community structure was altered at high Cu and cypermethrin levels

Abstract

Ciliated protozoa play important ecological roles in soils, yet few studies have investigated the effect of soil pollution on them. We determined the effect of copper (Cu) and cypermethrin on a soil ciliate community under microcosm conditions. Soils were treated with Cu or cypermethrin and the abundance and species richness of ciliates determined 15 days later. Cu treatment increased soil ciliates abundance at the highest concentration (960 mg kg^{-1}), as did cypermethrin at a treatment of 160 mg kg^{-1}. No negative effect on ciliate abundance was found for either substance due to increased numbers of tolerant species, particularly *Homalogastra setosa* and *Chilodonella uncinata* in the case of Cu and *Colpoda stenii* and *Colpoda inflata* for cypermethrin treatments. However, several species were absent at high treatment levels. Notably, *Halteria grandinella* was not found in Cu treatments above 240 mg kg^{-1}, whilst *Oxytricha setigera* was not found in cypermethrin treatments above 160 mg kg^{-1}. For *Homalogastra setosa*, there was an initial positive response to cypermethrin, but abundance then decreased at a treatment of 320 mg kg^{-1}, and treatment at 640 mg kg^{-1} eradicated the species from the microcosms. Accordingly, both substances affected the structure of the soil ciliate community at high concentrations.

Key words: Biodiversity; Pesticide; Protozoa; Soil; Toxicity; Trace metal.

Declarations of interest: none

1. Introduction

Ciliates are single-celled eukaryotic organisms that form one of the three functional groups of protozoa alongside flagellates and amoebae (Esteban and Fenchel, 2020; Finlay and Esteban, 2013). Ciliates are abundant phagotrophic microorganisms in soil, where they play important roles in food webs by controlling the communities of smaller microbes and recycling organic material from consumed prey (Esteban et al., 2006). Protozoa can be affected by pollutants, such as metals (Johansen et al., 2018; Madoni & Romeo, 2006), and pesticides (Mansano et al., 2020; Staley et al. 2015) which can thereby impact their ecological role. Despite their importance, protozoa have been subject to far less study than other soil organisms and there is a considerable gap in the understanding of how pollution may affect ciliate communities (Vilas-Boas et al., 2020), particularly in soil.

Heavy metal contamination of soil ecosystems can arise from the use of pesticides, sewage sludge, inorganic fertilizers, animal manures/slurries and through industrial waste and emissions (Wuana and Okiemmen, 2011), and is therefore considered to be widespread. Research on the impacts of trace metals in general, and copper (Cu) in particular, on protozoa in soil is scant (Johansen et al., 2018). Almost all studies to date have focussed on laboratory testing of trace metal toxicity, and only a few ciliates and amoeba species have been investigated (Bitencourt et al., 2016; Campbell et al., 1997; Díaz et al., 2006; Forge et al., 1993; Hao et al., 2016; Martín-González et al., 2006; Pratt et al., 1997; Martín-González et al., 2006). Moreover, microcosms have only occasionally been used to evaluate the changes in abundance and diversity of soil protozoa caused by elevated trace metal exposure (Ekelund et al., 2003; Johansen et al., 2018), despite such conditions more closely resembling those in the field. These studies indicated that considerable variation exists among protozoan species in sensitivity to trace metal toxicity, with certain distinct species demonstrating tolerance to elevated metal exposure (Díaz et al., 2006; Giller et al., 1998). Alterations in the structure of protozoan communities due to trace metal pollution are,

77 therefore, highly likely due to a loss or reduction in abundance of sensitive species and
78 concurrent rise in abundance of tolerant species. However, the extent of change at the
79 community level is difficult to determine given the current level of understanding.

80

81 Copper is an essential element for all living organisms, and is particularly required as a
82 cofactor for a number of enzymes (Krupanidhi et al., 2008). Cu is typically found at trace
83 levels in the environment, but can be toxic in excess, negatively impacting the activity of soil
84 enzymes (Wyszkowska et al., 2006), reducing microbial biomass, changing the diversity of
85 microbial communities, and altering the physiology of bacteria (Aoyama, 1993; Ekelund et
86 al., 2003). Cu-spiked soil experiments under laboratory conditions have indicated that at high
87 concentrations, Cu negatively affects mineralization by the soil microbial flora (Ekelund et al.,
88 2003; Giller et al., 1998), which would suggest a significant subsequent impact on nutrient
89 recycling.

90

91 The intensive use of pesticides creates a further risk for soil biology. As a consequence,
92 many pesticides have been evaluated for toxicity to microorganisms, including protozoa
93 (Adebavo et al., 2007; Ekelund et al., 1994; Ekelund, 1999; Mansano et al., 2020; Petz and
94 Foissner 1989; Todorov and Golemansky 1992; Xu et al., 2010). Cypermethrin is a synthetic
95 pyrethroid insecticide widely used to control a broad range of insect pests in households,
96 veterinary medicine and agriculture (Singh et al., 2012). Cypermethrin has a moderate
97 persistence in soil, but it is more persistent in soils with high organic matter content (Harris
98 and Chapman, 1981). The degradation rate of cypermethrin in soil increased in the presence
99 of microorganisms (Chapman et al., 1981; Xie and Zhou, 2008), but there has also been
100 increasing concern regarding the toxicity of cypermethrin to the soil microbial biomass and
101 its activity (Filimon et al., 2015). Studies have indicated that cypermethrin can cause a
102 reduction in the biomass and enzyme activities of microorganisms in the short term, but then
103 may stimulate microbial growth (Sechi et al., 2014; Tejada et al., 2015). To date, studies

have only considered the effect of this insecticide on freshwater ciliates (Dutta, 2015; Friberg-Jensen et al., 2003). Hence, there is crucial need to understand the toxicity of this insecticide to ciliated protozoa in soil due to the combination of their ecological importance and the widespread use of cypermethrin.

In the present study, we assessed the effect of Cu and cypermethrin on the abundance and species richness of soil ciliates in a microcosm system in order to determine how the ciliate community changed with increasing exposure to Cu and cypermethrin.

2. Materials and methods

2.1 Experimental soil

A sandy loam topsoil was obtained from a commercial supplier (Wicks, Northampton, UK). Selected physico-chemical parameters for the soil are given in Table 1. This soil had relatively high organic matter (OM) content for a mineral soil, which should have ensured sufficient nutrients for microorganisms. Additionally, soils with high OM can increase persistence of Cu and cypermethrin in the soil (Harris and Chapman, 1981). Thus, the use of this soil represents a scenario where the soil is expected to have a good microbial and ciliate community, and to be affected over a relatively long period by the contaminants.

2.2 Microcosm setup and sampling

For both Cu and cypermethrin, the appropriate volume of a solution necessary to raise the concentration in the soil by a predetermined amount was added to 2 kg of fresh soil. Soils were treated with a 10 mg mL^{-1} Cu (as $CuCl_2$) solution to provide 7 distinct Cu addition treatments of 0 mg Cu kg^{-1} soil (dry weight) as the control; 35 mg kg^{-1}; 70 mg kg^{-1}; 140 mg kg^{-1}; 240 mg kg^{-1}; 480 mg kg^{-1}; 720 mg kg^{-1} and 960 mg kg^{-1}. Similarly, a commercial

formulation containing 10 mg mL^{-1} of cypermethrin (PY Bug Killer concentrate, Vitax Ltd, UK) was used to provide cypermethrin treatments of 0 mg kg^{-1} (control); 10 mg kg^{-1}; 20 mg kg^{-1}; 40 mg kg^{-1}; 80 mg kg^{-1}; 160 mg kg^{-1}; 320 mg kg^{-1} and 640 mg kg^{-1}.

Cu and cypermethrin solutions were thoroughly mixed with the soil, and then divided into 4 replicate plastic containers to form microcosms consisting of 500 g (fresh weight) of soil. The experiment was conducted under laboratory conditions (19 ^{o}C ± 1-2 ^{o}C) for 15 days. Moisture content of the soil was adjusted to 60% water holding capacity at the start of the experiment using distilled water, and maintained at this level through the course of the experiment by the addition of sufficient distilled water to maintain a constant weight in each microcosm. For both substances, soil samples were collected from 0-5 cm 15 days after the soil was treated for evaluation of the ciliate communities.

2.3 Measurements of ciliates

To determine the abundance and species richness of soil ciliates, the method of Finlay et al. (2000) was followed, as outlined below. This method determines the potential abundance of free-living protozoa in samples following the re-wetting of air-dried soil with rainwater. As this method does not utilise additional carbon substrates or mineral nutrients, the community of protozoa that develops in the re-wetted samples is a function of the resources and inhibitors present in the original soil in the field (Finlay et al., 2000).

Sample treatment

Soil sampled 15 days after the application of Cu/cypermethrin was homogenised by thorough mixing in a clean 30 cm-diameter glass bowl. A sub-sample of soil (~50 g) was then taken and spread out as a layer in a clean 15 cm diameter glass Petri dish and dried at room temperature (18-22 ^{o}C) for 6 days. After air drying, soil was passed through a 4 mm

sieve to remove large objects such stones. This fraction of the soil was used for all ciliate-related work.

Incubation and ciliate counts

To facilitate the growth of ciliates, 5 g of the air-dried soil was placed into a 5 cm diameter sterile plastic Petri dish. This was replicated 3 times for each sample. Whatman® syringe filters with 0.2 µm diameter pore size were used to filter rain water to exclude ciliates and other microbes. A measured volume of filtered rainwater sufficient to produce slurry was then added to the soil samples in the Petri dishes. Samples were then incubated in the dark at 15 ^{o}C, and ciliates were investigated after 4 days and 10 days of incubation by removing a 50 µl sample from the water runoff. Sampled water was then placed in a glass Sedgewick-Rafter Chamber, and ciliate numbers in the 50 µl subsample were determined under a compound microscope at a magnification of 40-125x. This was repeated five times for each Petri dish of soil.

After counting, samples were used for enrichment culture in order to provide sufficient cells for detailed observation, including silver impregnations to aid identification, and to facilitate growth of species that were not observed during the counting process. Enrichment cultures were prepared by putting a soil inoculum (approximate 5 g of rewetted soil) into 50 ml sterile culture flasks that contained 20 ml of SES (Soil Extract added Salts) medium (http://www.ccap.ac.ukmedia/SES.pdf) and half a boiled wheat grain. The inoculated flasks were examined daily for 28 days to ascertain the ciliate species present.

Identification of ciliates

The species richness of ciliates was determined by identifying the specimens observed during the counting process and in the following enrichment cultures. Identification,

nomenclature and terminology of soil ciliates found was conducted according to the following taxonomic works: Foissner (1987; 1993; 2016), Foissner et al. (1991; 1992; 1994; 1995; 2002) and Kahl (1935). Live specimens were observed under a compound microscope with phase contrast (magnifications of 100-1000x). The ammoniacal silver carbonate impregnation (Fernández-Galiano, 1994) and the protargol (Foissner, 2014) staining methods were also used in order to reveal infraciliature of ciliates for species identifications.

Ciliate abundance and richness

The number of ciliates counted after 10 days of soil incubation (as explained above) were 6 and 9 times lower in the controls of Cu and cypermethrin treatments respectively than after 4 days. Consequently, only ciliates observed after 4 days of incubation were used to assess ciliate abundance. Total species richness was determined as the total number of species found by combining the species identified after both periods of incubation (i.e. 4 and 10 days) and in the enrichment cultures.

2.4 Data analysis

Statistical analysis was conducted with SPSS vs. 20 (IBM Inc.). Data were analysed statistically to determine the significance of differences between treatments in total abundance and richness of soil ciliates. The abundance of ciliate species showing appreciable change were then subject to further analysis as individual species. Data sets were analysed for homogeneity of variance with Levene's test prior to comparing means by one-way ANOVA. When this assumption was not met, data were $\log_{10}$ transformed to meet this assumption. Effects of individual treatments were determined by Turkey's HSD *post-hoc* test.

3. Results

3.1 Effects of copper (Cu) on the abundance of soil ciliates

There were fluctuations in the abundance of soil ciliates as treatment level increased (Figure 1), but the highest ciliate abundance (306 cells g^{-1} soil) was observed at the highest Cu treatment (960 mg kg^{-1}). Copper treatment had an overall statistically significant impact on the abundance of soil ciliates ($F_{(7, 24)} = 7.836$, $p < 0.001$), but *post hoc* testing showed the only treatment having a significant effect was the 960 mg kg^{-1} treatment, in which the abundance of ciliates was significantly higher than in all other treatments, including the control.

The abundance of individual ciliate species was examined to identify those with an appreciable response to treatment. From this, two species were identified, *Chilodonella uncinata* and *Homalogastra setosa*. Both of these species showed a significant increase in population abundance in the 960 mg kg^{-1} treatment compared to the control soil (Table 2). Indeed, it was due to the rapid increase in the abundance of these two species that the greatest number of soil ciliates was recorded at the highest Cu treatment.

3.2 Effects of copper on soil ciliate species richness and community structure

The mean ciliate species richness was relatively constant, but there was a slight decrease at the highest Cu treatment concentrations (720 mg kg^{-1} and 960 mg kg^{-1}, Figure 2). However, there was no significant effect of Cu on species richness ($F_{(7, 24)} = 1.033$, $p = 0.435$). Despite this, an effect of Cu on the structure of the soil ciliate community was observed at the highest treatment concentration; only 12 species in total were recorded in the 960 mg kg^{-1} treatment compared to a total of 17 species in the control (Table 3). Most notably, *Halteria grandinella*, *Kahlilembus attenuatus*, and *Oxytricha setigera* were found in the control and low to mid concentrations, but were absent in the highest concentrations. In contrast, *Kreyella minuta* was found infrequently except in the highest Cu treatment and, as previously

mentioned, the abundance of *Chilodonella uncinata* and *Homalogastra setosa* were significantly increased in the highest concentration. Hence, both the species composition and relative abundance of species in the community were affected by Cu treatment at high concentrations.

3.3 Effects of cypermethrin on the abundance of soil ciliates

The response of ciliate abundance to cypermethrin treatments was varied, initially decreasing, then increasing to the highest level at a concentration of 160 mg kg^{-1}, with 780 cells g^{-1} dry soil found, before abundance fell again (Figure 3). Overall, ciliate abundance was significantly affected by cypermethrin treatment ($F_{(7, 24)} = 13.157$, $p < 0.001$, $\log_{10}$ transformation of the dependent variable). However, the decrease in abundance as the cypermethrin treatment rose from 10 to 40 mg kg^{-1} was found to be non-significant compared to the control according to *post-hoc* testing. Furthermore, despite a marked increase in ciliate abundance as the cypermethrin concentration rose from 80 to 320 mg kg^{-1}, only the 160 mg kg^{-1} treatment differed significantly from the control. A very marked decline in ciliate abundance then occurred in the 640 mg kg^{-1} treatment, which resulted in a significant decrease in abundance in this treatment compared to the 80 to 320 mg kg^{-1} treatments, but not the control.

Ciliate species showing an appreciable change in abundance in response to treatment were again subject to further analysis as individual species. This demonstrated that cypermethrin had a positive and significant impact on *Homalogastra setosa* abundance at treatment concentrations of 80 and 160 mg kg^{-1} (Table 4). Above these concentrations, the abundance of this species decreased sharply and it was totally absent in the 640 mg kg^{-1} treatment. The results of *post-hoc* testing indicated that abundance of *H. setosa* in the 160 mg kg^{-1} treatment differed significantly to both the control and 80 mg kg^{-1} treatments.

Despite a lack of a significant difference between the control and the 320 mg kg^{-1} cypermethrin treatment, the abundance of soil ciliates tripled between the two. This was mainly attributed to a marked increase in the abundance of two colpodid species, *Colpoda steinii* and *C. inflata*, in the 320 mg kg^{-1} treatment (Table 4). Whilst no *Colpoda* were found in the control samples, the abundance of the two *Colpoda* species increased from the 80 mg kg^{-1} treatment. In the case of *C. steinii*, *post hoc* testing showed significant differences between the 320 mg kg^{-1} treatment and the two lower concentration treatments in which it was found, i.e. 80 and 160 mg kg^{-1}, but no significant difference between the 80 mg kg^{-1} and 160 mg kg^{-1} treatments. Significant differences in the abundance of *C. steinii* were found amongst the 80, 160, and 320 mg kg^{-1} cypermethrin treatments.

3.4. Effect of cypermethrin on soil ciliate species richness and community structure

In terms of mean ciliate species richness, there was a slight increase as cypermethrin treatments increased from the control to 160 mg kg^{-1}, but the mean number of species decreased slightly at higher concentrations, falling below the control in the two highest treatments (Figure 4). One-way ANOVA demonstrated a significant effect of cypermethrin on soil ciliate species richness ($F_{(7, 24)} = 4.405$, $p = 0.003$, $\log_{10}$ transformation of the dependent variable). Nevertheless, significant differences in ciliate species richness were only found between the 640 mg kg^{-1} treatment and the 20, 40, and 80 mg kg^{-1} treatments.

Ciliate community structure was also affected by cypermethrin treatment; in total, 21 ciliate species were found in the control, but only 12 ciliate species were recorded in the samples from the 640 mg kg^{-1} treatment. Several ciliate species were completely absent at the highest concentration (640 mg kg^{-1}), but were still found in the control and lower cypermethrin concentrations, i.e. *Arcuospathidium vermiforme*, *Halteria grandinella*,

285 *Homalogastra setosa,* and *Oxytricha setigera* (Table 5). As in the case of Cu, the frequency
286 with which some species were found increased at high cypermethrin treatments, especially
287 the two *Colpodid* species mentioned previously, and also a *Gonostomum* sp. and
288 *Pseudoplatyophrya nana*, which were found only in the three highest cypermethrin
289 treatments.

290

291 **4. Discussion**

292 **4.1 Effect of copper on the abundance and species richness of soil ciliates**

293 Research into the toxicity of trace metals to soil ciliates is sparse and the work that is evident
294 has nearly all been conducted in solutions amended with trace metal or in solution extracted
295 from metal contaminated soils, and has used common *Colpodid* species (Bowers et al. 1997;
296 Campbell et al. 1997; Díaz et al. 2006; Pratt et al. 1997). Only one investigation is evident in
297 which changes in protozoan abundance due to Cu was investigated actually in soil (Ekelund
298 et al., 2003). The results of these studies demonstrated that the ciliate strains used were
299 sensitive to trace metals, and the growth of ciliate species in tests was usually inhibited by
300 the presence of Cu (Bowers et al. 1997; Campbell et al. 1997; Díaz et al. 2006; Pratt et al.
301 1997). However, it is not clear how these results extrapolated to the far more complex
302 physical, chemical and biological environment of soil.

303

304 The present study investigated the effects of Cu on the soil ciliate community at the species
305 level in soil microcosms. The results demonstrated that 15 days after Cu treatment at a level
306 of 960 mg kg^{-1}, the abundance of soil ciliates was positively affected. However, no impact of
307 Cu on soil ciliate abundance in the range of Cu treatments from 0 to 720 mg kg^{-1} was
308 recorded. Ekelund et al. (2003) found that in a sandy soil, Cu additions up to 1,000 mg kg^{-1}
309 did not affect the abundance of all forms of protozoa 14 days after application. Both studies
310 demonstrate that protozoa are, in general, highly resistant to Cu toxicity, but there is a

discrepancy in terms of effects on abundance at high Cu concentrations between the studies. This may suggest that ciliated protozoa are more able to exploit conditions under high Cu levels than other protozoa. Alternatively, differences in the properties of the soils used in the two studies may also account for the reported differences in findings and this is a more likely cause given the influence of soil properties on Cu availability.

Studies on the tolerance of ciliate species from fresh- and wastewater to heavy metals under laboratory conditions have shown that whilst some species are sensitive, others are tolerant (Madoni et al., 1992; Rehman et al., 2008a; Rehman et al., 2010; Shakoori et al., 2004). The present study demonstrated that this also applies to soil systems; although the species richness of soil ciliates was not significantly affected by elevated Cu, there was a decrease in the total number of species from 17 in the control to 12 in the highest Cu treatment. *Halteria grandinella*, *Kahlilembus attenuatus*, and *Oxytricha setigera* were found to be amongst the most sensitive species. Of these, *H. grandinella* showed the greatest sensitivity to Cu, becoming less frequently found in treatments of 140 mg kg^{-1} and above, and absent at treatments of 480 mg kg^{-1} and above. By contrast, *Kreyella minuta*, *Chilodonella uncinata* and *Homalogastra setosa* were tolerant to Cu and increased in abundance or frequency at high treatment levels.

The rise in the abundance of tolerant species, specifically *H. setosa* and *C. uncinata,* was linked to the significant increase in the number of ciliate cells in the 960 mg kg^{-1} treatment compared to the control (303 compared to 126 cell g^{-1} dry soil, respectively). These two species clearly not only tolerate high Cu concentration, but also actively benefit from the changes in the soil induced by Cu treatment. Metal tolerance exhibited by protozoa and microorganisms relates to their metal resistance mechanisms (Diaz et al., 2006; Martín-González et al., 2006). Cu is tolerated by cells due to a combination of sequestration of free Cu by metal binding proteins, principally metallothionein (Tibbett et al., 2021; Zahid et al.,

2018) and the efflux of excess Cu via P_{1B}-ATPase pumps (Díaz et al., 2006; Tibbett et al., 2021). Both mechanisms could be contributing to Cu resistance in the species observed to be tolerant of Cu.

4.2 Effects of cypermethrin insecticide on the abundance and species richness of soil ciliates

Laboratory studies have shown that cypermethrin can have negative effects on the populations and activities of soil bacteria and fungi (Sechi et al. 2014; Tejada et al., 2015). Despite this impact on soil microorganisms, toxicity testing of pyrethroid insecticides on protists has been limited to a small number of freshwater species (Dutta, 2015; Friberg-Jensen et al., 2003; Hikal et al., 2015), and (as far as we are aware) the effect of cypermethrin on soil ciliates has not been reported up to now. Rather than causing a negative effect on ciliate abundance, the present study has demonstrated that ciliate abundance increased due to a cypermethrin treatment of 160 mg kg^{-1}. This is consistent with the findings of Friberg-Jensen et al. (2003), who reported that freshwater ciliate abundance showed a significant increase with cypermethrin treatment. Cypermethrin can increase bacterial populations in the soil (Gundi et al., 2005), which may explain the increased abundance observed in the present study. However, reduced top–down control of ciliates resulting from the toxic effects of cypermethrin to their predators has been reported in aquatic systems (Friberg-Jensen et al., 2003), and may also in part explain the oberved postive effect of cypermethrin on ciliate abundance.

Homalogatra setosa was favoured in cypermethrin treatments of 80 and 160 mg kg^{-1}, demonstrating that this species shows a tolerance to both Cu and pesticide contamination, but its growth in abundance was reversed at the higher cypermthrin concentrations tested. Indeed, the results strongly suggest that cypermethrin was toxic to *H. setosa* at 320 mg kg^{-1}

364 and 640 mg kg^{-1}, with the latter representing an approximate absolute lethal dose (LD_{100}). As
365 the abundance of *H. setosa* fell, the abundance of *C. stenii* and *C. iflata* increased. The
366 inhibitory effect of cypermethrin on *H. setosa* may have reduced competition for resources,
367 facilitating the increase in abundance of more tolerant species, which may account for the
368 increase in abundance of *C. stenii* and *C. inflata*.

369

370 Although no significant decline in the mean number of ciliate species in the soil was found
371 due to cypermethrin treatment, a loss of species was detected at high treatment levels; the
372 total number of species declined from 21 in the control to 12 in the samples from the 640 mg
373 kg^{-1} treatment. In addition to *H. setosa*, the other absent species in the 640mg kg^{-1} treatment
374 were *Arcuospathidium vermiforme*, *Halteria grandinella*, and *Oxytricha setigera*, suggesting
375 that these species also found cypermethrin toxic at this level. However, it is also possible
376 that changes in the ciliate community reflect changes in the microbial community on which
377 the ciliates feed. The extent to which the soil microbial community is affected by
378 cypermethrin is dependent on the dose and the properties of the soil (Tejada et al., 2015),
379 but impacts are usually still detectable four weeks after application (Sechi et al. 2014; Tejada
380 et al., 2015). Thus, in our experiment, cypermethrin should still have had an appreciable
381 effect on the soil microbiology at the 15 day point when samples were taken. As a
382 consequence, the ciliate community may have been subject to bottom-up effects related to
383 changes in food supply as well as any direct toxic effect of the cypermethrin. Whilst the lack
384 of a negative effect on the ciliate abundance suggests that prey supply to the community as
385 a whole may not have been affected, this does not mean that species with more specialised
386 feeding niches were not affected.

387

388 The present study also demonstrated that several ciliate species are highly tolerant to
389 cypermethrin and are able to take advantage of the environmental changes it induced, most
390 notably *C. steinii* and *Pseudoplatyophrya nana.* Petz and Foissner (1989) found that almost

391 all active ciliate cells in experimental plots 90 days after the insecticide lindane was applied
392 at high dose were from two species, *C. steinii* and *P. nana*. Hence, both species appear to
393 have a particular tolerance to pesticides and can benefit from their application.

394

395 **4.3 Ecological consequences of Cu and cypermethrin pollution**

396 Protozoa are ubiquitous in soils and can dominate the soil bacterivore community under
397 certain favourable conditions (Bonkowski et al., 2009), i.e. high bacterial numbers and
398 sufficient soil pore water (Clarholm et al. 2007). Protozoa play a particularly important role in
399 releasing nutrients and organic matter from the bacterial community due to the cursory
400 digestion of ingested food, which leads to the excretion of high-quality organic matter
401 (Clarholm et al. 2007). The robustness that the ciliate community has shown to perturbation
402 by two common soil pollutants demonstrated that a very high level of pollution would be
403 required to disrupt the important ecological functions of the community. To further illustrate
404 this point, whilst Cu concentrations higher than those used in the present study can be found
405 in contaminated soils, typical concentrations are in the range of 14 to 109 mg kg^{-1}. Hence, a
406 several fold increase in background concentrations would be required to affect the ciliate
407 community. In the case of cypermethrin, typical concentrations in agricultural soils are far
408 lower than those used in the present study, ranging between 34 to 70 µg kg^{-1} (Han et al.,
409 20178; Pelosi et al., 2021). Consequently, gross over application or accidental release would
410 be required to reach the concentrations used in the present study.

411

412 Despite the robustness shown by the community as a whole, species were found within the
413 community that were sensitive to high concentrations of both Cu and cypermethrin. The loss
414 of these species from the community and the observed increase in the abundance of tolerant
415 species, demonstrated that high concentrations of both pollutants can alter the structure of
416 the ciliate community. Individual ciliate species can respond to changes in the environment

that lead to the development of a suitable niche, which can lead to rapid increases in population of previously cryptic species (Finlay et al., 1997; Finlay and Esteban, 1998). Consequently, the increased abundance of tolerant species may have indicated that there had been shifts in their niche boundaries. Such a shift(s) could have arisen from the loss of sensitive species releasing resources, such as nutrients, as interspecific competition is reduced; to changes in the microbial community on which the ciliates preyed; the loss or reduction in ciliate predators, or a combination of all three.

Protozoan species can differ in their feeding strategies and food types (Esteban and Fenchel, 2020; Thurman et al., 2010). Hence, alteration in the composition of the ciliate community could potentially lead to a modification in the nature of the grazing pressure on the bacterial community. This in turn may influence nutrient cycling in the microbial loop. Consequently, investigation into the effect of soil pollution on the interaction between soil bacteria and ciliates is warranted.

5. Conclusions

Low treatment concentrations of both Cu and cypermethrin had no effect on the abundance of soil cilates. Higher concentrations of both substances (960 kg kg^{-1} in the case of Cu and 160 to 320 mg kg^{-1} for cypermethrin) increased ciliate abundance in the soil microcosms, but this positive effect was lost at the highest concentration for cypermethrin (640 km kg^{-1}). High concetrations of Cu and cypermethrin were also required to cause changes in the soil ciliate community structure under microcosm conditions. These changes manifested as a simplifcation of the ciliate community due to a loss of sensitive species. Concurrent with this was an increase in the abundance of tolerant, opportunistic species, which resulted in an overall increase in ciliate numbers, except at the highest cypermethrin treatment. Future

work is required to determine whether the effects on the cilate community resulted from bottom-up, top-down or a combination of both forms of control.

6. Acknowledgements

The authors acknowledge financial support from the Vietnamese Government through their PhD studentship funding initiative to HTTL.

7. References

Adebavo TA, Oio OA, Olaniran OA (2007) Effect of two insecticides Karate and Thiodan on population dynamics of four different soil microorganisms. Res J Biol Sci 2: 557-560

Aoyama M (1993) Effects of copper on the decomposition of plant residues, microbial biomass, and β-glucosidase activity in soils. Soil Sci Plan Nutr 39: 557-566

Bitencourt JAP, Pereira DC, da Silva Neto ID, Crapez MAC (2016) The toxic effect of copper on the association between ciliates *Euplotes vannus* and *Euplotes crassus* and their naturally associated bacteria isolated from a polluted tropical bay. Reg Stud Mar Sci 3: 25-32

Bowers N, Pratt JR, Beeson D, Lewis M(1997) Comparative evaluation of soil toxicity using lettuce seeds and soil ciliates. Enviro Toxicol Chem 16 (2): 207-213

Bonkowski N, Villenave C, Griffiths B (2009) Rhizoshpere fauna: the functional and structural diveristy of intimate interactions of soil fauna with plant roots. Plant Soil 321: 213-233

Campbell CD, Cameron CM, Warren A, Hope SJ (1997) Direct toxicity assessment of two soils amended with sewage sludge contaminated with heavy metals using a protozoan (*Colpoda steinii*) bioassay. Chemosphere 34: 501-514

Chapman RA, Tu CM, Harris CR, Cole C (1981) Persistence of Five Pyrethroid Insecticides in Sterile and Natural, Mineral and Organic Soil. B Environ Contam Tox 26: 513-519

Clarholm M, Bonkowski M, Griffiths B (2007) Protozoa and other prostista in soil. In: van Elsas, J.D., Jansson, J.K., Trevors, J.T. (Eds.), Modern soil Microbiology 2nd ed. CRC Press, Boca Raton, pp. 147-176

Díaz S, Martín-Gonzále, A, Carlos Gutiérrez J (2006) Evaluation of heavy metal acute toxicity and bioaccumulation in soil ciliated protozoa. Environ Int 32: 711-717

Dutta J (2015) Effect of Cypermethrin on the Growth of Ciliate Protozoan *Paramecium caudatum*. Toxicol Int 22: 100-103

Ekelund F (1999) The impact of the fungicide fenpropimorph (Corbel) on bacterivorous and fungivorous protozoa. J of Appl Ecol 36: 233-243

Ekelund F, Olsson S, Johansen A (2003) Changes in the succession and diversity of protozoan and microbial populations in soil spiked with a range of copper concentrations. Soil Biol Biochem 35: 1507-1516

Ekelund F, Ronn R, Christensen S (1994) The effect of three different pesticides on soil protozoan activity. Pestic Sci 42: 71-78

Esteban GF, Clarke KJ, Olmo JL, Finlay BJ (2006) Soil protozoa - An intensive study of population dynamics and community structure in an upland grassland. Appl Soil Ecol 33: 137-151

Esteban GF, Fenchel T M (2020) Ecology of Protozoa: the biology of free-living phagotrophic protists. Springer Nature Switzerland, 186 p

Fernández-Galiano D (1994) The ammoniacal silver carbonate method as a general procedure in the study of protozoa from sewage (and other) waters. Water Res 28: 495-496

Filimon MN, Voia SO, Popescu R, Dumitrescu G, Ciochina LP, Mituletu M, Vlad DC (2015) The effect of some insecticides on soil microorganisms based on enzymatic and bacteriological analyses. Rom Biotech Lett, 20(3): 10439-10447

Finlay B, Maberly S, & Cooper J (1997) Microbial Diversity and Ecosystem Function Oikos, 80(2): 209-213

Finlay BJ, Esteban GF (1998) Planktonic Ciliate Species Diversity as an Integral Component of Ecosystem Function in a Freshwater Pond. Protist, 149 (2): 155-165

Finlay, BJ, Black, HIJ, Brown, S, Clarke, KJ, Esteban, GF, Hindle, RM, Olmo, JL, Rollett, A, Vickerman, K (2000) Estimating the Growth Potential of the Soil Protozoan Community. Protist, 151, 69-80

Finlay BJ, Esteban, GF (2013) Protozoa In: Levin, S.A. (Ed.), Encyclopedia of Biodiversity 2nd ed. Cambridge, MA, Acadmic Press

Foissner W (1987) Soil protozoa: fundamental problems, ecological significance, adaptations in ciliates and testaceans, bioindicators, and guide to the literature. Prog Protist 2: 69-212

Foissner W (1993) Colpodea (Ciliophora). Stuttgart ; New York: G. Fischer Verlag

Foissner W (1994) Soil Protozoa as Bioindicators in Ecosystems under Human Influence. *In:* Darbyshire, J. F. (ed.). Soil protozoa. CAB International

Foissner W (2014) An update of 'basic light and scanning electron microscopic methods for taxonomic studies of ciliated protozoa'. Int J Syst Evol Microbiol 64: 271-292

Foissner W (2016) Terrestrial and semiterrestrial ciliates (Protozoa, Ciliophora) from Venezuela and Galápagos. Linz, Biologiezentrum des Oberösterreichischen Landesmuseums

Foissner W, Agatha S, Berger H (2002) Soil ciliates (Protozoa, Ciliophora) from Namibia (southwest Africa) : with emphasis on two contrasting environments, the Etosha region and the Namib Desert. Linz, Biologiezentrum des Oberösterreichisches Landesmuseum

Foissner W, Berger H, Kohmann, F (1991) Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. Band I: Cyrtophorida, Oligotrichida, Hypotrichia, Colpodea. Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft

Foissner W, Berger H, Kohmann, F (1992) Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. Band II: Peritrichia, Heterotrichida, Odontostomatida. Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft

Foissner W, Berger H, Kohmann, F (1994) Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. Band III: Hymenostomata, Prostomatida, Nassulida. Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft

Foissner W, Berger H, Kohmann, F (1995) Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems. Band IV: Gymnostomatea, Loxodes, Suctoria. Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft

Forge,TA, Warren A, Berrow ML, Darbyshire JF (1993) Protozoan bioassays of soil amended with sewage sludge and heavy metals, using the common soil ciliate *Colpoda steinii*. Biol Fertil Soils 16: 282-286

Friberg-Jensen U, Wendt-Rasch L, Woin P, Christoffersen K (2003) Effects of the pyrethroid insecticide, cypermethrin, on a freshwater community studied under field conditions. I. Direct and indirect effects on abundance measures of organisms at different trophic levels. Aquat Toxicol 63: 357-371

Giller KE, Witter E, McGrath SP (1998) Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: A review. Soil Biol Biochem 30: 1389-1414

Gundi VAKB,Narasimha G, Reddy BR (2005) Interaction effects of insecticides on microbial populations and dehydrogenase activity in a black clay soil. J Environ Sci Health B 40 (2): 69-283

Hao X, Lüthje F, Rønn R, German NA, Li X, Huang F, Kisaka J, Huffman D, Alwathnani HA, Zhu Y-G, Rensing C (2016) A role for copper in protozoan grazing - two billion years selecting for bacterial copper resistance. Mol Microbiol 102: 628-641

Han Y, Mo R, Yuan X, Zhong D, Tang F, Ye C, Liu Y (2017) Pesticide residues in nut-planted soils of China and their relationship between nut/soil. Chemosphere 180: 42-47

Harris CR, Chapman RA (1981) Persistence of Four Pyrethroid Insecticides in a Mineral and an Organic Soil. J Environ Sci Heal B 16(5 Pt B): 605-615

Hikal WM, Al-Herrawy AZ, El-Daly ES, Elowa SE (2015) Inhibitory Effect of Some Pesticides on the Freshwater Amoebae Vahlkampfia Ustina. Int J Environ Plan Manag 1: 63-68

Johansen JL, Rønn R, Ekelund F (2018) Toxicity of cadmium and zinc to small soil protists. Environ Pollut 242(Pt B):1510-1517

Kabata-Pendias, A (2010) Trace elements in soils and plants. CRC Press, Boca Raton, 253 p

Kahl A (1935) Urtiere oder Protozoa: 1. Wimpertiere oder Ciliata (Infusoria): Eine Bearbeitung der freilebenden und ectocommensalen Infusorien der Erde, unter Ausschluss der marinen Tininnidae. In: Dahl (ed.). Die Tierwelt Deutschlands. Jena, Gustav Fischer.

Krupanidhi S, Sreekumar A, Sanjeevi CB (2008) Copper & biological health. Indian J Med Res 128: 448-461

Madoni P, Esteban G, Gorbi G (1992) Acute toxicity of cadmium, copper, mercury, and zinc to ciliates from activated sludge plants. Bull Environ Contam Toxicol 49(6):900-905

Madoni P, Romeo MG (2006) Acute toxicity of heavy metals towards freshwater ciliated protists. Environ Pollut 141(1):1-7

Mansano AS, Moreira RA, Dornfeld HC, Freitas EC, Vieira EM, Daam MA, Rocha O, Seleghim MHR (2020) Individual and mixture toxicity of carbofuran and diuron to the protozoan Paramecium caudatum and the cladoceran Ceriodaphnia silvestrii. Ecotoxicol Environ Saf 201:110829

Martín-González A, Díaz S, Borniquel S, Gallego A, Gutiérrez JC (2006) Cytotoxicity and bioaccumulation of heavy metals by ciliated protozoa isolated from urban wastewater treatment plants. Res Microbiol 157: 108-118

566 Pelosi C, Bertrand C, Daniele G, Coeurdassier M, Benoît P, Nélieu S, Lafay F, Bretagnolle V,
567 Gaba S, Vulliet E, Fritsch C (2021) Residues of currently used pesticides in soils and
568 earthworms: A silent threat? Agric Ecosyst Environ 305: 107167.

569 Petz W, Foissner W (1989) The effects of mancozeb and lindane on the soil microfauna of a
570 spruce forest: A field study using a completely randomized block design. Biol Fertil Soils
571 7: 225-231

572 Pratt JR, Mochan D, Xu Z (1997) Rapid Toxicity Estimation Using Soil Ciliates: Sensitivity and
573 Bioavailability. B Environ Contam Tox 58: 387-393

574 Rehman A, Shakoori FR, Shakoori AR, (2008a) Heavy metal resistant freshwater ciliate,
575 *Euplotes mutabilis*, isolated from industrial effluents has potential to decontaminate
576 wastewater of toxic metals. Bioresour Technol 99: 3890-3895

577 Rehman A, Shuja R, Shakoori AR (2008b) Tolerance of two ciliates, *Stylonychia mytilus* and
578 *Paramecium caudatum*, isolated from industrial effluents to an organophosphate,
579 endosulfan and their potential use in bioremediation of insecticide-contaminated
580 wastewater. Pak J Zool 40: 255-261

581 Sechi V, D'Annibale A, Maraldo K, Johansen A, Bossi R, Jensen J, Krogh PH (2014) Species
582 composition of a soil invertebrate multi-species test system determines the level of
583 ectoxicity. Environ Pollut 184 : 586-596

584 Shakoori AR, Rehman A, Tariq R-Ul-H (2004) Multiple metal resistance in the ciliate
585 protozoan, *Vorticella microstoma*, isolated from industrial effluents and its potential in
586 bioremediation of toxic wastes. B Environ Contam Tox 72: 1046-1051

587 Singh AK, Tiwari MN, Prakash O, Singh MP (2012) A Current Review of Cypermethrin-
588 Induced Neurotoxicity and Nigrostriatal Dopaminergic Neurodegeneration. Curr
589 Neuropharmacol 10: 64-71

590 Staley ZR, Harwood VJ, Rohr JR (2015) A synthesis of the effects of pesticides on microbial
591 persistence in aquatic ecosystems. Crit Rev Toxicol 45(10):813-836

592 Tejada M, García C, Hernández T, Gómez I (2015) Response of soil microbial activity and
593 biodiversity in soils polluted with different concentrations of cypermethrin insecticide. Arch
594 Environ Contam Toxicol 69(1): 8-19

595 Tibbett M, Green I, Rate A, De Oliveira VH, Whitaker J (2021) The transfer of trace metals in
596 the soil-plant-arthropod system. Sci Total Environ 779(2):146260

Thurman J, Parry JD, Hill PJ, Laybourn-Parry J (2010) The filter-feeding Ciliates *Colpidium striatum* and *Tetrahymena pyrifirmis* display selective feeding behaviours in the presence of mixed equally-sized, bacterial prey. Protist 161: 577-588

Todorov MT, Golemansky VG (1992) Effect of pesticides fundasol, fuzamicin and lavendotricin on the growth of laboratory cultures of Protozoa. Acta Zool Bulg 45: 20-25

Vilas-Boas JA, Cardoso SJ, Senra MVX, Rico A, Dias RJP (2020) Ciliates as model organisms for the ecotoxicological risk assessment of heavy metals: A meta-analysis. Ecotoxicol Environ Saf. 199:110669

Wuana RA, Okieimen, FE (2011) Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation. ISRN Ecol: 2011, 1-20

Wyszkowska J, Kucharski J, Lajszner W (2006) The Effects of Copper on Soil Biochemical Properties and Its Interaction with Other Heavy Metals. Pol J Environ Stud 15: 927-934

Xie W, Zhou J (2008) Cypermethrin persistence and soil properties as affected by long-term fertilizer management. Acta Agric Scand B Soil Plant Sci 54: 314-321

Xu, H, Liu, G, Wang, L, Zheng, L (2010Evaluation on Joint Acute Toxicity of Bata-Cypermethrin and Chlorpyrifos to Freshwater Protozoan Community. 2010 4th International Conference on Bioinformatics and Biomedical Engineering, 1-4, doi: 10.1109/ICBBE.2010.5516279

Zahid MT, Shakoori FR, Zulfiqar S, Al-Ghanim KA, Shakoori AR (2018) Growth Characteristics, Metal Uptake and Expression Analysis of Copper Metallothionein in a Newly Reported Ciliate, *Tetrahymena farahensis*. Pak J Zool 50: 1171-1181

Tables

Table 1. Selected physico-chemical parameters of the topsoil prior to treatment with copper and cypermethrin. Values are means ± 1SE (n = 5).

Physico-chemical parameter	Content in soil
pH (H_2O)	6.48 ± 0.04
Sand (%)	77.30 ± 0.16
Silt (%)	14.07 ± 0.16
Clay (%)	8.63 ± 0.20
Loss on ignition (%)	17.02 ± 0.33
Total C (%)	6.49 ± 0.13
Total N (%)	0.25 ± 0.01
Total P (mg kg^{-1})	408.23 ± 5.19
Total K (mg kg^{-1})	429.82 ± 3.04
Total Cu (mg kg^{-1})	8.75 ± 0.30
Available Cu (mg kg^{-1})	0.10 ± 0.01

622 **Table 2**. The mean (± 1 SE) abundance (cells g^{-1} soil) of ciliate species showing marked

623 change 15 days after the treatment of soil with copper (mg kg^{-1} soil).

Cu treatment	*Homalogastra setosa*	*Chilodonella uncinata*
Control	50.0 ± 7.28	21.68 ± 6.96
960 mg kg^{-1}	100.57 ± 12.71	176.3 ± 28.89
$F_{(1, 6)}$	11.54	27.06
Sig	$p = 0.015$	$p = 0.002$

624

Table 3. Ciliate species isolated from soil microcosms 15 days after the application of copper treatments. Each replicate that the species was found in is represented by +.

Species	Control	35 mg kg^{-1}	70 mg kg^{-1}	140 mg kg^{-1}	240 mg kg^{-1}	480 mg kg^{-1}	720 mg kg^{-1}	960 mg kg^{-1}
Acineria sp.			++	+		+		
Arcuospathidium vermiforme Foissner, 1984	++++	++++	++++	+++	++++	+++	+	++
Blepharisma sp.				+				
Chilodonella uncinata (Ehrenberg,1838) Strand, 1928	+++	++++	++++	++++	++++	++++	++++	++++
Cinetochilum margaritaceum (Ehrenberg, 1831) Perty, 1849	+							
Colpoda cucullus (Miiller, 1773) Gmelin, 1790	+	+						
Colpoda inflata (Stokes, 1884) Kahl, 1931	++	+	+++	++	++	++	+	++
Colpoda steinii Maupas, 1883						+	++	+
Cyrtolophosis mucicola Stokes, 1885	++	+++	++++	++++	+++	++	+++	++++
Drepanomonas revoluta Penard, 1922				+				
Gastrostyla steinii Engelmann, 1862							+	
Gonostomum affine (Stein, 1859) Sterki, 1878	+++	+++	++	++++	++	+++	+++	++
Halteria grandinella (Muller, 1773) Dujardin, 1841	++	++	+++	+	+			
Hemisincirra filiformis (Foissner, 1982) Foissner, 1984	++			+		+		
Hemisincirra gellerti (Foissner, 1982) Foissner, 1984	++	++++	++++	+	++++	++++	++++	++
Homalogastra setosa Kahl, 1926	++++	++++	++++	++++	++++	++++	++++	++++
Kahlilembus attenuates (Smith, 1897) Foissner, Berger & Kohmann, 1994	++	++	++	++	++	++++	+	
Kreyella minuta Foissner, 1979		+	+		+	+		++++
Oxytricha setigera Stokes, 1891	++++	++++	++++	++	++++	++	+	
Oxytricha sp.							+	
Paragonostomum binucleata Foissner, Agatha & Berger, 2002				+				
Platyophrya vorax Kahl, 1926	++++	++++	++++	++++	++++	++++	++++	++++
Sathrophilus muscorum (Kahl, 1931) Corliss, 1960	++		+	+	++	+		+
Steinia platystoma (Ehrenberg, 1831) Diesing, 1866		+						
Trachelophyllum sp.	+	+		+				
Vorticella astyliformis Foissner, 1981	+		+		+		++	+
*Total species**	17	15	15	18	14	16	14	12

* Total species recorded from soil samples incubated 4 and 10 days and subsequent enrichment cultures

Table 4. The mean (± 1 SE) abundance (cells g^{-1} soil) of ciliate species showing marked change 15 days after the treatment of soil with cypermethrin (mg kg^{-1} soil).

Cypermethrin treatment	*Homalogastra setosa*	*Colpoda steinii*	*Colpoda inflata*
Control	157.10 ± 45.32	Not present	Not present
80 mg kg^{-1}	307.67 ± 78.26	6.52 ± 1.26	5.52 ± 1.26
160 mg kg^{-1}	615.82 ± 84.74	18.06 ± 4.09	11.54 ± 5.64
320 mg kg^{-1}	5.52 ± 3.41	278.06 ± 83.13	216.32 ± 64.87
$F_{(2, 9)}$	10.68	55.86	17.44
Sig	p = 004	p < 0001	*p = 0001*

Table 5. Ciliate species isolated from soil microcosms 15 days after the application of cypermethrin treatments Each replicate that the species was found in is represented by +

Species*	Control	10 mg kg^{-1}	20 mg kg^{-1}	40 mg kg^{-1}	80 mg kg^{-1}	160 mg kg^{-1}	320 mg kg^{-1}	640 mg kg^{-1}
Arcuospathidium vermiforme Foissner, 1984	++++	++++	++++	++++	++++	+++	+	
Chilodonella uncinata (Ehrenberg,1838) Strand, 1928	+++	++++	++++	++++	+++	++++	++	++++
Cinetochilum margaritaceum (Ehrenberg, 1831) Perty, 1849	+				+			
Colpoda cucullus (Miiller, 1773) Gmelin, 1790		+		+	+		+	+
Colpoda inflata (Stokes, 1884) Kahl, 1931	+++	++++	++++	++	+++	++++	++++	++++
Colpoda steinii Maupas, 1883	+	++++	+++	++	++++	++++	++++	++++
Cyrtolophosis mucicola Stokes, 1885	++++	++++	++++	++++	+++	++++	+++	++++
Gastrostyla steinii Engelmann, 1862		+	+	++++		+++	+++	
Gonostomum affine (Stein, 1859) Sterki, 1878	++++	++	++++	++++	++++	++++	++++	++++
Gonostomum sp.						+	+	++++
Grossglockneria acuta Foissner, 1980	+							
Halteria grandinella (Muller, 1773) Dujardin, 1841	+	++	+	+	++			
Hemisincirra filiformis (Foissner, 1982) Foissner, 1984	+				+			
Hemisincirra gellerti (Foissner, 1982) Foissner, 1984	+	++++	++++	++++	+	++++	++	+++
Hemisincirra interupta (Foissner, 1982) Foissner, 1984	+	++	+++		++++	+++	+++	++
Homalogastra setosa Kahl, 1926	++++	++++	++++	++++	++++	++++	+	
Kahlilembus attenuates (Smith, 1897) Foissner, Berger & Kohmann, 1994	+		++	+++	+			
Kreyella minuta Foissner, 1979	+	++	++	+		+		
Lepthopharynx costatus Mermod, 1914				++	+	+		
Oxytricha setigera Stokes, 1891	++++	+++	++++	++++	++++	+++		
Oxytricha sp1.	+							

Oxytricha sp2.							+	
Paragonostomum binucleata Foissner, Agatha & Berger, 2002			++		+++			
Platyophrya vorax Kahl, 1926	++++	++++	++++	++++	++++	++++	++++	++++
Podophrya sp.							+	
Pseudoplatyophrya nana (Kahl, 1926) Foissner, 1980						+	+	++++
Pseudoholophrya sp.			+	+	+			
Sathrophilus muscorum (Kahl, 1931) Corliss, 1960	+++	++++	++	++++	+++	+	++++	+
Spathidium longicaudatum (Buitkamp & Wilbert,1974) Buitkamp,1977	+				+	+		
Spathidium spathula (Muller, 1773) Moody, 1912		+				+		
Sterkiella histriomuscorum Foissner *et al.*, 1991							+	
Trachelophyllum sp.		++	+	++	++			
Urosomoida agiliformis Foissner, 1982					+			
Vorticella astyliformis Foissner, 1981	++	+	++	+	+++		+	
Total species	21	19	20	20	24	19	19	12

* Total species recorded from soil samples incubated 4 and 10 days and subsequent enrichment cultures

Figures

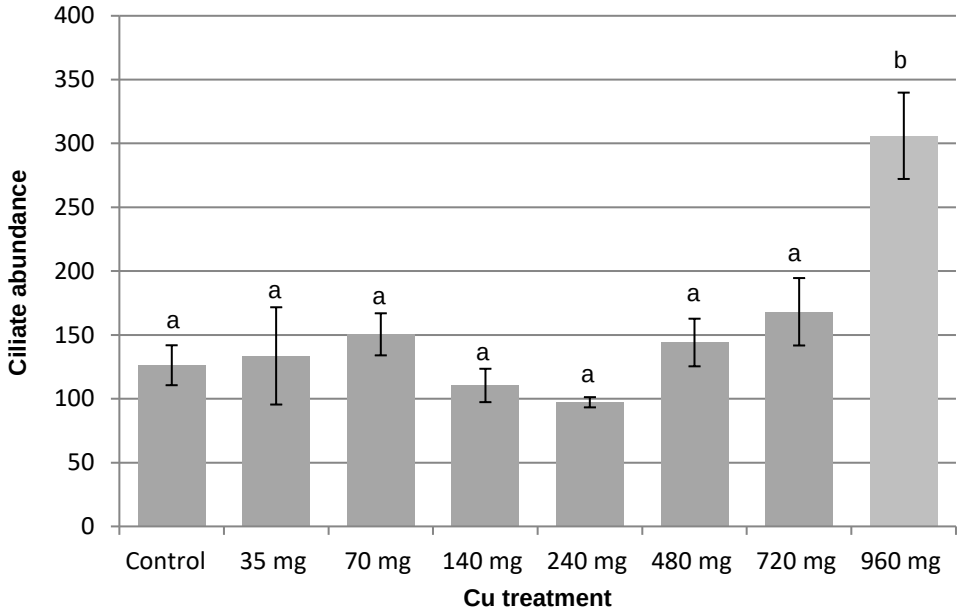

Figure 1. The abundance (cells g^{-1} soil) of ciliates (mean ± 1 SE) 15 days after the application of copper treatments (mg kg^{-1} soil) to soil. Treatments with same letters are not significantly different.

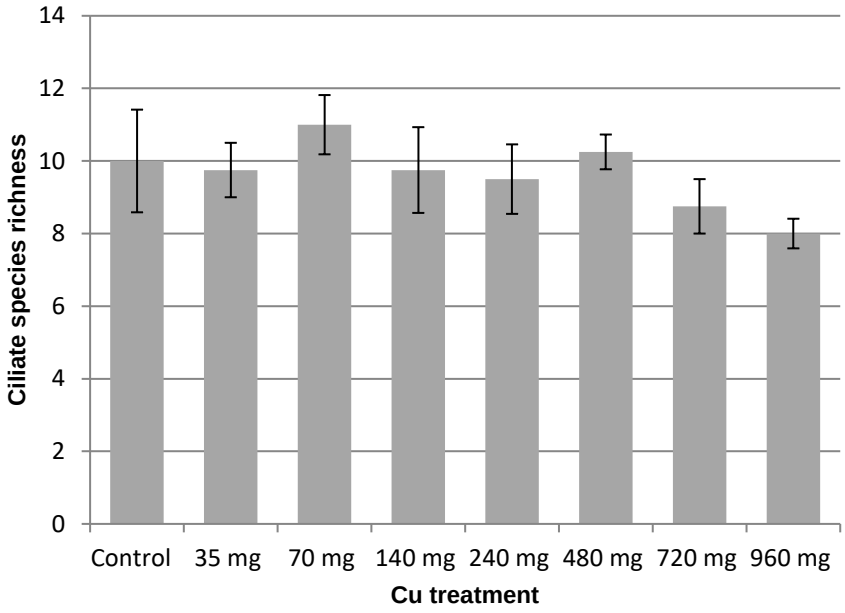

Figure 2. The total species richness of ciliates (mean ± 1 SE) in found in soil following treatment of the soil with Cu (mg kg^{-1} soil). No significant differences among treatments were found.

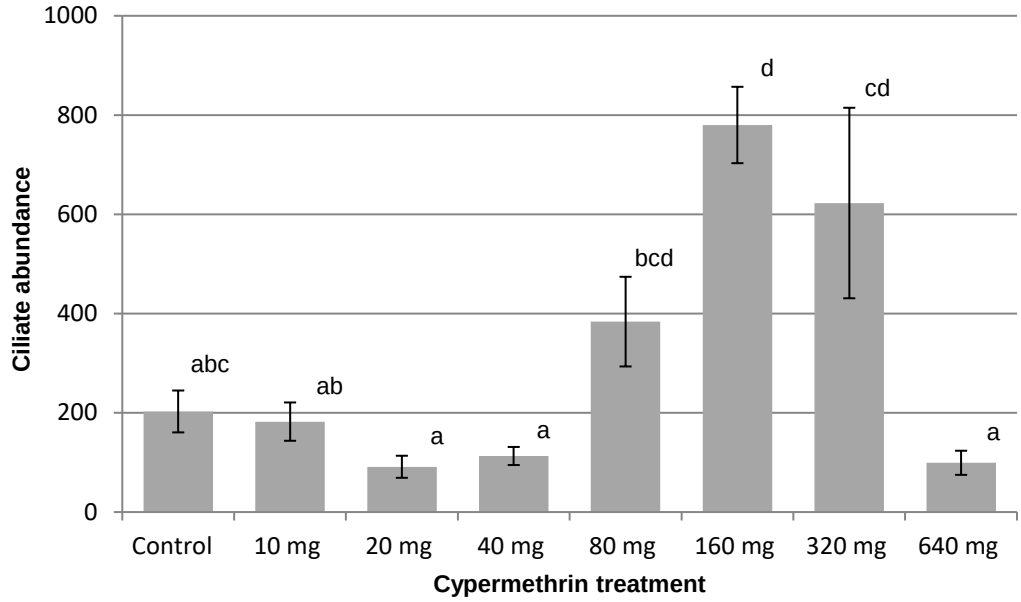

Figure 3. The abundance (cells g^{-1} soil) of ciliates (mean ± 1 SE) 15 days after the application of cypermethrin treatments (mg kg^{-1}) to the soil. Treatments with same letters are not significantly different

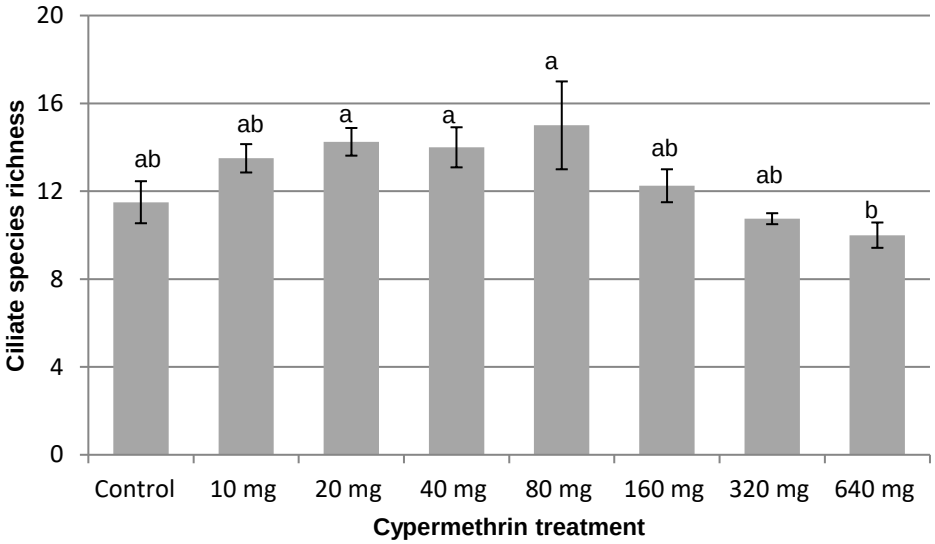

Figure 4. The total species richness of ciliates (mean ± 1 SE) in found in soil following treatment of the soil with cypermethrin (mg kg^{-1} soil). Treatments with the same letter are not significantly different.