Chemosphere 232 (2019) 180–185

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

The fungicide mancozeb affects soil invertebrates in two subtropical Brazilian soils

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HIGHLIGHTS

- Mancozeb offer a risk to key-groups of non-target species in subtropical soils.
- *F. candida* and *E. crypticus* were the most sensitive speciesto Mancozeb.
- Tests with earthworms were insufficient to protect other non-target invertebrates populations.
- A multicriteria approach (soil types and organisms) is needed for pesticides risk assessment.

ARTICLE INFO

Article history:
Received 12 February 2019
Received in revised form
20 May 2019
Accepted 21 May 2019
Available online 23 May 2019

Handling Editor: Willie Peijnenburg

Keywords:
Pesticides
Dithiocarbamate
Soil ecotoxicology
Soil fauna
Subtropical soils

ABSTRACT

Mancozeb is a dithiocarbamate non-systemic fungicide widely used to control fungal diseases of plants, commonly applied in apple orchards in Brazil. Instead of its common use, there are no reports about the risk to non-target organisms in Brazilian soils. We studied the risk of Mancozeb (in the commercial formulation Dithane® NT) for standard invertebrate species (*Folsomia candida*, *Eisenia andrei* and *Enchytraeus crypticus*) in two subtropical Brazilian soils, Oxisol and Ultisol, which are representative of apple production areas in Brazil. Reproduction and survival tests were carried out following ISO guidelines. Results showed that Mancozeb in Oxisol reduced the survival and reproduction of collembolans (LC_{50} 54.43 and EC_{50} 2.72 mg a.i. kg^{-1}) and enchytraeids (LC_{50} 6.97 and EC_{50} 3.56 mg a.i. kg^{-1}), in lowest values than those observed in Ultisol (*F. candida* $LC_{50} > 1000$ and $EC_{50} > 100$ mg a.i. kg^{-1}; *E. crypticus* LC_{50} 280.21 and EC_{50} 29.67). Effects to *E. andrei* were similar in both soils and indicated a lower sensitivity of this species to Mancozeb. The species *F. candida* and *E. crypticus* were more sensitive than *E. andrei*. These results reinforce the need to include other soil organisms besides earthworms, using chronical endpoints and considering different types of soils, to better predict the risk of pesticides for subtropical soils.

1. Introduction

Although pesticides have been used to control plant diseases and pests, the toxicity of these products can present a risk to in-soil fauna non-target species. Mancozeb is a dithiocarbamate non-systemic fungicide widely used as a contact fungicide to control fungal diseases of plants, commonly applied in apple orchards in Brazil. Even though active ingredients are frequently replaced by other new and more efficient pesticides, surprisingly forty-five years after the start of Mancozeb commercialization, market analysis data show that approximately U$740 million worth of mancozeb-containing products still were sold (Gullino et al., 2010). In Brazil, more than 30.000 tons of Mancozeb were commercialized just in 2017 (IBAMA, 2016). Dithane NT is a mancozeb based

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https://doi.org/10.1016/j.chemosphere.2019.05.179
0045-6535/© 2019 Published by Elsevier Ltd.

fungicide recommended in Brazil to application in many crops. In apple crops, is used to combat fungi diseases, with nearly thirty applications per harvest, 9 g ha^{-1} each application. The total application can reach more than 260 kg of Dithane ha^{-1} in apple crop with high plant density. Despite of the active ingredient Mancozeb has been widely used and commercialized beyond Brazil, in all European Union countries for example (EU, 2009) there are few data available about its toxicity to soil fauna. Some adverse effects in non-target organisms have been reported, such as negative influence on the community structure of oribatid mites and impact on feeding and growth performance of terrestrial isopod *Porcellionides pruinosus* (Al-Assiuty et al., 2014; Morgado et al., 2016).

Chronical terrestrial ecotoxicity tests with earthworms and arthropods have been used to pesticide registration and commercialization in Europe (EC, 2009a) whereas in Latin American countries these studies are scarce or inexistent (Niemeyer et al., 2017) and in the current legislation pesticide risk to key in-soil fauna groups is not assessed. For instance, concerning soil invertebrates Brazil and Argentina performs the risk assessment of pesticides just based on lethality tests with earthworms (IBAMA, 1996; SENASA, 1995). This approach can lead to an underestimated risk to other groups of in soil fauna, both due to the low sensitivity of lethality tests, not considering chronical exposure, and by not including other groups of soil organisms that exhibit different sensitivities to pesticides (Daam et al., 2011; Leitão et al., 2014).

Risk assessment of pesticides to soil invertebrates should be done using standardized tests. The impact of soil contaminants is measured through endpoints, such as effects on reproduction, using representative organisms, such as collembolans, earthworms and enchytraeids (Amorim et al., 2012; Chelinho et al., 2014; Pelosi et al., 2014).

To estimate the sensitivity of different in-soil fauna groups is crucial in pesticide ecological risk assessment (ERA), since its develop several contrasting ecosystem services. This subject was already defined as an important specific protection goal (EFSA, 2010) to maintain soil services and so, it already was highlighted in current legislation of European Union (EC, 2009a). Also, beyond ecotoxicity tests with different organisms, testing contrasting soils (artificial, tropical, subtropical, temperate) its important, since specific conditions, like temperature, rainfall regime, organic matter content, and others, could influence pesticides toxicity (Chelinho et al., 2011; Kamoun et al., 2018).

The aim of this study was to determine the ecotoxicity of the fungicide Mancozeb (commercial formula, Dithane® NT) to the soil invertebrates *Folsomia candida*, *Enchytraeus crypticus* and *Eisenia andrei* using standardized chronical ecotoxicity tests in two subtropical Brazilian soils.

2. Material and methods

2.1. Soils

Two subtropical soils, Oxisol and Ultisol, representative of apple orchard regions were sampled at Campo Belo do Sul (Santa Catarina State) and Vacaria (Rio Grande do Sul State) in Brazil. Soil samples were collected at a depth of 0.00–0.10 m in areas without historic of pesticides application in the last five years. The collected soil samples were air dried, sieved (2 mm mesh) and then stored at the laboratory until the use. Physico-chemical properties are shown in Table 1. Organic matter was determined by wet oxidation with potassium dichromate and measured by titration; Total organic carbon (TOC) was determined by dry combustion in a CHNS Vario EL Cube elemental analyzer; pH in water was determined in a soil solution ratio 1:1 wt:vol, with a glass electrode. Available Ca and Mg were extracted with KCl 1 Mol L^{-1} and measured by atomic absorption spectrometry. Available P and K were extracted by Melich 1 and measured by colorimetric spectrophotometry (P) and flame spectrometry (K). Available Cu were determined with HCl 0.1 M extractor, Fe with Ammonium Oxalate 0.2 M extractor (at pH 3.0) and were measured by atomic absorption spectrometry. The particle size distribution was determined with a Robinson pipette and with Calgon dispersant. All these methods were performed according to Tedesco et al. (1995). Water holding capacity (WHC) was determined according to ISO 11268-2 (ISO, 1998).

Table 1
Physical and chemical properties of the Oxisol and Ultisol soils collected in Vacaria, RS, and Campo Belo do Sul, SC-Brazil, respectively.

Properties	Oxisol	Ultisol
Organic matter (mg dm^{-3})	0.8	0.5
Total Organic Carbon (mg dm^{-3})	7.7	6.3
pH (water)	4.9	4.8
Cation-exchange capacity ($cmol_c$ dm^{-3})	10.0	8.6
Ca ($cmol_c$ dm^{-3})	6.9	4.0
Mg ($cmol_c$ dm^{-3})	2.1	1.4
P (mg dm^{-3})	13.9	2.1
K (mg dm^{-3})	272.0	141.0
Cu (mg dm^{-3})	10.6	18.3
Fe (mg dm^{-3})	77.7	55.3
Clay (%)	30.0	47.0
Sand (%)	24.0	18.0
Silt (%)	46.0	35.0
Water hold capacity (%)	78	83

2.2. Test species

Tests were conducted using laboratory cultured organisms of the species *F. candida* (Isotomidae, Collembola), *E. crypticus* (Enchytraeidae, Oligochaeta) and *E. andrei* (Lumbricidae, Oligochaeta). *F. candida* and *E. crypticus* were primarily obtained from Laboratory of Soil Ecology and Ecotoxicology, Center for Functional Ecology (CEF), Department of Life Sciences – FCTUC, Coimbra University – UC, Portugal. *E. andrei* was acquired from Minhobox® Corporation, Minas Gerais State, Brazil. Controlled cultures of these species (20 ± 2 °C, photoperiod 12 h: 12 h light/dark) have been maintained in laboratory. Collembolans were cultured in plastic containers filled with a moisturized substrate of plaster and activated charcoal (10:1), fed with biological dry yeast (*Saccharomyces cerevisiae*) three times a week. Enchytraeids were cultured in moisturized Tropical Artificial Soil (TAS) and fed with finely ground oats three times a week. The TAS consisted of a mixture of fine sand, kaolinitic clay (powdered kaolin), and coconut fiber, in a proportion of 70:20:10 d.w., respectively, with pH value adjusted to 6.0 ± 0.5 by $CaCO_3$ addition (Garcia, 2004). Earthworms were cultured in a moisturized substrate of defauned horse manure (free of antibiotics), coconut fiber and sand (70:20:10 w:w), pH 5.7 ± 0.3, receiving cooked oatmeal as an additional source of food once a week.

2.3. Experimental procedure

Range-finding lethality tests were carried out to determine the concentrations for the reproduction tests. All the ecotoxicity tests followed the ISO guidelines: for *F. candida*, ISO 11267 (ISO, 1999); for *E. crypticus*, ISO 16387 (ISO, 2004); and for *E. andrei*, ISO 11268-2 (ISO, 1998). All soils had their moisture adjusted to 50% of the water holding capacity (WHC) at the beginning of the experiments and organisms maintained at 20 ± 2 °C with photoperiod 12 h: 12 h light/dark. Different nominal pesticide concentrations were used in tests (Table 2).

Table 2
Summary of the tests indicating the test species, soil type and their respective nominal concentration range as active ingredient (a.i.) per kg soil (dry weight).

Test species	Test	Mancozeb concentrations (mg kg^{-1})	
		Oxisol	Ultisol
F. candida	LC_{50}	0, 1, 10, 100, 1000	0, 1, 10, 100, 1000
	EC_{50}	0, 2, 4, 8, 16, 32, 64, 128	0, 1, 10, 100, 1000
E. crypticus	LC_{50}	0, 0.1, 1, 10, 100, 1000	0, 0.1, 1, 10, 100, 1000
	EC_{50}	0, 0.2, 0.4, 0.8,1.5, 3, 6, 10	0, 15, 25, 50, 100, 200
E. andrei	LC_{50}	0, 1, 10, 100, 1000	0, 1, 10, 100, 1000
	EC_{50}	0, 10, 50, 100, 500, 1000	0, 1, 5, 10, 50, 100, 500, 1000

LC_{50} – Concentration causing 50% lethality; EC_{50} – Concentration causing 50% effect in reproduction.

Table 3
Nominal concentration (mg i.a. kg^{-1}) and chemical measured the concentration (mg i.a. kg^{-1}) of Mancozeb (Dithane® NT) in subtropical soils by gas chromatography.

Soil type	Nominal	Real
Oxisol	0	0.88
	0.2	1.48
	2	3.16
	10	6.98
	128	46.41
	1000	995.76
Ultisol	0	0.9
	1	1.28
	100	71.94
	1000	593.66

Collembolans survival and reproduction tests were carried out in glass vessels (175 mL capacity). Ten organisms (10–12 d old) were transferred to each replicate (n = 5) containing 30 g ww of contaminated or control soil. Dry yeast was added as food supply once a week. In the lethality test, adult survival was determined at day 14. In reproduction tests, after 4 weeks each test vessel was filled with distilled water, gently stirred with a spatula, causing flotation of the organisms. Through digital imaging and using the software IMAGE J (Schneider et al., 2012), adults and juveniles were manually counted.

For tests with enchytraeids, ten clitellate organisms were transferred to glass vessels (170-mL capacity) containing 30 g of contaminated or control soil (n = 5). Ground oat was added as food supply once a week. At the end of the tests (14 d for survival, 28 d for reproduction), 5 mL of alcohol (96°) and some drops of rose bengal were added to preserve the organisms, which were counted 48 h after, with a stereomicroscope (40x).

For tests with earthworms, ten clitellate organisms (250–600 mg) were transferred to plastic vessels (1000 mL capacity) containing 500 g of contaminated or control soil (n = 4). Survival was determined at day 14 with no food addition. In reproduction tests, horse manure was added as food supply once a week. Adults were removed at day 28 and the juveniles were counted at day 56 putting the replicates in a water bath at 60 °C to stimulate juveniles to emerge at the vessel's surface.

2.4. Chemical substance

The tested soils were contaminated in the laboratory using a commercial formulation of Mancozeb, the fungicide Dithane® NT, Dow Agro, aiming to assess its toxicity. The product characteristics are 800 g of active ingredient (a.i.) per kilo, Chemical Abstracts Service (CAS): 8018-01-7; Log KoW: 1.33; solubility 6.2 ppm at 25 °C. Mancozeb has low soil persistence with half-life pointed in literature as less than 2 days in aerobic soils (Xu, 2000) and in European legislation as less than one day (EC, 2009b).

In order to determine Mancozeb residues, chemical analyses by gas chromatography were performed by Federal University of Santa Maria (UFSM) - Chemistry Department Research and Analysis Center for Waste and Pollutants. Prior to assembling the tests, nominal concentrations representing low and high concentrations were selected to determine the real concentration of Mancozeb in the soil, independent of the test organism (Table 3).

2.5. Predicted environmental concentration (PEC) in soil and risk calculation

According with the new suggestion to in-soil fauna pesticides ERA (EFSA, 2017), a tiered approach based on lower, intermediate and higher tiers might be considered to evaluated potentially hazardous to organisms. Data of this paper could be considered as a lower tier and so, the available EC_s values were compared with an initial predicted environmental concentration (PEC) as the exposure scenario. Initial PEC was calculated following FOCUS (1997), which was recommended in EFSA Guidance (EFSA, 2017) as:

$$\textit{Initial PEC} = A^*(1 - f\text{int})/(100 * \textit{depth} * \textit{bd})$$

where: A = application rate (g ha^{-1}); f_{int} = fraction intercepted by crop canopy; depth = mixing depth (cm); bd = dry soil bulk density (g cm^{-3}).

A bulk density of 1.5 g cm^{-3}, and a mixing depth of 5 cm was assumed, as recommended for applications to the soil surface. As the fraction intercepted is assumed to be 0 in the lower tier (EFSA, 2017), using these assumptions, the concentration in soil immediately after a single application (mg kg^{-1}) becomes:

$$\textit{Initial PECS} = A/750$$

The recommended dose of the commercial product used (Dithane NT) for apple crops for each application is 2.000 g ha^{-1} (1.600 g mancozeb kg^{-1}) (MAPA, 2019). However, as there are several applications, the total amount in the end of the harvest could be much higher (>260 kg ha^{-1}). In terms of initial PEC, for a single application, the estimated value was 2.67 mg kg^{-1}.

The ratio between ecotoxicity tests (ECs or NOECs) and PECs could be performed in order to estimate the risk and its so-called toxicity-exposure ratio (TER). This value is compared with a trigger value (5): when TER is lower than the trigger value, risk is considered to in-soil fauna in this ERA step and further investigations must be required (intermediate and/or higher tier) (EFSA, 2017).

2.6. Statistical analyses

For lethality and reproduction tests, differences between contaminated and control soil were evaluated through analysis of variance (One-way ANOVA) followed by Dunnett test (M < control, $p < 0{,}05$), using the Statistica 7.0 Software (StatSoft, 2004). Through the Dunnett test, it is possible to establish NOEC (non-observed effect concentration) and LOEC (low observed effect concentration) when comparing concentrations with control (Concentration 0).

Concentrations causing 50% lethality (LC_{50}) in lethality tests were determined using PriProbit® 1.63 (Sakuma, 1998). In reproduction tests, concentrations causing 50% effect (EC_{50}) were determined by a nonlinear regression analysis, using the Statistica 7.0 Software (StatSoft, 2004). Histograms of the residuals and stem-and-leaf graphs were examined to ensure that normality assumptions were met. Variances of the residuals were examined to decide whether or not to weight the data, and to select potential models. These models included (1) logistic (Gompertz), (2) exponential and

(3) linear:

$$Y = a*\exp\left((\log(0,5))*(\log conc/x)\hat{b}\right) \quad (1)$$

$$Y = a*\exp(\log((a - a*0.5 - b*0.5)/a)*(\log conc/x)) + b \quad (2)$$

$$Y = ((-a*0.5)/x)*\log conc + a \quad (3)$$

where Y: value for a measurement endpoint (e.g. number of juveniles); a: control response; logconc: the log-transformed exposure concentration; x: EC_{50} predict for the data set; b: scale parameter.

3. Results

Ecotoxicity tests with in-soil organisms fulfilled the validity criteria of controls proposed by ISO guidelines to artificial soil and to subtropical soils (*F. candida*: mortality of adults <20%; reproduction rate of >100 instars per vessel; coefficient of variation of reproduction <30%; *E. crypticus*: mortality of adults <20%; reproduction rate of >25 juveniles per vessel; coefficient of variation of reproduction <50% and *E. andrei*: mortality of adults ≤10%; reproduction rate of ≥30 juveniles per vessel; coefficient of variation of reproduction <30%). Effects of Mancozeb were different depending on soil type and tested species (Table 4).

Collembolan species *F. candida* was sensitive to Mancozeb in Oxisol, showing effect in both lethality and reproduction tests (LC_{50} 54.43; EC_{50} 2.72 (2.03–3.40) mg a.i. kg^{-1}), while in Ultisol the toxicity was reduced (LC_{50} > 1000 and EC_{50} > 100 mg a.i. kg^{-1}). The same trend was observed to the species *E. crypticus*, which was more sensitive to Mancozeb in Oxisol (LC_{50} 6.97; EC_{50} 3.56 (1.39 ± 5.74) mg a.i. kg^{-1}) than in Ultisol (LC_{50} 280.21; EC_{50} 29.67 (16.10 ± 43.25) mg a.i. kg^{-1}). Non-acute effects on survival of earthworms were observed (LC_{50} > 1000 mg a.i. kg^{-1}), while for reproduction the EC_{50} values were >500 mg a.i. kg^{-1} for both tested soils.

Table 5 shows that, following the TER approach, *F. candida* and *E. crypticus* species were at risk in Oxisol, but not in Ultisol. *E. andrei* was not sensitive to PEC.

4. Discussion

Mancozeb reduced the reproduction of *F. candida*, survival and reproduction of *E. crypticus*, being the toxicity higher in Oxisol than in Ultisol. Besides the absence until the present of independent research of mancozeb toxicity to Collembola, consulting the Rapporteur Assessment Report (RAR) for ecotoxicological effects on non-target soil meso- and macrofauna (EFSA, 2018), was possible verify effects on *F. candida*. The study number 13DL1CR conducted in artificial soil by ECT Oekotoxikologie GmbH Germany following OECD guidelines (OECD, 2009) estimates an EC_{50} of 20.1 (20.0–20.3) mg mancozeb kg^{-1} soil dw. Even that artificial soils already normally has been indicated as showing lower toxicity values than reference natural soils in Europe (De Silva et al., 2009). Kamoun et al. (2018) indicates that toxicity of the pesticides deltamethrin, dimethoate, and chlorpyrifos to *F. candida* was higher in natural tropical soils than in OECD soil. Differences in natural soils of the present study could be one of the reasons for this higher toxicity. Notwithstanding there are no reports in the literature about enchytraeids sensitivity to dithiocarbamates (like Mancozeb). Since this group still is not required by European Union in pesticides ERA, there is no available information at RAR document for this group. Kuperman et al. (2004) has pointed enchytraeids as more sensitive than collembolans and earthworms to manganese – one of the main components of Mancozeb, which could explain the effects observed in this study.

For *E. andrei*, there was no lethal effect even at the highest concentration for both soils (LC_{50} > 1000 mg a.i. kg^{-1}). Concentrations posing risk to earthworm's reproduction were higher than that observed for collembolans and enchytraeids in Oxisol and in Ultisol. Low toxicity of Mancozeb has been reported to earthworms, as shown to *Perionyx excavatus* (LC_{50} 460–544 mg a.i. kg^{-1} dry soil; De Silva et al., 2010) and *Eisenia fetida* in a natural soil (AC_{50} 4 mg kg^{-1} and LC_{50} 8 mg a.i. kg^{-1} dry soil, respectively; García-Santos and Keller-Forrer, 2011; Reinecke et al., 2002). In the same way, Vermeulen et al. (2001) found LC_{50} of 1262 mg a.i. kg^{-1} to *E. fetida* in artificial soil, and concluded, with the endpoints growth and cocoon number, that Mancozeb had low toxicity. These studies corroborate our results indicating the low sensitivity of the earthworms to Mancozeb.

Earthworms have been used as test organisms in ecotoxicology for a long time (Kula and Kokta, 1992; Pelosi et al., 2014). In some Latin American countries, including Brazil and Argentina, lethality test with earthworms is required to register new pesticides (IBAMA, 1996; SENASA, 1995). However, such regulations do not include chronical endpoints, such as reproduction of earthworms, or other soil invertebrates in such evaluation, as recommended by the European Union (EC, 2009a). It can be a limitation to protect soil organisms and ecosystem processes since some authors have shown the low sensitivity of earthworms to Mancozeb.

Several soil characteristics are important to predict bioavailability of contaminants, such as pH, redox potential and presence of cation competitors (Ca^{2+}, Fe^{2+}, and Mg^{2+}) or concentration of

Table 4
Toxicity of Mancozeb for soil invertebrate organisms in Oxisol and Ultisol. LC_{50} values represent an effect on survival and EC_{50} values on reproduction. Values are given with the corresponding confidence interval of 95%. All values are expressed in mg a.i. kg^{-1} dry soil, using the commercial product Dithane® NT.

Test	Days	Collembola		Enchytraeids		Earthworms	
		Oxisol	Ultisol	Oxisol	Ultisol	Oxisol	Ultisol
Lethality							
LC_{50}	14	54.43	>1000	6.97	280.21	>1000	>1000
	28	55.78	>100	–	–	>1000	>1000
NOEC	14	1	1000	0.1	100	1000	1000
	28	4	100	–	–	1000	1000
LOEC	14	10	>1000	1	1000	>1000	>1000
	28	8	1000	–	–	>1000	>1000
Reproduction							
EC_{50}		2.72 (2.03 ± 3.40)	>100	3.56 (1.39 ± 5.74)	29.67 (16.10 ± 43.25)	570.13 (459.27 ± 680.99)	547.30 (385.14 ± 709.47)
NOEC		<2	100	0.2	<15	<10	5
LOEC		2	1000	0.4	15	10	10

Table 5
Effective concentration affecting 50% of reproduction of the tested species (EC_{50}) in Oxisol and Utisol, initial predicted environmental concentration (PEC) of mancozeb in one application in apple orchard, and the toxicity-exposure ratio (TER). When TER <5, the population is considered at risk.

Organism	Soil	EC_{50}	Initial PEC	TER
F. candida	Oxisol	2.72	2.67	1.02[a]
	Ultisol	>100		37.45[b]
E. crypticus	Oxisol	3.56		1.33[a]
	Ultisol	29.67		11.11[b]
E. andrei	Oxisol	>500		187.26[b]
	Ultisol	>500		187.26[b]

[a] TER <5.
[b] TER >5.

organic and inorganic binders where a metal could be connected or adsorbed (Azevedo and Chasin, 2003). Mancozeb is composed basically by manganese (20%) and zinc, which could be less available in soils with free negative charge – with fewer cation competitors. Differences observed between the Mancozeb toxicity in Oxisol and Ultisol could be related to cation competition, favoring lower adsorption and consequently higher availability of Mancozeb in Oxisol than in Ultisol, since pH, clay and organic matter content were similar between them. Furthermore, soil texture could be another reason for higher toxicity in Oxisol, which has less clay and more sand content in comparison to the Ultisol, possibly favoring higher availability of Mancozeb.

The United States Environmental Protection Agency (EPA) and the European Union have allowed Mancozeb application because they concluded that its use is acceptable to modern agriculture (Reis and Reis, 2015). However, as shown in this paper, other groups of organisms besides earthworms need to be considered and investigated for different tropical and subtropical soils. Reproduction tests showed higher sensitivity of collembolans and enchytraeids than earthworms, and Mancozeb in the Oxisol showed higher toxicity than in the Ultisol. Even that DT_{50soil} of Mancozeb in soil has been estimated in less than one day (EC, 2009b), it can cause effects on fauna in-soil key groups, requiring further investigations at community or field levels. Effects under different soils needs to be considered in the risk assessment.

The guarantee of survival of several groups of soil organisms has direct effect on the provision of soil functioning and derived ecosystem services offered by the soil community, promoting soil quality, such as decomposition and biological regulation (Moreira and Siqueira, 2006; Odum and Barrett, 2011; Townsend et al., 2010). Minimizing the risk of pesticides use is an important goal in the maintenance of soil functionality and environmental quality. We must consider the class of soil and the sensitivity of different groups of non-target organisms before the determination of concentrations and for license of pesticides. Furthermore, risk to other vertebrates including humans cannot be discharged. Dithiocarbamates fungicides has already pointed as potential thyroid disruptors in vertebrates (Axelstad et al., 2011; Ksheerasagar and Kaliwal, 2003). Pandey and Mohanty (2015) also conclude that pituitary–thyroid axis of avian population is highly sensitive to mancozeb, even in environmentally equivalent concentrations. This sensibility can cause alterations of reproductive and metabolic homeostasis.

5. Conclusion

The risk of Mancozeb to soil fauna varied among the representing groups of soil fauna and type of soils, which reinforces the need to evaluate different scenarios of pesticides application.

Mancozeb affected the reproduction and survival of soil invertebrates, being *F. candida* and *E. crypticus* more sensitive than *E. andrei*. Earthworms showed to be less sensitive than collembolans and enchytraeids despite their great use as an indicator of pesticide risks around de world.

Our results reinforce the need to include further evaluations embracing soil organisms and different types of soils, besides chronical endpoints such as reproduction, to better predict the risk of pesticides for subtropical soils.

Acknowledgments

L. S. C. C. thanks the Coordination of Superior Level Staff Improvement (CAPES) for a M.Sc. grant. L. C. I. O. F. thanks the CAPES for a Post-Doctor grant (Project nº 079/2012 of "Ciência Sem Fronteiras - CAPES" programme).

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