Environmental Pollution 159 (2011) 1844–1851

Contents lists available at ScienceDirect

Environmental Pollution

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

Combined effects of soil moisture and carbaryl to earthworms and plants: Simulation of flood and drought scenarios

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ARTICLE INFO

Article history:
Received 19 November 2010
Received in revised form
21 March 2011
Accepted 24 March 2011

Keywords:
Soil moisture
Carbaryl
Synergism
Antagonism
Non-target species

ABSTRACT

Studying tolerance limits in organisms exposed to climatic variations is key to understanding effects on behaviour and physiology. The presence of pollutants may influence these tolerance limits, by altering the toxicity or bioavailability of the chemical. In this work, the plant species *Brassica rapa* and *Triticum aestivum* and the earthworm *Eisenia andrei* were exposed to different levels of soil moisture and carbaryl, as natural and chemical stressors, respectively. Both stress factors were tested individually, as well as in combination. Acute and chronic tests were performed and results were discussed in order to evaluate the responses of organisms to the combination of stressors. When possible, data was fitted to widely employed models for describing chemical mixture responses. Synergistic interactions were observed in earthworms exposed to carbaryl and drought conditions, while antagonistic interactions were more representative for plants, especially in relation to biomass loss under flood-simulation conditions.

1. Introduction

In the last decades, several factors have had an impact on ecosystem sustainability. Among them, anthropogenic activities (e.g. agricultural practices) and environmental (including climatic) changes are important causes for such an imbalance. Global climatic changes are increasingly redirecting ecotoxicological studies, and there has been an increment on the knowledge about interactions between natural and chemical stressors, and the way they affect organisms and their performance. The response of soil fauna and flora to chemicals is dependent on the environmental conditions under which they are exposed. Environmental conditions cannot only increase the organism susceptibility to pollutants, but also become stress factors themselves (Holmstrup et al., 2007; Sjursen and Holmstrup, 2004; Spurgeon et al., 2005; Svendsen et al., 2007).

Biota play an important role in maintaining soil quality and functioning, since they intervene on the decomposition of dead organic material and nutrient cycling (Bardgett et al., 2005). The species used in this work represent different groups of organisms in terms of function, trophic level, life history strategy and route of exposure to chemicals.

Earthworms are one of the most important biocomponents of ecosystems, contributing to maintaining soil structure and fertility, promote plant growth, and aid in important soil processes such as carbon and nitrogen cycling (Edwards and Bohlen, 1996). Higher plants are considered to be versatile tools for identifying and monitoring the effects of pollutants on soil (Gong et al., 2001; Loureiro et al., 2006). Plants can be used as bioindicators for toxicity assessment in both aquatic and terrestrial ecosystems (Azevedo et al., 2005; Gorsuch et al., 1991). In this context, the monocotyledonous *Triticum aestivum* and the dicotyledonous *Brassica rapa* are amongst those species more commonly used in environmental risk assessment, while being representative of economically relevant plants.

Soil available water is a key factor in determining soil fauna and plant fitness (Fragoso and Lavelle, 1992; Lavelle and Spain, 2001), while influencing the activity and habitat selection of soil organisms, as well as the behaviour and toxicity of anthropogenic contaminants towards edaphic species and plants (Højer et al., 2001; Martikainen and Krogh, 1999). Some studies have been carried out on the influence of soil moisture on soil fauna dynamics, evaluating changes in sensitivity to soil moisture after chemical exposure or vice-versa or even the combined assessment simultaneously (Bindesbøl et al., 2005; Friis et al., 2004; Højer et al., 2001; Holmstrup, 1997; Holmstrup et al., 2007, 1998; Long et al., 2009; Maraldo et al., 2006; Sjursen and Holmstrup, 2004; Sjursen et al., 2001; Sorensen and Holmstrup, 2005). Such approaches are crucial to understand scenarios of drought and flood, which have become more frequent over the last decades.

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doi:10.1016/j.envpol.2011.03.029

Analysing the effects of pharmaceutical mixtures has become common practice in toxicology, with conceptual models such as concentration addition (CA) and independent action (IA) amongst those more widely used. Such models have also been transposed to environmental research, for predicting effects of chemical mixtures or combinations of natural and chemical stressors (e.g. Holmstrup, 2008; Loureiro et al., 2009; Pestana et al., 2009). The CA concept described by Loewe and Muischnek (1926) is based on the assumption that the individual components of the mixture have similar mechanisms of action. The IA principle relates to independent modes of action of the mixture components and was firstly described by Bliss (1939). Recently, both models were successfully employed as part of the European project NoMiracle (2004–2009), for describing the combined effects of chemicals and natural stressors (Ferreira et al., 2008, 2010; Long et al., 2009).

The aim of this study was to investigate and predict the toxicity of carbaryl under different soil moisture contents, thus simulating drought and flood scenarios. Earthworms and plants were used as test-organisms. Dose–response curves for single stressors exposure was modelled using the independent action (IA) concept, and tested for possible deviations for synergism or antagonism. The carbamate insecticide carbaryl (1-naphthol N-methyl carbamate) was chosen as test-chemical, due to the fact that it is widely used in both agricultural and domestic applications, while being considered a potential neurotoxicant to non-target species (Gambi et al., 2007). The inhibition of cholinesterase (ChE) activity by carbaryl is well documented for different animal species (e.g. Caselli et al., 2006; Ferrari et al., 2004; Gambi et al., 2007; Gupta and Sundararaman, 1991). The persistence of carbaryl in plants has also been investigated, with fruit trees having shown to be able to accumulate this pesticide (Galhotra et al., 1985; Iwata et al., 1979; Rao and Ramasubbaiah, 1988). In addition, other studies have also reported the side effects of carbaryl on plant growth (e.g. apple trees; (Jones et al., 1991; Murthy and Raghu, 1990)).

2. Materials and methods

2.1. Test substance and test species

Carbaryl (CAS No 63-25-2) was purchased from Sigma–Aldrich Ltd. (99.8% purity). Carbaryl stock solution was prepared using acetone and applied to pre-moistened soil one day before the experiment started, in order to allow the evaporation of acetone.

All experiments were carried out using the natural standard soil LUFA 2.2 from Speyer, Germany (Løkke and van Gestel, 1998). Lufa 2.2. soil is considered a standard sandy-loam soil (17% silt, 6% clay and 77% sand), with 4.4% of organic matter, a carbon/nitrogen ratio of 14, pH 5.8, water holding capacity of 55% (weight per volume) and a cation exchange capacity of 11.2 cmol/kg.

The earthworm *Eisenia andrei* Bouché was kept in laboratory cultures, in plastic boxes with a mixture of Sphagnum peat (50%) and horse dung (50%) as substrate, with pH 6.0 ± 0.05 adjusted with powdered calcium carbonate ($CaCO_3$). Organisms were fed weekly with horse dung. Cultures were maintained at 16:8 h light:dark photoperiod and at 20±2 °C.

Seeds of *B. rapa* were purchased from Carolina Biological Supply Company (US) and *T. aestivum* from a local supplier (Aveiro, Portugal).

2.2. Single exposure – chemical stressor

2.2.1. Earthworms

Tests were performed in accordance to the OECD 207 guideline (OECD, 1984). Ten adult worms (clitellated), with individual fresh weight between 300 and 600 mg, were exposed to different carbaryl concentrations (20, 40, 60, 80, 100 mg/kg) at four replicates each. In addition to the negative control, a solvent control was prepared for comparison with 100 ml acetone/Kg. The chemical exposure test was carried out in a climatic chamber at 20±2 °C; 16 h light/8 h dark, and soil moisture adjusted to 60% water holding capacity (WHC). After 7 and 14 days of exposure, surviving worms were counted, and at the end of the test (14 day), earthworms were pooled weighted and their mean biomass (mg) reported. For the earthworms, loss of weight was calculated using the equation:

$$LW = \frac{\left(Fw_i - Fw_f\right)}{Fw_i}$$

where LW is the mean loss of weight; Fw_f is the mean weight after 14 days; Fw_i is the mean weight at the beginning of the test.

2.2.2. Plants

The plant tests were performed following the protocol ISO 11269-2 (ISO, 1995). For each species, ten seeds were placed per plastic pot with 500 g of soil at a depth of 1 cm from the soil surface. Four replicates/pot per treatment were used. Carbaryl exposure treatments ranged from 50 to 150 mg/kg. In addition to the negative control, a solvent control was prepared with 100 ml acetone/Kg. Bioassays were carried out at 20±2 °C, at 12000 lx, in a 16:8 (light:dark) photoperiod. The test duration was 14 days after 50% of seeds had emerged in the control soil. In the first 7 days, seeds' germination was reported. The soil moisture was maintained by capillary action, through a fibreglass wick (between 5 and 10 mm ø) located at the pot's bottom (Loureiro et al., 2006). At the end of the tests, individual growth (shoot length), fresh and dry weight were recorded and the hydric content (HC) calculated using the equation:

$$HC = \frac{FW - DW}{FW} \times 100$$

where DW is the plant dry weight and FW the plant fresh weight.

2.3. Single exposure – natural stressor

The tests with earthworms and plants were adapted from that described in the OECD 207 (OECD, 1984) and ISO 11269-2 (ISO, 1995), respectively. Earthworms and plants were exposed to different soil moisture contents, simulating drought (10, 20 and 40% of the WHC), as well as flood conditions (80, 100 and 120% of the WHC). In both approaches, a control (60% of the WHC), similarly to that described in the chemical exposure bioassays. To control moisture levels during the experiments, soil pots were weighted daily and water added when needed.

2.4. Combined exposure

The procedures for the tests with earthworm and plants were carried out in accordance to that described in the OECD 207 (OECD, 1984) and ISO 11269-2 (ISO, 1995), with further adaptations. Dry soil was contaminated with different carbaryl concentrations dissolved in acetone, left to evaporate for one day and then moistened with deionized water in order to obtain 10, 20, 40, 60 (control), 80, 100 and 120% WHC.

All procedures were carried out as previously described for the single stressors exposures.

2.5. Statistical analysis

One way (ANOVA), followed by Dunnett's test, was used to analyse differences between control and treatments. Whenever data were not normally distributed and data transformation did not correct for normality, a Kruskal–Wallis ANOVA on Ranks was performed (Zar, 1996), followed by the Dunn's method when significant differences were found. Differences between control and solvent control were analyzed using a *t*-test or a Mann–Whitney Rank test when normality failed ($\alpha = 0.05$). For this statistical analysis, the software package SigmaStat was used (SPSS, 1995).

EC_{50} values were calculated using a sigmoidal (logistic, 3 parameter) equation (SystatSoftwareInc, 2002). LC_{50} values were calculated through the Probit method (SPSS, 2003). Data from the mixture exposures were analysed by comparing the observed data with the expected mixture effects from the IA reference model using the MIXTOX model of Jonker et al. (2005). Under this approach, in the second step, the IA model was extended according to Jonker et al. (2005), using deviation functions to describe synergistic/antagonistic interactions, dose level, and dose-ratio dependency. For that a nested framework was built with the extra parameters needed for the deviation functions. Data are fitted to the models using the method of maximum likelihood, resulting on model fits that can be statistically compared through likelihood testing. The effect patterns were then deduced directly from the parameter values (Jonker et al., 2004, 2005).

When dose–response curves where not established for one of the stressors or parameter EC50/LC50 values for carbaryl were compared between soil moisture treatments to detect shifts in toxicity.

3. Results

3.1. Single-exposure

There were no significant differences of the parameters measured between the control and solvent control; therefore the solvent control data will be used to compare the results obtained. The EC50 and LC50 values calculated from the single exposures to