Environment International 35 (2009) 318–324

Contents lists available at ScienceDirect

Environment International

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

Effects of natural and chemical stressors on *Enchytraeus albidus*: Can oxidative stress parameters be used as fast screening tools for the assessment of different stress impacts in soils?

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ARTICLE INFO

Article history:
Received 11 April 2008
Accepted 7 August 2008
Available online 25 September 2008

Keywords:
Enchytraeids
Phenmedipham
Copper
Abiotic factors
Enzymatic and non-enzymatic antioxidants
Oxidative stress

ABSTRACT

Enchytraeids are important organisms of the soil biocenosis. They improve the soil pore structure and the degradation of organic matter. These organisms are used in standardized testing, using survival and reproduction (6 weeks) as endpoints. The use of biomarkers, linked to ecologically relevant alterations at higher levels of biological organization, is a promising tool for Environmental Risk Assessment. Here, enchytraeids were exposed for different time periods (two days and three weeks) to different soils (OECD artificial soil, different compositions in its organic matter, clay or pH value, and LUFA 2.2 natural soil) and different chemicals (Phenmedipham and copper). The main question addressed in the present study was if the effects of chemicals and different soil properties are preceded by alterations at the sub-cellular level, and if these endpoints may be used reliantly as faster screening tools for the assessment of different stress conditions in soils. The parameters measured in *E. albidus* whole body were: lipid peroxidation (LPO), total glutathione (TG), as well as the enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione *S*-transferase (GST). The results showed that biomarker responses in *E. albidus* were significantly affected by the soil type (GST, CAT, GPx, GR and LPO) and the duration of exposure in OECD artificial soil (GST, GPx, GR, CAT and LPO) but not in LUFA 2.2 natural soil. For the abiotic factors studied, after 2 days, low pH decreased significantly the TG levels and the activities of CAT and GR,and low OM also significantly decreased CAT and GR activities. After 3 weeks, differences in soil properties caused a decrease in GR and GPx activities, whereas increased GST activity was observed due to low organic matter and pH. Copper significantly increased the activities of CAT, GPx and GR, and decreased the activity of GST after 2 days as well as inscreasing LPO levels after 3 weeks. Phenmedipham increased LPO levels, associated with increased levels of TG as well as increased activities of CAT and GPx and decreased GST activity after 3 weeks exposure. This study shows that both abiotic and chemical stresses could be followed through biomarker analysis and that some of these determinations are potential endpoints in a quick soil contamination assessment procedure.

1. Introduction

Enchytraeids are part of the saprophagous mesofauna of the litter layer and the upper mineral soil, and contribute to vital processes of this environmental compartment. These terrestrial organisms directly improve the pore structure of the soil and are indirectly involved in the regulation of the organic matter degradation (Amorim et al., 2005a). They have been used in ecotoxicology and Environmental Risk Assessment (ERA) to assess the effects of single chemicals or to evaluate soil quality (ISO, 2003; Jänsch et al., 2005). The biological endpoints often used, survival and reproduction, are consistent and important to predict threshold values for policy makers and to screen polluted soils for toxicity. However, these methods are time consuming and may underestimate effects occurring at the molecular and sub-cellular levels which may have impacts later in time, if they are important enough to decrease vital processes (e.g. survival, growth, reproduction, defences against chemical insults) in a sufficient number of individuals.

Over the past few years, increasing emphasis has been placed on the use of biomarkers as early-warning tools for monitoring both environmental quality and the fitness of organisms inhabiting contaminated ecosystems (Stegeman et al., 1992).

The interest in biomarkers gave rise to studies on antioxidant defences (Di Giulio et al., 1989; Winston and Di Giulio, 1991) that play a crucial role in cell homeostasis avoiding DNA damage, enzymatic inactivation and peroxidation of cell constituents due to increased reactive oxygen species (ROS) production (Halliwell and Gutteridge, 1999). When xenobiotics cause physiological responses to deviate beyond typical

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doi:10.1016/j.envint.2008.08.004

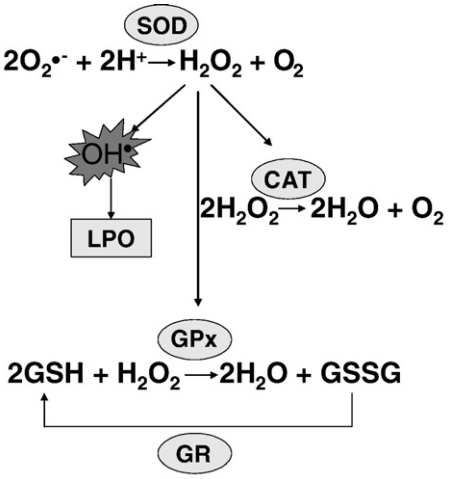

Fig. 1. Response to an oxidative stress challenge: the superoxide anion ($O2^{\bullet}-$) is metabolised by SOD into molecular oxygen and hydrogen peroxide (H_2O_2), which is then deactivated by CAT; H_2O_2 and organic hydroperoxides are also metabolised by GPx in the presence of GSH; GSH is then regenerated by GR; GST conjugates electrophilic compounds with reduced glutathione; H_2O_2 can also give rise to the hydroxyl radical ($OH^{\bullet}$) that leads to lipid peroxidantion (LPO).

ranges, then individual fitness may be impaired (Calow, 1991; Calow and Forbes, 1998). Biomarkers that measure toxic effects at the sub-cellular level have been shown to provide rapid quantitative predictions of a toxic effect upon individuals in laboratory studies. Therefore, they should be applied as complementary tools to demonstrate possible links between sublethal biochemical alterations and ecologically relevant effects in natural populations inhabiting contaminated ecosystems. To correlate the alterations on individuals to adverse effects at higher levels of biological organization, the biomarker response should be associated with the impairment of growth, reproductive output or metabolic function that directly affects the organism (Depledge and Fossi, 1994).

Generally, biomarker responses are considered to be intermediates between pollutant sources and higher level effects (Suter, 1990). When these compensatory responses are activated, the survival potential of the organism may already have begun to decline because the ability of the organism to mount compensatory responses to new environmental challenges may have been compromised (Depledge and Fossi, 1994). In the normal healthy cell, reactive oxygen species (ROS) and prooxidant products are detoxified by antioxidant defences that comprise water and lipid soluble low molecular weight free radical scavengers (like GSH) and specific antioxidant enzymes (Halliwell and Gutteridge, 1999). The balance between prooxidant endogenous and exogenous factors and antioxidant defences in biological systems can be used to assess toxic effects under stressful environmental conditions. Marked increases in ROS production (either directly, or indirectly) can overcome antioxidant defences, resulting in increased oxidative damage to macromolecules and alterations in critical cellular processes, which is designated oxidative stress. Oxidant-mediated effects with a potential suitability as biomarkers include either adaptive responses, such as increased activities of antioxidant enzymes and concentrations of non-enzymatic compounds, or manifestations of oxidant-mediated toxicity such as oxidations of proteins, lipids and nucleic acids, as well as perturbed tissue redox status (Winston and Di Giulio, 1991; Filho, 1996). The response to an oxidative stress challenge is depicted in Fig. 1.

Establishing the linkage between biomarkers and higher level effects, by choosing the appropriate useful biomarker for discriminating different stresses that are relevant for risk assessment is now the goal of this line of research. Recently, some studies have shown that some biomarkers (e.g. acetylcholinesterase, lactate dehydrogenase, glutathione redox status, lipid peroxides) are in fact related with ecological relevant parameters (Castro et al., 2004; Moreira-Santos et al., 2005; Moreira et al., 2006; Stanek et al., 2006).

The choice of an appropriate biomarker or groups of biomarkers for biomonitoring purposes requires knowledge of a variety of factors that influence the biomarker response (Mayer et al., 1992; Peakall and Shugart, 1993). A number of biotic and abiotic factors can influence the extrapolation of individual biomarkers to the field monitoring of contaminant effects at population and community levels (Adams, 1990; Lagadic et al., 1994). Therefore, the main goal of the present research study was to investigate the effects of different stress conditions (natural versus artificial soils; different soil properties and 2 chemical compounds) on several biomarkers indicative of prooxidant/antioxidant status of *Enchytraeus albidus*. The selection of the two chemicals, copper and Phenmedipham, was due to their different properties and due to previous knowledge of effects at population level (Amorim et al., 2005a, b,c, 2008b). Copper is a redox cycling metal able to produce ROS, present in soils worldwide and phenmedipham is an organic substance and a commonly used herbicide. Known effects of these chemicals on *E. albidus,* include avoidance behaviour, survival and reproduction (copper chloride: LC_{50}>320 mg/kg, AC_{50}=132.6 mg/kg, EC_{50}=97 mg/kg; Phenmedipham: AC_{50}=50.7 mg/kg, LC_{50}=50 mg/kg and EC_{50}=29.4 mg/kg). Additionally, the effect of exposure time on the biomarker response was also investigated, since these responses may have a transient temporal feature while others can persist for weeks or months with a continued exposure of the organisms.

2. Materials and methods

2.1. Test organism

The test species used here was the enchytraeid *E. albidus* (Henle, 1837), one of the largest species of the genus *Enchytraeus* (adults reach 15–40 mm). This species was maintained in laboratory cultures, being bred in moist soil (50% OECD soil, 50% natural garden soil), at 18 °C with a photoperiod of 16:8 h (light:dark), and fed once a week with finely ground and autoclaved rolled oats (Cimarrom, Portugal). Details of the culturing process are given in Römbke and Möser, (2002).

2.2. Test soils

Two standard soils were used: natural LUFA 2.2 soil, from Speyer, Germany (Løkke and van Gestel, 1998) and OECD artificial soil (OECD, 1984). Different OECD artificial soils were produced, manipulating its composition in terms of percentage of clay, sand, peat content and pH value. The main characteristics of the test soils used are presented in Table 1.

2.3. Test chemicals

Phenmedipham, an herbicide, was applied as the formulation Betosyp formerly known as Betanal (STÄHLER AGROCHEMIE, 157 g/L a.i.) to LUFA 2.2 soil in the following nominal concentrations: 10 and 32 mg a.i./Kg soil DW (Dry Weight). Copper chloride (di)hydrated ($CuCl_2{\cdot}2H_2O$; purity, 99%; molecular weight 170.48 g/mol; Merck, Darmstadt, Germany), was added as an aqueous solution to the soil to give a final concentration of 320 mg/kg DW. The contamination of all test substrates was done by mixing the aqueous solution of the test chemical into the pre-moistened LUFA 2.2 soil. After homogenous mixing, sub-samples of soil were introduced into the individual test vessels. In the case of the metal, the soil was allowed to equilibrate three days before the start of the test as recommended by McLaughlin et al. (2002). All concentrations are given as active ingredient (a.i.) per kg soil (dry weight).

Test concentrations were selected based on previous results (Amorim et al., 2005a, b,c, 2008b) and the criterion was to select a concentration where chronic effect (reproduction, within the EC_{50} range) was observed, in order to link biomarkers with higher level effects.

2.4. Test procedure

Fifteen adult worms with well-developed clitellum were selected and introduced in a glass vessel (covered afterwards with a parafilm layer with a few holes for airing),

Table 1
Main characteristics of the test soils, showing approximate values for: particle size distribution (sand, clay, silt), organic matter content (OM), pH and Water Holding Capacity (WHC)

Soil Type	Sand (%)	Clay (%)	Silt (%)	OM (%)	pH ($CaCl_2$ 0.01 M)	WHC
LUFA 2.2.	79	13	8	2.36	5.6	48
OECD St.	70	20	–	10	6.3	80
OECD-clay	20	70	–	10	6.4	107.5
OECD-pH	70	20	–	10	5	59
OECD-OM	72.5	22.5	–	5	6.8	47.5