Environmental Pollution 234 (2018) 804–811

Contents lists available at ScienceDirect

Environmental Pollution

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

Integration of behavioral tests and biochemical biomarkers of terrestrial isopod *Porcellio scaber* (Isopoda, Crustacea) is a promising methodology for testing environmental safety of chars☆

Suzana Madžarić [a, b], Monika Kos [c], Damjana Drobne [c], Matej Hočevar [d], Anita Jemec Kokalj [c, *]

[a] CIHEAM - Mediterranean Agronomic Institute of Bari (MAIB), Via Ceglie 9, 70010, Valenzano, BA, Italy
[b] Department of Environmental Science, Parthenope University, Centro Direzionale, Isola C4, 80143 Napoli, Italy
[c] University of Ljubljana, Biotechnical Faculty, Večna Pot 111, 1000 Ljubljana, Slovenia
[d] Institute of Metals and Technology, Lepi Pot 11, 1000 Ljubljana, Slovenia

A R T I C L E I N F O

Article history:
Received 8 September 2017
Received in revised form
16 November 2017
Accepted 7 December 2017

Keywords:
Biochar
Hydrochar
Terrestrial isopods
Avoidance behavior
Acetylcholinesterase
Glutathione S-transferase

A B S T R A C T

We investigated how different carbonized materials, or chars (olive mill pomace biochar (BC) and urban greens pruning residues hydrochar (HC)) affect an important member of soil fauna, the terrestrial isopod *Porcellio scaber*. The selection behavior of isopods towards chars after a 48 h exposure was studied in (i) soil-char amended tests with single and multiple choices, and (ii) tests with chars offered as pure material. Finally, we exposed the isopods to char-amended soils for a period of 14 days to follow the effect on food consumption, body mass and activities of enzymes that are commonly altered upon stressor exposure (acetylcholinesterase, AChE, and glutathione S-transferase, GST). We showed that isopods are able to select between char amended and un-amended soil and different forms of char amendments: a clear preference for BC, and avoidance of HC were evidenced. The preferences remained the same when the chars were sterilized leading to the conclusion that initial microorganism composition was not the reason for selection, but selection was governed by other chars' physico-chemical properties. It remains to be elucidated which of these properties were the dominant reason for the selection. We also showed that isopods intentionally use BC as food at a similar rate to alder leaves. Medium-term exposure to HC resulted in adverse effects on isopods because it led to reduced feeding and growth, in addition to increasing GST activity, although no alterations in AChE activity were found. We suggest that behavioral tests with *P. scaber* could be used as a fast, reliable and economically feasible screening method for determining the safety of chars for the soil environment. Results represent significant contribution in the field of char toxicity testing, highlighting the importance of tests with isopods as important members of soil meso fauna, with the aim of influencing environmental policies and quality standards.

1. Introduction

The impact of adding pyrolyzed organic carbon (biochar) to agricultural soils has been widely studied with respect to soil quality, crop yields and carbon sequestration (Lehmann, 2007; Lehmann et al., 2011; Luo et al., 2011), while in recent years hydrochar resulting from hydrothermal carbonization process has been receiving increasing attention. Biochar is product of pyrolysis, where carbon-based biomass (e.g. agricultural residues) is heated (300–700 °C) in the absence of oxygen. This results in a wide range of carbon rich products, mostly depending from the type of feedstock used (Lehmann and Joseph, 2009; Verheijen et al., 2010). By contrast, hydrochar is obtained by hydrothermal carbonization where biomass is heated in the presence of liquid water at lower temperatures (up to 220 °C) under high pressure. Also the characteristics of hydrochar depend on the type of feedstock (Libra et al., 2011). Hydrochar is generally characterized by a lower pH compared to biochar, smaller surface area and it degrades faster in the soil due to the higher presence of functional groups caused by the less intensive carbonization process (Schimmelpfennig and

☆ This paper has been recommended for acceptance by B. Nowack.

* Corresponding author.
E-mail address: anita.jemec@bf.uni-lj.si (A. Jemec Kokalj).

https://doi.org/10.1016/j.envpol.2017.12.024

Glaser, 2012).

A very important consideration prior to the wide application of chars in agriculture is how they affect the natural state of the soil, with the aim of minimizing the impact on soil biodiversity and function (McCormack et al., 2013). In agroecosystems, soil fauna contribute significantly to soil functioning and provision of ecosystem services and they are the key drivers determining the decomposition of organic materials (Scheunemann et al., 2015). Thus, soil fauna can contribute to the stability or decomposition of carbonized materials amended into the soil, enhance incorporation and movement of chars through the soil profile and perform top-down control effects on organisms lower in the soil food web (Lehmann et al., 2011; Ameloot et al., 2013). The understanding of soil fauna – char interaction is needed for assessment of the possible negative effects of chars on soil fauna and, conversely, the possible role of soil fauna on the fate of chars in the soil.

It has been previously shown that biochar and hydrochar affect terrestrial invertebrates. For example, biochar affected the growth and survival, as well as the soil selection behavior and mass change of earthworms *Eisenia fetida* (Liesch et al., 2010; Li et al., 2011) and the soil selection behavior of earthworms *Aporrectodea caliginosa* (Tammeorg et al., 2014). Inhibitory effects on survival and reproduction of *Folsomia candida* (Collembola) and *Enchytreus crypticus* (Enchytraeidae) were associated with biochars that had a high pH and high carbonate content (Marks et al., 2014). Hydrochar had a negative effect on the abundance of collembola *Protaphorura fimata* (Reibe et al., 2015) and affected the behavior of earthworms *E. foetida* and *E. andrei* (Busch et al., 2011).

To our knowledge, no effects on terrestrial crustaceans have been reported yet. Terrestrial isopods (Crustacea: Isopoda) significantly contribute to organic matter decomposition and nutrient recycling in the soil. Despite these important roles, as yet they have not received enough attention regarding the possible effects of char application in the soil. Isopods are generally considered to be beneficial since they enhance nutrient cycling by grinding of organic material and transporting them to moister soil microsites (Paoletti and Hassall, 1999). Their fecal pellets affect soil structure and they represent “hot spots” of microbial activity (Snyder and Hendrix, 2008).

Terrestrial isopods have been successfully used in toxicity testing with many different types of chemicals and substances, from metals (Drobne, 1997), pesticides (Drobne et al., 2008) to nanomaterials (Novak et al., 2012). One of the commonly used tests with isopods is the soil selection behavior (avoidance) test (Loureiro et al., 2005; Žižek and Zidar, 2013; Škarková et al., 2016). As pointed out by Domene et al. (2015a), the soil selection test allows detection of a preference or avoidance behavior for agricultural practices as the addition of chars (i.e. it can be driver for char feedstock selection or tailored char production). Growth and food consumption are the most widely used endpoints of isopod responses to environmental contaminants (Drobne and Hopkin, 1994). Therefore these responses were also selected in our study, along with some other stress exposure biomarkers that are considered very sensitive markers of exposure to stress.

Biochemical biomarkers, such as the enzyme activities of acetylcholinesterase (AChE) and glutathione S-transferase (GST), have also proven to be valuable tools for evaluating the exposure and effects of contaminants on isopods in combination with whole organism responses (Jemec et al., 2010). AChE is a serine hydrolase having a primary physiological role in hydrolyzation of the neurotransmitter acetylcholine in cholinergic synapses. It is crucial for the functioning of nervous system and it is the target for some organophosphorus and carbamate pesticides (Frasco et al., 2006; Nunes, 2011). Additionally, a number of AChE physiological roles not related to the regulation of neurotransmission have been reported in invertebrates, such as in fertilization, embryogenesis (Cariello et al., 1986), tissue regeneration (Lenique and Feral, 1976; Fossati et al., 2015), brood rearing (Kim et al., 2017), response to stress (Lehtonen et al., 2006), and xenobiotic defense (Kang et al., 2011; Kim et al., 2012). Glutathione S-transferases presents a group of multifunctional enzymes participating in cellular detoxification of exogenous chemicals, endogenous toxic metabolites and lipid hydroperoxides formed due to reactive oxygen species generation (Halliwell and Gutteridge, 2007). The increase of GST activity indicates that cellular processes related to detoxification took place. Therefore this enzyme activity is frequently used as a measure of stressor exposure in environmental studies (Elumalai et al., 2002).

The overall aim of the present study was to implement a new combination of laboratory bioassays to assess chars effect on isopods *P. scaber*. Two exemplary chars were used: olive mill pomace biochar (BC), and urban greens pruning residues hydrochar (HC). We first investigated the effect of short-term exposure (48 h) on the selection behavior of isopods towards different types of chars, either amended with soil or offered as pure material. Furthermore, we assessed the chars effects after 14 days exposure on isopod food consumption, animal mass change, and the activities of AChE and GST. Considering functional aspects, avoidance behavior test gives us sound base to discuss whether or not certain habitat is going to be colonized by test organism or not. On the other hand, long-term exposure provides information whether the colonization of such environment would result in adverse effects for isopods.

2. Materials and methods

2.1. Chemicals

The following chemicals were used in experiments (being of the highest commercially available grade, 99% or higher): Triton X-100, dibasic and monobasic potassium phosphate, sodium hydrogencarbonate (NaHCO3), 5,5′ dithiobis-2-nitrobenzoic acid (DTNB), acetylthiocholine chloride (ACh-Cl), 0,1-chloro-2,4-dinitrobenzene (CDNB), L-glutathione (reduced form, GSH), bovine serum albumine analytical standard (BSA) (Sigma - Aldrich, Germany) and BCA Protein Assay Reagent A and BCA Protein Assay Reagent B (Pierce, U.S.A.).

2.2. Test organisms

Isopods (*Porcellio scaber*) used in experiments originated from unpolluted site in Lukovica, Slovenia. Rearing was conducted at University of Ljubljana (Department of Biology) in laboratory climate chamber (22 ± 1 °C, 80% relative air humidity, with a 16/8 h light/dark period). Their diet consisted of fallen leaves from different trees, potatoes and carrots. Test organisms were acclimatized in the laboratory for at least a month before the experiments. Adult individuals of both sexes were used for testing (30–60 mg body weight, with intact antennae). Molting individuals and gravid females were not used in the experiments.

2.3. Soil and chars

Standardized, non-sterile natural soil Lufa 2.2 (LUFA Speyer, Germany) was used for the testing, with following characteristics: loamy-sand texture, clay content of 7.2 ± 1.2%, organic matter 1.77 ± 0.2%, pH 5.5 ± 0.2 (in 0.01 M $CaCl_2$), Cation Exchange Capacity 10.1 ± 0.5 meq/100 g and water holding capacity (WHC) 41.8 ± 3.0 g (H_2O/100 g dry soil). Biochar (BC) was produced from air-dried olive mill pomace (olive mill waste) using an experimental reactor at the Mediterranean Agronomic Institute of Bari