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Effects of food salinization on terrestrial crustaceans *Porcellio scaber*

Pavlína Škarková[a], Monika Kos[b], Damjana Drobne[b], Milada Vávrová[a], Anita Jemec[b,*]

[a] Brno University of Technology, Faculty of Chemistry, Purkyňova 118, 612 00 Brno, Czech Republic
[b] University of Ljubljana, Biotechnical Faculty, Department of Biology, Večna pot 111, 1000 Ljubljana, Slovenia

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ABSTRACT

Salinization of soil as the main cause for soil degradation is a global issue. Despite this, the data concerning the effects of different salts on terrestrial organisms are still missing. Our aim was to address the effects of four different salts (NaCl, KCl, $NaNO_3$, and KNO_3) on terrestrial isopods *Porcellio scaber.* Two types of experiments were performed. In the first experiment, the isopods were exposed to 1, 2, and 5 g salt/kg dry leaf for 14 days and afterwards animal mortality, feeding activity, moult and growth were monitored. In the second experiment, a 48-h soil selection tests was done to investigate their preference/avoidance to certain salts. The feeding rate and growth efficiency of isopods decreased in a dose-dependent manner after 14 days exposure to KNO_3 and $NaNO_3$. We suggest that nitrate (NO_3^-) may be the predominant reason for these effects, because (i) the effects were only observed in the case of NO_3^--salts, and (ii) K^+ and Na^+ induced no effects in the case of Cl^--salts where the concentrations of these two cations were even higher as in the case of NO_3^- salts. 48-h soil selection test showed that isopods were able to choose between NaCl dosed and unamended soil: the isopods preferred to spend time on NaCl spiked soil (up to 5 g salt/kg dry soil). In the case of KNO_3 and $NaNO_3$ no significant soil selection by isopods was found. In these two exposures isopods spent approximately 50% of their visits on both sides of the soil. The survival of isopods decreased in a dose independent manner when exposed to $NaNO_3$, KNO_3, and KCl. We conclude that $NaNO_3$, KCl, and KNO_3 affect the isopods, while no effects were caused by NaCl under the exposure conditions of this study. This study implies that also other salts besides commonly investigated NaCl should be tested when the potential hazard of soil salinization to terrestrial organisms is in question.

1. Introduction

Salinization is the process that leads to an excessive increase of water-soluble salts in the soil (Várallyay et al., 2006). Soil salinization is a global problem and has been recognised as one of the major reasons for soil degradation (Tóth et al., 2008; Com., 2012; Environment Canada, 2015; USDA, 2015). This phenomenon is either a result of erosion of parental material and natural processes or is induced by anthropogenic activities, such as the salting of roads during winter in temperate and subarctic regions (Environment Canada, 2015), the use of fertilizers, excessive irrigations, (Tóth et al., 2008) or the use of snow additives for the production of artificial snow for winter tourism (Rixen et al., 2003). Besides sodium chloride (NaCl), the snow additives also contain calcium chloride ($CaCl_2$), potassium chloride (KCl), urea, magnesium chloride, sodium acetate, calcium magnesium acetate, ammonium nitrate and ammonium sulfate (Peters, 2006). Potassium and sodium nitrate (KNO_3, and $NaNO_3$) are common composites of fertilizers (Haifa, 2014).

An excessive amount of salts in the soil has been shown to affect terrestrial organisms, such as earthworms (Owojori and Reinecke, 2009, 2014; Owojori et al., 2008, 2009a,b; Guzyte et al., 2011), enchytraeids (Owojori et al., 2009b), collembolans (Owojori et al., 2009a) and plants (Rixen et al., 2003; Hu and Schmidhalter, 2005). Most of the studies conducted up until now have focused on soil contaminated with NaCl or KCl or natural saline soil (Owojori et al., 2009a). Most commonly observed effects of NaCl on earthworms (*Eisenia fetida*) were decreased survival (28 days LC_{50} = 5.436 g NaCl/kg soil and 5.623 g NaCl/kg soil), decreased growth (28 days EC_{50} in range from 2.512–4.985 g NaCl/kg soil) and decreased cocoon production (28 days EC_{50} in range from 1.00–2.02 g NaCl/kg soil) (Owojori et al., 2008; Guzyte et al., 2011). In a recent study, Owojori and Reinecke (2014) showed that besides NaCl, other salts like $NaNO_3$, Na_2HPO_4, Na_2SO_4, KCl, $CaCl_2$, and $MgCl_2$ can cause saline-induced effects on earthworms. Among these the most toxic

* Corresponding author.
E-mail addresses: xcskarkova@fch.vutbr.cz (P. Škarková), monika.kos@bf.uni-lj.si (M. Kos), damjana.drobne@bf.uni-lj.si (D. Drobne), vavrova@fch.vutbr.cz (M. Vávrová), anita.jemec@bf.uni-lj.si (A. Jemec).

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to earthworms *E. fetida* was $NaNO_3$ (28 days LC_{50} 5.354 g/kg soil), while NaCl was among the least toxic (28 days LC_{50} = 6.428 g/kg soil) (Owojori and Reinecke, 2014).

Other relevant terrestrial invertebrates potentially exposed to salt-affected areas are isopods such as the species *Porcellio scaber* Latreille, 1804 (Isopoda, Oniscidea, Porcellionidae), which live predominately in forests and grasslands of temperate climates, but are also very common in urban and agricultural areas (Harding and Sutton, 1985). They have been recognized as a suitable ecotoxicity test organism because their biology and physiology is well-known, it is relatively easy to maintain them as a laboratory culture and handle individual animals in the experiment and, above all, it is possible to obtain individual toxicity data at different levels of biological complexity (Hassall et al., 2005). These organisms have been used successfully in toxicity testing on a variety of organic pollutants (Drobne et al., 2008) and inorganic pollutants, such as metals (Drobne and Hopkin, 1995; Zidar et al., 2003) and nanomaterials (Drobne et al., 2009). Generally, a decreased feeding rate of isopods denote an adverse effect of the test chemical on the isopods (Drobne and Hopkin, 1995). At the moment there is a lack of knowledge regarding the effects of salinity on these organisms.

The aim of the present study was to assess the effects of four commonly used salts in agriculture ($NaNO_3$ and KNO_3) and in ice-melting compounds (NaCl, KCl) on terrestrial isopods *P. scaber*. NaCl is also the predominant salt in most saline environments (Owojori et al., 2008). Isopod feeding behaviour, moult and mortality were monitored. A 48-h soil selection tests was done to investigate their selection of certain salts. The prime goal was to determine the differences in adverse effects caused by different salts.

2. Materials and methods

2.1. Test chemicals

Four analytical grade salts, namely NaCl, $NaNO_3$, KCl, and KNO_3, were purchased from Sigma–Aldrich (Germany). The concentrations tested in this paper (1, 2 and 5 g salt/kg dry leaf or dry soil) were selected based on previous work done by other researchers in this field (Owojori and Reinecke, 2009). These authors exposed the earthworms to a concentration range 1–4 g NaCl/kg dry soil corresponding to electrical conductivity (EC) in range from 0.12 to 1.31 dS/m. These exposures already induced adverse effects on earthworms. The EC of the soil in our case (soil choice experiments) was in the same range (0.32–1.87 dS/m, Table 1). Furthermore, these EC values are in range of those reported for natural saline soils (0.08–1.62 dS/m) (Owojori and Reinecke, 2010).

2.2. Test species

Isopods *P. scaber* originated from the synchronized laboratory culture at the Department of Biology, University of Ljubljana (Večna pot 111, Ljubljana). Cultures of *P. scaber* were derived from individuals collected from an unpolluted site in Polhov Gradec, Slovenia (46°3′0″N, 14°18′0″E). Animals were kept in a climate chamber at 22 ± 1 °C with a 16/8 h light/dark period (120 and 16 lx, respectively; measured using LI-1000 Data Logger, LI-COR, Nebraska, USA), caged in glass containers with moist loamy sand and peat at the bottom. They were fed fallen leaves from various trees, with periodical additions of potatoes, fresh vegetables, and apples. Only adult animals (30–60 mg fresh body mass) of both sexes were used for the test. Particular attention was paid to include only the specimens with intact antennae. Moulting individuals and gravid females were excluded (Zidar et al., 1998).

2.3. Experimental design for 14-days feeding exposure

2.3.1. Food preparation

Partially decomposed hazel leaves (*Corylus avellana*) were collected in the Karavanke region, Slovenia (46°21′32.29″N, 14°16′36.12″E), for the purpose of the experiment. Leaves were air dried at room temperature (24 ± 1 °C) and stored in a cardboard box until use. Bigger leaves with minimally damaged leaf lamina were straightened and the serrated leaf edge was cut off. Leaf lamina were cut into pieces of 120 ± 20 mg. NaCl, $NaNO_3$, KCl, and KNO_3 were individually dissolved in bideionized water (MilliQ, Millipore, Billerica, Massachusetts, USA [pH 5.7, ρ = 18.5 MΩcm]), to obtain nominal concentrations of 1, 2, and 5 g salt/kg dry leaf. The nominal mass concentrations were also converted to molarity for a better comparison to previously published data (Supplementary information Table S1). For 100 mg of leaf, 100 μL of deionised water (control) or freshly prepared salt solution was applied to the bottom side of the leaf and spread over the surface as evenly as possible, using a paintbrush. After application, leaves were allowed

Table 1
Electrical conductivity and pH measured in the test soils at the beginning (day 0) of the soil selection test with *Porcellio scaber* (EC, electrical conductivity) (*n* of independent measurements = 3).

Test groups	Mass concentration (g/kg)	Molar concentration (mM)	Mean EC ± SD (dS/m)	Mean pH ± SD
Control	0	0	0.10 ± 0.00	6.18 ± 0.10
NaCl	1	17.11	0.46 ± 0.00	5.86 ± 0.00
	2	34.22	0.81 ± 0.03	5.77 ± 0.02
	5	85.55	1.87 ± 0.05	5.71 ± 0.01
KCl	1	13.41	0.43 ± 0.01	5.79 ± 0.03
	2	26.83	0.74 ± 0.01	5.71 ± 0.01
	5	67.07	1.67 ± 0.08	5.64 ± 0.01
$NaNO_3$	1	11.77	0.35 ± 0.01	5.95 ± 0.07
	2	23.53	0.60 ± 0.01	5.78 ± 0.01
	5	58.83	1.25 ± 0.03	5.73 ± 0.01
KNO_3	1	9.89	0.32 ± 0.01	5.80 ± 0.01
	2	19.78	0.55 ± 0.02	5.72 ± 0.03
	5	49.45	1.18 ± 0.04	5.69 ± 0.01