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Effects of NaCl and seawater induced salinity on survival and reproduction of three soil invertebrate species

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HIGHLIGHTS

- The effects of salinisation on soil ecosystems due to sea level rise were assessed.
- Soil invertebrates were exposed to NaCl and seawater in OECD soil.
- Increased sensitivity observed: *H. aculeifer* ≪ *E. crypticus* ≈ *F. candida*.
- Soil invertebrate sensitivity to NaCl depends from the exposure pathway.
- Adverse effects were found for soil conductivity values below the limit defined for saline soils.

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ABSTRACT

The increase of global mean temperature is raising serious concerns worldwide due to its potential negative effects such as droughts and melting of glaciers and ice caps leading to sea level rise. Expected impacts on soil compartment include floodings, seawater intrusions and use of saltwater for irrigation, with unknown effects on soil ecosystems and their inhabitants. The present study aimed at evaluating the effects of salinisation on soil ecosystems due to sea level rise. The reproduction and mortality of three standard soil invertebrate species (*Folsomia candida*, *Enchytraeus crypticus*, *Hypoaspis aculeifer*) in standard artificial OECD soil spiked with serial dilutions of seawater/gradient of NaCl were evaluated according to standard guidelines. An increased sensitivity was observed in the following order: *H. aculeifer* ≪ *E. crypticus* ≈ *F. candida* consistent with the different exposure pathways: springtails and enchytraeids are exposed by ingestion and contact while mites are mainly exposed by ingestion due to a continuous and thick exoskeleton. Although small differences were observed in the calculated effect electrical conductivity values, seawater and NaCl induced the same overall effects (with a difference in the enchytraeid tests where a higher sensitivity was found in relation to NaCl). The adverse effects described in the present study are observed on soils not considered saline. Therefore, the actual limit to define saline soils (4000 μS cm^{-1}) does not reflect the existing knowledge when considering soil fauna.

1. Introduction

Over the past years, the increase of global mean temperatures is causing the decrease of the snow cover and of the ice stocks. These events have originated a rising of the sea level (IPCC, 2007a) from an increase of 1.5 to 1.7 mm $year^{-1}$, observed in the last century, to an increase of 3 mm $year^{-1}$ in the last decade (IPCC, 2013). The IPCC forecast for this phenomenon is between 40 and 62 cm until 2100 (IPCC, 2013) enhancing the risk of drought and flooding events (IPCC, 2007b). At a global scale, the most affected regions will be the arid and semi-arid parts of Australia, South America, Asia and Europe (European Soil Portal, 2012). In Europe, countries near the Mediterranean and Caspian Seas have been the most affected with an increase of 1 million hectares of saline soils in 2002 (Commission of The European Communities, 2002) to an estimated 3 million hectares in 2012 (European Commission, 2012). In Spain, about 3% of the 3.5 million hectares of irrigated land is affected by soil salinisation limiting the local agriculture (Van-Camp et al., 2004). Along with Spain, France, Greece, Italy and Portugal (among others) have extensions of 250, 3.5, 450, 25 thousands hectares of saline soils, respectively (Eckelmann et al., 2006). The rise of sea level will lead to soil salinisation mainly due to seawater (constituted by free ions of sodium – 31% – and chloride – 55%; Wiesenburg and Little,

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1987–1988) intrusions (IPCC, 2007b) and irrigation with saltwater (Wang and Li, 2012) since the freshwater availability and quality will be reduced (IPCC, 2007b). Besides being induced by global climate change, soil salinisation can also occur due to land irrigation with saltwater where a high evapotranspiration, higher temperatures and/or dry climate are found (leading to an increase of salt concentration in water and also the deposition of those salts on soils; IPCC, 2007b). The accumulation of soluble salts on soils negatively affects their fertility (European Soil Portal, 2012). Crop yields can be further affected due to the increase of pest species and/or diseases, risk of disappearance of less resistant species to salinity, with potential loss of biodiversity (IPCC, 2007b). Soil salinisation can occur through natural processes like the increased evapotranspiration (primary salinisation) and/or induced by human activities like the increase withdraws from aquifers (secondary salinisation) (European Soil Portal, 2012). The classification of saline soils depends not only on the amounts of salts dissolved, but also on the pH and the exchangeable sodium percentage (ESP). Soils with a pH lower than 8.5 and ESP lower than 15% are considered saline when the electrical conductivity (EC) is equal or higher than 4000 $\mu S\ cm^{-1}$ (Micheli et al., 2002).

Terrestrial and aquatic communities are differently affected by salinisation but all suffer a change on their species abundance and diversity with a dominance of salt-tolerant species (Davis et al., 2003; Andronov et al., 2012). Despite this, some species are known to tolerate salinity due to special morphological or physiological traits. For example, the spider *Arctosa fulvolineate* and the beetle *Merizodus soledadinus* can accumulate amino acids which originate an increase in the osmolality of body fluids (Foucreau et al., 2012; Hidalgo et al., 2013), while different species of amphipods can regulate the internal concentration of salts by their release in the urine (hypo-osmotic or isosmotic urine; Morritt, 1988).

Despite the existing knowledge on the impacts of salinity on coastal ecosystems in freshwater and plant species (James et al., 2003), soil organisms and their responses toward soil salinity have been neglected so far. From the few studies conducted, the most studied soil fauna groups are earthworms and nematodes. Besides the avoidance behavior to natural saline soils by earthworms (Owojori and Reinecke, 2009) and the complex relationship between nematodes and salt (with tolerance of *Caenorhabditis elegans* to salt in the presence of food and avoidance in its absence) (Adachi et al., 2010), effects in a long-term experiment are described for earthworms. Owojori et al. (2009) exposed two earthworm species (*Eisenia fetida* and *Aporrectodea caliginosa*) to a natural saline soil with electrical conductivities (EC) between 0.08 and 1.62 dSm^{-1} and found significant effects on growth, mortality and reproduction. Both species showed a higher sensitivity when considering reproduction (with the production of cocoons only in the control). In the same study, reproduction of springtails (*Folsomia candida*) and enchytraeids (*Enchytraeus doerjesi*) were also evaluated with significant effects on reproduction found. Complete cessation of reproduction was observed for springtails at 1.62 dSm^{-1} and for enchytraeids at 1.31 dSm^{-1} and above.

Effects like those reported above can impair soil functioning due to the relevant role that soil fauna has on key ecological processes like organic matter decomposition, nutrient cycling and maintenance of soil structure (Lavelle et al., 2006). Therefore it is essential to better comprehend salinisation impacts on soil fauna, to better perceive the effects of this stressor on soil functions underlying key ecosystems services.

The present study had three main objectives: (1) to evaluate the effects of exposure to sodium chloride (NaCl) and seawater on reproduction of three standard soil test-species; (2) to evaluate the use of NaCl as a surrogate of exposure of soil organisms to seawater and (3) to derive safety levels of salinity to soil fauna. In order to fulfill these aims, standard reproduction tests using three standard soil invertebrates (the springtail *Folsomia candida*, the enchytraeid *Enchytraeus crypticus* and the mite *Hypoaspis aculeifer*) were performed using artificial OECD soil spiked with a gradient of concentrations of salt (sodium chloride) or a gradient of seawater dilutions, the latter presenting equivalent electrical conductivity values to the former.

2. Materials and Methods

2.1. Test soil and concentration range for NaCl and seawater

The artificial OECD soil, used in all assays, was prepared mixing 5% of air dried and sieved sphagnum peat, 20% of kaolin clay and 75% of quartz sand. The pH was adjusted with $CaCO_3$ to 6.0 ± 0.5 (OECD, 2008). In order to evaluate the use of sodium chloride (NaCl) as a surrogate of seawater, two sets of tests were performed. A gradient of NaCl concentrations or seawater dilutions was used as shown in Table 1. NaCl concentrations were prepared diluting a stock solution of sodium chloride (Merck KGaA, 64271 Darmstadt, Germany) in distilled water. Seawater was collected from Praia da Barra in Aveiro, Portugal, filtered through cellulose nitrate membranes (0.20 μm) and kept at 4 °C until used. The NaCl gradient was prepared using a multiplication factor of 1.37 starting from 0.5 gKg^{-1} DW and ending at 4.5 gKg^{-1} DW (Table 1). The range of concentrations was performed based on the reproduction tests performed by Owojori et al. (2009) for springtails and enchytraeids. A different concentration range was used for the mite test due to the lower sensitivity observed in this species in the range finding test performed earlier (where no effects were observed on concentrations of up to 4.5 gKg^{-1} DW of NaCl). The seawater dilutions were prepared mixing seawater with distilled water. The concentrations of NaCl and the seawater dilutions were prepared in order to obtain an equivalent range of electrical conductivity (furthermore referred as conductivity) values. In case of mite tests, only six seawater dilutions were prepared since the equivalent conductivity values of the last three NaCl concentrations were higher than the conductivity of the soil when mixed with pure seawater (Table 1).

Table 1
Range of NaCl concentrations, seawater dilutions (SW) and the corresponding measured conductivity values used in the reproduction tests with *Folsomia candida*, *Enchytraeus crypticus* and *Hypoaspis aculeifer* (NaCl – salt concentrations; Cond – measured conductivity in the solution; SW – seawater dilutions; DW – dry weight.).

Folsomia candida and *Enchytraeus crypticus*	NaCl ($g\ Kg^{-1}$ DW)	0	0.5	0.7	0.9	1.3	1.8	2.4	3.3	4.5
	Cond ($\mu S\ cm^{-1}$)	107.4	262	305	372	494	634	825	1057	1415
	SW (%)	0	5	8	10	14	19	25	33	45
	Cond ($\mu S\ cm^{-1}$)	134.5	309	388	456	615	742	988	1264	1677
Hypoaspis aculeifer	NaCl ($g\ Kg^{-1}$ DW)	0	1.6	2.6	4.1	6.6	10.5	16.8	26.8	42.9
	Cond ($\mu S\ cm^{-1}$)	108.5	629	841	1276	1963	2760	4250	6830	10130
	SW (%)	0	17	24	38	59	91	100	–	–
	Cond ($\mu S\ cm^{-1}$)	180.5	729	997	1395	1973	3040	3320	–	–

2.2. Test Organisms

All test organisms were obtained from cultures maintained in the Soil Ecology and Ecotoxicology Laboratory from the Department of Life Sciences, University of Coimbra, Portugal. Springtails and mites were fed three times per week after the change of the culture medium. Mites were fed with "cheese mites" (*Tyrophagus putrescentiae*) while springtails were fed with dry yeast (ISO-11267, 1999; OECD-226, 2008). Enchytraeids were fed once a week and were maintained in agar plates (ISO-16387, 2003). All cultures were maintained at 20 ± 2 °C with a 16:8 (light:dark) photoperiod.

2.3. Reproduction Tests

2.3.1. Springtails (F. candida)

The tests were performed according to ISO guideline (ISO-11267, 1999). Briefly, ten individuals, between 10 and 12 d old, were placed into each vessel with 30 g fresh weight of the test soil. The springtails were fed with 2 mg of dry yeast at the beginning of the test and at the 14th day. After 28 d, the content of each vessel was transferred to a larger vessel and then flooded with water and gently stirred with a spatula. A few drops of dark-blue ink were added to increase contrast of the springtails. The surface of the vessels was then photographed and the organisms counted using Image Tool software (software available at http://compdent.uthscsa.edu/dig/itdesc.html).

2.3.2. Enchytraeids (E. crypticus)

The enchytraeid reproduction test was performed according to ISO-16387 (2003). Ten individuals were placed in each vessel with 20 g dry weight of the test soil and fed once a week with 25 mg of autoclaved rolled oats. After 28 d, 5 ml of ethanol (96%) were added to the vessels and then filled with water (one centimeter above the soil level) and a few drops of Bengal red 1% were added to stain the organisms. The vessels stood for 24 h and the enchytraeids were then counted as described by Chelinho et al. (2014).

2.3.3. Mites (H. aculeifer)

Reproduction tests with mites were performed according to OECD-226 (2008) in vessels placing ten females with an age of 28–35 d in 20 g dry weight of the test soil. The mites were fed at the beginning and twice a week with a tip of a spatula of cheese mites (*Tyrophagus putrescentiae*) during 14 d. After those 14 d, the mites were extracted using a Macfadyen high-gradient extractor and counted under a stereomicroscope.

All tests described above were maintained at 20 ± 2 °C with a photoperiod of 16:8 (light:dark).

2.4. Data analysis

A one-way analysis of variance (ANOVA) was used to detect differences between NaCl or seawater treatments. Post hoc comparisons (Dunnet's test) were used to find differences between the control and the concentrations/dilutions of NaCl or seawater. The software used was Statistica 7.0 (http://www.statsoft.com/). ANOVA assumptions, normality and homoscedasticity, were checked using Shapiro-Wilk's and Levene's tests, respectively. Whenever these assumptions were not fulfilled, data were transformed (using logarithmic, square root or exponential transformations according to the type of data). Effect concentrations (EC's) causing 20 and 50% reduction in reproduction were calculated for each test species (for both NaCl and seawater tests) using non-linear regression models, according to Environment Canada (2007). The software used in all analyses was Statistica 7.0 (http://www.statsoft.com/). For comparison purposes, values are expressed in $\mu S\ cm^{-1}$.

3. Results

The validity criteria for the control defined for each test by the respective guideline were fulfilled. For springtails (ISO-11267, 1999) percentage of adult survival in the control was in average 93%, with a mean of 345 juveniles produced per test vessel and an associated coefficient of variation (CV) of 16%, for tests with NaCl. And, when using seawater, springtails showed a percentage of adult survival, average number of offspring and respective CV of 90%, 433 and 20%. For enchytraeids (ISO-16387, 2003) average number of individuals in the control and corresponding CV were 712 and 7%, respectively. Using seawater, enchytraeids showed an average number of individuals and respective CV of 519 and 29%. For mites (OECD-226, 2008) the mean female survival in the control was 96% and the mean number of juveniles produced in the control was 235, with a CV of 11% for NaCl. For seawater the values of the same parameters were 94%, 160 and 28%, respectively. Furthermore, no relevant adult mortality was observed in any of the concentrations tested on the six tests for springtails and mites (NaCl: average adult survival of 91% and 99% for springtails and mites, respectively; seawater: average adult survival 93% and 91% for springtails and mites, respectively). Adult mortality was not measured on enchytraeids tests since the adults and juveniles presented a similar body size making it hard to distinguish. The last concentration of NaCl tested with mites presented a significant reduction on adult survival, but this concentration had conductivity higher than the soil mixed with pure seawater.

3.1. Effects of exposure to NaCl

The three species tested showed different responses to a 28-d exposure to NaCl (Fig. 1). Enchytraeids, at concentrations equal to or higher than 0.7 $g\ Kg^{-1}$ NaCl DW (295 $\mu S\ cm^{-1}$), showed a gradual decrease on the number of juveniles, only statistically significant at concentrations equal to or higher than 1.8 gKg^{-1} NaCl DW (616.5 $\mu S\ cm^{-1}$; One Way ANOVA, Dunnet test, $p < 0.05$; Fig. 1A). On the other hand, for *F. candida* the number of juveniles only decreased after 1.3 gKg^{-1} NaCl DW (493.5 $\mu S\ cm^{-1}$) and it was only statistically significant at concentrations higher than 3.3 gKg^{-1} NaCl DW (1091 $\mu S\ cm^{-1}$; One Way ANOVA, Dunnet test, $p < 0.05$; Fig. 1B). Mites showed similar number of juveniles in relation to the control until 10.5 gKg^{-1} NaCl DW (2740 $\mu S\ cm^{-1}$). Only in the last three concentrations (with conductivity values higher than the soil mixed with pure seawater) a statistically significant decrease on the number of juveniles was observed (One Way ANOVA, Dunnet test, $p < 0.05$; Fig. 1C).

Despite the different responses to the salt concentrations, enchytraeids and springtails showed similar sensitivities with EC_{50s} of 883.5 ± 99.2 $\mu S\ cm^{-1}$ and 986.5 ± 202.6 $\mu S\ cm^{-1}$, respectively; and EC_{20s} of 562.2 ± 133.6 $\mu S\ cm^{-1}$ and 797.7 ± 162.1 $\mu S\ cm^{-1}$, respectively. Mites were the least sensitive with an EC_{50} of 6027.6 ± 350.3 $\mu S\ cm^{-1}$ and EC_{20} of 4570.4 ± 452.8 $\mu S\ cm^{-1}$ (Table 2).

3.2. Effects of exposure to seawater

When exposed to seawater, the response along the gradient was irregular for the three tested species. Enchytraeids showed a reproduction response along the first six dilutions tested (until 25% SW; 985 $\mu S\ cm^{-1}$) similar to the control. Only at dilutions equal or higher than 33% SW (1293.5 $\mu S\ cm^{-1}$) a statistically significant decrease on the number of juveniles was observed (One Way

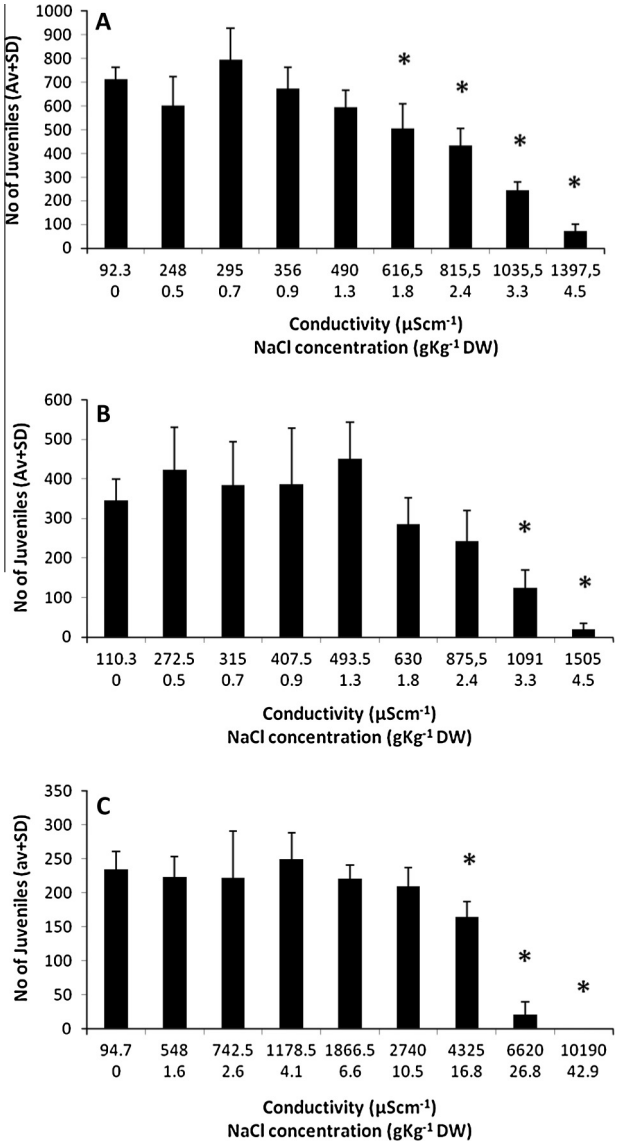

Fig. 1. Mean number of juveniles (+standard deviation) of *Enchytraeus crypticus* (A), *Folsomia candida* (B) and *Hypoaspis aculeifer* (C) when exposed to artificial soil spiked with a range of NaCl concentrations (conductivity values shown on the *x* axis are means of the conductivity values measured at the beginning and at the end of the test). *mean statistically different from control, One Way ANOVA, Dunnet test, $p < 0.05$.

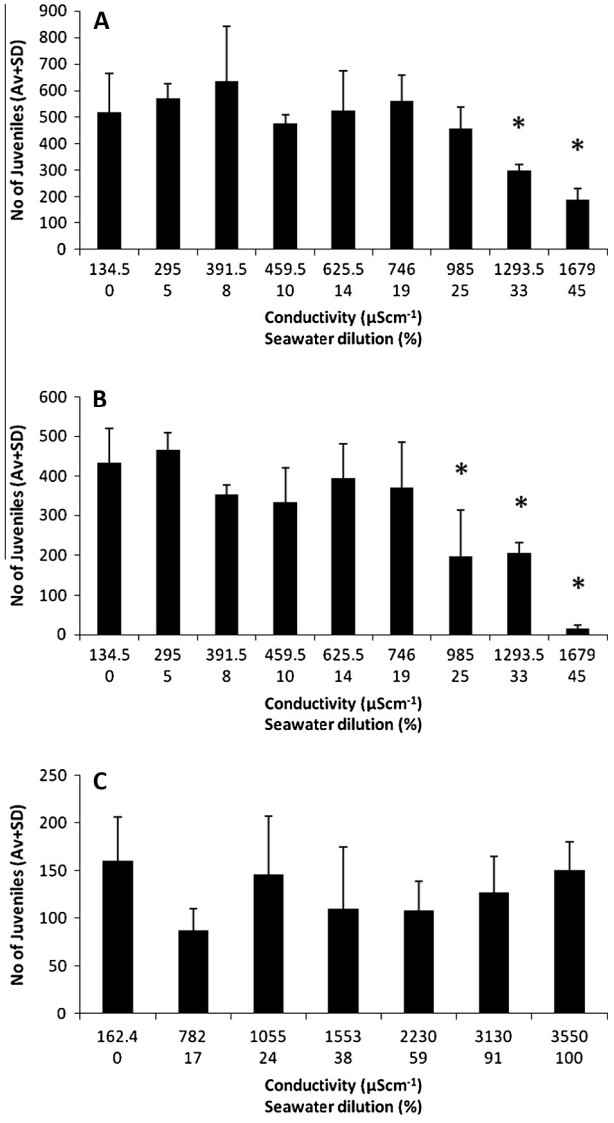

Fig. 2. Mean number of juveniles (+standard deviation) of *Enchytraeus crypticus* (A), *Folsomia candida* (B) and *Hypoaspis aculeifer* (C) when exposed to artificial soil spiked with a range of seawater dilutions (conductivity values shown on the *x* axis are means of the conductivity values measured at the beginning and at the end of the test). *mean statistically different from control, One Way ANOVA, Dunnet test, $p < 0.05$.

ANOVA, Dunnet test, $p < 0.05$; Fig. 2A). Like enchytraeids, the reproduction outputs found in springtails until 19% SW (746 µS cm^{-1}) were similar to the control. At dilutions equal or higher than 25% SW (985 µS cm^{-1}) a statistically significant decrease on the number of juveniles was observed (One Way ANOVA, Dunnet test, $p < 0.05$; Fig. 2B). Mites did not show a clear dose-response and no statistically significant effects of seawater were found (One Way ANOVA, Dunnet test, $p > 0.05$; Fig. 2C).

The toxicity parameters derived for the seawater exposure revealed larger differences between enchytraeids and springtails than those obtained for NaCl exposure (Table 2) with EC_{50s} of 1449.0 ± 322.2 µS cm^{-1} and 857.0 ± 264.4 µS cm^{-1}; and EC_{20s} of 1083.3 ± 252.3 µS cm^{-1} and 511.1 ± 318.2 µS cm^{-1} respectively for *E. crypticus* and *F. candida*.

Table 2
EC (Effect Conductivity) values calculated for the three tested species (*Folsomia candida*, *Enchytraeus crypticus* and *Hypoaspis aculeifer*) after exposure to sodium chloride and seawater in artificial soil. Data in brackets refer to upper and lower confidence limits (n.d. – not determined).

		Conductivity NaCl (µS cm^{-1})	Conductivity Seawater (µS cm^{-1})
E. crypticus	EC_{20}	562.2 (428.6–695.8)	1083.3 (828.9–1337.7)
	EC_{50}	883.5 (785.2–982.7)	1449.0 (1126.8–1771.2)
F. candida	EC_{20}	797.7 (635.6–959.8)	511.1 (192.9–829.3)
	EC_{50}	986.5 (783.9–1189.1)	857.0 (604.7–1109.2)
H. aculeifer	EC_{20}	4570.4 (4117.5–5023.2)	n.d.
	EC_{50}	6027.6 (5677.4–6377.9)	n.d.

4. Discussion

4.1. Effects of exposure to NaCl and seawater

Mites were the least sensitive organisms with EC_{50s} and EC_{20s} about six times higher than springtails and enchytraeids when exposed to NaCl (Table 2). Mites were also the least sensitive organisms, with no significant reproduction impairment in all seawater dilutions tested. Springtails and enchytraeids showed similar sensitivities when considering exposure to NaCl, while for exposure to seawater, a higher sensitivity of springtails was found (Table 2).

Terrestrial invertebrates are able to osmoregulate due to different mechanisms: (1) reduced permeability of the "skin", varying from an almost "waterproof" to a very permeable cuticle; (2) excretion done by specialized organs and gut; (3) cellular regulation; (4) uptake systems; and (5) reduction of the water loss in respiration (Willmer, 2006). The observed high tolerance of mites to salt might be due to their morphological features since in general they present a continuous exoskeleton (O'Connor, 2003) with a rigid dorsal shield in the case of the tested species *H. aculeifer* (Jänsch et al., 2005), that may provide them with a possibility of a lower exposure to substances present in soil pore water, such as salts. In fact, it has been described in other mite species that the cuticle plays an important role in osmoregulation and protection against pathogens (Cook et al., 2008). Osmoregulation in *H. aculeifer* is not well studied, but the presence of specialized organs in osmoregulation have been described in other mite species like the presence of coxal glands (Bayartogtokh and Chatterjee, 2010) and sclerotized rings in the cuticle (Witalinski et al., 2002). The regulation of the hemolymph osmolality by the production of amino acids (Foucreau et al., 2012; Hidalgo et al., 2013) and the regulation of the presence of salt in their bodies by its release in the urine (Morritt, 1988) has also been described in other organisms with a rigid exoskeleton. Examples of those are the spider *Arctosa fulvolineata* (Foucreau et al., 2012), the beetle *Merizodus soledadinus* (Hidalgo et al., 2013) and some talitroidean amphipods (Morrit, 1988). The presence of a similar or even one or more of these mechanisms in the tested species could have facilitated its survival and reproduction under saline conditions.

The similar sensitivity of springtails and enchytraeids was not expected since enchytraeids are soft-bodied organisms thus should be more exposed to salt via soil pore water, while springtails possess an exoskeleton that would offer a higher protection against harmful substances present in soil pore water (Peijnenburg et al., 2012). Although not described for the tested enchytraeid species, it is known that some annelid species (*Enchytraeus albidus* and *Heterochaeta costata*) are able to survive in saline conditions by (1) releasing an hypotonic urine and (2) due to an impermeabilisation of their membranes (Generlich and Giere, 1996), physiological avoidance mechanisms that could explain the lower sensitivity (than expected) of enchytraeids. In fact, another species of enchytraeids (*E. albidus*) has shown a high tolerance to salinity since the transport of active amino-acids and sugars is done through an ATPase-dependent Na-gradient which is activated in the presence of sodium and inactivated in its absence (Siebers and Bulnheim, 1977). The accumulation of these substances, adjusting the fluid osmolarity helps the enchytraeids to survive in saline conditions (Patrício Silva et al., 2013).

Springtails should have been more tolerant than enchytraeids, which was not observed in the present study. The fluid exchange in springtails occurs by drinking and via the ventral tube (collophore; Hopkin, 1997), and can be increased by the fact that springtails have their bodies segmented causing a higher contact with the soil pore water in the thinner part of the exoskeleton. Furthermore, their unexpected higher sensitivity can also be related with dehydration (the reduction of water uptake by the ventral tube with the increase of salinity in *Tomocerus* sp. and *Orchesella villosa* has been described; Eisenbeis, 1982).

Some data are available for the effects of soil salinisation to invertebrate species. Owojori et al (2014) also found a high tolerance of mites to sodium chloride presenting an EC_{50} of 10400 $\mu S\ cm^{-1}$. Although this value is higher than the value obtained in the present study, both show a higher tolerance of this species toward sodium chloride. Princz et al. (2012) also performed reproduction tests but using the oribatid mite *Oppia nitens*. In this case, mites were also the most tolerant species tested (in comparison with *Eisenia andrei* and *F. candida*) but this species showed an EC_{25} (2900 $\mu S\ cm^{-1}$) lower than our EC_{20} (4570 $\mu S\ cm^{-1}$). The difference in the obtained values can be due to the fact that in Princz et al. (2012), a natural salt contaminated soil was used while in the present study the tests were conducted with artificial soil OECD. Given the small differences in the EC values, in these three studies conducted, mites show a high tolerance toward salt. Owojori et al. (2009) also reports statistically significant effects on the reproduction of enchytraeids (*E. doerjesi*) and springtails (*F. candida*) using a natural saline soil at electrical conductivities starting at 1030 $\mu S\ cm^{-1}$. In fact, springtails and enchytraeids showed statistically significant effects (One Way ANOVA, Dunnet test, $p < 0.05$) on similar conductivity values when looking to the results of seawater exposure obtained in the present study (1293.5 for enchytraeids and 985.0 $\mu S\ cm^{-1}$ for springtails). When comparing the results obtained by Owojori et al. (2009) – 1030 $\mu S\ cm^{-1}$ – with the ones obtained in the present study for NaCl exposure, for springtails significant effects were found at similar conductivity values (1091.0 $\mu S\ cm^{-1}$ in the present study) but enchytraeids showed a higher sensitivity (616.5 $\mu S\ cm^{-1}$) in the present study. This difference in sensitivity can be related to the presence of other substances (not only sodium chloride) on the seawater composition that may have affected differently the test-species. Owojori et al. (2009) also found no reproduction on conductivity values of 1620 $\mu S\ cm^{-1}$ and 1310 $\mu S\ cm^{-1}$ for springtails and enchytraeids, respectively, although testing a natural saline soil. Princz et al. (2012) also reported results for springtails showing an EC_{25} of 1300 $\mu S\ cm^{-1}$, higher if compared with the results obtained in the present study (EC_{20} of 797.7 $\mu S\ cm^{-1}$). As for the mites, the differences on those values can be due to the different soils used as explained previously.

4.2. NaCl as a surrogate for seawater effects on soil communities

Sodium chloride, as a major constituent of seawater (Wiesenburg and Little, 1987–1988), should be a good surrogate of seawater effects in soil organisms. In fact, for springtails, both substances showed similar effects with differences of about 15% between both EC_{50s}. Despite this, enchytraeids were slightly more sensitive to sodium chloride than to seawater with about 40% of variation between both EC_{50s}. Owojori and Reinecke (2014) already described that the hazardous effects of soils mixed with different salt solutions in earthworms are not correlated with the conductivity value but rather with the composition of the solution. Nevertheless in the present study and although the small differences described in this study for the test species, NaCl and seawater generally caused the same overall effects consisting in a lower and similar tolerance of springtails and enchytraeids and a higher tolerance of mites. Despite this, the use of sodium chloride as a surrogate of seawater exposure can lead to an overestimation of the toxic potential of the latter to some soil fauna groups, and therefore its use is more advisable in a screening phase when evaluating the overall effects of seawater.

4.3. Safety levels for salinity to soil communities

Soils are considered saline when they present an electrical conductivity equal or above 4000 μS cm^{-1} (Micheli et al., 2002). In fact, mites were only affected by salt deposition at those conductivity values (statistically significant effects on reproduction starting from 4325 μS cm^{-1}). In opposition, springtails and enchytraeids were already affected by NaCl and seawater at conductivities much lower than 4000 μS cm^{-1} (the maximum value tested was 1679 μS cm^{-1} for seawater and 1505 μS cm^{-1} for NaCl). The results described in the present study were obtained using an artificial soil and would much probably be quite different when using a natural saline soil. However, it has already been reported that enchytraeids and springtails did not reproduce in soils of conductivity values under 2000 μS cm^{-1} (Owojori et al., 2009) using a natural saline soil.

The actual limit to define saline soils was obtained taking into consideration effects on plants (Micheli et al., 2002). However, as explained above, soils presenting those conductivity values would already affect soil fauna as proved by the results obtained in this study and the results obtained by Owojori et al. (2009) and Owojori and Reinecke (2009). Integrating the existing information about salinisation effects on soil fauna, and since this group is essential to the maintenance of soil ecological functions, the threshold to define saline soils should probably be changed. In order to define a more accurate value, more tests should be performed using, for example, field collected soils and field collected species, community tests, and tests under different conditions, especially to test the interactions between abiotic factors (e.g. different conductivity values, temperature, humidity). Then, this information should be combined, for instance using a Species Sensitivity Distribution (SSD) approach, to derive hazard salt concentrations to define protective levels for soil.

5. Conclusions

Seawater and sodium chloride exposure did not affect the mortality of the tested species but differently affected their reproduction. Mites were the least sensitive organisms while springtails and enchytraeids showed similar sensitivity. The same overall effects were obtained with both substances (seawater and NaCl) but a slightly higher sensitivity was found for enchytraeids to NaCl exposure. Seawater composition could be the explanation for the different sensitivity observed. Nowadays, the limit posed to define a saline soil – 4000 μS cm^{-1} – may not reflect the knowledge that already exists for effects of salt exposure to soil organisms. Indeed, in the present study, at conductivity values below that threshold value, soil invertebrates were negatively affected. Therefore, harmful consequences for soil ecosystem inhabitants are expected in a real exposure scenario.

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