

EFFECTS OF SALINITY ON EARTHWORM (*EISENIA FETIDA*)Gintare Guzyte<sup>1</sup>, Gintare Sujetoviene<sup>2</sup>, Jurate Zaltauskaite<sup>3</sup><sup>1,2,3</sup>Vytautas Magnus University, Vileikos str. 8, LT-44404 Kaunas, Lithuania.  
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**Abstract.** Road salts negatively affect environment ecosystems. Sodium chloride (NaCl) from runoff is responsible for the increased salinity of soil surface and ground waters. The aim of the study was to assess the effects of salinity on earthworm *Eisenia fetida* growth, reproduction and survival. To achieve this, experiment was conducted using OECD artificial soil spiked with NaCl (0, 1000, 2000, 4000, 6000 and 8000 mg NaCl kg<sup>-1</sup>). Growth was assessed at day 7, 14, 28, mortality and cocoon production - at day 56. Total mortality of worms was found in 6000 mg NaCl kg<sup>-1</sup> and 8000 mg NaCl kg<sup>-1</sup> tests. The calculated LC<sub>50</sub> for mortality after 28 days was 5623 mg NaCl kg<sup>-1</sup>. Weight of worms was significantly affected by NaCl. Dispersion analysis showed that NaCl concentration had statistically significant influence on weight of worms ( $P < 0.05$ ). The calculated EC<sub>50</sub> (effect concentration at which a 50 % reduction in a measured parameter) for growth was 2512 mg NaCl kg<sup>-1</sup>. Cocoon production was significantly affected by increased NaCl substance. There was no cocoon production in soil with 4000 mg NaCl kg<sup>-1</sup> and higher concentrations. It is concluded that sodium chloride negatively effects lifecycle of earthworms. It is essential to find less toxic de-icers on purpose to ensure transport safety.

**Keywords:** sodium chloride, salinity, earthworm, *Eisenia fetida*, mortality, reproduction, growth.

## 1. Introduction

Salinisation is among the major degradation processes endangering the potential use of European soils. Despite of naturally occurring salinisation, deicers for removal of ice from roads are essential to wintertime road maintenance in many countries.

Deicing chemicals are widely used to clear the pavement snow Lithuania and many other countries, because of their simple operation, low price, and excellent snow-melting effect. The use of chemical deicers such as sodium chloride (NaCl) has increased significantly during the past three decades. In USA 15 million tons and in Canada 5 million tons of road salts are applied to roads every year (Mayer *et al.* 1999; Robidoux and Delisle 2001). In Finland 100 000 tons of salt mainly as sodium chloride is used annually (Joutti *et al.* 2003).

In Lithuania, on purpose to ensure transport safety about 140 000 tons of sodium chloride is used during winter time, and about 10-680 g salt is applied to 1 m<sup>2</sup> road (Kadūnas and Arustienė 2010).

With the increasing application of deicing chemicals, their negative effects on ecosystem and environment have appeared increasingly. Sodium chloride (NaCl) from road runoff is responsible for the increased salinity of soil surface and ground waters still several months after the last road treatment. Chloride accumulation can compro-

mise soil structure and increase soil erosion, reduce soil fertility, to have an effect on soil chemistry. Road salts negatively affect environmental ecosystems – crop yield (Grewal 2010; Li *et al.* 2010; Zhang *et al.* 2010), soil microorganisms (Yuan *et al.* 2007; Ibekwe *et al.* 2010), plants (Gadallah and Ramadan 1997; Joutti *et al.* 2003; Ruhland and Krna 2010) and soil organisms (Owojori *et al.* 2008). There has been a growing concern of the environmental effects resulting from de-icing.

The environmental hazard of chemicals is evaluated primarily by chemical analyses. However, chemical data alone are insufficient to assess the potential environmental hazards of chemicals.

Bioassays can help define the ecotoxicological effects of environmental contaminants. The bioassay approach integrates the biological effects and factors such as pH, bioavailability and interactions between compounds. In this respect bioassays representing different trophic levels such as plant, microbiological and enzymatic tests could be used.

The aim of this paper was to assess NaCl salt toxicity to the earthworm *Eisenia fetida* and to lifecycle parameters. This species was chosen because it is recommended to used as soil invertebrate species in toxicity testing. NaCl salt was chosen because it is predominantly used as deicers for ice removal from road.

## 2. Research methods

### 2.1. Earthworm characteristics

*Eisenia fetida* specimens used for this study were age-synchronized from a culture kept in the laboratory of Vytautas Magnus University, Lithuania. Adult worms (18-20 weeks old) of between 250 and 500 mg weight were used in the experiments, using 10 earthworms in each sample. Each sample had 2 replicates. The worms were acclimatized for 24 h in OECD (Organisation for Economic Co-operation and Development) soil and were fed during this period with the same type of food to be used during the experiment consisting of oats.

### 2.2. Soil characteristics

A bulk sample of OECD test soils was prepared by mixing 10 % of finely ground sphagnum peat, 20 % kaolin clay and 70 % quartz sand, adjusted with CaCO<sub>3</sub> to pH 6.0 ± 0.5 according to OECD guidelines (OECD, 2004). The maximum water holding capacity (WHC) of soil was determined by using the procedure described by ISO (ISO 1996). The maximum WHC was adjusted to 60 %.

### 2.3. Experiment

Technical grade sodium chloride (NaCl, purity, 99.9 %) was added in solution form to 500 g of soil sample in the following concentrations: 0, 1000, 2000, 4000, 6000 and 8000 mg NaCl kg<sup>-1</sup> by dry weight soil. The concentrations were chosen in this way to include nominal concentrations of NaCl found in roadside soils in Lithuania and elsewhere.

The spiked soils were placed in rectangular plastic vessels of 2 l allowed to equilibrate for 2 days before earthworms were introduced. Ten worms per container and three per treatment were used. The worms were introduced into each container by placing them on the surface and allowing them to burrow in. The test containers were covered with perforated lids to limit water loss due to evaporation and kept in 16 h light, 8 h dark at 20 ± 1 °C in a climate chamber for 28 days. Worms were fed weekly with ground, sieved and dried oats (0.5 g per worm).

Sampling was done at day 7, 14 and 28 after worms were introduced to the substrates. Mortality and growth were monitored on each sampling day while cocoon production was assessed at day 56. LC<sub>50</sub> (lethal concentration at which a 50 % reduction in a measured parameter) and EC<sub>50</sub> (effect concentration at which a 50 % reduction in a measured parameter) were calculated. Growth was determined by individually weighing all surviving worms from each container and comparing the mean weight with initial values. Mortality was assessed by stimulating the worm with wooden stick and an earthworm was confirmed as dead if no response could be observed. Worms not found during sampling in test soil was assessed as dead. The number of cocoons was determined at the end of the exposure by wet sieving the substrates (through 1.0 mm sieve).

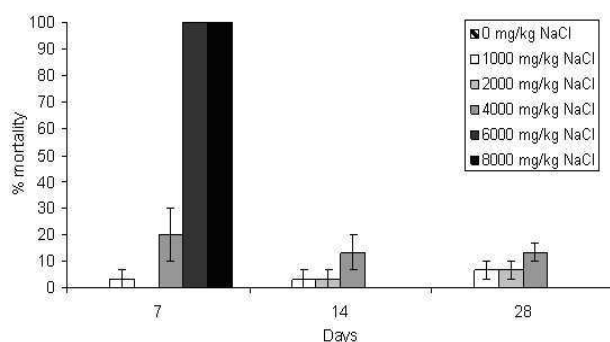
Soil moisture content and pH<sub>H2O</sub> were monitored on each sampling day. Moisture loss was replenished by adding equivalent amount of deionised water.

### 2.4. Statistical analyses

Calculations were performed using the statistical software package Statistica 7.0. Growth data per sampling time, cocoon number, and mortality data were analyzed using the Mann-Whitney U test. The EC<sub>50</sub> values of NaCl for growth and cocoon production were calculated by using the linear interpolation method. The LC<sub>50</sub> value for NaCl was calculated by using the Probit Analysis.

## 3. Results and discussion

Data on the mortality of earthworm exposed to different concentrations of NaCl in soil are presented in Fig. 1. There was no mortality in control soil (0 mg NaCl kg<sup>-1</sup>). Total mortality of worms occurred in treatments exposed to 6000 and 8000 mg NaCl kg<sup>-1</sup> at day 7 (Fig. 1). Significant higher mortality of worms was recorded in soil containing 4000 mg NaCl kg<sup>-1</sup> in comparison with 1000 and 2000 mg NaCl kg<sup>-1</sup>. The calculated LC<sub>50</sub> for mortality after 28 days was 5623 (confidence intervals, CI, 5438 - 5854) mg NaCl kg<sup>-1</sup>.



**Fig 1.** Mean (± SE) percentage mortality of three groups of 10 worms (*Eisenia fetida*) each exposed to NaCl for 28 days

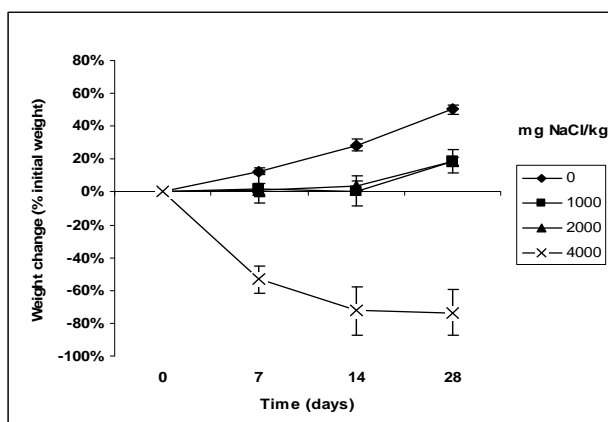
Our results is in agreement with results observed by Bright and Addison (2002). In 1000 and 2000 mg NaCl kg<sup>-1</sup> soil substrate there were no statistically significant differences in mortality while the 4000 mg NaCl kg<sup>-1</sup> concentration had a significant higher effect on mortality. High mortality resulting from increased NaCl has been reported by Owojori *et al.* (2008) where total mortality was found in 8000 mg NaCl kg<sup>-1</sup> soil substrate. High salinity has caused acute toxicity and worms not survive.

Generally, there was a decrease in weight of worms in all treatments except 0 mg NaCl kg<sup>-1</sup> at day 7 (Fig. 2). The mean weight in 0 mg NaCl kg<sup>-1</sup> was significantly higher in comparison with all exposure groups ( $P < 0.05$ ).

The mean weight of worms exposed to 1000 and 2000 mg NaCl kg<sup>-1</sup> changed slightly at day 14 and after 28 days only 18 and 19 % increase in the weight, respectively, was found.

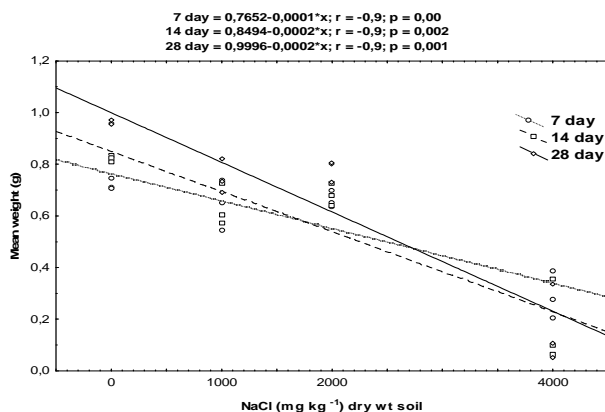
The mean weight of worms exposed to 4000 mg/kg NaCl and higher concentrations was considerable and significantly lower in comparison with the control (0 mg/kg NaCl) soil ( $P < 0.05$ ). At day 7 the mean weight decreased 53 % in 4000 mg NaCl kg<sup>-1</sup>. After 14 and 28

days the mean weight decreased 72 % and 74 %, respectively (Fig 2). In the exposure treatments defined adaptation to NaCl concentrations because weight change with time decreased and remained fairly steady.



**Fig 2.** Mean ( $\pm$  SE) weight change (% of initial weight) of worms (*Eisenia fetida*) exposed to sodium chloride in OECD substrates during 28 day period

The highest negative effect on weight was found at 6000 and 8000 mg/kg NaCl actually at the exposure beginning. These concentrations caused acute toxicity and worms not survive. Dispersion analysis showed that NaCl concentration had statistically significant influence on weight of worms ( $P < 0.05$ ). Correlation between the mean weight of worms and NaCl concentrations showed that at all treatment days the mean weight decreased with increasing NaCl concentration ( $P < 0.05$ ) (Fig. 3). The calculated  $EC_{50}$  for growth was 2512 (CI, 1798-3232) mg NaCl  $kg^{-1}$ .



**Fig 3.** Correlation between the mean weight of worms (g) and sodium chloride concentrations

Under the influence of 2000-4000 mg NaCl  $kg^{-1}$  concentrations the mean weight of worms was statistically lower after 28 days than in the control ( $P < 0.05$ ). The mean weight of worms was more significantly (weight was lower 74 % after 28 exposure days) affected in soil containing 4000 mg NaCl  $kg^{-1}$ . The  $EC_{50}$  of 2512 mg NaCl  $kg^{-1}$  for growth observed here was lower than 4681 and 4985 mg NaCl  $kg^{-1}$  found by Bright and Addison (2002) and Owojori *et al.* (2008) respectively. In the experiment ob-

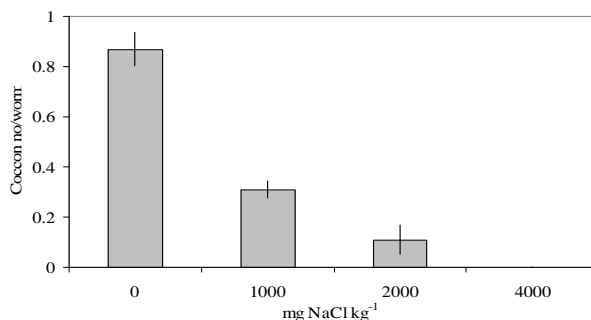
served value was also lower than 3586 mg NaCl  $kg^{-1}$  found by Owojori *et al.* (2009). The reason could be that under such low concentrations (1000-2000 mg NaCl  $kg^{-1}$ ) at the beginning of exposure (at day 14) worms contended with stressor and adapted to low toxicity and as a consequence earthworm weight increased (at day 28).

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The mean cocoon number produced over exposure period at different NaCl concentrations is shown in Fig. 4. Linear regression between weight of worm and NaCl concentration showed that the NaCl toxicity had significantly negative influence on reproduction (Fig. 4). Worms exposed to 4000, 6000 and 8000 mg NaCl  $kg^{-1}$  did not produce any cocoons ( $P < 0.05$ ). The calculated  $EC_{50}$  for reproduction was 1288 (CI, 949-1688) mg NaCl  $kg^{-1}$ .

The effect of NaCl on cocoon production was similar to those presented in the literature. Cocoon production was affected at concentrations far lower than those affecting growth and mortality. This is in accordance with the results of other study (Owojori *et al.* 2008) which showed that cocoon production was more sensitive parameter than mortality and growth in assessing the toxic salinity effect to *E.fetida*.

Twenty eight-day  $EC_{50}$  for cocoon production of 1288 mg NaCl  $kg^{-1}$  was observed in our study. This value is comparable with those available in the literature. Owojori *et al.* (2008) found 28-day  $EC_{50}$  for cocoon production of 2020 (CI, 1467-2636) mg NaCl  $kg^{-1}$ , while Bright and Addison (2002) found  $EC_{50}$  of 1884 mg NaCl  $kg^{-1}$  soil substrate. No successful reproduction occurred in replicates with a NaCl concentration above 3000 mg NaCl  $kg^{-1}$  (Kerr and Stewart 2004).



**Fig 4.** Mean ( $\pm$  SE) number of cocoons per worm produced after exposure of worms (*Eisenia fetida*) to sodium chloride

Although the presented data can be considered as a step forward in the assessment of the potential risks of salinity in terrestrial environments, further research is needed to evaluate the influence of soil parameters on the toxicity of salinity and to quantify the effect of ageing on bioavailability.

#### 4. Conclusions

Our results showed that increased salinity had harmful effects on growth, mortality and reproduction of the earthworms. Of these parameters, cocoon production was the most sensitive parameter. We determined 28 day LC<sub>50</sub> for mortality and growth of 5623 and 2512 mg NaCl kg<sup>-1</sup>, respectively. Weight of worms was significantly affected by NaCl and dispersion analysis showed that NaCl concentration had statistically significant influence on weight of worms ( $P < 0.05$ ). Cocoon production was significantly affected by increased NaCl substance. There was no cocoon production in soil with 4000 mg NaCl kg<sup>-1</sup> and higher concentrations. It is concluded that sodium chloride negatively affects life cycle of earthworms. Field studies are needed to confirm the observed biological effects under natural conditions.

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