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Can Winter Road De-Icing Affect Mortality of Organisms in Aquatic Ecosystems? An Experimental Approach

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Abstract: Road maintenance in winter using de-icing agents, which is widely used in Eastern Europe, is the cause of water salinization in neighbouring environments, which might lead to biodiversity loss in aquatic ecosystems. In this study, we investigated NaCl toxicity to test young organisms: *Daphnia magna* and *Poecilla reticulata* (standard tests organisms). The salinity of NaCl was measured by electrolytic conductivity (EC). It was statistically demonstrated that the test solutions should be prepared using natural water. For *D. magna* the NOEC was 7.17 mS/cm and the LC50 9.76 mS/cm. *Poecilla reticulata* showed resistance to salinity up to a conductivity of 25.2 mS/cm, and no lethal effects were recorded for any individual in the test population. The study showed that winter salinities recorded in inland waters (without emergencies such as sudden influx of pollutants due to industrial accidents) are unlikely to affect fish but may be hazardous to small plankton. However, the high dare of *D. magna* may result in a reduction of planktivorous fish.

Keywords: *Daphnia magna*; salinity; toxicity; LC50; road de-icing; climate change

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1. Introduction

Periods of mild winters, characterized by minor temperature drops and little snowfall, have become increasingly common in the temperate climate zone. Not only do the ice-free winters affect water quality, but they also cause aquatic organisms in reservoirs to remain unnaturally active or change their behavioral patterns and may modify community structure [1,2]. The occurrence of mild winters is influenced by climate change, induced by human activity leading to the effect of global warming. Short duration of ice also results from anthropogenic impacts, such as road de-icing agents runoff [3].

In many countries of eastern and central Europe, even in the case of minor snowfalls, winter road maintenance is performed with de-icing agents containing sodium chloride. Its use, however, is associated with negative environmental effects, as it is dissolved in the melting snow and ice and subsequently flushed by overland flow reaching reservoirs and lakes. Consequently, it also affects groundwater and soils near traffic routes. It is estimated that the salinity of watercourses in the vicinity of transport routes during winter can increase to 1300 µS/cm for watercourses in sparsely developed areas [4] and up to 5000 µS/cm for sites in the vicinity of mines, industrial facilities, or accident sites [5,6].

Human-induced salinization caused by the application of sodium chloride for de-icing has become an increasing threat to freshwater ecosystems as it can cause irreversible changes. The salinization can result from a magnitude of human-induced activities ranging from agricultural practices, mining, vegetation removal, to river regulation. However, one of the most common causes inducing salinity of water bodies is runoff from saline routes (through de-icing salt) [6]. The most dangerous effect of salinization is the disruption of the water and mineral economy of organisms caused by an increase in osmotic pressure, but even more importantly an increased mobility of heavy metals [7]. It is therefore important to identify the problems posed by the use of de-icing agents.

In this study we investigated the effect of salinity on aquatic organisms. We assessed the sensitivity of *Daphnia magna* and *Poecilla reticulata* to salinity levels as a basis to determine whether salinization of water resulting from saline inflows from traffic routes can be hazardous to aquatic biotopes. These test species can act as excellent model organisms to study the effect of salinity on aquatic biota because of their well-recognized sensitivity to environmental changes and the fact they are ubiquitous in freshwater systems [8,9]. Because solutions of compounds with distilled water are used in the laboratory, and natural materials are used in ecological studies, we examined the sensitivity of test organisms to two types of solutions.

We also compared our research results with those of other researchers. We did it in order to confirm the correctness of our research. Until the present times many scientists have studied the effects of salinity on aquatic ecosystems [1,6,8,10–14]. However, we did detailed data on the sensitivity of *P. recticulata* compared to *Daphnia magna*, as representatives of the standard organisms used as test organisms in assessing the environmental harm of substances, which had not been already completely established. We did not find a specifically determined survival rate for *P. recticulata* too. Very small organisms were selected because they respond most rapidly to environmental changes. At a time of climate change and rapidly varying habitat conditions, it is critical to determine their potential impact on biodiversity and ecological structure.

2. Materials and Methods

To study the effects of salinity on the survival of aquatic organisms, we analysed the toxicity of sodium chloride on selected test organisms. The behaviour of standard species: *Daphnia magna* (Crustacea, order *Cladocera*, size around 1–1.5 mm), and *Poecilia reticulata* (guppy, small aquarium fish, family *Poecillidae*, size around 10 mm), which in this study represent zooplankton and nekton organisms. The above-mentioned organisms were collected from lab culture. *D. magna* and *P. recticulata* are used as standard in environmental toxicity testing of substances [8,10,15,16]. Solutions were prepared with: (1) distilled water (EC 0 mS/cm) and (2) natural water, with an initial conductivity of 390 µS/cm. Natural water was obtained from a waterwork mains, fed from an underground intake: the treatment process removed excess iron compounds and disinfectants were not used. This water is intended to simulate natural water, free of any toxic substances that may affect the viability of the test organisms.

The test population (10 U) was exposed to NaCl solutions characterized by variable conductivity. Initially, NaCl concentrations corresponding to the salinity of physiological salt (0.85%; 18.04 mS/cm) were used in the tests for *D. magna*. Physiological salt was selected as a reference because of its widespread suitability for keeping cells alive. Solutions of conductivity used in our tests were: 7.17; 8.01; 8.54; 9.65 mS/cm (variant 1 and variant 2), and from 16.05 to 23.80 mS/cm (only variant 1). *Poecilla reticulata*, on the other hand, were tested in the conductivity range from 7.17 mS/cm to 25.2 mS/cm (variant 2). We abandoned testing variant 1 for *P. recticulata* because of the demonstrated high sensitivity of *D.magna* to distilled water solutions. A full explanation of the order of NaCl concentrations used is described in the Results and Discussion section (Section 3).

The test conditions were chosen to create favourable living conditions for the sample organisms that would not interfere with the analysis results. According to the guidelines [15,16], the recommended temperature for toxicity tests should be "appropriate for the given species". For *Daphnia magna* a temperature between 15–20 °C is recommended, and for freshwater fish the range 18–22 °C is suggested. Therefore, the toxic effect of salinity was tested at 15 °C for *D. magna* and 20 °C for *P. reticulata*. The organisms had access to light according to the time of day but were not directly exposed to it. As recommended, photoperiod ranges of 14 h and an intensity of 600–800 lux were prevailing. According to OECD guidelines for freshwater fish [16], a maximum load of 0.8 g wet weight of fish/L was not exceeded in testing. The toxic effect was analysed for 24–30 h duration of the experiment. However, testing for *D. magna* in variant 1 was completed after 40 min because all of the sample organisms had died. Variant 2 stopped after 30 h because the results allowed to determine the LD50 index. For *P. recticulata* the test was discontinued after 30 h also as no organism reactions in each analysed salinity were noticed. Young organisms and those in very good condition were selected for the experiment—during which the organisms did not take any food to avoid changes in water parameters. The guidelines according to ISO 6341:2012 and ISO 7346-2:1996 and OECD 203 and the Dobosikova methodology [10,16,17] were applied.

The reactions of the test organisms and the mortality rate were observed during the experiment: LC50 ("Lethal Concentration"—concentration of substance, which causes the death of 50% of the population) and NOEC ("No Observed Effect Concentration"—the highest concentration of a substance that causes no observed adverse changes in the test population) Statistical analysis of the obtained results was performed by means of Statistica software, along with variable regression method to verify the representativeness of the tests performed for both variants of the solutions. For this purpose, 3 variables were taken into account: salinity concentration, type of water (natural or distilled), and quantity of living organisms. For the calculation of the regression of the variable, 44 test results from each variant for *D. magna* were used. The test results concerned the conductivity ranging from 7.17 mS/cm to 9.65 mS/cm. The results of the statistical research concerned the period of time in which the changes in the number of the *Daphnia* population took place (Table 1).

Table 1. Results of the regression analysis. Checking the representativeness of the research on both analyzed variants using the "b" and "b *" coefficients.

1-Distilled Water; 2-Natural Water	b*	Standard Error from b*	b	Standard Error from b	t (42)
1	−0.01759	0.15427	−0.0744	0.65289	−0.11403
2	−0.42656	0.13956	−0.8428	0.27576	−3.05649

b—unstandardized regression coefficient. b*—standardized regression coefficient.

3. Results and Discussion

As given in the Methods section (Section 2), NaCl concentrations corresponding to saline (0.85%; EC 18.04 mS/cm) were initially used in this study. However, it turned out (Figure 1) that all test organisms died after a maximum period of 40 min, which suggested very high toxicity of the test solutions. Since studies on the salinity of inland watercourses conducted in 2020 [4] showed an increase in EC during the winter period to a maximum of 11.39 mS/cm, lower concentrations, i.e., from 7.17 to 9.65 mS/cm, were selected for further studies on the effect of salinity on aquatic organisms. The effect of the type of water used to prepare the test solutions on the toxicity found also became subject to analyses.

Therefore, solutions prepared using distilled water (variant 1) and natural water (variant 2) were used in the studies. NaCl concentrations were chosen that could show measurable results in acute toxicity examinations. According to Schuytema et al. [11], the salinity corresponding to the sodium chloride toxicity value for half of the *Daphnia magna* test population is 8.38 mS/cm on average, so this value was considered as a reference in subsequent tests. Following values of solutions conductivity were further: 7.17; 8.01; 8.54; 9.65 mS/cm.

Figure 2 shows the dynamics of change in the survival of *Daphnia magna* over time, in solutions prepared using distilled water. It can be observed that the NaCl concentration has little influence on the mortality of *Daphnia magna* (similar lethal effects at different concentrations—Figure 2). The results presented here do not allow to verify whether the mortality of *Daphnia magna* is caused by salinity, whose concentration exceeds the adaptive capacity of this species, or whether the mortality is caused by the high osmotic pressure of the solution.

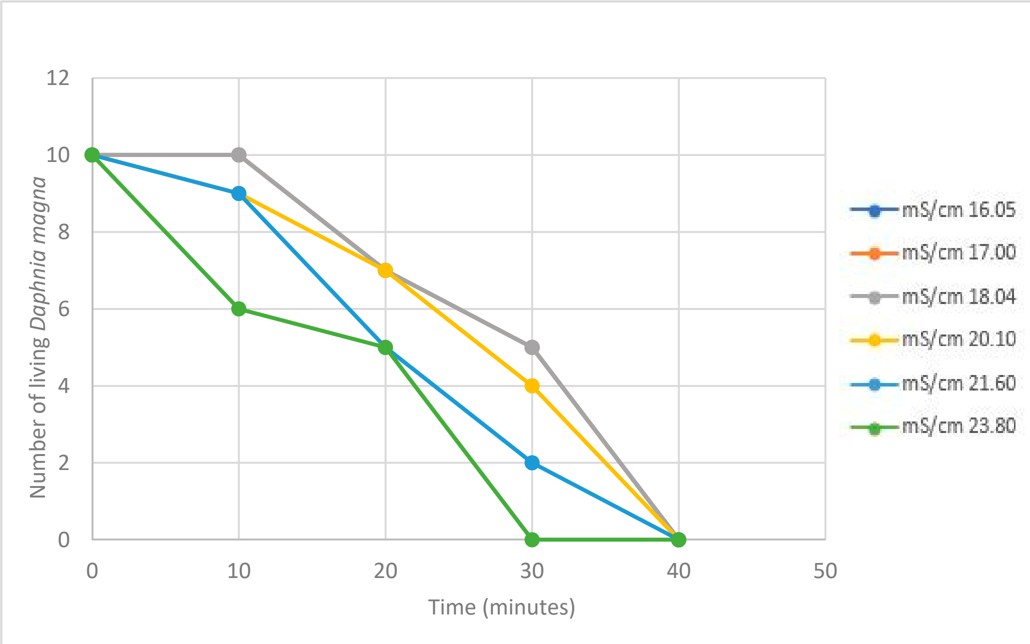

Figure 1. Dependence of the number of living (survival) of *Daphnia magna* individuals on the exposure time in NaCl solutions—variant 1 (distilled water).

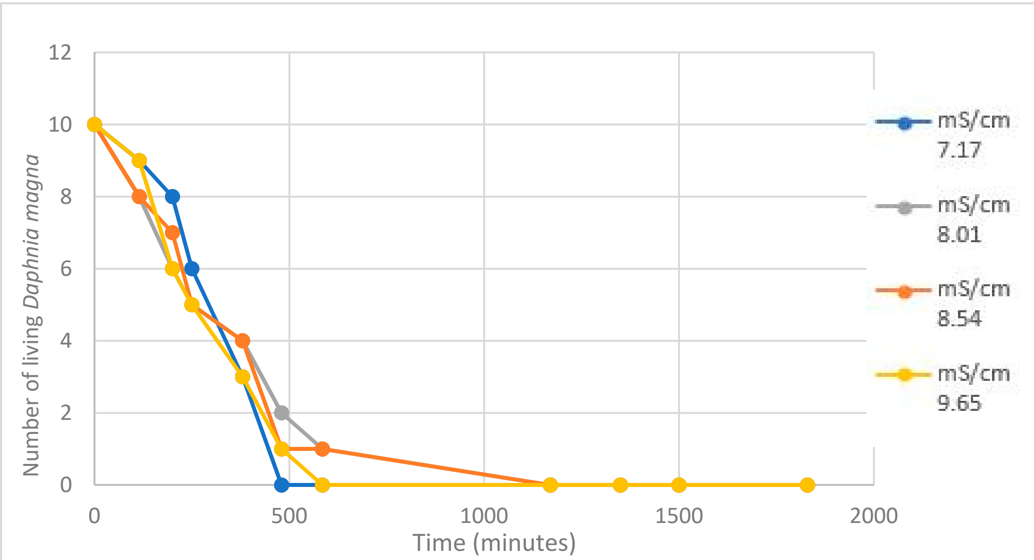

Figure 2. Dependence of the number of living (survival) of *Daphnia magna* individuals on the exposure time in NaCl solutions—variant 1 (distilled water).

Figure 3 shows the survival rate of *Daphnia magna* in the tested solutions prepared using natural water. The data show that the salinity affects the mortality of *D. magna*. Organisms tolerate salinity only within a certain range of values. The LC50 value varies from 9.65 mS/cm for 1150 min to 8.54 mS/cm for 1900 min. According to the results obtained, the NOEC value was set as 7.17 mS/cm.

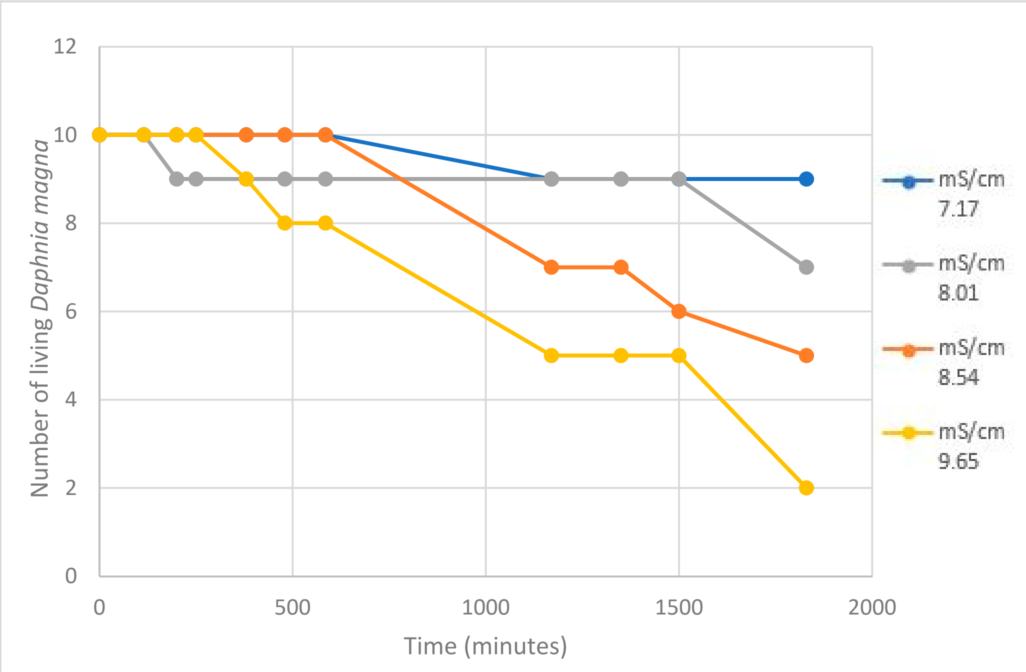

Figure 3. Dependence of the number of living *Daphnia magna* individuals on the exposure time in NaCl solutions—variant 2 (natural water).

In Table 1 we present the results of dependent variable regression for 44 tests performed (N). The coefficients "b" and "b*" indicate the correlation between the three variables (number of living organisms and salinity and type of water). The coefficient b indicates that as the salinity concentration increased in successive samples, the number of test organisms decreased by the value of "b" (increase in mortality). The calculation shows that "b" and "b*" have negative values, and this means a negative correlation. In this case, the higher the salinity concentration, the fewer organisms remain alive. The natural water variant achieves significantly greater negative values (negative correlation) than the distilled water variant. In addition, the standard errors from b and from b* are also noticeably higher for the distilled water variant. This means that the obtained results in variant 2 can be considered as representative (salinity has a statistical effect on the lethality of the tested organisms) in contrast to the results obtained in variant 1.

Moreover, on the basis of Figure 3, it can be concluded that the survival rate of *Daphnia magna* decreases with increasing salinity concentration. *D. magna* survival in the sample with the lowest salinity (conductivity at the level of 7.17 mS/cm) is 90% after 48 h of measurements, while the survival of *D. magna* in the sample with the highest salinity (conductivity at the level of 9.65 mS/cm) is only 20% after 48 h of measurements.

The above results are similar to the results of Shuteyama et al. research [11] who found same tolerance to salinity by *Daphnia magna*. They also showed 80% of the survival rate of young 7-day-old *Daphnia magna* in saline water with a conductivity of 3.71 mS/cm, and as low as 60% in saline water with a conductivity of 5.06 mS/cm. This proves that the increase in salinity had a statistical effect on the mortality of *Daphnia magna*.

The *Poecilla reticulata* survival in NaCl aqueous solutions with conductivities (mS/cm) of 7.17; 10.35; 12.55; 16.18; 19.00; 22.2; 25.2 was also investigated. Since it was noticed that the results of tests of NaCl solutions with distilled water were not representative (statistical analysis), only variant 2—i.e., solutions prepared with natural water—was used for tests with *Poecillia recticulata* (Figure 4). It was considered that the determined NOEC for *D. magna* (7.17 mS/cm) would be the lowest to be used for *P. recticulata* tests. The duration of the tests was limited to 30 h. Subsequent solutions were selected based on salinity values in different regions of the Baltic Sea [18] and considering salinity of inland waters (below 11.39 mS/cm). Higher salinity values were also considered, due to the potential for occasional contamination of surface waters with high inflow loads of mineral salts as a result of anthropogenic activities.

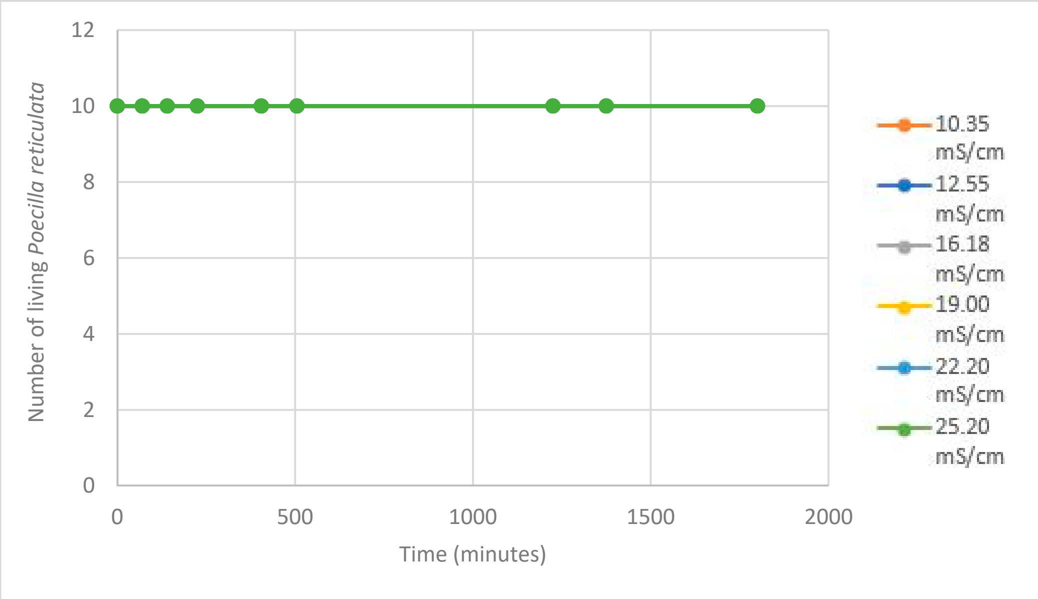

Figure 4. Dependence of the number of living individuals of *Poecilla reticulata* on the duration of exposure in NaCl solutions—variant 2 (natural water).

In the range of NaCl concentrations tested in this study, it was not possible to determine NOEC and LD50 for *P. recticulata*: none of the test organisms showed signs of mortality (Figure 4—the lines for all analyses the same course). The effects of NaCl concentrations that realistically occur in inland waters adjacent to transportation routes were also analysed.

Sodium chloride is a common substance found in the natural environment. It plays an important role in regulating the water balance, the nervous system, maintaining stable blood pressure, toxins removal from the body, or the proper functioning of the digestive system. Its surplus can have a harmful effect on the functioning of organisms and the entire ecosystem. Increased salinity can impair food uptake by small aquatic organisms and dehydrate them. The toxic effect of NaCl depends primarily on the duration of exposure to a given concentration of the compound [19]. Sufficiently high exposure may result in the death of the organism, which in the environment seems to be the main cause of the ecosystem's biodiversity loss [12,13]. Since anthropogenic activities may contribute to the increased salinity of aquatic environments, the effects of NaCl solutions on the survival of the test organisms, *Daphnia magna* and *Poecilla recticulata*, were investigated.

Determined LC50 value for *Daphnia magna* does not differ from the results carried out by Schuyteama et al. [11], who determined the upper EC value for the survival rate to be 8.38 mS/cm for a similar test duration. According to the results obtained, the NOEC value was set as 7.17 mS/cm. As reported by Schuyteama et al. [11], salinity with an EC value of 7 mS/cm does not increase mortality, but it does stop the reproductive processes of *D. magna*.

We conducted survival tests in two variants of NaCl solutions: with distilled water (1) and natural water (2). It was demonstrated that the increase in salinity in both tested variants increased the mortality among *Daphnia magna* populations. However, higher mortality of test organisms was observed in solutions prepared using distilled water. This is related to the increase in osmotic pressure of the solution to a toxic value. The reason for the high mortality of sample organisms may also be due to the lack of other chemical compounds, including micronutrients, necessary to maintain viability and survival. A similar dependence of the effects of distilled water on living organisms was also noted by Vatsos, Thompson, and Adams [20] in the case of studies on *Flavobacterium*, as well as by Abbott [21] conducting studies on crabs of the genus *Uca* (*Uca pugnax*), or Young [22] reffering to the fish species. A NaCl solution in distilled water increases the osmotic pressure significantly, which in turn causes plasmolysis and dehydration of living cells. However, since saline solutions have been found to be highly harmful (Figure 1), another reason seems to be relevant. Natural water also contains small amounts of many mineral salts, which have a protective effect on living cells. This allows us to draw a conclusion that studies on the toxic effects of salinity on aquatic organisms, the results of which are related to natural conditions, should be carried out using natural water.

The regression analysis confirmed that the studied effect of salinity on the survival of *Daphnia magna* is unrepresentative for the study performed with solutions prepared with distilled water (Table 1). Salinity has a significant statistical effect on mortality only for natural water: in this variant (2), higher salinity causes higher mortality of test organisms. In the variant with solutions prepared with distilled water (1), in addition to excessive salinity, the increase in *D. magna* mortality may also be due to a lack of essential macro- and micronutrients. We conducted further tests with solutions prepared from natural water.

In the present study, for solutions with conductivities of 5.18 and 7.15 µS/cm, no detrimental changes in the abundance and behaviour of *D. magna* were observed. Therefore, the NOEC was determined for *D. magna* at 7.15 mS/cm (variant 2), corresponding to a dissolved NaCl concentration of 4.55 g/L. Salinity toxicity for *D. magna* was recorded after a 16-h incubation: a lethal effect was recorded for half of the test population at LC50 = 9.76 mS/cm. In a study by Gonçalves et al. [14] an LC50 for *D. magna* of 5.9 g/L NaCl was determined, corresponding to a salinity of about 9.3 mS/cm. The difference between the results obtained is small but may be due to the use of different conditions during the tests. Gonçalves et al. treated *D. magna* with food, thus reducing the environmental pressure. Feeding the test organisms, as well as higher concentrations of *Daphnia* metabolic products, may therefore have a beneficial effect on culture conditions and water parameters, resulting in higher resistance of aquatic organisms to pollution.

According to a study conducted by Chervinski [23], *Poecilla reticulata* is able to tolerate an abrupt change in salinity levels without lethal effects (NOEC) if it does not exceed 19.5 g/L (corresponding to a conductivity of 30.43 mS/cm—in our study the highest conductivity for *Poecilla reticulata* were found—in our research—to be 25.20 mS/cm). Mortality at the 100% rate was achieved when salinity increased to 27.3 g/L (corresponding to an EC of 32.96 mS/cm) after 24 h. In the inland waters (as well as the Baltic Sea), such salinity values are virtually non-existent.

It is likely that the NaCl concentrations used are not toxic to fish, due to their very high salinity tolerance. Salinity which is harmful to *D. magna* does not seem to be harmful to *P. reticulata*. Therefore, the level of salinity that may occur in inland waters in areas adjacent to traffic routes during the winter road maintenance period does not pose a risk to fish.

A study by Bednarczyk and Frąk [4] shows that the salinity of inland watercourses varies over the year. This is influenced, among others, by the regulated character of the rivers, presence of single- and multi-family residential buildings in the catchment and the vicinity (and their number) of transport routes. Strong industrialisation of the catchment may also be a cause of periodical increase in water salinity, resulting from area and point source run-off. It is not common to reach conditions where the salinity of surface water is equal to the NOEC for *Daphnia magna*. Figure 5 shows the average salinity level of the River Długa (Poland) in comparison with the determined NOEC and LC50 values. The River Długa is located in the vicinity of 3 large roads of national and international importance, which are maintained with de-icing salts during periods of low temperatures and snowfall.

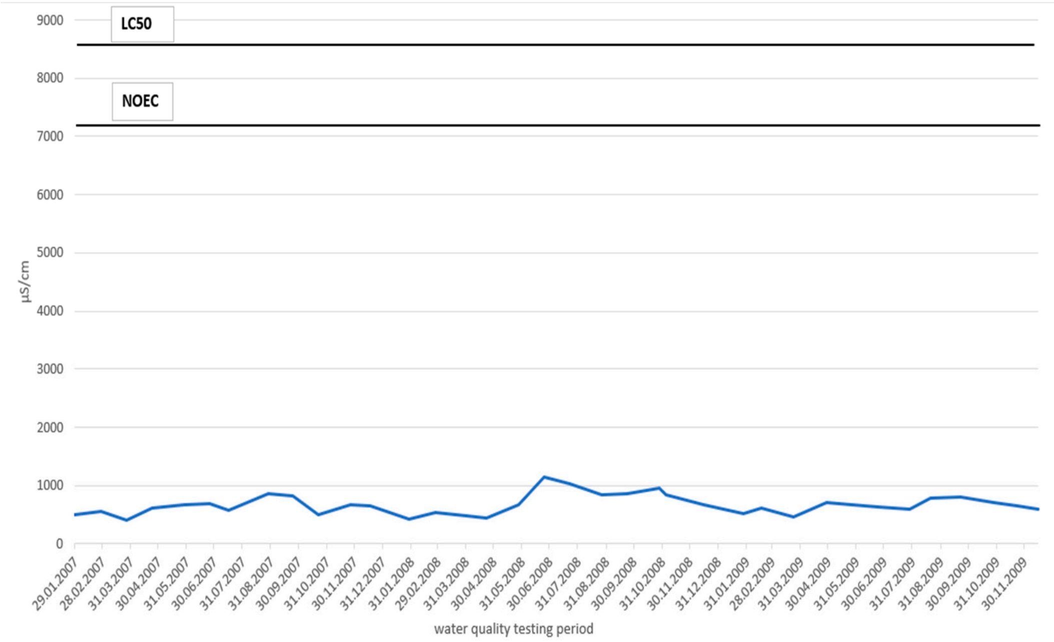

Figure 5. Comparative graph of electrolytic conductivity in the river Długa in 2007–2009 with LC50 and NOEC for *Daphnia magna*.

It can be observed (Figure 5) that the harmful salinity levels are much higher than those recorded in the average inland watercourse, even for species more sensitive than *Poecilla reticulata* and *Daphnia magna*. This indicates that an average salinity level does not necessarily imply an ecological disaster and a deterioration of living conditions for aquatic organisms. However, mobile species may avoid unfavourable conditions and move to less contaminated areas.

This does not mean, however, that the problem of inland water salinity should be underestimated. The fact that organisms do not die as a result of salinization does not mean that there are no negative effects on their immune systems or their reproduction rate. It is also important to note that a toxic substance is less harmful if it occurs individually. If, in addition to excessive salinity, other pollutants appear in the water, then their synergistic toxicity will outweigh the harmfulness of salinity alone [24].

4. Conclusions

The paper shows that winter road de-icing does not necessarily affect the mortality of organisms in adjacent aquatic ecosystems. *Daphnia magna* would, in most cases, be able to cope with the average salinity of inland waters. Only highly saline anthropogenic waters would adversely affect the population of this species. *Poecilla reticulata*, as a representative of small freshwater fish, also does not indicate a high sensitivity of this group of organisms to the recorded salinity of inland water ecosystems. However, fish populations may be indirectly affected by salinity, as high mortality rate of *Daphnia* can deprive fish of a food source and affect their population.

The salinity of inland waters may increase, e.g., due to emergency inflows of mine water or sewage from storm sewers. Specific loads flowing from urban centres [25] may pose a threat to the aquatic ecosystem biotope. It is important to note that in highly polluted waters, for example, fish lose their sense of smell, and this result in impaired migration and subsequent death [26]. It is important to be aware of the threat posed by the use of sodium chloride-based de-icing agents, as well as inadequate protection of rivers against untreated surface runoff entering through buffer zones (which are in short supply in urban areas) from streets and pavements [27]. This supports the need for additional research on the extent to which road salt pollution introduced into ecosystems alters freshwater communities.

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