Rapport de stage individuel

4ème

Impact of roads salts pollution on zooplanktons species

European Regional Centre for Ecohydrology
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Organizacja Narodów Zjednoczonych do Spraw Oświaty, Nauki i Kultury · ERCE Europejskie Regionalne Centrum Ekohydrologii pod auspicjami UNESCO

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Introduction

Liquid tight floor and paved roads are majors' characteristics of urbans landscapes and anthropogenic area with human presence. Roads, especially in urban areas, are the cause of pollutions source, like hydrocarbons, chemical input or roads salts pollution. Salinization of freshwater ecosystems is an emergent environmental and ecological issue worldwide. Indeed, lands which are localized especially in the north hemisphere (United States, Canada, Poland, France etc...) are faced with snowfall, and use the salt to melt the ice or the snow layer to avoid and prevent roads accidents, and to fluidify the traffic in winter. Therefore, with the melting snow, salts particles enter the surrounding landscape, to adjacent soil and waterbodies. Research on the persistence of roads salts in the environment and its cumulative effects has raised concerns about the long-term implications of this large-scale anthropogenic input (ATHENA TIWARI AND JOSEPH W. RACHLIN).

Moreover, it has been shown that different concentrations of NaCl (the most common salt used during winter) can affect the normal osmoregulatory and physiological processes of individual aquatic invertebrates. Despite these findings, the responses of invertebrate communities to this stressor in the natural environment have not been well-defined (B.J BLASIUS AND R.W MERRIT). As the main topic of the internship will be based on zooplankton communities, it will be significant to know that zooplankton constitute an important component of food web in water ecosystems. They can also reduce the risk of toxic algal blooms contaminating the water needed for consumption or recreation, by controlling the amount of phytoplankton (top-down effect). However, greater knowledge of the factors affecting the hatching and growth of zooplankton is needed to maintain adequate surface water quality.

Therefore, we may wonder which salts have the most important impact on zooplankton community to compare to the sodium chloride (NaCl)? Which zooplankton species is the most resistant to salt pollution? Indeed, it can be useful to know if the organism which eat the most phytoplankton, die with low salt concentration or not, compared to the other. Afterwards, is there a link between the concentration of chloride in salts and the death-rate in zooplankton communities? Finally, what are the alternatives to solve this problem? The main aim of the internship, and therefore my role, is to determine the lethal dose when 50% of the zooplankton communities die (EC50), according to 3 salts used in Lodz area (Poland), to take part in my tutor research. This lethal dose will be determined on 4 invertebrate's species, which are *Thamnocephalus Platyurus, Daphnia magna, Ceriodaphnia dubia* and *Brachionus calyciflorus,* based on ecotoxicology concept (S. SZKLAREK). For that, TOXKITS will be used. They are microbiotests in kits containing all necessary materials, including the test organisms, to perform simples, rapids, sensitives, and reproducible toxicity tests at low cost. Toxkits microbiotests are suited for toxicity testing of chemicals and wastes released in aquatic and terrestrial environement. Moreover, some researches will be made to know which kind of alternative can be set up to prevent this emergent environmental and ecological issue worldwide (KAYLA D. COLDSNOW ET AL.).

I) Host organization presentation

The research centre where the internship was performed, is the ERCE PAS (European Regional Centre of Ecohydrology of the Polish Academy of sciences) under the auspices of UNESCO (United Nations of Education, Sciences, Culture Organization) which is linked to the Polish Academy of Sciences since 2008. The 8th of March 2006, an agreement was signed between Polish Minister of Sciences and High Education and the Director General of the UNESCO and ensued the creation of ERCE. This centre is situated in Lodz, the 3rd biggest city of Poland under the direction of Professor Maciej Zalewski.

The different missions are particularly, to develop the concept of ecohydrology in an empiric way, and to develop biotechnologies and systemic approach with a cooperation at a world scale in the institution of UNESCO International Hydrological Programme and European Commission projects. Moreover, the centre is also focussed on the innovative methods based on the ecohydrology concept for aquatic ecosystems and the improvement of the catchment area sustainability. The last important mission is the networking for the sharing of ingenious ecohydrology solutions and the enhancement of environmental consciousness of people in the current society. Indeed, the centre is in charge to publish the journal Ecohydrology & Hydrobiology jointly with Elsevier. It includes original research papers, reviews and communications from ecohydrology discipline, therefore, it's the interplay between ecological and hydrological processes.

The research centre work also on these different projects at the international scale, like the project "AMBER" (Adaptive Management of Barriers in European Rivers), "EKOROB" (Ecotones for reducing diffusion pollution), "ENABLE" (Enabling green-blue infrastructure in complex social-ecological regions system solutions to wicked problems).

My place in the institute was under the deputy director, among the research unit for fluvial

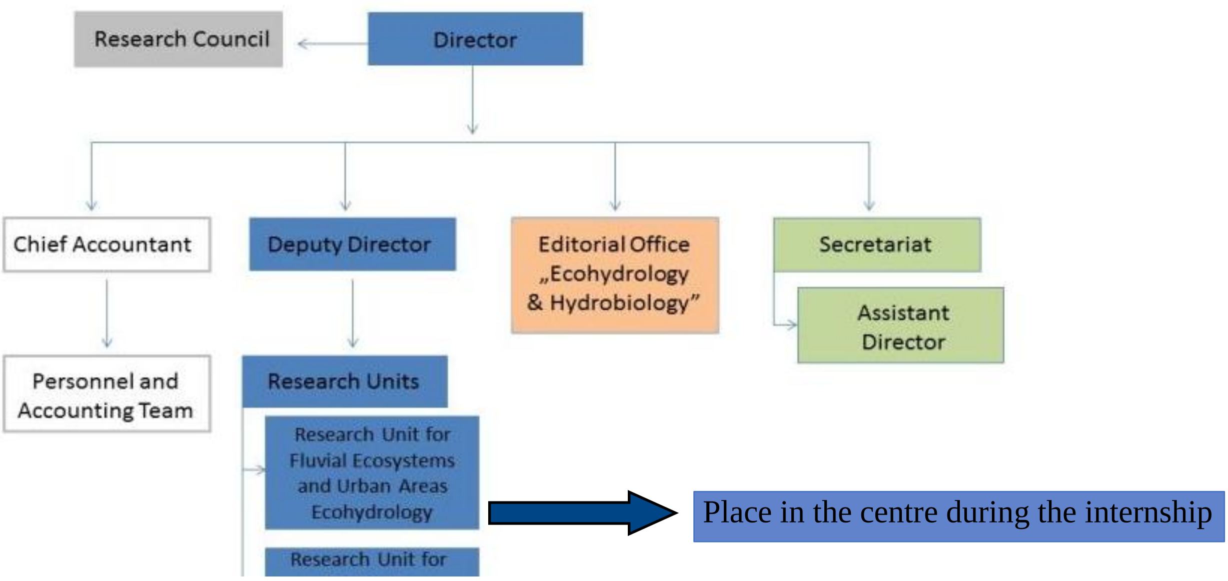


Fig. 1: Organization chart of the European Regional Centre for Ecohydrology

ecosystems and urban areas ecohydrology (Fig. 1).

II) Study concepts

2.1) Ecotoxicology concept

The notion of ecotoxicology appeared in the 1970s, with the basic principles of science which were those of toxicology. A false conception is always presenting not only among scientists from other disciplines, but also among biologists who consider the place of ecology and ecotoxicology among biological science. Indeed, at the limit of the biological discipline called reductionist (relation between biology and physico-chemic science), and the field of ecology in the strict sense, is the study of individuals taken in isolation, and the influence of the environment factors on their various physiological functions (BERNARD FELZT). Another major confusion with environmental toxicology of is made (Fig. 2). It is based on research carried out most often at the lowest levels of biology, from the molecule, to the cell, the vital organs and at best the isolated individuals.

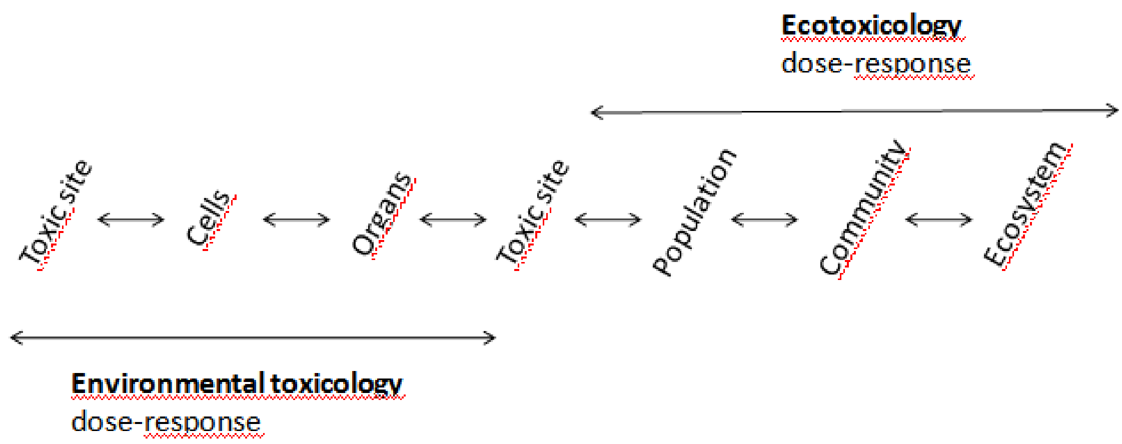


Fig. 2 Respective area of the environmental toxicology and ecotoxicology

The ecotoxicology appears because of toxicologists's interest in environment. As a result of its multidisciplinary nature and the diversity of scientific fields on which it is based, the specificity of the ecotoxicology approach has in the past given rise to different interpretations and different definition. As it lies at the crossroads between toxicology and ecology, it can be defined by science that assesses, monitors and predicts the fate of pollutants in the environment. But it is incomplete, because it forgets the major purpose of the discipline. Indeed, this discipline must assess the ecological consequences of pollutions, and predicts the effects of other pollutants in order to implement preventive measures that eliminate the risk of damage. (FRANÇOIS RAMADE).

Either ecotoxicology is the science that studies toxic pollutants in population, ecosystems, even up to the biosphere. This concept "encompasses not only effect assessment alone, but also integrates environmental chemistry with aquatic toxicology" (KARL FENT)**.** The experimental testing is based on analysis of dose-effect relationships, and estimation of effect concentrations, such as the exposure concentration at which 50% effect is observed within a certain period (EC50). Thanks to ecotoxicology, the environmental regulation gets a lot of benefits with efficient elements to note maximal acceptable chemical concentration based on "testing-based approach" of ecotoxicology. Handbooks have related the different kind of tests that have been developed to collect data about chemicals concentration, but the role of this concept extends beyond simple data collection. Moreover, the well-known environmental pollutants, at least in Europe and North America, are coming under the control of regulatory authorities and are declining. This part of ecotoxicology is now more or less completed. Although there is some work to do because monitoring polluted sites, evaluating new chemical substances, and developing alternatives scenarios will require a considerable effort, they are not expected to call for major scientific innovation and discovery. (NICO M. VAN STRAALEN). Moreover, non-ecologists often reply that ecology lacks the predictive power of physics and chemistry. Consequently, ecotoxicology can hardly profit from ecology because of the complexity of this discipline. Indeed, it contains so many relations and interactions between biotics and abiotic factors, and therefore is hardly predictable. *“Nature is not only more complex than we think. It is more complex than we can think”* Frank E. Egler.

2.2) Ecotoxicity Microbiotests

Since 1940, when the first bioassays were published and developed, the number of available toxicity assays has grown considerably. Indeed, this increase is due to the useful information and data that these tests can provides on toxicity of chemicals for the environment (C. R. JANSSEN ET AL.).
These kinds of bioassay are defined like environmental toxicity tests with invertebrates, in conjunction with measures of invertebrate’s community structure and chemical analyses of the ecosystem. The toxicity tests methods were developed with a variety of invertebrates, including *Daphnia magna* and *Ceriodaphnia dubia* that will be used during the internship.

These microbiotests are a combination of standard methods with toxicity tests, ecological analyses, and analytical chemistry. They present considerable advantages over existing standard single methods tailored for the assessment of specific areas. Moreover, toxicology and ecology discipline are integrated in invertebrates ecotoxicity testing and often implies combinations of laboratory testing, field studies, in situ exposures, and analytical chemistry (analyse of chemical). Afterward, this type of testing is a widely accepted method in ecotoxicology, because of the link between toxicology, ecology and several general characteristics of ecotoxicity micro biotests.

To argue again that these biotests involve many advantages, any aquatic ecosystems and wetlands which are degraded because of anthropogenic activities, can be asses by this kind of tool to lead environmental quality guidelines for preservation, protection and conservation of aquatic ecosystems. Moreover, they allow to monitor the implementation of regularization efforts and purification measures. They are an ecologically relevant group of organisms to test in order to adequately protect aquatic ecosystems. Moreover, the maintain of invertebrate’s cultures is simple in the lab, requires minimal area, and don't need flow conditions which simplifies the cultures of these organisms. They are tolerant when the environmental conditions change, such that dissolved oxygen, temperature and pH. Therefore, microbiotests are applicable in several research subjects. Additionally, the spatial mobility of aquatic invertebrates is limited, therefore, invertebrates which are collected, have a good representation of specific sites conditions (ADRIENNE J. BARTLETT AND WARREN NORWOOD).

Degradation of benthic area is a common beneficial use and they are used to develop and monitor programs to restore benthic conditions. Indeed, one survival strategy of zooplankton community to cope to unfavourable environment, is the production of dormant eggs. They are stored in sediments to create an egg bank where they might hatch when environmental conditions become better. But there is a lack of knowledge and references about the impact of salt on these eggs banks. This lack of knowledge can be address by microbiotests, and especially by tests on zooplankton communities that will be used for my supervisor project in link with my internshipth, like *Thamnocephalus Platyurus, Daphnia magna, Ceriodaphnia dubia* and *Brachionus calyciflorus* (S. SZKLAREK).

III) Significance of the project

From a certain dose, the salt become a pollutant which is toxic for aquatic and terrestrial organisms. Moreover, 51% of the salt production in the world is used for the salting road in cold country (especially in the northern hemisphere). Indeed, for example, some lakes in the United States could become so much salted in 2050, that there could be lots of impacts on aquatics species, with 10 millions of tons applied in the 1980s. Currently, because of road salt application, the salinity levels of many lakes, streams, and wetlands exceed the chronic and acute thresholds established for chloride (Cl^-) (KAYLA D. COLDSNOW ET AL.).

3.2) Roads salts pollution impact on macroinvertebrates

Many macroinvertebrate taxa (Ephemeroptera, Plecoptera, and Trichoptera) show tolerance to road salt. The diversity of macroinvertebrate stream communities did not vary significantly with differences in chloride concentration. A study was made by two scientists (Blasius and Meritt) who found that insects have a NaCl EC50 value in excess of aquatics systems concentrations measured. Indeed, concentrations used for the experiment were 1000 to 10000 mg/L of NaCl, whereas concentrations were 9 mg/L to 16 mg/L of NaCl in the stream site.

Moreover, a link between water temperature and salinity tolerance in Trichopterans has been shown. An experiment shown that trichopteran have a lower salinity tolerance when the temperature is high. When the salt is sown on the road during winter, a seasonal salt-pulse occurs at spring melt, when water is still cold and oxygen levels are high. During the summer there may be another salt-pulse concentration due to evaporation, that stress Trichopterans and lead a decrease in survival. Indeed, some species of the Limnephilidae family in larva stage are only found in winter, however, never in warmer months and in thermal springs (ATHENA TIWARI AND JOSEPH W. RACHLI).

Despite the evidence of macroinvertebrates resistance and resilience to roads ssats pollution, it has been shown that this pollution can alter zooplankton communities like Cladocerans, such as *Daphnia,* which are less resilient facing road salt pollution. Zooplankton are an important species, especially in the regulation of phytoplankton and to keep ecosystems services. Moreover, there is a lack of information on the impact of road salt pollution on zooplankton species with other salts than NaCl. Therefore, it's for why the present study will focus on zooplankton species and not on macroinvertebrates species.

3.2) Roads salts pollution impact on zooplankton

As a result, the salinity of surface water bodies is increasing that represents a threat for water ecosystems, not only in the winter periods (ATHENA TIWARI ET AL.). It was calculated that 60% of applied road salt during winter is washed to water systems during the snow melt period and winter rainfall. The other 40% are retained in the soil or in groundwater.

In Poland, the regulation and a limitation for the introduction of Cl^- into water bodies was established just for industrial sewage. However, no lows or national regulations was found to limit the introduction of chloride into water bodies through meltwater and stormwater. Therefore, aquatics ecosystems may be threatened especially in urban area with an increase of Cl^- concentration in the aquatic environment. This threat applies mainly on the zooplankton community. Indeed, zooplankton abundance and diversity decrease as salinity increases, contrary to macroinvertebrates. As above, experiments manipulating salt concentrations generally observe an increase in macroinvertebrates and a decline in Cladocerans communities. Cladocerans species (*Thamnocephalus Platyurus, Daphnia magna, Ceriodaphnia dubia* and *Brachionus calyciflorus)* are major consumers of phytoplankton. Their decline generally leads to trophic cascades with an increase in phytoplankton and a degradation of freshwater communities structure with a loss of ecosystem services (KAYLA D. COLDSNOW ET AL). The genus *Daphnia* in Cladocerans order, is one of the more primordial groups of zooplankton. They are essential to aquatic ecosystems as a preferred food source for many planktivorous fish and a primary consumer of phytoplankton. Therefore, their roles are vitals and primes in freshwater communities and food webs, and it is essential to evaluate if zooplankton, especially Daphnia, can adapt to high salinities resulting from road salt contamination to maintain a good quality of aquatics ecosystems (S. SZKLAREK).

It has been found that for a temperature of 20°C and a concentration higher than 1000 mg/L of Cl^-, there is a significant increase of Cladocerans mortality in adult individuals. A study was made by Coldsnow in 2017 with *Daphnia pulex* that were exposed to similar salinity (NaCl) in environment. After two-and-a-half-month exposure, these Cladocerans were tested to find an evolution on their tolerance to the salinity. Results showed that *Daphnia pulex* evolved their tolerance to roads salts, but their evolution has a limit. However, there is a lack of information about the impact of high chloride concentrations that can appear in the environment (above 3000-4000 mg/l) caused by salting

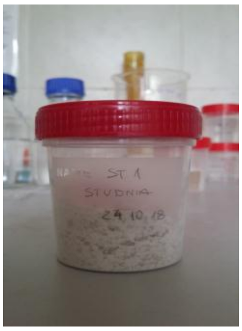
road, on zooplankton species (KAYLA D. COLDSNOW ET AL.).
Due to the toxic rate of NaCl on zooplankton communities and environment, the need to identify other less toxic salts can't be negligible. A study has reported that other salts such as $CaCl_2$ and $MgCl_2$, used as pH regulators or corrosion inhibitors, may have lower toxicity than NaCl against *Daphnia* species or wider against Cladocerans, even though all salts (beside NaCl) possess an "ecolabel" (product which have a lower impact on environment). However, the lack of information about the impact of roads salts, except for pure NaCl, on a wider group of zooplankton species prove the significance of the present study (S. SZKLAREK).The main topic of the internship will provide information about the difference between the common used roads salts (NaCl) and others type of roads salts. Thanks to the calculation of EC50 value for different salt, the impact that they have on zooplankton communities can be interpreted. Moreover, this study will bring knowledge on which species among tests organisms is the most resistant to salt pollution, on control and preventing approaches, and on the link between chloride and zooplankton's death-rate. After seeing fundamental concepts and the importance of the topic, we will see in the second part, the material and the methods that was used to do the experiment.

IV) Materials and Methods

The present study aims to have more knowledge about the impact of 3 road salts commonly used in Poland, on 4 zooplankton species (*Thamnocephalus Platyurus, Daphnia magna, Ceriodaphnia dubia* and *Brachionus calyciflorus)* which are present in Poland water bodies. The main goal is to determine the EC50 value for these salts. In all, 24 experiments were done. Indeed, all experiments was done twice as it will be explained latter.

4.1) Presentation of salts

The different kind of salts (Fig. 3) which have been tested during the internship are commonly used for road salting during winter in Poland. They were bought on the market like salting company, to make the experiments with the similar components that are used during the winter.



NaCl

Ice Go

Lodolamacz

Fig. 3 The different salts that are tested during the experiments

To test the impact of different salts concentrations on zooplanktons (aquatics organisms), dilutions were done. For all salts, the initial tested concentrations in the first step is the same (**1,52 g/ 100 ml of salt**), because it's the highest salt concentration that has been found in the environment during the melting snow, in the urban area of Lodz, Poland. A scale was used to weigh the exact weight of salt to put it in the 100ml tube. Afterwards, a dilution series (100% - 50% - 25% - 12.5% and 6.25%) of the effluent sample is prepared by the serial dilution procedure (Annex 1). Each dilution is made by diluting the previous concentration by half (Fig. 4). They are diluted with EPA or ISO Medium solution that are described in the next part.

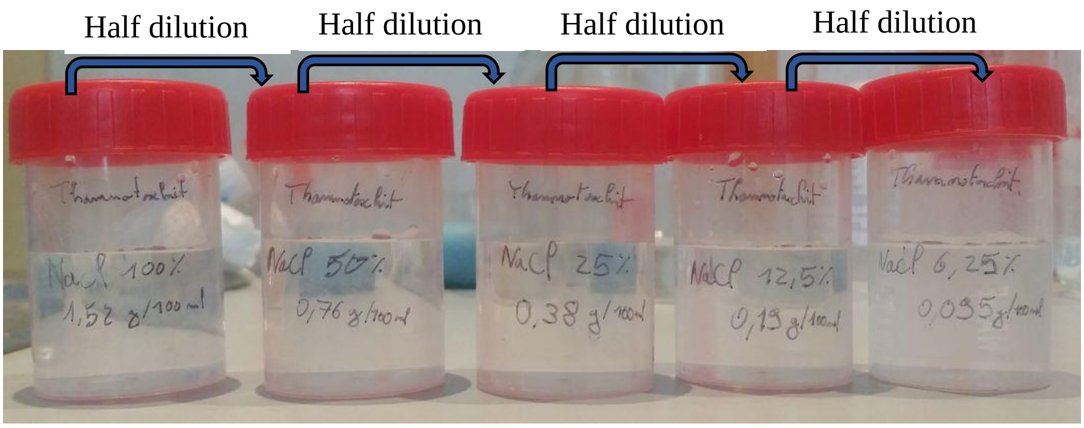


Fig. 4 Presentation of dilutions series for the first round of experiments

4.2) The Chromatography

Each testing salt is different from the others. Indeed, their concentration in Chloride are different. Knowing that the chloride (Cl-) is the chemical compounds in the salt that present the biggest impact for zooplankton community (KAYLA D. COLDSNOW ET AL.), it's important to know what the proportion in Chloride of each salt is. The knowledge of this parameter is also important to compare each salt and to prove the link or not between chloride concentration and the death-rate of each organism. Therefore, to know the concentration for each ion (Na+, Cl-, K+, Mg+) which composed the salts, a chromatography was done using a Dionex® ion chromatograph (Fig. 5). The ions curve was sent to a software to know the exact concentration of each ions.

Fig. 5 Dionex® ion chromatograph

A dilution series was made in the same way that for the preparation of salts solutions (100% - 50% - 25% - 12.5% and 6.25%). The samples were done as the following way:

First, five samples must be done according to the dilution series, for each salt. All the samples of one salt must be one next to the others, starting with lowest concentration to the highest. Each group of salt dilution are separated by a sample of distil water, to wash the Dionex® ion chromatograph after each salt analyses. It can be important to notice that salts dilutions are filtrated before to put 8 ml of them in tubes (Fig. 6) for the chromatograph.

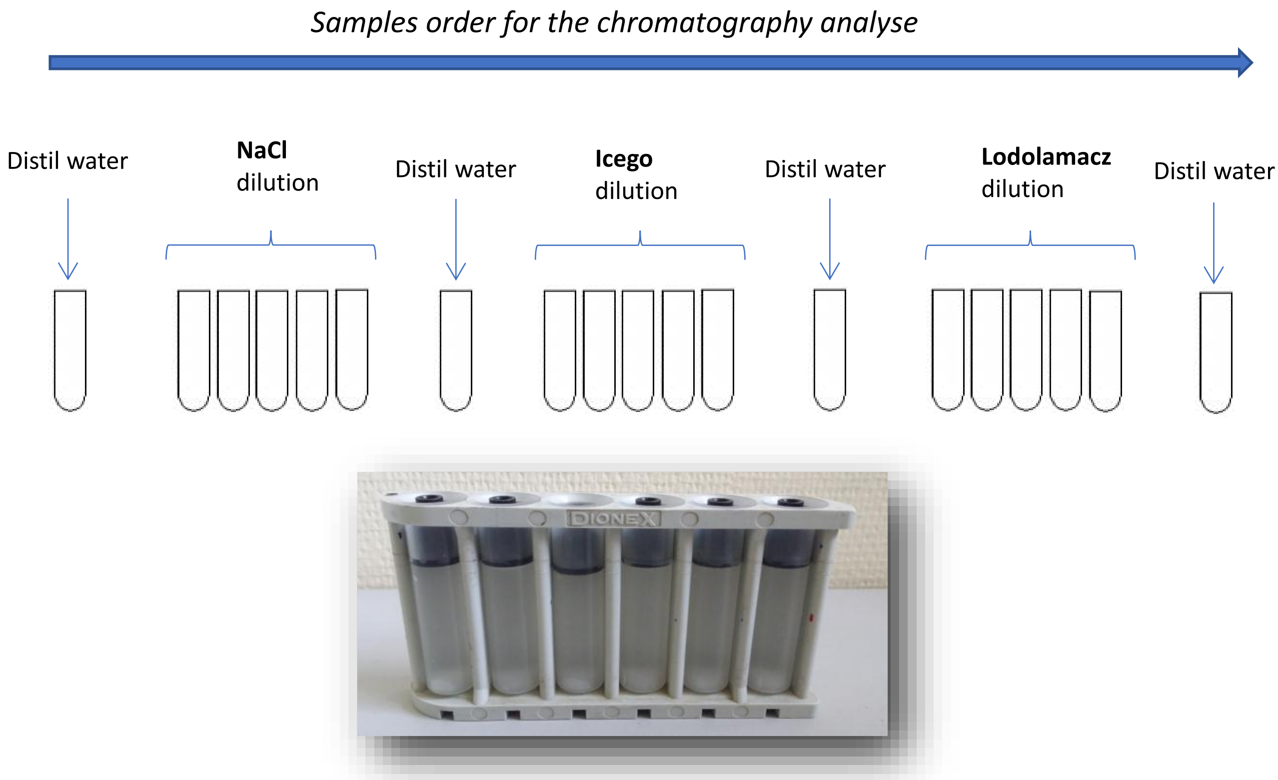


Fig. 6 Sample order for the chromatograph analyses and test tubes holder composed by distil water and the first salt dilutions

4.2) Presentation of the hatching step

TOXKITS are microbiotests in kits containing all necessary materials, including the test organisms to perform simple, rapid, sensitive and reproducible toxicity tests at low cost. Toxkit microbiotests are suited for toxicity testing of chemicals and wastes, released in aquatic as well as terrestrial. The principle is a 24 h EC50 bioassay is performed in a multiwells test plate using instar zooplanktons larvae which are hatched from cysts. Therefore, the first step is the hatching of zooplanktons eggs which are placed in test tubes at 5°C. Below is presented the hatching step for each zooplankton.

Therefore, for ***Thamnocephalus platyurus, Brachionus calyciflorus*** and ***Ceriodaphnia dubia*** (Annex 2), a standard freshwater which is a “moderately hard water” according to the US EPA formula, is prepared. Vials with concentrated salt solutions provided in the kit are used with deionized water to prepare one litter of standard (artificial) freshwater. This solution mixed to deionized water with known standard volume is used to allow the cysts hatching. It’s called « diluted medium ». After this step, these eggs are placed in a petri dish for the incubation during different times according to the type of zooplanktons (Fig. 7). Indeed, the incubation time for these three organisms are:

- *Thamnocephalus platyurus*: incubate at 25°C for 20-22 hours.
- *Brachionus calyciflorus*: incubate at 25°C for 16-18 hours
- *Ceriodaphnia dubia*: inubate at 25°C for 72 hours

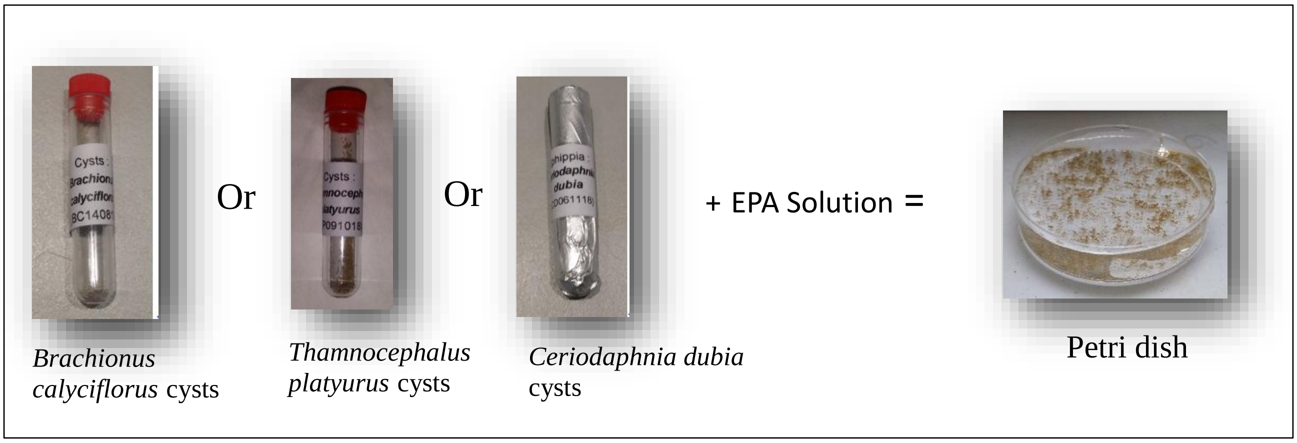


Fig. 7 Presentation of the procedure for the eggs hatching

Afterwards, for ***Daphnia magna*** (Annex 2), the standard freshwater is different than above. Indeed, a "reconstituted" natural freshwater is prepared for this organism, according to the formula recommended by the International Standardization Organization (ISO). As above, vials with concentrated salt solution are used to prepare 2 litters of standard (artificial) freshwater with deionized water. After this step, the cysts are washed by tap water using a filter (it can be notice that ***Ceriodaphnia dubia*** eggs are also washed by tap water) and they are put in a petri dish as above to allow the cysts hatching (Fig. 8). The incubation time for these organisms are different from the others:

- *Daphnia magna*: incubate at 25°C for 80 hours

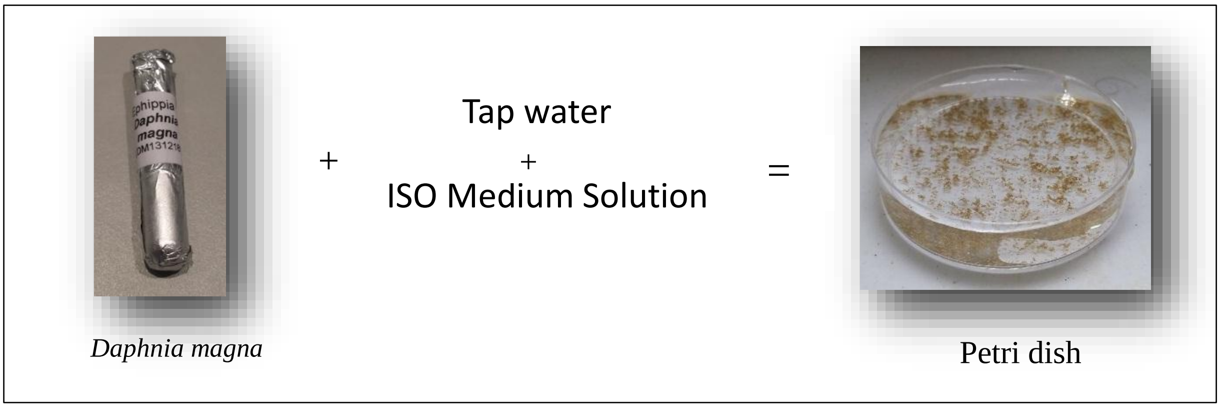


Fig. 8 Presentation of the procedure for Daphnia magna eggs hatching

4.3) Presentation of the plates and first results

Each toxicant dilution is filled into all multi-well plate. As in the diagram, the wells are labelled from A to D vertically and from 1 to 6 horizontally. The toxicants are filled all the time from the control solution (just EPA or ISO) in column 1 to the highest concentration of salt in column 6. They are filled in each wells of their respective column.

As the presentation of zooplankton, the multiwells plates (Fig. 9) and the adding are different according to the type of organism. Indeed, for ***Thamnocephalus platyurus, Brachionus calyciflorus*** and ***Ceriodaphnia dubia*** when the multi-wells plates are filled, there is the step of adding larvae. For that, a micropipette is used to transfer all larvae into the multi-well plate in two steps. First, the larvae are transferred from the petri dish into the wells labelled D1 to D6. They are rinsing wells, to minimize the dilution of toxicant solutions in the test's wells. Approximately 50 larvae must be transferred in each rising well. Secondly, the larvae are transferred from the rinsing well into the test wells in their respective columns. These transfers must be made with a dissection microscope because of the organism's size and to count exactly how many larvae exit the micropipette, to confirm transfer of 10 organism per tests well. Afterwards, a parafilm strip is placed on the top of each multiwells plate to put it in the incubator at 25°C in darkness for 24 hours for each test organism.

Fig. 9 Presentation of the multiwells plates to add Thamnocephalus platyurus, Brachionus calyciflorus and Ceriodaphnia dubia

Afterwards, for ***Daphnia magna*** the adding is different. Indeed, these multiwells plates present 24 test's wells and the volume to add is 10 ml in each well. Indeed, *Daphnia* being the biggest test organisms, they need more place to live. Also, the number of larvae to add is at least 20 *Daphnia magna* in each rising well and 5 in each test's wells. The transfer is done in the same way than for the others. Unlike above, there is 2 incubation time with a respective duration of 24 hours and 48 hours. Therefore, there is also 2 reading of results to determine the 24 hours EC50 value and the 48 hours EC50 value.

When the incubation time has passed, there is the counting step. As above, the dissection microscope is used because of the organism's sizes. It's important to noticed that toxicity test is acceptable if the percentage of mortality for tests organisms in the controls is not higher than 10%.
For each column, the total number of dead and alive larvae is counted, apart from rising wells, to

know the percentage of dead larvae in each salt's concentrations. With these results, EC50 values can be known approximately but not so much precise. That's why a second-round experiment is done as below.

4.4) Second-round experiments

Knowing that the main goal is to determine the EC50 24h value for each salt, the same experiment must be made two times with more specific concentrations around and close to this value. Indeed, after the first-round experiment for each salt and each organism, EC50 values are just known approximately, but because of the little data that we have around this latter and to be more precise, a second-round experiment must be done.

Therefore, after the first test, we must check what are the concentrations value that include 50% of dead test organisms.

For all second-round experiments, 5 concentrations between the highest and the lowest concentration which includes the EC50 value must be done, and one control to have one complete plate. We can notice that the highest and the lowest concentration which includes EC50 are included in these 5 concentrations.

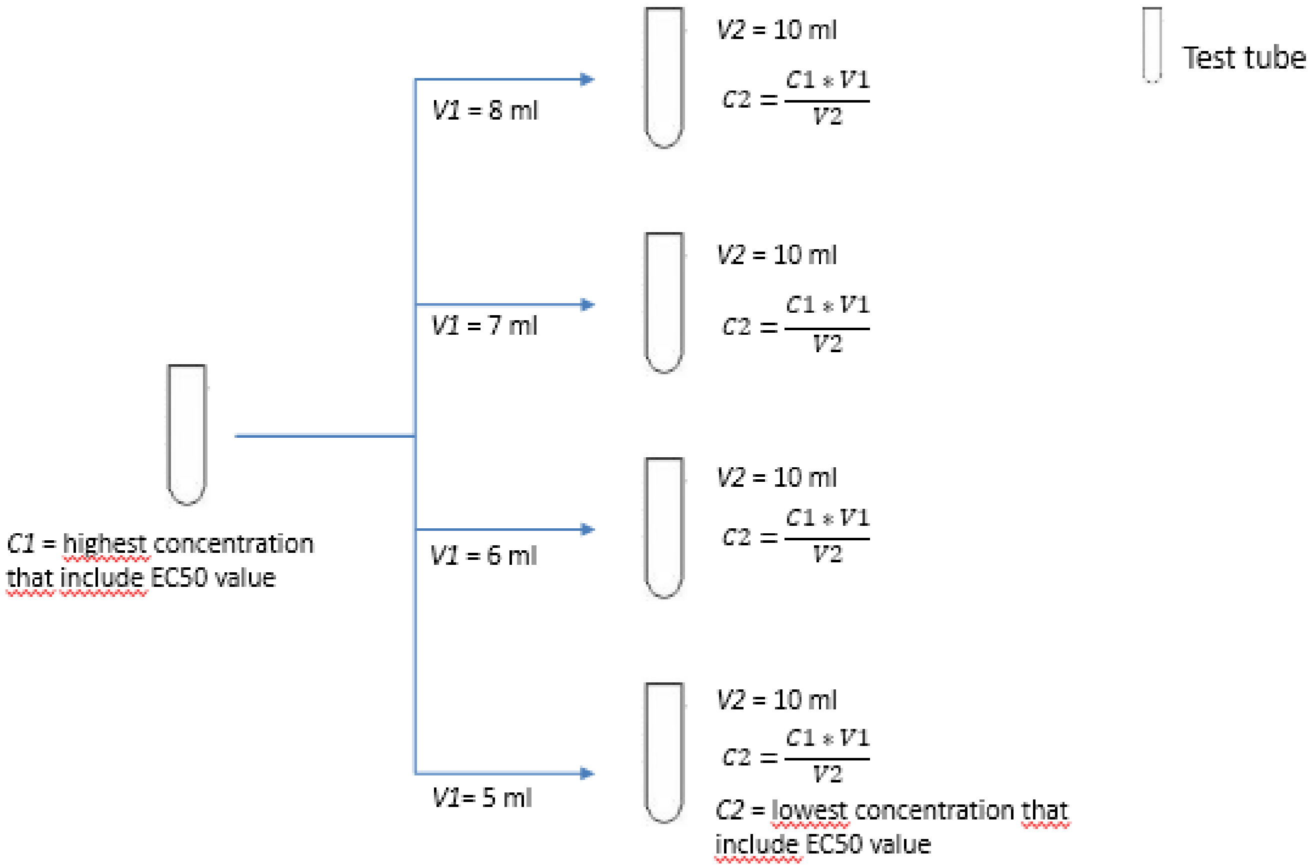


Fig. 10 Diagram of the dilution series for the second round experiment

The values (concentrations and volumes Fig. 10) are known except for the concentrations *C2*. Indeed, these concentrations must be known, because they step in the research of the EC50 value. Therefore, the first goal is to find concentrations *C2*. Knowing that there is the relation “ $\boldsymbol{C}1 * \boldsymbol{V}1 = \boldsymbol{C}2 * \boldsymbol{V}2$ “the unknown concentration *C2* is equal to:

$$\boldsymbol{C}2 = \frac{\boldsymbol{C}1 * \boldsymbol{V}1}{\boldsymbol{V}2}$$

After finding all new concentrations, the experiment can be made and based on the same protocol than in the part “presentation of the pates and first results”, to be closer to the EC50 value for all organisms. Therefore, the results sheet can be completed with more data for finding the EC50 value more precisely.
It can be notice that the first adding volume start at 8 ml and not at 9 ml to have 5 new concentrations and one control

4.5) Calculation of the LC50 value

There are many procedures for calculating 50% effect thresholds. Indeed, data treatment program on Excel software, to calculate the 24h EC50 value for the different microbiotests are available. First, calculations were done to determine the mean percentage mortality in the control and in each test concentration. Afterwards, the determination of the 24h EC50 is done by an appropriate statistical method moving average, depending on the mortality values in the dilution series.

Other models may be used depending on the shape of the dose-response curve. Indeed, thanks to the curve’s equation, the concentration when 50% of tests organisms die can be determine. As the objective is to obtain the best fit to the data.

V) Results

It will be show in the first part the results of the chromatography to see what their respective concentration in chloride are, and therefore, which salt can be the most impacting for zooplankton communities. Afterwards, the result of different dose-response curve for each salt according to each organism will be described to know the EC50 values, and which salt can be the most impacting. This part will be focus only on EC50 values that is the most relevant lethal concentration to compare with the other test organism.

5.1) Chloride concentration of each salts

First, testing salts are diluted with EPA (moderate hard water that is prepared with salt concentration), therefore, concentration of some ions according to the chromatography can be higher than concentrations salts in the test tubes.

Ions	Cl^- (mg/L)	Na^+ (mg/L)	Mg^{2+} (mg/L)	Ca^{2+} (mg/L)
NaCl	13151,1	5807,6	155,8	322,3
Icego	50,0	425,2	151,9	325,6
Lodolamacz	15408,7	2462,0	115,6	5561,6

Table 1. Ions concentration of each salt

According to the results (Table. 1), the concentration of chloride for Icego is very low (50,0 mg/L of chloride for 15200 mg/L of Icego). Indeed, it's 263 times lower than the chloride concentration in NaCl and 308 times lower than the chloride concentration in Lodolamacz. Therefore, Icego is the salt used on the road during the winter with the lowest concentration of chloride. Afterwards, for a concentration of 15200 mg/L for each salt, NaCl contains 13151,1 mg/L of Cl^- and 15408,7 mg/L of Cl^- for Lodolmacz. Therefore, Lodolamacz contains the highest concentration of chloride. However, according to the results, the chloride concentration for Lodolamacz is higher than the salt solution concentration. It's because the salt was diluted with EPA solution which presents a low concentration of chloride. Therefore, it can make that the result can be a little higher than the initial salt concentration (15200 mg/L).

About the other ions, the results show that the proportion of Mg^{2+} are close for each salt and low compared to the other ions. However, there is a significant difference between the concentrations of Na^+. Indeed, NaCl contains the highest concentration of Na^+ (5807,6 mg/L), followed by Lodolamacz (2462,0 mg/L) and by Icego (425,2 mg/L of Na^+). Moreover, the proportion of Ca^{2+} present a significative difference according to Lodolamacz, that present a concentration of calcium 17 times higher than for the two other salt.

Therefore, according to the concentration of Na^+ for NaCL, and the concentration of Ca^{2+} for Lodolamacz, it shows that NaCl is **sodium chloride**, and Lodolamacz is **calcium chloride.**

5.2) The most common salt NaCl

According to the results about the impacts of the most common salt (Table. 2), it shows that the EC50 values are between 5239,7 mg/L for *Daphnia magna* and 3245,6 mg/L for *Thamnocephalus platyurus*. These values don't vary too much with this salt to compare to the other two.

Organisms	*Thamnocephalus Platyrurus*	*Brachionus calyciflorus*	*Ceriodaphnia dubia*	*Daphnia magna*
EC50 Value (mg/L)	3245,6	3364,9	4238,8	5239,7

Table 2. NaCl EC50 24h value for each organism

By referring to the dose-response curves of *Thamnocephalus platyurus* and *Brachionus calyciflorus* (Fig. 11), their EC50 value don't varies greatly (3245,6 mg/L for *Thamnocephalus platyurus* and 3364,9 mg/L for *Brachionus calyciflorus*). Indeed, the variation between these two values is 119 mg/L and shows almost the same response curve. Indeed, *Thamnocephalus platyurus* dose-response curve shows that the mortality of this specie starts to increase when the concentration of NaCl is around 950 mg/L, in the same way that *Brachionus* dose-response curve. Their mortality increases almost in the same way, except that the mortality of *Brachionus calyciflorus* is smaller than the other species when the concentration is around 3800 mg/L, causing a curve that varies less quickly than the other. However, the highest percentage of mortality is reached when the concentration of NaCl is around 7600 mg/L for these two organisms.
Afterwards, the EC50 value for *Cerodaphnia dubia* is 4238,8 mg/L, what makes it the second most important for this salt. The increase of the mortality for this spiece of zooplankton start at 1900 mg/L of NaCl, with an EC50 equal to 2500 mg/L (the value when 5% of the testing population already died). The mortality increases until the concentration reaches 7600 mg/L as *Thamnocephalus platyurus* and *Brachionus calyciflorus.*

Finally, the test organism which present the most important EC50 value for this salt is *Daphnia magna,* with 50% of the population that dies when NaCl concentration reach 5239,7 mg/L. According to the dose-response curve (Fig.11), the test organisms *Daphnia magna* starts to die for a higher salt concentration. Indeed, the mortality of this species start to increase at 3800 mg/L and reach the highest death-rate when the salt concentration is equal to 7600 mg/L as the other organisms above. It can be noticed that *Daphnia magna* have the highest EC50 value for the fastest increase in mortality. Moreover, the incubation time "24 hours" has been chosen to have more significant results and to compare more easily with the others test organisms.

Therefore, *Daphnia magna* can be the most resistant species in contact with this salt compared to the others testing species, presenting the higher EC50 value. Moreover, for each test organisms, 100% of mortality is reach for the same NaCl concentration (7600 mg/L) with lower variation between EC50 values than for other salts.

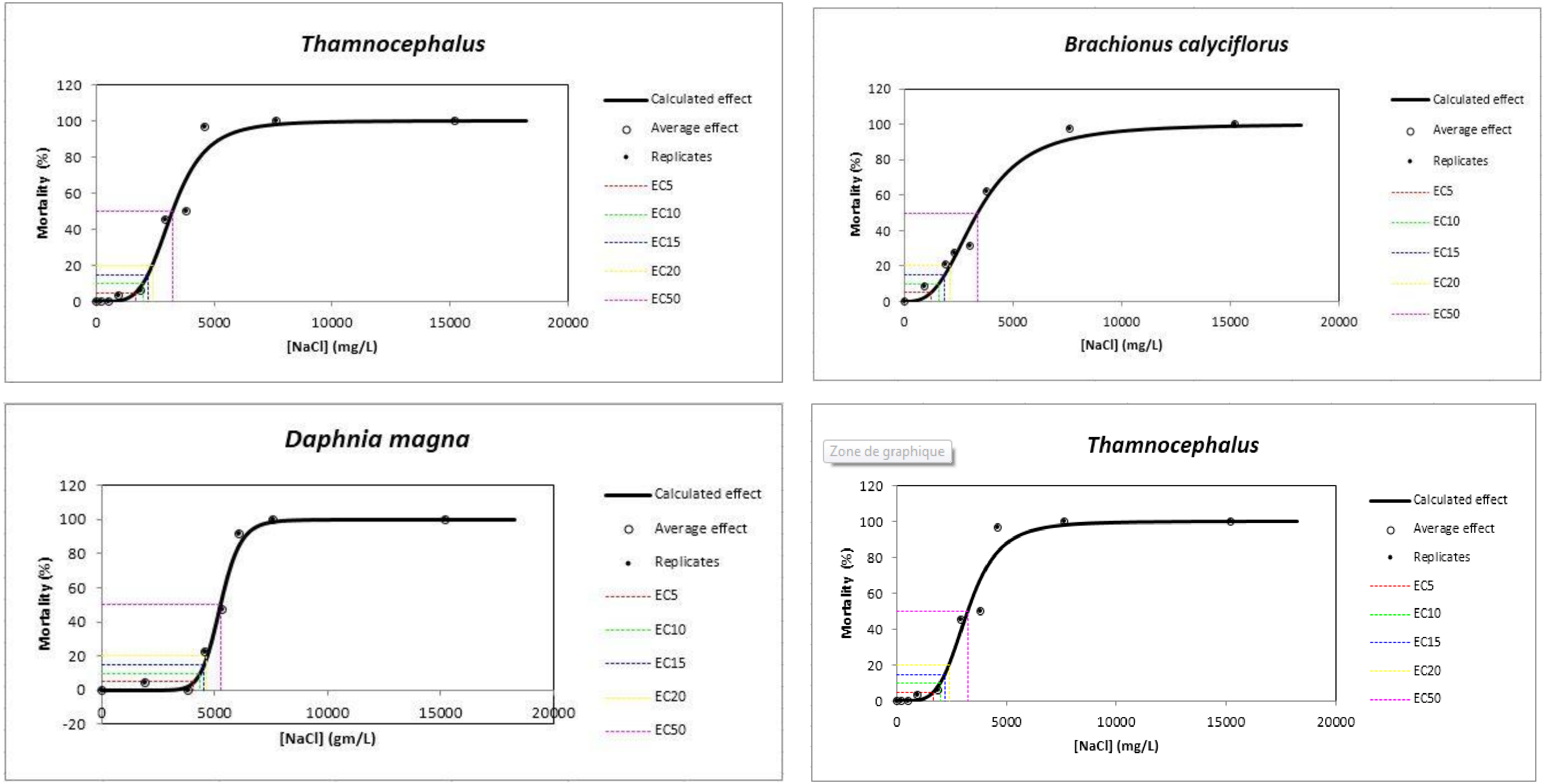


Fig. 11 Response curve of each organism according to NaCl concentration

5.2) Lodolamacz salt

According to the results (Table. 3), it shows that EC50 values varies from 1343,8 mg/L to 4394,7 mg/L. These results show also that EC50 values of each salts don't exceed 5000 mg/L according to each test zooplanktons species.

Organisms	*Thamnocephalus Platyrurus*	*Brachionus calyciflorus*	*Ceriodaphnia dubia*	*Daphnia magna*
EC50 Value (mg/L)	1343,8	2919,6	3934,8	4394,7

Table 3. NaCl EC50 24h value for each organism

First, according to the dose-response curve (Fig. 12) of *Thamnocephalus platyurus*, the mortality increases fast with an EC5 almost the same that the EC50. Indeed, with an EC5 equal to 683,7 mg/L and an EC50 equal to 1343,8 mg/L, there is a variation of 660,1 mg/L between the moment they start to die and when the EC50 value is reached. To compare to the others salts and the others test organisms, this species present the lowest EC50 value in contact with Lodolamacz. Moreover 100% of the mortality is reached when the concentration is equal to 3800mg/L of Lodolamacz. It can be noticed that 3800 mg/L of salts is the lowest concentration when a testing species reach 100% of mortality, all salts confused.

Afterwards, the second lowest EC50 for all salts confused, occurs for *Brachionus calyciflorus.* Indeed, the EC50 for this species is equal to 2919,6 mg/L. They start to die around 950 mg/L of Lodolamacz and reaches 100% of the mortality at 7600 mg/L in the same way that for the dose-response curve with NaCl. The difference with NaCl dose-response curve and why the EC50 is not the same, occurs between these two values. Indeed, compared to NaCl with the same concentration, the number of dead organisms is higher, that's why the EC50 value is lowest.
Finally, for *Ceriodpahnia dubia* and *Daphnia magna*, their EC50 value are respectively 3934,8 mg/L and 4394,7 mg/L. The difference between these two values is not important because they show a response curve which occur in the same way. Indeed, the species start to die around 1900 mg/L and reach 100% of the death-rate around 7600 mg/L.

Therefore, when the species are in contact with Lodolamacz, they present the lowest EC50 values compared to the other salts. Moreover, as with NaCl, *Daphnia magna* present the highest EC50 compared to the other species.

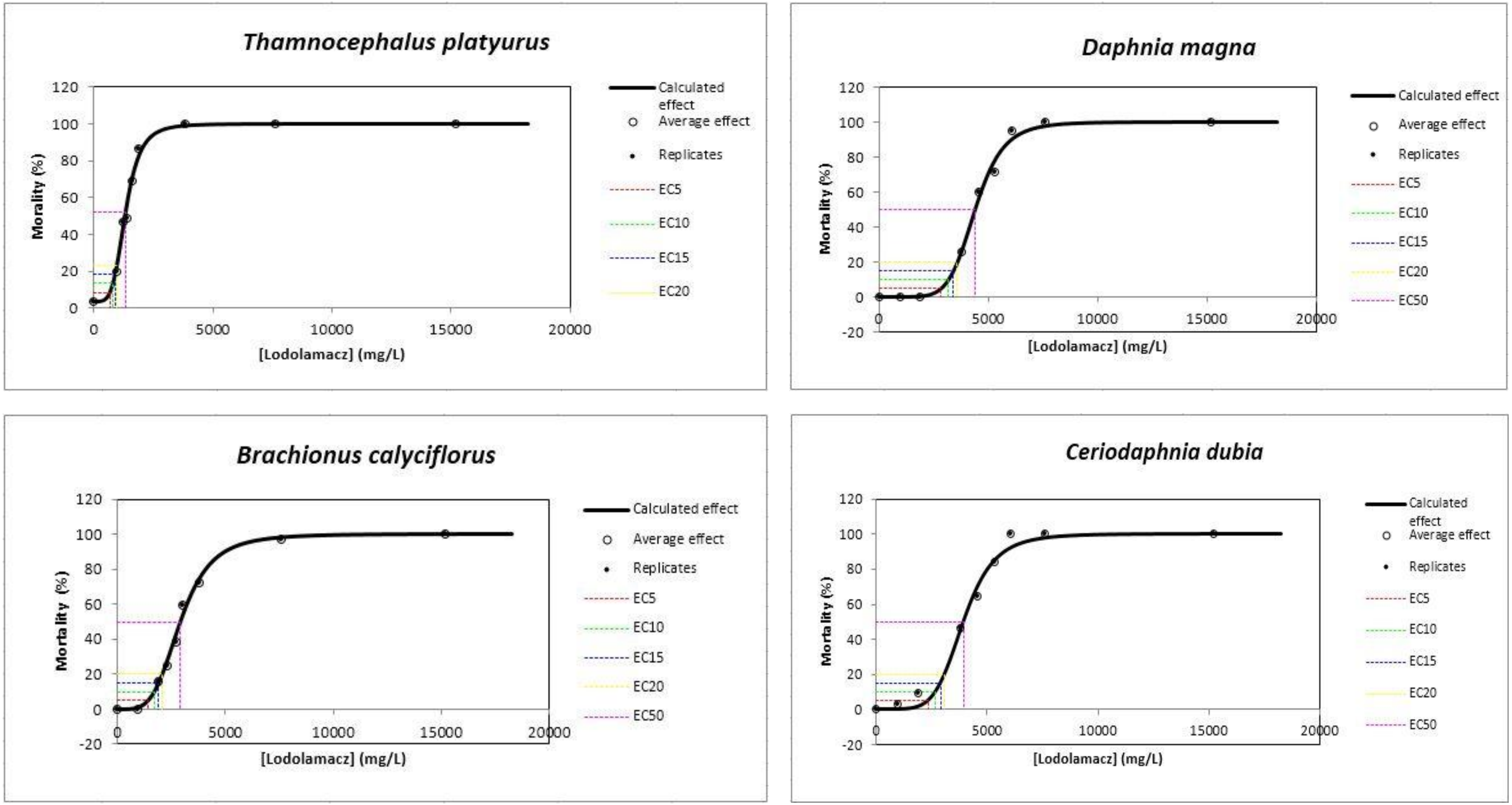


Fig. 12 Response curve of each organism according to Lodolamacz concentration

5.3) Icego salt

According to the following table, EC50 values for each salt according to test organisms is higher than the other salts. Indeed, for each organism, this value exceeds 15 200 mg/L.

Organisms	*Thamnocephalus Platyrurus*	*Brachionus calyciflorus*	*Ceriodaphnia dubia*	*Daphnia magna*
EC50 Value (mg/L)	18696,7	18721,5	20996,5	20290,3

Table 4. Icego EC50 24h value for each organisms

To compare EC50 values of each organisms (Table. 4), the results show that *Ceriodpahnia dubia* has the most important. Indeed, 50% of the population of *Ceriodpahnia dubia* died when the concentration of Icego reach 20996,5 mg/L. The dose-response curve for *Ceriodaphnia dubia* shows that these organisms start to die when the concentration of Icego reach 7600 mg/L. They start to die before *Thamnocephalus platyurus* and *Daphnia magna*, but their mortality curve increases slower than their latter. Indeed, the highest percentage of mortality is reach around 30000 mg/L. That's why their EC50 value is highe,r even if they start to die around 7600 mg/L.

The organism which has the second higher EC50 value is *Daphnia magna*. The EC50 value for these organisms is 20290,3 mg/L. Its dose-response curve looks like *Ceriodaphnia dubia,* except that the death-rate increases faster. Indeed, according to the dose-response curve, these organisms start to die around 15200 mg/L (the highest concentration of salt find on the field) of salt and reach the highest percentage of mortality around 30 000 mg/L as *Ceriodaphnia dubia*.

Afterwards, *Thamnocephalus platyurus* and *Brachionus calyciflorus* have a different dose-response curve compared to the other organisms. First of all, for *Thamnocephalus platyurus* the highest score of mortality is reach for an Icego concentration around 21300 mg/L, but the fact is, that the concentration when this zooplankton community start to die is 15200 mg/L. This specie has the fastest response curve in contact to this salt, with a variation of 6100 mg/L between the point when they start to die and the point when they reach 100 percent of death-rate. Finally, *Brachionus calyciflorus* in contrary to *Thamnocephalus platyurus,* has the slowest dose-response curve with a EC50 of 18721,5 mg/L, almost equal to *Thamncephalus platyurus* (18696,7 mg/L). Indeed, 5% of the population already died when the concentration reaches 3500 mg/L, and the graphic doesn't allow us to be sure when it reaches the highest percentage of mortality. That would be relevant to do another experiment with this salt and this organism, with higher concentration of Icego, to know exactly when the response curve reaches 100% of death-rate.

Therefore, according to the different dose-response curves in the and the different EC50 values, *Ceriodaphnia dubia* has the most important EC50, that means it can be the most resistant organisms against this salt. Also, the EC50 of each organism according to this salt are higher to compare to the other salts. It can mean that Icego present the lowest impact on these zooplankton community.

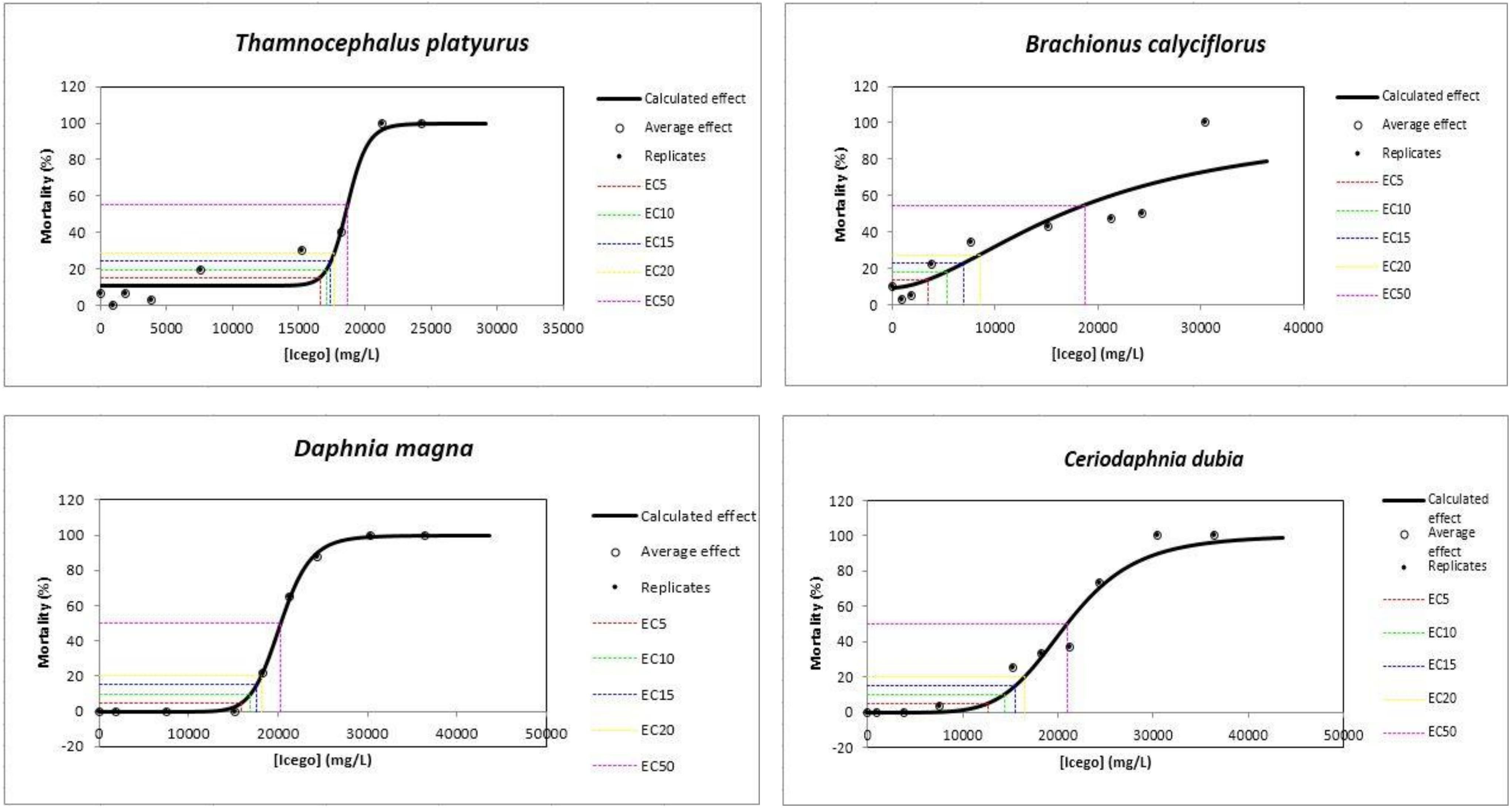


Fig. 13 Response curve of each organism according to Icego concentration

5.4) Comparaison of LC50 value

After having described all dose-response curves for each organism and each salt, it's relevant to compare more specifically what is the salt which leads the highest percentage of mortality in the different zooplankton's communities.

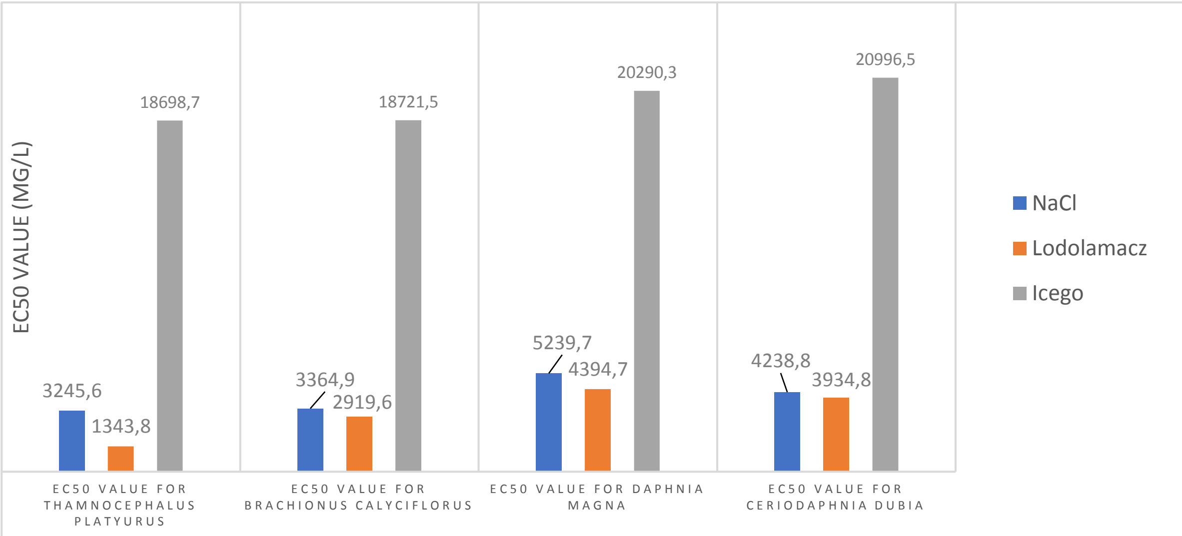


Fig. 14 Comparaison of EC50 values of each salt according to each organism

According to the results (Fig 14), Icego present the highest EC50 values that can be 5 to 6 times bigger than NaCl and 5 to 9 times bigger than Lodolamacz. To make the ranking, depending on the figure above, the salt which cause the death of 50% of the testing zooplankton population with the lowest concentration is Lodolamacz following by NaCl and finally by Icego.

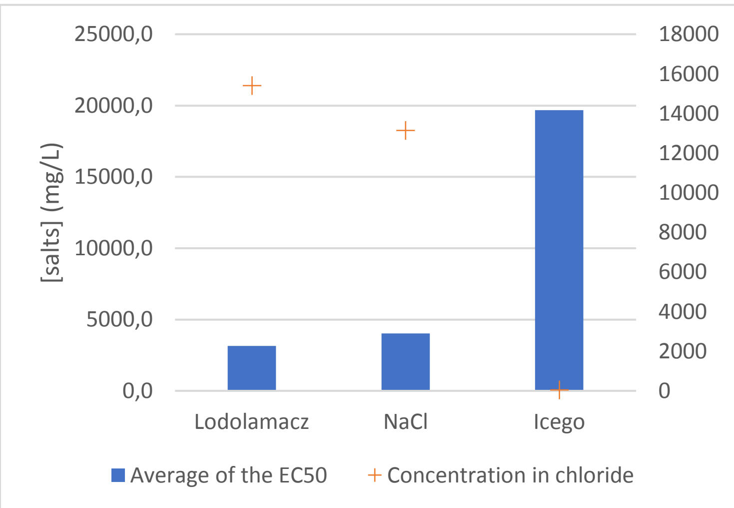


Fig. 15 Comparaison between the EC50 values of each salt and their concentration in chloride

To argue the results above, there is the average of the different EC50 values for each salt as well as the different concentration of chloride (Fig. 15). The average of the different EC50 values with Icego is equal to 19676,8 mg/L. It presents the highest concentration to compare to the others. However, the difference between the average for NaCl and Lodolamacz is not so important (874,1 mg/L) compared to Icego. All these results are coherent with those above.
Moreover, it shows that the average of EC50 values and the concentration chloride average for each salt evolve in the reverse way. Indeed, Lodolamacz contains the highest concentration in Cl^- and in same way, it presents the lowest EC50. On the opposite, Icego which present the highest average for the EC50 value, it contains the lowest concentration in Cl^-. Therefore, this figure show that more the chloride concentration is high, more the EC50 is low, and therefore, there may be a link between the proportion of Cl^- in salts and the zooplankton death-rate.

Discussion

Compared to the results of the chromatography, the ranking of the different chloride concentration is that Lodolamacz present the highest concentration of chloride, followed by NaCl and finally by Icego. The hypothesis can be that Lodolamacz present the most important impact on zooplankton, followed by NaCl and finally by Icego. To answer to this hypothesis and to the first problematic “which salt is the most impacting on the zooplankton communities”, EC50 values are used. Indeed, more EC50 value is low, more the impact on organisms is high. According to Fig. 15, it shows that the lowest EC50 value for all testing organisms confused occurs when they are in contact with the salt “Lodolamacz”. It means that 50% of the population of zooplankton die with a lower concentration of Lodolamacz than with the other. Therefore, Lodolamacz is the most impacting salt for zooplankton communities. As for NaCl, which present the second highest concentration of chloride, close to that one of Lodolamacz, it’s the second most impacting salt for zooplankton. Finally, as for Icego, its concentration in Cl^- s very inferior and the average of EC50 is very superior compared to the two other salts. That makes him the salt that present the lowest impact on zooplankton communities.
Compared to NaCl, because it was one of the problematics, it was said that the other salt presents a lowest impact than NaCl. However, to complete the lack of knowledge about the other salt used during the winter, this study shown that it’s not the case for all salt. Indeed, with Lodolamacz, it was shown that it presents a biggest impact on zooplankton communities. However, as for Icego, it presents a lowest impact on these communities compared to the sodium chloride NaCl. Moreover, to answer at the second issues, if there is link between the concentration of chloride in water and the death-rate in zooplankton communities, the study shows that they vary proportionally. Indeed, according to what it was said above, to the Fig. 15, and to the chloride concentration values presented in the Table. 2, it shows that more the concentration of chloride is important, more the EC50 values are low and more the zooplankton death-rate is high. Lodolamacz contains the highest concentration of chloride, and it is the most impacting, followed by NaCl and finally Icego.
However, are they more efficient than NaCl?

First, when salt is sown on the snow, this disruptor is dissolved with water. However, to be dissolve in the water, the salt needs energy, and will therefore draw the "heat" of the snow, which will then cool. This is called an endothermic character, that is, salt absorbs heat to be dissolve, heat it finds in the snow. Except that the saltwater, as we saw earlier, has a lower melting temperature than pure water and freezes below 0 ° C. The water salt mixture cannot become solid, only around -21°C for sodium chloride (NaCl) and around -32°C for calcium chloride (Lodolamacz). Afterwards, water salt mixture stabilizes at the ambient air temperature (WANG YZNCHUN ET AL.). According to this, Lodolamacz is more efficiency than NaCl, because water salt solution with Lodolamacz becomes solid at a lower temperature than NaCl, but it’s more impacting on zooplankton communities. However, about Icego, its concentration in Cl^-, Na^+ and Ca^{2+} are so low, that we may wonder if it really works to melt the snow and if it can be considered as a salt. This problematic can be a subject for a new study.

Afterwards, it’s significant to know which test organism is the most resistant to salt pollution. Indeed, zooplanktons being consumers of phytoplankton, that leads to a decline of chlorophyll concentration in the water, a better water transparency and it can reduce the eutrophication phenomenon and avoid the degradation of water ecosystems. However, these heterotroph biomasses don’t consume the same level of chlorophyll. Indeed, the size ratios between predator (zooplankton) and pray (phyto-plankton) are fixed with certain limits. Prey species rarely exceed their predators in size, but also there is a minimum size of prey, below which capture is inefficient for the predator (TOM FENCHEL). Therefore,

higher the zooplankton size is, higher the phytoplankton consumption. To return at the initial problematic, which test organism is the most resistant to salt pollution, it could be interesting if zooplanktons with bigger size are the most resistant to salt pollution. Indeed, as they have a higher consumption of phytoplankton, they are more efficient to avoid algal bloom. Knowing that *Daphnia* has the largest zooplankton size (WANG YZNCHUN ET AL.) and according to the Fig. 14, it shows that *Daphnia magna* is the most resistant test organisms and the biggest.
Therefore, if salts don't exceed a certain concentration in water, *Daphnia* have the highest probability to stay in the surface water. As they are more efficient to avoid algal bloom than other zooplanktons species, they can reduce eutrophication phenomenon and allow to have a better water quality.

Finally, knowing that salt pollution is a current and a future problem for aquatics ecosystems, it's relevant to know that there are solutions to overcome this problem. According to the present study, it's difficult to find a real and available solution that works, because the main topic wasn't to find solutions, but to increase knowledges about the impact of road salts used during the winter on zooplankton communities. However, according to the results, it's possible to find a way to reduce the impact of salt pollution. Indeed, Icego being the salt that present the lowest impact on zooplankton communities, it would be relevant to use it all the time. However, this salt is not very effective to melt the snow because of its low chloride concentration. Therefore, knowing the different EC50 value of each salts, it could be possible to set up a study of salts combinations to have an efficient salt mixture on the road during the winter, with a lowest impact on zooplankton communities. Moreover, the combination with sand and salts can be established. Indeed, the sand allow to recover the adequate adhesion for cars with no impact on the environment, and the salt got the power to melt the snow. However, one drawback can occur about the sand use. Indeed, the accumulation of sand on the road can obstruct the rainwater pipe, but it can be also collected and reused. It can be a sustainable solution about the snow treatment in urban areas during the winter. Therefore, a study of salt combination and sand/salts combination together could be a sustainable alternative to salt pollution.
Afterwards, a study was made on the optimisation of salting roads using an evolutionary algorithm. Indeed, according to the local urban geography, some road sections are warmer than others. Therefore, the optimisation of salting roads can be based on the temperature of routes. Indeed, using a dataset based on predicted temperature distribution, some financial saving can be made by reduce the salt level on these roads or even not salting the warmer roads. The real data reveal that this method can reduced the total distance travelled by 10% of the conventional salting roads (HISASHI HANDA, DAN LIN ET AL.).
Finally, another study was made and show the link between salt concentration and the coefficient of friction between a road surface and a vehicle tire according to the temperature. Indeed, instead of controlling salt amount to melt a given amount of ice or snow, it would be more relevant to control the proportion of salt is needed. In that way, it will prevent surface road to become too slippery and use only a fraction of salt as compared to current practises (HTAISTO HAAVASOJA ET AL.).

Conclusion

In Poland, there is just a regulation and a limitation for the introduction of Cl^- into water bodies, for industrial sewage. However, there are no lows or national regulations to limit the introduction of Cl^- into water bodies through meltwater and stormwater. Therefore, aquatics ecosystems may be threatened especially in urban area with an increase of Cl^- concentration in the environment. This threat applies mainly on the zooplankton community.
The present study of the internship brings information about the difference between the common used road salt (NaCl) and others type of road salt, especially about the impact that they have on zooplankton communities thanks to the calculation of EC50 values for several zooplanktons species tested for several roads salt. Moreover, it was also interesting to focus on which is the most resilient community and what are solutions to solve this current problem.

Therefore, the present study allowed to bring more knowledge about salts that are used in Poland during the winter, especially about their impact on zooplanktonic wildlife. Indeed, it was found that Lodolamacz, one of the salts used in winter, has a higher impact than the most common salt NaCl. However, the other salt Icego has a very low impact compared to the two others, but with a low efficiency to melt the snow and the ice, because of its low chloride concentration.
Afterwards, it was shown that *Daphnia magna* is the organism which is the most resistant to salt pollution. Knowing that it's the zooplankton species which consumes the most phytoplankton, if salts don't exceed a certain concentration in water, *Daphnia magna* have the highest probability to stay in the surface water. As they are more efficient to avoid algal bloom than the other zooplankton species, this allows to avoid eutrophication phenomenon and to have a better water quality.
Moreover, thanks to studies with different solutions to reduce the salt pollution impact on the environment. Indeed, according to the present study that we conducted, another study subject can be done, knowing each EC50 for each organism, to find combinations of salts that can have a lower impact on aquatics ecosystems. Moreover, mixture with sand can be done to reduce the amount of salt and increase the grip of the road. Afterwards, a study was based on the road temperature to reduce the salt levels sown on roads. With a dataset based on road temperature distribution, it allows to reduce the salt rate on these roads or even not salting these roads. Finally, another study shown that it was better to control proportions of salt needed, to prevent surface road to become too slippery and use only a fraction of salt as compared to current practises. It can be done according to vehicles tires, salt concentration and the coefficient of friction.

This internship was very interesting and knowledge-rich for me. I learnt the concept of Ecotoxicology and the use of macroinvertebrates bioassays. I also learnt a lot of things about current problems of road salting, especially in north countries, on aquatic ecosystems. I acquired knowledge about ecology, toxicology and in laboratory technique to how done microbioassays. I achieved the main objective project. Indeed, I provided new data about the impact of roads salt used in Poland compared to the most common salt NaCl. This comparison was made thanks to the calculation of EC50 for each test organisms. Moreover, the present study allowed to find also which is the most efficient on roads, according to the different ions concentration which composes it, and which zooplankton species is most resistant to salt pollution.

During the internship, the different spots was done only by myself and it was rewarding, because I learnt how to work in an autonomous way and reading a lot of publications. Working completely autonomous was also a good experience to manage the study as I wanted. This experience taught me new work way and methods. I adapted myself to work more open-minded and in their way. After this internship and this experience abroad, I convinced I am really interested about ecological subject, ecological processes, and in aquatic environment interactions, with a goal to solved problems caused by humans. For the next internship, I will orientate myself more in fish biomanipulation that is also a subject that interested me a lot, and to make more field trip to be in the centre of fish interactions and learn more about biomanipulation.

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Glossary

EC50: The concentrations of the chemical in water that kills 50% of the test animals during the observation period is the EC_{50} value.

Bloom: Great increase of phytoplankton, algae or cyanobacteriae as answer of high level of nutrients.

Cyanobacteria: Bacteria which produce energy thanks to photosynthesis.

Macroinvertebrates: animals that neither possess nor develop a vertebral column, that humans can see with naked eyes.

Zooplankton: Zooplankton (pictured below) are a type of heterotrophic plankton that range from microscopic organisms to large species, such as jellyfish.

Heterotrophes: Organisms which need organic matter for their development.

Heutrophication: Nutrients accumulation in water bodies that leads to an imbalance in aquatic environment.

Nutrients: Chemical elements and compounds necessary for the plant to growth and reproduced. It includes the macronutrients (N, P, K, C, O etc) and the trace minerals (Fe, Cu, Mo etc)

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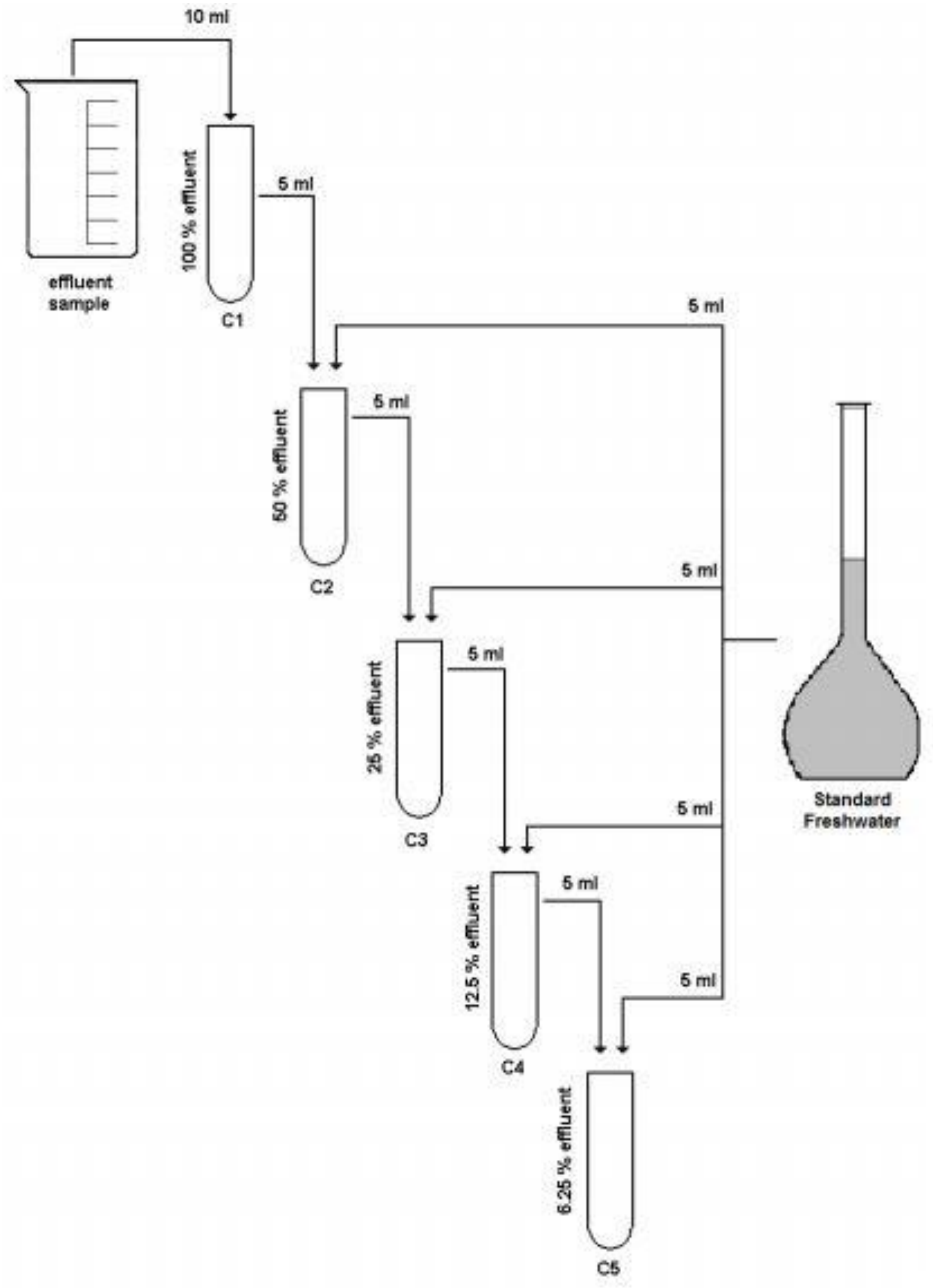
Annex

Annex. 1 serial procedure dilution for each salt



Annex. 2 The different test organismes *(Thamnocephalus platyurus, Brachionus calyciflorus, Daphnia magna* and *Ceriodaphnia dubia)*

Thamnocephalus platyurus

*Source:*https://www.flickr.com/photos/44150996@N06/6758680949

Brachionus calyciflorus

*Source:*http://www.plingfactory.de/Science/Atlas/KennkartenTiere/Rotifers/01RotEng/source/Brachionus%20calyciflorus1.html

Ceriodaphnia dubia

*Source:*http://cfb.unh.edu/cfbkey/html/Organisms/CCladocera/FDaphnidae/GCeriodaphnia/Ceriodaphnia_dubia/ceriodaphniadubia.html

Daphnia magna

*Source:*https://fr.wikipedia.org/wiki/Daphnie

35 ALLÉE FERDINAND DE LESSEPS
37200 TOURS

Bastien SALABERT
2018-2019

Titre: Impact of roads salts pollution on zooplanktons species

Résumé: The main topic of the study is the impact of 3 roads salts pollution on 4 zooplanktons species to complete knowledges about this subject. Microbiotests was used to show the impact of the different salts. A comparison was made between these salts to know which one present the highest impact on zooplankton species. It was shown that it's not NaCl which present the highest impact but another used salt.

Mots Clés: Microbiotests, EC50, zooplanktons, roads salts, impact

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