Ecobiological study of *Artemia salina* L. and first determination of the toxicity of ammonium sulphate

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Abstract: The objective of this work is firstly an ecobiological study, which aims to determine whether salinity is favorable for a good reproduction rate of cysts in the *Artemia salina* L., a species known for its use in aquaculture. Secondly, a short-term test of ammonium sulfate toxicity on its vitality is carried out as its environment is permanently subjected to sulphate-laden waste. The work consists of subjecting the species to different salinities in order to produce a good yield in cysts, which once recovered, hatching and breeding is maintained until individuals reach the adult stage. A toxicological study using toxicity test (CL50) is then conducted to study the resistance of these adults with various concentrations of ammonium sulphate. The results obtained from the ecobiological study show a mean similar to the natural environment ($p > 0.05$) with a favorable concentration for the reproduction of the species at a salinity of about 75 g dm^{-3} . The toxicity test indicates a CL50 equal to 75 mg dm^{-3} after 24 hours. It is obvious that *Artemia salina* L. can be cultivated by controlling its reproduction by varying the salinity rate for its use during different seasons of the year, thus ammonium sulphate from waste is considered as a limiting factor.

Key words: *Artemia salina* L., salinities, reproduction, toxicity, ammonium sulphate

Introduction

Ecological factors, such as salinity and temperature, play a very important role in the development of zoo planktonic species (Hoffmeyer 1994; Calbet et al. 2001; Vieira and Bio 2011; Lei et al. 2009; Bennabi et al. 2015). Thus, these crustaceans are characterized by a variation of their organic fluids in ionic compounds, that is, it is interesting to study their interactions with their environment (Parekh and Tank 2015).

Indeed, these species along with fish, being inhabitants of limnic ecosystems, cannot escape from the effects of industrial pollution and they are widely used to assess the health of ecosystems (Parekh and Tank 2015).

The salines of Bethioua (western Algeria) constitute a favorable environment for the development of crustacean species, of which *Artemia salina* L. remains the characteristic species of this environment. Unfortunately, this habitat is subjected to continued deterioration after various releases concentrated in sulphate generated by a salt factory built to improve the economic sector in the region (Bennabi et al. 2015).

Artemia salina L. easily adapts to changes in salinity and pH and to laboratory conditions, which justifies the selection of this species for acute toxicity testing to

evaluate the impact of the factory on its survival in its natural environment (Kherraz-Chemlal et al. 2017).

Static toxicity tests provide faster and more repeatable dose-response curves for estimating the toxic effects of chemicals on aquatic organisms. Typically, acute toxicity tests are used to estimate the exposure concentration resulting in 50% mortality (LC50) of the organisms tested during a specified exposure period (Parekh and Tank 2015).

In this context, the objective of our work is to study the toxicity of ammonium sulphate on its survival in order to determine tolerable thresholds for its viability.

Material and Methods

Biological material

Artemia salina L. is a crustacean widely distributed in saline and hyper saline lakes around the world. Due to its privileged position in the food chain of fish larvae and shrimp post-larvae, this biological material has attracted the attention of scientists and has been the subject of extensive research. It is able to survive in saline environments (hyper-halines) in salt lakes, lagoons and salt marshes locally called “Sebkha”. This species occupies biotopes with a tropical, subtropical or temperate

Fig. 1. Location of the collection area of *Artemia salina* L. species

climate (Lavens and Sorgeloos 2000). When the living conditions of the environment are no longer favorable after hydration to give birth to a larva called nauplius, in natural conditions *Artemia salina* L. is able to produce cysts, which are able to feed on algae, protozoa and detritus (Abatzopoulos et al. 2002).

Sampling

The species was collected from the Bethioua salt mines with geographical coordinates $0^{\circ}17'50.92''$ W and $35^{\circ}41'38.70''$ N. They are part of the Oran Province, Bethioua District and the municipality of Boufatis. This salt is located in the south of a depression called "Arzew Salt Lake" (Fig. 1) with an area of 2700 hectares and characterized by a Mediterranean climate. Winter rains fill the lake and dissolve the salt layer lining the ground. The lake is currently under the direction of the National Salt Company (NSC).

Toxicity test

To obtain healthy adult species for the performance of the toxicity test, *in vitro* breeding from the cysts was performed.

First, to obtain a maximum number of cysts, seven concentrations of crude NaCl (50, 75, 100, 125, 150, 175, 200 g dm⁻³) were prepared in beakers each containing 10 females. A control was performed in parallel with females placed in a beaker containing saline wa-

ter. Egg recovery was carried out according to the technique described by Person-Le Ruyet (Person-Le Ruyet 1976; Trigui et al. 2016).

Once the cysts were recovered, hatching and rearing followed to obtain biological material for the toxicity test. The toxicity test was performed according to the OECD (1992) method. To do this, we performed a 72 hour acute toxicity test on *Artemia salina* L. specimens recovered from our farm. Six concentrations of ammonium sulphate were prepared (50, 75, 100, 125, 150 and 200 mg dm⁻³) to test the tolerance of this species and to determine the LC₅₀ (OECD 1992).

Statistical Analysis

The collected data were expressed as mean values ($\pm$ standard deviation). The data analysis was carried out using IBM SPSS Statistics, version 23.0 (IBM Corp. 2015). A one-way analysis of variance was used, followed by a significant Dunett's posteriori test. The differences are considered significant if $p < 0.05$, highly significant if $p < 0.01$ or very highly significant if $p < 0.001$.

Results and discussions

Cysts Production

After being subjected to different concentrations, *Artemia salina* L. produced a maximum number of (87 ± 4) cysts at salinity equal to 75 g dm⁻³ compared

Fig. 2. Average egg numbers at different salinity conditions (NaCl concentration)

Explanation: significant differences ($p < 0.05$) are marked with an asterisk, highly significant differences ($p < 0.01$) with a double asterisk, and very highly significant differences ($p < 0.001$) with a triple asterisk.

to other concentrations where the number of cysts was less important. This is equal to (60 ± 3) , (61 ± 3) , (49 ± 4) , (40 ± 3) , (27 ± 2) , (24 ± 1) respectively for 50, 100, 125, 150, 175 and 200 g dm⁻³ (Fig. 2). Thus, *Artemia salina* L. salines of Bethioua present their optimal production in cysts at salinity of the order of 75 g dm⁻³.

The statistical analysis shows a non-significant difference in the number of cysts produced in the control (natural environment) and the concentrations 50 g dm⁻³ ($p = 0.135$), 75 g dm⁻³ ($p = 0.900$) and 100 g dm⁻³ ($p = 0.149$). On the other hand, this difference is highly significant with the highest concentrations, namely 125 g dm⁻³ ($p = 0.022$), 150 g dm⁻³ ($p = 0.004$) and 175 g dm⁻³ ($p = 0.001$), and strongly significant for the highest concentration of our experiment was 200 g dm⁻³ ($p = 0.000$) (Fig. 2).

For species of the same genus, Browne et al. (2000) and Triantaphyllidis et al. (1995) found that the optimal salinity for cyst production of Tangu's *Artemia parthenogenetica* ranged from 60 to 100 g dm⁻³, while for the *Artemia franciscana* L. species of San Francisco Bay the optimal salinity for the production of cysts was between 100 and 140 g dm⁻³.

Outbreak and rearing

The cyst hatching was performed in an aquarium at a NaCl concentration equal to that of seawater (36 g dm⁻³) at 25°C. The hatching of the cysts is stimulated by the phenomenon of osmosis, which favors the bursting of the wall, and the birth of naupliis. They (240 individuals), are fed with rice flour and put in breeding at a concentration of 75 g dm⁻³ until the adult stage (Sorgeloos et al. 2001).

Toxicity test

The 240 individuals of *Artemia salina* L. obtained were exposed to different concentrations of ammonium

Fig. 3. Evolution of mortality according to the different concentrations of ammonium sulphate

sulphate at the rate of 40 individuals per concentration. Generally, the toxicity is expressed as a lethal concentration, which causes the death of 50% of the group of experiment species (LC₅₀) (Libralato et al. 2016).

The results obtained indicate variability in the resistance of the species to the different concentrations of ammonium sulphate (Fig. 3). Its tolerance is observed at a concentration equal to 50 mg dm⁻³ where 25% of the individuals survived even after 72 hours of exposure. On the other hand, 50% of the individuals showed no resistance after 24 hours of exposure to the other concentrations.

After 48 hours, 75% of individuals died at concentrations of 100, 125, and 150 mg dm⁻³, while not all individuals resisted after this period at 200 mg dm⁻³.

After 72 hours of experimentation, there was total death of all individuals remaining in the other concentrations (75, 100, 125, and 150 mg dm⁻³).

Our short-term toxicity test showed a LC₅₀ equal to 75 mg dm⁻³ for the duration of 24 hours of exposure.

In other studies the effect of toxicity on *Artemia salina* L., Abu Shaala et al. (2015) have demonstrated a LC₅₀ = 23.27 mg dm⁻³ for the toxicity of diuron on the morphology of the species. For the effect of copper sulphate, Manfra et al. (2015) found a LC₅₀ = 23.31 mg dm⁻³ and Libralato et al. (2016) performed toxicity tests for copper sulfate pentahydrate on the species with a LC₅₀ = 6.6 mg dm⁻³ after 24 hours. However, our work is the first to highlight the toxicity of ammonium sulphate on *Artemia salina* L. in western Algeria.

The toxicity of each chemical differs from one organism to another (Patel et al. 2013). Delisle et al. (1977) and Greenwood et al. (1984), studied sulphates and found that high values of this parameter can be observed in polluted streams, at the level of industrial waste areas (mining industry, pulp and paper mills, tanneries, textile mills). It can also be found in agricultural

activities involving the excessive use of insecticides and fungicides based on ammonium sulphate fertilizers (Derwich et al. 2010).

Conclusion

In the light of the present results, the reproductive dynamics in *Artemia salina* L. vary according to the ecological factors and the conditions of the environment in which it survives.

The species in question is a perfect food for marine life and good nutrition.

Although the optimum rate of salinity favoring reproduction is 75 g dm^{-3} , the most effective method of reproduction is not necessarily heterosexual reproduction. Indeed, under optimal conditions, parthenogenesis reproduction remains the most efficient from the point of view of the number of eggs hung.

Our study consists of examining the effect of certain pollutants, such as ammonium sulphate $(\text{NH}_4)_2\text{SO}_4$ on *Artemia* behavior.

As in most living things, adult individuals are the most resistant to attack from a polluted environment. But our study on the ammonium sulphate toxicity, where sulphate is the dominant element of the rejections generated by the salt plant on the species biotope, reveals that even at this stage the species cannot withstand a concentration over 75 mg dm^{-3} .

Therefore, the conservation of this environment remains a primary necessity if one wants to develop a subsidiary of cyst production that constitutes a basic element in the aquaculture sector in order to improve the socio-economic framework of the neighboring populations.

Aquaculture has become one of the major sectors of food production to meet the needs of individuals and its development today ensures half of the production of fish consumed in the world. It should, however, be part of a sustainable and responsible process.

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