

## Evaluation of the Acute Toxicity of an Ammonium Sulfate Fertilizer in Two Species of Anuran Tadpoles: *Bufo mauritanicus* (Schlegel 1841) and *Rana ridibunda* (Boulanger 1913)

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### ABSTRACT

As part of the ecotoxicological tests on anuran tadpoles, the authors propose to study the sensitivity of different stages towards a nitrogenous compound frequently used in Morocco (Ammonium sulphate). For this purpose, the acute toxicity tests on tadpoles of two species (*Bufo mauritanicus* and *Rana ridibunda*) belonging to different stages of development were carried out. The lethal effects after 96 hours caused by the pollutant used as a reference (potassium dichromate) as well as ammonium sulfate increase with concentration and vary according to the stage of development. Significant differences were observed in the sensitivity of the tadpoles to pollutants according to the stage of development. The tadpoles belonging to the early stages of development (stage 24 according to Gosner, 1960) show greater acute effects on pollutants than the tadpoles belonging to the advanced stages of development (Stage 36 according to Gosner, 1960). After 96 hours, the lowest effective concentration (LOEC) is observed in the tadpoles belonging to the early stages of development (Stage 24 according to Gosner, 1960) in *Rana ridibunda* exposed just to the lowest concentration used (20 mg/l of ammonium sulphate). Therefore, ammonium sulfate may play a role in the local decline of amphibians.

**Keywords:** anuran tadpoles, ecotoxicological tests, ammonium sulfate, *Bufo mauritanicus*, *Rana ridibunda*.

### INTRODUCTION

One of the most serious global environmental problems is the loss of biological diversity. The human activities today threaten many species on the verge of global extinction (Díaz et al., 2020). Populations of several species have declined worldwide and amphibians are considered highly threatened (Grant et al., 2016, IUCN 2019, Bókony et al., 2020). Amphibians are vulnerable to environmental disturbance (Becker et al., 2010; Bovo et al., 2018; Ribeiro-Jr et al., 2018) due to their basic characteristics, such as their high skin permeability (Freitas et al., 2019) which offers limited activity capacity and increased vulnerability to the biological or chemical agents (Yan et al., 2008). Therefore, amphibians have been considered ideal bio indicators of the aquatic and

agricultural ecosystems (Feng et al., 2004). Their tadpoles are essential links to the various links in the food chain; they are both prey and predators (Junges et al., 2012); therefore, they play an important role in the freshwater and terrestrial ecosystems (Bókony et al., 2020).

It has been noted that the decline in amphibians is linked to the use of agrochemicals (Hayes et al., 2010; Polo-Cavia et al., 2016, Zhao et al., 2019). In Morocco, ammonium sulfate (a nitrogenous fertilizer which could possibly modify the biotic and abiotic environment) is the most used synthetic chemical in agricultural areas for soil fertilization. It should be noted that studies and diagnostics (PCN, 2016; Naamane et al., 2020) carried out point out the problem of fertilizer use in an empirical way, the theoretical needs do not match the volumes of fertilizers actually used,

which could be a source of the surface water pollution due to run-off of these fertilizers and thus a threat to amphibians.

The studies conducted by Grant et al. (2016) in North America on 83 amphibian species (including anurans) revealed that the declines are not related to any particular threat on a continental scale but are the result of the locally induced processes. As a result, more emphasis needs to be placed on local solutions to this globally shared phenomenon. In Morocco, *Bufo mauritanicus* and *Rana ridibunda* are widespread and abundant species, living in various habitats, including agricultural sites. In this study, these anuran tadpoles are used for the first time as test animals in order to evaluate the toxic and lethal effects of ammonium sulfate in an aquatic environment. A short-term exposure assessment was carried out to evaluate the toxicity of this suspected threat (ammonium sulfate) in the tadpoles of *Bufo mauritanicus* and *Rana ridibunda*. The main objective was to assess the acute toxicity of ammonium sulfate at different concentrations, on different species of anuran tadpoles, belonging to different stages of development, in order to check whether mortality depends of the nature of the species, the concentration of the pollutant and the stage of development. This will allow us to provide reliable data regarding the effects of ammonium sulfate on local anuran species and standardize these tests later.

## MATERIALS AND METHODS

### General presentation of the study area

The swamp covered by this study is located in Tit Mellil (a village in the province of Médouna in the Casablanca-Settat region (Morocco)). It is located at 33°3' N and 7°28' W. It is an area without any sign of pollution, but to better characterize the water quality of this swamp, some of

the physicochemical parameters were measured. The measurements were conducted at 4 points, (2 points on the banks: P1, P2 and 2 others in the center: P3, P4) on June 30, 2019. These 4 samples were chosen in order to have an overall picture of the swamp.

The choice of sampling of anuran tadpoles (*Bufo mauritanicus* and *Rana ridibunda*) on this swamp was guided in particular by its accessibility and for its good to excellent physical and chemical properties.

### Physicochemical parameters analyzed

Concerning the physicochemical quality of the swamp water, the measurements of temperature, dissolved oxygen, pH, conductivity and turbidity were carried out *in situ* using a Multi-parameter model MU61004. The analysis of the nitrogen compounds ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ), phosphates ( $\text{PO}_4^{3-}$ ), suspended matter (SM), and sulfates were carried out in the laboratory (Table 1) using the standard methods: AFNOR, and Rodier.

### Toxicity tests

The tadpoles of *Bufo mauritanicus* and *Rana ridibunda* were captured with a strainer between April and July and driven to the laboratory. Upon arrival, they were placed in plastic basins containing 10 L of water (one tadpole/100 ml), and were fed with green salad *ad libitum*. After 7 days (acclimatization period), and based on the study carried out by Baran et al. (2013), the tadpoles were distributed in glass crystallizers ( $V=2\text{L}$ ) containing 1 L of dechlorinated tap water at a rate of 10 tadpoles per crystallizer or 1 tadpole per 100 ml. The tadpoles were divided according to their developmental stages into 3 categories for *Bufo mauritanicus* and only into 2 categories for *Rana ridibunda* (because there is no tadpoles advanced development stage (development stage 36 (according to Gosner 1960)) in the swamp.

**Table 1.** Methods and parameters studied

| Parameters                             | Methods  |
|--|--|
| Orthophosphates ( $\text{PO}_4^{3-}$ ) | Spectrometric determination using ammonium molybdate (AFNOR 2001). |
| Ammonium ( $\text{NH}_4^+$ )           | Indophenol blue spectrophotometric method (AFNOR 2001)             |
| Nitrate ( $\text{NO}_3^-$ )            | Spectrometric determination using sodium salicylate (Rodier 2009)  |
| Sulfate ( $\text{SO}_4^{2-}$ )         | Nephelometric method (AFNOR 2001)                                  |
| Suspended Matter (SM)                  | Filtration method (AFNOR 2001)                                     |

The first includes the tadpoles belonging to the early stages of development (stage 24 according to Gosner 1960), the tadpoles without internal gill limbs, with an average size of  $0.94 \text{ cm} \pm 0.05$  for *Bufo mauritanicus* and *Rana ridibunda*, the second includes the tadpoles belonging to the development stage 27 (according to Gosner 1960), the tadpoles with two legs (leg bud), of average size  $1.81 \pm 0.11 \text{ cm}$  for *Bufo mauritanicus* and  $3.02 \pm 0.19 \text{ cm}$  for *Rana ridibunda*, and the third includes the tadpoles belonging to development stage 36 (according to Gosner 1960), legs with toe paddles, with an average size of  $2.47 \pm 0.08 \text{ cm}$  for *Bufo mauritanicus*.

For carrying out the acute toxicity tests on the Anuran tadpoles having the same stage of development and coming from the same source, as recommended by USEPA (2002) and highlight a lethal effect following the daily exposure to dechlorinated water containing the pollutant and renewed every 24 hours. Standard pollutant, i.e. potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) was used as a control, to which only the *Bufo mauritanicus* tadpoles were exposed. This pollutant is known for its particularly oxidizing nature. In addition, an agrochemical widely used in Morocco, ammonium sulfate ( $(\text{NH}_4)_2\text{SO}_4$ ) was employed, to which the tadpoles of two species (*Bufo mauritanicus* and *Rana ridibunda*) were exposed. For 96 h, the tadpoles of different stages were subjected to 5 increasing concentrations of the pollutant, potassium dichromate and ammonium sulfate (20, 40, 80, 160, 320 mg/l). The concentrations selected are based on the previous studies conducted for ammonium nitrate. These concentrations were the maximum concentrations that should appear in water bodies after fertilizer run off applied in agricultural landscapes (Schollefield et al. 1996). For each concentration, and each stage of development, three identical tests were performed. The controls for each stage of development were planned to compare the sensitivity of different development stages to these agrochemicals and to provide more realistic data on the effects of ammonium sulfate on the local anuran species.

The crystallizers were placed under conditions of natural photoperiod, and constant aeration was ensured by bubblers. The physicochemical parameters (temperature, dissolved oxygen, pH and electrical conductivity) were measured daily using a Multi-parameter analyzer model MU61004.

The dead individuals (no reaction to a slight push) were counted, removed from the crystallizers and stored in 10% formalin for further pathology study. Toxicity was assessed by visual observation of survival and expressed as a percentage.

## Data processing

Microsoft office Excel 2007 software was used for data processing as well as a statistical method: analysis of variance test (ANOVA test) which was carried out using R version 3.6.1 software (2019-07-05) the objective of which is to check whether mortality depends of the nature of the species, the concentration and the stage of development. Another statistical method was used to determine LC 50 objectively by a nonlinear regression, using TRAP (The Toxicity Relationship Analysis Program) version 1.30 (June 25, 2015).

## RESULTS AND DISCUSSIONS

### General assessment of the quality of swamp water

By comparing the results the physicochemical parameters of the swamp waters with the values of the Moroccan surface water standard, we can consider that the quality of the swamp water is generally excellent.

### Eco-toxicological tests

The physicochemical characteristics of the solutions recorded during the acute toxicity tests are grouped in Tables 2 and 3. The temperature and oxygen values appear relatively constant and identical to the controls; however, there is a correlation between the pollutant concentration and the conductivity and pH concentration. An increase in the concentration of the pollutant leads to an increase in conductivity and a decrease in pH; this is due to dichromate for the crystallizers containing potassium dichromate and sulfates and ammonium ions for those with ammonium sulfates.

The results of acute toxicity tests obtained in tadpoles of two species, belonging to different developmental stages: early developmental stages, the tadpoles without limbs with internal gills (Stage 24); the tadpoles belonging to development stage 27, the two-legged tadpoles (leg bud), and finally the tadpoles belonging to development

**Table 2.** Physicochemical characteristics of solutions during the acute toxicity tests for potassium dichromate

| Measured parameters     | Witnesses | Concentrations of potassium dichromate |          |          |          |          |
|-------------------------|-----------|--|----------|----------|----------|----------|
|                         |           | 20 mg/l                                | 40 mg/l  | 80 mg/l  | 160 mg/l | 320 mg/l |
| Temperature, °C         | 21.6±0.3  | 21.6±0.3                               | 21.6±0.3 | 21.6±0.3 | 21.6±0.3 | 21.6±0.3 |
| pH                      | 8.28±0.2  | 8.28±0.2                               | 8.25±0.3 | 8.14±0.3 | 7.42±0.3 | 6.54±0.3 |
| Conductivity (µs/l)     | 1.68±0.2  | 1.74±0.2                               | 1.77±0.2 | 1.84±0.2 | 1.96±0.2 | 2.04±0.2 |
| Dissolved oxygen (mg/l) | 9±0.2     | 9±0.2                                  | 9±0.2    | 9±0.2    | 9±0.2    | 9±0.2    |

**Table 3.** Physicochemical characteristics of solutions during the acute toxicity tests for ammonium sulfates

| Measured parameters     | Witnesses | Concentrations of potassium dichromate |          |          |          |          |
|-------------------------|-----------|--|----------|----------|----------|----------|
|                         |           | 20 mg/l                                | 40 mg/l  | 80 mg/l  | 160 mg/l | 320 mg/l |
| Temperature, °C         | 20.6±0.3  | 20.6±0.3                               | 20.6±0.3 | 20.6±0.3 | 20.6±0.3 | 20.6±0.3 |
| pH                      | 8.28±0.2  | 8.28±0.2                               | 8.25±0.3 | 8.22±0.3 | 8.15±0.3 | 7.42±0.3 |
| Conductivity (µs/l)     | 1.68±0.2  | 1.74±0.2                               | 1.78±0.2 | 1.81±0.2 | 1.95±0.2 | 2.3±0.2  |
| Dissolved oxygen (mg/l) | 9±0.2     | 9±0.2                                  | 9±0.2    | 9±0.2    | 9±0.2    | 9±0.2    |

stage 36, the two-legged stage (leg with toe paddle) reveals a significant difference in sensitivity between these different development stages as well as the concentrations (Table 4 and 5). The stages of development that showed a significant difference are stages 24 and 36 and concerning the concentrations are 0, 20, 40, 160 and 360 mg/l of pollutant. It was noted that no mortality was observed during the experimental period for the control groups.

For the exposure duration of 96 h, there was no mortality in the *Bufo mauritanicus* tadpoles exposed to the low concentration (20 mg/l of ammonium sulfates) and which belong to different development stages (Fig. 1). On the other hand, the same concentration of ammonium sulfates resulted in a mortality of 10% in the *Rana ridibunda* species (Fig. 2) for the early stage of development (stage 24), which indicates that it may be less tolerant to sulfates than *Bufo mauritanicus* and this could explain its low abundance in the swamp.

Concerning the tadpoles of *Bufo maritanicus* exposed to ammonium sulfate, the concentration

of the pollutant: 40 mg/l has no observed effect (NOEC) for the both stages of development (24 and 27) whereas, for stage of development 36, NOEC is noted at 160 mg/l (Fig. 1). Indeed, during this no-observed-effect concentration, the tadpoles were all alive and seemed to be feeding normally. This resistance may be due to more effective detoxification mechanisms than those in the early development stages (Bucciarelli et al. 1999).

The authors observed the lethal effects caused by ammonium sulfate that increase with stage of development and the concentrations (Fig. 3 and 4). For the *Bufo mauritanicus* tadpoles exposed to ammonium sulfate, the lowest effective concentration (LOEC) is 80 mg/l for developmental stages 24 and 27 while it increases to the concentration of 320 mg/l for developmental stage 36.

For *Rana ridibunda*, the lowest effective concentration (LOEC) is the concentration 20 mg/l of ammonium sulfate to which the tadpoles in development stage 24 were exposed to and it changes to the concentration of 80 mg/l for development

**Table 4.** ANOVA test to check whether the morality varies according to the type of species, the stages of development and or the concentrations

| Specification  | SS<br>(Sum-of-squares) | df<br>(Degrees of freedom) | MS<br>(Mean squares) | F value  | Pr (>F)   |     |
|----------------|------------------------|----------------------------|----------------------|----------|-----------|-----|
| Species        | 0.0596                 | 1                          | 0.0596               | 1.9293   | 0.1671    |     |
| Pollutants     | 0.0334                 | 1                          | 0.0334               | 1.082    | 0.3001    |     |
| Stages         | 1.9290                 | 2                          | 0.9645               | 31.2215  | 7.399e-12 | *** |
| Concentrations | 18.4678                | 5                          | 3.6936               | 119.5617 | <2.2e-16  | *** |
| Residuals      | 4.1396                 | 134                        | 0.0309               |          |           |     |

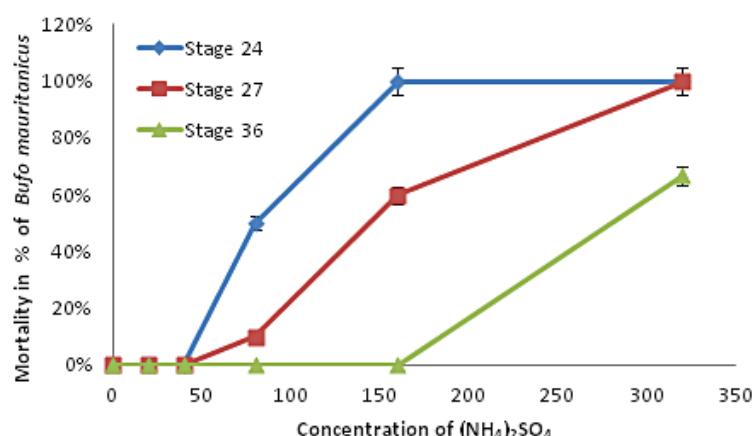
**Table 5.** ANOVA test inside the different variables (Species, stages, and concentrations)

| T test                            | Estimate  | Std. Error | t value | Pr (> t ) |     |
|-----------------------------------|-----------|------------|---------|-----------|-----|
| (Intercept)                       | 0.315509  | 0.019832   | 15.9091 | < 2e-16   | *** |
| Specie – <i>Bufo mauritanicus</i> | -0.027546 | 0.019832   | -1.3890 | 0.16714   |     |
| Specie – <i>Rana ridibunda</i>    | 0.027546  | 0.019832   | 1.3890  | 0.16714   |     |
| Pollutant – potassium dichromate  | 0.017593  | 0.016913   | 1.0402  | 0.30012   |     |
| Pollutant – ammonium sulfate      | -0.017593 | 0.016913   | -1.0402 | 0.30012   |     |
| Stage – S 24                      | 0.162500  | 0.020714   | 7.8450  | < 2e-16   | *** |
| Stage – S 27                      | -0.035648 | 0.020714   | -1.7210 | 0.08756   | .   |
| Stage – S 36                      | -0.126852 | 0.023918   | -5.3035 | < 2e-16   | *** |
| Concentration – C 0               | -0.313194 | 0.032751   | -9.5628 | < 2e-16   | *** |
| Concentration – C 160             | 0.290972  | 0.032751   | 8.8842  | < 2e-16   | *** |
| Concentration – C 20              | -0.300694 | 0.032751   | -9.1811 | < 2e-16   | *** |
| Concentration – C 320             | 0.645139  | 0.032751   | 19.6980 | < 2e-16   | *** |
| Concentration – C 40              | -0.279861 | 0.032751   | -8.5450 | < 2e-16   | *** |
| Concentration – C 80              | -0.042361 | 0.032751   | -1.2934 | 0.19809   |     |

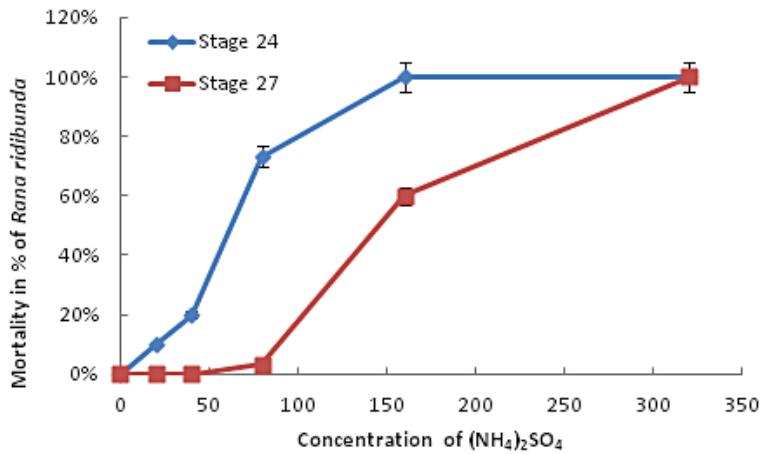
stage 27 (Fig. 2). A decrease in locomotors and food intake performance was also observed in the tadpoles exposed to pollutants compared to controls. The decrease in food intake may be due to a loss of activity or energy investment in the detoxification mechanisms (Schmuck et al. 1994). When animals are exposed to adverse conditions, they exhibit the escape behaviors or activate physiological adjustments in an attempt to stabilize the body (Salla et al. 2015). Energy stores, glycogen and, to a lesser extent, protein can be depleted in response to exposure of amphibians to environmental contaminants, when the body tries to maintain homeostasis by compensatory metabolic mechanisms, thus using energy reserves (Dornelles et Oliveira 2014). Moreover, chemical pollutants are capable of even causing deregulation of the endocrine system and thus affecting the hormonal activity. Wild leopard frogs

(*Rana pipiens*) exposed to a chemical exhibited developmental delay and hermaphroditism (Hayes et al., 2002).

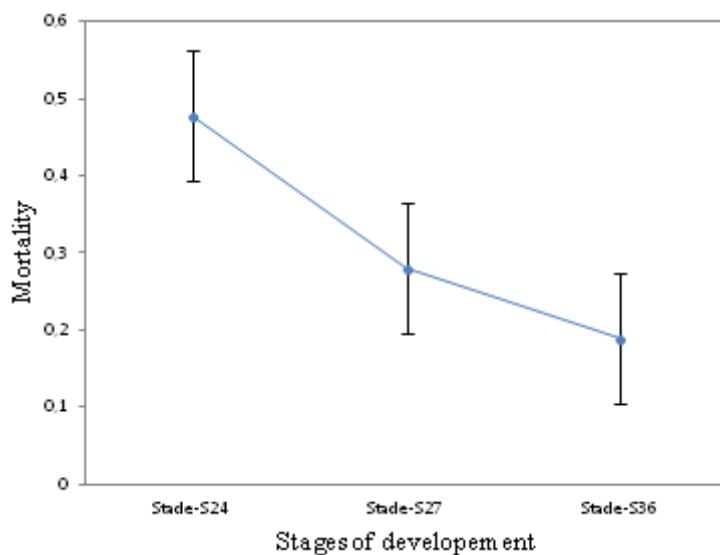
Exposure to an agrochemical (ammonium nitrate) has already been shown to reduce the activity or feeding rate of various amphibian larvae (Burgett et al., 2007). Several mechanisms have been suggested to explain the toxicity of ammonia (Marco and Ortiz-Santiestra, 2009). Exposing tadpoles to moderate levels of ammonia can reverse the ammonia diffusion gradient, and animals can absorb ammonia from the water. In these cases, the costly urea synthesis becomes a common mechanism for detoxifying ammonia (Wright and Wright, 1996). This additional energy investment therefore alters the swimming activity. We also know that the ammonium ion affects the nervous system of many vertebrates by replacing the potassium cations in the nervous membranes



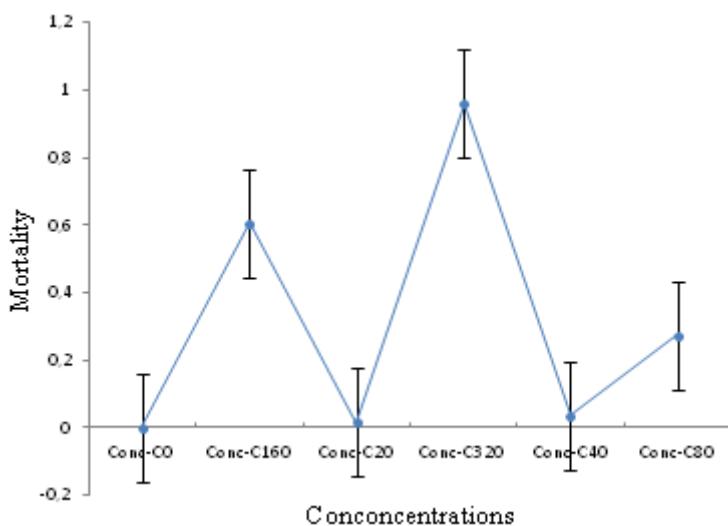
**Fig. 1.** Mortalities recorded in the acute toxicity test in the *Bufo mauritanicus* tadpoles at the development stage 24, 27 and 36 exposed to ammonium sulfate ( $(\text{NH}_4)_2\text{SO}_4$ ) for 96 hours



**Fig. 2.** Mortalities recorded in the acute toxicity test in the *Rana ridibunda* tadpoles at the stage 24 and 27 exposed to ammonium sulfate ( $(\text{NH}_4)_2\text{SO}_4$ ) for 96 hours



**Fig. 3.** Graphical ANOVA for mortality according to the stage of development



**Fig. 4.** Graphical ANOVA for mortality according to concentration

(Randall et Tsui, 2002). In fish, this mechanism causes dysfunction of Mauthner's cells (giant neurons involved in rapid reflex responses), which ultimately causes an increase in latency preventing the escape from predators (McKenzie et al., 2009). In addition, the replacement of potassium by ammonium at the sarcolemmal membrane can cause depolarization of the white muscle (McKenzie et al., 2009), which includes most of the axial musculature and is responsible for performing the rapid movements involved in the escape response in fish (Sherkov, 1970).

The main behaviors of tadpoles are feeding and avoiding predators (Denver, 2019). However after the exposure of the *Bufo mauritanicus* and *Rana ridibunda* tadpoles to ammonium sulfate, they may be more susceptible to predation in addition to poor foraging. In addition, more physiological studies are needed to identify the mechanisms involved in the exposure to ammonium sulfate in tadpoles.

The same observation can be noted for the lethal concentration which affects 50% of the population (LC50). It increases with the stage of development and varies slightly between the two species (Table 6). We can also note significant lethal effects (100% mortality), *Bufo mauritanicus* tadpoles exposed to ammonium sulfate (Fig. 1) showed 100% mortality from concentration of 160 mg/l for the early development stage (Stage24), and it is the same for *Rana ridibunda* (Fig. 2). The stage 27 has 100% mortality from the concentration of 320 mg/l ammonium sulfate for both species (*Rana ridibunda* and *Bufo mauritanicus*) and recently stage 36 which seems to be the most tolerant shows a fair mortality of 67% at the maximum tested concentration of 320 mg/l ammonium sulfate for *Bufo mauritanicus*. This high sensitivity is due to incomplete

differentiation of tissues and organs in tadpoles in the early stages of development (Herkovits et al. 1978). Since the development of anurans is characterized by the appearance of new phenotypic characters over time (Fabrezi et al., 2019). In addition, in the anurans tadpoles belonging to the advanced stage of development, the gills are internal but for the first stages of development, the gills are external and in direct contact with water (Gosner, 1969); therefore, during this stage of development, a negative effect of ammonium sulfates on osmoregulation could be significant.

The tolerance of tadpoles to toxic substances increases with their stage of development, and this has also been reported in previous studies (Ortiz-Santaliestra, 2006) in which tadpoles were exposed to chemicals.

In addition, the differences in tolerance between the species of anurans are also remarkable and agree with the studies carried out by García-Muñoz et al. (2011) including 4 species of anurans (*Bufo bufo*, *Bufo calamita* (*Epidalea calamita*), *Pelodytes ibericus* and *Pelophylax perezi*) were exposed to a fertilizer (ammonium nitrate) at numerous concentrations. The tolerance of tadpoles to toxic substances is seen to increase with their stage of development, and this has also been reported in previous studies. Mann et al. (2009) in his study reported that nitrates are often without any effect on different species of anurans (Gosner stage 25) at concentrations up to 100 mg/l. Additionally, Miaud et al. (2011) showed that calamine toad tadpoles (Gosner stage 25) are highly resistant to the nitrate exposure for an exposure time of 72 h at 1 g/l (no mortality). This resistance was also observed for the tadpoles in the early stages of development (stages 10–12) exposed to 200 mg/l for 15 days (Ortiz et al. 2004). In this study, the exposure to ammonium sulfate

**Table 6.** Estimate of lethal concentration that affects 50% of the population (LC 50) for the 2 species

| Species                  | Pollutant                                       | Development stage | LC 50 (mg/l) | 95% LCL (The lower control limit ) | 95%UCL (The upper control limit ) | Standard error |
|--------------------------|---|-------------------|--------------|------------------------------------|-----------------------------------|----------------|
| <i>Bufo mauritanicus</i> | (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> | Stage 24          | 80.00        | 80.00                              | 80.00                             | 0.00           |
|                          |   | Stage 27          | 147.23       | 145.78                             | 148.67                            | 0.45349        |
|                          |   | Stage 36          | 294.73       | 294.73                             | 294.73                            | 0.00           |
|                          | K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>   | Stage 24          | 71.322       | 60.502                             | 82.14                             | 3.39           |
|                          |   | Stage 27          | 173.09       | 168.37                             | 177.81                            | 1.48           |
|                          |   | Stage 36          | 188.52       | 188.52                             | 188.52                            | 0.00           |
| <i>Rana ridibunda</i>    | (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> | Stage 24          | 68.071       | 67.615                             | 68.52                             | 0.14           |
|                          |   | Stage 27          | 151.02       | 148.37                             | 153.66                            | 0.83           |

had a lethal effect demonstrated by the increased mortality of the exposed tadpoles. This reveals that it can be seriously dangerous for the survival of anurans. The results reveal that anurans are very sensitive to the negative effects of ammonium sulfate which is in agreement with the studies carried out by Sparling et al. (2010); Hayes et al., (2010); Hoffmann (2010); Polo-Cavia et al. (2016) who have already mentioned the negative effects of agrochemicals on the anuran tadpoles. In addition, the earlier the development stage is, the greater the vulnerability of the tadpoles to the pollutant, which also strengthens the previous studies (Castañaga et al., 2009; Aronzon et al., 2011). This demonstrates the need to use the early stages of development when assessing the effects of one or more chemicals, such as ammonium sulfate, on the tadpoles of anurans.

In addition, the studies by Ortiz-Santiestra et al. (2012) showed that the mortality of the *Salamandra salamandra* tadpoles related to ammonium nitrate was five times higher at high than at low density. Overcrowding (group effect) can lead to strong trophic competitiveness within the species. Some more vigorous tadpoles then monopolize food to the detriment of the community to finally metamorphose. The release of growth inhibitors by the most developed tadpoles in water with feces is also envisaged, and these proteinaceous substances would be absorbed by the smallest tadpoles (Hourdry et al., 1985). Thus, the tadpoles of anurans living in temporary ponds are possibly more impacted by chemicals than those living in permanent ponds.

Ultimately, these results suggest that the decline in the population of anurans could be caused by contamination by ammonium sulfate in their aquatic environment with a dependence of the mortality on the concentration of the pollutant, the stage of development and probably the nature of the species. On the other hand, the effects of ammonium sulfate measured in the laboratory on the tadpoles of the two species of anurans may be different from the stress experienced in the field as a result of synergistic effects with other environmental factors. Variations in the abiotic and biotic factors as well as different ecological conditions can act in complex ways to influence the morphology, behavior, growth and development of tadpoles (Denver, 2019). Finally, more research efforts are therefore needed to improve our knowledge of how ammonium sulfate can impact the decline of the amphibian population.

## CONCLUSION

At the end of this work, which aimed to assess the toxicity of ammonium sulfate in the anuran tadpoles, the results reveal that the pollution by ammonium sulfate from anthropogenic sources such as agriculture, entering surface waters by runoff, has acute toxicological effects in anurans. The presence of ammonium sulfate has a negative impact since it modifies the way in which the tadpoles interact with their environment, which results in a drastic reduction in the number of survivors.

Furthermore, this sensitivity varies according to the stage of development, the concentrations and slightly with the species. Significant differences between the different stages of development as well as the concentrations studied were noted. The higher the exposure concentration, the more the resistance to the pollutant decreases; however, it was noted that the stage of development has a decisive influence on the sensitivity of species. The earlier the stage of development, the more the nuisance of the pollutant or the fragility of the tadpoles increases. Therefore, the results make it possible to deduce that the ammonium sulphate has an impact on the survival of tadpoles and this impact depends on various factors.

The contamination of surface water with ammonium sulfate, may therefore play a role in the decline of the local amphibian population; however, additional studies on the toxicity of ammonium sulfate in anurans, its ecological implications, as well as measurements of sulfate and ammonium concentrations in the areas close to agricultural activities which allows to have relevant concentrations of this pollutant for the environment, are recommended in order to subsequently implement a local conservation action plan to combat the worldwide decline in the amphibian populations.

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