JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2023, 24(5), 103–109
https://doi.org/10.12911/22998993/161445
ISSN 2299–8993, License CC-BY 4.0

Received: 2023.02.12
Accepted: 2023.03.15
Published: 2023.04.01

Evaluation of the Effects of Three Chemical Fertilizers on *Artemia salina*

Naamane Ayoub[1*], El Birgui Kaouthar[2], Rafii Soumaya[3], Merbouh Chaimaa[1], Iounes Nadia [1], El Amrani Souad[1]

[1] Laboratory of Ecology and Environment, Faculty of Sciences Ben M'Sik, Hassan II-University, Avenue Cdt. Driss El Harti, B.P. 7955 Sidi Othmane, Casablanca, Morocco

[2] Laboratory of Chemistry-Biochemistry, Environment, Nutrition and Health Laboratory, Faculty of Medicine and Pharmacy, Hassan II-University, Rue Tarik Ibnou Ziad, Casablanca, Morocco

[3] Laboratory of Cellular and Molecular Pathology, Faculty of Medicine and Pharmacy, Hassan II-University, Rue Tarik Ibnou Ziad, Casablanca, Morocco

* Corresponding author's e-mail: mr.naamane@gmail.com

ABSTRACT

As part of eco-toxicological tests on *Artemia salina*, the impact of the most commonly used fertilizers in Morocco was assessed (Ammonium Sulfate, Ammonium Nitrate, and a mixture of Nitrogen 10%, Phosphorus 30% and Potassium 10%), with Potassium Bichromate ($K_2Cr_2O_7$) as reference substance at different concentrations (10, 20, 40, 80, 160, 320 mg/l), for 24 hours. The results of the statistical study revealed that the lethal effects caused by the three agrochemicals increase with the concentration and vary from one substance to another in a significant way. There is a higher sensitivity to ammonium sulphate (the concentration that kills 50% of the animals: 37.32 mg/l ± 6.09) followed by ammonium nitrate the concentration that kills 50% of the animals: 110.7 mg/l ± 7.11), and a mixture of Nitrogen 10%, Phosphorus 30% and Potassium 10% (the concentration that kills 50% of the animals: 143.13 mg/l ± 23.12). Therefore, *Artemia salina* exhibits sensitivity to agrochemicals and can be considered as a good biomonitoring tool for future toxicological analysis of agrochemicals.

Keywords: ecotoxicological tests, *Artemia salina*, chemical fertilizers, biomonitoring.

INTRODUCTION

Nowadays, the rapid growth of the world population and the environmental conditions have contributed to the creation of great pressure on the agricultural sector to ensure greater food production (Gill et al., 2022). Indeed, to meet food needs, agriculture uses large quantities of water as well as fertilizers and pesticides. It is therefore one of the most important sources of pollution of aquatic environments (ONU, 2019; Zambrano et al., 2022), affects the quality and quantity of water, its current and future sustainability (Commission européenne, 2017) and also harms aquatic biodiversity (Barreau et Magnier, 2019).

In addition, the agricultural sector is an essential pillar of the economy and society in Morocco, contributing nearly 13% of GDP (DEPF, 2019). However, to improve food productivity and protect crops, scientific studies carried out locally show an extravagant and unjustified use of fertilizers to the detriment of the preservation of natural resources (ADA, 2018; Naamane et al., 2020; Barua et Eslamian, 2021). This kind of human intervention is one of the causes of water pollution (Barua et al., 2021), causing deterioration of aquatic systems (Piren-Seine, 2019).

Other studies have revealed the negative effects of agrochemical contamination on the diversity of organisms with complex life cycles such as amphibians (Naamane et al., 2020; Suarez et al., 2021; Zambrano et al., 2022).

A survey carried out in the agricultural areas of Casablanca-Settat showed that among the chemical fertilizers most used for soil fertilization are mixture of Nitrogen10%, Phosphorus 30% and Potassium 10% (NPK 10-30-10), ammonium nitrate and ammonium sulphate (Naamane et al., 2020). These chemical fertilizers, which can be found in fresh water because of contamination, could reach the ocean environment as the final destination of contaminated waters.

Anthropogenic inputs of chemical fertilizers accelerate the pollution of surface waters with a negative effect on aquatic biodiversity. To test their short-term effects, one of the most sensitive species was used: *Artemia salina.*

Artemia salina (AS) is a small crustacean living in saline aquatic environments (Munteanu et al., 2011) and is characterized by rapid development and a transparent body as well as a variety of development parameters that can be easily monitored (Da Fontoura et al., 2021). Its permanent presence (Artemia cysts) and its rapid results allow it to be among the model organisms for experimental studies related to bioassays (Rajabi et al., 2015).

In this study, *Artemia salina* is used for the first time as a test animal to assess the toxic effects causing lethal effects of chemical fertilizers in the aquatic environment. The main objective is the evaluation of the toxicity of three chemical fertilizers (Ammonium sulfate, Ammonium nitrate, mixture of Nitrogen 10%, Phosphorus 30% and Potassium 10%: NPK10-30-10) after a short-term exposure (24h) in *Artemia salina* at different concentrations in order to help understand the ecological risk of agrochemicals on surface waters.

MATERIALS AND METHODS

Agrochemicals

Chemical fertilizers (NPK 10-30-10, ammonium nitrate 33.5% N and ammonium sulphate 21% N) are obtained from farmers in the Casablanca-Settat study region.

Animals and breeding conditions

The cysts of *Artemia salina* were obtained from an aquarium shop and whose origin is the company ARFIDEL. Nauplii: Stage II were obtained after 30 hours by hatching the cysts in separating funnels. Aeration and constant and continuous lighting were provided by a bubbler (RS-8801Pump 10W) and a lamp (70W) as well as a salinity of 35% (artificial sea water) at a pH of 8-9. After 30 hours with a temperature of 25 ± 3 °C (Fig. 1), bubbling was stopped to harvest the nauplii (Castro et al., 2003).

Figure 1. Preparation of brine shrimp for hatching

Toxicity testing

To perform acute toxicity tests on nauplii at stage II, and to demonstrate a lethal effect following exposure for 24 hours, 5 nauplii were transferred into pillboxes containing 10 ml of artificial seawater in the presence of a standard pollutant as a control, potassium dichromate ($K_2Cr_2O_7$). For 24 hours, stage II brine shrimp were subjected to 6 increasing concentrations of the pollutant, Ammonium Sulfate NPK 10-30-10, and Ammonium Nitrate and Potassium Dichromate: 10, 20, 40, 80,160 and 320 mg/l. Concentrations chosen are based on previous studies performed (Ortiz-Santaliestra et al., 2012; Karaca et al., 2017). These concentrations of agrochemicals would correspond to the maximum concentrations that are expected to appear in water bodies following runoff from applied fertilizers in agricultural landscapes (Ortiz-Santaliestra et al., 2012 ; Karaca et al., 2017). For each concentration, three identical tests were carried out, with three repetitions as well as controls for each product tested.

During the exposure period, there was no aeration and the nauplii were not fed and they were placed in an incubator at 20 °C ± 2 in the dark.

Mortality was considered as the inability of nauplii to swim for 15 s. *Artemia* mortality/immobilization was counted and the LC50 (lethal concentration that causes 50% mortality) was calculated.

Data processing

The lethal concentrations that cause 50% mortality (LC50) were analyzed by linear regression using the probit method using Microsoft Office Excel 2010 software. Mann Whitney test and Anova test were carried out to check if there is a significant difference between the different repetitions and the different pollutants used. Results were presented as mean ± standard deviation.

RESULTS AND DISCUSSION

The results of the acute toxicity tests obtained in *Artemia salina* show that there is no significant statistical difference for the different repetitions carried out (P value > 0.05).

The LC50 value of Potassium Bichromate ($K_2Cr_2O_7$) during the 24 h duration of the test is 39.06 mg/L ± 3.44. The LC50 value of this toxic substance in acute toxicity tests on invertebrates is expected to be between 0.067 and 59.90 mg/L according to USPEA recommendations (Albendín et al., 2021). The LC50 value of Potassium Bichromate ($K_2Cr_2O_7$) found is within this range, which means that the population of *Artemia salina* was optimal for performing tests with the different agrochemicals.

For an exposure period of 24 hours the results reveal the negative impact of the agrochemicals used (ammonium sulphate, ammonium nitrate and NPK) compared to the controls in which no mortality was recorded during the experimental period in *Artemia salina* (Fig. 1). It is reported the presence of a very significant difference in sensitivity in *Artemia salina* for the different agrochemicals tested in *Artemia salina*, with a higher sensitivity to Ammonium Sulfate compared to Ammonium Nitrate and NPK which did not show a significant difference in mortality between them (Fig. 2).

The observed lethal effects caused by the different agrochemicals increase significantly with increasing concentration used (Fig. 3, 4 and 5). Additionally, body length and swimming speed showed a concentration-dependent decrease after 24 h of fertilizer exposure. Swimming inhibition can be caused by malformation of the gills. As important morphological and ethological parameters, body length and swimming speed have been widely used in the toxicity assessment of nanoparticles. A similar phenomenon was reported by the study carried out by Zhu et al. (2018) on *Artemia salina* reporting that body length and swimming speed showed a concentration-dependent decrease after exposure to O-SWCNTs (oxidized single walled carbon nanotubes) for 24 h with gill malformation.

Regarding *Artemia* exposed to ammonium nitrate and NPK, the concentration of 10 mg/l did not record any mortality. Indeed, during this concentration with no observed effect, all *Artemia* still have the ability to swim. Nevertheless, the 10 mg/l concentration of ammonium sulphate is the lowest concentration causing the death of brine shrimp while it goes to the concentration of 20 mg/l for ammonium nitrate and NPK.

For LC50 (Fig. 6), the results of the statistical study also reveal a significant difference (P Value < 0.05) in sensitivity of brine shrimp to different

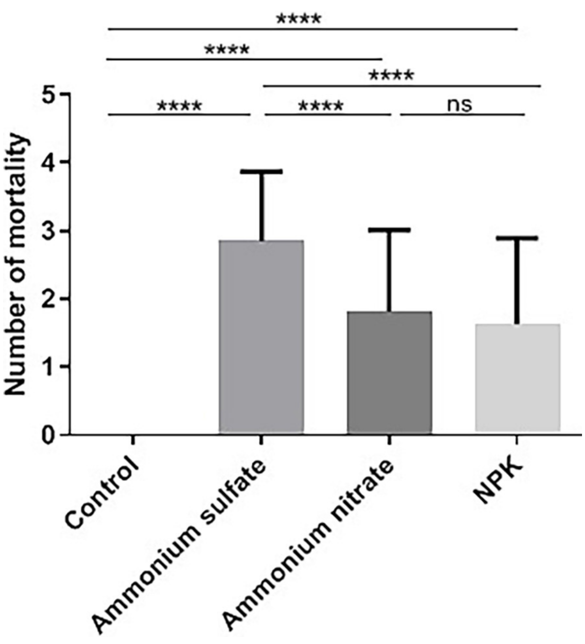

Figure 2. Estimation of the effect of the different chemical substances compared to the control in Artemia salina, *** – significant difference (P value < 0.05), ns – no significant difference (P value = 0.43)

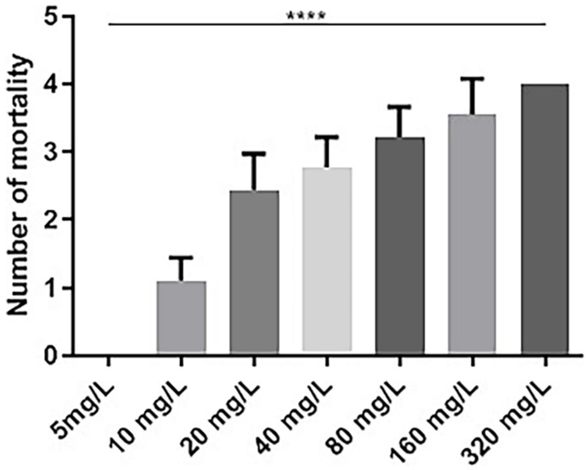

Figure 3. Estimation of the effect of different concentrations of ammonium sulphate in Artemia salina

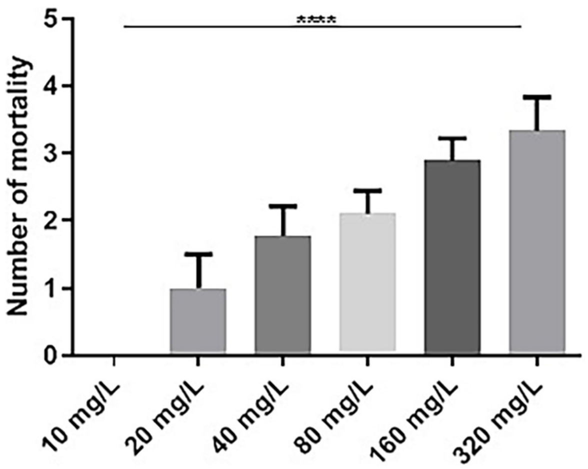

Figure 4. Estimation of the effect of different concentrations of ammonium nitrate in Artemia salina

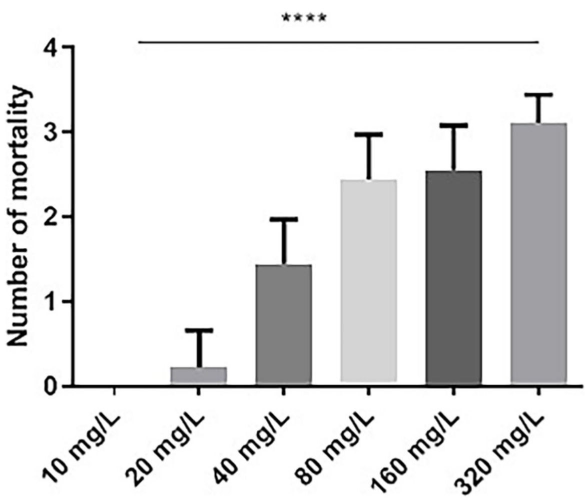

Figure 5. Estimation of the effect of different concentrations of NPK in Artemia salina, *** – significant difference

agrochemicals. A concentration of 37.32 mg/l ± 6.09 of ammonium sulphate generates 50% mortality and it goes to the concentration of 110.7 mg/l ± 7.11 for ammonium nitrate while it goes to the concentration of 143.13 mg/l ± 23.12 for NPK.

These chemical fertilizers can be ingested, accumulated and excreted by *Artemia salina*. When the application of chemical fertilizers exceeds the recommended limit, they can modify and alter the environment that can in turn negatively affect human health.

These chemical fertilizers that end up arriving in rivers, lakes or the sea are responsible for the phenomenon of eutrophication and the massive death of fish and other aerobic organisms (Idrissi, 2006; Piren-Seine, 2019; Mustafa et al., 2023), which has become a big problem in Morocco (Idrissi, 2006). The study carried out by Wenninso (2016) reveals that chemical fertilizers, more specifically urea and NPK, combined with livestock manure (sheep, cattle, poultry) have an impact on the quality of surface water which can go from a state mesotrophic to a state of degradation. Water contaminated with fertilizers causes low dissolved oxygen in surface waters, causing fish and other aquatic life to suffocate (Mustafa et al., 2023). In addition, disturbing changes in the food chain including the loss of local species can be noted (Idrissi, 2006). Therefore, the presence of fertilizers in surface waters harms biodiversity, with consequences for a large number of aquatic animals and plants.

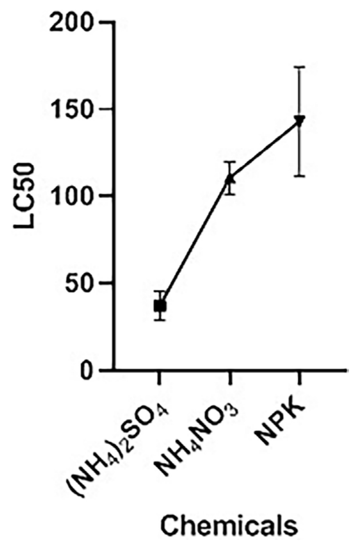

Figure 6. Estimate of the lethal concentration of ammonium sulfate, ammonium nitrate and NPK used which affects 50% of the population (LC 50) in Artemia salina

The toxicity of chemical fertilizers for aquatic animals is considerable. Exposure to agrochemicals (ammonium sulphate and ammonium nitrate) has been shown to have a negative impact on aquatic species such as anuran tadpoles: *Bufo mauritanicus*, *Rana ridibunda* (Naamane et al., 2020) and *Rana sylvatica* (Burgett et al., 2007) which are considered good bioindicators of aquatic ecosystems (Feng et al., 2004). Indeed, a high level of nitrates should have serious consequences on aquatic life (Isaza et Rodgers, 2021).

Nitrogen-based fertilizers are commonly used in agriculture and enter aquatic ecosystems through runoff (Burgett et al., 2007). The study carried out by Naamane and al. (2020) on anuran tadpoles from the same study region (Casablanca Settat) showed following a short-term exposure that a concentration of 80 mg/L of ammonium sulphate generates a 50% mortality in *Bufo mauritanicus* for the earliest stage of development that is considered the most susceptible (Stage 24). This concentration increases to 68.07 mg/l for *Rana ridibunda*. In addition to survival, tadpole activity decreased with increasing ammonium sulphate concentration.

For ammonium nitrate, the study carried out by Burgett et al. (2007) on *Rana sylvatica* revealed that survival as well as activity decreased with increasing ammonium nitrate concentration. In addition, a 50% mortality is generated by a concentration of 75±25 mg/L of ammonium nitrate. Nitrates used in agriculture have been implicated in the decline of frogs and salamanders in Canada at water concentrations greater than 60 mg NO_3/L, nitrate has lethal effects on the tadpoles of many amphibians (P.G.C., 2001). Limb and tail malformations with a significantly higher degree ($p < 0.01$) of nuclear damage in toads (*Sclerophrys regularis*) were observed after exposure to nitrate (Said et al., 2022). For fry, 50% mortality is generated by a concentration of 1121 mg NO_3-N/L for lake trout and 1903 mg NO_3-N/L for lake whitefish (McGurk et al., 2006).

Exposure of *Daphnia magna* to phosphorus at increasing concentrations (0.5; 1; 2; 4; 8 mg/l) led to a progressive reduction in survival, growth, longevity as well as delayed egg laying size (Zvidzai, 2017).

The study carried out by Gueddouche (2017) on a bioindicator invertebrate, the snail *Helix vermiculata* which was exposed by contact for 8 weeks to fresh lettuce leaves soaked in fertilizer (NPK and urea) showed that these fertilizers have an effect on energy reserves (carbohydrates, proteins and lipids), growth, an induction of the activity of antioxidant enzymes such as GST (glutathione S-transferase), GPx (glutathione peroxidase) and LDH (lactate dehydrogenase), in parallel with an increase in the level of GST, LDH and GPx, to a decrease in the level of AChE (acetylcholinesterase). Fertilizer application that exceeds the recommended limit alters the soil which harms beneficial soil microorganisms. In the presence of soil bacteria, nitrogen fertilizers can be converted into nitrous oxide which is a greenhouse gas. These nitrogen oxides (NO, N_2O, NO_2) are responsible for heavy air pollution. Although these chemical fertilizers help plants grow faster, these plants are not strong and healthy because they don't have enough time to establish strong roots and stems, or better quality fruits and vegetables. Weak roots and stem lack a good immune system and the weak plant will be more susceptible to pests and diseases (Mustafa et al., 2023).

The nitrate that is used in these fertilizers is a major component of polluted water that causes major and fatal illnesses in humans. Consumption of water contaminated with nitrates and nitrites causes methemoglobinemia in children, blue discoloration of the skin and cancer and even damage to the respiratory and vascular system (Mustafa et al., 2023).

The poor practices of these agrochemicals in crops affect the physico-chemical quality of water, and therefore have a negative effect on the biodiversity of aquatic environments. They lead to flows of chemical nutrients and pathogens towards water resources and upset ecosystems in a damaging way (Lawani et al., 2017), and consequent loss of habitats and degradation of environmental quality (Suarez et al., 2021).

According to the results, even if chemical fertilizers make it possible to maximize the output, an inefficient management of these acts on the balance of the ecosystems. It is the source of pollution of surface water, groundwater and soil and can enter the food web when absorbed by plants which will then be consumed by animals or humans. These agrochemicals affect both the environment and human health. Indeed, in the face of a rapidly increasing world population, a changing climate, increasing natural disasters, this negative impact of chemical fertilizers will be accentuated if we do not carry out fundamental changes that do not cause ecological and environmental imbalances.

Artemia salina plays an important role in the aquatic ecosystem. It is more sensitive to exposure to agrochemicals than some organisms that are considered good bioindicators of aquatic ecosystems. In addition, it has a higher sensitivity to ammonium sulfate compared to ammonium nitrate and NPK. Besides its use in aquaculture, *Artemia salina* which is characterized by a simple anatomy, a short life cycle and a small size can be considered as a good biomonitoring tool for future toxicological analyses. Finally, to ensure food security while protecting the environment and human health, the excessive use of fertilizers for high yields is not sustainable in the long term. The adoption of measures to avoid or reduce effects that are potentially serious or irreversible is necessary. Responsible use and management of fertilizers in agriculture at the farm level would help achieve the Sustainable Development Goals, including enhancing food security and nutrition, combating hunger and malnutrition, reducing pollution and improving food safety.

Additional research efforts are needed to improve our knowledge of the effects of agrochemicals on *Artemia* in particular.

CONCLUSION

At the end of this work, which aims to assess the toxicity of 3 chemical fertilizers (NPK, ammonium sulfate, and ammonium nitrate) in *Artemia salina*, the results reveal that pollution by agrochemicals presents acute toxicological effects in brine shrimp. *Artemia* show a high sensitivity to agrochemicals and they showed a significantly higher sensitivity to ammonium sulphate compared to ammonium nitrate and NPK.

To assess the ecological risk of agrochemicals, it is therefore recommended to study their toxicity on *Artemia salina* this would allow taking corrective measures to reduce or use these products judiciously, in order to eliminate the harmful effect of these synthetic chemicals on the environment. Further research is needed to increase the information available on the effects of agrochemicals on aquatic systems in general by testing with other taxa and with organisms at different stages of development.

REFERENCES

1. ADA. 2018. Guide for investors in the agricultural sector in Morocco. Agricultural Development Agency. (in French)
2. Albendín, M.G., Aranda, V., Coello, M.D., González-Gómez, C., Rodríguez-Barroso, R., Quiroga, J.M., Arellano, J.M. 2021. Pharmaceutical products and pesticides toxicity associated with microplastics (Polyvinyl chloride) in *Artemia salina*. International Journal of Environmental Research and Public Health, 18(20), 10773.
3. Barua, P., Eslamian, S. 2022. Exploitation of agrochemicals and its effect on health of farmers and environment on south-easter n coast of Bangladesh. Frontiers of Agriculture and Food Technology, 11(2), 1–9.
4. Barreau, S., Magnier, J. 2019. Fighting against nitrogen pollution and preserving aquatic environments: measures of the Sixth Regional Action Programs nitrate. International Office for Water (in French)
5. Burgett, A.A., Wright, C.D., Smith, G.R., Fortune, D.T., Johnson, S.L. 2007. Impact of ammonium nitrate on wood frog (*Rana sylvatica*) tadpoles: effects on survivorship and behavior. Herpetological Conservation and Biology, 2(1), 29–34.
6. Castro, B.T., Castro, J.M., Castro, G.M. 2003. Artemia, 67–81. In T. Castro B. (Comp.) Alimento vivo para organismos acuáticos. AGT, México.
7. Commission européenne. 2017. Agriculture and Sustainable Water Management in the EU; Commission Sta_ Document; European Commission: Brussels, Belgium.
8. Da Fontoura, L.P., Puntel, R., Pinton, S., de Ávila, D.S., da Rocha, J.B.T., de Souza, D.O., Roos, D.H. 2021. A toxicological comparison between two uranium compounds in *Artemia salina*: Artificial seawater containing $CaCO_3$. Marine Environmental Research, 163, 105221.
9. DEPF 2019. Department of studies and financial forecasts. The Moroccan agricultural sector: structural trends, challenges and development prospects (In French)
10. Feng, S., Kong, Z., Wang, X. et al. 2004. Acute toxicity and genotoxicity of two novel pesticides on amphibian, *Rana* N. Hallowell. Chemosphere, 56, 457–463.
11. Gueddouche, M. 2017. Effect of certain fertilizers on a species of gastropod, *Helix vermiculata:* Biochemistry and biomarkers (Doctoral dissertation, Laarbi Tebessi Tebessa University, Algeria. (in French)
12. Gill, R., Naeem, M., Ansari, A.A., Chhikara, A., Bremont, J.F.J., Tuteja, N., Gill, S.S. 2022. Agrochemicals in Soil and Environment: Conclusions and Future Perspectives. Agrochemicals in Soil and Environment, 609–612.
13. Idrissi, L. 2006. Nitrates and nitrites Pollutants that threaten health and the environment. Laboratory Technologies, 1, 10.
14. Isaza, D.F.G., Rodgers, E.M. 2021. Nitrate in Fresh Waters. In Nitrate Handbook, 185–208.

15. Karaca, B., Özdemir, N., Kalayci, T.E. 2017. Some Toxic Effects of Ammonium Sulphate Fertilizer to *Rana macrocnemis* Tadpoles From Rize Black Sea Region, Turkey. Biyolojik Çeşitlilikve Koruma, 10(3), 107–113.
16. Lawani, A.N., Kelome, N.C., Agassounon Djikpo Tchibozo, M., Hounkpe, J.B., Adjagodo, A. 2017. Effects of agricultural practices on the pollution of surface water in Benin Republic. Larhyss Journal, 30, 173–190. (in French)
17. McGurk, M.D., Landry, F., Tang, A., Hanks, C.C. 2006. Acute and chronic toxicity of nitrate to early life stages of lake trout (*Salvelinus namaycush*) and lake whitefish (*Coregonus clupeaformis*). Environmental Toxicology and Chemistry: An International Journal, 25(8), 2187–2196.
18. Munteanu, C., DumitraŞCU, M., Biosafety, S.C. 2011. *Artemia salina*, Balneo. Research Journal, 2(4).
19. Mustafa, G., Hayat, N., Alotaibi, B.A. 2023. How and why to prevent over fertilization to get sustainable crop production. Sustainable Plant Nutrition, 339–354.
20. Naamane, A., Sadiq, A., Belhouari, A., Iounes, N., El Amrani, S. 2020.Survey on the use of fertilizers and pesticides among farmers in the Casablanca-Settat region. Moroccan Review of Agronomic and Veterinary Sciences, 8(3), 279–285. (in French)
21. Naamane, A., Kamtass, S., Namoussi, S., Iounes, N., Belhouari, A., El Amrani, S. 2020. 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). Journal of Ecological Engineering, 21(7), 1–10.
22. Ortiz-Santaliestra, M.E., Fernández-Benéitez, M.J., Marco, A. 2012. Density effects on ammonium nitrate toxicity on amphibians. Survival, growth and cannibalism. Aquatictoxicology, 110, 170–176.
23. ONU. 2019. United Nations World Water Development Report: Leaving No One Behind.
24. P.G.C. 2001. Toxic effects induced by the addition of nutrients. Government of Canada publications. (in French)
25. Piren seine. 2019. Agriculture and its effects on water and aquatic environments. Arceau-idf. (in French)
26. Rajabi, S., Ramazani, A., Hamidi, M., Naji, T. 2015. *Artemiasalina* as a model organism in toxicity assessment of nanoparticles. DARU Journal of Pharmaceutical Sciences, 23(1), 1–6.
27. Said, R.E.M., Said, A.S., Saber, S.A.L., ElSalkh, B.A.E. 2022. Biomarker responses in *Sclerophrys regularis* (Anura: Bufonidae) exposed to atrazine and nitrate. Pollution, 8(4), 1387–1397.
28. Suarez, R.P., Goijman, A.P., Cappelletti, S., Solari, L.M., Cristos, D., Rojas, et al., 2021. Combined effects of agrochemical contamination and forest loss on anuran diversity in agroecosystems of east-central Argentina. Science of The Total Environment, 759, 143435.
29. Wenninso C.D. 2016. Impact des engrais chimiques (urée, NPK) et des déjections d'animaux (moutons, bœufs, volailles) sur la qualité de l'eau brute (cas du barrage de Ziga). Mémoire pour l'obtention du master en ingénierie de l'eau et de l'environnement option : eau et assainissement. Institut International d'Ingénierie de l'Eau et de l'Environnement. Ouagadougou –Burkina faso
30. Zambrano-Fernández, S., Zamora-Camacho, F.J., Aragón, P. 2022. Direct and indirect effects of chronic exposure to ammonium on anuran larvae survivorship, morphology, and swimming speed. Chemosphere, 287, 132349.
31. Zhu, B., Zhu, S., Li, J., Hui, X., Wang, G.X. 2018. The developmental toxicity, bioaccumulation and distribution of oxidized single walled carbon nanotubes in *Artemia salina*. Toxicology Research, 7(5), 897–906.
32. Zvidzai, C.C. 2017. The effects of phosphorus pollution on the growth, reproduction and dynamics of the population of Daphnia magna. Thesis presented with a view to obtaining the Master's degree. Mentouri Constantine Brothers University, Algeria. (in French)