PREDICTION OF WATER POLLUTION LEVEL USING ACUTE TOXICOLOGICAL TESTS

Belinda Hoxha
University of Elbasan "A.Xhuvani", Faculty of Natural Sciences, Department of Chemistry, Elbasan, Albania, belinda.hoxha@uniel.edu.al, belindahoxha@hotmail.com
Marilda Osmani
University of Elbasan "A.Xhuvani", Faculty of Natural Sciences, Department of Chemistry, Elbasan, Albania, marilda.osmani@uniel.edu.al, marilda.86@gmail.com
Armela Mazrreku
University of Elbasan "A.Xhuvani", Faculty of Natural Sciences, Department of Chemistry, Elbasan, Albania, armela.mazrreku@uniel.edu.al, armelamazreku@hotmail.com

Abstract: Water pollution can be defined as the accumulation in high quantity of chemical, biological and physical materials in water bodies, which might destroy the quality of water life. There are a wide range of pollutants that alter the natural water parameters. Environmental toxicology is a multidisciplinary field that deals with the study of the adverse effects of various chemical, biological and physical agents in living terrestrial/water organisms. Ecotoxicology deals with the study of the adverse effects of chemical toxicants in the population and in different levels of ecosystem. A living organism might be exposed toward a specific toxicant in different stages of its life cycle. The toxicity might differ when considering the given organism in within its food web. Bioaccumulation occurs when the organism accumulates the toxicants in its fatty tissues. This process is usually limited in the areas affected by the environmental toxicants. The adverse effects of such chemical agents might influence a specific organism or its whole community and thus decreasing the species diversity.

Ecotoxicological studies use living organisms to predict the effects of different chemicals in various environments, including water systems. Water toxicology is the study of the effects of anthropogenic chemicals on water organisms. Some non-volatile compounds might be soluble in water and thus the water organisms are exposed to them. Acute and chronic toxicity tests are performed for different living organisms. Daphnia is one of many ecologically important species of fresh water systems. It can be reproduced with parthenogenesis in optimal conditions, but during the periods with less food supplies and during the cold period of time, males appear in the offspring and the sexual reproduction occurs. In laboratory continuously optimal conditions only female organisms can be born, and making thus possible to have a genetically identical population. Daphnia sp. is one of the organisms used to foresee the possible impact of the chemicals in water bodies.

This study takes in consideration the acute tests using *Daphnia sp*, as an indicator of possible effects in water of different concentrations of nickel solutions. These organisms, which are natural inhabitants of water systems, are raised in laboratory conditions in accordance with their natural habitat. Various nickel concentrations are used to test Daphnia behavior and mortality. During the acute tests the living organisms of less than 24 hours of life are exposed to nickel solutions for a period of time not more than 24 hours. The acute test goal is to find the chemical concentration that immobilizes 50% of the tested organisms in 24 hours. These tests are performed according to OECD guides and standards.

The laboratory data are then elaborated in order to determine the highest concentration of the chemical that shows the highest mortality of the organisms, as well as the lowest concentration with no effect in the population. Experiments show that Daphnia organisms exhibit various behaviors in different chemical concentrations.

Keywords: ecotoxicology, Daphnia sp., chemical concentration, mortality, acute tests

1. INTRODUCTION

Water pollution refers to the contamination of water bodies mainly as a result of various anthropogenic activities, which contribute with a wide range of water toxicants and chemicals. This leads to poor water quality and as a consequence adverse effects on living organisms and ecosystems appear. There are several types of water pollutants, including inorganic, organic and biological ones (Wong 2012). Among inorganic toxicants, a special attention was paid to heavy metals, such as nickel, because of their adverse environmental effects even in low concentrations in water bodies (Salomons et al 1995). The leaching from the metals on contact with drinking water or the dissolution from the nickel ores are considered the main anthropogenical contributors of nickel in water. It might occur in various chemical forms in groundwater, mostly in complex forms, depending on water pH. Its mobility in the soil increases with the increase of acid rain, and as a consequence might lead to an increase of nickel in water (IPCS

1991). The exposure for a long period of time to concentrations lower than lethal of nickel in water leads to several effects on invertebrates (FAO 1984).
The goal of ecotoxicology is to predict the effect of toxic chemicals on living organisms (Altenburger 2011) and the whole environment (Truhaut 1977). Toxicity tests are used to evaluate the adverse effects of a certain chemical on a specific organism (Rand and Petrocelli 1985). Therefore, these tests offer a valuable tool on assessing the fate of a certain toxicant in the environment, and predicting the specific bioindicator organism for the chemical (Adams and Rowland 2003). Ecotoxicology uses the LC 50 of a chemical, which is the estimated concentration of the chemical at which 50% of the population is expected to die. The dose/response curve represents the number of organisms affected against the dose of the contaminant (Sparling 2018).
Daphnia is one of many ecologically important species of fresh water systems. It can be reproduced with parthenogenesis in optimal conditions, but during the periods with less food supplies and during the cold period of time, males appear in the offspring and the sexual reproduction occurs (Zaffagnini 1987). In laboratory continuously optimal conditions only female organisms can be born, and making thus possible to have a genetically identical population. Daphnia sp. is one of the organisms used to foresee the possible impact of the chemicals in water bodies (Hodgson 2004).
This study takes in consideration the acute tests using *Daphnia sp*, as an indicator of possible effects in water of various concentrations of nickel solutions. These organisms, which are natural inhabitants of water systems, are raised in laboratory conditions in accordance with their natural habitat. Different nickel concentrations are used to test Daphnia behavior and mortality. During the acute tests the living organisms of less than 24 hours of life are exposed to nickel solutions for a period of time not more than 24 hours. The acute test goal is to find the chemical concentration that immobilizes 50% of the tested organisms in 24 hours. These tests are performed according to OECD guides and standards.

2. MATERIALS AND METHODS

The focus of this study was the effect of soluble nickel on Daphnia sp. immobilization for 24 h and the evaluation of LC50. The organisms were collected from their natural habitat and then are raised in similar laboratory conditions with their natural habitat. The oxygen saturation was kept > 60% during the whole experiment. This testing procedure is based on the ISO: 6341 standard (1989) and OECD (1984) guideline for testing the chemicals. According to the standard, the LC50 is the concentration of the chemical that immobilizes 50% of the exposed animals in 24h. Therefore, Daphnia sp., are exposed to different concentrations of nickel solutions for 24 h in a controlled atmosphere of 20±2°C.
A stock solution of nickel was used to prepare different concentrations of this testing chemical. The concentrations used in the experiment are of $0.1\cdot10^{-4}$ M, $0.3\cdot10^{-4}$ M, $0.8\cdot10^{-4}$ M, $0.9\cdot10^{-4}$ M, $1\cdot10^{-4}$ M, $2.5\cdot10^{-4}$ M, $3\cdot10^{-4}$ M, $4\cdot10^{-4}$ M, and $5\cdot10^{-4}$ M. These concentrations are prepared with the appropriate dilution with the dilution solution composed of 25 ml of $CaCl_2$ (8.88 gr/l), 25 ml of $MgSO_4$ (4.93 gr/l), 25 ml of $NaHCO_3$ (2.59 g/l) and 25 ml of KCl (0.23 gr/l), and then distilled water is added till 1 liter of solution. NaOH or HCl are used to adjust the pH.
During the experiment four parallel tests are conducted for each concentration. Every single test used approximately 4 young organisms of Daphnia. Afterwards, the beakers with the animals are incubated in 20°C for 24 hours. The immobilized individuals are then counted in each sample beaker using a microscope.

3. RESULTS AND DISCUSSIONS

The mobilization of Daphnia individuals were tested with 10 different concentrations of nickel solution, with 4 parallel tests for each concentration. The total number of the organisms used in each concentration was 16, with 4 individuals in each testing Becker.
The tested organisms were incubated for 24 h at approximately 20°C. Afterwards the Daphnia were counted using a microscope, type Motic DMI43 Digital Stereo Microscope with camera. The non-moving organisms for at least 15 seconds are considered immobilized. The table 1 is a summary of the experiment, showing the total number of the mobile individuals and the percentage of the immobilized organisms in each concentration.

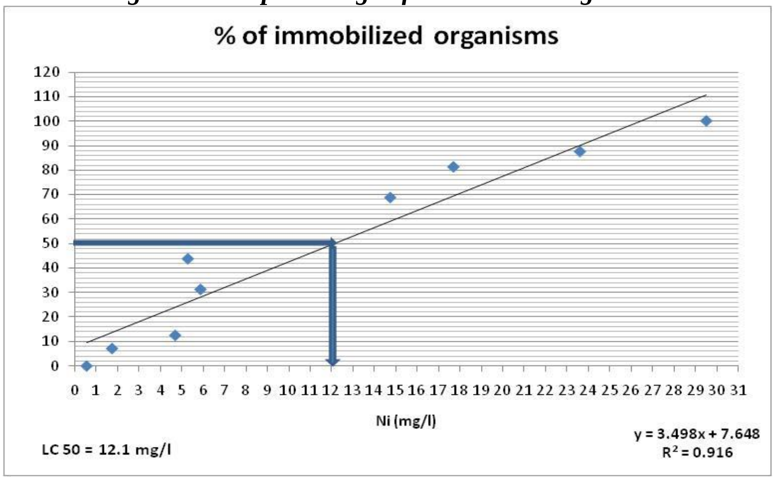
Table. 1. Mobile organisms in different nickel concentrations

Ni solution (mol/L)	**Number of mobile Daphni sp. in each test Becker**				**T**	**P**
	1	**2**	**3**	**4**		
0 (control)	4	4	4	4	16	0
$0.1 \cdot 10^{-4}$	4	4	4	4	16	0
$0.3 \cdot 10^{-4}$	4	3	3	3	13	7.14286
$0.8 \cdot 10^{-4}$	3	4	3	4	14	12.5
$0.9 \cdot 10^{-4}$	2	3	3	1	8	50
$1 \cdot 10^{-4}$	3	2	3	3	11	31.25
$2.5 \cdot 10^{-4}$	2	1	1	1	5	68.75
$3 \cdot 10^{-4}$	1	1	1	0	3	81.25
$4 \cdot 10^{-4}$	0	1	1	0	2	87.5
$5 \cdot 10^{-4}$	0	0	0	0	0	100

T is the number of the mobile Daphnia individuals; **P** is the percentage of the immobilized organisms in each concentration

As it can be seen from the figures in the table 1, the when the concentration is of $0.1 \cdot 10^{-4}$ M (which corresponds to 0.59 mg/l of soluble Ni), no effect appears on the living organisms. Meanwhile, at the concentration of $5 \cdot 10^{-4}$ M (corresponding to 29.5 mg/l of soluble Ni), no mobile organisms were observed. Thus, the increase of nickel solution concentration leads to the decrease of the mobile organisms. This suggests that the increase of the concentration would have an impact on the sensitivity of Daphnia individuals towards the chemical.

Figure 1. The percentage of immobilized organisms



The graph in figure 1 represents the percentage of the immobilized organisms depending on nickel content in mg/l in each of the testing solutions. The nickel content in mg/l was calculated based on its molar concentration of the testing solutions. The figure 1 shows a linear relationship between the daphnia mortality and nickel content in testing solutions. Concentrations lower than $0.1 \cdot 10^{-4}$ M, which corresponds to 0.59 mg/l of Ni show no adverse in daphnia individuals and thus resulting in 0% of mortality. On the contrary, 100% of the organisms resulted immobilized on concentrations of $5 \cdot 10^{-4}$M, corresponding to 29.5 mg/l Ni, or higher.

LC50 was calculated based on the equation in figure 1. Thus, 50% of Daphnia individuals would be immobilized in nickel concentration of 12.1 mg/l.

The determination of the relative toxicity of nickel solutions in daphnia organisms is performed using the probit analysis according Finney (1952).

Table 2. Probit analysis (Finney 1952)

Calculation of LC 50 using probit analysis	
Log 10 dose (based on mg/l Ni)	Empirical probit
-0.229	0
0.25	3.52
0.67	3.82
0.73	4.85
0.77	4.5
1.17	5.5
1.25	5.88
1.37	6.18

Figure 2. LC 50 calculation using the Probit analysis

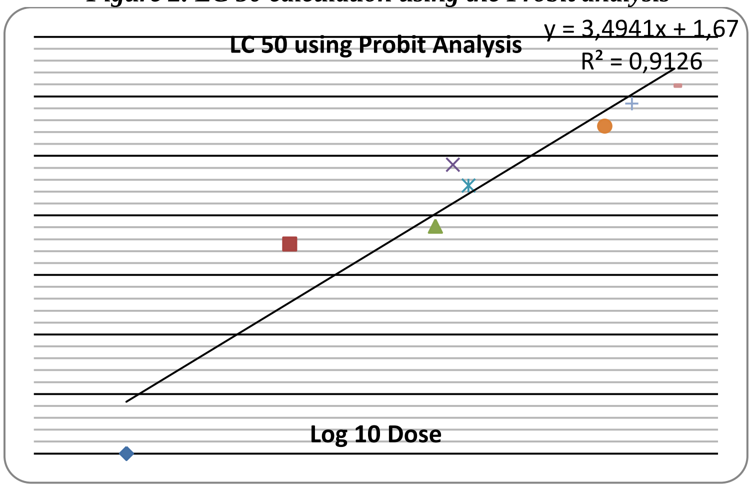


Figure 2 represents the LC 50 of the daphnia individuals using probit analysis, according the data on table 2, with 95% of confidence. The analysis shows that the LC 50 for Daphnia individuals in nickel solution is 8.438 mg/l Ni, with an upper level of 11.36 mg/l Ni and the lower level of 6.267 mg/l Ni. Meanwhile, the calculation of LC 50 based on the equation in figure 2 is evaluated as 13.8 mg/l Ni.

4. CONCLUSIONS

Water quality takes a special focus nowadays. Toxicity testing represents a tool in the prediction of the toxic level of a certain chemical and thus representing a serious risk for the water environment and water life. This experimental study showed some preliminary data on the effect of Ni on Daphnia sp. as bio-indicator species. The experimental LC 50 was 12.1 mg/l of soluble Ni, and the statistical LC 50 was 13.8 mg/l. This suggests that concentrations higher than 12.1 mg/l Ni would lead to Daphnia mortality higher than 50%. In concentrations higher than 29.5 mg/l Ni, 100% of the individuals were immobilized. Summarizing, the increasing of the nickel concentrations leads to higher mortality of daphnia sp individuals. Therefore, the environmental adverse effects of this chemical in water bodies and water life could be predicted.

REFERENCES

Adams, W.J. and Rowland, C.D. (2003). Aquatic toxicology test methods. In *Handbook of Ecotoxicology*, D. J. Hoffman, B. A. Rattner, G. A. Burton Jr., and J. Cairns Jr., Eds. Lewis Publisher

Altenburger, R. (2011). *Metal ions in toxicology: effects, interactions, interdependencies*. RSC Publishing

FAO. (1984). *Water quality criteria for European freshwater fish*. Report on nickel and freshwater fish, EIFAC Technical Paper 45

Finney, D.J. (1952). Probit Analysis, 2nd edition. *Journal of the Institute of Actuaries*, 78 (3): 388-390

Hodgson E. (2004) *A Textbook of Modern Toxicology* (3rd ed.) Wiley-Interscience

IPCS. (1991). *Nickel*. Geneva, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 108).

ISO 6341. (1982). *Water Quality - Determination of the Inhibition of the Mobility of Daphnia magna Straus* (Cladocera, Crustacea)

OECD. (1984). *GUIDELINE FOR TESTING OF CHEMICALS. Daphnia sp., Acute Immobilization Test and Reproduction Test* http://www.oecd.org/dataoecd/17/21/1948249.pdf

Rand, G. M., and Petrocelli, S.R. (1985). *Fundamentals of aquatic toxicology: Methods and Applications*. Hemisphere Publishing

Salomons, W., Forstner, U., Mader, P. (1995). *Heavy metals: Problems and solutions*. Springer-Verlag

Sparling, D.W. (2018). *Basics of Ecotoxicology*. CRC Press

Truhaut, R. (1977). Eco-toxicology – Objectives, principles and perspectives. *Ecotox Environ Safe* 1: 151-173

Wong, M.H. (2012). *Environmental contamination: Health risks and ecological restoration*. Taylor & Francis Group

Zaffagnini F. (1987). Reproduction in Daphnia. *Memorie Istituto Italiano Idrobiologia* 45: 245–284.