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TOXICITY TESTS USING LIVING ORGANISMS

SUMMARY

Toxic chemicals pose a serious and continuous risk for specific parts of the food chain, leading to the extinction of different communities. The effects of toxins might be direct or indirect. The sub lethal effects lead to the change of the behavior of an organism. The acute or chronic tests might be performed for water or terrestrial organisms, according to the OECD standards, in different levels of the food chain and thus it is possible to predict the pollution effect in the ecosystem.

This study has taken in consideration the acute and chronic tests using *Daphnia sp*. The goal of the research is the behavior evaluation of these organisms, common inhabitants of water systems, towards the change of the concentration of different chemicals. These organisms are taken from their natural habitat and then raised in laboratory conditions, in accordance with their natural habitat needs. Acute tests are performed using different concentrations of specific chemicals. Physical-chemical parameters of their natural water habitat are taken in consideration as well.

The experimental laboratory data are then elaborated in order to determine the highest concentration of the tested chemical that shows adverse effects on *Daphnia* population, as well as the lowest concentration with no effect. The laboratory results are evaluated and compared with other similar studies.

INTRODUCTION

The increase of the anthropogenic activities is associated with the increase of the toxic pollutant concentration in different media. As a consequence, the determination of bio indicator species towards different levels of toxic substances in a media receives a special importance. Ecotoxicology is the

study of the toxic effects caused by natural or synthetic pollutants on the components of the ecosystem (Truhaut, 1977). Toxic chemicals represent a serious and continuous risk to particular parts of food chain, and thus leading in disappearance of different communities. The toxin effect could be direct or indirect. Sublethal effects lead in changes of the behavior of an organism (Relyea and Hoverman, 2006). Moreover, toxins might modify the spread of particular individuals in a given population (Newman and Dixon, 1996), but they even influence the ratio between the predator and prey, depending on the population affected by the toxins (OSU, 2011).

The toxicity tests can be performed for terrestrial or aquatic organisms, based on the standards established by the OECD, in different levels of the food chain. These tests include acute and chronic toxicity tests (HSUS, 2011). Acute toxicity tests measure the adverse effects of the tested chemical that results from the animal single or a multiple exposures in a short period of time. Chronic toxicity tests are used to show the health adverse effects from the exposure to the chemical, usually at lower doses, for a long period of time. EC 50 indicates the concentration of the tested chemical where 50% of the population shows a response after a specified exposure period. LC 50 indicates the mean lethal concentration of the chemical that causes 50% of mortality in the tested animal in a single exposure. LD 50 is defined as the amount of a chemical, which causes the death of 50% of the tested animals during a short-time exposure (acute toxicity) (Hodgson 2004; Schultz 2013).

Daphnia sp is an important component of zooplankton in fresh water bodies. *Daphnia* are small arthropods of about 1 – 5 mm. They usually populate the fresh water bodies, but some species might live in sea water as well. They feed on algae, bacterial flora and smaller zooplankton. They are an important part of the diet of small fish and insects. The individual ontogenesis is direct without the larval phase. Throughout the year it has one or some biological cycles, during which the parthenogenic generations alternate with bisexual generations (Zaffagnini 1987; Omstead and LeBlanc, 2009). Moreover, *Daphnia* is recommended by OECD for laboratory testing of the influence of different chemicals in natural systems.

Environmental issues, especially the water problems are of really concern nowadays in Albania. The goal of the study is to demonstrate the role of toxicology tests with *Daphnia sp* in the evaluation of different levels of toxins in aquatic environments. *Daphnia* tested were collected from the temporary ponds with large content of organic matters in vicinity of Elbasan, one of the most polluted in the country. This paper presents some preliminary data on acute toxicological tests with *Daphnia sp*, part of a major research. Potassium dichromate solutions are used to assess *Daphnia sp* susceptibility toward this chemical.

MATERIALS AND METHODS

The study has considered the immobilization tests with *Daphnia* for 24h and the evaluation of LC50. The *Daphnia* individuals were collected from the temporary ponds with large content of organic matters in vicinity of Elbasan and then are tested in laboratory conditions similar with those in nature. The O_2 saturation is >60%. The procedure is based on the ISO: 6341 standard (1989) and OECD (1984) guideline for testing the chemicals. The standard determines the initial concentration that immobilizes 50% of exposed *Daphnia* in 24 h. This concentration is known as LC50 24h. The *Daphnia* individuals are exposed to different chemical concentrations for 24 h in a controlled atmosphere of 20 ± 2° C.

The procedure involves the use of Potassium dichromate as a testing chemical, with various concentrations prepared just before the experiment. Different concentrations of 0.5, 0.7, 1.2, 1.7 and 2 mg/l of dichromate are prepared using a stock solution (10 mg/l) with the appropriate dilution with the dilution solution. The dilution solution is prepared mixing 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 with distilled water till 1 liter of solution. The pH is adjusted properly with NaOH or HCl.

Four parallel testing beakers are prepared for each concentration, and in each of them are placed approximately 4 *Daphnia* young individuals. Then, the samples are incubated in 20°C for 24 hours. After this period of time, the immobilized individuals are counted using a microscope for each sample and each concentration.

DISCUSSION

The environmental issues regarding the water quality are taking special attention nowadays. Ecotoxicological tests use living organisms to predict the possible effects of certain chemicals in environment. This study uses *Daphnia sp* collected from temporary ponds rich with nutrients to test the ecotoxicology of Potassium dichromate. Six different concentrations (as mg/l) of this chemical were used, with 4 parallel tests for each concentration. The total number of individuals for each concentration is 20, with 5 individuals in each testing Becker.

Daphnia individuals in each beaker were incubated for 24 h at approximately 20° C. Then, after 24 h, the *Daphnia* were counted using a microscope, type *Motic DMI43 Digital Stereo Microscope* with camera. The *Daphnia* are considered immobilized when they do not show any movement for at least 15 seconds. Table 1 shows the number of the mobile individuals in different concentrations of the tested chemical.

Table 1. Mobile *Daphnia sp* in different dichromate concentrations

$K_2Cr_2O_7$ (mg/l)	Number of mobile Daphnia magna in each test Becker				T	P
	1	**2**	**3**	**4**		
0 (control)	5	5	5	5	20	0
0.5	5	4	4	3	16	20
0.7	4	4	3	2	14	30
1.2	3	2	2	2	9	55
1.5	3	2	2	1	8	60
1.7	0	2	2	1	5	75
2	**0**	**0**	**1**	**0**	**1**	**95**

T is the number of mobile *Daphnia sp* at each concentration; **P** is the percentage of the immobilized *Daphnia* at each concentration.

As it can be seen by the table, with the increasing of concentration the number of the mobile *Daphnia* decreases. This indicates that the increase of chemical concentration enhances the *Daphnia* sensitivity towards it.

Figure 2 is a plot of mortality of *Daphnia sp* in the selected concentrations of tested dichromate. It shows a linear relationship between the concentration and *Daphnia* mortality.

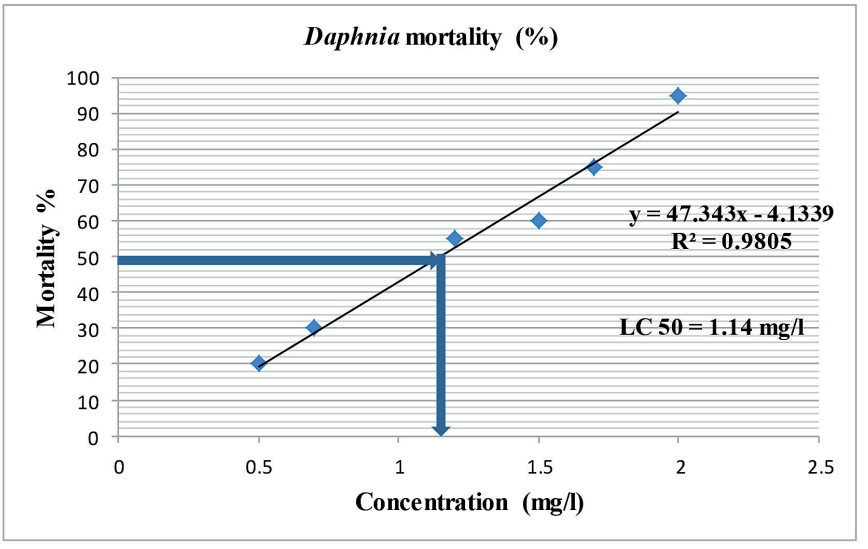

Figure 1. LC 50 of *Daphnia* in different dichromate concentrations.

LC 50 is calculated from the equation in Figure 1. It is evaluated as 1.14 mg/l of Potassium dichromate, which corresponds to 403.2 mg/l of dissolved Chromium, showing the 50% of the immobilized *Daphnia*. Concentrations lower than 0.5 mg/l dichromate resulted in 0% of the immobilized individuals, which corresponds to 176 mg/l of the dissolved Chromium in water. More than 95% of the individuals resulted dead in concentrations higher than 2 mg/l dichromate, which corresponds to 707.1 mg/l of dissolved Chromium in water.

The figure 2 represents LD50/LC50, which is determined using the Probit analysis (Finney 1952) with a 95% of confidence level. According to this statistical calculation the LD 50/LC 50 for *Daphnia sp.* in the presented experiment would be 0.995 ppm of the tested chemical (approximately 351.9 mg/l Cr). Meanwhile, the lower level of the chemical for LD50/LC50 is 0.788 ppm (approximately 278.7 mg/l Cr) and the upper level is 1.257 ppm (approximately 444.6 mg/l Cr). These results are in concordance with the ISO 6341 standard (1989) for the dichromate effects in *Daphnia* individuals for a 24 h exposure period. The Chromium is calculated as dissolved in water.

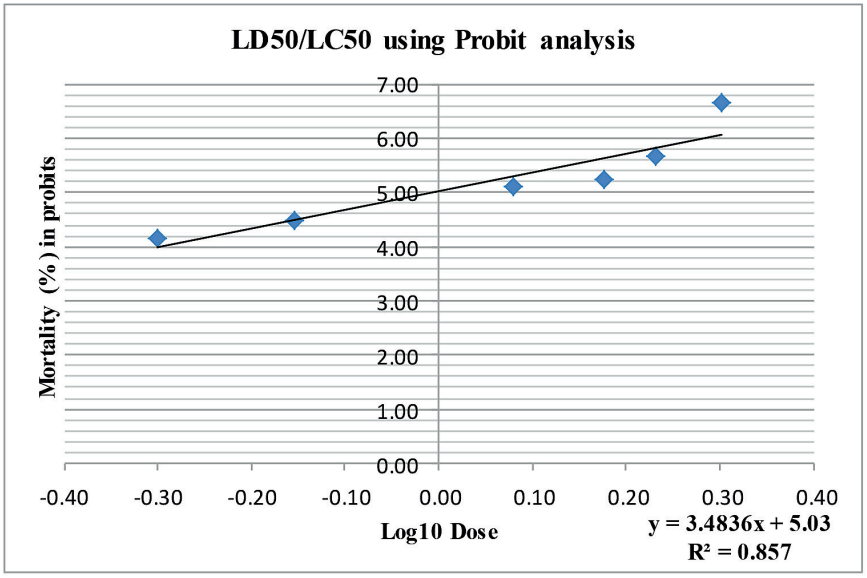

Figure 2. LD50/LC50 of *Daphnia sp* using Probit analysis.

CONCLUSIONS

Daphnia sp. has a great advantage in toxicity experiments, and it is quite easy to cultivate it in the laboratory. This study includes preliminary data of an ongoing experimental project, which aims in predicting the effect of certain environmental pollutants in water bodies using *Daphnia sp.* as bioindicator.

Daphnia individuals were collected from the temporary ponds with large content of organic matters in vicinity of Elbasan. The study shows that increasing concentrations of the selected chemical affect the mobility of *Daphnia*. Therefore, the possible environmental effects of this particular anthropogenic pollutant in water bodies, with origin from industrial sources in the area could be predicted.

Several environmental parameters or the combinations of some of them with anthropogenic chemicals are subject of further ecotoxicological studies with *Daphnia* sp. That would allow the evaluation of the pollutants' impact on natural water systems.

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