

## PRELIMINARY ECOTOXICITY TEST ON *LEMNA MINOR* OF SAMPLES COLLECTED IN 15.10.2020 IN ROMANIA

### 1 Introduction

The samples collected in February 2021 from Romania, Moldova Nouă area, were tested in order to determine their ecotoxic effects towards the aquatic macrophyte, *Lemna minor*.

### 2 Materials and methods

The assay involved the exposure for 7 days of a total number of 10 duckweed fronds, using 2 colonies of 3 fronds each and 2 colonies of 2 fronds each, for each sample. The water samples were tested without diluting the samples, using a volume of 10 mL from each sample, while the sediment and soil samples were tested by adding 0.1 g sediment / soil to 10 mL culture media, obtaining a concentration of 1% (equal to 10 mg/mL or 10000 mg/L). The assay also included a negative control (duckweed in culture media) and a positive control (0.5% ZnCl<sub>2</sub>). All samples and the two controls were tested in triplicates.

### 3 Results

None of the tested samples showed ecotoxic effects to duckweed, although some of the samples showed a reduction of green frond number (Figure 1).

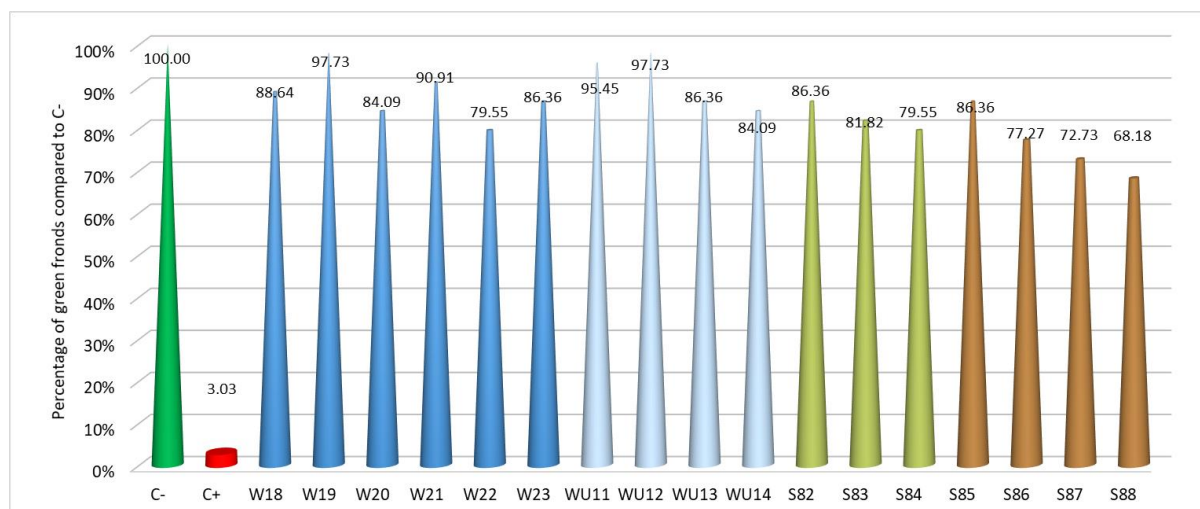


Figure 1. Percentage of green fronds for each tested samples, water, sediment and soil, compared to the negative control.

From the surface water samples, the lowest number of green fronds was observed for sample ID W22 (water from Nera river, from Socol village), representing 79.55% compared to the negative control. Regarding the well water samples, the lowest number of green fronds was observed in the case of sample ID WU14 (groundwater from well from Moldova Noua city), representing 84.09% compared to the negative control (Figure 2).

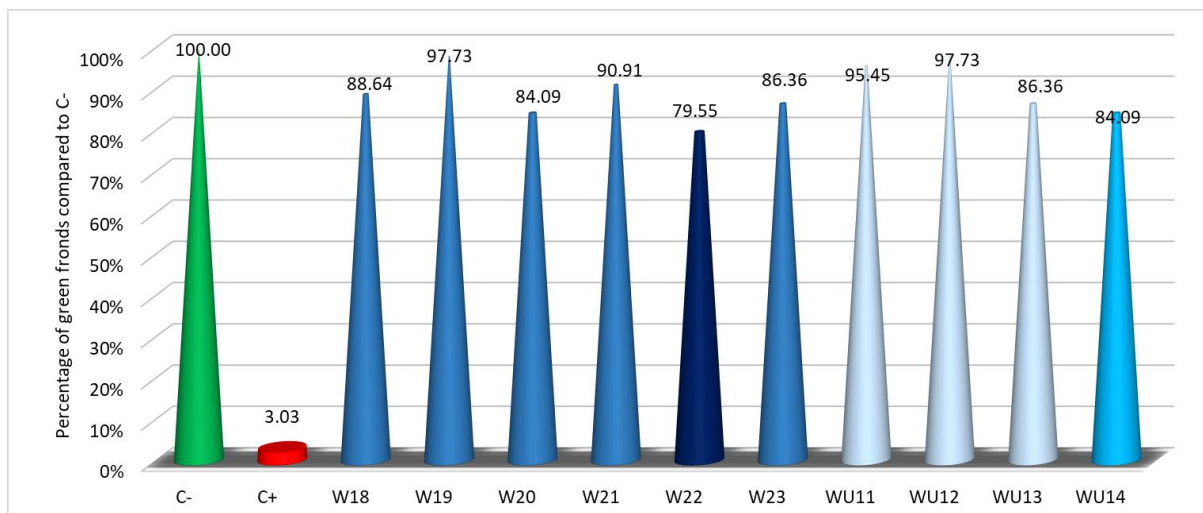


Figure 2. Percentage of green fronds for each tested water samples, both surface and groundwater, compared to the negative control.

Regarding the sediment samples, the lowest number of green fronds was observed for sample ID S84 (sediment from Nera river), representing 79.55% compared to the negative control. The soil sample with the lowest number of green fronds was the sample with ID S88 (soil from 600 m distance in N-V direction inside the Bosneag tailing pond), representing 68.18% compared to the negative control (Figure 3).

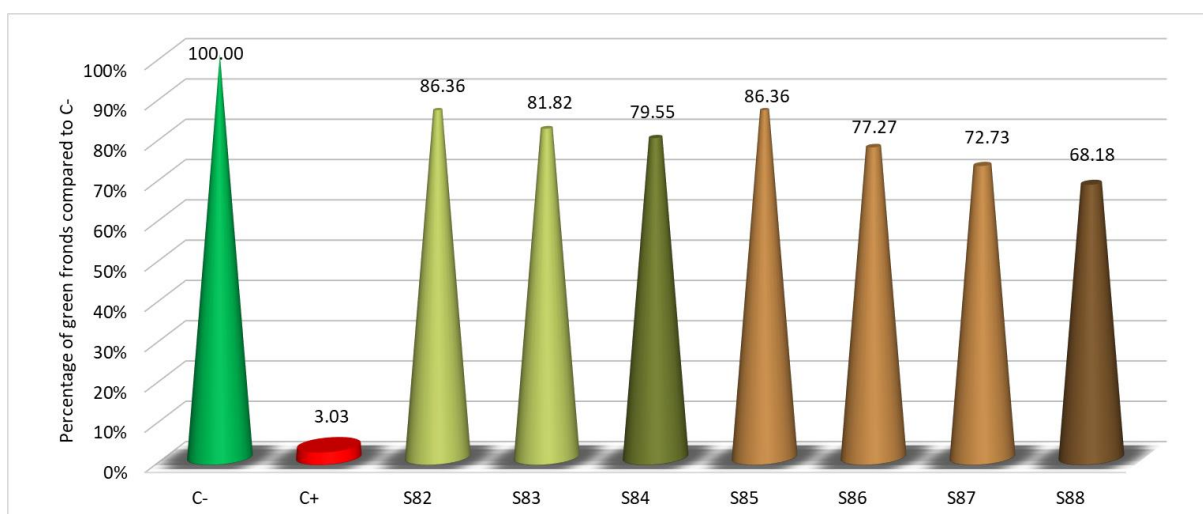


Figure 3. Percentage of green fronds for each tested sediment and soil samples compared to the negative control.

## 4 Interpretation of results

As the water samples were tested undiluted, these samples are considered as not toxic to *Lemna minor*. Even more, the lowest number of green fronds observed for water samples, which represents 79.55% (W22) from the negative control, is only 1.25 times smaller than the negative control.

The soil samples could be tested in a more concentrated dose, but this is not necessary as the tested concentration is a high-enough concentration in order to reveal significant toxic effects. Regarding the sediment and soil samples, the lowest number of green fronds observed for sediment / soil samples, which represents 68.18% (S88) from the negative control, is only 1.46 times smaller than the negative control. According to both U.S. Environmental Protection Agency [1] and United Nations [2], a  $EC_{50}$  value higher than 100 mg/L is considered as practically non-toxic. Thus, the tested concentration, of 10000 mg/L, is a high enough concentration to be classified as nontoxic even if it would represent the  $EC_{50}$  value.

## 5 References

1. U.S. Environmental Protection Agency. Analysis Phase: Ecological Effects Characterization. In Technical Overview of Ecological Risk Assessment; EPA: Washington, DC, USA, 2017. Available online: <https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/technical-overview-ecological-riskassessment-0> (accessed on 28.11.2019).
2. United Nations. Part 4 Environmental Hazards. In Globally Harmonized System of Classification and Labelling of Chemicals (GHS), 5th ed.; United Nations: New York, NY, USA; Geneva, Switzerland, 2013; pp. 219–243.



### Cooperation beyond borders.

Interreg-IPA Cross-border Cooperation Romania-Serbia Programme is financed by the European Union under the Instrument for Pre-accession Assistance (IPA II) and co-financed by the partner states in the Programme.

**Project RoRS 337- ROMania Serbia NETwork for assessing and disseminating the impact of copper mining activities on water quality in the cross-border area (RoS-NET2)**