PRELIMINARY RESULTS ON THE ECOTOXIC EFFECTS OF ACETIC ACID TO COMMON DUCKWEED (*LEMNA MINOR* L.)

Bianca-Vanesa BOROS[1*], Nathalie Iris GRAU[2], V. OSTAFE[1]
[1] West University of Timisoara, Department of Biology–Chemistry, Pestalozzi 16, Timisoara, 300315, Romania & Advanced Environmental Research Laboratories, Oituz 4, Timisoara 300086, Romania
[2] RWTH Aachen University, Institute for Environmental Research, Worringerweg 1, 52074 Aachen, Germany
**E-mail address: bianca_boros2006@yahoo.com*

Abstract: *Acetic acid has a wide range of applications in fields as food industry, in chemistry, in chemical industry and in medicine. Due to its numerous applications in several industries, the pollution of the environment with acetic acid is possible, thus the assessment of its ecotoxicity is imperative. The ecotoxicity of acetic acid was assessed using a growth inhibition assay on duckweed (Lemna minor L.) followed by the determination of fresh and dry weight. The growth inhibition assay and the weight and measurements were conducted in accordance with the OECD 221 guideline on duckweed cultivated under standard conditions. Ten concentrations of acetic acid were tested (0.5%, 0.25%, 0.05%, 0.025%, 0.005%, 0.0025%, 0.0005%, 0.00025%, 0.00005% and 0.000025%). For each replicate, 35 fronds of duckweed were maintained under standard conditions for 7 days, when the number of green fronds, fronds with chlorosis and colonies were assessed. The fronds were then weighted after blotting on paper for the fresh weight measurements. For the dry weight measurements, the fronds were maintained at 60˚C until no weight change was recorded. The average specific growth rate and water content were also calculated from the obtained data. Dose-response curves were plotted based on both total number of fronds and percent inhibition of growth rate with the concentrations of acetic acid, thus enabling the calculation of the median effective concentration (EC_{50}) and median inhibition of growth (ErC_{50}). The results showed that the four highest concentration caused a 100% growth inhibition of duckweed. The total number of fronds, green fronds and colonies increased with the decrease in concentration, while the number of fronds with chlorosis decreased. The five highest concentrations inhibited the growth rate similarly to the positive control, while the five lowest concentrations tested had very low inhibitory effect. Only the highest two concentration cause a decrease of percent water content. Regarding the two dose-response curves, the calculated EC50 and ErC50 values were similar, being 38.8 mg/L and 39.4 mg/|L, respectively. All results showed that acetic acid did affect the common duckweed fronds.*

Keywords*: ecotoxicity, common duckweed, acetic acid*

INTRODUCTION

Acetic acid or ethanoic acid is a major chemical product with a global consumption in 2018 of over 16 million tons. The growth of its application in products such as adhesives, coatings, resins, vinyl alcohols, cellulosic fibers, etc. led to its economic development (DIMIAN *et al.* 2019). Due to its wide range of applications in fields as food industry, in chemistry, in chemical industry and in medicine (JOHNSTON *et al.* 2006, MADHUSUDHAN 2016) the pollution of the environment with acetic acid is possible, thus the assessment of its ecotoxicity is imperative.

Due to the pollution of the environment, the ecotoxicological research is under rapid development (KAHRU *et al.* 2010). Ecotoxicological assays are used in the environment hazard assessment framework, usually based on a standardized protocol or a guideline such as the OECD (Organization for Economic Cooperation and Development), US EPA (United States Environmental Protection Agency) and ISO (International Organization for Standardization) guidelines (CRANE *et al.* 2008).

The ecotoxicological research developed as aquatic and terrestrial, the terrestrial lagging the aquatic one (KAHRU *et al.* 2010). There is a widespread desire, especially in

Europe, to minimize the vertebrate testing such that vertebrates, such as fish, would be used only if necessary (CRANE *et al.* 2008).

In the aquatic segment of ecotoxicological assessment, duckweed represents a model organism due to its small size and vegetative reproduction. The duckweed ecotoxicity assay, a growth inhibition assay, is standardized under the form of guidelines formulated by OECD (OECD-221 2006), EPA (EPA-850.4400 2012) and ISO (ISO-20079 2005, ISO-20227 2017).

The duckweed growth inhibition assay involves the growth in monoculture of exponentially growing duckweed in media with the tested substance. The effects of the tested chemical is observed at different concentration over a period of 7 days. The substance-related effects on vegetative growth can be assessed on different variables such as frond number, fresh or dry weight, frond area, etc. (OECD-221 2006). These gravimetric parameters can be determined quickly and cheaply for other plant species (DATCU and SALA, 2018; DATCU *et al,* 2019).

MATERIAL AND METHODS

The ecotoxicological effects of acetic acid were analyzed on common duckweed (*Lemna minor* L.). The duckweed culture was maintained under standard laboratory conditions as described in OECD and EPA guidelines (OECD-221 2006, EPA-850.4400 2012).

The experimental treatments consisted of ten acetic acid (Carl Roth, cat. no. 3738.4) doses as solutions of acetic acid and culture medium of concentrations ranging from 0.000025% to 0.5% acetic acid. The ten tested concentrations were: 0.5%, 025%, 0.05%, 0.025%, 0.005%, 0.0025%, 0.0005%, 0.00025%, 0.00005% and 0.000025%.

The ecotoxicological effect of acetic acid to common duckweed were assessed through a growth inhibition assay conducted according to the OECD guideline (OECD-221 2006), the chosen positive control being 0.5% zinc chloride (Carl Roth, cat. no. 3533).

Each concentration was tested in duplicate on 35 fronds of common duckweed (five colonies with 3 fronds and 5 colonies with 4 fronds) which were maintained under standard laboratory conditions for 7 days. At the end of the testing period, the total number of fronds and colonies, the number of green fronds and the number of fronds with chlorosis were assessed. The percent inhibition of growth rate was calculated in accordance with the OECD standard (OECD-221 2006). A dose-response curve was plotted using the total number of fronds and the tested concentrations of acetic acid and the EC_{50} value was calculated for acetic acid. The ErC_{50} value of acetic acid was calculated by plotting the acetic acid concentration and the percent inhibition of growth rate. The dose-response curves were plotted using Microsoft Office Excel 365 with Solver Add-in.

The fresh weight, dry weight and water content were also assessed. The fresh weight of the fronds was measured by blotting the fronds on a paper towel before weighting, while the dry weight was measured after placing the fronds in an oven at 60˚C for several days, until no change in weight over time was observed. The water content was calculated as the difference between fresh and dry weight of fronds and it was expressed as a percentage.

RESULTS AND DISCUSSIONS

The study aimed to analyze the ecotoxicological effects of acetic acid to common duckweed. The number of green fronds decreased as the concentration of acetic acid increased, while the number of fronds with chlorosis increased with the concentration (Figure 1).

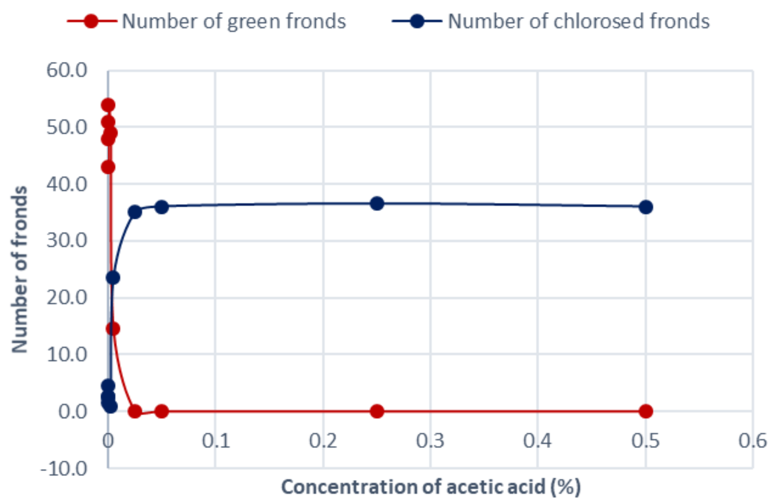

Figure 1. The effect of acetic acid on the number of green fronds and fronds with chlorosis

Both the total number of fronds and the number of colonies decreased as the concentration of acetic acid increased (Figure 2). The decrease of both frond number and colony number signifies that the higher concentrations did cause chlorosis of the fronds but didn't cause the break-up of the colonies.

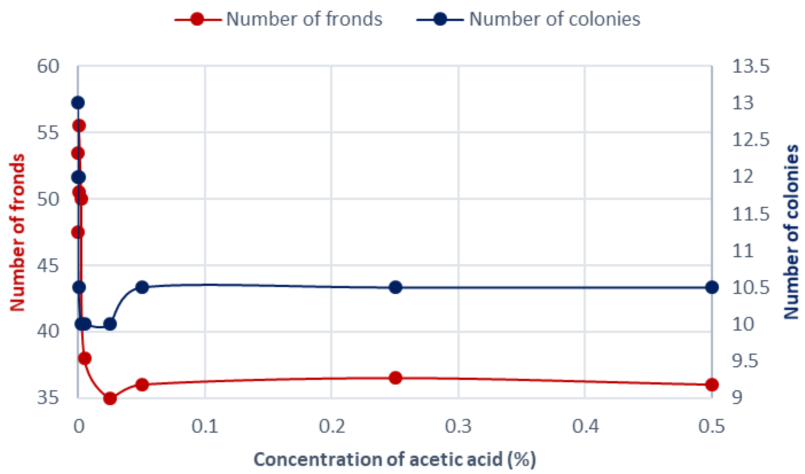

Figure 2. The effect of acetic acid on the total number of fronds and colonies

The four highest acetic acid concentrations caused the chlorosis of all fronds, while the 0.005% concentration allowed the survival of some green fronds, the green frond number reaching a plateau for the lowest five concentrations (Figure 3). At 0.5% acetic acid, no green fronds were observed, while the positive control of zinc chloride of the same concentration allowing the survival of some green fronds. A possible explanation might be the fact that the apparently higher toxicity of acetic acid is due to the pH modification of the testing medium.

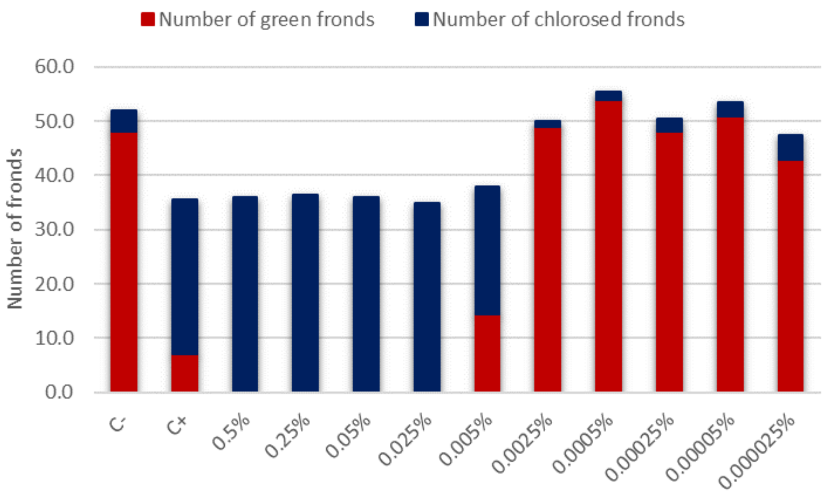

Figure 3. The number of green fronds and fronds with chlorosis for the ten tested concentrations of acetic acid in comparison with the negative and positive controls

The percent inhibition of growth rate of the highest four concentrations of acetic acid were similar to that of the positive control, starting to decrease at lower concentrations. At 0.0005% and 0.00005% the percent inhibition values were negative, showing that the growth rate was promoted (Figure 4).

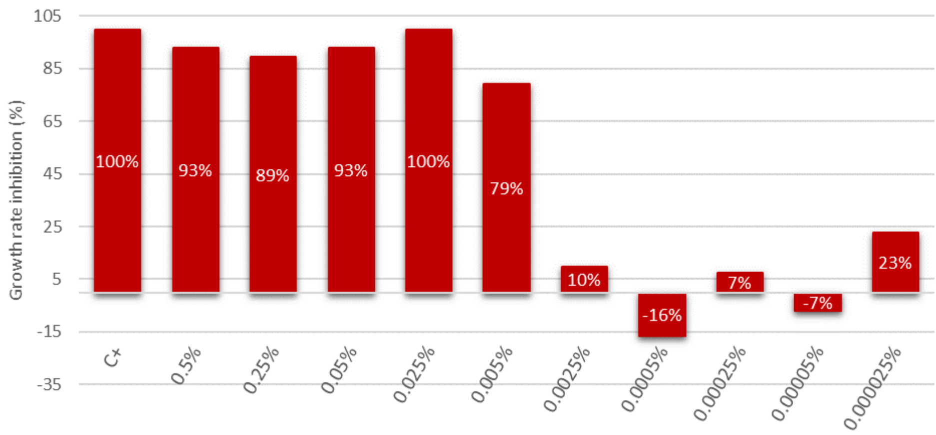

Figure 4. Percent inhibition of growth rate of the ten tested concentrations of acetic acid in comparison with the positive control

Both the fresh and dry weight of fronds showed an increase at 0.025% acetic acid, then decreasing with the increase of the concentration (Figure 5). The fresh weight was on average ten times higher than the dry weight of the fronds.

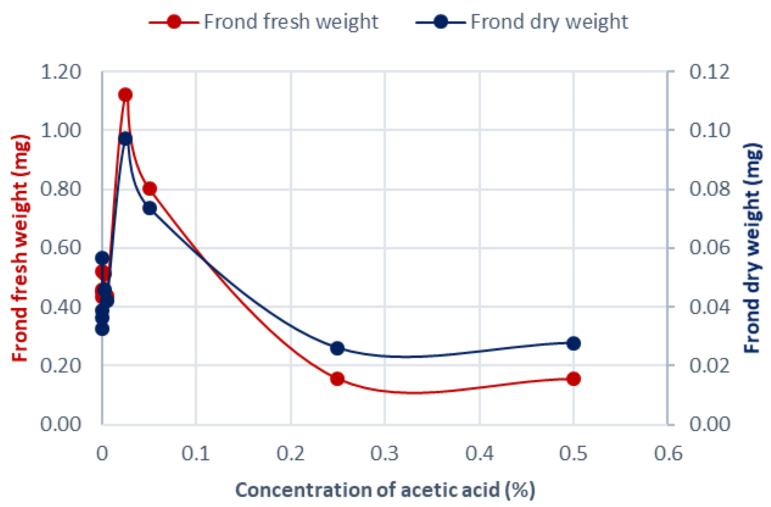

Figure 5. The effect of acetic acid on the fresh and dry weight of fronds

The percent water content was similar to the control values, with the exception of 0.5% and 0.25% acetic acid where it was decreased (Figure 6).

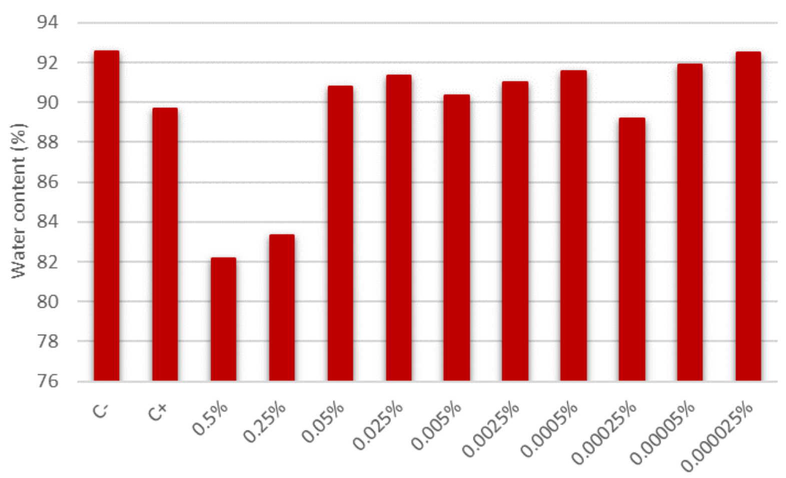

Figure 6. Percent water content of the ten tested concentrations of acetic acid in comparison with the negative and positive controls

The dose-response curve plotted using the total number of fronds, enabled the calculation of EC_{50} for acetic acid. The obtained EC_{50} value was 38.8 mg/L (0.00388%) (Figure 7).

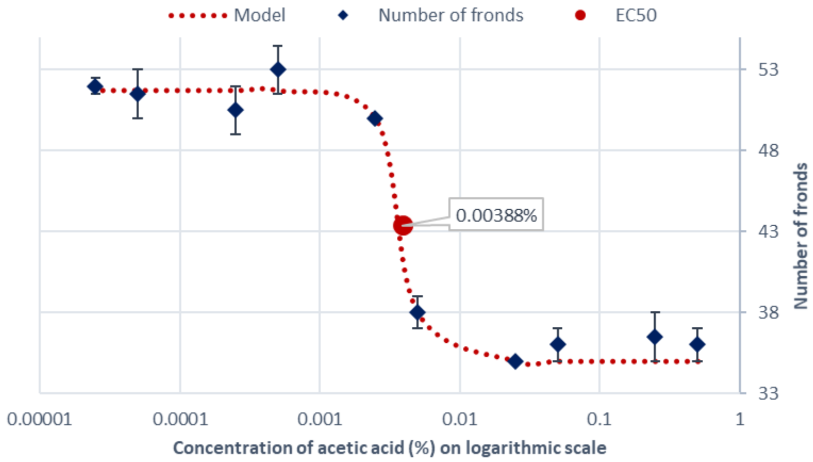

Figure 7. Dose-response curve based on the total frond number and EC_{50} of acetic acid. The model represents the fitted data calculated from the total frond number

From the dose-response curve plotted using the percent inhibition of growth rate the ErC_{50} was calculated to be 39.4 mg/L (0.00394%) (Figure 8), a similar value to that of EC_{50} (38.8 mg/L).

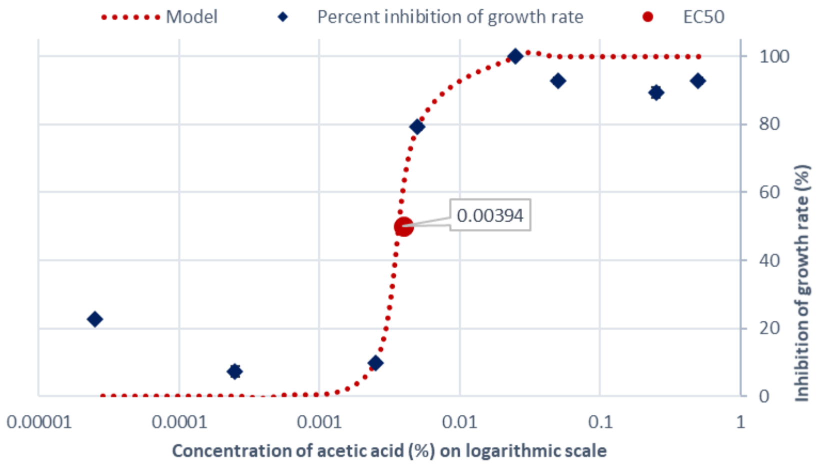

Figure 8. Dose-response curve based on percent inhibition of growth rate and ErC_{50} of acetic acid. The model represents the fitted data calculated from the percent inhibition of growth rate

Compared to the positive control, the EC_{50} value of acetic acid was 4 times smaller than the value of zinc chloride (INCE *et al.* 1999). Acetic acid proved to be less toxic to duckweed than phenol, acetonitrile and metals such as zinc, cobalt, copper and chromium. Compared with sodium dodecyl sulphate, acetic acid had a similar EC_{50} value, but compared to sodium chloride it had about a hundred times smaller mean effective concentration (Table 1).

Table 1

EC_{50} values of different substances to common duckweed. The toxicity ranking is in accordance with UNECE GHS (Globally Harmonized System of Classification and Labelling of Chemicals) document, revision 6 from 2015

EC_{50} (mg/L)	Substance	Toxicity according to GHS (Rev.6) (2015) of UNECE	Reference
3981.4	Sodium chloride		(GODOY *et al.* 2017)
51.6	SDS (sodium dodecyl sulphate)	Category Acute 3	(FORNI *et al.* 2008)
38.8	Acetic acid	Category Acute 3	our results
15.7	Cobalt chloride	Category Acute 3	(INCE *et al.* 1999)
12	Phenol	Category Acute 3	(LARSON *et al.* 2008)
9.6	Zinc chloride	Category Acute 2	(INCE *et al.* 1999)
8.5	Copper nitrate	Category Acute 2	(INCE *et al.* 1999)
3.6	Acetonitrile	Category Acute 2	(LARSON *et al.* 2008)
1.5	Potassium dichromate	Category Acute 2	(INCE *et al.* 1999)

Our scientific literature study revealed that there are not many articles published on the topic of toxicity of acetic acid on duckweed species. The number of similar articles in Romania is very small.

However, the effects of other acid such as gibberellic acid, indole-3-acetic acid, salicylic acid, jasmonic acid and abscisic acid was assessed. The growth of common duckweed was severely inhibited by abscisic acid and moderately by jasmonic acid. No apparent growth-promoting effects were observed for gibberellic acid, indole-3-acetic acid and salicylic acid, while a slight decrease in root length was observed for indole-3-acetic acid and salicylic acid (UTAMI *et al.* 2018).

The number of articles on the ecotoxicological effects of acetic acid to other organisms is also very small. There are a few articles that study indirectly the effect of acetic acid on aquatic microorganisms, plants and animals. Its effect was tested on marine bacteria *Vibrio fischeri* through the Microtox toxicity assay, the calculated median effective concentration being 71.8 mg/L at 5 min, 72.9 mg/L at 15 min and 73.3 mg/L at 15 min (DE MORAIS *et al.* 2015).

CONCLUSIONS

The study was realized to assess the potential ecotoxicity of acetic acid, due to its wide range of applications.

Regarding frond number, both green frond number and total frond number increased with decrease in acetic acid concentration, as well as colony number, while the number of fronds with chlorosis decreased.

The percent inhibition of growth rate of the ten tested concentrations of acetic acid showed that the five highest concentrations inhibited the growth rate similarly to the positive control, while the five lowest concentrations tested had very low inhibitory effect, some even promoting frond number.

The percent water content of the ten tested concentrations of acetic acid revealed that only the highest two concentrations of acetic acid inhibited the water content in comparison with the lowest concentrations and both controls.

Regarding the two dose-response curves, the calculated EC_{50} and ErC_{50} values were similar, being 38.8 mg/L and 39.4 mg/|L, respectively.

By comparing our results to literature data, it was observed that acetic acid has a Category Acute 2 level toxicity, according to the UNECE GHS (Globally Harmonized System of Classification and Labelling of Chemicals) document, revision 6 from 2015. The toxicity of acetic acid proved to be four times larger than that of zinc chloride and about a hundred times smaller than that of sodium chloride.

All results showed that acetic acid had an inhibitory effect on the common duckweed fronds, making it a potentially ecotoxic substance, as its toxicity is enhanced by the pH modification of the culture media.

ACKNOWLEDGEMENTS

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