ECOTOXICOLOGICAL RESEARCH OF SPIROHYDANTOINS AND THEIR DERIVATIVES TOWARDS *VAUCHERIA LITOREA*

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Abstract

This article is presenting a study on the toxic effects of various synthetic organic compounds (spirohydantoins and their derivatives) towards Vaucheria litorea. In addition, a comparative analysis of the effects of the tested products towards Vaucheria litorea and Ulva lactuca has also been made. The tests are conducted in accordance with the international standards adopted by the Organisation for economic co-operation and development (OECD). The statistical manipulation, Dose-Response Curves (drc) and ANOVA analyses of the data obtained are conducted with R language for Statistical Computing. The comparison graphics about the results received for the two alga species are created with the Tableau public software for interactive data visualization.

Keywords: *Spirohydantoins, Ecotoxicology, Vaucheria litorea, Ulva lactuca, R language, drc, ANOVA, Tableau*

1. INTRODUCTION

Vaucheria litorea is a yellow-green algae from Xanthophyceae family [1]. The alga is a common intertidal species of coastal brackish waters and salt marshes in the world – Europe, North America, Eastern Pacific [2]. The alga together with the similar specie *Vaucheria dichotoma* is a common alga in the Black Sea [3].

The toxic effects of the following compounds: cyclopentanespiro-5-hydantoin /1,3-diazaspiro[4.4]nonane-2,4-dione/ (compound 1, table 1), cyclopentanespiro-5-(2,4-dithiohydantoin) /1,3-diazaspiro[4.4]nonane-2,4-dithione/ (compound 2, table 1), 1-aminocyclopentane-1-carboxylic acid (compound 4, table 1) and cyclohexanespiro-5-hydantoin /1,3-diazaspiro[4.5]decane-2,4-dione/ (compound 5, table 1) towards Sea lettuce (*Ulva lactuca*) have been investigated in previous work of ours. It has been found out that only compound 1 shows deleterious effect on the tested plant [4].

The aim of the current study is to investigate the possible toxic effects of various synthetic organic compounds (spirohydantoins and their derivatives) towards *Vaucheria litorea*, as well as to compare data obtained with those related to *Ulva lactuca*.

2. MATERIALS AND METHODS

2.1. Synthetic compounds

All chemicals used for the synthesis are purchased from Merck and Sigma-Aldrich. The spirohydantoins and their derivatives are synthesized in accordance with the methods listed in table 1. The products obtained are characterized via physicochemical parameters, IR and NMR spectral data. The results obtained from these analyses are identical with those previously published in the literature (see the references pointed in table 1).

2.2. Ecotoxicological tests

The trials are conducted in accordance with OECD (Organisation for Economic Co-operation and Development) guidelines for chemicals testing – Manual 201 (Alga, Growth Inhibition Test) [5] and determination of the bleaching percent of tested algae.

The plants have been collected from the natural sea water from Bulgarian Black sea coast. The algae has been grown in tubes of 16 mm length and 15 mm width in a clima-room in sterile conditions at 24 °C, by the constant light of 80 µmol/m^2s [6].

The sea water is mixed with tested chemicals in 10-20 different test concentration (5 replicates for each concentration). The initial concentration is the saturated concentration of the given test compound in the natural sea water (see table 2). The next concentrations are with a decreased ratio of -0.01. The control variant is used with pure natural sea water. Morphological changes are possible to be distinguished macroscopically as a percentage of algae bleaching (less intensity of green color or the absence of green color) [6].

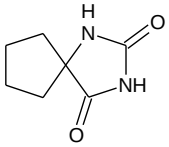
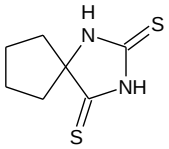
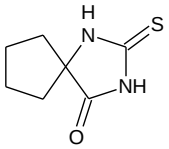
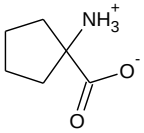
Statistical manipulation is conducted with R language for Statistical Computing [7]. Dose-Response Modeling for calculation of the values of LD_{50}, LOAEL (Lowest Observed Adverse Effect Level) and NOAEL (No Observed Adverse Effect Level), as well as Dose-Response Curves are made by R language additional package drc [8]. ANOVA analyses is conducted with R language [7] in order to reveal if there are significant differences between toxicological values of the tested variants.

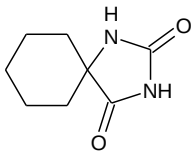
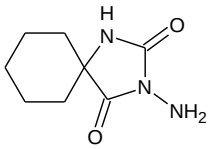
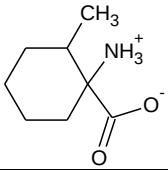
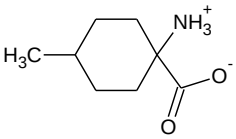
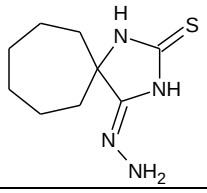
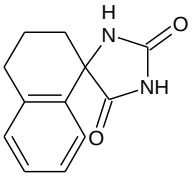
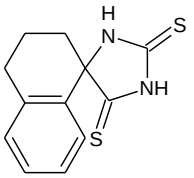
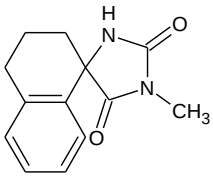
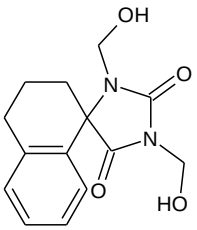
The Tableau Public software for interactive data visualization [9] is used to create the comparison graphics of results obtained for two alga species [10].

3. RESULTS AND DISCUSSION

Various spirohydantoins and their derivatives are prepared for the purposes of this study. The structures, systematic names and methods for synthesis of the compounds are presented in table 1.

Table 1. Spirohydantoins and their derivatives used for the ecotoxicological tests

Compound	Structure	Systematic name	Synthetic procedure
1		1,3-diazaspiro[4.4]nonane-2,4-dione	[11]
2		1,3-diazaspiro[4.4]nonane-2,4-dithione	[12] [13]
3		2-sulfanylidene-1,3-diazaspiro[4.4]nonan-4-one	[12] [14]
4		1-aminocyclopentane-1-carboxylic acid	[14]

5		1,3-diazaspiro[4.5]decane-2,4-dione	[11]
6		3-amino-1,3-diazaspiro[4.5]decane-2,4-dione	[15] [16]
7		1-amino-2-methylcyclohexane-1-carboxylic acid	[14] [17]
8		1-amino-4-methylcyclohexane-1-carboxylic acid	[14] [17]
9		4-hydrazinylidene-1,3-diazaspiro[4.6]undecane-2-thione	[18]
10		3',4'-dihydro-2'*H*-spiro[imidazolidine-4,1'-naphthalene]-2,5-dione	[19] [20]
11		3',4'-dihydro-2'*H*-spiro[imidazolidine-4,1'-naphthalene]-2,5-dithione	[12]
12		1-methyl-3',4'-dihydro-2'*H*-spiro[imidazolidine-4,1'-naphthalene]-2,5-dione	[21]
13		1,3-bis(hydroxymethyl)-3',4'-dihydro-2'*H*-spiro[imidazolidine-4,1'-naphthalene]-2,5-dione	[20]

The Dose-Response Curves that describe the toxicity of the compounds obtained towards *Vaucheria litorea* and *Ulva lactuca* (see the Materials and methods part) are presented on figures 1-16.

Toxic indexes calculated/NOAEL (LD_{05}), LOAEL (LD_{25}) and LD_{50}/ are summarized and shown on figures 17-19 and table 2.

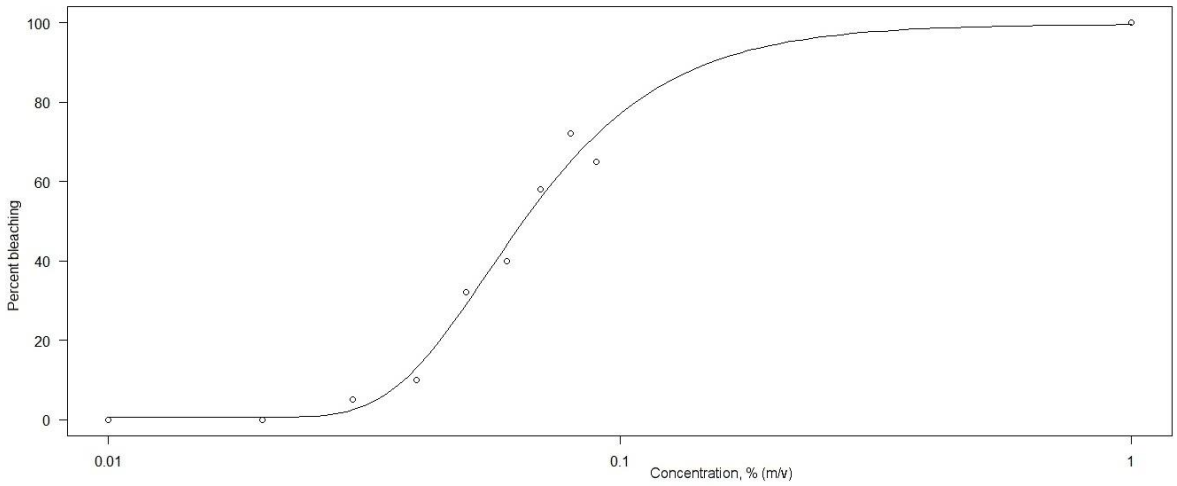


Fig. 1. Dose-Response Curve of Compound 1 / *Vaucheria litorea*

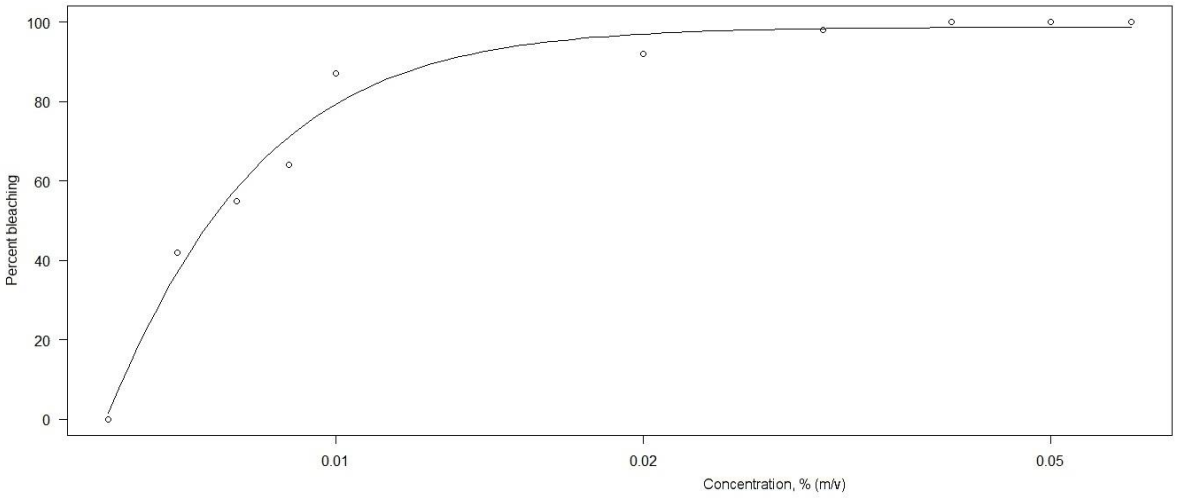


Fig. 2. Dose-Response Curve of Compound 3 / *Vaucheria litorea*

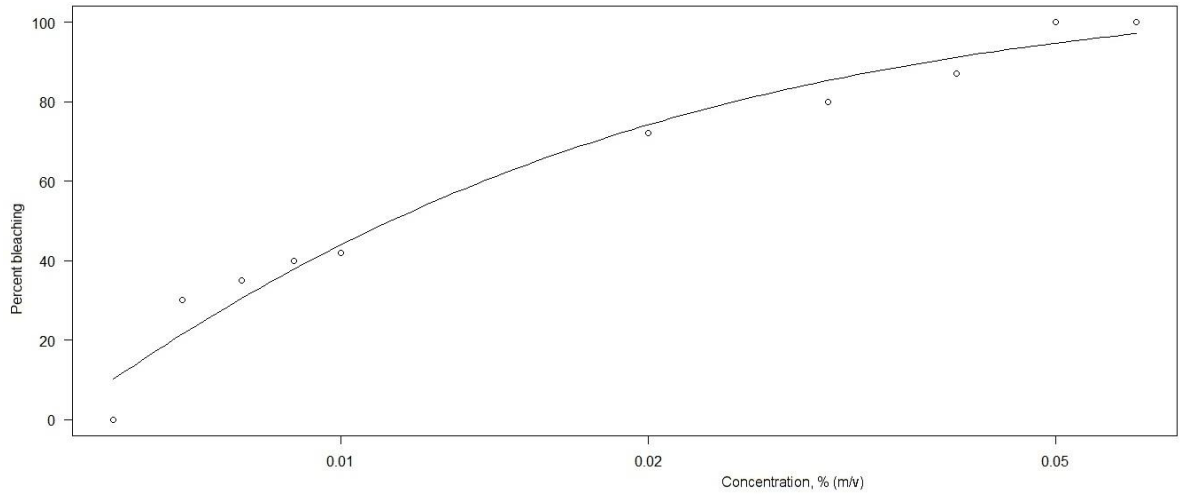


Fig. 3. Dose-Response Curve of Compound 3 / *Ulva lactuca*

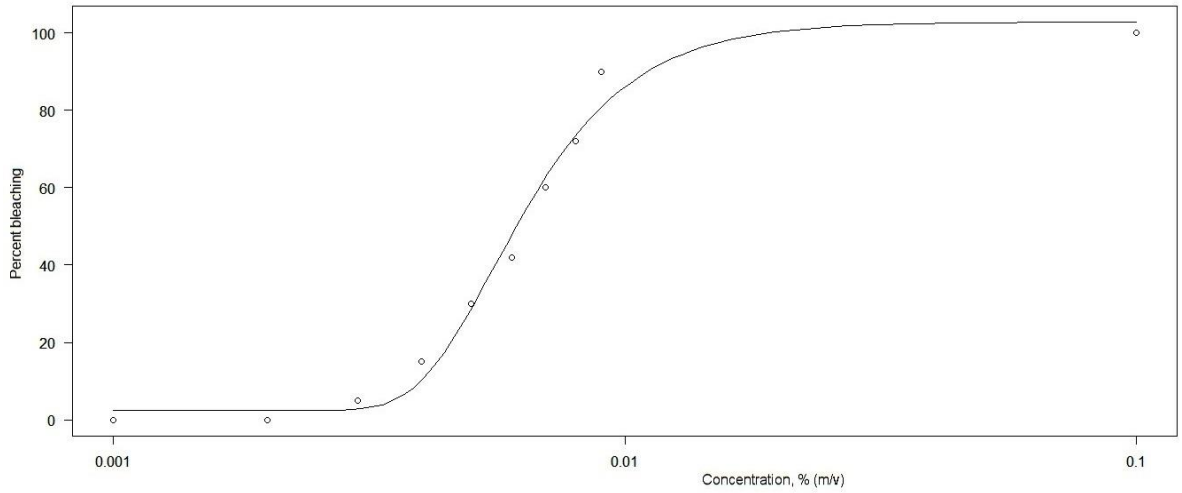


Fig. 4. Dose-Response Curve of Compound 5 / *Vaucheria litorea*

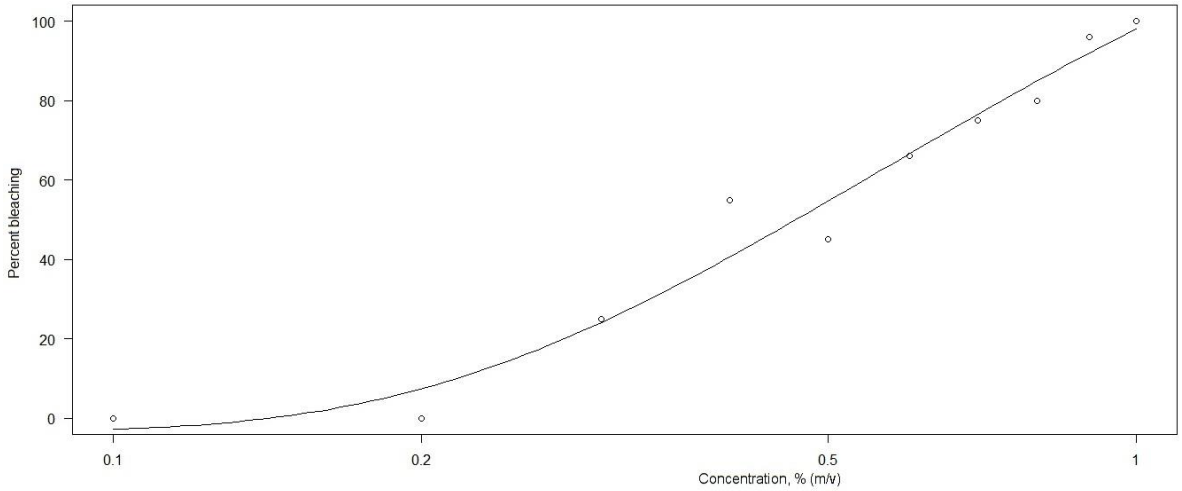


Fig. 5. Dose-Response Curve of Compound 6 / *Vaucheria litorea*

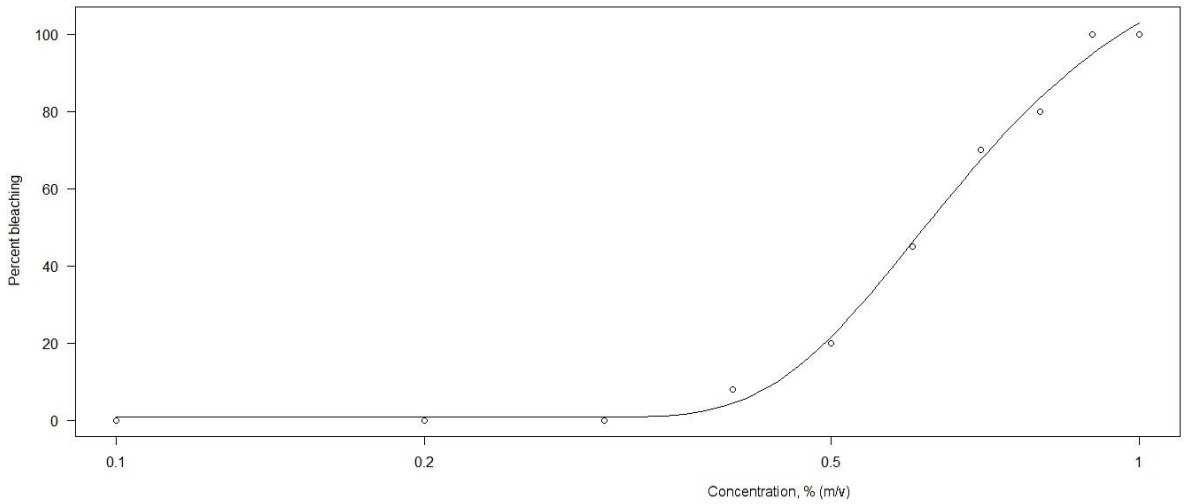


Fig. 6. Dose-Response Curve of Compound 6 / *Ulva lactuca*

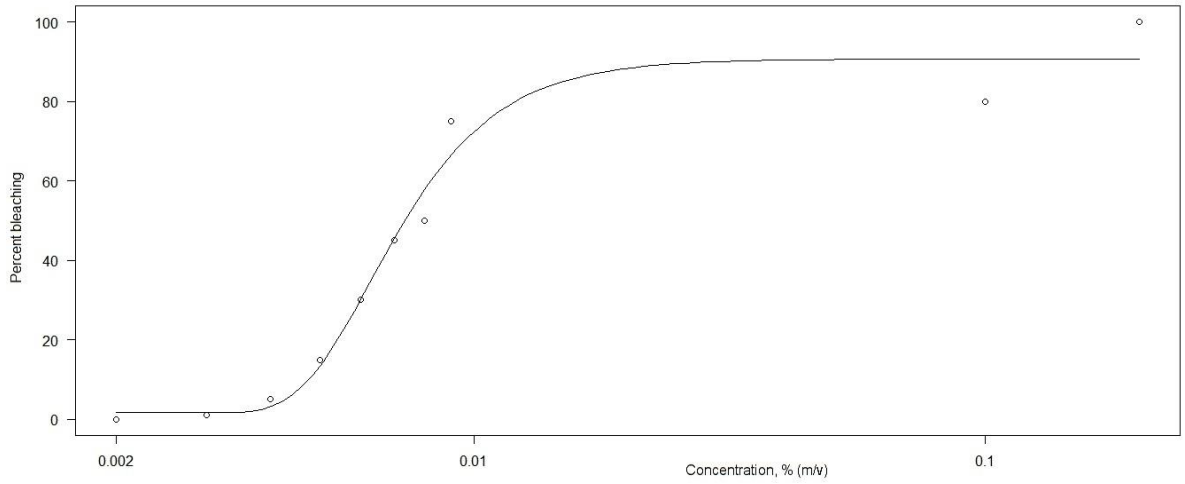


Fig. 7. Dose-Response Curve of Compound 7 / *Vaucheria litorea*

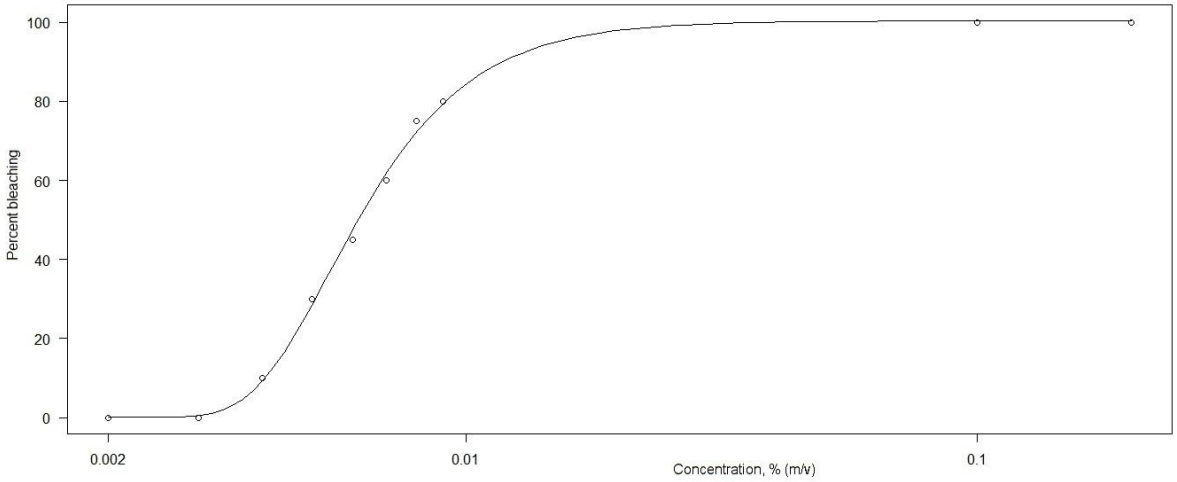


Fig. 8. Dose-Response Curve of Compound 7 / *Ulva lactuca*

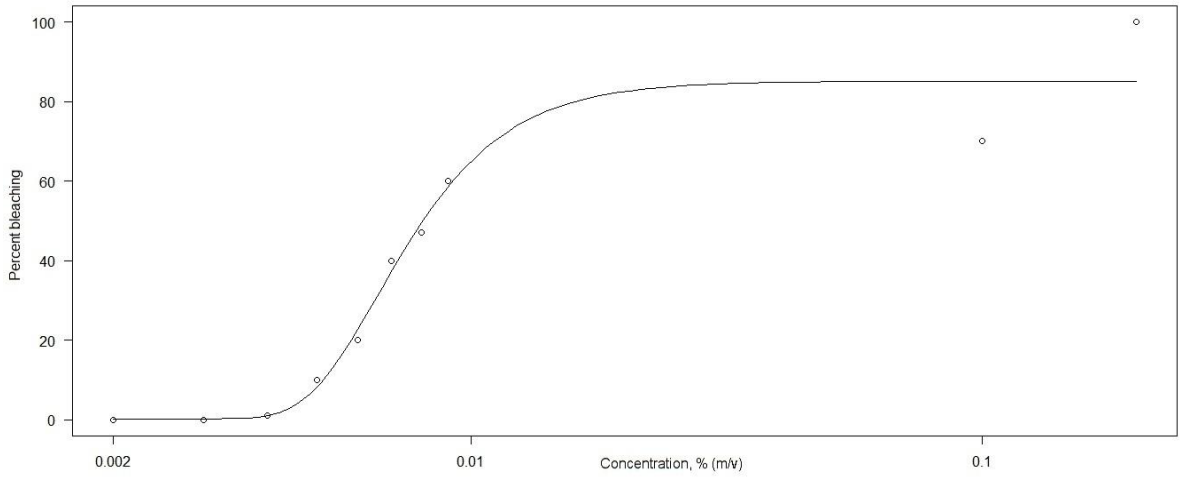


Fig. 9. Dose-Response Curve of Compound 8 / *Ulva lactuca*

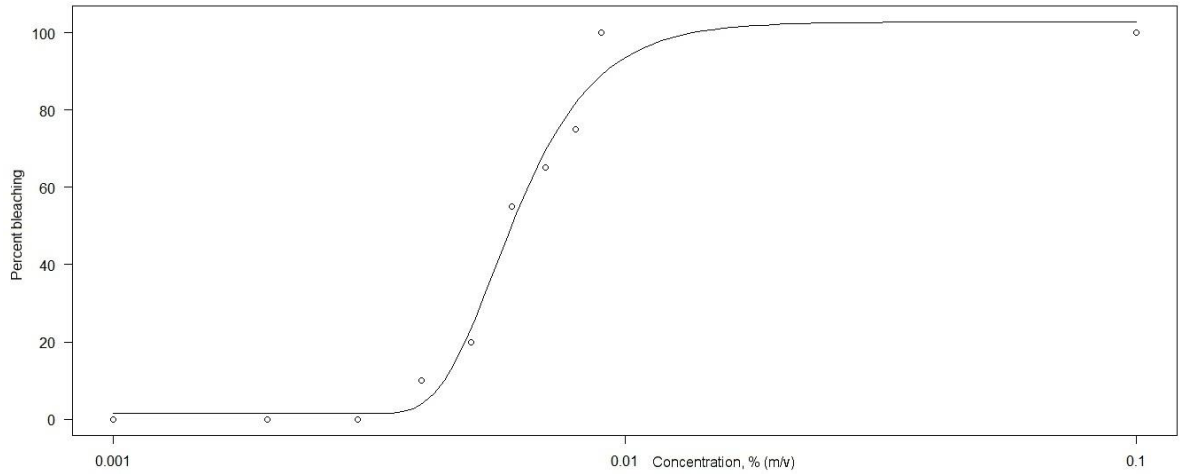


Fig. 10. Dose-Response Curve of Compound 9 / *Vaucheria litorea*

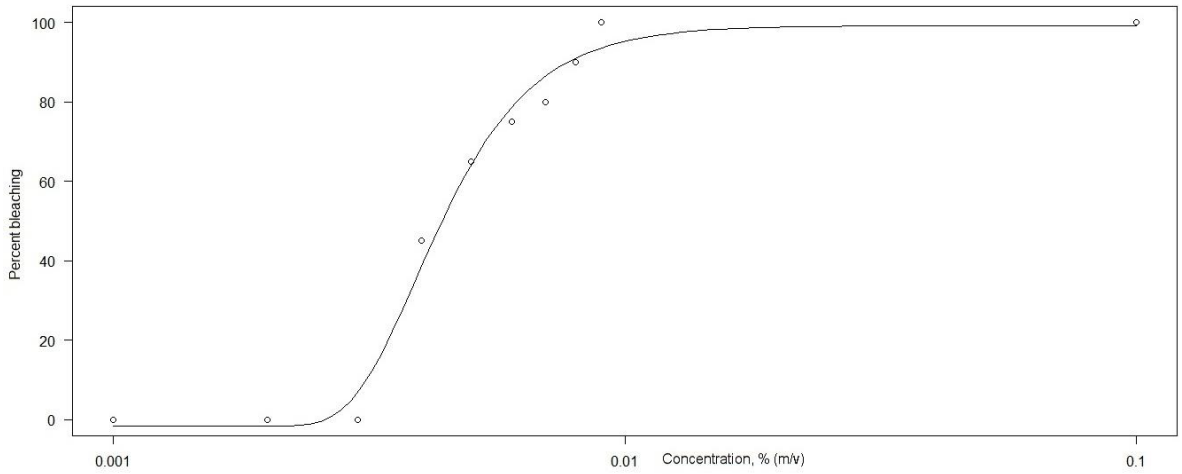


Fig. 11. Dose-Response Curve of Compound 9 / *Ulva lactuca*

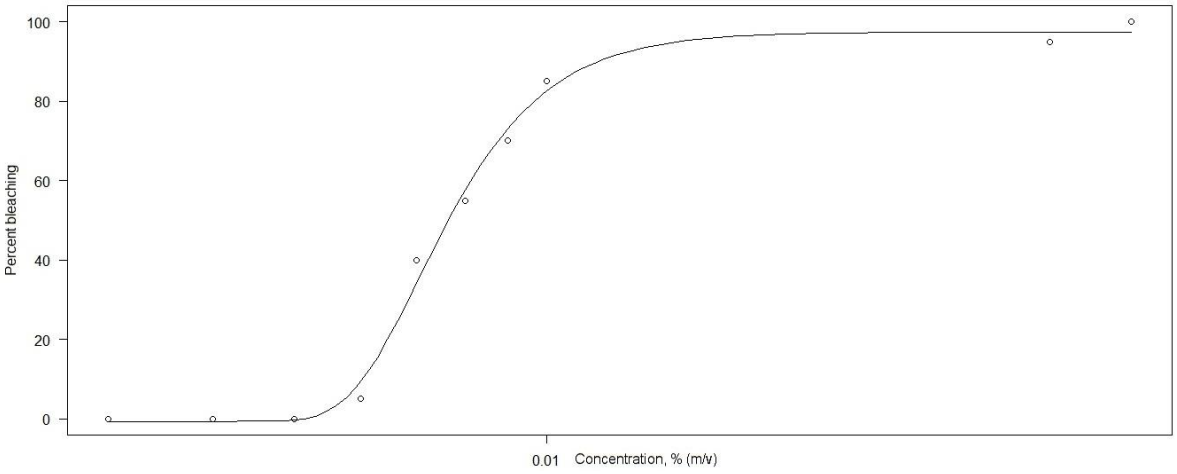


Fig. 12. Dose-Response Curve of Compound 10 / *Ulva lactuca*

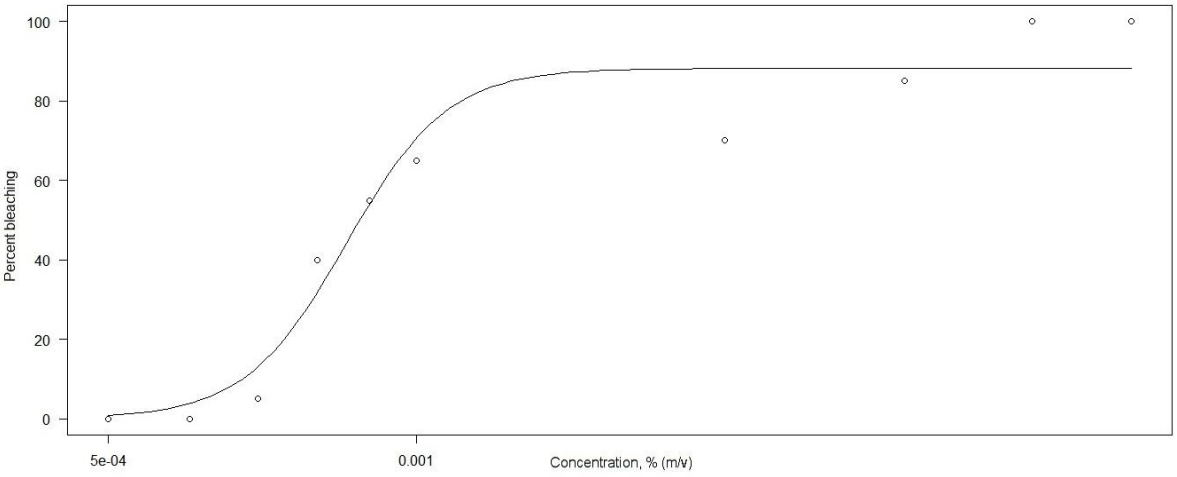


Fig. 13. Dose-Response Curve of Compound 11 / *Ulva lactuca*

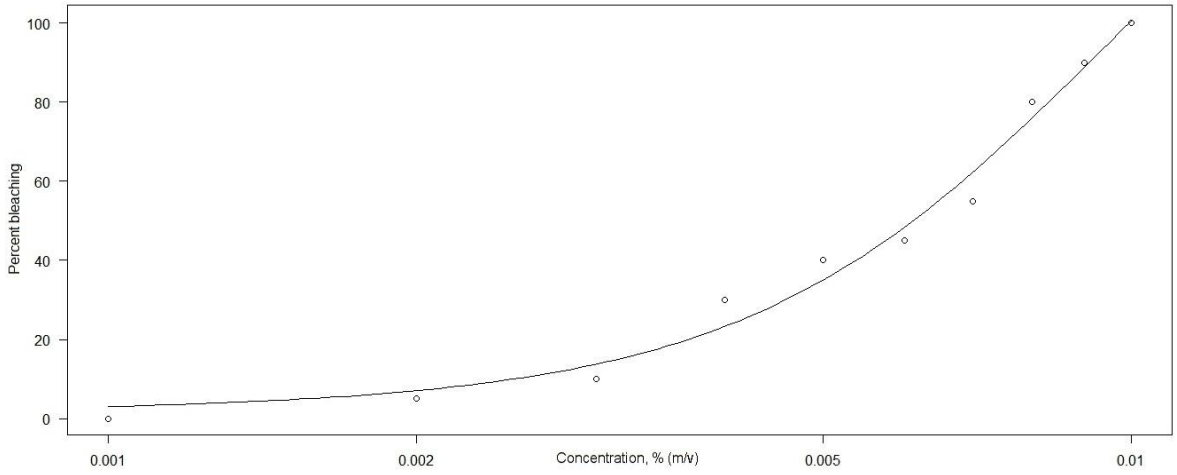


Fig. 14. Dose-Response Curve of Compound 12 / *Ulva lactuca*

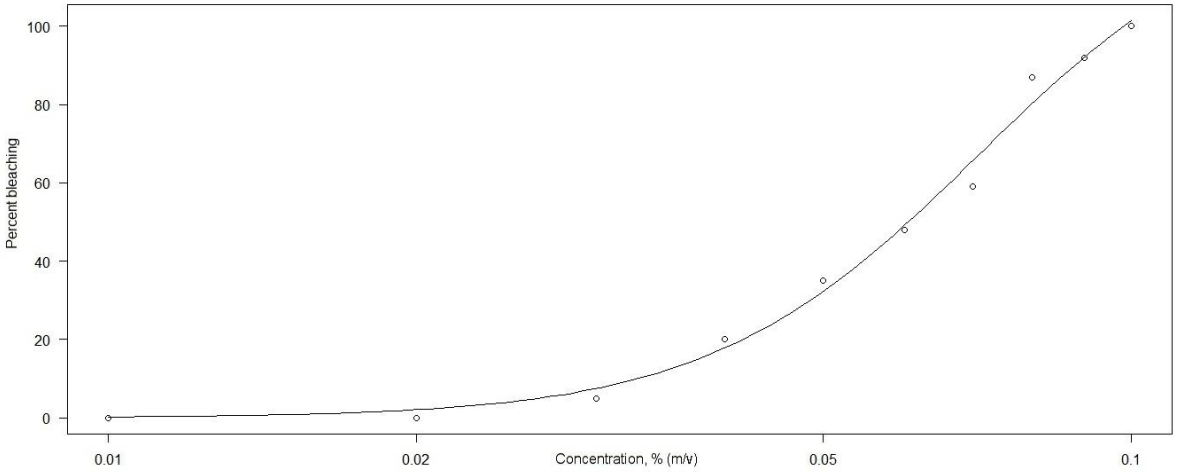


Fig. 15. Dose-Response Curve of Compound 13 / *Vaucheria litorea*

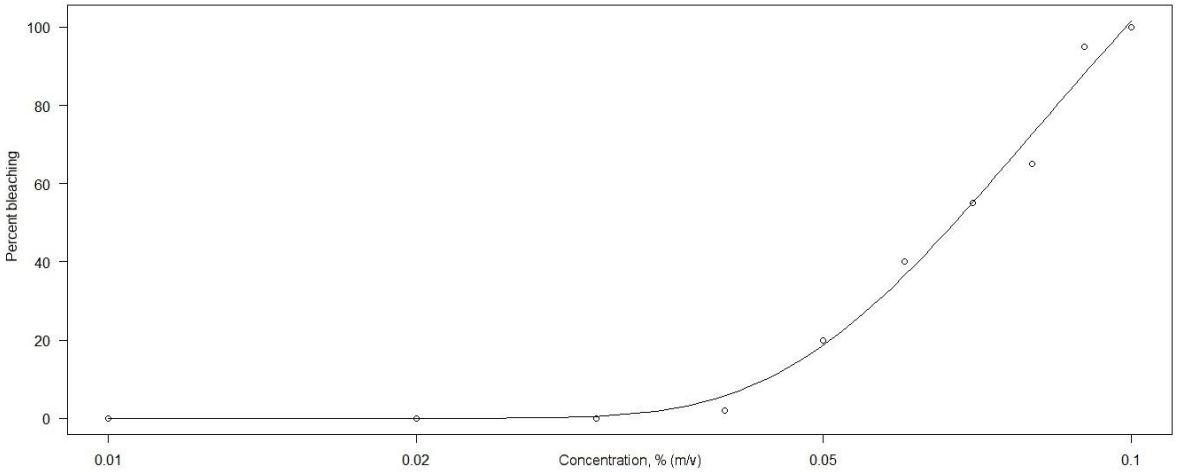


Fig. 16. Dose-Response Curve of Compound 13 / *Ulva lactuca*

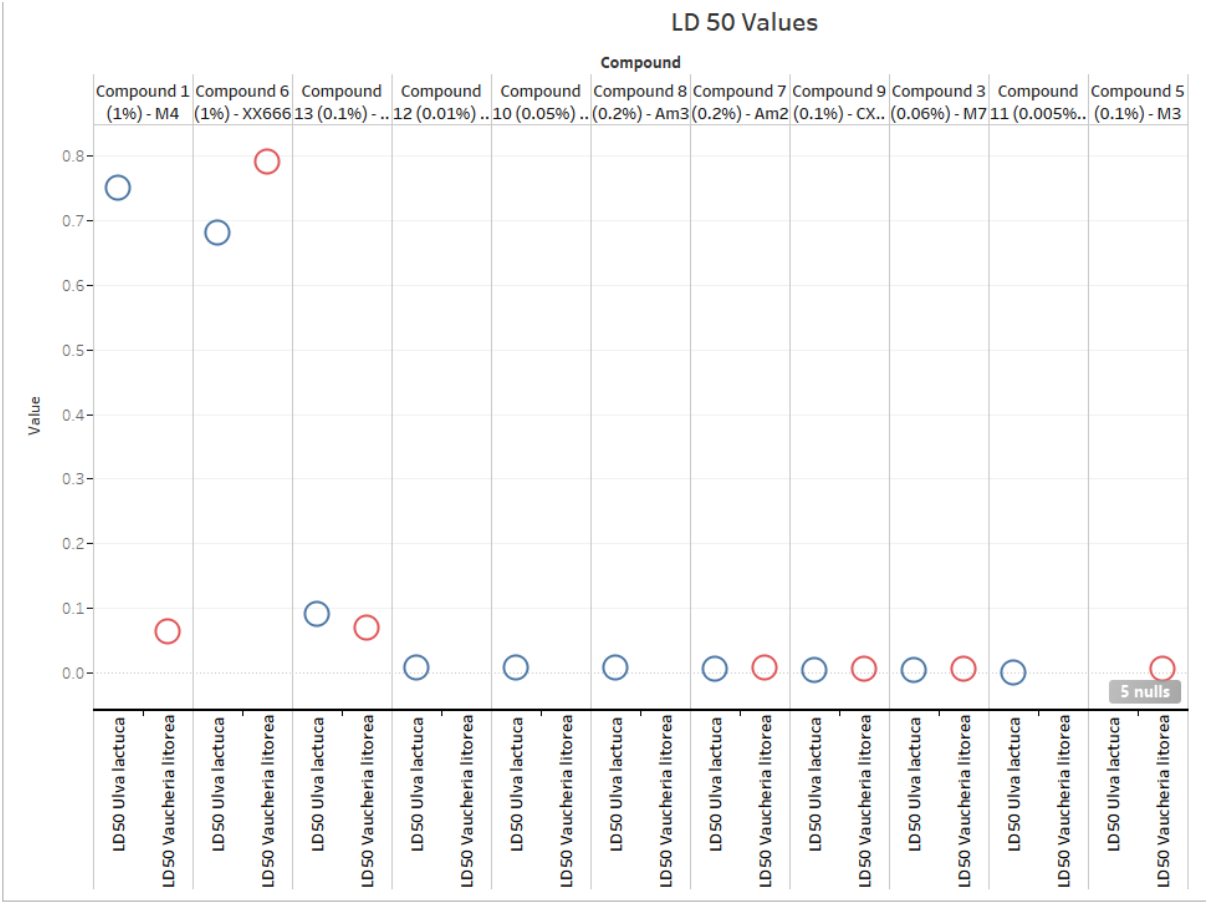


Fig. 17. Comparison on the base of LD_{50} values for *Ulva lactuca* and *Vaucheria litorea*

As per LD_{50} values shown in figure 17 it is obvious that Compound 1 is a lot less toxic to *Ulva lactuca* than *Vaucheria litorea* ($p < 0.05$). The same tendency can be seen as per LOAEL and NOAEL values with classical Dose-Response Curves.

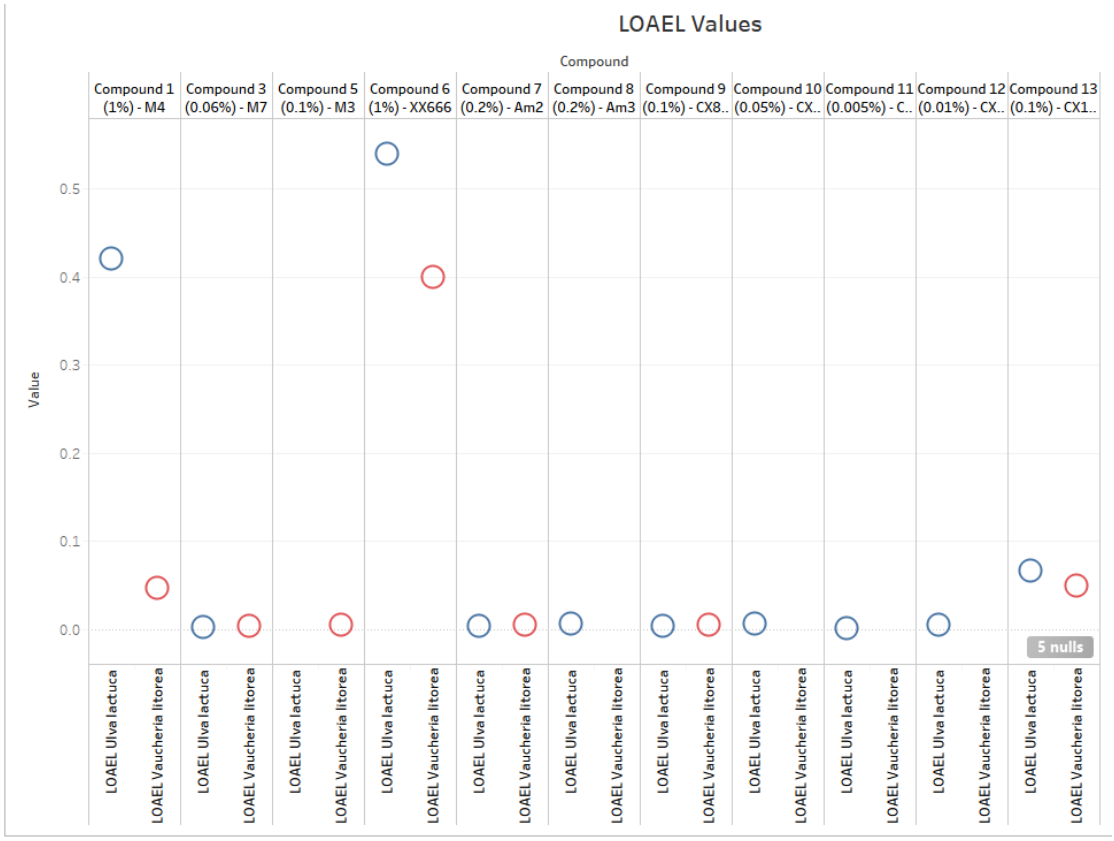


Fig. 18. Comparison on the base of LOAEL values (LD_{25}) for *Ulva lactuca* and *Vaucheria litorea*

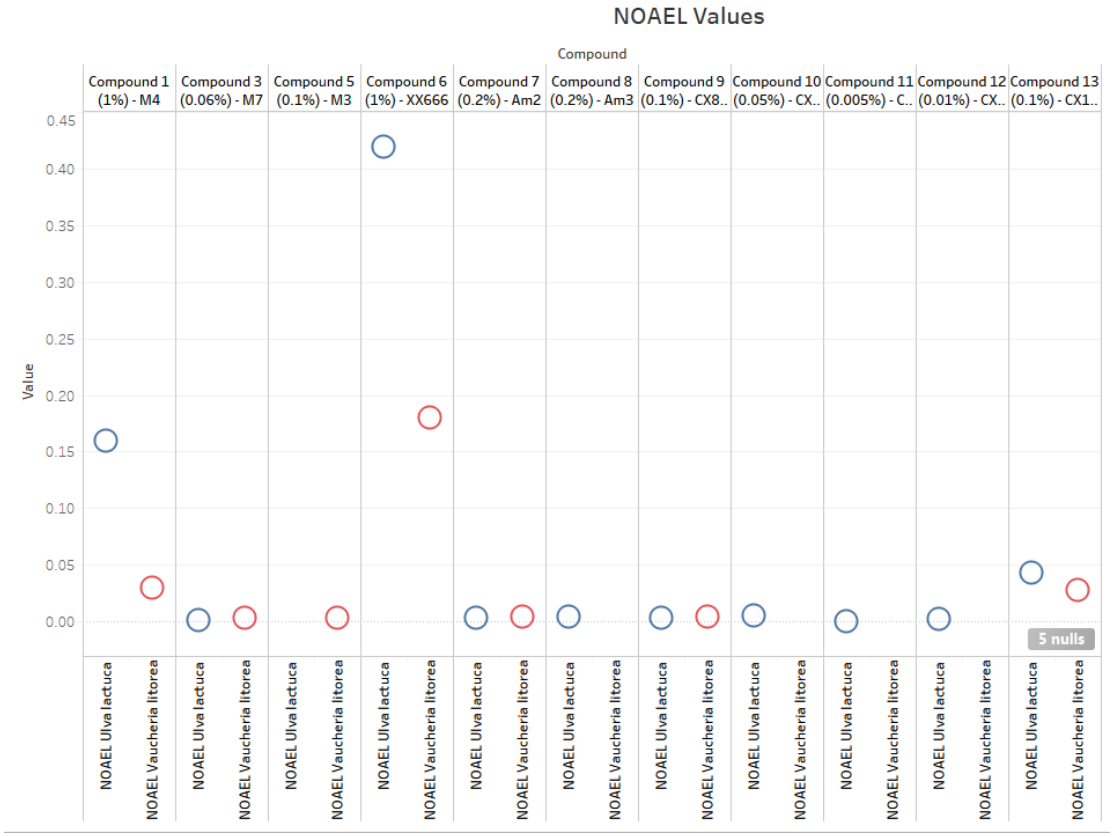


Fig. 19. Comparison on the base of NOAEL values (LD_{05}) for *Ulva lactuca* and *Vaucheria litorea*

Table 2. Toxicity indexes of the tested compounds

Compound	***Ulva lactuca***			***Vaucheria litorea***		
(Concentration)	**NOAEL**	**LOAEL**	**LD_{50}**	**NOAEL**	**LOAEL**	**LD_{50}**
Compound 1 (1 %)	0.165 [4]	0.427 [4]	0.75 [4]	0.03	0.047	0.064
Compound 2 (0.025 %)	- [4]	- [4]	- [4]	-	-	-
Compound 3 (0.06 %)	0.001	0.002	0.004	0.003	0.0044	0.0056
Compound 4 (0.1 %)	- [4]	- [4]	- [4]	-	-	-
Compound 5 (0.1 %)	- [4]	- [4]	- [4]	0.003	0.0052	0.0067
Compound 6 (1 %)	0.42	0.54	0.68	0.18	0.40	0.79
Compound 7 (0.2 %)	0.003	0.004	0.006	0.004	0.0052	0.0075
Compound 8 (0.2 %)	0.004	0.006	0.0074	-	-	-
Compound 9 (0.1 %)	0.0028	0.0035	0.0043	0.0042	0.0051	0.006
Compound 10 (0.05 %)	0.0056	0.0066	0.0075	-	-	-
Compound 11 (0.005%)	0.00061	0.00075	0.00085	-	-	-
Compound 12 (0.01 %)	0.0021	0.0053	0.0082	-	-	-
Compound 13 (0.1 %)	0.043	0.066	0.09	0.028	0.05	0.07

* - No toxicity observed.

The ANOVA analysis conducted reveal that there is no significant difference between LD_{50}, LOAEL and NOAEL values in relation to the tested two alga species ($p > 0.05$). It can be noted from the figures above that Compound 1 and Compound 6 have the lowest toxicity towards *Ulva lactuca*. However, Compound 1 is significantly more toxic towards *Vaucheria litorea*. There is no significant difference of the toxicity of Compound 6 in relation to the tested two alga species ($p > 0.05$) as per LD_{50} and LOAEL values. However, as per NOAEL values it is evident that - $p < 0.05$. The Dose-Response Modeling of the same compound also produces a "flat" Dose-Response Curve - with greater difference between LD_{50} and LOAEL than between LOAEL and NOAEL towards *Ulva lactuca*. However, the Dose-Response Curve is exponential for *Vaucheria litorea*.

The other compounds also manifest such an intense toxicity with some exceptions:

- Compounds 12, 10, 8 and 11 are very toxic towards *Ulva lactuca* but have no toxicity towards *Vaucheria litorea*. Compounds 8, 10 and 11 have a classical Dose-Response Curve while Compound 12 - exponential with relatively close differences between NOAEL, LOAEL and LD_{50} values.
- Compound 5 is the opposite – it reveals a severe toxicity towards *Vaucheria litorea* but no toxicity towards *Ulva lactuca*.

Unlike the others, Compound 3 has an unusual logarithmic Dose-Response Curves for both, *Ulva lactuca* and *Vaucheria litorea*. For the latter, the curve is very steep, with the greatest gap being between NOAEL and LOAEL values, rather than between LOAEL and LD_{50} values.

4. CONCLUSIONS

The results show *Ulva lactuca*'s higher sensitiveness towards the tested chemical compounds as compared to *Vaucheria litorea*. The Compound 5 does not express any toxicity towards *Ulva lactuca* while the other compounds except Compound 1 and 6 are able to cause bleaching of the tested alga at very low concentrations.

Concerning *Vaucheria litorea*, Compound 8, 10, 11 and 12 show no toxicity. Concerning *Ulva lactuca*, Compound 6 causes bleaching at very high concentrations unlike the other compounds (1, 3, 5, 7, 9 and 13) which are highly toxic to the plant.

ACKNOWLEDGMENTS

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