ACITE ALGICIDAL TOXICITY OF DIMETHYL SULFOXIDE TOWARDS *CYCLOTELLA MENEGHINIANA*

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Abstract: *In vitro* and *in silico* trials for acute algicidal toxicity of dimethyl sulfoxide were performed. The *in silico* tests were carried out with ECOSAR 2.2 and VEGA QSAR software. Both software products perform QSAR predictions as a general for algae i.e not for specific species and both software calculated LC 50 values (acute toxicity) of DMSO towards algae in the range of 4000 – 5000 ppm.ECOSAR point EC50 from acute toxicity test to be 4620 ppm or 0.46 % concentration while Vega QSAR (Toxicity model (IRFMN) 1.0.1) point EC50 value of 4.42 ppm. However, the predicted compound is outside the Applicability Domain of the model and the result may be not reliable. According to algae classification toxicity model (ProtoQSAR-Combase), DMSO is classify as a non-toxic but, again the predicted compound is outside the applicability domain of the model and the result may be not reliable. Towards the same algorithm EC50 is 635.22 ppm or 0.063 % concentration.The online version of EpiSuite - Episuite.dev or EPI Suite™ BETA version 1.0, created and maintained by US Environmental Agency (EPA), give EC50 for freshwater algae = 5660 ppm or 0.56 % concentration which is fairly close to the value of ECOSAR 2.2 which is logically due to the fact that ECOSAR 2.2 is also created and supported by EPA. However, conducted *in vitro* trials with dimethyl sulfoxide for *Cyclotella meneghiniana* show its extremely low toxicity (LC 50 near 260000 ppm) and up to the 5000 ppm the tested compound do not cause any acute toxicity to the tested aqua plants. NOAEl or LC50 was 85 700 ppm, LOAEL or LC25 was 170 800 ppm, LC50 was 257 400 ppm and LC90 was 585 000 ppm. These results confirm other similar investigations of toxicity of DMSO towards other algae species such as *Raphidocelis subcapitata*, *Chlorella vulgaris* and *Selenastrum capricornutum* which also show very low similar toxicity ofdimethyl sulfoxide.The conducted tests in the present research reveal that different algae species can respond differently towards the same chemical and the in silico tests which give general toxicity towards algae cannot be applied in all cases.QSAR analyses of DSMO with ECOSAR and VEGA QSAR show dramatic differences between real and in silico trials towards specific algae species which can be the base for future perfection of the QSAR algorithms towards toxicity of aqua organisms. The low algae toxicity of DMSO makes this chemical very suitable as emulsifying agent of different synthetic or natural compounds with a large application in the medicine, veterinary medicine, agriculture and pest management.
Keywords: dimethyl sulfoxide, *Cyclotella meneghiniana,* algae, toxicity, ECOSAR, VEGA

1. INTRODUCTION

Dimethyl Sulfoxide is a common for many organic compounds often used for dissolving drugs and toxic substances which are not soluble in the water. The compound has penetration and enhanced penetration of other substances across biologic membranes, free radical scavenging, and anticholinesterase activity (Martin et al., 1967; Brayton, 1986). Dimethyl Sulfoxide was firstly isolated by Russian chemist Alexander Saytzeff in 1866 and is amphipathic substance with highly polar domain and two apolar methyl groups, making it soluble in both aqueous and organic media. This make is one of the most common solvents for the in vivo administration of several water-insoluble chemicals (Santos et al., 2003). It is commonly derived as a by-product from wood pulp in the production of paper. Except being typical solvent, it has numerous applications in the medicine primarily anti-inflammatory agent (Capriotti and Capriotti, 2012). Dermal application of DMSO in medicine is used for relief of pain in the case of arthritis and connective tissue injuries (Swanson, 1985). The compound is also used in the cardiac disease healing, ulcerative colitis, wound healing respiratory stimulation and dieresis (Jacob and de la Torre, 2015). In the agriculture DMSO can be used for increasing the effectiveness of pesticides when is used as a solvent (Olinger and Kerr, 1969). It was found also that dimethyl sulfoxide can express significant growth inhibition towards Botrytis cinerea and Fusarium graminearum at 0.5 % concentration while it is well tolerated by lettuce and tomato plants (Petruccelli et al., 2020; Li et al., 2025). In the in vitro conditions growth rate and sclerotial production of Sclerotium rolfsii decreased when DMSO is added into media (Melhuish and Bean, 1971). Other in vitro tests proves that DMSO concentrations greater than 2.5% significantly reduced the germination rate of Tilletia laevis teliospore on water agar (Ansari et al., 2015) and at 50 % water concentration dimethyl sulfoxide completely inhibit the growth of Aspergillus parasiticus and do not affect the colour, structure and chemical characteristic of the wood (Osman et al., 2017). Dimethyl sulfoxide was found even to be protective agent ozone injury of agricultural plants (Weidensaul, 1982). The substance also can express herbicidal activity towards purple nutsedge (Cyperus rotundus L.) (Anderson and Dunford, 1966). In biology and chemistry DMSO is also commonly used for extraction of chlorophyll which can explain its herbicidal properties (Shinano et al., 1996; Vimala and Poonghuzhali, 2015). However was found that Seven of eight pure, axenic cultures of marine planktonic algae produced significant amounts of DMSO. These findings suggest that DMSO is associated with phytoplankton activity in surface which cause DMSO to occurs at concentrations of >10 nmol per liter in the surface ocean and in rivers and lakes (Andreae, 1980; Malin and Kirst, 1997). Dimethyl sulfoxide despite of being used frequently as vehicle solvent in ecotoxicity bioassays was found to be toxic in 1 % concentration (NOAEL) towards microalgae Raphidocelis subcapitata, microcrustacean Daphnia magna and the rotifer Brachionus calyciflorus. Concentration of 0.01% DMSO is safe for the tested organisms (Andrade-Vieira et al., 2022). From several organic solvents as methanol, ethanol, acetone, N,N-dimethylformamide, and dimethyl sulfoxide, DMSO was the least toxic towards species of marine microalgae: Bacillariophyceae; Skeletonema costatum and Chaetoceros calcitrans, Prasinophyceae; Tetraselmis tetrathele, Haptophyceae; Isochrysis galbana and Pavlova lutheri, Dinophyceae; Prorocentrum minimum, Raphidophyceae; Heterosigma akashiwo, Euglenophyceae; Eutreptiella sp., Chlorophyceae; Dunaliella tertiolecta (Okumura et al., 2001). The compound was found to have now toxicity towards freshwater algae Scenedesmus quadricauda and Anabaena flos-aquae at 0.729% (volume/volume) concentration (Abou-Waly, 2000). Photosynthetic oxygen evolution and 14CO2 fixation by 3 blue-green algae, Anabaena flos-aquae, Oscillatoria sp., and Anacystis nidulans, and green alga, Chlorella pyrenoidosa, were inhibited by dimethyl sulfoxide at concentrations above 1% (Cheng et al., 1972). Cyclotella meneghiniana is a freshwater diatom which is a cosmopolitan algae recorded typically in freshwater basins around the world – rivers, lakes, dams and ponds (Roubeix and Lancelot, 2008). This phytoplankton is commonly used in ecotoxicological trials both for non-organic and organic chemicals (Olguín et al., 2004; von der Au et al., 2020; Nguyen et al., 2024) and various another tests as investigation about photosynthesis under different light conditions (Chukhutsina et al., 2013), mechanism of the effect of flow velocity (Zhang et al., 2025), differential effects of phosphorus form and nitrogen-phosphorus ratio or level of salinity concentrations on the growth of cells (Tuchman et al., 1984; Han, et al., 2025)

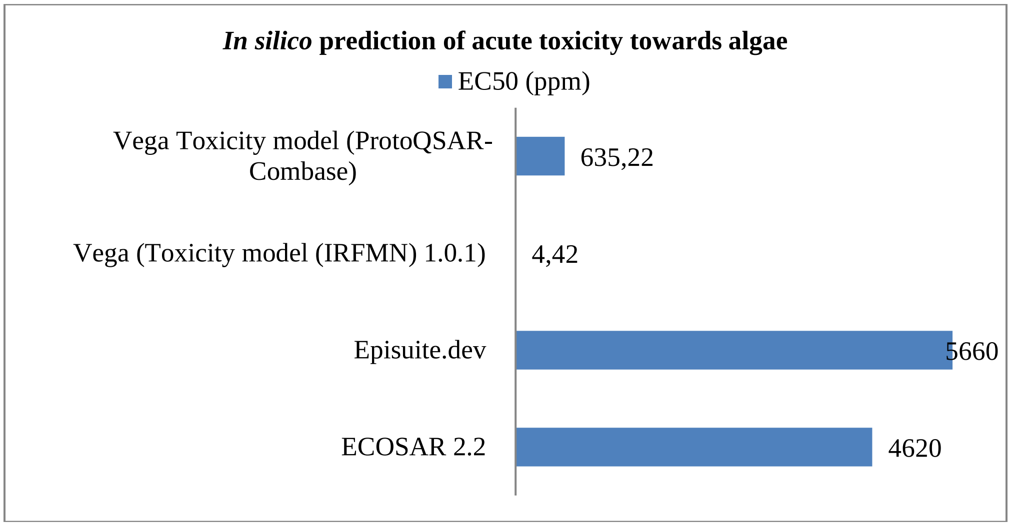
2. MATERIALS AND METHODS

The *in silico* studies of algicidal action of dimethyl sulfoxide were carried out by ECOSAR 2.2 (Sanderson et al., 2003; De Roode et al., 2006), VEGA (Benfenati et al., 2013) and a newly developed QSAR instrument by US Environmental Protection Agency Episuite.dev or EPI Suite™ BETA version 1.0 which purpose is to replace the old EPI Suite software (part of which is ECOSAR 1.11) via online user interface (Banjare et al., 2021). Both platforms are widely used in the ecotoxicological *in silico* studies and predictions. Freshwater algae of *Cyclotella meneghiniana* naturally grow in artificial ponds in Agricultural University, town of Plovdiv, Bulgaria was used in the current test. Water solutions of DMSO in different concentrations were used and algae were placed into them. Each concentration was examined in 5 repetitions and solutions were made with the same water in which algae naturally grows. The phytoplankton was additionally concentrated with the laboratory centrifuge at 5000 revolutions per second for 5 minutes and placed into micro plates. The observations were conducted 96 hours after the start of the test. Dose – Response analysis was performed by R language for statistical computing to establishing the NOAEL (EC05), LOAEL (EC25) and EC50 values (Knezevic et al., 2007; Ritz et al., 2015)

3. RESULTS

ECOSAR 2.2 point EC50 from acute toxicity test to be 4620 ppm or 0.46 % concentration while Vega (Toxicity model (IRFMN) 1.0.1) point EC50 value of 4.42 ppm. However, the predicted compound is outside the Applicability Domain of the model and the result may be not reliable. According to Algae Classification Toxicity model (ProtoQSAR-Combase), DMSO is classify as a “Non-toxic” but again the predicted compound is outside the Applicability Domain of the model and the result may be not reliable. Towards the same algorithm EC50 is 635.22 ppm or 0.063 % concentration. The online version of EpiSuite - Episuite.dev or EPI Suite™ BETA version 1.0 which purpose is, give EC50 for freshwater algae = 5660 ppm or 0.56 % concentration which is fairly close to the value of ECOSAR 2.2. The results are presented in the Figure 1 below

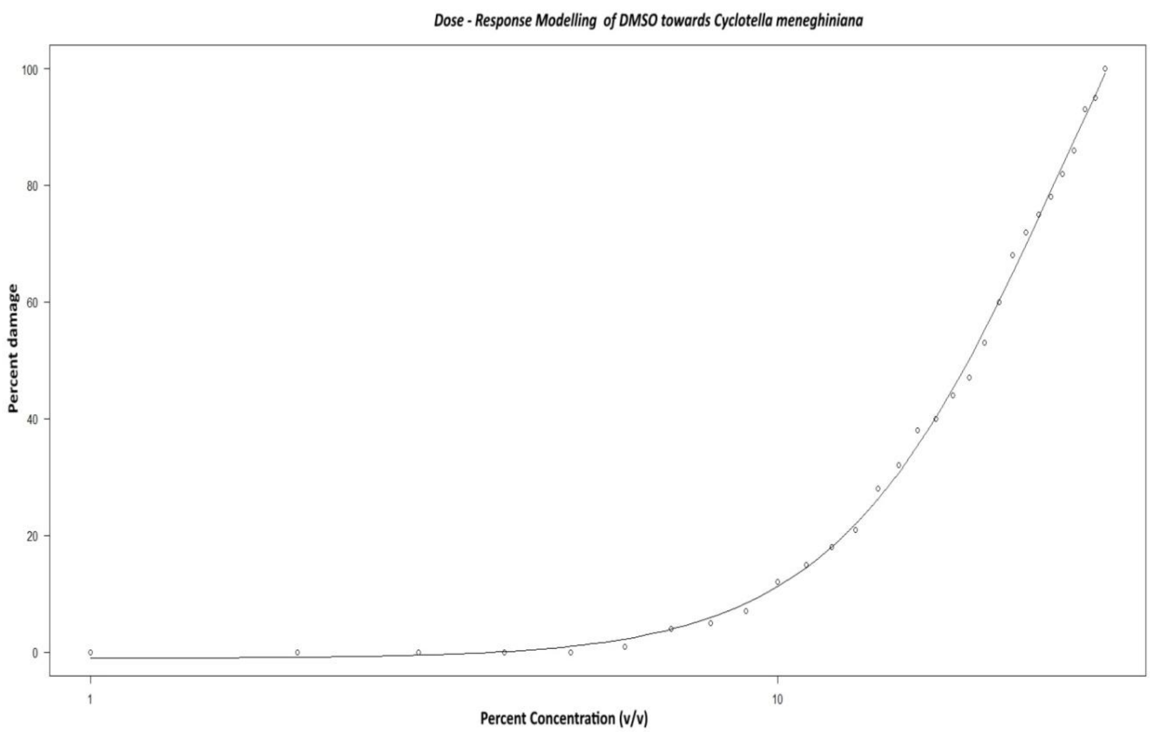
Figure 1: Acute toxicity of dimethyl sulfoxide towards algae specie by tested QSAR software



Source: Author research

Figure 1 clearly show dramatic differences of toxicity predictions of the VEGA performed by two different algorithms and differences between Vega and ECOSAR.
In the figure below (Figure1) is presented Dose – Response Curve of dimethyl sulfoxide towards *Cyclotella meneghiniana* conducted with R language, drc package:

Figure 2: Toxicity of dimethyl sulfoxide towards Cyclotella meneghiniana

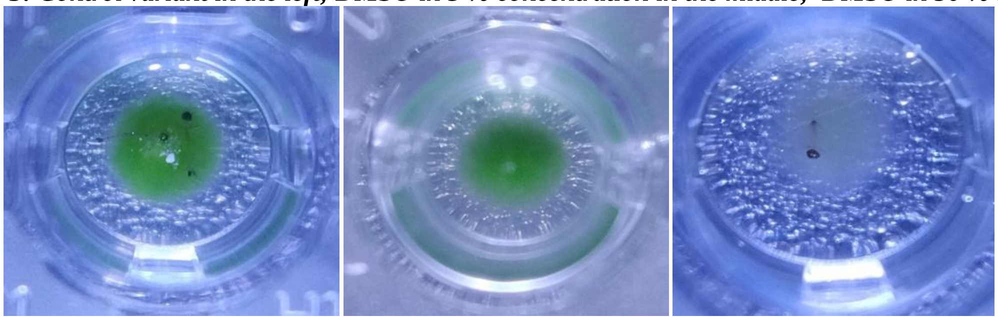


Source: Author research

The calculated LC (Lethal Concentration) values are:

- NOAEC or LC05 = 8.57 % (v/v)
- LOAEL or LC25 = 17.08 % (v/v)
- LC50 = 25.74 % (v/v)
- LC90 = 58.5 % (v/v)

Figure 3: Control variant in the left; DMSO in 5 % concentration in the middle; DMSO in 50 % in the right



Source: Author research

Up to the 5 % concentration (v/v) dimethyl sulfoxide do not cause any acute toxic effect to the *Cyclotella meneghiniana* and algae were identical to the control variant. The established LC50 was approximately 26 % concentration or 260000 ppm, while LC90 was approximately 60 %. Over 30 % concentration of whitening (death) of the algae cells was seriously visible.

4. DISCUSSIONS

The conducted tests in the present research reveal that different algae species can respond differently towards the same chemical and the *in silico* tests which give general toxicity towards algae cannot be applied in all cases. DMSO was found to be extremely low toxic towards *Cyclotella meneghiniana*, although towards *Raphidocelis subcapitata* was relatively close toxicity of LC = 2.13 % (Andrade-Vieira et al., 2022). The low toxicity action of DSMO was reported also for *Chlorella vulgaris* and *Selenastrum capricornutum*, which are also very commonly used algae species in the ecotoxicological researches (El Jay, 1996).

5. CONCLUSIONS

The conducted in vitro trials of dimethyl sulfoxide towards *Cyclotella meneghiniana* confirm its very low toxicity towards some algae species. However QSAR analyses of DSMO with ECOSAR and VEGA show dramatic differences between real and *in silico* trials towards specific algae species which can be the base for future perfection of the QSAR algorithms towards toxicity of aqua organisms. More real trials with different algae species are needed for correction of the *in silico* software and at the given state, the results from QSAR analyses towards algae toxicity cannot be accepted as a credible.

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