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THE EFFECTS OF CHITOSAN, CHITOOLIGOSACCHARIDES AND DERIVATIVES ON AQUATIC ORGANISMS

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Abstract: *The contamination of the aquatic environment with chitosan, chitooligosaccharides and derivatives is possible, potentially producing toxic effects on aquatic organisms, due to their production and use in numerous fields of application. In the present study, both an experimental approach and a computational approach were used for the assessment of the effects of chitosan, chitooligosaccharides and derivatives with different properties. For the experimental approach, Lemna minor was the target aquatic organisms, while for the computational approach, Tetrahymena pyriformis, Daphnia magna and fathead minnow were the target organisms. The Lemna minor EC50 values highlighted the higher toxicity of D-glucosamine, a "slightly toxic" chitooligosaccharide, in comparison with the other tested samples which were "practically nontoxic". The results obtained in the experimental approach were in agreement with the results obtained in the computational approach. The predictions obtained using admetSAR2.0 emphasized that the analyzed chitooligosaccharides and derivatives were not toxic towards the three target organisms. The predictions obtained using ADMETLab2.0 showed that the lowest values obtained for the three target organisms were observed for totally acetylated chitooligosaccharides. The effects of the tested chitosan, chitooligosaccharides and derivatives were dependent on parameters such as molecular weight, deacetylation degree, acetylation pattern and solubility.*

• Introduction

Taking into account their production and use in numerous fields of application, the contamination of the aquatic environment with **chitosan**, **chitooligosaccharides (Cos)** and **derivatives** is possible, potentially producing toxic effects on aquatic organisms.

Lemna minor, *Tetrahymena pyriformis*, *Daphnia magna* and *Pimephales promelas* (fathead minnow) are **aquatic organisms** used as target organisms in several ecotoxicity assay, both experimentally, and computationally.

• Material and method

EXPERIMENTAL STUDY

Four chitosan samples and four chitooligosaccharide samples were analyzed in the experimental approach:

- Low molecular weight (MW) chitosan (ChiS)
- Medium MW chitosan (ChiM)
- High MW chitosan (ChiL)
- 50% deacetylation degree (DaD) chitosan (Chi50)
- D-glucosamine hydrochloride (G)
- N-acetyl-D-glucosamine (NAG)
- Chitobiose dihydrochloride (2G)
- Carboxymethyl chitosan (CMChi)

The target organism was the common duckweed (*Lemna minor*) a growth inhibition assay being conducted.

COMPUTATIONAL STUDY

In the computational approach the considered materials were:

- Homo-chitooligosaccharides
- Hetero-chitooligosaccharides
- Derivatives

The Simplified Molecular Input-Line Entry System (SMILES) were used as entry data for the prediction tools: admetSAR and ADMETlab.

The target organisms were *Tetrahymena pyriformis*, *Daphnia magna* and *Pimephales promelas.*

• Conclusions

The novelty of this study consists in experimental and computational assessment of several ecotoxicological data for chitosan samples with variable MW and DaD, chitooligosaccharides and derivatives.

The experimental data revealed that D-glucosamine hydrochloride was "slightly toxic", while all the other investigated molecules were "practically non-toxic".

These results were in very good correlation with the results of the computational approach: the investigated molecules were not considered to produce toxicity against crustaceans, fish and *Tetrahymena pyriformis*. The lowest values of IGC_{50} for *Tetrahymena pyriformis*, LC_{50} for fathead minnow and LC_{50} for *Daphnia magna* are revealed by totally acetylated COs.

• Results and discussions

EXPERIMENTAL STUDY

The results obtained in the experimental approach allowed the plotting of dose-response curve for the tested samples, the EC_{50} values being determined (Fig. 1).

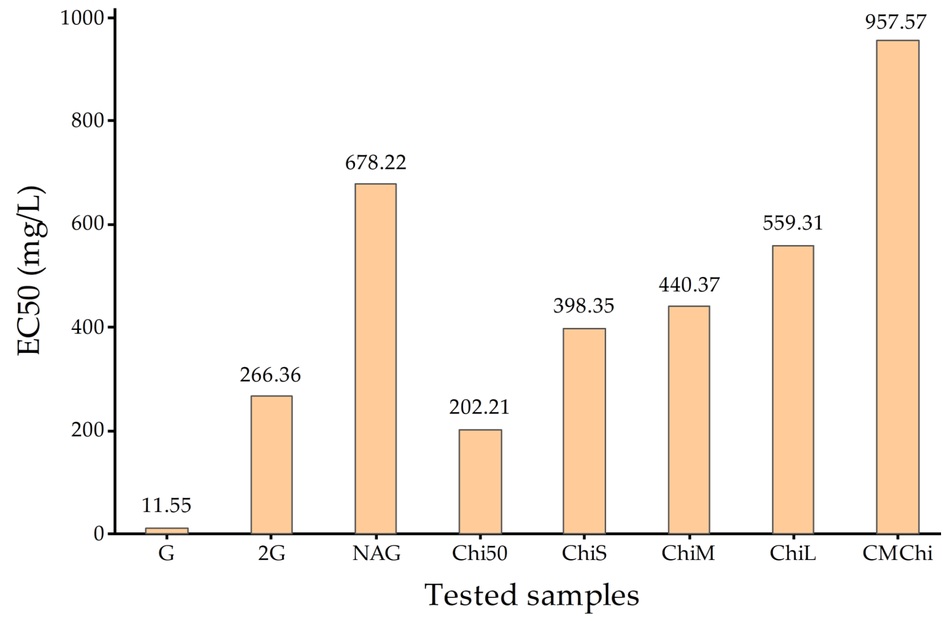

Fig. 1. Comparison of EC_{50} values of tested chitooligosaccharides, chitosans and their derivatives.

Based on their EC_{50} values, the analyzed samples were categorized into the U.S. EPA aquatic toxicity categories (Table 1). D-glucosamine hydrochloride was slightly toxic, while the other samples were practically nontoxic.

Table 1. Aquatic toxicity categories according to U.S. EPA.

Aquatic Toxicity Category	EC_{50} (mg/L)
very highly toxic	<0.1
highly toxic	0.1-1
moderately toxic	1-10
slightly toxic	10-100
practically nontoxic	>100

COMPUTATIONAL STUDY

The **admetSAR** tool predicted no toxic effects towards the three target organisms of totally acetylated COs, COs derivatives, partially deacetylated and totally deacetylated COs in both neutral and cation forms.

The **ADMETlab** tool predictions emphasized the follwing:

- the lowest values obtained for the three target organisms were observed for totally acetylated chitooligosaccharides
- the effects of the tested chitosan, chitooligosaccharides and derivatives were dependent on parameters such as molecular weight, deacetylation degree, acetylation pattern and solubility