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Toxicity Evaluation of Surface Cleaning Preparation Using Different Test Methods

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Abstract **— Surface cleaning preparations pose a serious threat to the environment. Toxicity of a pre-manufactured preparation SCP-1 was tested on bacteria, algae and higher plants and was expressed as a minimal inhibitory concentration (MIC). Obtained results showed that among unicellular and more complex test-organisms MIC values differed 10–100 fold suggesting that an application of complex test-organism battery is necessary to evaluate the toxicity of SCP-1 thoroughly. MIC values were different from the critical micelle concentration; this indicates that the SCP-1 mechanism of action might not involve membrane disruption and/or destabilization.**

Keywords **— Critical micelle concentration, surface cleaning preparations, test-organism battery, toxicity.**

I. Introduction

Surface cleaning preparations (SCPs) or detergents are substances with cleaning/solubilisation properties. Components of SCPs are: surface-active agents (surfactants), builders, boosters, fillers and auxiliary compounds. SCP components that are the biggest threat to the environment are surfactants [1]. Surfactants are well known for their toxicity to organisms. Different surfactant classes have different target structures in cells. For example, anionic surfactants tend to bind to bioactive molecules like peptides, enzymes, DNA; cationic surfactants bind to the inner membrane of the cell. As a result biological function of the target structure is influenced and this can cause cell death [2].

The use of SCPs is steadily growing and contamination with these substances is becoming a serious problem. Surfactants or residues of these chemicals reach the environment via the use of sewage sludge on land, effluents from wastewater treatment plants and direct industrial discharges [1]. Even after treatment wastewater can contain a considerable amount of detergents and surfactants [3]−[4].

Toxicity of SCPs is measured using a wide range of toxicity tests. The most often used test-organisms are aquatic organisms − microorganisms, aquatic plants, benthic organisms, phytoplankton, zooplankton and vertebrates [5]−[7]. Terrestrial higher plants and microorganisms are also used for this purpose [8].

The ranges of sensitivity of different test-organisms and toxicity tests are large and diversified. It is emphasized that a battery of several tests is required to assess toxicity more thoroughly [9]−[10]. Toxicity values of the used surfactant often correlate with its critical micelle concentration (CMC) [11]. CMC is a concentration of surfactant or detergent at which it forms micelles. Surface tension of solutions decreases at CMC [12]. It is important to evaluate the toxicity of SCPs and to predict the possible effect on the environment by comparing toxicity of tested preparations on various test-organisms.

This study was aimed at assessing ecotoxicological behavior of complex SCP (SCP-1. Gram-negative bacteria as a single strain and in consortium, algae, crustaceans and higher plants were tested using standard protocols and modified methods. Toxicity of SCP-1 towards test-organisms was expressed as a minimum inhibitory concentration (MIC). These organisms are often used as test-organisms [13]−[18].

II. Materials and Methods

A. Surface Cleaning Preparation

SCP-1 tested in this study was pre-manufactured. It contained propanol, wax emulsion, mineral oil and quaternary ammonium compounds and exhibited strong disinfecting properties. The full composition of the preparation was not disclosed by the manufacturer.

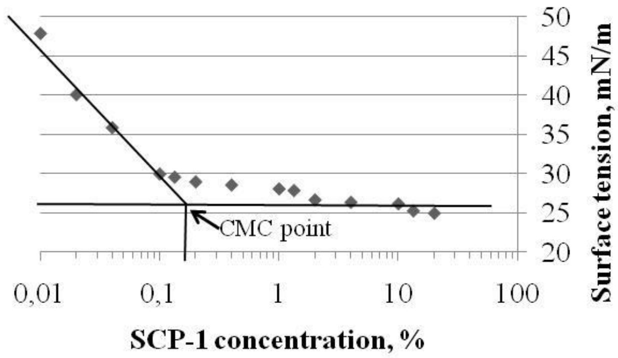

Fig. 1. Dependence of surface tension on the concentration of SCP-1.

SCP-1 was characterized by critical micelle concentration (CMC). CMC was determined by measuring surface tension depending on SCP-1 concentration (Fig. 1). Surface tension measurements were done using tensiometer *Krüss K6* (*Krüss GmbH*), fitted with a platinum ring 19 mm in diameter. Samples were prepared for measurement by diluting the appropriate concentration of standard solution and equilibrating for 24 h. Solutions were placed in a shallow glass dish 50 mm in diameter and the platinum ring was inserted in the middle of the container to avoid edge effects and equilibrated for 90 min. The ring was raised by manual operation of a torsion mechanism and tension readings at the instant of surface detachment were noted [19]. All measurements were taken in triplicate at 22 °C and the mean results had standard deviations less than ±2 mN/m. It can be seen that the CMC of SCP-1 is approximately 0.17 %.

B. Microorganisms and Growth Conditions

Bacterial consortium (consisting of 8 bacterial strains (5 strains representing *Stenotrophomonas maltophilia* cluster and 3 strains of *Pseudomonas* spp.), as well as single bacterial culture of *Pseudomonas fluorescens* AM11) was used in experiments with microorganisms. Bacterial consortium had been previously isolated from hydrocarbon-contaminated soil. Bacterial consortium was prepared by overnight cultivation in Bushnell Haas broth (medium composition: $MgSO_4 \cdot 7H_2O$ − 0.41 g·L^{-1}; $CaCl_2$ − 0.02 g·L^{-1}; KH_2PO_4 − 1.00 g·L^{-1}; K_2HPO_4 − 1.00 g·L^{-1}; NH_4NO_3 − 1.00 g·L^{-1}; $FeCl_3 \cdot 6H_2O$ − 0.08 g·L^{-1}) with 0.5 % molasses at 28 °C under aerobic conditions with agitation 140 rpm.

P. fluorescens AM11 culture was prepared by overnight cultivation in minimal nutrient broth (medium composition: $Na_2HPO_4 \cdot 12H_2O$ − 6.0 g·L^{-1}, KH_2PO_4 − 3.0 g·L^{-1}, NaCl − 0.5 g·L^{-1}, $(NH_4)_2SO_4$ − 0.3 g·L^{-1}, $FeSO_4 \cdot 7H_2O$ − 0.002 g·L^{-1}, Na_2MoO_4 − 0.001 g·L^{-1}) amended with 1 % glucose at 28 °C under aerobic conditions with agitation 140 rpm.

C. Microdilution Method

Microdilution was performed in 96-well microplates. Bacterial consortium described previously was used as inoculum (initial concentration $3.1 \cdot 10^6$ CFU/mL). SCP-1 concentrations tested ranged from 0.012 % to 0.8 %. Mixtures of 750 µL of preparation dilutions in Bushnell Haas (BH) broth and 50 µL of inoculum were prepared. 200 µL of each mixture was transferred to a microplate well in triplicate. The results were expressed as the difference between the optical density (OD) at 620 nm at the beginning of cultivation and after 48 h.

D. Determination of Specific Growth Rate using Microdilution Method

Experiments were carried out overnight in microplates with *P. fluorescens* AM11 culture. Bacterial culture concentrated five times was prepared by centrifuging 10 mL of culture at 3000 rpm for 10 min and discarding 8 mL of supernatant. 100 µL of inoculum and 100 µL of diluted SCP-1 (0.006−0.2 %) was transferred to microplate wells in triplicate. OD at 620 nm was measured in *Tecan Infinite 200 PRO* series microplate reader for 22 h every 11 min. OD_{620} measurements of exponential growth phase were used to calculate the specific growth rate of bacterial culture (1).

$$\mu = \frac{4.02hOD_{620} - 0.55hOD_{620}}{3.47h} \quad (1)$$

E. Germination and Early Seedling Growth Tests

Wheat (*Triticum* spp.) and cress (*Lepidium sativum*) were used as test-organisms. Filter paper was put in a Petri dish with 10 cm diameter. SCP-1 was diluted in sterile distilled water. 10 mL of diluted SCP-1 samples 0.2 %, 0.4 %, 0.6 %, 0.8 %, 1 %, 1.2 %, 1. 5% and control experiment without SCP-1 addition were poured on the filter paper. Three Petri dishes with each SCP-1 concentration were prepared for both plant species. Afterwards 10 seeds were put on the filter paper. Petri dishes were closed and put in darkness for 7 days, at 20 °C. Germinated seeds and grains were counted and the height of shoots was measured after the incubation. Germination was expressed as percent of germinated seeds. Seedling height was expressed as relative height − ratio of seedling height in presence of SCP-1 to control height.

F. Range Finding Test for Algae Selenastrum Capricornutum

Range finding test was performed using SCP-1 concentrations 0.01−0.00001 %. SCP-1 was diluted in algal culture medium provided by *Algaltoxkit F*^{TM} (*MicroBioTests Inc.*, Belgium). Flasks with 90 mL of each dilution were prepared. 0.9 mL of *S. capricornutum* stock suspension ($1 \cdot 10^6$ per 1 mL) was added to each flask to obtain initial concentration of $1 \cdot 10^4$ algae per 1 mL in each of the toxicant concentrations. 25 mL of the algae-toxicant dilutions from each flask were transferred to 3 long cells. The cells were closed and incubated for 72 h in an incubator at 21 °C and 10000 lux sideway illumination. OD_{670} measurements of algal suspensions in the long cells were performed after 24 h, 48 h and 72 h exposure to the toxicant. Relative OD_{670} change was calculated (2).

$$\frac{(70hOD_{670} - 24hOD_{670})_{suspension\,with\,SCP-1}}{(70hOD_{670} - 24hOD_{670})_{control}} \quad (2)$$

G. Range Finding Test for Crustacean Thamnocephalus Platyurus

SCP-1 dilutions with concentrations 0.01−0.00001 % were prepared in water. 1 mL of each dilution was added to a multiwell plate in quadruplicate. *T. platyurus* cysts provided by *Thamnotoxkit F*^{TM} (*MicroBioTests Inc.*, Belgium) were pre-hydrated in 1 mL of 12.5 % standard freshwater (medium composition: $NaHCO_3$ − 96 mg L^{-1}, $CaSO_4 \cdot 2H_2O$ − 120 mg L^{-1}, $MgSO_4 \cdot 7H_2O$ − 123 mg L^{-1}, KCl − 4 mg L^{-1}) for 30 min. Pre-hydrated cysts were transferred to Petri dishes with 10 mL 12.5 % standard freshwater. Cysts were incubated at 25 °C for 21 h under continuous illumination (4000 lux). Approximately 10 *T. platyurus* larvae were transferred to each well of a multiwell plate. Plate was tightly covered both with parafilm and lid and incubated at 25 °C in darkness, for 24 h. After 24 h incubation the number of dead larvae was recorded and the mortality (%) of larvae was calculated.

H. Statistical Analysis

Mean values and standard deviations were calculated using *Microsoft Word Excel*. The significance of differences among the treatments was calculated using the t-test in program R (significance level − 0.05).

III. Results

A. Bacterial Consortium

Growth of consortium was detected in SCP-1 concentrations 0.012−0.025 %. (Fig. 2). No growth was observed in the set with 0.05 % SCP-1. Therefore MIC for bacterial consortium, tested by microdilution method was 0.05 %.

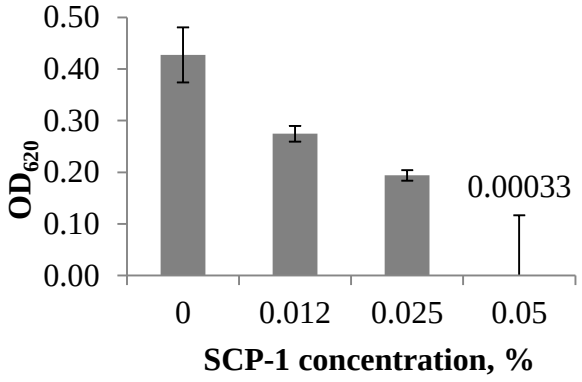

Fig. 2. Optical density (OD_{620}) of bacterial consortium and SCP-1 solutions after 48 h incubation.

B. Bacteria Pseudomonas Fluorescens AM11

P. fluorescens AM11 was used in further experiments to compare the response of a separate strain to the response of the bacterial consortium to SCP-1.

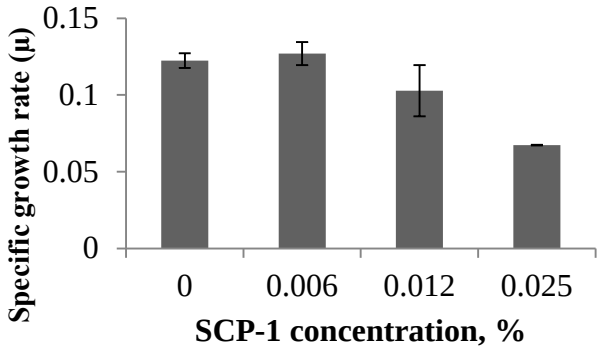

Fig. 3. *P. fluorescens* AM11 specific growth rate in suspensions with SCP-1 after 22 h incubation.

Specific growth rate (μ) of *P. fluorescens* AM11 was tested in SCP-1 concentration range 0.006−0.2 %. Bacterial growth was detected in suspensions with 0.025 %, 0.012 %, 0.006 % SCP-1 (Fig. 3). The μ values were inversely proportional to SCP-1 concentration. Corresponding μ values were 0.0672 ± 0.0002, 0.103 ± 0.017, 0.127 ± 0.008. The μ value in set with no SCP-1 was 0.122 ± 0.005. No growth was detected and therefore no μ value could be calculated in set with 0.05 % SCP-1. Hence, MIC for the specific growth rate of *P. fluorescens* AM11 was 0.05 %.

C. Algae S. capricornutum

After testing microorganisms further experiments were conducted with more complex, i.e., eukaryotic organism − algae *S. capricornutum*.

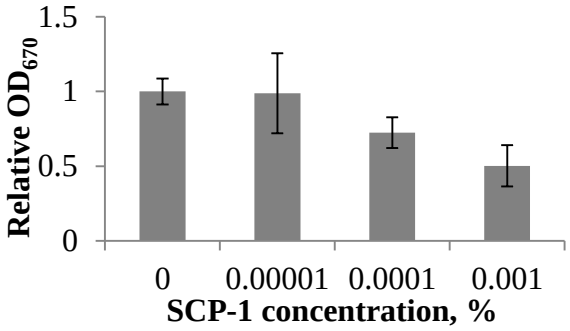

Fig. 4. Relative optical density (OD_{670}) of *S. capricornutum* suspensions with SCP-1.

SCP-1 concentrations tested in the experiment with *S. capricornutum* ranged from 0.00001 % to 0.01 %. Growth was detected in the concentration range from 0.001 % to 0.00001 %, which was inversely proportional to SCP-1 (Fig. 4). MIC for *S. capricornutum* was 0.01 %.

D. Wheat and Cress

To evaluate the response of higher plants to SCP-1, wheat and garden cress were tested in a germination test.

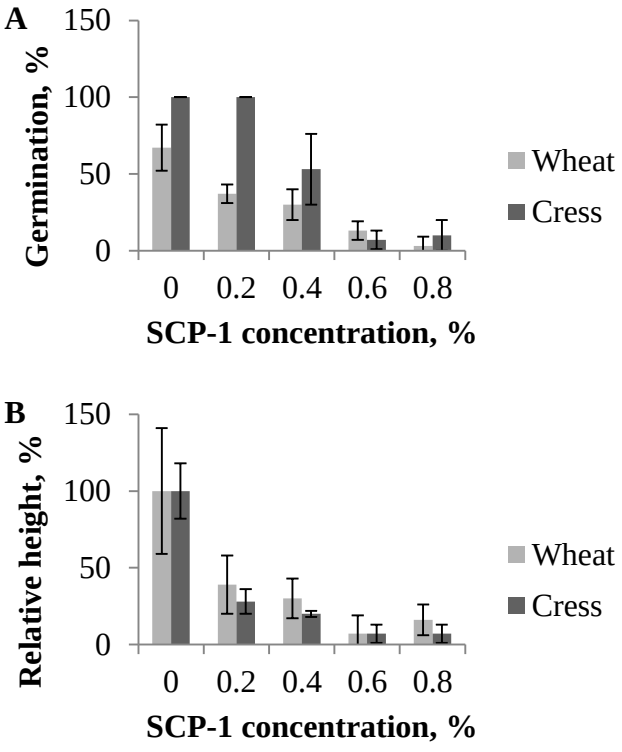

Fig. 5. Germination rate (A) and relative height (B) of wheat and garden cress in presence of SCP-1.

Wheat and cress growth was characterized using two criteria − germination and shoot height. Tested SCP-1 concentrations ranged from 0.1 % to 3 %.

Germination of both cress and wheat was observed in sets with 0.4 % SCP-1. 53 % of cress and 30 % of wheat seeds were germinated in the presence of 0.4 % SCP-1 (Fig. 5A). Germination of cress was 100 % in sets with no SCP-1 and

0.2 % SCP-1. MIC for wheat and cress in vegetation experiments was 0.6 %.

Shoot height for wheat and cress increased with decreasing SCP-1 concentration (Fig. 5B). The longest seedlings were detected in set with 0.2 % SCP-1 — the relative wheat length was 39 ± 19 % and relative cress length was 28 ± 8 %.

E. Crustacean Thamnocephalus Platyurus

T. platyurus growth was detected in sets with SCP-1 concentrations of 0.001 % and below, therefore the MIC of SCP-1 for the tested crustaceans was determined to be 0.001 %.

IV. Discussion

The summarized results of the conducted experiments presented in Table I reveal that MIC of SCP-1 differs depending on the used test-organism and the applied method.

TABLE I

Summary of the Obtained MICs for the Various Test Organisms and Methods Used

Test-organism	Method	MIC
Bacterial consortium	Microdilution	0.05 %
P. fluorescens AM11	Microdilution - specific growth rate	0.05 %
Wheat *Triticum spp.*	Germination test	0.6 %
Garden cress *L. sativum*	Germination test	0.6 %
S. capricornutum	Range finding test	0.01 %
T. platyurus	Range finding test	0.001 %

According to previous experimental studies, freshwater crustacean *T. platyurus* often has the highest surfactant sensitivity [20]−[21]. This observation also applies to the conducted study. Experiments comparing the bacterial consortium and the single strain revealed that the SCP-1 MIC of consortium is equal to that of *P. fluorescens*. These results showed that the possible co-metabolism of consortium did not enhance the degradation of SCP-1 and consortium growth as it is seen other studies [22]. SCP-1 MIC for cress and wheat was significantly ($p < 0.05$) higher than for other test-organisms. Lower detergent toxicity in higher plants compared to bacteria *Vibrio fischeri* and crustacean *Daphnia magna* has been observed previously [9]. Overall the MIC values differed 5−600 fold between various test-organisms (from 0.001 % for *T. platyurus* to 0.6 % for wheat and cress). Taken together, our results support the observations in other studies and indicate the necessity to use a complex test-organism battery to evaluate the toxicity of surface cleaning preparations thoroughly. The response of a single test-organism might serve only as a preliminary indicator of toxicity, which can not substitute the whole data set from various test organisms [9]−[10]. Surfactant concentrations in the environment can reach various values, for example, 0.003 % in sediments or 0.2 % in freshwater [23]−[24]. The wide range of MIC values observed in this study (0.001−0.6 %) indicates that the test-organism battery used might be applied to evaluate the toxicity of SCP-1 in different environments.

It has been reported previously, that toxicity of surfactants often correlates with their CMC; this indicates that the toxicity is related to destabilization and/or destruction of cell membrane [11]. Most of the MICs acquired in our experiments are below SCP-1 CMC value showing that this is not always the case. This possibly could be explained by mechanism that does not affect the cell membrane yet allows surfactant molecules to cross the cell membrane and to interact with cytoplasmic components directly [25]. The wide range of observed MICs may arise from different detergent component interactions with different cell types. As an example, these dissimilarities may be caused by differences in lipid and protein content between plasma membranes or the manner in which DNA and RNA is protected in test-organism cells [26]. However further studies are needed to confirm this hypothesis.

V. Conclusion

1. MIC values differed 5–600 fold between various test-organisms (from 0.001 % for *T. platyurus* to 0.6 % for wheat and cress). These results prove that multiple test-organisms are needed to fully evaluate impact of SCP-1 on the environment.
2. The obtained MIC values did not match the CMC; this indicates that SCP-1 mechanism of action might not involve disruption and/or destabilization of cell membranes.

Acknowledgement

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Dagnija Vecstaudža, Reinis Rutkis, Māris Kļaviņš, Olga Muter. Virsmu tīrīšanas līdzekļa toksiskuma novērtēšana, izmantojot dažādas testa metodes.

Virsmu tīrīšanas līdzekļi ir būtiski vides piesārņotāji. Svarīgs posms jaunu tīrīšanas līdzekļu testēšanā ir to toksiskās ietekmes novērtēšana ar dažādiem testa organismiem un metodēm. Šajā pētījumā veikts virsmu tīrīšanas līdzekļa SCP-1 toksiskuma novērtējums, izmantojot vairākus testa organismus: Gram-negatīvām baktērijām (*Pseudomonasfluorescens* AM11 kultūru, *Pseudomonas* spp. un *Stenotrophomonas* spp. celmiem konsorcija sastāvā), saldūdens aļģēm *Selenastrum capricornutum*,, žaunkājvēžiem *Thamnocephalus platyurus*, augstākajiem augiem — kviešiem *Triticum* spp. un kressalātiem *Lepidium sativum).* Kā toksiskuma rādītājs tika izvēlēta SCP-1 minimālā inhibējošā koncentrācija (MIK). Iegūtie rezultāti norāda uz MIK atkarību no testa organisma sugas. MIK vērtības savā starpā atšķiras 5–600 reizes — no 0.001 % testa organismam *T. platyurus* līdz 0.6 % kviešiem un kressalātiem. Piesārņotāju koncentrācijas var sasniegt dažādus līmeņus atkarībā no to fizikāli ķīmiskajām īpašībām un apkārtējās vides īpatnībām. Rezultāti apstiprina nepieciešamību ekotoksikoloģiskos pētījumos izmantot testa organismu bateriju, kas satur dažādu trofisko līmeņu pārstāvjus. Virsmaktīvo vielu toksiskums bieži tiek saistīts ar to kritisko micelāro koncentrāciju (KMK), t. i., vielas koncentrāciju, kurā vielas molekulas veido micellas. Korelācijas cēlonis ir virsmaktīvo vielu izraisītā šūnu membrānu destabilizācija. Pētāmā preparāta KMK ir 0.17 %. Veikto eksperimentu rezultāti neapstiprina viennozīmīgu KMK ietekmi uz SCP-1darbības mehānismu testa organismos.