



## Evaluation of phytotoxic effect of wastewater contaminated with anionic surfactants

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#### Summary

Among anionic surfactants, two of the major groups in current use are linear alkylbenzene sulphonates and alkyl ether sulphates. They pass into the wastewater treatment plants, where they are usually aerobically degraded, and next their remnants and biodegradation products enter the environment. The influence of wastewater containing the anionic surfactants at concentrations from 0.5 to 58 mg L<sup>-1</sup> on seed germination was investigated. The tested wastewater came from the lab-scale biodegradation experiments conducted in the continuous flow system. Four different plants: mustard (*Sinapsis alba*), cress (*Lepidium sativum*), rye (*Secale cereale*) and wheat (*Triticum aestivum*) were employed in the tests. In order to evaluate the inhibition of seed germination, the germination index was calculated. It occurred that sodium alkylbenzene sulphate inhibited seed germination of the rapidly growing plants (mustard and cress), starting with 10 mg L<sup>-1</sup> in the effluent, while sodium alkyltrioxyethylene sulphate exerted the toxic effect at the concentrations above 30 mg L<sup>-1</sup> for all tested plants. Mustard (*Sinapsis alba*) was most sensitive to the anionic surfactants exposure and could be used as a bioindicator within the phytotoxicity tests concerning anionics.

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## 1. Introduction

Linear alkylbenzene sulphonates (LAS) and alkyl ether sulphates (AES) are one of the most often utilised groups of the anionic surfactants nowadays. They are used in a broad spectrum of domestic applications such as household cleaners, dishwashing liquids and fabric care (powders and liquids) (1). The basic structure of the LAS molecule is a benzene ring connected to an alkyl chain and a sodium sulphonate group:  $R-C_6H_4-SO_3-Na$ . The group of LAS consists of the homologues of different lengths of the alkyl chain and of different position of the benzene ring on the fixed alkyl chain (2). The commercial LAS usually have a linear alkyl chain consisting of 10 to 14 carbon atoms. Petrovic and Barcelo (2004) reported that the average chain length of LAS dissolved in the wastewater influent is 11.3 carbon atoms (3). Alkyl ether sulphates (AES), which are also called alcohol ethoxysulphates, are synthesised by the sulphonation of the ethoxylated alcohols. Their chemical structure is as follows:  $RO-(CH_2-CH_2-O)_n-SO_3-Na$ . The alkyl chain consists of 12 up to 18 carbon units, while the number of the ethoxylate groups is between 1 and 12, on average three (3).

The average concentration of LAS in the domestic wastewater varies from 1 to 25  $mg L^{-1}$ , whereas the presence of AES was reported at the level from 0.3 to 5  $mg L^{-1}$ . At the same time, the concentrations of both anionics in the industrial wastewater are significantly higher. For example, AES was found at the concentrations between 46 and 298  $mg L^{-1}$  in the wastewater coming from one of the surfactant formulating companies (3). It was found that the removal efficiency of both mentioned above anionics was generally higher than 95% in the activated sludge wastewater treatment plants (3). However, a certain amount of the surfactants is sorbed onto the solid fraction and discharged in the original or partly degraded form with the excess sludge. According to the literature data, concentration of LAS in the sewage sludge comprised three orders of magnitude, ranging from 100  $mg kg^{-1}$  even to 30  $g kg^{-1}$  (3).

The mechanism of linear alkylbenzene sulphonate breakdown involves the degradation of straight alkyl chain, sulphonate group and, finally, benzene ring. The breakdown of alkyl chain starts with the oxidation of terminal methyl group ( $\omega$ -oxidation) through alcohol, aldehyde to carboxylic acids (4). The reactions are catalysed by alkane monooxygenase and two dehydrogenases. The carboxylic acids can then undergo  $\beta$ -oxidation and two carbon atoms enter the tricarboxylic acid cycle as acetyl-CoA. The second stage of the LAS breakdown is a loss of sulphonate group. Three mechanisms have been proposed for desulphonation, which was thoroughly discussed in (4) by Scott and Jones (2000). Phenylacetic or benzoic acids obtained after the loss of alkyl and sulphonate groups are converted into catechol within microbial oxidation. The  $\omega$ -oxidation of alkyl chain and cleavage of the benzene ring require molecular oxygen, therefore under anaerobic conditions the degradation *via* these pathways is unlikely (4). As a result, the readily biodegradation of

LAS under aerobic conditions was confirmed, but LAS biodegradation under anaerobic conditions has not been proved till now. The biodegradability of LAS under anaerobic conditions has been directly investigated in few studies. In some of them, it was indicated that LAS decomposition could undergo anaerobically, if preceded by a period of aerobic exposure. The scientists suggested that the aerobic exposure allowed for  $\omega$ -oxidation of the terminal carbon of alkyl chain. The initial oxidative attack is the only step that requires molecular oxygen. Once formed, the sulphophenyl carboxylates can be biodegraded *via*  $\beta$ -oxidation and aromatic ring reduction and cleavage under strictly anaerobic conditions. Angelidaki et al. (2000) found that 14-25% of LAS fed into the anaerobic digester was transformed and this transformation was limited by bioavailability of the surfactant due to the sorption and toxic effects caused by elevated surfactant concentrations (5).

Commercial LAS usually contains dialkyltetralin sulphonates (DATS). DATS can be produced during the synthesis of linear alkylbenzene (LAB), followed by sulphonation, where LAB is converted to LAS. DATS structure is more complex than LAS due to the formation of *cis/trans* isomers. DATS are biodegraded to the intermediates called tetralin sulphonate carboxylates (DATSI). Moreover, LAS are generally degraded more rapidly than DATS (6).

Alkyl ether sulphates (AES) are readily biodegradable under aerobic as well anaerobic conditions (3). However, the literature data concerning the biodegradation pathways are limited. Microbiological decomposition of AES is generally believed to start with an oxidative conversion of one of the terminal methyl group into the carboxylic group ( $\omega$ -oxidation), and then to be followed by  $\beta$ -oxidation. The ethoxylate chain is shortened as a result of  $\omega$ -oxidation, too.

The half-lives estimated for LAS varied from 1 to even 87 days in the terrestrial environment, depending on the initial concentration, adaptation of the microorganisms, bioavailability and soil properties (2,7-8). Several studies concerning the effect of LAS on the germination or growth of the terrestrial plants in the hydroponic culture reported that LAS was not toxic at the concentrations of 5-10 mg L<sup>-1</sup> and toxic at the concentrations of 10-40 000 mg L<sup>-1</sup> (9). Taking the influence of LAS on the terrestrial organisms in soil into account, it occurred that the adverse effect started at 10-50 mg kg<sup>-1</sup> approximately for microorganisms and at 90 mg kg<sup>-1</sup> approximately for plants (10-12). Alkyl ether sulphates, in contrast to linear alkylbenzene sulphonates, were very seldom an object of investigations. Also, the data concerning the ecotoxicity of LAS biodegradation products are limited.

The aim of this work was to evaluate the influence of wastewater effluent containing different amount of LAS or AES and their potential biodegradation products on seed germination and root length of the selected terrestrial plants. The wastewater came from the lab-scale activated sludge system and contained from 0.5 to 58 mg L<sup>-1</sup> of anionic surfactants expressed as methylene blue active substances (MBAS). At the same time, phytotoxicity of the water solutions of the tested anionic surfactants was investigated. The bioassays with the use of four plants: two mono-

cotyledonous *Secale cereale* and *Triticum aestivum* and two dicotyledonous *Sinapsis alba* and *Lepidium sativum* were performed.

## 2. Materials and methods

### 2.1. The characteristics of the tested materials

The following anionic surfactants were tested: sodium alkylbenzene sulphonate  $C_{10-13}H_{21-27}C_6H_4SO_3Na$  (A2), representing linear alkylbenzene sulphonates (LAS), and sodium alkyltrioxyethylene sulphate  $C_{12-14}H_{25-29}O(C_2H_4O)_3SO_3Na$  (A3), representing alkyl ether sulphates (AES).

Experimental materials were the treated wastewater containing one of the two tested anionics as well as the potential products of its microbiological decomposition. The wastewater came from the lab-scale biodegradation experiments performed with the use of activated sludge in the continuous flow system simulating the biological part of the wastewater treatment plant. The biodegradation processes were conducted at ambient temperature ( $22 \pm 1^\circ C$ ) and constant aeration flow rate. The experimental setup was precisely described elsewhere (13). The influent was the synthetic wastewater containing one of the tested anionic surfactants at the level of  $250 \text{ mg MBAS L}^{-1}$ . The biodegradation experiments were carried out at four different volumetric loading rates of anionics: 6.6, 15, 25, 56  $\text{mg MBAS L}^{-1} \text{ h}^{-1}$ . The samples of the purified wastewater were taken when at least five residence times passed and the steady state in the chamber was achieved. The concentrations of anionics in the treated wastewater varied from 0.5 to 58  $\text{mg MBAS L}^{-1}$ . In Table 1, the average concentration of anionics in the effluent obtained in each biodegradation experiment are shown.

Table 1

Working conditions of biodegradation processes of A2 and A3 in the activated sludge system

Run no.	Tested anionic	Volumetric loading rate $\text{mg MBAS L}^{-1} \text{ h}^{-1}$	Concentration of anionic in the effluent $\text{mg MBAS L}^{-1}$
1	A2	6.6	1
2	A2	15	7
3	A2	25	10
4	A2	56	58
5	A3	6.6	0.5
6	A3	15	2
7	A3	25	4
8	A3	56	36

At the same time, the seed germination tests towards the water solutions of anionics A2 and A3 were performed, too. These measurements aimed at the estimation of the effect, which the anionic surfactant alone (without its potential biodegradation products) exerts on the tested plants. The seeds of terrestrial plants were exposed to sodium alkylbenzene sulphonate or sodium alkyltrioxyethylene sulphate at the concentrations adequate to their values in the effluent (Table 1).

## 2.2. Analytical procedure

The concentration of anionic surfactants was determined by the methylene blue method and expressed as Methylene Blue Active Substances (MBAS) (14). Anionics form ion pairs with methylene blue that are extracted by chloroform and determined spectrophotometrically at 652 nm.

## 2.3. Seed germination assay

The seed germination tests were conducted with the use of four plants: mustard (*Sinapsis alba*), cress (*Lepidium sativum*), rye (*Secale cereale*) and wheat (*Triticum aestivum*). Seeds of all tested plants were purchased from the garden material stores. Before being used for bioassays, their germination potential was examined at  $25 \pm 1^\circ\text{C}$  in darkness. Germination level above 90% was obtained. It guaranteed viability of the seeds. The undamaged seeds of almost identical size were employed in the bioassays.

The seed germination assays were carried out on a filter paper in Petri dishes containing 5 ml of the appropriate solution. Two different types of solutions were applied. The first one was the treated wastewater, which came from the lab-scale biodegradation experiments and contained one of the tested anionic surfactants together with its potential biodegradation products. The second one was the water solution of each of the investigated anionics. The short description of the tested materials is presented above (The characteristics of the tested materials). Also, the control tests with distilled water were performed. The bioassays were conducted for 48 h at  $25 \pm 1^\circ\text{C}$  in darkness. After that time, the number of germinated seeds as well as root length were measured. The tests were performed in four replications for each plant and for each of two tested materials. The conditions of bioassays are summarised in Table 2.

Table 2

## The summary of seed germination test conditions

Test	Description
Test type	static
Test species	<i>Sinapsis alba</i> , <i>Lepidium sativum</i> , <i>Secale cereale</i> , <i>Triticum aestivum</i>
Pretreatment	No
Temperature	25 ± 2°C
Light	No
Test vessel	100 × 10 mm Petri dish, Whatman N°1 filter paper
Solution volume	5 ml
Number of seeds	10 per dish
Replicates	four
Control	distilled water
Test duration	48 h
Endpoint	Seed germination rate (primary root 5 mm or more)

The percentage of relative seed germination (RSG), relative root growth (RRG) and the germination index (GI) were calculated according to (15). RSG is the ratio of a number of seeds germinated in the test sample (with surfactant) to the number of seeds germinated in the control, while RRG is the ratio of mean root length in the test sample to mean root length in the control. Germination index (GI) is defined as follows:

$$GI(\%) = \frac{RSG \cdot RRG}{100}$$

### 3. Results and discussion

#### 3.1. Phytotoxic effect of treated wastewater containing sodium alkylbenzene sulphonate (A2)

Figures 1 and 2 present the comparison of the germination index obtained within the seed germination tests performed towards different samples of the processed wastewater containing A2 and water solutions of A2. It occurred that for cereals at volumetric loading rate from 6.6 to 25 mg MBAS L<sup>-1</sup>h<sup>-1</sup> (run no. 1-3), the germination index varied from 90 to 110% (Fig. 1). The concentration of MBAS in the treated wastewater was relatively low and did not exceed 10 mg MBAS L<sup>-1</sup>. GI calculated for A2 water solutions was also about 100%. Neither A2 nor the potential products of its biodegradation exerted the phytotoxic effect on the terrestrial plants in these conditions. However, the higher A2 concentration, at the level of 58 mg MBAS L<sup>-1</sup>, the

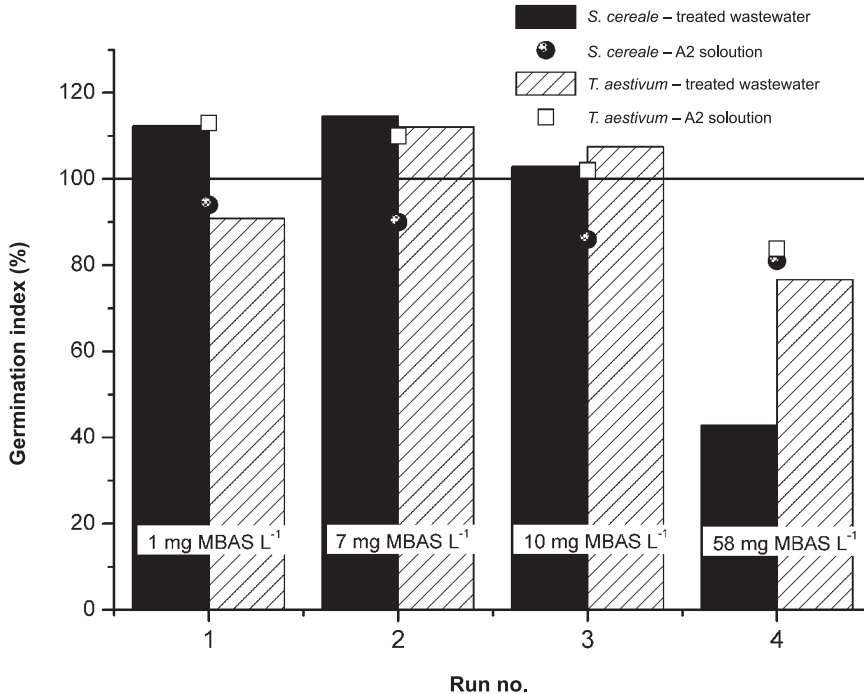


Fig. 1. The comparison of germination index (GI) calculated for cereals towards A2.

treated wastewater inhibited seed germination for both tested cereals. GI was equal to 76.5% towards *Triticum aestivum* and 43% towards *Secale cereale*. At the same time GI estimated for the water solution of A2 was about 83% for *Triticum aestivum* and 80% for *Secale cereale*. The biodegradation products of A2 could cause this difference, which is especially well seen in the bioassays with the rye.

The germination index calculated for the dicotyledonous plants (mustard and cress) towards the wastewater contaminated with A2 gradually decreased with an increase of volumetric loading rate and A2 concentration (Fig. 2). At two runs, no. 1 and no. 2, GI for *Lepidium sativum* was significantly higher than for *Sinapsis alba*. In spite of the fact that both plants belong to the same family *Cruciferae*, mustard occurred to be more sensitive to anionics than cress. The germination index of *Sinapsis alba* decreased from 86% to even 25%, while in the bioassays with *Lepidium sativum* it decreased from 165 to 30%. These results indicated that the presence of A2 and its potential biodegradation products inhibited the germination of some dicotyledonous plants starting already with the concentration of 10 mg MBAS L<sup>-1</sup> (Fig. 2).

It was also observed that the difference between the values of GI obtained within the tests towards the treated wastewater containing A2 and the water solu-

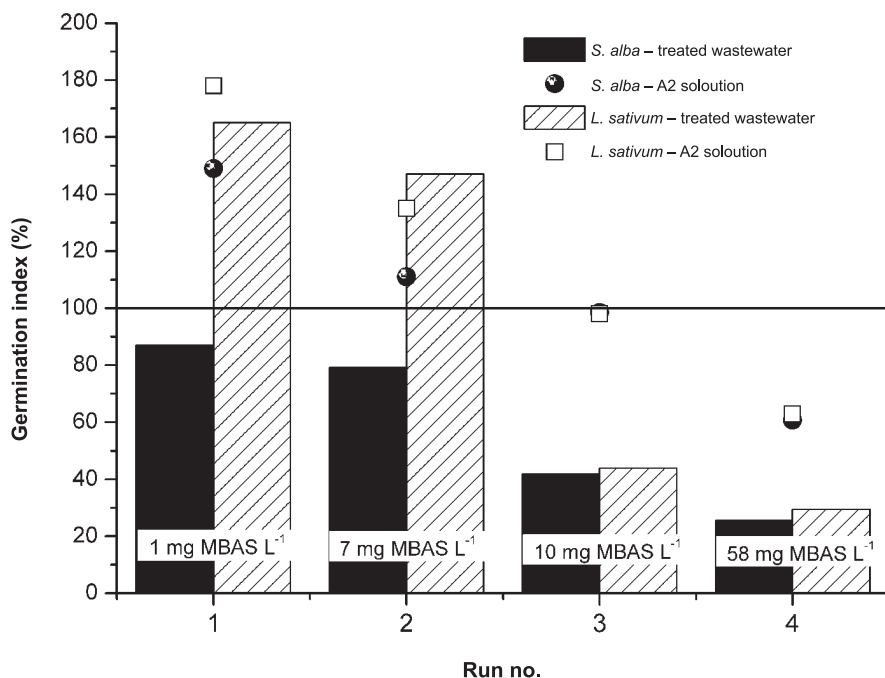


Fig. 2. The comparison of germination index (GI) calculated for the rapidly growing plants towards A2.

tion of A2 was higher for the rapidly growing plants (mustard and cress) than for cereals (compare Figures 1 and 2). In runs no. 3 and no. 4, the germination index calculated for the rapidly growing plants was about twice higher for the water solution of A2 than GI determined for the treated wastewater containing A2 at the same amount. It indicates that the potential products of biodegradation of linear alkylbenzene sulphonates can exert the inhibition effect on seed germination of some dicotyledonous plants. At the highest tested A2 concentration (58 mg MBAS L<sup>-1</sup>) in water solution, the inhibition of seed germination of both rapidly growing plants was also relatively high and GI was at the level of 60%.

Klopper and Sams (1996) found that the minimum values of no-observed effect concentrations (NOEC) estimated for LAS towards different terrestrial plants varied from 16 to 27 mg kg<sup>-1</sup> (12). Mieure et al. (1990) reported that the lowest observed effect concentrations estimated for LAS within the phytotoxicity studies were at the level of 10 mg L<sup>-1</sup> (9). In our study, the inhibition of seed germination was also observed at concentration equal to 10 mg MBAS L<sup>-1</sup> with regard to the rapidly growing plants. Nevertheless, taking the cereals into account, the inhibition effect was observed only at the highest concentration tested (58 mg MBAS L<sup>-1</sup>).

Klopper and Sams (1996) also denounced that one of the lowest EC50 found in literature for terrestrial plants exposed to LAS was 50 mg kg<sup>-1</sup> towards *Avena sativa*

and mustard (*Sinapsis alba*) (12). Their observations, proving that *Sinapsis alba* is very sensitive to linear alkylbenzene sulphonates exposure, were confirmed by the results presented in this study (compare Figures 1 and 2).

### 3.2. Phytotoxic effect of treated wastewater containing sodium alkyltrioxyethylene sulphate (A3)

In Fig. 3 the values of the germination index for cereals towards the wastewater with A3 as well as the water solution of A3 are presented. Generally, the germination index slightly decreased with the increase of A3 concentration in the treated wastewater or the water solution. In case of *Triticum aestivum*, the inhibition of seed germination was rather weak because the germination index decreased from about 115 to 75% within the assays towards the treated wastewater containing A3. At the same time, the value of germination index estimated for the second cereal *Secale cereale* was 49% in run no. 8, when the concentration of A3 was equal to 36 mg MBAS L<sup>-1</sup>. Addi-

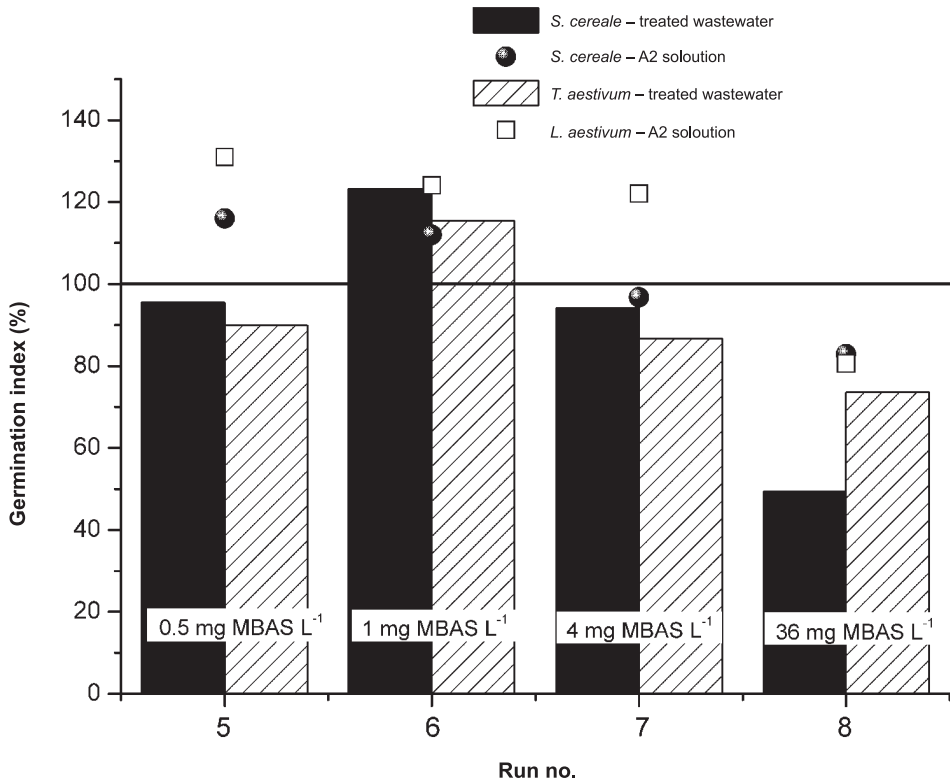


Fig. 3. The comparison of germination index (GI) calculated for cereals towards A3.

tionally, it was observed that the GI calculated for the water solutions of A3 was usually higher than in the wastewater containing the same amount of A3. In comparison to the tests with A2, these differences were seen not only at higher volumetric loading rates. It suggested that the potential biodegradation products of A3 could also inhibit seed germination. Due to the presence of ethoxylate groups in A3 molecule, its biodegradation process is not just a simple  $\beta$ -oxidation leading to simple organic acids, but there is a possibility to form more complicated organic compounds containing oxygen. One must be also aware that AES was more easily removed from wastewater in the activated sludge systems than LAS (13).

Comparing the germination index determined for the rapidly growing plants, significant difference between the value of GI obtained for *Lepidium sativum* and *Sinapsis alba* was noticed (Fig. 4). Taking the results of bioassays with *Lepidium sativum* into account, the phytotoxicity effect was observed only for the treated wastewater containing the highest amount of A3 (run no. 8). In this case, GI was equal to 66.7%. At the same time, within the toxicity tests performed with *Sinapsis alba*, the germination index decreased gradually from about 90 to 36%. The lowest value of GI (36%) estimated in run no. 8 for *Sinapsis alba* indicated that over half of the tested seeds exposed to the treated wastewater with A3 were not able to germinate. These results also confirmed that *Sinapsis alba* is more sensitive than *Lepidium*

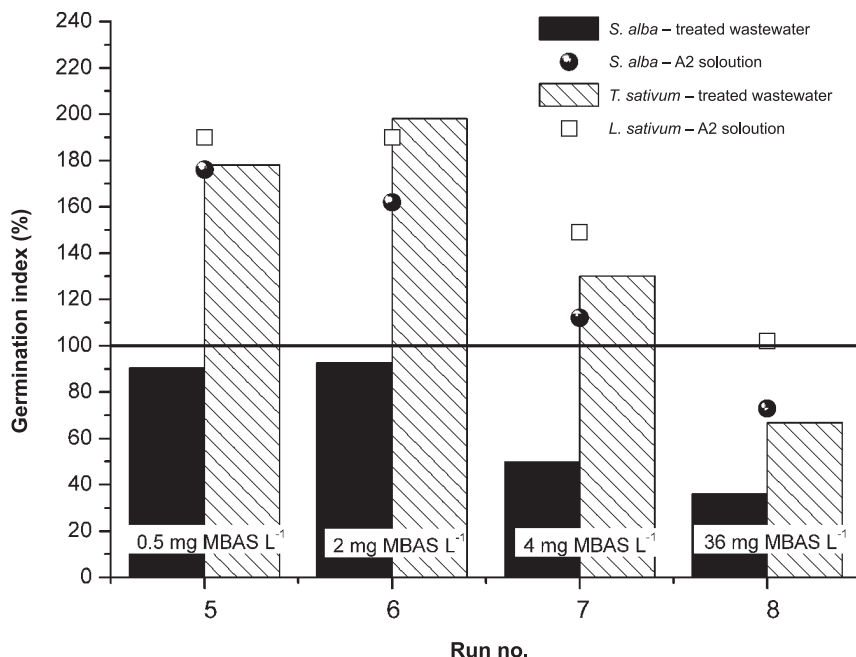


Fig. 4. The comparison of germination index (GI) calculated for the rapidly growing plants towards A3.

*sativum* to anionic surfactants and could be used as a bioindicator within the tests aimed at the evaluation of anionics phytotoxicity.

#### 4. Conclusions

Both tested compounds, representing the most commonly used groups of anionics i.e. linear alkylbenzene sulphonates and alkyl ether sulphates, may exert an inhibitive effect on seed germination of the terrestrial plants. This effect was observed at the concentrations in the effluent from 10 mg MBAS L<sup>-1</sup> for sodium alkylbenzene sulphate and at the concentration above 30 mg MBAS L<sup>-1</sup> for sodium alkyltrioxyethylene sulphate. At the highest tested anionics concentrations in wastewater (36 or 58 mg MBAS L<sup>-1</sup>), the germination index was, dependently on the plant, in the range from 25 to 76%. It indicates how important the proper wastewater treatment is and not exceeding the concentration limit for anionics in the effluent (5 mg L<sup>-1</sup>). In spite of the fact that the highest concentrations are rarely found in the effluent, they are often reported in the sewage sludge, which is willingly utilised as a fertilizer.

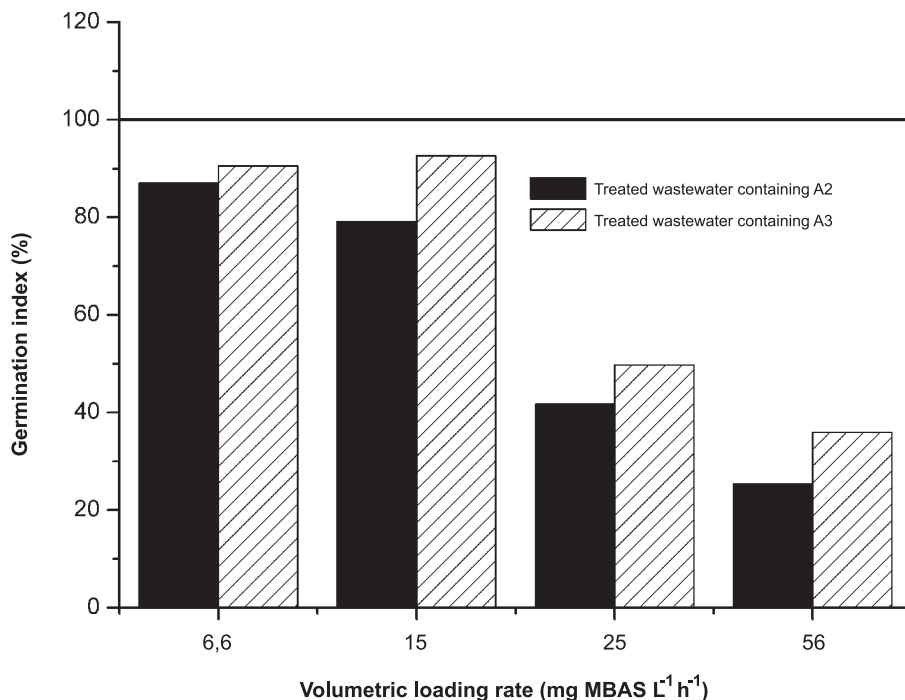


Fig. 5. The comparison of germination index (GI) calculated for mustard (*Sinapsis alba*) towards A2 and A3.

In the water solutions of anionics, the values of GI obtained at 36 or 58 mg MBAS L<sup>-1</sup> were higher than in the effluent and ranged between 60 and 100%, which means that the presence of biodegradation products of anionics in the wastewater might enhance their phytotoxicity.

In order to compare the toxic effect of A2 and A3, the GI values of bioassays with *Sinapsis alba* are presented in Fig. 5. Nevertheless, the direct comparison of phytotoxicity of A2 and A3 is difficult because of different concentrations of these substances in the treated wastewater. The previously obtained results indicated that A2 could exert stronger phytotoxic effect than A3 (16). The performed experiments confirmed that phytotoxicity of chemicals differs dependently on the plant used in the bioassays. Therefore, in order to investigate properly the ecotoxicity of the compounds with regard to plants, at least three or four plants should be tested.

Among the tested plants, mustard (*Sinapsis alba*) appeared to be so sensitive to anionic surfactants exposure that it could be used as a bioindicator within the tests concerning this group of synthetic surfactants.

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