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Effects of five sulphonamides on duckweed (*Lemna minor*) after prolonged exposure time and their dependency on photoradiation

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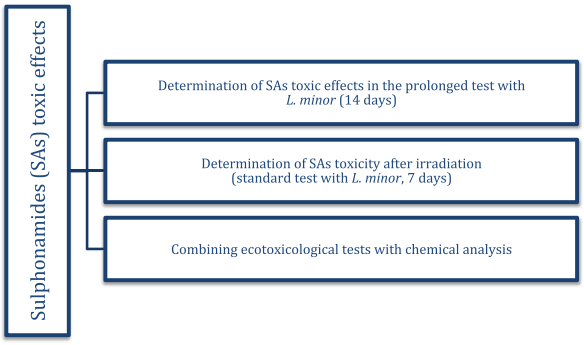
HIGHLIGHTS

- Toxicity of five SAs to duckweed after prolonged exposure times was determined.
- Shift in the order of toxicity of the SAs was observed in the prolonged test.
- After irradiation concentration and toxicity of all the SAs decreased within time
- Potentially produced photodegradation products less toxic than native forms
- SAs toxicity in the prolonged exposure might be explained by different mode of action.

GRAPHICAL ABSTRACT



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ABSTRACT

Sulphonamides (SAs) are one of the most commonly used veterinary drugs and therefore their residues are regularly found in the environment. So far scientific attention has mostly been paid to the evaluation of their acute ecotoxicological effects with data on long-term effects for non-target organisms still largely missing. Therefore, the main aim of this study was to evaluate the potential toxicities of five sulphonamides to duckweed (*Lemna minor*) after prolonged exposure time (14 days). To elucidate whether their phytotoxic effects result from potential photodegradation products, the toxicity of standard solutions of selected sulphonamides was also investigated in a standard 7-day test but after irradiation (by keeping them under the test conditions) for the selected time (after 7 and 14 days). The ecotoxicological tests were accompanied by chemical analyses to be able to link the observed effects to the concentrations and nature of the exposed compounds. The results showed a shift in the toxicity of SAs: a strong decrease in toxicity for the two most toxic sulphonamides (sulphamethoxazole and sulphadimethoxine) and a slight increase in toxicity for three other SAs (sulphadimidine, sulphathiazole, sulphamerazine) in the prolonged test. However, a decrease in the toxicity and concentration of all the SAs was observed when stock solutions were irradiated prior to the toxicity experiment, which suggests that the observed effects towards *L. minor* of five SAs in the prolonged test cannot be directly associated with the degradation of these compounds under the test conditions but with their different mode of toxic action towards these organisms.

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1. Introduction

Sulphonamides (SAs), for more than fifty years, have been widely consumed antibiotics in both human and veterinary medicine mainly due to their low cost and broad spectrum of activity (Sukul and Spiteller, 2006). A vast number of these pharmaceuticals are not completely metabolized, hence a high proportion of them are excreted unchanged in feces and urine and enter the environment leading to their ubiquitous occurrence in aquatic and terrestrial environments (at the ppt or ppb level) (Baran et al., 2011; García-Galán et al., 2008; Kümmerer, 2009; Sukul and Spiteller, 2006), with maximal sulphonamide concentrations presented in Table 1A (Appendix). Moreover, based on the conducted research (according to the guidelines OECD 301, ISO 11734, ISO 9408), it was proved that SAs are neither biodegradable in aerobic nor anaerobic conditions (Al-Ahmad et al., 1999; Alexy et al., 2004; Gartsier et al., 2007; Ingerslev and Halling-Sørensen, 2000), which is in agreement with the fact that they are detectable in effluent waters from WWTPs at concentrations even up to 3 $\mu g\ L^{-1}$ (Senta et al., 2008) (Table 1A). They are also resistant to hydrolysis (Białk-Bielińska et al., 2012). Available literature data confirms that SAs are only slightly susceptible to direct photolysis (as their maximum absorbance of UV light is in the range of 260–280 nm) in aquatic or terrestrial environments (Boreen et al., 2004, 2003; Wolters and Steffens, 2005). In contrast, it has been proved that these chemicals might be eliminated from the environment *via* indirect photolysis initiated by natural photosensitizers, which are widely present in aquatic environments (Niu et al., 2013; Trovó et al., 2009; Wolters and Steffens, 2005). However, it must be highlighted that the obtained results indicate that this process does not lead to the complete mineralization of SAs, which confirms that new degradation products are produced that can have even higher toxicity than the parent compounds (Boreen et al., 2004; Niu et al., 2013; Trovó et al., 2009; Wolters and Steffens, 2005). For these reasons, as well as due to the fact that they are continuously released into the environment, the residues of SAs and their transformation products might be present in the environment for long periods of time, hence they might have an impact on the living organisms present there.

The knowledge of the potential effects of SAs on the environment, however, is still very limited as shown in papers (Białk-Bielińska et al., 2014, 2013; García-Galán et al., 2009; Park and Choi, 2008; Santos et al., 2010). Although these data indicate a possible negative impact on organisms in different environmental compartments, data evaluating a broader selection of SAs and also considering more complex scenarios such as long-term effects are scarce (Białk-Bielińska et al., 2014, 2013; Santos et al., 2010). The available literature data on the chronic toxicity of SAs have been reviewed in detail in our previous paper (Białk-Bielińska et al., 2013), however, the most important and more recent information on this topic has been summarized and presented in Fig. 1. According to these data and information presented by Bártíková et al. (2016), who just recently reviewed the available data on the toxicity of veterinary drugs to plants, there is no information about the long-term effects of these compounds to higher plants.

Therefore, the main aim of this study was to evaluate the biological effects of five selected sulphonamides (sulphadimidine (SDIMID), sulphathiazole (STZ), sulphamerazine (SMR), sulphamethoxazole (SMX), sulphadimethoxine (SDM)) to the duckweed *Lemna minor* in a prolonged version of the standard (7 days) test with a total exposure time of 14 days. The selection rational for the test organisms and test compounds was based on data gained in a previous study (Białk-Bielińska et al., 2011), where the distinct phytotoxicity of twelve sulphonamides to duckweed was observed. Therefore, these five SAs were selected based on their differing toxicity (low toxicity to high toxicity) to be able to detect the potential toxicity shifts in a broader spectrum of activity of these compounds. Moreover, the selected SAs belong also to one of the most commonly detected in environmental samples; hence they might be of the highest concern while evaluating their effects on organisms living in the environment. Furthermore, these representatives are known to be susceptible to photolysis (for example, the photolysis rates of SMX at initial concentrations of 1.0, 5.0 and 10 mg L^{-1} determined by Niu et al. (2013) were: 1.8×10^{-2} and $1.4 \times 10^{-2}\ min^{-1}$ and $3.9 \times 10^{-3}\ min^{-1}$ with direct photolysis half-lives ($t_{1/2}$) of 39, 50 and 178 min, respectively). For these reasons, we proposed to verify two different hypotheses in our study:

i) SAs undergo photodegradation under the test conditions of the *L. minor* test, which might influence the assessment of their ecotoxicity to this representative of higher plants;
ii) SAs are toxic to *L. minor* in the standard 7-day test, but after the prolongation of the exposure time their toxicity could decrease due to suspected photodegradation and hence the lower concentration of bioavailable fraction of native forms of SAs, or increase due to the production of photodegradation products more toxic to *L. minor*.

In order to verify the proposed hypotheses, besides performing the prolonged (14 days) test with *L. minor*, additional 7-day standard tests were performed with spiked SA solutions which had been artificially aged (irradiated) for 7 and 14 days under test conditions. All ecotoxicological tests were accompanied by chemical analyses using HPLC-UV and LC-MS/MS techniques to be able to link the observed (toxic) effects to the actual concentrations and nature of the exposed compounds.

2. Experimental

In general, in this study two sub-sets of tests were performed:

i) the analysis of the toxic potential of sulphadimidine (SDIMID), sulphathiazole (STZ), sulphamerazine (SMR), sulphamethoxazole (SMX), sulphadimethoxine (SDM) (Table 2A) in the prolonged test version (14 days) with *L. minor*;
ii) the evaluation of the toxic potential of these compounds in the 7-day test after ageing them in an environmental chamber for 7 and 14 days.

2.1. Chemicals and reagents

Standards of STZ, SMR, SMX, SDM, SA and SN as well as acetonitrile (ACN) and methanol (MeOH) - all HPLC grade, and salts used for the test media, were purchased from Sigma–Aldrich (Steinheim, Germany). SDIMID was obtained from Serva (Heidelberg, Germany).

Standard stock solutions (1000 $\mu g\ L^{-1}$) of SAs were prepared by dissolving each compound in MeOH and were stored at −18 °C in the dark. All stock solutions were reconstituted in the test medium after evaporating the MeOH in a stream of nitrogen.

2.2. The experiment set-up of the prolonged test (14 days) towards L. minor

For each of the selected SAs five different concentrations were tested in order to evaluate their effects towards *L. minor* after prolonged exposure time. These concentrations were selected based on the results obtained in the 7-day standard test (Białk-Bielińska et al., 2011), and their concentration ranges are presented in Table 1. Prior to the exposure (day zero of the test) stock solutions of each sulphonamide at five different concentrations were prepared in the test medium (Steinberg medium, pH 5.5 ± 0.2). The prolonged growth inhibitory test with *L. minor* was performed according to the modified procedure of OECD 221 (2006), as there is no such reference/standardized procedure for the evaluation of long-term effects on duckweed available in the literature. For this reason also a positive control with a reference substance (3,5-dichlorophenol, 3,5-DCP), recommenced in the procedure OECD 221 (2006) for the standard test, was investigated. It consisted of the ecotoxicity evaluation of this compound also at five different concentrations (30, 10, 5, 1 and 0.5 mg L^{-1}) prepared in the test medium.

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