PERGAMON

Chemosphere 40 (2000) 835–838

CHEMOSPHERE

Surfactant (Genapol OX-80) toxicity to *Selenastrum capricornutum*

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Received 3 February 1999; accepted 25 June 1999

Abstract

A non-ionic surfactant (Genapol OX-80) was proposed as a means of reducing destructive crayfish activity in rice. In order to study the toxicity of this product on algae, a test in accordance to the ISO 8692 protocol (1989) was performed. The growth inhibiting effect of the pure formulation of Genapol OX-80 was studied on *Selenastrum capricornutum* in the concentration range of 0.01–1.0 mg/l. The result indicated a 72 h EC_{50}-value of 0.5 mg/l. The suggested field concentration of approximately 50 mg/l is therefore two orders of magnitude above the algal EC_{50}-value. From our findings it is expected that the impact on the rice field algae will not be negligible.

Keywords: Algae; EC_{50}; Crayfish; Rice

1. Introduction

The Louisiana red swamp crayfish, *Procambarus clarkii*, is an exotic species in Spain and Portugal. In both countries, soon after introduction, crayfish populations increased without control, invading most of the rice fields and other wetland areas (Correia, 1993). To prevent damage to rice production, farmers have repeatedly tried to eradicate crayfish populations by means of xenobiotic chemicals. Such methods proved to be ineffective and had a devastating impact on useful species (Velez, 1980; Roqueplo and Hureaux, 1989). A better solution is the use of crayfish as a food resource, which would control the size of crayfish populations, with a simultaneous socio-economical profit (Chien and Avault, 1979; Huner, 1988; Caño and Ocete, 1994; Huner et al., 1992; Huner, 1995).

In this context, the use of a non-ionic surfactant was proposed as a means of reducing destructive crayfish activity in rice fields (Anastácio et al., 1995; Fonseca et al., 1996). The use of Genapol OX-80 could bring several advantages (Jørgensen et al., 1997):

1. Crayfish would not be killed and could be harvested at a later stage.
2. Bioaccumulation is an important environmental concern and is expected to be significantly lower when using Genapol OX-80 in comparison with the use of xenobiotics such as dimethoate or parathion.
3. The toxicity of Genapol OX-80 for several animal species is relatively small (Cabral et al., 1996), especially in comparison with pesticides commonly used in rice fields in order to control crayfish populations (Chang and Lange, 1967; Brown and Avault, 1975; Baker, 1975).

Although, studies on the toxicity of Genapol OX-80 to several animals have been performed, there is still the

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0045-6535/00/$ - see front matter
PII: S0045-6535(99)00275-1

need for knowledge on the toxicity to other organisms common in the rice fields. Some algae (particularly filamentous) can be used directly as food for crayfish (Sanguaruang, 1988; Momot, 1995; Ilhéu and Bernardo, 1967), or indirectly, i.e., they can sustain a diverse macroinvertebrate community. Moreover, the algae are sometimes responsible for oxygen depletion during the night, though releasing large quantities of oxygen during daytime. Algal crash, especially when occurring after severe algal blooms, can reduce oxygen and therefore water quality (Sfriso et al., 1990). For a rice-crayfish double-cropping system such as the one being simulated, water quality is fundamental. Actually, maintaining reasonable dissolved oxygen concentrations is one of the major concerns of the aquaculturists in general (Boyd et al., 1978), and the crayfish farmers in particular (Huner, 1988; Huner and Barr, 1991). For these reasons we decided on testing the toxicity of Genapol OX-80 to an algae commonly used in standard toxicity tests. Our purpose is to determine if a strong impact on algal populations in the rice field is predictable.

2. Materials and methods

2.1. Chemicals

Genapol OX-80, a non-ionic surfactant, was obtained from Hoechst Portuguesa and used as model compound during this study. The active ingredient of Genapol OX-80 is a mixture of polyglycol ethers of fatty alcohols – CH_3–$(CH_2)_{12\text{-}15}$–$O(CH_2CH_2O)H$. The model compound was tested in the following concentrations: 0.01, 0.05, 0.1, 0.5 and 1.0 mg/l, in the pure formulation, i.e., without the use of additional solvents.

2.2. Algal culture

Non-axenic uniculture of the test organism *Selenastrum capricornutum* was obtained from the Norwegian Institute of Water Research culture collection in Oslo, Norway, and maintained in culture. Inocula were taken from pre-cultures setup 1–3 days before the experiment and propagated under the same test conditions as the subsequent test. The initial cell densities were adjusted to approximately 10^4 cells/ml.

2.3. Toxicity test

The toxicity tests were performed in open 250 ml Erlenmeyer flasks covered with laboratory film under ISO standard 8692 (ISO 8692, 1989) conditions. Dilution water for media preparation was of ultrapure quality produced from distilled water by means of Milli-Q apparatus. Both control and test flasks were inoculated so an initial concentration of 10^4 cells/ml was reached. Six replicates of controls (untreated) and three replicates of each test concentration were applied. All the flasks for the toxicity test were incubated at 22 ± 1°C and exposed under a shaking procedure to white fluorescent light with an intensity of 8 Klux to ensure exponential algal growth. After a test period of three days (72 h), the algal density was quantified by using a modified version of the whole water extract fluorescence method described by Mayer et al. (1997). Extracts were prepared by mixing 1/3 culture sample (final v/v) with 2/3 ethanol (99.9%) in brown glass containers to avoid the extracted chlorophyll being degraded by light. As a further precaution the containers were covered with aluminium foil. The mixture was left to extract for 24 h while being continuously shaken. The fluorescence of the chlorophyll extract was measured on a Perkin Elmer Luminescence Spectrometer LS50B with an excitation wavelength of 430 nm and an emission wavelength of 671 nm. Triplicate measurements were made on each sample. pH in all samples was measured using a glass electrode.

2.4. Calculations

The results of the toxicity test were quantified in terms of average growth rates calculated from chlorophyll measurements. Inhibition was calculated from relative average growth rates as: $I = 1 - \mu_{\text{average}}/\mu_{\text{average, control}}$. EC_{50}-value was determined by weighted non-linear regression analysis directly on the data using the Weibull equation to describe the concentration-response relationship (Nyholm et al., 1992). A regression program developed by Andersen (1994) which calculates confidence intervals by proper inverse estimations and which also takes into account the covariance with the control response was used.

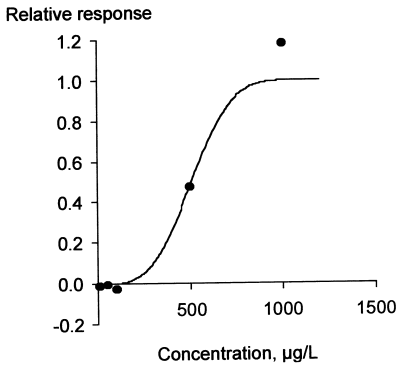

Fig. 1. Concentration–response curve of the non-ionic surfactant Genapol OX-80, on *Selenastrum capricornutum*.

Table 1
Acute toxicity of the non-ionic surfactant Genapol OX-80 to several organisms, ranked with decreasing toxicity

Organism	Type of test	Value (mg/l)	Reference
S. capricornutum (planktonic algae)	72 h EC_{50}	0.5[a]	Present study
Chironomus riparius (mosquito larvae)	96 h LC_{50}	2.8	Cabral (1999)
Gambusia holbrooki (small fish)	96 h LC_{50}	2.9	Cabral et al. (1996)
Daphnia magna (small crustacean)	96 h LC_{50}	3.6	Cabral et al. (1996)
Physa acuta (gastropod)	96 h LC_{50}	8.0	Cabral et al. (1996)
P. clarkii (big crustacean)	96 h LC_{50}	140.9	Cabral et al. (1996)

[a] It may also be considered a chronic test due to the number of generations involved.

3. Results and discussion

The results indicated that the 72 h EC_{50}-value for *S. capricornutum* is of 0.509 mg/l (Fig. 1 and Table 1), with upper and lower confidence limits, respectively, of 0.510 and 0.507 mg/l. A minor positive effect on algal growth is apparent for the lowest concentrations tested.

Several authors refer the possibility of growth stimulating properties of surfactants (Lewis, 1990; Ernst et al., 1983; Parr and Norman, 1965). This might be an explanation for the positive effects on *S. capricornutum* observed for the lowest concentrations tested (Fig. 1).

The EC_{50}-values of non-ionic surfactants to *S. capricornutum* found in the literature range from 0.21 mg/l (Lewis and Hamm, 1986) to 50 mg/l (Yamane, 1984). Our value of 0.5 mg/l lies within this range, though it is probably quite low, compared to the majority of available EC_{50}-values; for a review of EC_{50}-values, see Lewis (1990). From Table 1 we can compare the effects of Genapol OX-80 on several aquatic organisms at different trophic levels. It is clear that *S. capricornutum*, one of the suggested reference algal species in the ISO guideline, is the most sensitive life form among the tested species. In fact, the target organism, i.e., *P. clarkii* is the least sensitive one, with a LC_{50} several orders of magnitude above the EC_{50}-value for the algae. Furthermore, it is worth mentioning that the algal toxicity test is performed over several generations. This means that the derived EC_{50}-value is considered chronic when compared to the rest of the values in Table 1.

The algal EC_{50}-value of 0.5 mg/l presented in this study is two orders of magnitude lower than the suggested Genapol OX-80 field concentration of 50 mg/l. Unfortunately, for several reasons, extrapolation to predict field results on algae is troublesome. Several authors refer that the surfactant field effects are usually lower than the laboratory effects (Lewis, 1990; Lewis and Hamm, 1986). Moreover, the sensitivity to a surfactant may differ by three orders of magnitude depending on the algae species being tested. This is very clear from Yamane et al. (1984) in which they end their paper with the statement "The effects of surfactants on algae, therefore, must be regarded as being species specific". We should therefore notice that in the field the most abundant group are the filamentous green algae, particularly the *Pitophora* sp. (Anastácio et al., 1999). This is a considerably different species from *S. capricornutum* which is planktonic, therefore any conclusions about field effects should be taken with caution.

A considerable effort is being made to use standard toxicity measurements and we believe that this will justify the use of *S. capricornutum* instead of more common algae in the area. The toxicity of several surfactants to a certain algal species may differ by four orders of magnitude (Lewis, 1990). Therefore, the use of a single algae species for comparative purposes seems recommendable. Moreover, there are less algal toxicity studies of non-ionic surfactants compared to other types of surfactants (e.g., cationic and anionic) (Lewis, 1990). As a final remark, from our findings it is expected that the impact on the rice field algae will not be negligible with a field concentration of 50 mg/l. The hypothesis of the development of a new product should be considered if it is going to be used in field conditions.

Acknowledgements

This work was sponsored by the EU in the scope of the R&D project AIR 3-CT-94-2432 and also by JNICT and the PRAXIS XXI Program (Portugal).

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