Toxic and interactive toxic effects of agrochemical substances and copper on *Vibrio fischeri*

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Abstract

The toxic and interactive toxic effects of two agrochemicals (fosthiazate and metalaxyl-M) and copper were investigated in this study on the photobacterium *Vibrio fischeri*. The toxicities of all tested compounds were generally comparable. The order of toxicity was: copper > fosthiazate > metalaxyl-M. The interactive effects of fosthiazate and metalaxyl-M mixtures were found to be additive for most of the concentration combinations tested. For the binary mixtures of fosthiazate and copper the interactive effect was antagonistic for all concentration combinations tested. Both agrochemicals showed a similar toxic response, while the toxicity of copper increased significantly by the increase of exposure time and a steep increase of copper toxicity was observed with a small increase of its concentration.
Keywords: pesticides, agrochemicals, copper, bioassays, Vibrio fischeri, Microtox, interactive effects.

1 Introduction

The toxicity of various solid and liquid substances to aquatic life forms has started to be the subject of considerable research in the last decade. Quantification of the different compounds based on standard chemical analysis

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methods indicates the concentration of toxic compounds that released in the environment. Furthermore, the purpose of regulating acts issued by national and international authorities regarding the management and disposal of liquid and solid wastes to the environment is to protect both human health and ecosystem. However, most of these acts are relied on physicochemical analyses for the determination of physical and chemical parameters of waste or of receiving water body. As a result, the assessment of water quality contaminated with chemicals has been based on chemical analyses and the compliance or not of the measured concentrations with limit concentrations imposed by the legislation.

The toxicity tests in addition to chemical analysis can be used for the assessment of the direct effect of the compounds on the terrestrial and aquatic environment. Furthermore, chemical analysis methods do not take into consideration possible synergistic or antagonistic effects of the various compounds on aquatic life forms [1, 2]. Among the anthropogenic substances that are released in the environment, agrochemicals pose a serious risk of toxic effects on the ecosystem. Agrochemicals may be transported from the treated soils in aquatic environment by waterborne runoff or by direct deposition from the atmosphere [3]. Microtox test is among the widely used bioassays for the toxicity determination of various compounds, including agrochemical substances [3–6]. The aim of this study was the evaluation of toxic response of two agrochemicals and copper, when act alone or in combination, using the Microtox test.

2 Materials and methods

The agrochemicals examined for their toxic properties were: fosthiazate and metalaxyl-M using their commercial formulations of Nemathorin and Ridomil, respectively. The toxicity of copper was also evaluated using copper chloride dehydrate, ($CuCl_2{\cdot}2H_2O$) provided by J.T. Baker, Holland. The toxicity of agrochemicals and copper was evaluated using the bioluminescence bacteria *V. fischeri* (Microtox test) that were in freeze-dried form (SDI, USA) and activated prior to use by a reconstitution solution. Since *V. fischeri* is a marine organism, an adjustment of the osmotic pressure of the samples was applied to obtain samples with 2% salinity, using a concentrated salt solution (solution containing 22% NaCl in deionized water). The light emission of the test organisms obtained by their direct contact with the samples was measured using the Microtox 500 analyzer (SDI) within exposure times of 5, 15 and 30 min. The data processing was performed using the MicrotoxOmni software (SDI). The IC_{50} values (the tested compound concentration that caused 50% inhibition on the bioluminescence of *V. fischeri*; expressed as mg/L of fosthiazate, metalaxil-M or Cu) of the tested compounds were evaluated using the 45% basic test [7].

The interactive toxic effects between fosthiazate and copper or metalaxyl-M on *V. fischeri* were also investigated and the evaluation of the results was performed by statistical analysis. The concentrations of the tested compounds used were obtained from the IC_{50} estimation experiments of each compound. The theoretically expected effect of the binary mixtures was evaluated using a simple mathematical model based on the theory of probabilities [1]. According to this model, if P_1 is the inhibition caused by a certain concentration of chemical A_1 and P_2 the inhibition caused by a certain concentration of chemical A_2, then the theoretically expected additive inhibition P(E), when those concentrations of the two chemicals are applied together, is given by the following equation:

$$P(E) = P_1 + P_2 - P_1 \cdot P_2/100 \qquad (1)$$

The null hypotheses were that the observed values were higher or lower than the theoretically predicted values, for synergistic and antagonistic effects, respectively. The result was considered to be antagonistic or synergistic only if the observed effect was significantly lower or higher, respectively, than the theoretically predicted value at the 0.05 level of significance [8].

3 Results and discussion

The toxicities of the single agrochemicals and copper were first examined by applying several dilutions for each one of them and the IC_{50} value was evaluated as an endpoint. The IC_{50} values and the corresponding confidence ranges of the tested compounds, calculated using MicrotoxOmni software, are presented in Table 1, while the bioluminescence inhibition of *V. fischeri* caused by the tested compound concentrations are given in Figure 1 (dose response curves).

Table 1: IC_{50} values and the corresponding confidence ranges for the tested compounds on *V. fischeri*, for exposure time 5 and 30 min.

Compound	**5 min IC_{50}, mg/L (C.R)**	**30 min IC_{50}, mg/L (C.R)**
Fosthiazate (Nemathorin)	0.15 (0.13-0.18)	0.20 (0.17-0.25)
Metalaxyl-M (Ridomil)	0.57 (0.33-0.97)	0.88 (0.35-2.21)
Copper ($CuCl_2 \cdot H_2O$)	0.37 (0.32-0.44)	0.18 (0.17-0.19)

C.R.: confidence range

As it is shown in Table 1, the toxicity of both agrochemicals was almost constant within 5 and 30 min exposure time, while the toxicity of copper for 30 min exposure time was significantly higher than that of 5 min exposure time. The toxicities of copper and fosthiazate were generally comparable, while the toxicity of metalaxyl-M was slightly lower than those of the other two tested compounds. The toxicity of copper may be affected from chemical species formed by the hydrolysis reactions of metals and is mainly correlated to the free metal ion concentration [2]. Electrostatic interactions between ions presented in the solutions may cause a slow response of the bacteria. The diffusion of copper species through the cell membrane may be possibly delayed and the action might not be completed within a short exposure time (i.e. 5 or 15 min). The assessment of IC_{50} values of various pesticides for 5 and 15 min showed that in most cases their action was completed within 15 min of exposure time; however certain pesticides might present a longer time effect, indicating that the type of the chemical might affect the test organism by different ways [4, 9]. Furthermore, the dose response curves of fosthiazate and metalaxyl-M showed a similar pattern, while a steep increase of copper toxicity was observed with small increase of its concentration.

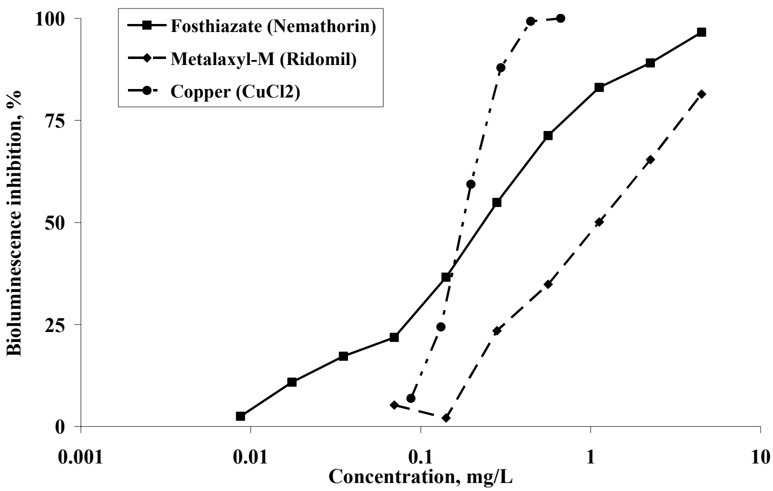

Figure 1: Bioluminescence inhibition of *V. fischeri* caused by the tested concentration of agrochemicals and copper, for exposure time 30 min (dose response curves).

After having tested the effects of the compounds alone on *V. fsicheri*, the interactive effect between fosthiazate and metalaxyl-M or copper were evaluated. The theoretical expected interactive effects, as calculated by Equation 1, and the observed effects for the combine action of fosthiazate and metalaxyl-M, as well as between fosthiazate and copper are illustrated in Figures 2 and 3, respectively.

The interactive effect between fosthiazate and metalaxyl-M was additive in most cases (Figure 2). For the concentration combinations B and G the observed bioluminescence inhibition was significantly lower than the corresponding theoretically expected, indicating an antagonistic action, while for the other concentration combination the effect was additive.

As shown in Figure 3, the observed bioluminescence inhibition of *V. fischeri* for the binary mixtures of fosthiazate and copper was significantly lower than the theoretically expected for all concentration combinations, indicating an antagonistic action. Similar results were found by Kungolos *et al.* [10] for the examination of interactive toxic effect of fosthiazate and copper on the crustacean *Daphnia magna*. The antagonistic action between fosthiazate and copper could be attributed to the presence of additives contained in commercial formulation of fosthiazate, that may potentially reduce the bioavailability of copper.

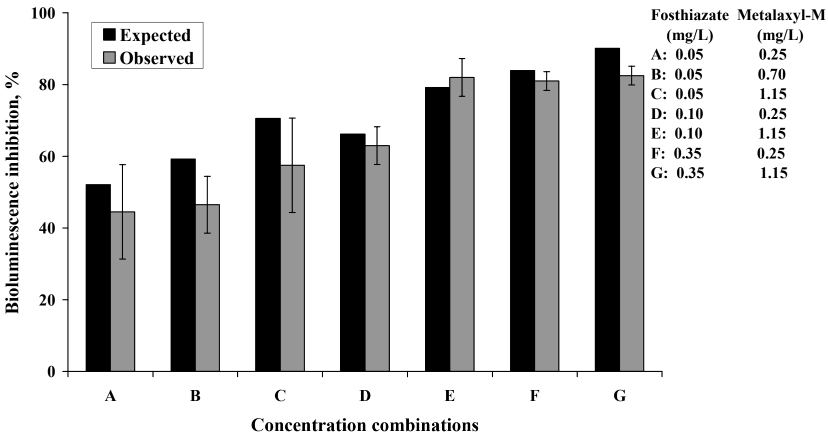

Figure 2: Comparison between theoretically expected and observed inhibitions for the combined effect of fosthiazate and metalaxyl-M on bioluminescence of *V. fischeri*.

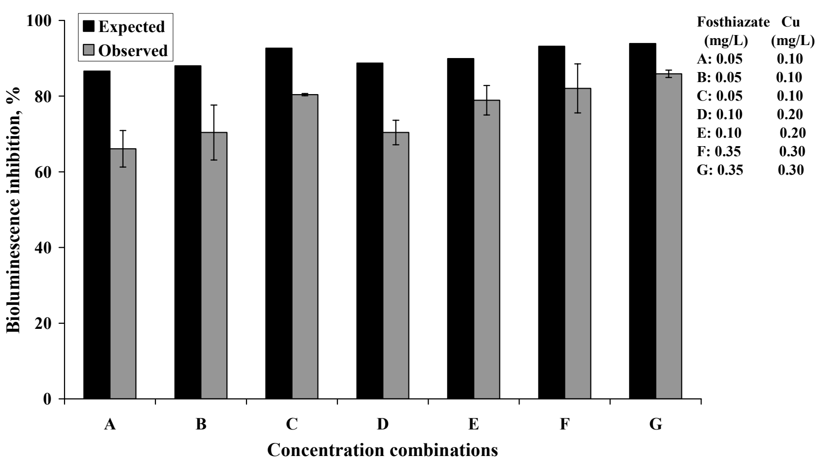

Figure 3: Comparison between theoretically expected and observed inhibitions for the combined effect of fosthiazate and copper on bioluminescence of *V. fischeri*.

4 Conclusions

The interactive effects of fosthiazate and metalaxyl-M mixtures on *V. fischeri* were found to be additive for most of the concentration combinations tested. For the binary mixtures of fosthiazate and copper the interactive effect was antagonistic for all concentration combinations tested. Both agrochemicals showed a similar toxic response; their toxicity was not significantly varied by the exposure time, while their toxicity was not sharply increased by the increase of concentration. On the other hand, the toxicity of copper increased significantly with the increase of exposure time and a steep increase of copper toxicity was observed with small increase of its concentration.

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