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Bioassay of Pyrethroid Insecticide Esfenvalerate Using Fractal Analysis of *Daphnia Magna* Motion.

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ABSTRACT

The article presents the data on the impact of pyrethroid insecticide esfenvalerate assessment at the concentration of 3 mcg • l^{-1} (the Russian standard of this pesticide content in water) on the nature and complexity of Daphniamagna movement. The analysis is performed using the system "TrackTox", implementing computer vision algorithms. It is shown that in the presence of esfenvalerate (at 30 min exposure to toxicant) the swimming activity stimulating response is observed for Daphnia. The statistically significant increase in the swimming speed of Daphnia takes place from 0.58 cm • s^{-1} in control group to 0.98 cm • s^{-1} in experimental group. Also, the complexity of Daphnia movement is changed. The complexity of movement was evaluated through fractal analysis of daphnia swimming trajectories before and after the introduction of pesticide. Using the method of cell counting, it was stated that the trajectory of individual Daphnia has a fractal character, which may be described by the fractal dimension close to 1.4. It was found that the fractal dimension of Daphnia swimming trajectories is significantly increased from 1.39 in normal conditions to 1.46 in the presence of esfenvalerate. The presence of a pronounced Daphnia response to the presence of pesticides in water at a controlled norm suggests the possibility of this bioassay use for the detection of low concentrations of pesticides, as for the rapid assessment of aquatic toxicity in environmental monitoring, so as for the construction of early warning biological systems.

Keywords: esfenvalerate, *Daphnia magna*, bioassay, computer vision, fractal analysis, fractal dimension.

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INTRODUCTION

Esfenvalerate belongs to the pyrethroid insecticides - one of the most common and widely used pesticides in the world [1]. Preparations based on esfenvalerate exhibit demonstrate a strong striking activity, as in an outer contact so as in the contact with the digestive system of arthropods. Esfenvalerate affects the nervous system of arthropods blocking sodium channels in nerve cells, and leaves these channels open. This may manifest itself through the change of behavior, excitement, tremors, convulsions, paralysis, and eventually death [2].

It is important to take into account that water ecosystems are in a close relationship with agricultural landscapes within which they are located. As a result, pyrethroid insecticides may get into rivers and lakes, making a negative impact on water ecosystems with a surface and groundwater runoff from fields. These insecticides are highly toxic to non-target aquatic animals, especially for crustaceans and insects with similar mechanisms of injury that terrestrial arthropods have [3, 4]. For example, 48-HrLC_{50} esfenvalerate for Cladocera may make only 0.24 mg • L^{-1} [2]. The efficacy and duration of synthetic pyrethroids action is largely determined by their photostability. For esfenvalerate photostability (DT50) in water makes 10 days under the natural sunlight irradiation [5].

It is possible to assess the impact of pollutants on aquatic ecosystems possible using an integral index of environment pollution - biological testing [6]. Among the methods of biological testing an important place is occupied by the determination of toxicity environment with the use of lower crustaceans and especially with Daphniamagna Straus, 1820. Daphnia biological testing methods are widely used for environmental monitoring, both in Russia and abroad. The mortality of crustaceans is preferably used as a test reaction, and the observations on the changes in fertility and offspring quality are carried out during the establishment of chronic toxic effects [6-10]. But before the death of test objects toxicants influence on the change of their behavioral activity. Under the influence of pollutants Daphnia have an increase in motor activity, or vice versa, the activity slows down. Therefore, the list of recorded parameters may be extended by using additional information about a test object, for example, based on its behavioral responses, such as swimming speed, orientation and spatial distribution [11-14].

In addition to these reactions, one may use the characteristic of trajectory complexity for the purpose of biological testing. This characteristic may be expressed in terms of the fractal dimension [15]. Fractal is a mathematical set with a self-similar property, i.e. uniformity in different scales of measurement. Self-similarity is a very common property of natural systems: basins of major rivers, the spatial structure of microorganism colonies, etc. - they have the structural flexibility. Often, in this regard, they talk about the fractality of natural objects. In its turn, the fractal dimension is the measure of a subject geometric complexity [16]. In practice, box-counting method is often used to determine the cell count.

Considering the things mentioned above, the purpose of this work is to evaluate the possibility of fractal analysis concerning Daphnia magna trajectory use to assess the toxicity of the of insecticide esfenvalerate.

MATERIAL AND METHODS

D. magna crustacean from laboratory monoculture grown in the incubator "B-4" (Energolab, Moscow, Russia) were used as an object of study as described in [17]. In this study, Daphnia motion detection was carried out using the hardware-software complex "TrackTox" implementing computer vision algorithms. The processing and analysis of the data was performed using a specialized program. The experimental scheme is shown on Fig. 1.

A single daphnia using a micropipette was transferred from uterine culture into a clear plastic test chamber (98 mm × 40 mm × 9 mm) with 25 ml of the cultivation water (Fig. 1 p. 1), then the chamber was placed in the toxicity analyzer "TrackTox" (p. 2) located in thermostatically controlled conditions (20 ± 2 °C). Daphnia were incubated in an acclimatization chamber for 2-3 minutes, after which the measurement of swimming activity in normal conditions was performed during the following 30 minutes, the so-called "control group" (p. 3). After that the required amount of the toxicant esfenvalerate was added in the container (p. 4) to reach the concentration of 3 g • L^{-1} (MPC of this pesticide in water, approved in Russia). Then the swimming activity was re-measured, the so-called "Experimental group" (p. 3) during 30 minutes. During the whole experiment, the data for Daphnia swimming activity were displayed on the screen (p. 5) and the end of the experiment they were saved in a text file available for subsequent processing and statistical analysis (p. 6). 40 measurements of D. magna swimming activity were performed: 20 in normal conditions and 20 with the addition of the pesticide esfenvalerate at the concentration of 3 mcg • L^{-1}.

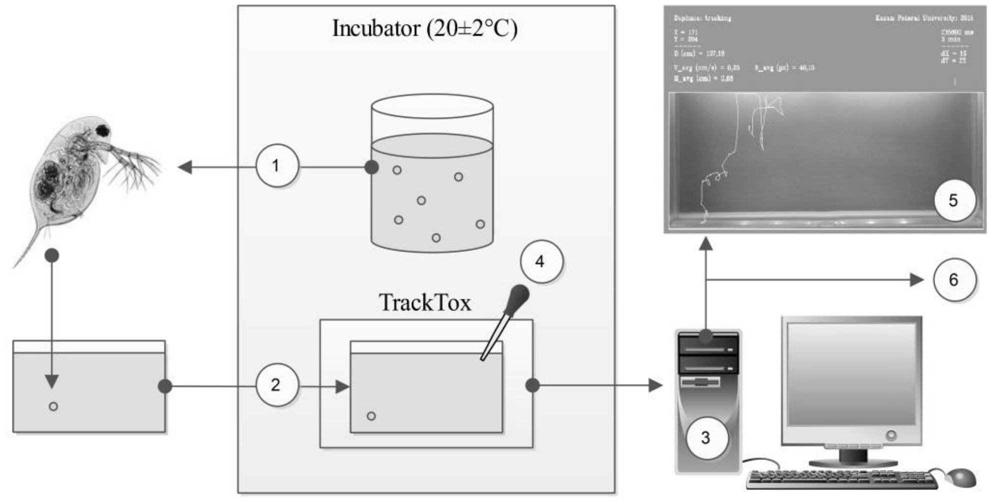


Figure 1: The scheme of daphnia swimming activity definition in the experiment.

The fractal nature of Daphnia motion was established by the method of cell counting (box-counting method) [18]. Due to the significantly smaller width of a test chamber in comparison with its length and height, the assumption was made about a two-dimensional character of Daphnia movement. The principle of the method is that the dimension of the set of elements composing the trajectory of movement is determined by the rate of the number of squares (boxes) increase, containing all the elements of the set - BC, wherein there will be the squares which will be empty. The number of non-empty BC cells depends on the shape of the trajectory and the dimensions of the square cell δ. It is postulated that BC is proportional to $(1/\delta)^{FD}$ (the smaller the size of the cells is, the more they need to cover the trajectory). The value of FD grade is the dimension of an object. The value of fractal dimension is calculated as follows according to this method:

$$FD = -\lim_{\delta \to 0} \frac{\ln(BC)}{\ln(\delta)}. \qquad (1)$$

In practice, the index of the fractal dimension is defined as the slope ratio of the regression line according to ln (BC) dependence on ln (δ).

According to the abovementioned scheme, we analyzed the trajectory of Daphnia as the initial time interval equal to 1 min. The plane containing Daphnia trajectory, was covered with a uniform grid containing $X = 2^b$ cells on each side, with a cell size of $\delta = 1/X$. The value b was ranged from 0 to 6, the physical size of a single cell were varied from 98.0 to 1.5 mm. Next, the number of cell intersections of different scale was determined by Daphnia - BC trajectory. Based on these data an approximating line was developed, in the case of the close location of points to the line, the conclusion on the fractal nature of Daphnia trajectory was made. An approximate fractal dimension of Daphnia trajectory (FD*) was determined using the software "TrackTox" (Fig. 2) according Katzand George formula [19]:

$$FD^* = \frac{\ln(N)}{\ln(N) + \ln(D/L)}, \qquad (2)$$

where L is the overall length of daphnia swimming trajectory during the time interval of 1 min (the total number of analyzed trajectories in 1 experiment corresponded to 30); N - the number of segments from which this trajectory is consisted; D - the longest distance between any two points of the trajectory (corresponds to the diameter of a circumscribed circle around a trajectory).

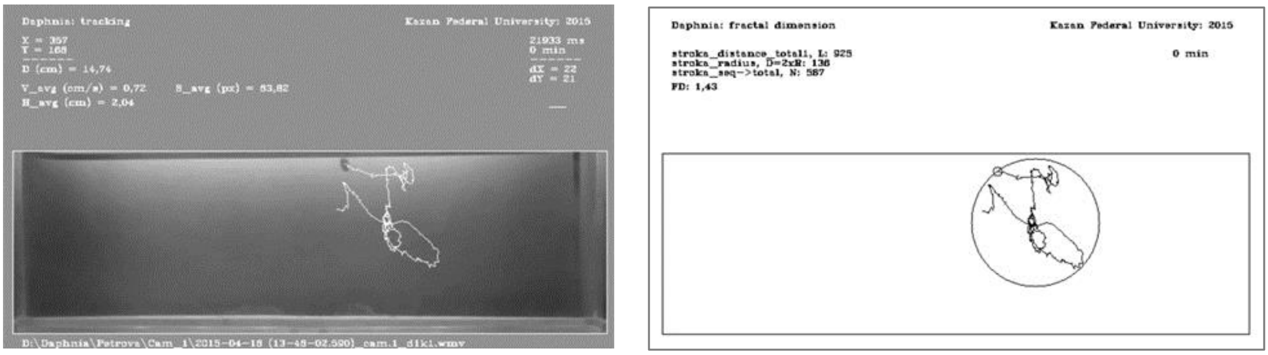


Figure 2: Sample of Daphnia trajectory processing using the computer program for an approximate determination of a fractal dimension

The data is presented in a medium-sized, standard errors of average and median. Differences between samples installed by using non-parametric U-Mann-Whitney criterion. Statistical data processing performed in the program Statistica 10 (StatSoft, Tulsa, USA).

MAIN PART

The performed studies demonstrated that D. magna reacts to the appearance in the medium of esfenvalerate within a short time interval of 30 minutes, increasing its swimming activity (Fig. 3). The average rate of daphnia made 0.59 ± 0.01 cm • s^{-1} in the control group and 0.92 ± 0.03 cm • s^{-1} in the experimental group (the median rate made 0.58 and 0.98 cm • s^{-1}, respectively). The rates were significantly different by Mann-Whitney test ($U = 4915$, $P < 0.001$).

A similar was demonstrated by Daphnia in our previous studies with esfenvalerate [20]. Also, the obtained results are consistent with the data on the changes in the swimming activity of Daphnia in the presence of pesticides, known from the literature. In particular Zeinetal studies [21] examined the effect of an acetylcholinesterase inhibitor introduction (as the component of many pesticides) on D. pulex. It was shown that low concentrations cause a similar increase in the rate of daphnia swimming.

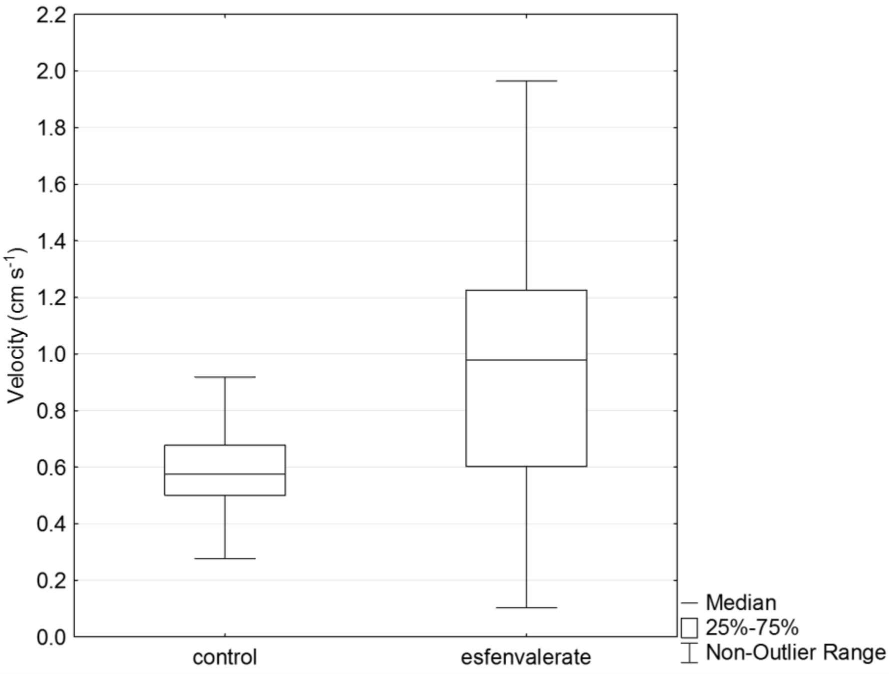
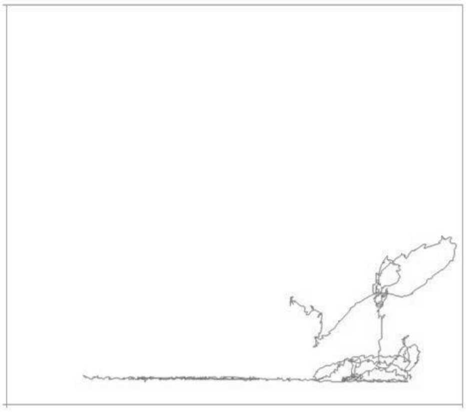


Figure 3: Daphnia swimming activity in a control and in an experimental group

Using the method of cell counting, it was found that the trajectory of daphnia swimming has the character close to the fractal ones. Fig. 4 shows an example of Daphnia fractal dimension trajectory calculation procedure by the method of cell counting.

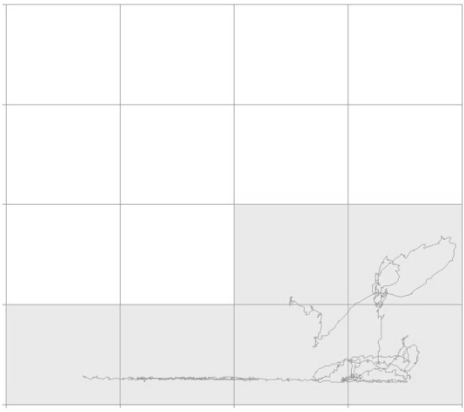
According to the parameters X and BC found by this method the regression equation was developed (Fig. 5). A clearly pronounced linear character of the points location on the graph shows the fractal character of Daphnia movement trajectory. The fractal trajectory dimension calculated according to the regression equation, made 1.36. A similar value of the fractal dimension for daphnia in normal conditions (FD = 1.30), calculated by the same method is described in literature [15].



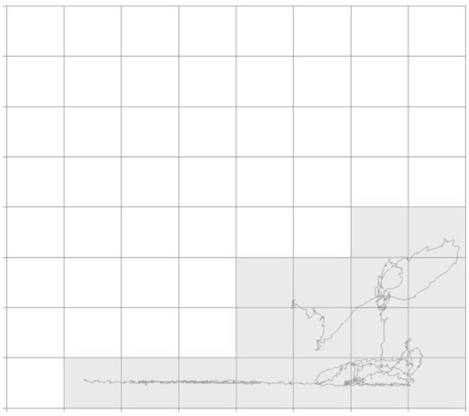
***Trajectory example;BC* = 0**

***X* = 2^0; *BC* = 1**

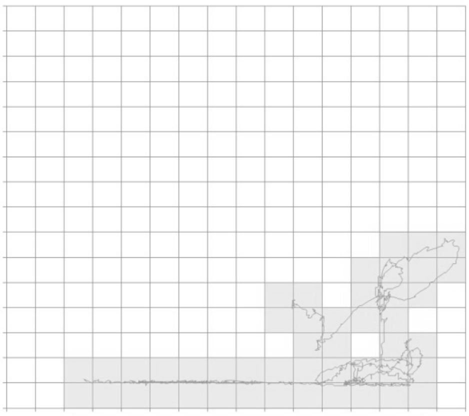
***X* = 2^1; *BC* = 2**



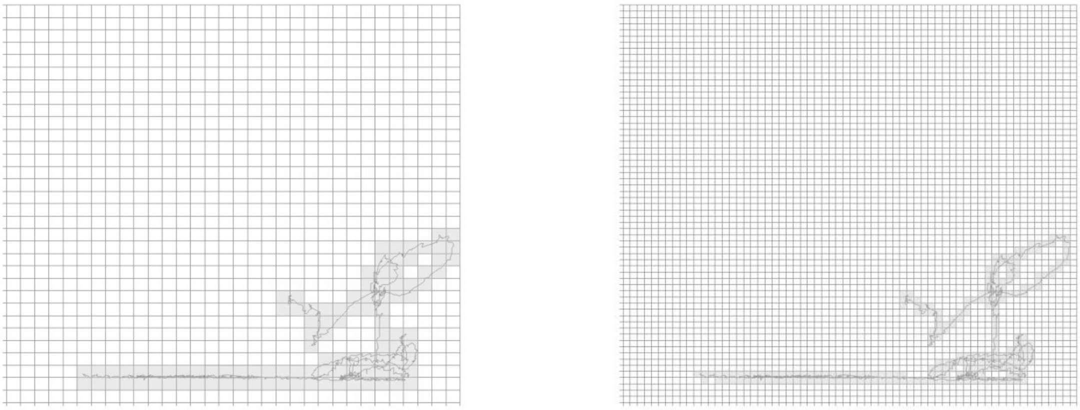
***X* = 2^2; *BC* = 6**



***X* = 2^3; *BC* = 17**



***X* = 2^4; *BC* = 47**



$X = 2^5$; $\bar{BC} = 106$ $X = 2^6$; $\bar{BC} = 231$

Figure 4: The procedure of Daphnia trajectory fractal dimension calculation by cell counting method (explanation in a text)

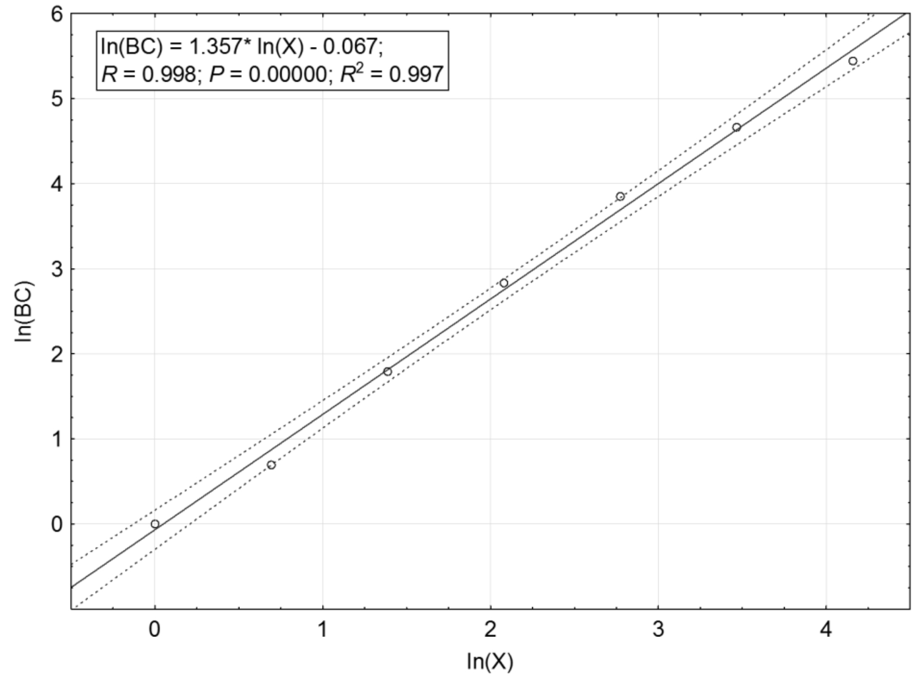


Figure 5: Application of the cell count for Daphnia fractal trajectory dimension calculation (explanation in the text)

At the introduction of a toxicant and speed increase the change the circumscribed circle size around the swimming trajectories of Daphnia took place. An average diameter of a circle was increased by 26% from 4.1 cm to 5.2 cm. This is also reflected in the change of the trajectory fractal dimension. Fig. 6 presents the summary of the calculated series of experiments concerning the fractal dimensions of Daphnia trajectories in the control groups (under normal conditions) and experimental groups (with the pesticide esfenvalerate).

It may be noted that the value of the fractal dimension is statistically significant ($U = 8007$, $P < 0.001$) and was increased from 1.39 in normal conditions to 1.46 in the presence of esfenvalerate. The literature presents the data confirming the increase in the fractal dimension of swimming daphnia trajectory under the influence of pesticides. For example, in the work written by Shimizu et al. [15] FD increase was shown for D. magna after the exposure in organophosphorus (Dichlorvos) solutions and carbamate (Propoxur) pesticide solutions. It was also observed that the reaction depends on the exposure time and the used concentration.

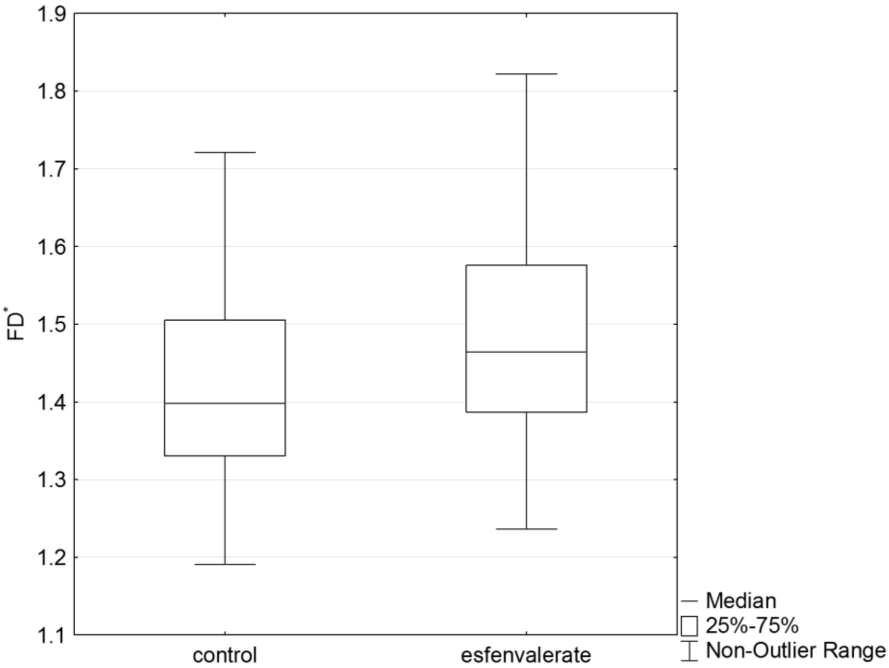


Figure 6: The fractal dimensions of daphnia swimming trajectories, calculated according to the formula 2: in the control and in the experience with esfenvalerate pesticide

CONCLUSION

For the first time the study of pyrethroid insecticide of esfenvalerate effect on the complexity of the swimming trajectories of D. magna was revealed. It was shown that during 30 min. of exposure with the toxicant the complexity of the Daphnia trajectory is increased, expressed through a fractal dimension. The fractal dimension was significantly increased from 1.39 in normal conditions to 1.46 in the presence of esfenvalerate.

SUMMARY

Thus, the study results showed the fractal nature of the swimming trajectory D. magna and demonstrated that the complexity of the trajectory may be assessed using the fractal dimension value. In terms of operational biological testing (30 min) the presence of a pyrethroid insecticide esfenvalerate causes the stimulation of Daphnia swimming activity. The presence of severe Daphnia reactions on the presence of esfenvalerate in water at MPC level suggests the possibility of using this biological test for the detection of low concentrations of pesticides, both for the rapid assessment of aquatic toxicity in environmental monitoring, as well as for early warning biological systems development at some contamination.

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