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Simple and Rapid Method for the Detection of Early Signs of Toxicity in *Daphnia magna* Straus

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Swimming, as a spontaneous activity in aquatic animals, is an indication of the vitality of the animal and represent the integrated result of many physiological vital processes, and is considerably affected upon exposure to any stress conditions or exposure to toxic chemicals. This behavior has long been used as a criterion in determining sublethal impairment in fish (Brett, 1967 and Sprague ,1971) as well as in different higher vertebrates (Reiter, 1978 and Reiter and MacPhail 1979). Water flea *Daphnia magna* Straus, is the recommended and perhaps, the most widely used test organism for toxicity evaluation (ISO 1982). Tremendous number of papers could be found regarding the use of daphnids in such evaluation, through the estimation of the median lethal (or effective)concentration (LC_{50} or EC_{50}) or Time (LT_{50}) values. In spite of the importance, of these measurements, the primary disadvantage, is that concentrations (or time of exposure) should be high enough to induce mortality in daphnids, otherwise no signs or symptoms of intoxication could be noticed or measured according to the procedures available today. Such high concentrations are seldom encountered in the environment.

In this work, a simple, rapid and reliable procedure for toxicity evaluation using *D. magna* was designed so that early signs of intoxication due to exposure to low, nonlethal concentrations of toxic water pollutants could easily be detected.

MATERIALS AND METHODS

Experimental part of this work comprises three steps: the estimation of the LC_{50} value of the toxicant to *D. magna* . Exposure of daphnids to sublethal concentrations in the light of the numerical value of LC_{50} and

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measurement of the spontaneous activity of daphnids as an indicator to their exposure to toxic water pollutants.

Glyphosate herbicide (N-phosphonomethyl glycine, 48% active ingredient) was used as the toxic water pollutant. The reason for this choice is that a project on the environmental effect of this herbicide is being done in our laboratory, using daphnids as the test organism . In order to estimate 48-hr LC_{50} value of the herbicide, a series of dilutions (10, 20, 30 , 40 up to 100) mg/L was made in aged (chlorine- free) tap water, 200 ml from each dilution was placed in a 250-glass beaker before the addition of daphnids. This water was changed after 24 hr with freshly prepared herbicide solution, to avoid the possible decrease in concentrations due to degradation or loss of the herbicide. Temperature during all parts of the study was $21\pm2^{o}C$.

A pilot study for the estimation of LC_{50} value was performed on groups of ten female daphnids (*D. magna* Straus) age 24 hr, that were obtained from parthenogenesis (laboratory stock culture). Daphnids were placed in acid washed glass beakers containing the above mentioned herbicide concentrations, Another group of ten daphnids was retained in the same way in aged tap water (free from contaminant) to serve as a control. Very little amount of food (1 ml of resuspension of dried powdered grass/beaker) was added during exposure. Observation of toxicity was started after 24 hr and continued for 48 hr, dead daphnids were immediately removed from water, oxygen was constantly supplied by means of small air pumps to exclude the effect of oxygen depletion. Immobilization of daphnids was considered as the end point in toxicity measurement, The experiment was repeated 15 times on three replicates each time.

After estimation of the numerical value of the median lethal conc., Another series of graded concentrations of glyphosate below the LC_{50} value (i.e. 5,10,15,20, 25 & 30) mg/L was used in the same manner, where 10 female daphnids of the same age (24 hr), were placed in each concentration and after 24 hr of exposure to the toxicant, daphnids were taken for the measurement of their activity. A group of 10 daphnids was retained as a control and was taken for activity measurement in the same manner. This procedure was repeated 13 times with 3 replicate each.
Measurement of spontaneous activity (as indicator of exposure) was performed using glass petri dishes (8.6 cm diameter). Each dish was

placed on a piece of millimetric graph paper, filled with aged (chlorine-free) tap water -oxygenated prior to addition- to a depth of exactly one centimeter, so that the activity of the animals is restricted to horizontal dimensions only and the movement of the animal is expressed as units of square centimeters and will be called "activity".

Daphnids (that survived from the previous experiment i.e. 24 hr exposure) were transferred individually (one at a time) by a wide-mouth dropper, placed in the dish, and observed for exactly one minute, through which the number of square centimeters traveled was counted (which in fact, corresponds to cubic centimeters, but using units of cm^3 is misleading since no vertical movement is available to the animal), and considered as a function of its spontaneous activity or viability. This observation was repeated for at least 25 times for each daphnid at the session, the average value was then calculated for each daphnid, and mean value for all survivors from a particular concentration was then calculated. The experiment was repeated 8 times with 3 replicate each.

In order to find out whether the diameter of the petri dish may affect the mobility of daphnids, the same procedure was applied using larger glass petri dishes of (19.5) cm diameter, this part was repeated 2 times with 3 replicates each. On the other hand, there was some doubt that turbidity in water may interfere with the swimming pattern or behavior of the animals. Therefore, water, in part of this experiment was rendered turbid by the addition of 50 mg/L bentonite clay prior to its addition to petri dishes (both sizes) and is used in the same manner and this part was repeated 3 times with 3 replicates each.

The whole procedure of activity evaluation was applied to all living daphnids from all concentrations used as well as the control group. Stock cultures as well as experimental vessels were subjected to normal photoperiodism (i.e. indirect sun light, about 12 hr light: 12 hr dark). pH and salinity of water were monitored using pH meter (Philips, PW9421) and digital EC meter (Palin Test, Singapore) respectively.

RESULTS AND DISCUSSION

Physicochemical parameters were constant through out the study period. The average values were: pH (8.15), electrical conductivity (295) µS/cm, total hardness (45 mg /L as $CaCO_3$) and dissolved oxygen was constantly about 7 mg/L.

Forty eight hour LC_{50} value of glyphosate herbicide in this work was found to be 20 mg/L, according to the direct method , and 21.88 mg/L using probit analysis method (Finny,1971;Stephenson et al, 1991). Lower and higher values were reported by other workers (Atkinson, 1985; WHO,1994). In the second part of the experiment, daphnids were exposed to mild concentrations (5 - 30 mg/L) of the herbicide, which normally caused few mortality within 24 hr exposure especially at the lower concentrations, and the survivors showed no observable sings of intoxication. Table 1 shows the percentage of survival of daphnids in different concentration levels for both 24 & 48 hr of exposure

Table 1 Percentage of survival of *D. magna* in different concentration levels of the herbicide.

Conc. mg/L	Percentage of survival	
	24 hr	48 hr
Control	100	100
5	100	96.7
10	83.3	70.0
15	80.0	70.0
20	78.3	50.7
25	61.7	45.0
30	45.0	33.3

The survivors were taken for individual activity measurements. Figure 1 illustrates average values of activity (i.e. number of square centimeters) of daphnids survived from exposure to different concentrations in clean and turbid water, dotted lines represent the activity in turbid water which is evidently much lower than activity in clear water. The sensitivity and the reliability of this method could be recognized from Fig.1 since, the activity of the exposed daphnids was considerably lower than control, showing a clear concentration – effect relationship.

The mean value of daphnid's activity after 24 hr exposure to different concentrations are listed in Table 2, for both clear and turbid water, using smaller petri dishes (8.6 cm diameter).

It is evident that daphnids exposed to the highest concentration of the toxicant, were the least active, crossing only 51.7 % of the number of squares that the control daphnids crossed. The reduction in activity due

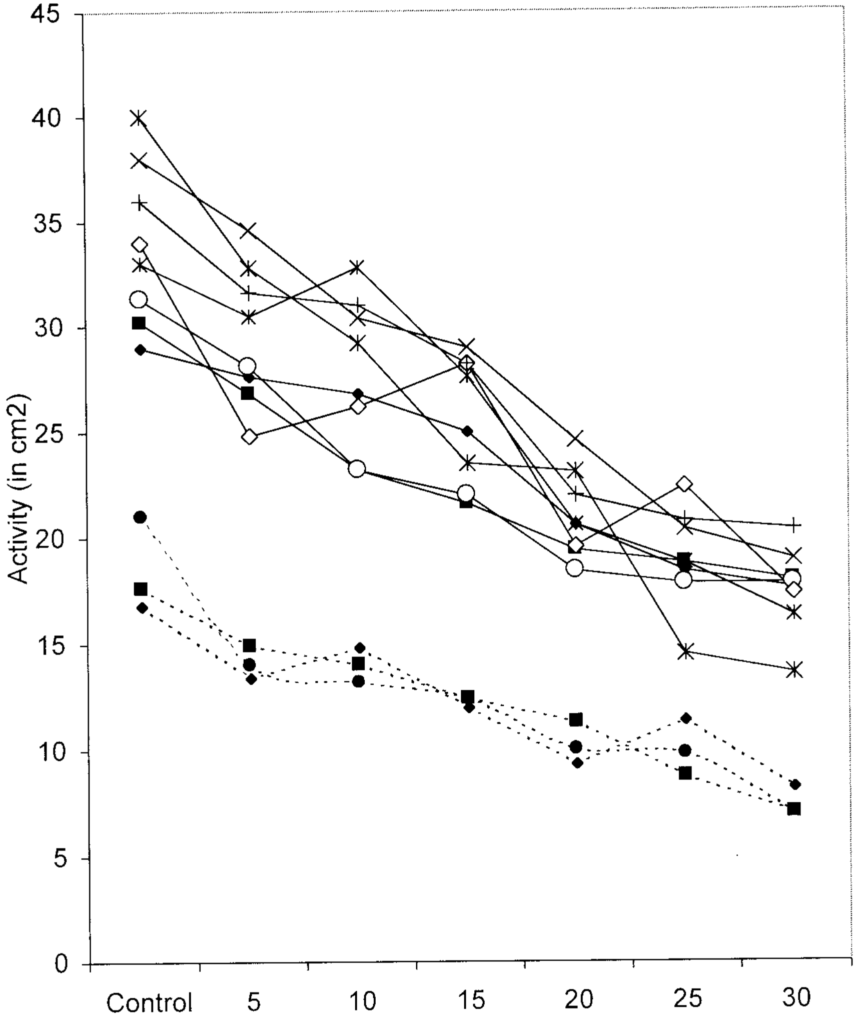

Figure 1. Average values of activity with respect to different exposures (dotted lines represent the activity in the presence of bentonite clay as turbidity). Each segmented line is a separate run.

to exposure to 15, 20, 25 and 30 mg/L of the toxicant, were statistically significant at P <0.01, (Table 2), however exposure to 5 & 10 mg/L were statistically insignificant with respect to control. Diameter of the larger dish (which represent the field of swimming or activity) did not show any significant difference, the readings were very close to those of the smaller one, therefore they were not included.

Table 2. Mean values of daphnid's activity in clear and turbid water after 24 hr exposure to the toxicant.

Conc.	Activity (in number of squares) ± S.D.	
	Clear water	Turbid water
control	34.8±5.3	17.1± 2.4
5	29.7±4.1	15.5±1.9**
10	28.4±4.3	14.9±2.1**
15	25.7±5.2**	12.2±4.2**
20	21.1±4.5**	11.0±2.2**
25	18.7±4.7**	10.2±2.1**
30	18.0±3.5**	7.4±1.9**

Impairment was more obvious in turbid water, since control daphnids in turbid water crossed only 17.1 squares (Table 2) which represent 49.1% of the number of squares that the control daphnids in clear water crossed, consequently daphnids exposed to different levels of the toxicant showed an increasing reduction in activity, the lowest value of 7.4 squares was reached at 30 mg/L in turbid water. Reduction of activity was statistically significant ($P<0.01$) for all levels used in turbid water. Explanation is not yet clear, however, change in the physical characteristics of water could play a role in such reduction.

Spontaneous activity of animals is the final product of nervous system performance, as well as the general condition of the animal. Among many devices-automated and nonautomated- that are used to measure this form of behavior, is the open field method (WHO,1986) yet this measurement of such activity is mainly applicable to vertebrates.

Many authors have expressed the need for faster, simpler and less expensive tests to determine the toxicity of chemical products, among such recently developed tests is the enzymatic inhibition bioassay (Janssen and Persoone, 1992, 1993). Dodson and Hanazato (1995) used a video system to digitize three dimensional swimming track of individual zooplank-tonic animals.

Thus this technique could certainly be taken as a rapid, sensitive and inexpensive method for evaluating sublethal toxicity of pollutants.. Sensitivity of the method and reproducibility of the results were relatively high, however data regarding these two parameters will be published in the near future.

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