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Evaluation of Freshwater Toxicity with *Hydra* as a Test Animal

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The emphasis in assessing pollution in freshwater ecosystem has been demonstrated with hydra, a freshwater solitary polyp of Cnidaria. It yields a unique plasticity in auto-regenerating, cell replacement, and cell renewal system. Heavy metal Cadmium is an extremely potential toxic element for living and even a very minute dose (0.5 ppm) of Cadmium-chloride can initiate noticeable damage to the body organization of hydra within a short time. Two methods have been employed in measuring the toxicity of hydra. One is the determination of median lethal concentration (LC50) of Cadmium-chloride by Probit Analysis method, while the second one involves the progressive changes in morphological structures through scoring procedures. The second method is more accurate in determining the lethal toxicity. Rural people may use this method for detection in toxicity hazards in freshwater more easily and precisely without having any statistical knowledge.

Key words: Hydra, ecosystem, heavy metal, progressive change, toxicity

INTRODUCTION

Water is an essential natural resource required for all life activities and ecological functions. It is used by man for irrigation, navigation, industries, electricity generation, and a variety of domestic and commercial purposes. But water quality is continuously degraded by the activities of modern civilization mainly through industrial effluents and agriculture pesticides The regular entry of pesticidial and industrial pollutants into the natural resources eventually causes physical, chemical, and biological deterioration of the water when its natural purifying capacity is exceeded.

In India, the problem has assumed an acute phase due to unawareness of most farmers about the hazardous effects of pesticides that they use regularly. For quick control measures, they frequently use these pesticides above the recommended dose resulting in adverse effects on environment and its associated biota.

On the other hand, the continuous discharge of effluents containing non-essential heavy metals and their chemical compounds at an unprecedented and constantly increasing rate, even beyond permissible level from various industries into adjoining aquatic milieu might result in accumulation, and subsequent magnification to dangerous level because of their toxicity, water solubility, longevity, and non-degradable nature. Aquatic life is strongly influenced by physical and chemical properties of water body. It is known that heavy metals and pesticides are potentially harmful to aquatic lives.

Hydra vulgaris is an important and common component of freshwater ecosystems and is sensitive to a wide range of pollutants (Mookerjee and Dutta 1959;

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Aditya 1981, 1983,1986; Kalafatic 1997; Trottier et al. 1997; Pollino and Holdway 1999; Holdway et al. 2001). It is a diploblastic, sedentary coelenterate occurring only in clean and clear freshwater bodies. It is easy to culture in the laboratories in large number and used as a bio-monitoring tool for detecting water quality of fresh-water ecosystem (Pascoe & Edwards 1989; Beach & Pascoe 1998; Fu et al. 1991a, 1991b). Hydra can also be useful for assessing toxicity (Adams 1984; Herring et al. 1988; Peters et al. 1991). Since the animal is diploblastic, all of its cells are in intimate contact with surrounding medium and make hydra a potentially useful indicator for pollution. *In vitro* hydra assay can predict the developmental toxicity hazard chemicals and rank them accordingly (Fu et al. 1990; Newman et al. 1990; Johnson and Newman 1990). In laboratory condition, it is difficult to determine the mortality of hydra through external appearance, the assessment of LC50 value of the toxicants becomes inaccurate. So, an alternative approach, based on the observation of physiological responses of hydra can be useful for measuring toxicity (Wilby 1988). The objectives of this study are to determine toxicity to hydra using LC50 value calculation by means of Probit Analysis procedure and simultaneously a physiological response-based technique for the same, and also to ascertain the result in the large scale (in nature).

MATERAIALS AND METHODS

Laboratory Study

Hydra has been used in toxicity testing biological material because of its sensitivity and ubiquitous nature in many aquatic ecosystems. The specimens were collected from the ponds and swamps of Santiniketan and adjoining areas. These were cultured in M-solution (Sugiyama and Fujisawa 1977) in the laboratory within large Petri dishes (6" diameter, depth 1") and kept in B. O. D. incubator at 21 ± 1° C at 16 h light, and 8 h dark cycles. They were fed with *Artemia nauplii* on alternate day. After feeding, the culture medium was replaced with fresh ones to avoid contamination of water. Prior to the experiment, hydras are acclimatized to the laboratory condition at least for 3 months. The stock solution of 2000 ppm (2000 mL/1) of Cadmium-chloride (Qualigens, about 95% $CdCl_2$) was prepared by using distilled water as diluents.

The first method for evolution of toxicity response of hydra was the median lethal concentration (LC50) by Probit Analysis technique. For the detection of 24 h LC50 value of Cadmium-chloride, 10 Petri dishes (6" diameter, depth 1") were taken and in each one was kept 25 matured, non-budding, one day-starved hydras. Then, each Petri dish containing hydras was exposed to different concentrations (0.30 ppm, 0.40 ppm, 0.50 ppm, 0.60 ppm, 0.65 ppm, 0.70 ppm, 0.75 ppm, 0.80 ppm, 0.85 ppm, 0.90 ppm) of Cadmium-chloride (prepared in M-solution) for 24 h. After 24 h of exposure the hydras were counted and 50% of the specimens were found dead, and the concentration was considered to be the lethal concentration LC50, (killing 50% of the organisms compared to the control over a 24 h exposure period). With the same procedure 48 h, 72 h, and 96 h exposures were done to the assess lethal toxicity of hydra. Throughout the experiments, food were not supplied and the toxicant containing culture medium was replaced with freshly prepared toxicant every day. All the tests were repeated 3 times with freshly prepared solution having 3 replicas per test concentrations.

The second method for measuring toxicity was conditioned by progressive changes of structure through scoring procedure. The observations under microscope on the appearance of hydra had been recorded at specific concentration of $CdCl_2$ at different hours until the total degradation by dissociation of cells of hydra took place. At higher concentration of toxicant, the disintegrable rate of the test animal was more frequent. The progressive effects of toxicants were recorded by rating the morphological status of the individual, and by assigning a score from 6 (normal) to 0 (disintegrable / cell dissociate). In measuring the toxicity of the second method (progressive changes of structure through scoring procedure), 10 Petri dishes (6" diameter, depth 1") were taken and each one was kept with 5 matured, 24 h starved non budding hydras. The first 6 Petri dishes containing hydras were exposed to 9 different concentrations of Cadmium-Chloride (0.1 ppm, 0.2 ppm, 0.3 ppm, 0.4 ppm, 0.5 ppm, 0.6ppm, 0.7 ppm, 0.8 ppm, and 0.9 ppm), respectively for 96 h, and the remaining Petri dish containing hydras served as the control set (0 ppm). During the experiment, no foods were supplied to the treated and normal specimens. For accurate result, daily change of fresh toxicant along with 3 replicas were necessary.

The changes in gradual morphological structures of hydra (score procedure) were selected from the different concentrations and from the different time exposures, and some treated specimens were sent back to the normal culture medium to observe whether they could recover from stress or not. The specimens were observed for 10 to 15 days to underline the recovery phases. Photographs had been taken with the help of Leitz microscope (Germany) attached with SDC-310 digital camera (Korea).

Field Study

During field study, "Population counting" is 1 of the unique techniques in determining a bio-indicator species, and accordingly, the following is accomplished for hydra. Only matured and healthy (apparently same age) hydras were collected and counted for measuring same amount of vegetation (weight/area) in a specific field.

Two different ponds, one was normal (Deer park pond; where it is free from any sorts of pollutants) and another was polluted pond (Lalbandh pond; where bathing and washing of clothes were seen in many parts of the pond) was selected in the same locality of Santiniketan for this study. Randomly submerged weeds were collected from different sites of those ponds, and in each site same numbers of submerged weeds were collected along with pond water in a large glass jar for population counting. From each site, 5 sets of glass jars were used for minimizing the statistical errors. The hydras were collected by conventional technique and counted for each glass jar.

Collection of Hydra: At first, the weeds were picked up randomly from the surface of the ponds (sub-littoral zone) and kept in glass jars along with pond water. After an hour or so, when the water settled due to the sedimentation of clay particles, the jars were then put near direct sunlight and observed closely. The hydra could be seen attached to the weeds or glass jars with its basal disc or could be floating on the surface of the water. Finally, hydras were collected very carefully by pasture pipette and kept in glass petridishes and were cultured in laboratory.

RESULT

Laboratory Study

The specimens that had been exposed to different concentrations at $CdCl_2$ at different time periods for evaluation of LC50 value produced the following results. The 24 h LC50 value happened to be 0.64 ppm whereas 0.15 ppm was the 96 h LC50 value (Table 1).

In the second method for measuring toxicity, morphological change in the structure of hydra has been observed at different hours until the degradation or cell dissociation occurred. The change in morphological structures was recorded by assigning a score of which 6 was the normal score and 0 was the score of disintegrated hydra (Table 2).

Table 1. Median lethal concentration (LC50) of cadmium chloride calculated from different time exposure by Probit Analysis method

Toxicant	24 h LC50	48 h LC50	72 h LC50	96h LC50
Cadmium chloride	0.64 ppm	0.41 ppm	0. 26 ppm	0.14 ppm

Table 2. Key for assessing progressive toxicity effects of animal morphology through scoring rate

Score	Morphology
6	Extended tentacles and body reactive
5	Largely extended tentacles, body partially contracted
4	Clubbed tentacles, body slightly contracted
3	Tentacles (clubbed) and body shortened
2	Rudimentary tentacles (very shortened), body shortened
1	Dead but intact (body almost ball shaped, almost no tentacles)
0	Disintegrate (cells dissociate)

The morphological changes of hydra (by score-procedure) in different concentrations at different time exposures are represented in Table 3.

Some of the specimens that were exposed to different concentrations of the toxicant at different periods for assessing progressive toxicity effects of animal morphology were sent back to the normal culture medium to observe as to whether they could recover from the stress or not. It has been observed that in the case of Cadmium-chloride, up to score 3 level of morphological changes in hydra could be returned to the culture medium for recovery. Gradually, the specimens overcome the stress and attain normal morphological appearance within 10 to 15 days if proper food is supplied. It has been also observed that disintegration rate of test animal was more frequent due to higher concentration of toxicant (Table 3).

Table 3. Median score recorded at different cadmium chloride concentration at different exposure time

Conc. (ppm)	Exposure time (h)			
	24	48	72	96
0	6	6	6	6
0.1	6	5	5	3
0.2	5	3	2	0
0.3	4	2	0	0
0.4	4	1	0	0
0.5	3	0	0	0
0.6	2	0	0	0
0.7	1	0	0	0
0.8	0	0	0	0
0.9	0	0	0	0

It was clear from Table 3 that score 3 level was achieved at 24 h exposure in 0.5 ppm, at 48 h in 0.2 ppm, and at 96 h in 0.1 ppm concentration of Cadmium-chloride. Therefore, 0.5 ppm of Cadmium-chloride (score 3 level) may be considered to be the Maximum Tolerance Limit (TLm) of hydra for 24 h exposure, whereas 0.1 ppm is to be the TLm value of hydra for 96 h exposure.

Field Study

In normal pond, it has been found that the maximum population of hydra was seen in 4 sites whereas no hydra was seen (collected) from the 3 sites of the polluted pond. It has also been observed that maximum vegetation (weight/area) of hydra occurred in normal pond rather than in the polluted pond. It has also been observed that either no or very low number of hydra was found in the sites where bathing and washing of clothes are regularly done (Table 4.)

DISCUSSION

Bio-indicator can be used to measure a wide range of responses to chemicals at the biochemical, cellular or tissue levels. Responses may also be measured at physiological, behavioural or population levels. From the result of confined environment (Laboratory study), it has been observed that the tentacles are the sensitive and reactive portions of hydra's body. The tentacles are beset with batteries of matured nematocysts (Fig. 1), which help in food catching and also act as useful indicator for any kind of change in environment (Kovacevic et al. 2001). When an effective dose of Cadmium-chloride solution has been introduced in a fresh culture medium containing hydra, within an hour it has been noticed that hydra's tentacles are extended maximally for recognizing any foreign particles entering to the ecosystem. In later phase, the morphological features of the tentacles are noted to have changed. These become clubbed (aggregation of cnidoblast cells at the tip of the tentacles), shortened due to release of discharged nematocysts and other cells, and finally the tentacles are almost eradicated (Fig. 2). The bodies are also contracted and at score 1 level it assumes ball shaped appearance (Fig. 2e & 2f), and finally the cell dissociation and disintegration (Fig. 2g) start.

Table 4. Population counting of Hydra from two different ponds

Normal pond		Polluted pond	
No. of sites	No. of individuals	No. of sites	No. of individuals
1.	10 ± 1.90	1.	-
2.	07 ± 1.41	2.	02 ± 1.41
3.	14 ± 1.41	3.	-
4.	10 ± 1.10	4.	06 ± 1.10
5.	05 ± 1.48	5.	-
6.	09 ± 1.41	6.	-

From a comparative study of the 2 methods for assessing toxicity test of hydra, it has been seen that evaluation of the toxicity of hydra by the Probit Analysis technique (LC50) is not so accurate (here 24 h LC50 of Cadmium-chloride is 0.64 ppm) as compared to the second one (24 h TLm value is 0.5 ppm). Determination of the acute lethal toxicity of hydra by Probit Analysis method is very difficult to achieve because the exact point of death of the animal could not be deciphered from its appearance. It has also been seen that sometimes the animals retain an inert state without showing any visible sign of movement but are not dead totally. In rare or some extreme cases, however, the hydra could recover from stress if they are brought back to normal culture medium. Since it is difficult to determine the mortality of hydra through external appearance, the assessment of LC50 value of $CdCl_2$ becomes inaccurate. So, the alternative approach for measuring toxicity is the "progressive changes of animal morphology (structure) through scoring procedure". Assessing toxicity of Hydra through scoring procedure has several advantages over the Probit Analysis technique because, by this method, the particular stage of toxicity could be determined by the gradual change in morphological pattern and recovery rate of hydra. This method yields sensitive indication of toxicity since morphological changes can be detected at lower concentration than those causing mortality but within same timescale. This method also ensures the ability of hydra to recover from the pollutant-induced

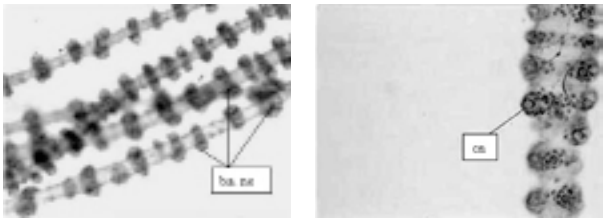

Figure 1. Tentacles in a normal Hydra. Tentacles with batteries of nematocysts a) lower magnification (X 40); b) higher magnification (X 100) ba. ne = batteries of nematocysts; cn = cnidoblast

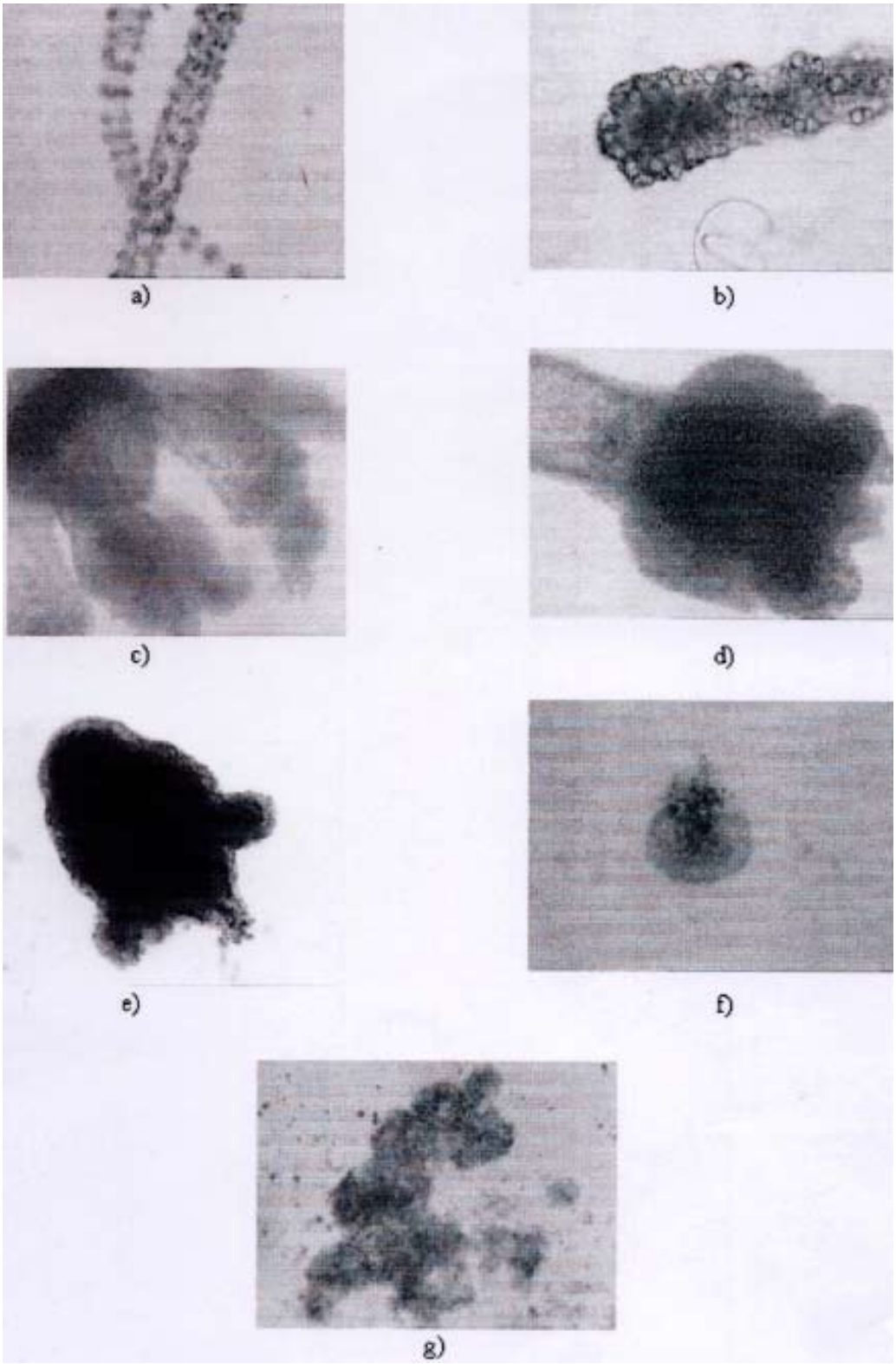

Figure 2. Progressive changes of Hydra (X 40) by scoring procedure a) Score 5: Extended tentacles (with the nematocysts in-groups) b) Score 4: Clubbed tentacle (clubbed tentacle with the cluster of cnidoblasts) c) Score 3: Tentacles and body shortened (note the tentacles and cone of hypostome) d) Score 2: Rudimentary tentacles (very shortened), body shortened e) Score 1: Dead but intact (early stage) f) Score 1: Dead but intact (ball shaped body; late stage) g) Score 0: Disintegration (cells dissociate).

damage (Karntanut and Pascoe 2000). This recovery could be obtained due to their unique cell replacement system. In addition, scoring procedure through progressive changes in structure may help not only in identifying different mechanisms of toxicity caused by different classes of toxicant, but also serves as a mode of evaluating the toxicity in respect to potential hazards.

Bio-indication is a scientific analysis of field-collected ecological information, with the aim of using that information to make inferences about the quality of the environment at the place under investigation. From the result of population counting method, it has been seen (Fig. 4) that a lot less amount of hydra vegetation is found in sites where bathing and washing of clothes are regularly done.

In the present context, maximum contaminant level of Cadmium-chloride in drinking water by Environmental Protection Agency is 0.005 ppm (EPA 1995), whereas enforcement standard of Cadmium-chloride of Public Health Ground water Quality is 0.5 ppm (CELDs 1994). So, it is extremely important that one should have a concept about the need of keeping the environment unpolluted for one's safety. In India, particularly in rural areas, economically and technologically it is very difficult to determine the purity of drinking water. Many people live below the poverty line and they do not have any knowledge about the detection of pollutants and the resultant hazards. They are also very unconcerned about these.

CONCLUSSION

The present investigation clearly demonstrates that hydra responds to even a subtle change in freshwater ecosystem. And the contamination of freshwater can be measured easily and quickly mainly by Indian rural people through the observation of progressive changes in structure of hydra without having any knowledge of the statistic. Therefore, hydra may be utilized easily as a bio-monitoring tool for detecting the purity of drinking water in ponds and lakes, and in other fresh-water ecosystem mainly in rural areas where economic and technological developments remain inadequate. The repeated use of bio-indicators in the monitoring program may be helpful to detect environmental changes at an early stage or to evaluate the efficiency of measures taken to improve environmental quality.

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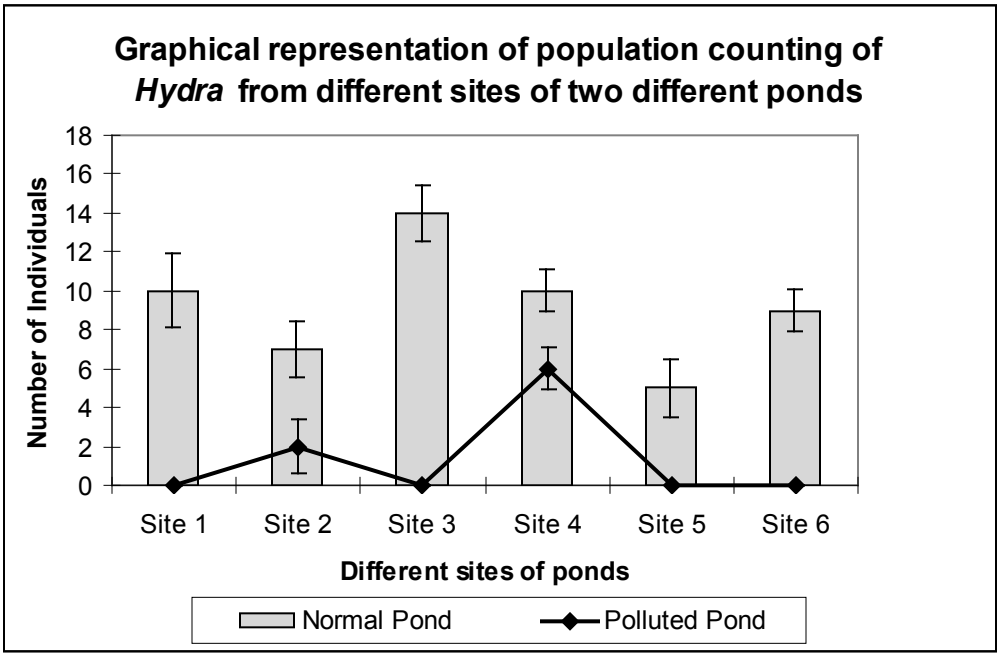

Figure 3. Tentacle at recovery stage. Less number of different cells, nematocysts and cnidoblasts a) lower magnification (X 40); b) higher magnification (X 100) im. cn = immature cnidoblast

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