Toxicological studies of pesticides on cytoplasmic streaming in *Nitella*

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In the present study, changes in velocity of cytoplasmic streaming in the giant internodal cells of *Nitella* for varying concentration of the pesticides, 2,4-D, dieldrin, malathion, methyl parathion and endosulfan, were measured. Marked decrease in the velocity of cytoplasmic streaming was found at the concentrations of 0.01, 0.1, 1, 10 and 100 mM. Dieldrin was the most toxic to all the pesticides investigated, followed by methyl parathion, endosulfan, malathion and 2,4-D. Threshold values for dieldrin, methylparathion, endosulfan, malathion and 2,4-D as indicated by the onset of decrease in the normal cytoplasmic streaming velocity were less than 6.25 × 10⁻⁶, 2.5 × 10⁻⁵, 5 × 10⁻⁵, 5 × 10⁻⁵ and 1.25 × 10⁻⁵ M respectively. Cessation of streaming was noticed above 1 mM in dieldrin and above 10 mM when exposed to methylparathion and endosulfan. Cessation of streaming was not seen up to 100 mM concentration of 2,4-D and malathion.

**Keywords:** Aquatic pollution, Biomonitoring, Cytoplasmic streaming, *Nitella*, Pesticides

Frequent uses of pesticides pose a serious threat to aquatic ecosystems. Since their removal from the aquatic system is difficult, effects of pesticides on non-target species have become important. Effects of pesticides on various algal species have been studied earlier with respect to growth, photosynthesis and nitrogen fixation. None of these studies exploited cytoplasmic streaming in the Characean alga, *Nitella*, while studying toxicological effects of pesticides.

Pesticides reach water either directly or indirectly through run off from agricultural fields, spray drifts, rain water, sewage and effluent from industries manufacturing pesticides or using them in their processes. Toxic chemicals such as pesticides, heavy metals etc. may affect the metabolic or physiological processes common to life in general and can inhibit or stimulate vital process detectable at the molecular, cellular, tissue, organism or population level. Thus, pollution monitoring (biomonitoring) has become an essential component for pollution assessment as it acts as backbone for future line of action for remediation strategies.

Numerous methods for toxicity testing with plants have been devised, end points of the most common are based on rate of population growth (in microalgae), sexual reproduction/zoospore germination/vegetative growth of sporophyte and germ tube growth (in macroalgae), rate of germination/ rate of root elongation/rate of growth in hydroponic culture and rate of growth in sediments in non-vascular plants. Apart from these, measuring the inhibition of test algae by ¹⁴C assimilation. Reduction in the velocity of cytoplasmic streaming has been also used for measuring toxicity of natural and wastewater for heavy metals, industrial effluents and sewage for the first time by us. The objective of present study was to prove use of reduction in cytoplasmic streaming velocity in *Nitella* as a new biomonitoring tool for pesticide pollution in aquatic bodies.

*Nitella* sp. with giant algal cells (2-5 cm long multinucleate internodal cells), exists as sub-aquatic meadows, was collected from a freshwater pond at Budkal, Faridabad, India and maintained in glass aquaria (75 x 40 x 30 cm) containing wet soil mixed with humus (5 cm thick layer). Growth of phytoplanktons over the cells was prevented by co-culture of zooplanktons like *Cyclops* and *Daphnia*. Healthy culture of *Nitella* could be established in a few months. Prior to experiment, internodal cells of medium size (about 4 cm long and 1 mm in diam.) were separated from the main stem and kept in artificial pond water (APW) containing 0.1 mM each of KCl, CaCl₂ and NaCl. Pesticides (malathion, methylparathion and endosulfan) were used as commercial grade and obtained from Bayer (India) Ltd. available in the form of emulsifying concentrate or wettable powder except 2,4-D (99% pure) that was obtained from Sigma Aldrich Corp. USA. Different concentrations (0.01, 0.1, 1.0, 10 and 100 mM) of stock solutions of pesticides were made using artificial pond water (APW). We used perfusion chamber method in order to avoid shock that otherwise leads to the cessation of streaming during change from one concentration to another. The perfusion chamber was made up of a plain glass plate (8 x 4 cm) with all the four sides...
glued by glass rods (8 mm diam.). Another glass rod was glued asymmetrically so as to partition the whole inside area into two compartments in ratio, 4:1 (Fig. 1).

Cytoplasmic streaming velocity can be measured by exposing a giant algal cell to APW and/or test solution(s) in perfusion chamber (Fig. 1) followed by light microscopic observations (under 100x magnification) and recording of the time taken by cytoplasmic particles to move a fixed ocular distance (with a stopwatch). In lieu of one of the eyepiece, an ocular with scale was inserted with a stage micrometer and found that one scale of the ocular corresponded to 115 μm. Observations made to record the time (Δt) taken for medium size cytoplasmic particles to cross six (centrally located) scales of the ocular. The velocity was, therefore, 690 μm/Δt. The internodal cell of Nitella (preconditioned in APW for 24 hr) was kept in the large chamber and brought to position under objective of the microscope. Initial readings were taken in APW. After this 1 ml APW was taken out from the smaller chamber with the help of pipette and discarded. It was followed by addition of 1 ml of test solution (pesticides solution) in smaller chamber. The cotton bridge was kept over the barrier of two chambers which led to the equilibrium of effective concentration. Observations were made after 15 min as it was found that time taken by test solution to get equilibrated between the chambers was approximately 15 min. In order to eliminate the inherent though small differences in the streaming velocity in APW (V_{APW}) in internodal cells arising from age, season and other ungovernable parameters we have used the percentage relative decrement in streaming velocity (V_{PRD}) for quantitative analyses. Formula used was

\[ V_{PRD} = \frac{V_{APW} - V}{V_{APW}} \times 100, \]

where \( V_{APW} \) is average recorded time in artificial pond water and \( V \) is recorded time in different test solutions taken by particles to traverse the fixed distance (ie, 690 μm). For each averaged value of \( t \), 10 recordings were taken on one internodal cell. Each sequence was repeated on three internodal cells. The three values of relative percentage variation in streaming velocity for the three internodes were again averaged and were represented in the experimental data.

The gradual increase in pesticides concentrations (0.01, 0.1, 1, 10 and 100 mM) had shown visible quantitative changes in cytoplasmic streaming velocity expressed as V_{PRD} (Table 1). The decrease in V_{PRD} values at different concentrations of pesticides test solutions in comparison to control indicate extent of toxicity. For the first time, cessation of streaming was observed at 10 mM concentration of dieldrin indicating its highest toxicity followed by methylparathion (50.7%), endosulfan (63.1%), 2,4-D (83.6%) and malathion (87.1%). At 100 mM concentration of pesticides cessation of cytoplasmic streaming could be observed with methylparathion and endosulfan. Surprisingly, in 2,4-D and malathion (100 mM) cytoplasmic streaming continued (77.9 and 77.2 % respectively) indicating their lesser toxicity. Order of toxicity for minimum used concentration (0.01 mM) of pesticides was 93.2% for dieldrin, 94.8% for 2,4-D, 97.1% for endosulfan, 98% for methylparathion and 99% for malathion (Table 1). At this concentration it

![Fig. 1—Perfusion chamber](image)

**Table 1—Effect of different concentrations of pesticides on V_{PRD} values**

<table>
<thead>
<tr>
<th>Conc. (mM)</th>
<th>Dieldrin</th>
<th>Methyl Parathion</th>
<th>Endosulfan</th>
<th>Malathion</th>
<th>2,4-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.01</td>
<td>93.2 ± .03</td>
<td>98.2 ± 41</td>
<td>97.1 ± 1.15</td>
<td>99 ± 2.1</td>
<td>94.8 ± .57</td>
</tr>
<tr>
<td>0.1</td>
<td>83.1 ± .03</td>
<td>91.8 ± 30</td>
<td>84.4 ± 1.4</td>
<td>98 ± 2.1</td>
<td>91 ± .65</td>
</tr>
<tr>
<td>1</td>
<td>79.3 ± .03</td>
<td>88.5 ± 26</td>
<td>74.1 ± 1.26</td>
<td>96.2 ± 2.0</td>
<td>86.7 ± .43</td>
</tr>
<tr>
<td>10</td>
<td>CEASED</td>
<td>50.7 ± 1.6</td>
<td>63.1 ± 1.21</td>
<td>87.1 ± 2.1</td>
<td>83.6 ± .62</td>
</tr>
<tr>
<td>100</td>
<td>CEASED</td>
<td>CEASED</td>
<td>CEASED</td>
<td>77.2 ± 2.0</td>
<td>7.5 ± .38</td>
</tr>
</tbody>
</table>
was interesting to observe a decrement of about 6% in \( V_{\text{PRD}} \) for 2,4-D, which was only next to dieldrin (about 7%) that may be the organomercury compound. Besides, cessation of cytoplasmic streaming velocity, threshold concentration was also studied for these pesticides. Threshold concentration (below 0.01 mM), at which no effect or response was observed, for the pesticides was 6.25 \( \times 10^{-6} \) for dieldrin; 2.5 \( \times 10^{-5} \) for methylparathion; 5 \( \times 10^{-5} \) for endosulfan; 5 \( \times 10^{-5} \) for malathion and 1.25 \( \times 10^{-6} \) for 2,4-D.

Any correlation between changes in cytoplasmic streaming and pesticides is not known so far in terms of mode and site of action. However, at molecular level cytoplasmic streaming is known to be regulated as a result of actin-myoosin interactions, so anything that affects streaming either directly or indirectly affects the actin-myoosin interaction in moving cytoplasm. The organelles in moving cytoplasm are attached to actin filaments by myosin molecules, which use the energy of ATP hydrolysis to slide along the actin filaments pulling the organelles with them. Streaming is inhibited if depletion of free Mg\(^{2+}\) and ATP occurs. Cytoplasmic streaming in Nitella is susceptible to change in pH, temperature, mechanical shock, extracellular concentration of potassium and calcium and viscosity of bathing medium. These changes occur at interface of the cytoplasm where actin filaments are spread and the endoplasm containing the dispersed myosin exists. These changes are also affected by change in membrane potential by means of acto-myoosin interactions. The cytoplasmic streaming velocity changes reflects the cumulative effects of all the toxicants/stresses present in the aquatic habitat.

Organo-mercury compound, 2,4-D, is an auxin analog that stimulates destructive growth in higher plants. Algae do not have auxin growth regulation, but react to inhibition of photosynthesis and energy transport process. Similarly, chlorinated insecticide (dieldrin) has been reported to inhibit enzyme activity and photosynthesis and alters the cell membrane permeability. Endosulfan is known to cause hypomagnesemia and hypocalcemia. Organophosphates (malathion and methylparathion) are reported to decrease the activity of glucose dehydrogenase. They also decrease the activity of mitochondrial enzymes such as isocitrates, succinate, malate dehydrogenase, cytochrome-C-oxidase and Mg\(^{2+}\) ATPase which would be due to the masking of active sites or due to blocking of sulphhydryl groups. The gradual decrease in streaming with exposure to the studied pesticides might be associated with the depletion of ATP and magnesium ion directly or indirectly, which are required for actin-myoosin interaction regulated movement of the cell organelles.

Biological monitoring of water pollution is conducted to determine the deleterious effect of toxic substances and is preferred over chemical monitoring because (1) the list of chemicals to be monitored is unending, (2) biological effect often occur at concentrations below analytical capabilities. Many of the pollutants are present at such a low concentration that instrument sensitivity is too poor to determine the microquantity of pollutants, (3), the toxicants and other traces may act quite differently in mixture than individually. Such toxicants affect the ecosystem in a synergistic manner which cannot be detected by chemical analysis alone, and (4) chemical nature of toxicant is highly dynamic in environment with time and space, whereas biological system can integrate all environmental variables over a long period of time in terms of effects can be easily measured and quantified. Scientists have used several methods (microplate technique, algistatic method and standard bottle test technique, EC\(_{50}\) value tests) for bioassay. These methods are too laborious and time-consuming for routine tests. Our observations suggested that change in cytoplasmic streaming velocity was the first visible (though microscopic) metabolic signal of cell’s response against the environmental stress and in comparison to other referred techniques was easy, quick and cheap.

The order of toxicity obtained for pesticides from our study (dieldrin > methylparathion > endosulfan > malathion > 2,4-D). From the findings of our experiment, during present study and earlier lab studies and their comparison with established ADI and LD\(_{50}\) values of these pesticides as well as with values given by WHO (1996), USEPA (1979), BIS (1991), it appears that the techniques based on the alterations in the cytoplasmic streaming velocity in Nitella with reference to aquatic pollutants could be possibly introduced as a new biomonitoring technique for aquatic pollution.

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References
4 Rana B C, Studies on chemical and biological treatment of zinc smelter- effluent evaluation through the growth of test alga, Pollution and biomonitoring, (TMH Publishing Co. Ltd., New Delhi) 1995, 63.
8 Shimmen T & Tazawa M, Control of cytoplasmic streaming by ATP, Mg^{2+} and cytochalasin B in permeabilized characean cell, Protoplasma, 115 (1983) 18.