5. SUMMARY

The extensive and intensive use of parathion and methyl parathion, as replacements to more persistent organochlorine insecticides, in Indian agriculture results in the accumulation of large quantities of PNP in soils due to microbial or chemical hydrolysis. Any interference of PNP, a priority pollutant, and its reduction metabolites, PNSP and PAP, with the normal activities of microalgae and cyanobacteria could result in potentially serious consequences on the overall fertility of soils. Very limited information is available on the nontarget effects of PNP and its metabolites towards microalgae and cyanobacteria in soil. The present investigation was therefore aimed at toxicity evaluation, and determining alterations, if any, in biochemical activities of *Chlorella vulgaris*, *Scenedesmus bijugatus*, *Nostoc muscorum*, and *Nostoc linckia*, all isolated from soil. The effect of toxicants on siderophore production in cyanobacteria, and the potential of all the isolates in degrading PNP were also determined.

The linear regression model has been recommended over the other conventional models of probit and logit for the estimation of effective concentrations (EC) that cause a certain percentage of inhibition in microbial
toxicity tests. Therefore, the EC50 (effective concentration causing a 50% inhibition) values of PNP and its metabolites, PNSP and PAP, towards selected soil isolates of microalgae and cyanobacteria were determined following the linear regression analysis. Growth yield, in terms of optical density of the cultures, was used as the toxicity criterion. The EC50 values of the selected toxicants towards the test organisms were in the range of 32 to 227 μg/ml. The growth response of any test culture for the EC figure of different toxicants was found to be variable indicating the differential toxicity pattern. Such a wide range in EC values emphasizes the need for a prior determination of EC values of a toxicant before establishing its toxicity pattern toward a nontarget microorganism.

The impact of PNP and its metabolites on cellular constituents in all the test cultures at EC25 and EC50 was determined. In general, each of the three phenolic compounds significantly affected production of chlorophyll a, carotenoids, phycocyanin, DNA and RNA. Microalgae were more sensitive to the phenolic compounds than cyanobacteria. The nontarget effects of PNP and its reduction metabolites on above toxicity criteria was greatly affected.

Any disturbance in carbon and nitrogen cycles may lead to alterations in the energy budget of the cell. Pollutants affect not only the rate of carbon flow in a given metabolic pathway but also the contribution of different pathways to the total metabolism of an organism. The protein profiles of the organism can be considered a diagnostic tool in assessing the physiological status of the organism as a whole. Following are some of the biochemical parameters employed to study the impact of the toxicants on selected microalgae and cyanobacteria.
The total carbohydrates, activities of enzymes in carbon metabolism such as aldolase, dehydrogenases (succinate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase), and starch phosphorylase, were measured in the presence of toxicants at both the EC levels. The overall response of the toxicants showed a differential response in all the four test cultures. The data on the impact of PNP and PAP on metabolism of carbohydrates indicated that these two toxicants up to EC25 greatly enhanced the activities of different enzymes. The observed inhibition in the enzyme activities at EC50 might be due to possible degradation of these enzyme protein molecules, and this was further confirmed by increased proteolytic activity and consequent increase in amino acids in amino acid pool. PNSP, on the other hand, was inhibitory to the activities of enzymes involved in carbohydrate metabolism.

Free amino acids, total proteins, and enzymes implicated in the nitrogen metabolism such as alanine aminotransferase, aspartate aminotransferase, glutamate dehydrogenase, nitrate reductase, glutamine synthetase, protease, were measured in presence of the toxicants at EC25 and EC50 levels. The contents of free amino acids were unaffected while total proteins decreased significantly when the cultures were exposed to PNP and PAP. Such a decrease in total protein content may be ascribed to enhanced proteolysis under the impact of toxicants’ stress. However, PNSP significantly increased the total proteins together with the enzymes involved in nitrogen metabolism. The enhanced activities of alanine aminotransferase and aspartate aminotransferase, in particular, indicate the implications of ketogenesis essential for the production of energy during citric acid cycle. The observed inhibition toward the activity of nitrate reductase by PNP and PAP at both the levels may
be due to decline in the rate of enzyme synthesis. The decreased activity of glutamate dehydrogenase was due to inhibition in glutamate oxidation. The results suggest that PNP and its reduction metabolites, PNSP and PAP, cause marked changes in enzymatic activities associated with nitrogen metabolism.

Iron is the most essential of the trace elements, and is required for photosynthesis and nitrogen fixation. Due to the presence of two valencies (Fe$^{2+}$ and Fe$^{3+}$), iron is involved in the most oxidation-reduction reactions in the biological system. The acquisition of iron by microorganisms requires the production of high-affinity chelating agents known as siderophores which are essential for solubilizing iron in a biologically available form. Siderophore produced by species of *Nostoc* was characterized by employing TLC and IR spectroscopy, and was tentatively identified as schizokinen. Siderophore was assayed in all the test cultures in the presence of both EC25 and EC50 levels of the toxicants. Siderophore production in the presence of toxicants was greatly inhibited. However, when the culture medium was supplemented with glucose (0.5%), succinate (0.5%), or ATP (10 μM), the test cultures were significantly relieved of the toxicants' stress.

The biodegradation of PNP at 180 μM and 360 μM was carried out using species of *C. vulgaris*, *S. bijugatus*, *N. muscorum* and *N. linckia*. The rate of biodegradation was in direct relation to the inoculum density of the cultures. At the time of PNP disappearance, the yield of nitrite was about 80 to 85% of added PNP. The degradation of PNP was fairly rapid with PNP-grown cells than with uninduced cells.

PNP biodegradation was also studied using immobilized cells of each of the test cultures employing calcium alginate beads to entrap the cells. The
rate of PNP degradation by immobilized cells was more or less similar to the
degradation rate in free cells, and was directly related to the stocking density of
cells in alginate beads. There was no loss or decrease in PNP-degrading ability
of the beads stocked with test cultures when stored at 4°C even for 8 months.

To sum up, following are the salient findings of the present study
relating to interactions between the selected toxicants and the strains of
microalgae (C. vulgaris, S. bijugatus) and cyanobacteria (N. muscorum and N.
linckia) isolated from soil.

• The effective concentration (EC) values of PNP and its metabolites,
PNSP and PAP on growth of selected microalgae and cyanobacteria were in the range of 32 to 227 μg/ml.

• The cellular constituents such as chlorophyll a, carotenoids, phycocyanins,
DNA and RNA were significantly in low concentrations in cultures treated with
the phenolic compounds.

• PNP and its reduction metabolites, greatly altered the activities of enzymes involved in carbon and nitrogen metabolism.

• PNP exerted significant toxicity towards siderophore production in cyanobacteria than its reduction metabolites.

• PNP was degraded fairly rapidly by the selected cultures, liberating stoichiometric amounts of nitrite.

• Cells of the test cultures immobilized in alginate beads were equally active in degrading PNP.