1. INTRODUCTION

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1.1 POLLUTANTS IN THE ENVIRONMENT

Impact of human activities on the environment has reached a global scale. Soil matrix and water bodies, in the past couple of decades, have become the sinks for chemical and radiation pollutants. This is due to the direct and continuing impact of industrial revolution. The industrial effluents released into the environment normally get diluted to low concentrations, but gradually their levels are rising in the air. Residues of pesticides and fertilizers, aimed at increased agricultural output, add considerably to the chemical contamination of soil and water bodies. The widespread use of aerosols and chlorofluorocarbons has resulted in increased incidence of UV light on the earth surface by depleting the ozone layer.

The earth can act as a protecting or buffering agent by absorbing these polluting materials irrespective of their source or origin. However, when these chemical and radiation pollutants reach a certain threshold value they may disrupt various biological processes which probably will lead to an imbalance in the ecosystems (UNEP, 1972; UNESCO/UNEP, 1977).

1.1.1 Pesticides in the Environment

The fertility of soil depends not only on its physical constituents and its stock nutrients, but also on the intensity of the biological processes that take place within it. Soil microflora carry out various processes which are favourable to the growth of plants; for example, they fix atmospheric nitrogen and break down animal and plant remains into components which are more easily available to the plants (Alexander, 1961). Thus, soil appears to be in a state of biological equilibrium. But, this
equilibrium is delicately maintained in the soil. Each disturbance in the environment presents a risk of modifying the activity of microflora and consequently the fertility of the soil.

Widespread use of pesticides has become imperative in modern agriculture. Even though most of the pesticides are degraded quickly by various soil microorganisms, a considerable portion of them persist in the soil for a long time. Pesticide residues in the food chain have been of great concern. It is feared that continuous exposure to minute concentrations of pesticides might cause unforeseen chronic illness or genetic damage to the organic (living) body of the ecosystem.

Soil, which acts as a sink for various radiation and chemical pollutants, may also act as a perennial source of terrestrial metal pollution. Mercury compounds, which have been found to be very effective fungicides, insecticides, herbicides and bactericides, get into the soil. Several activities, not directly related to mercury may also account for the release of substantial amount of it into the environment. These include burning of fossil fuels, production of steel, cement and phosphate, and the smelting of metals. In addition, mercury compounds are used in photography and research laboratories. Industrial effluents are yet another source of mercury contamination.

It has been shown by many researchers that phenyl mercury can cross the placental barriers and affect the developing mammalian embryo (Gale and Ferm, 1971; Gale and Hanlon, 1976). Matsumura et al (1971) demonstrated metabolic conversion of phenyl mercury to diphenyl mercury which is more stable and toxic. Mutagenicity of mercury compounds in prokaryotes and eukaryotes has been studied by many
workers (Fiskesjo, 1979; Gayathri and Krishnamurthy, 1985). Ceresan, a mercurial fungicide containing phenyl mercury acetate has been shown to be mutagenic in Drosophila (Gayathri and Krishnamurthy, 1985). Adverse effects of ceresan has been studied in *E.coli* by Tewary (1982). Ceresan has also been proposed to intercalate with nitrogenous bases of DNA in *A.vinelandii* (Saini, 1987).

1.1.2 UV Radiation in the Environment

Life has evolved in this world in which major source of energy essential for biological processes is in the form of radiation. Radiation impinging on the earth’s surface is utilized by living organisms in a variety of ways. For example, sunlight provides heat and energy for photosynthesis. Most of these radiations are not only harmless but are actually necessary for life. However, high energy radiation such as UV radiation reaching the earth’s surface is known to produce deleterious effects on all forms of life.

Ultraviolet radiation ranging from 180 to 400 nm has been divided into three parts. Short wave UV which covers 180-285 nm is termed as far-UV or UV-C, whereas the wavelength ranging between 285-315 nm has been grouped into near-UV or UV-B. And the rest, upto 400 nm, is UV-A. UV radiation, which constitutes an important part of solar spectrum is filtered out by stratospheric ozone layer, particularly in the region of 200-310 nm. The stability of ozone layer in stratosphere has become a subject of great concern in the recent years. According to the recent estimates, at the beginning of 21st century the density of the ozone layer may drop by 3 to 5% (Druzhinina, 1990). Many researchers connect this deplorable situation with
the overall rise in air pollution due to chemical pollutants such as aerosols and chlorofluorocarbons. This has resulted in the increased incidence of UV-B on the earth’s surface. Artificial devices such as sun lamps and therapeutic lamps which emit UV radiation are also responsible for environmental contamination. Most of the known effects of UV radiation are damaging, therefore the possibility of increased exposure to solar UV radiation gives particular cause for concern.

1.1.3 UV Radiation: DNA as a Target

The primary target for the lethal and mutagenic effects of far-UV radiation (UV-C) in living organism is DNA. It has been shown that nucleic acids show very strong absorption characteristic in the UV-C region (around 260 nm). Absorption of UV radiation energy results in the electronic excitation of the molecule. This facilitates the formation of various photoproducts such as cyclobutane rings (pyrimidine dimers, particularly thymine dimers) which are chemically very stable (Brash and Haseltine, 1982). In addition to these thymine dimers, formation of several other lesions such as DNA - protein cross links (Smith, 1978; Larcom and Rains, 1985), pyrimidine-purine adducts (Bose et al, 1982) and pyrimidine - pyrimidine adducts (Varghese and Wang, 1967) have also been reported.

It has been shown that cyclobutane rings act primarily by inhibiting DNA template activity (Barskaya and Kaptiskaya, 1983). Irradiation of xeroderma fibroblasts with UV leads to a decreased rate of incorporation of $^3$H-Tdr in DNA (Rosenstein, 1984).
In the living organisms, DNA functions as a store house of genetic information. Therefore, damage caused to this molecule either by chemical mutagens or by irradiation will have grave consequences. However, it has been observed that living organisms are equipped with some repair mechanisms which accomplish the task of correcting the damage in DNA molecule and maintaining the genetic integrity. Three repair mechanisms have been reported.

1.1.3.1 Photoreactivation

It refers to a phenomenon in which pyrimidine dimers are monomerized by using visible light energy in the range between 300-500 nm with the help of DNA photolyase (Smith, 1978). Cyclobutane dimers in DNA, polynucleotides or oligonucleotides greater than about 10 nucleotides in length are the only substrates for DNA photolyase (Sutherland, 1981). It has been shown that photoreactivation proceeds without making any nick in the substrate (DNA) either in the presence or absence of light.

DNA photolyase purified from a variety of prokaryotes, including E.coli, has been shown to possess FAD as intrinsic chromophore (Eker, 1980; Eker et al, 1981; Sancar and Sancar, 1984). On the other hand, the enzyme (DNA photolyase) isolated from yeast cells has been shown to consist of two non-identical subunits, none of which when alone is catalytically active (Friedberg, 1988). However, mixing of both the subunits restores enzyme activity (Boatwright et al, 1975). It has been reported that DNA photolyase loses its activity when the cells irradiated with UV, are incubated in the dark (Resnick and Setlow, 1972).
1.1.3.2 Excision repair

It is a coordinated series of biochemical reactions which eliminate pyrimidine dimers during the postirradiation cell growth in the absence of photoreactivating light. It has been reported that in the living organisms excision repair is the major repair pathway employed for the removal of UV induced damage (Friedberg et al., 1977; Smith, 1978; Friedberg, 1985; Rubin, 1988). The process starts with the incision of DNA phosphodiester backbone on the 5'-phosphate side of the dimer. Biological evidence shows that the incision step of the excision repair pathway is a 'SOS' response in E.coli (Walker, 1985). The incision step has been described using UvrABC enzyme complex which constitutes a part of 'SOS' system in E.coli (Sancar and Rupp, 1983).

The UvrA protein identifies DNA damage by recognizing a major distortion in the three dimensional shape of the DNA double helix (Rubin, 1988). The binding of UvrA protein to the damaged DNA results in unwinding of the double helix. UvrB protein binds to UvrA-DNA complex which inturn forms a site for the binding of UvrC protein. The formation of UvrABC-DNA complex is followed by DNA incision. It has been reported that several other cellular proteins, such as UvrD protein, enhance the efficiency of both DNA incision and the removal of damaged oligonucleotides during excision repair (Kumura et al., 1985). The resulting gaps are filled following the synthesis of new DNA, by attaching recA protein to the single stranded region and the dimer containing segment is replaced. The repair process is completed by a ligase which joins newly synthesized DNA to the phosphodiester backbone of parental DNA.
1.1.3.3 Postreplication repair

This pathway primarily operates through a combination of replication and recombination processes in which DNA fragments are synthesized in lengths corresponding to the predicted distances between pyrimidine dimers in the parental strands. This leads to the formation of gaps in the daughter strand which undergoes several rounds of replication and recombination before diluting out DNA lesions and makes a new viable strand (Ganesan and Smith, 1971; Smith, 1978). It has been shown that these gaps opposite to the pyrimidine dimers are different from those produced in excision repair. The gaps produced in the latter are located on the opposite template and can be repaired by repair synthesis. On the contrary the gaps produced during postreplication repair are located on the opposite, noncoding DNA strand and are therefore, not repairable by repolymerization of the missing bases (Mookerjee and Bhattacharjee, 1984). Mistakes are occasionally inserted into the genomic molecule during repair processes which might result in mutations. Hence, repair events are viewed as a means of organic evolution (Smith, 1978).

1.2 NITROGEN FIXATION

Among many biological processes which contribute to the healthy upkeep of ecosystems, biological fixation of atmospheric nitrogen is of prime importance. It is a fundamental process by which most organisms obtain nitrogen for their metabolism. The fixed nitrogen is the source for proteins, nucleotide biosynthesis and several other nitrogen requiring metabolic pathways. Diazotrophically growing organisms utilise a considerable amount of energy to reduce molecular nitrogen into ammonia (Roberts and Brill, 1981). To maintain a dynamic equilibrium between atmospheric nitrogen and
the contents of fixed nitrogen in the ecosystem, ammonia is converted into nitrogen
gas by the process of denitrification. Nitrogen gas, thus produced, is lost in the
atmosphere. Nevertheless, the biological world stays ahead of nitrogen deficiency as
the rate of fixation of nitrogen is higher than the rate of denitrification.

Microorganisms such as *Rhizobium* carry out nitrogen fixation by symbiotic
association with the roots of leguminous crops (Newcomb, 1980). The other
group of prokaryotes include non-symbiotic microorganisms such as *Clostridium,*
*Cyanobacterium* and the members of Azotobactereaceae. They can fix atmospheric
nitrogen independently in the soil and water.

1.2.1 *Azobacter vinelandii*

*Azobacter vinelandii*, a member of the family Azotobactereaceae has been
selected as a model microbe system for the present study. It is a free living aerobic,
gram negative, nitrogen fixing bacterium which for centuries has been used as a
biofertilizer (Apte and Shende, 1981). In addition to its ability to fix atmospheric
nitrogen, *Azobacter* can also produce vitamins, antibiotics and growth hormones
such as indole acetic acid (Apte and Shende, 1981).

1.2.2 Nitrogenase Complex

Nitrogenase complex which is responsible for the reduction of dinitrogen to
ammonia consists of two distinct components in *Azotobacter vinelandii*. One, usually
called Fe protein with a molecular weight of 60,000 daltons, consists of two apparently
identical subunits together with a cluster of four iron and four labile sulphur atoms
(Orme-Johnson, 1977). The second component, the MoFe protein (200 kDa), consists
of four subunits (Burris et al, 1980). Because reduction of nitrogen takes place on the MoFe protein, it was suggested that this component should be called as dinitrogenase or true nitrogenase and Fe protein as dinitrogenase reductase (Hagerman and Burris, 1978).

Nitrogenase complex has an absolute requirement for ATP. It has been observed that to reduce one molecule of dinitrogen, nitrogenase complex requires six molecules of ATP. The process starts with the attachment of MgATP to dinitrogenase reductase (Fe protein) which causes a decrease in the membrane potential from -300 mV to about -400 mV (Zumft et al, 1974). This is accompanied by the unfolding of the strand of dinitrogenase reductase so that Fe moiety is exposed in such a fashion that it enables chelating agents to react directly with Fe. Electrons now flow from dinitrogenase reductase to dinitrogenase with the concomitant hydrolysis of MgATP molecules. After the transfer of each electron, components of dinitrogenase reductase: dinitrogenase complex dissociate from each other (Hagerman and Burris, 1978). This is followed by the transfer of electrons to the molecular nitrogen.

1.2.3 Oxygen Toxicity

The toxicity of oxygen is a common phenomenon among all organisms. It has been reported that the complete reduction of oxygen to water, which requires four electrons, proceeds most readily by a stepwise pathway and generates partially reduced intermediates such as superoxide radicals (O₂⁻) (Hamilton, 1974). The superoxide radicals are too reactive to be tolerated within a living system (Fridovich, 1975, 1979).
The primary defence is provided by superoxide dismutase (SOD) which catalytically scavenges \( \text{O}_2^- \) and converts it to \( \text{H}_2\text{O}_2 \) and oxygen (McCord et al, 1979). Hydrogen peroxide, which is dangerously reactive, is removed by catalase and peroxidase which convert it to water and oxygen (McCord and Fridovich, 1969).

1.2.3.1 Superoxide dismutase

Facultative anaerobes such as *E.coli* possess only FeSOD as a constitutive enzyme when cultured under anaerobic conditions (Hassan and Fridovich, 1977). But on exposure to high oxygen conditions MnSOD is induced (Hassan and Fridovich, 1978). A similar case has been noticed with *Aquaspirillum magnetotacticum* cells growing under anaerobic conditions where FeSOD contributed 88\% of the total activity. However, increased aeration caused induction in the synthesis of MnSOD relative to that of FeSOD (Short and Blakemore, 1989). A very high content of FeSOD has been reported in *Rhizobium japonicum*, an aerobic nitrogen fixing bacterium, which can be induced under increased aeration (Stowers and Elkan, 1981). Buchanan and Lees (1980) have described the purification, characterization and intracellular localization of MnSOD in *Azotobacter chroococcum*. On the other hand *Azotobacter vinelandii* possesses a high concentration of only FeSOD. So far MnSOD is not reported from this species.

1.2.4 Oxygen Protection

The most striking feature of nitrogenase complex is the sensitivity of this enzyme complex to oxygen. Both the components of nitrogenase system viz., dinitrogenase and dinitrogenase reductase, are irreversibly damaged by oxygen and
special anoxic handling techniques are required for their purification (Postgate, 1974). However, in *Azotobacter* the crude extract of nitrogenase complex has been shown to be relatively resistant to oxygen; but in purified form it was found to be oxygen sensitive (Robson and Postgate, 1980). Mossbauer studies (Kelly and Lang, 1970; Smith and Lang, 1974) as well as EPR (Palmer *et al.*, 1972; Smith *et al.*, 1972; Evans *et al.*, 1973) have indicated that exposure of MoFe proteins to oxygen results in a sequence of changes which is probably associated with various oxidation states of the Fe atoms (Smith *et al.*, 1972; Evans *et al.*, 1973). X-ray absorption edge fine structure spectroscopy (EXFAS) indicates that Mo-O bonding in MoFe protein is formed only after oxygen inactivation (Cramer *et al.*, 1978). All these studies indicate that *Azotobacter* needs oxygen to grow but must keep it away from its nitrogenase. To accomplish this task, *Azotobacter* has some unique adaptations. Some of these are discussed below.

### 1.2.4.1 Morphological protection

*Azotobacter vinelandii* cells grown under oxygen stress conditions form aggregates which probably result in only a portion of the cells experiencing deleterious effect of oxygen. This helps the cells to restore nitrogenase activity. Besides this, *Azotobacter* cells have also been shown to possess an extensive internal membrane system which divides each cell into separate aerobic and nitrogen fixing regions (Oppenheim and Marcus, 1970). The nitrogen fixing machinery is in close association with the membranes or particulate fragments which protect it when oxygen concentration is too high (Patel *et al.*, 1973).
1.2.4.2 Conformational protection

Exposure of *Azobacter* cells to high oxygen concentration causes nitrogenase system *in vivo* to assume a conformation in which it is protected from molecular oxygen. In the conformationally protected stage, nitrogenase complex has been shown to be in a totally inhibited stage. Activity of the enzyme returns rapidly when aeration is lowered, without *de novo* synthesis of nitrogenase (Yates, 1970). Recent studies have shown that conformational protection plays an important role when aeration is extremely high (Post et al. 1982, 1983). The conformational protection of nitrogenase results from association of the same with an iron sulphur protein (Haaker and Veeger, 1977), which is also called as Shethna protein. This iron sulphur protein, which is unique to *Azotobacter*, forms a complex with both the components of nitrogenase system. Isolation of an oxygen stable complex has shown that this iron-sulphur protein forms a 1:1:1 or 1:2:1 complex with Fe and MoFe proteins of nitrogenase (Wang et al, 1985). This complex is formed *in vivo* when respiratory protection is overburdened (Robson and Postgate, 1980). However, it is believed that conformational protection is a passive mechanism of protecting nitrogenases (Ramos and Ramos, 1985a,b).

1.2.4.3 Respiratory protection

Another important nitrogenase protection mechanism in *Azotobacter* is through high respiratory rate which depends on the composition and functioning of the respiratory chain. The respiratory chain of *A. vinelandii* includes several flavin dependent dehydrogenases, ubiquinone Q and a chain of cytochromes. These are arranged in the form of a complex branched chain (Haddock and Jones, 1977). Similar systems are reported in other aerobic microorganisms as well (Anraku and Gennis,
1987). Branching of respiratory chain which occurs after cytochrome b₁ provides two alternate routes of electrons to oxygen. The branch, cytochromes c₄+c₅ → o → O₂ is associated with energy conservation, whereas the other branch which forms a major pathway of electrons from cytochrome b₁ to O₂ via cytochrome d is not coupled with ATP synthesis. The presence of a third terminal oxidase cytochrome a₁ has also been demonstrated by photochemical action spectra studies (Jones and Redfearn, 1964). Besides cytochromes c₄+c₅ → o → O₂ energy conserving branch, the second energy conservation site is provided by an obligatory central segment, ubiquinone Q, which supplies electrons to cytochrome b₁. The third energy conserving site is located at NADH dehydrogenase. Thus, the complete respiratory chain of *A. vinelandii* can be represented as shown below.

\[
\begin{array}{ccc}
\text{NADH} & \rightarrow & 4 \text{ Fe-S/FMN} \\
\text{Malate} & \rightarrow & \text{Fe-S/FP} \\
\text{NADPH} & \rightarrow & \text{Fe-S/FP} \\
\end{array}
\]

\[
\begin{array}{ccc}
& \rightarrow & \text{c₄+c₅} \\
\rightarrow & a₁ & \rightarrow O₂ \\
\rightarrow & b₁ & \rightarrow d \\
\rightarrow & & \rightarrow O₂ \\
\end{array}
\]

Fe-S = Iron sulphur protein; FMN = Flavin mononucleotide; FP = Flavoprotein; Q = Ubiquinone Q; b₁, c₄+c₅, d, o = cytochromes. 1, 2 and 3 are energy conserving sites.

**Cytochromes c₄+c₅:** The isolation and purification of c₄+c₅ from *A. vinelandii* has revealed that cytochrome c₄ is a monomer having two haeme units per molecule (Swank and Burris, 1969). The molecular weight of cytochrome c₄ has been shown to be 24000 ± 2000 daltons. On the other hand, cytochrome c₅ is a dimer with a molecular weight of 24400 ± 1000 daltons. Low temperature spectroscopic studies have demonstrated that cytochrome c₄ is the main c type cytochrome present in the
phosphorylating membranes of *A. vinelandii*, whereas cytochrome c₅ is found only in trace amounts (Jurtshuk and Yang, 1979). The redox potential of both the c type cytochromes (c₄ + c₅) is in the range of 0.30 - 0.32 mV (Neumann and Burris, 1959).

**Cytochrome o**: Cytochrome o serves as a terminal oxidase in the energy conserving branch of the respiratory chain in *A. vinelandii*. It shows absorption maxima in the photochemical action spectrum at about 412-420, 534-544 and 560-572 nm (Hoffman *et al*, 1979; Edwards *et al*, 1981). The spectral overlap of cytochrome o with other b or c type cytochromes makes resolution of composite absorption bands difficult. Nevertheless, cytochrome o is generally recognized by the distinct spectral features of CO compounds. A peculiar feature of cytochrome o is its strong sensitivity for KCN. It has been reported that cytochrome o purified from *A. vinelandii* in the oxidized form reacts with KCN and leads to the formation of a compound absorbing at 433 nm (Yang and Jurtshuk, 1978b). The oxidized cytochrome shows a high spin signal of g = 6.0 which is abolished by KCN (Anraku, 1988). Although cytochrome o appears to be the major oxidase, its actual substrate is not known (Yang, 1986).

**Cytochrome b₁**: This cytochrome shows maximum absorption in the range of 559-562 nm. The characteristic feature of cytochrome b₁ is that it serves as an electron donor for the cyanide insensitive pathway (Poole, 1983).

**Cytochrome d**: Cytochrome d terminates a non-energy transducing branch of the respiratory chain in *A. vinelandii* (Jones and Redfearn, 1967b). In the oxidized form it forms a complex compound with cyanide at the physiological temperature (Kauffman and Van Gelder, 1974). Optical as well as EPR studies have demonstrated that
cytochrome d has a strong affinity for molecular oxygen and is directly involved in its reduction to water (Hata et al, 1985).

It has been reported by many scholars that the relative concentration of the components of respiratory chain depends on the availability of molecular oxygen. In oxygen limited cells, cytochromes c_4+c_5 are the most abundant species. When cells are grown with excess of oxygen, the activity of cytochromes c_4+c_5 pathway decreases and cytochrome b_1 → d → O_2 (the non-energy conservating) pathway becomes predominant and maintains a very high respiratory rate (Swank and Burris, 1969; Haaker and Veeger, 1976). *Azotobacter* has been shown to possess one of the highest respiratory quotients among all the nitrogen fixing organisms studied so far (Haddock and Jones, 1977). The high respiratory rate maintains a low dissolved oxygen concentration inside the cell and protects nitrogen fixing machinery (Robson and Postgate, 1980).

*Azotobacter* faces a problem of utilizing oxygen very fast without producing much of ATP so as not to upset the regulatory factor in the nitrogen fixation. This is achieved by uncoupling of phosphorylation at various places along the electron transport chain to drive the electron flow to oxygen without producing ATP. Spectroscopic studies also suggest that cytochrome d is a major non-energy conserving pathway which is probably involved in the protection of nitrogenase (Yang, 1984). However, the exact role of the aerobic pathway localized by cytochrome d in protecting nitrogenase from damaging effects of oxygen is not known.
1.3 PRESENT WORK: AIM AND SCOPE

The foregoing account has briefly discussed the importance of nitrogen fixation in maintaining a food web in an ecosystem. Environmental contaminants (e.g. chemical and radiation) which are sunk into the soil and water matrices can adversely affect the process of biological nitrogen fixation. Dissection of such a problem in terms of fundamental scientific research is of prime importance for a successful conservation of environment and a proper upgrading of its quality. Hence, it is imperative to study the impact of chemical and physical pollutants on the nitrogen fixing ability of Azotobacter vinelandii which is strictly an aerobic, free living and soil inhabiting organism.

Oxygen, which is exceedingly useful in energy yielding metabolism and biosynthesis also constitutes a threat to the living cells when in excess. One important aspect of O$_2$ toxicity is the O$_2^-$ which are produced during increased aeration by univalent reduction of oxygen. The superoxide radical thus generated during various biological reactions is one of the active species of oxygen which produces various cytotoxic effects in the living organisms (Fridovich, 1974a,b). Aerobic nitrogen fixing microbes need oxygen to grow but can fix nitrogen only at low oxygen concentrations. One of the most intriguing characteristics of nitrogen fixing organisms is the irreversible inactivation of both the components of nitrogenase complex by oxygen.

Methyl viologen (1,1’dimethyl, 1,4’bipyridinium dichloride), widely used as a herbicide, augments the production of $O_2^-$ in chloroplasts (Bartels and McCullough, 1972). It has been observed that methyl viologen is easily reduced to semiquinone (methyl viologen radical) which then reacts with oxygen to generate $O_2^-$ (Hassan and
The decay of methyl viologen radical is very rapid and is essentially completed in 3 μmin (Farrington et al., 1973).

When methyl viologen is administered to E. coli it subverts electrons from normal electron transport pathway. The result is an increase in cyanide resistant respiration and increase in the rate of production of O$_2^-$ (Hassan and Fridovich, 1979). Under conditions of constant aeration, methyl viologen elicits dramatic increase in the biosynthesis of MnSOD and the cells are rendered resistant to the lethal effects of hyperbaric oxygen (Hassan and Fridovich, 1977). From these studies it can be concluded that methyl viologen is an effective source of O$_2^-$. Keeping this in view, in the present study, methyl viologen was used to induce oxygen toxicity in Azotobacter vinelandii, and the role of respiratory chain and superoxide dismutase in the protection of nitrogenase complex from oxygen toxicity was studied.

The present study has been carried out in two parts:

1.3.1 Part A

In part A, the impact of chemical and physical pollutants on the nitrogen fixing ability of Azotobacter vinelandii has been studied. Two prime variables, ceresan, a mercurial fungicide and ultraviolet radiation have been chosen for the exploration of the mechanism of their interaction with Azotobacter. The interaction profiles of these two stresses (individually and in combination) with the model system have been studied.
The objective of the present study is to provide qualitative information about the effects of ceresan and UV radiation on the nitrogen fixing ability of *Azotobacter vinelandii*. The environmental stresses may influence the activity of nitrogenase complex which is responsible for the nitrogen fixing ability of *A. vinelandii*. Therefore, nitrogenase activity was assayed under the influence of above mentioned environmental pollutants.

DNA biosynthesis is the major biochemical pathway which controls almost every biochemical cycle in the living system. Any perturbation caused by chemical or physical pollutants may disturb the whole system. Keeping this in view modulation of DNA biosynthesis under these stresses was studied.

Alterations in translational processes will directly reflect upon the cellular enzyme titre. Keeping this in view, protein biosynthesis profiles were obtained to understand the perturbations imparted by these environmental variables.

1.3.2 Part B

Part B deals with the role of superoxide dismutase and the respiratory chain in protecting nitrogenase from oxygen toxicity in *Azotobacter vinelandii*.

The initial studies were carried out to determine the sublethal dose levels of methyl viologen. Based on this, future experiments were planned. It has been proposed that SOD protects nitrogenase from oxygen damage. To check this, nitrogenase and SOD were assayed during the generation of O₂.
Methyl viologen, when comes in contact of oxygen, generates $O_2^-$ and thus induces cyanide resistant branch of the respiratory chain. Under such conditions, the levels of cytochrome b$_1$ and d also should increase. This was spectroscopically determined, by checking the role of individual cytochromes in scavenging $O_2^-$. 

Further, to test the involvement of respiratory chain in the protection of nitrogenase from oxygen toxicity, respiratory chain was inhibited with KCN and the rate of respiration was monitored in methyl viologen treated membranes using an oxygen electrode.
Soil matrix and water bodies, which support a variety of microorganisms, facilitate many biological processes which are essential for the sustenance of the higher living components of ecosystem. Unfortunately in the past couple of decades, these soil and water bodies have become the sinks of chemical and radiation pollutants. These pollutants, when reach a certain threshold, deleteriously act upon the microbes which ultimately results in the disruption of various useful biological processes. A fuller understanding of the interaction of these chemical and physical pollutants with the microbial component is a fundamental necessity to cope with the problem of environmental protection. In this part of the study an attempt has been made in this direction.

Two pollutants, ceresan (an organomercurial fungicide) and UV radiation (253 nm), have been employed in this study. These variables have been used singularly and in combination. The nitrogen fixing soil bacterium *Azotobacter vinelandii* has been chosen as a model organism for study. The parameters monitored are growth of cells, DNA biosynthesis, protein biosynthesis and nitrogenase activity.