GENERAL

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The agricultural and industrial revolution has resulted in many materials which are anthropogenic and do not resemble the natural ones. These materials may be synthetic products or their metabolites or wastes of industrial production activities. Major sources of xenobiotic compounds are from agrochemical industries, petroleum industries, metal processing and aromatic industries. Almost daily media carry alarms about the threat of pesticides to our health and environment. No doubt we are living in hazardous conditions due to combination of the environment pollutants like heavy metals, organic, inorganic compounds, plastics etc. The pesticides are the most significant among them (Hermann et al., 1992). Pesticides are added to the soil for the purpose of killing or injuring some form of life (Prakash and Arora, 1998). Perhaps hazardous pesticide management is the most challenging task before the people of life science.

Serious contamination issues arise at waste disposal sites close to agricultural fields and at pesticide production facilities, due to inappropriate handling and improper storage (Hernandez et al., 1997). Another problem is buildup of wastes. In undeveloped countries, there are about a hundred thousand metric tons of obsolete pesticides that are no longer usable. These pesticides have simply expired; their storage conditions are very poor with inadequate safety measures, resulting in improper containment, leaks, filtration into soil and water bodies, and accidental spills. Environmental hazards and health risks caused by obsolete pesticides could therefore, potentially affect many countries (Martinez, 2004).

The increased use of pesticides in the agricultural systems caused the contamination of soil with toxic chemicals. When pesticides are applied, the possibility exists that these chemicals may exert certain effects on non-target organisms including soil micro-organisms (Slavikova and Vadkertiova, 2003; Khairella, 2006). The use of pesticides has become an integral essential part of
modern agriculture. Pesticides are often applied several times during one crop season and a part always reaches the soil. The wide use of pesticides has created numerous problems, including the pollution of the environment (Digrak and Kazanini, 1999). The influence of pesticides on soil microorganisms is dependent on physical, chemical and biochemical conditions, in addition to nature and concentration of the pesticides (Aurelia, 2009).

Pesticides definitely have been proved to be of great value in reducing the damages caused by the pests that affect the agricultural crops, livestock and vector born human diseases. But adverse affects of pesticides are also a cause of concern. Most of the chemicals which are used as pesticides are not only highly selective, but are generally toxic to many non-target organisms like soil micro flora, domestic animals, aquatic animals, birds, including man (Murphy, 1980; Okon 1985). Extensive work has been done on the toxicity of heavy metals and pesticides on higher organisms like rats (Bernandin et al., 1981, Suke et al., 2008), Cats (Cholak and Hubbard, 1944), rabbits (Carbon and Friberg, 1957), Fishes (Balavenkatasubbaiah, 1983; Pandey et al., 2001, Faromb et al., 2008) and amphibians (Zaheer et al., 2003). It has also been reported from our laboratory that studies on mammalian species have shown that some of the pesticides play an important role in chronic poisoning in human beings, and in animals causing potent effects on the development of follicles of ovary, spermatogenesis, enzyme activities and biochemical constituents (Math and Kaliwal, 1995; Nanda and Kaliwal, 1995; Dhondup and Kaliwal, 1997; Soratur and Kaliwal, 1997; Asmathbanu and Kaliwal, 1997; Hiremath and Kaliwal, 2000; Jadramkunti and Kaliwal, 2001; Mahadevswnamy et al., 2001; Baligar and Kaliwal, 2001; Radhika and Kaliwal, 2001, Sudheer and Kaliwal, 2006; Shreelakshmi 2007; Shreelaksmi and Kaliwal, 2007; Sudheer and Kaliwal, 2009; Mudaraddi and Kaliwal, 2009).

The use of animal models is not convenient as it is difficult to get and rear a particular type of animal and economically also it would work out costlier. During the recent years the toxicity testing of environmental pollutants
is being shifted towards the microbial models. Protozoa, algae, fungi and bacteria are being extensively used as models for the toxicity testing for their sensitivity to various environmental pollutants and many microbial systems are being standardized as the indicators of pollutants (Kulkarni et al., 2004). Harmful effects of contaminants on the ecosystem and humans can not be assessed by standard chemical analysis of environmental samples, therefore toxicity tests using live organisms or cells represent a vital part of environmental monitoring. Many different biological methods based on native or genetically modified microorganisms as test-species, have already successfully been applied to environmental toxicity/genotoxicity assessment. An important reason is the modern 3R concept (reduction, replacement, refinement) in toxicology and ecotoxicology, which promotes the application of microorganisms in biotests due to simple cultivation in axenic cultures and due to the lack of ethical problems (Logar and Vodovnik, 2007). The first biotests for environmental monitoring were based on multi-cellular eukaryotic organisms, in particular fish and mammals (Inouye, 1994; Farre and Barcello, 2003). As they were relatively expensive, time-consuming, difficult to standardize and ethically questionable, the need for alternative biological methods for environmental monitoring based on 3R strategy (Reduction, replacement, refinement) soon became evident (Hartung et al., 2003). The development and standardization of toxicity tests based on prokaryotic (bacteria) or eukaryotic (protozoa, unicellular algae, yeasts) microorganisms instead of higher organisms has enabled fast and inexpensive screening of environmental samples for toxic and genotoxic effects (Rogers, 2006; Logar and Vodovnik, 2007).

The microbial biomass plays a very important role in the soil ecosystems where they fulfill a critical role in nutrient cycling and decomposition (De Lorenzo et al., 2001). The prokaryotes compose an interesting group of microorganisms, which can be used as instruments for scientific investigations. This can be explained by the fact that they possess intrinsic properties, such as reduced time of generation and relatively low cost of culture and maintenance
Pseudomonas is a versatile genus and previous reports suggested that this genus could degrade a number of chemicals like p-nitrophenol and parathion (Doughlas et al., 1974), carbaryl (Vandana et al., 2005), malathion (Hashmi et al., 2004), bethozaxin (Wallace and Dickinson 2004), chlopyrifos (Ajaz et al., 2005), Trichlory butoxyethyl ester (Fulejar et al., 2009), propiconazole (Sarkar et al., 2009). Escherichia coli is a common bacterium of the intestinal tract of warm blooded animals. It is an important parameter for the metabolic and genetic characterization of cells of more complex organisms (Kappke, 2005). The most dominant genera of bacteria isolated from soil were Escherichia, Shigella, Xanthomonas, Acetobacter, Citrobacter, Enterobacter, Moraxella and Methyllococcus. The bacterial isolates identified in the study reported by Bahig et al., (2008) were mostly represented by gram-negative bacteria, which have been often found in wastewater polluted soils. The role of microorganisms is mostly indirect, but still significant. They are used as artificial factories (expression systems) for recombinant purposes. Since Escherichia coli provide the most popular expression system, much research has been done to maximize the expression levels of recombinants in this system (Logar and Vodovnik, 2007). It was for these obvious reasons that Escherichia coli was used in our studies and compared to the soil isolate Pseudomonas aeruginosa for its bioremediation ability.

To avoid the problems of handling and cell separation, which are associated with the use of free bacteria, cell immobilization is one of the most attractive alternatives. Nowadays, the most widely accepted technique is the immobilization technique which is successfully employed in various industrial applications and also in environment field like xenobiotics, biosensors, purification of water etc. immobilization systems have advantages over free systems like continuous usage, to maintain the microbes in log phase, reducing the culture time, better storage, efficiency, longevity, productivity etc study of immobilization technique for environmental applications opens newer ideas to manage environmental problems (Prasad, 2009). The main advantages of the
immobilized whole micro-organisms are their higher operational stability, high cell density and their use in continuous reactors (Hulst et al., 1985). Recently, immobilized cell processes have received increasing attention in the field of waste water treatment. A considerable amount of information is available on the advantages of immobilization (Cheetam 1980; Chibata et al., 1986).

Remediation efficiency of xenobiotic pollutants by microbial cells remains a major challenge to environmental engineers and biotechnologists. Current methods of removing pollutants from waste water including microbial degradation adsorption onto different matrices, chemical oxidation, solvent extraction or irradiation. One of the cheapest possible solutions to resolve the pollutant contamination problems is by bioremediation using immobilized cells (Beshy et al., 2002). The main advantages in the use of immobilized whole cells are their higher operational stability, ease of use in continuous reactors, high cell density and ability to scale up (Cassidy et al., 1996). Among the various matrices available for cell immobilization, alginate beads are more predominant, because it is simple and cheap, readily available and non-toxic to cells and the production methods do not require drastic conditions (Carvalho et al., 2003; Patil et al., 2005; Kulkarni and Kaliwal, 2008; Kulkarni and Kaliwal, 2009). The entrapment of cells in alginate is a promising method for microbial degradation of toxic substance (Kewloh et al., 1989).

The method of cell immobilization seems to more promising in the development of biotechnology for the removal of xenobiotic bearing effluents (Murugesan, 2003). The immobilized cells also have valuable applications in industries for the production of biochemical's (Bisping and Rehm, 1988). Since the entrapped cells remain viable for a considerable duration they would be a better alternative against free cells for the bioremediation of variety of organics from effluents (Karigar, 2006). Immobilized cells have been suggested for many applications (Denkova et al., 2004). Advantages of immobilized cell technology include continuous utilization, retention of plasmids bearing cells, prevention of interfacial inactivation, stimulation of production and excretion of secondary metabolites and protection against turbulent environment (Nath
Immobilization of cells will lead to effective enhancement of cell concentration, survival and increase of process efficiency (Groboillot et al., 1994).

Assessing the side-effects of the pesticides on soil microbial ecosystems is important to maintain soil fertility and to prevent critical damage to the agricultural ecosystems (Anderson, 1978; Francis et al., 1987). As soil microbial parameters, such as microbial population, biomass, and community structure could be affected by natural stresses and fluctuate in the environment, the side-effects caused by pesticides should be evaluated by comparing them with those caused natural stresses (Domsh et al., 1983; Itoh et al., 2003). It is well known that pesticides cause deleterious effects in all living organisms. Biochemical indices are sensitive index to the changes due to pesticide toxicity and can constitute important diagnostic tool in toxicological studies (Dabrowska and Wlasow, 1986). The biochemical responses of non target organisms to pesticides can be used to predict early warning of pesticide in non-target animals and these responses are quite effective for rapid detection (Pant et al., 1987; Mandal and Lahiri, 1989).

Oxidative stress is a disbalance between reactive oxygen species (ROS) generation and detoxification resulting in the increased levels of enzyme activity. ROS are of increasing interest in environmental toxicity as they may provide insights to toxicity mechanisms and may identify novel biomarkers. ROS can modify and inactivate proteins in a variety of ways (Fagan et al., 1999; Choy et al., 2008). It is commonly recognized that *Escherichia coli* is the most suitable model system for the investigation of the cell response to oxidative stress (Semchyshyn et al., 2004). When organisms or cells are exposed to low levels of certain harmful physical or chemical agents, the organisms acquire an induced tolerance against the adverse effect. (Benjamin et al., 1998; Flahaut et al., 1996). The effect of hydrogen peroxide on the activity of Sox RS and Oxy R regulon enzymes in different strains of *Escherichia coli* has been studied by Semchyshyn et al., (2004). Yao et al., (2006), have reported the response of stress enzymes in *Escherichia coli*, *Pseudomonas* and *Bacillus subtilis* on exposure to Acetemaprid.
The stress proteins in *Escherichia coli* have been characterized using heat, radiation, heavy metals, oxidizing agents, nutrient starvation, the SOS response, and organic solvents (Van et al., 1987; Lindquist 1991; Martin 1994; Kobayashi et al., 1998; Asghar et al., 2005). The fact that specific patterns of proteins are expressed for a particular stress has led to the use stress proteins to monitor the environmental samples for the presence of particular pollutants (Belkin et al., 1996; Sanders et al., 1993). Such unique proteins could be purified and be used as biomarkers of the xenobiotics in the environment (Kulkarni and Kaliwal, 2009). Comparison of gene/protein expression profiles of different test microorganisms and higher organisms may provide useful information about the possibility of extrapolation of the effects of toxic chemicals across species. Determining similarities and dissimilarities in toxicity mechanisms across species would give the answer where the extrapolation of chemical hazards from one species to another is technically valid. The knowledge about conservation of toxicity mechanisms in organisms will therefore, enable to choose appropriate model organisms at lower levels of biological organization (e.g. microorganisms) for relevant monitoring of specific environmental toxicants. The use of genomic, proteomic and metabolomic techniques may also provide the possibility to predict toxic potential of unknown chemicals by comparing specific patterns of expression, reflecting the mode of action of unknown chemical, with expression profiles of known toxicants.

Bioremediation is the use of microorganisms, or biologically active agents to degrade, sequester, or conjugate environmental pollutants. Advantages of bioremediation include ease and timing of application, ability to target specific pollutants, decreased sludge volume, and decreased ecological hazard (Eerd et al., 2003) and is a common method for the removal of organic pollutants because of its low cost and low collateral destruction of indigenous animal and plant organisms (Liu et al., 2007). Studies of microbial biodegradation are useful in the development of strategies for detoxification of pesticides by microorganisms (Qiu et al., 2006; Hernandez and Salinas, 2010).
Microbial degradation of hazardous waste offers a promising strategy by which such chemicals may safely be detoxified and bioremediation is an effective method that could be improved as a toll for cleaning of natural environment and the degrading genes present in the main genome or plasmid or both which are specified to multipotent strain are excellent and effective tool in bioremediation (Hernández, 2002; Nazarian and Amini, 2008). Most of the soil isolates that were tolerant to methomyl were gram negative (Gordon and Debson, 2001). It was reported that *Pseudomonas, Alcaligenes PA-10* and *Agrobacterium radiobacter* degraded flouranthene. Gram negative bacteria degraded bromoxynil both in batch and continuous culture (Muller and Gabreil, 1999). Odokuma and Akubuenyi (2008) have reported that the organochlorine, pesticides lindane and dieldrin were more toxic than organophosphate pesticides, pirimphos methyl and malathion to *Nitrosomonas, Nitrobacter* and *Thiobacillus*. The carbamates benomyl and methomyl are equally as toxic as the organochlorines to these microorganisms. Degradation of pesticides is usually beneficial, since the reactions that destroy pesticides convert the residues in the environment to inactivate, less toxic, harmless compounds (Lan et al., 2006). Microorganisms play an important role in soil ecosystems, especially in nutrient cycles. Studies have been conducted regarding the side effects of pesticides on soil microbial populations, biomass and functions (Hill, 1978; Francis et al., 1987) and means by which the xenobiotic compounds are removed from the environment thus preventing from pollution problems (Digrak and Oczelik, 1998). Soil microbial flora has also been considered to be an important parameter for characterizing soil microbial ecosystems (Ritz et al., 1996; Itoh et al., 2003). The soil microorganisms are capable of degrading a wide variety of chemical compounds, from polysaccharides, amino acids, proteins, lipids to more complex materials such as pesticides (Hernández, 2002; Ahmed and Ahmad, 2006). Diverse bacterial genuses are adapted to develop in polluted soils with pesticides. These microorganisms present enzymes involved in the hydrolysis of P-O, P-F, P-S and P-C bonds, which are found in a wide variety of organophosphate pesticides (Singh and Walker 2006). Some soil
isolated bacteria are capable of degrading pesticides such as methyl parathion and ethylic parathion, normal doses of pesticides has only slight effects on soil microflora and microorganisms are capable to recover rapidly (Digrak, 2001; Aurelia, 2009; Sarkar, 2009).
METHOMYL

Methomyl (S-methyl-1-N (methyl carbamoyl) oxy] thioacetimidate is a broad spectrum insecticide. Methomyl was first synthesized in the United States in October, 1968 by E.I. Dupont de Nemours and Co. for use as an insecticide in commercial plantings of chrysanthemum, cabbage and cauliflower. Methomyl is effective in two ways, as a "contact insecticide", because it kills target insects upon direct contact, and as a "systemic insecticide" because of its capability to cause overall "systemic" poisoning in target insects, after it is absorbed and transported throughout the pests that feed on treated plants. Methomyl CAS Registry Number 16752-77-5, with chemical formula C$_5$H$_{10}$N$_2$O$_2$S bearing molecular weight 162.23 belongs to family N-methyl carbamate group of insecticide. Trade names include Lannate, nudrin, Mesomile, Dash, Larvin.

Structural formula of methomyl

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{S} & \quad \text{O} & \quad \text{H} & \quad \text{N} & \quad \text{CH}_3 \\
\end{align*}
\]

Methomyl is produced by reacting S-methyl-N-hydroxythioacetamide (MTHA) in methylene chloride with gaseous methyl isocyanate at 30-35°C (Tamimi et al., 2006). Methomyl is a white crystalline solid with slight sulfurous odour in its pure state. It has a density of 1.2946 (at 24°C), vapour pressure $5 \times 10^{-5}$ mg Hg at pH 7.0 or less. Methomyl is soluble in water (57.9 g L$^{-1}$), Isopropanol (220 g/kg), and in Toluene (30 g/kg). Methomyl is active against various insects. Methomyl is a highly toxic compound in EPA toxicity class 1. It is classified as Restricted Use Pesticide (RUP) by EPA because of its high acute toxicity to humans.
Methomyl is a carbamate used for fruits and vegetables, being well identified, the mode of action of the carbamates is by the inhibition of acetylcholine esterase and the signs and symptoms of poisoning are typically cholinergic with lacrimation, miosis, convulsions and death (Barakat, 2005). It has been classified by the WHO (World Health Organization), EPA (Environmental Protection Agency) and EC (European Commission) as a very toxic and hazardous pesticide (Malato et al., 2002).

Methomyl is highly toxic via the oral route, with reported LD₅₀ value of 17 to 24 mg/kg in guinea pigs. Symptoms of methomyl exposure are similar to those caused by other carbamates and cholinesterase inhibitors (Baron, 1991). These may include weakness, blurred vision, headache, nausea, abdominal cramps, chest discomfort, constriction of pupils, sweating, muscle tremors, paralysis etc. These pesticides are potent acetyl choline esterase inhibitors, and thus various hazardous effects in humans and more target organisms can occur due to their toxicity (Tago et al., 2006). It is moderately toxic via inhalation with a reported 4-hour inhalation LC₅₀ in male rats of 0.3 mg/Kg (Sterens and Sunner, 1991). Methomyl has low persistence in the soil environment, with a reported half-life of approximately 14 days. Methomyl is highly toxic to birds. The oral LD₅₀ of methomyl is 28 mg/kg in hens. Methomyl is moderately to high toxic to fish and highly toxic to aquatic invertebrates (US EPA, 1987). The LD₅₀ for 90% pure formulation of methomyl is 11.0 to 22.0 mg/kg in mule deer (Tucker, 1970). Methomyl is metabolite of thio-dicarb and acetamidate is suspected oncogen which is a metabolite of methomyl in animal tissues (EPA, 1996).

The chronic exposure of male rat reproductive system of methomyl (17 mg/kg for 2 months) shown by degeneration in tissue structure and seminiferous tubules upto cellular destruction (Mohgoub and D1-medony, 2001). Methomyl is genotoxic is Swiss CDI mice and has shown DNA damage and mutagenic chromosomal aberration in mouse spleen (Hemavathy and
It has been reported that in vitro studies of methomyl affects the level of androgenicity, estrogenicity and aromatase activity (Anderson et al., 2002). Lohitnavy and Sinhaseni reported that methomyl (3.5 and 7 mg/kg) caused increase in lactate dehydrogenase in spleen and suggested that methomyl has cytotoxicity in rats. Gupta et al., (1994) have reported that methomyl alters energy related metabolic compounds with loss of membrane permeability in muscle tissue.

Methomyl being highly soluble in water has low sorption affinity to soils and therefore, easily cause ground water contamination (Strathman and Stone, 2001). Due to the long half-life of methomyl in ground water, the stringent pesticide standard of water bodies and protection of human health, the treatment technologies of contaminated water bodies are required urgently (Chang et al., 2007). Reports regarding methomyl effects are scanty. Hence, more studies are essential to elucidate the exact toxic potential of methomyl. Therefore, the present investigation was undertaken to elucidate the effects of methomyl to *Escherichia coli* and soil isolate- *Pseudomonas aeruginosa* on biochemical contents, enzyme activity, protein profiling and its bioremediation aspects.