Chapter 6.

Summary
6. SUMMARY

Environmental toxicology is now being globally considered a developing field in environmental research owing to the hazardous impact of different synthesized and released chemicals from different sources. Heavy metal(s) are widespread pollutants of great concern as they are non degradable and thus persistent, although some heavy metals are essential trace elements. Cadmium is a hazardous environmental contaminant, widely distributed in the environment due to its widespread use in industries such as battery production and electroplating. The continuous release of toxic metals into the environment mostly due to industrialization has been a global threat. Among many different kinds of toxic metals, cadmium (Cd) has been identified as a significant pollutant due to its toxicity and solubility. Cd\(^{2+}\) is easily taken up by cells and thus can seriously damage the cellular functions in several ways, as a potent oxidative agent, inhibitor of DNA replication, and making the DNA more susceptible resulting in single strand DNA breaks. In bacteria, Cd reduces growth rate, lengthens lag phase, lowers cell density and may even cause death at certain concentration.

Additional hazards are coming up due to the release of nanosized Cd particles, from the industries involved in manufacturing quantum dots for both medical diagnostic imaging and targeted therapeutics. Such particles have attracted a great deal of attention owing to their potential interference in biological processes. Engineered nanoparticles (NPs) are now being released into the environment in many ways and are emerging as potential environmental contaminants. There are various concerns relating to the possible impacts on public health and the environment that can arise when the smaller counterparts of materials are released. Few data are available regarding the consequences of the release of NPs into the environment and their effect on microorganisms. One area of concern is the bioavailability and toxicity of NPs to bacteria, as bacteria contribute a major role on the functional part of an ecosystem. Because different metal-NPs have different chemical and morphological properties, it is troublesome to designate a model to describe a general pattern of toxicity towards bacteria. Metal or metal-NPs damage in bacterial cells during their internalization to cytosol across cell membranes. However, it is very difficult to ascertain whether metal or metal-NPs are internalized through healthy cell membranes or whether cells underwent damage and the metal or metal-NPs go inside by simple diffusion. It could be assumed that smaller particle would pierce through porins like channel. It is uncertain that metal or metal-NPs can invade the cytoplasm until the membranes are perforated.

Thus, many different mechanisms of damage have been hypothesized, depending on features of NPs, experimental conditions, dose and test microorganisms. Among the various metal NPs, Cd-NPs are used in purposes like bioimaging and biosensing. Cytotoxic effects of Cd-NPs have been reported both in prokaryotic and eukaryotic systems, however, the exact mechanisms are yet to be elucidated. In
general, induction of intracellular oxidative stress seems to be a key event of the toxicity mechanisms of many nanomaterials.

Upon environmental release, Cd or its NPs could inhibit bacterial processes, as evidenced by laboratory studies. Thus, it warrants a better understanding of the consequences of released NPs in the environment. As bacteria perform many critical roles in ecosystem, any negative effect of toxic metals like Cd or its nanosized forms \textit{i.e.} NPs at hazardous concentration to the bacterial community affect the system in a long run. Laboratory study on the interactions between bacteria and metals or its NPs could show the avenue to find their toxicological effect on microbiota. At the same time, bacteria as single cell organisms are good test models to study the metal or metal-NPs toxicity study. \textit{E. coli} became one of the utmost popular models used for studying the effects of metal stress, owing to its generation time and rapid biological response to toxicants. There are few reports on the effect of metal-nanoparticles on bacterial cell cycle at molecular level. Observing the interference of Cd and Cd-NPs on cell cytotoxicity, hypothesis is arisen to find the effect of Cd and Cd-NPs on cell septum formation as target site. At least 14 proteins are involved in this process, all of which assemble into a ring-like structure at mid cell. Among those essential division proteins FtsZ and FtsQ are reported to be highly conserved. These proteins play crucial role in septum formation by interacting with other cell division proteins. However, reports are scanty for documenting the expression of \textit{ftsZ} and \textit{ftsQ} in transcriptional and translational level under metal or its nanoparticle stress.

Considering the above mentioned background present work was aimed to find the toxicological effect of Cd and Cd-NPs on the growth, cell morphology and on the expression of major cell division proteins, FtsZ and FtsQ at transcriptional and translational level using \textit{E. coli} as a model organism. Emphasis was given to find out the relation between intracellular reactive oxygen species (ROS) level and toxic effect of Cd or its nanosized particles.

The synthesized Cd-NPs were characterized by TEM, DLS, AFM and XRD study. The average sizes of the CdO NPs and CdS NPs were finding to 22 nm and 3 nm respectively. Both the CdCl\textsubscript{2} and synthesized Cd-NPs inhibited the growth of \textit{E. coli} cells both with the function of dose and exposure time. Both the Cd NPs were found to inhibit the growth of \textit{E. coli}, when added to the culture at mid-log phase, the viable cell number declined with increasing concentration of NPs. Cadmium at 0.3 mM was found to inhibit more than 50\% of bacterial growth whereas it was completely ceased at 0.6 mM when CdCl\textsubscript{2} was added at mid-log phase without causing significant cell death even after 6 h of exposure. At mid log phase 25 μg/mL CdO NPs inhibit ~50\% of growth in \textit{E. coli}, whereas 40 μg/mL completely ceased the growth. When the mid-log phase cells were challenged to CdS NPs, the complete growth inhibitory effect was found at 25 μg/mL and 50\% inhibition was found at 13 μg/mL. Cadmium can exert toxic effects in different ways on organisms. Even though Cd does not
participate in any biological process, but its influx or contact into the cells or tissues of the organisms it may affect severely depending on the concentration. Toxic effects exerted by Cd in microbes include enzyme dysfunction, inhibition of DNA-mediated functions, affecting ecological interactions, and changing pathogenicity. Cell length of *E. coli* also increased due to both CdO NPs and CdS NPs exposure. With the progress of exposure time to NPs, cells became more filamentous and showed filamentation associated clumping. Gradual decrease in fluorescence peak intensities of CdS NPs bacteria mixture with exposure time and a significant emission peak corresponding to CdS NPs on bacterial cell pellets signifies that NPs might be deposited on the cell surface or entered into the cells. Further, AFM study of bacterial cell morphology reveals a severe surface damage of the Cd or Cd NPs treated cells. Morphological changes of cells to filamentous form indicate the possible interference of Cd or Cd NPs in the process of cell division. AFM study of the bacterial cell morphology also indicates NPs attachment on the bacterial cell.

Various proteins are required for cell division and septum formation at the division site in bacteria. Among those essential division proteins FtsZ and FtsQ are reported to be highly conserved in bacteria. Both the proteins play a pivotal role in septum formation by interacting with other cell division proteins. Expression level of FtsZ decreased with Cd exposure time both at transcription and translational level. The mRNA/16s rRNA ratio and relative mRNA expressions both in case of *ftsZ* and *ftsQ* decreased sharply with the time of both CdO NPs and CdS NPs exposure. Similarly, expression level of both FtsQ and FtsZ proteins gradually decreased with time of both CdO NPs and CdS NPs exposure. Changes in FtsZ and FtsQ expression level or its improper function under different stress conditions interfered with cell division thus caused multinucleated filamentous structure of bacterial cell. The Cd or Cd-NPs treated cells, when stained with DAPI, showed filamentous multinucleated bead structure suggests that due to Cd or Cd NPs treatment proper cell septum formation was inhibited without affecting nucleoid segregation.

Cadmium, in general, imposes an oxidative challenge to cells exposed to mild or severe Cd stress, however, in response to such stress cells try to protect its housekeeping processes and restore cellular homeostasis, which help the cells to developed resistance against such prolonged stress. In this present study, peroxidase and SOD activities were measured to determine the intracellular ROS level. Activities of both the enzymes in Cd or Cd NPs treated cells significantly decreased with exposure time, suggested increasing intracellular ROS level. The accumulation of ROS resulted in redundant free radicals which would oxidize bio-molecules and as a consequence lead to metabolic dysfunction. There are contradictory reports that attribute the toxicity mechanism of metal-NPs on the basis of their size, morphology, or electrostatic attraction. Toxic effect or the magnitude of toxicity may vary with the type of metal-NPs, ambient conditions and species of microorganisms tested. It is hypothesized that metal-
NPs induce generation of ROS which is responsible for membrane damage or damage the cells directly. An important factor of toxicity due to ROS generation is the proximity between bacteria and metal or its NPs. Charged groups on the particle surface and adsorption of ions can alter the charge on a particle surface. Bacterial cell envelope possesses a negative charge. It seems that positively charged metal ions directly or released from its NPs should have the highest attraction to negatively charged cell surfaces. In the present study the order of toxicity showed CdS NPs (~3 nm) > CdO NPs (~22 nm) > Cd-salts or bulk inorganic forms of cadmium, which might be due to size difference or its physico-chemical features. These findings demonstrate the toxic effects of Cd or Cd-NPs in bacteria, by affecting their morphology and cell division as a result of intracellular oxidative imbalance. The inverse correlation between declined cell growth and elevated ROS level suggests that oxidative stress might be the major route by which Cd or Cd-NPs induce cytotoxicity in bacteria.

Considering ecotoxicological impact of Cd-NPs, attention should be taken before releasing such particles into the environment by developing a scientifically defensible fact profile for the purpose of risk assessment. The matters those to be thought are that the current analysis of risk assessment of metal-NPs identified a number of limitations. It is currently impossible to systematically link reported features of metal-NPs to the observed effects for identifying effective hazard. For dose-response analysis, it warrants further to investigate the no effect threshold, to determine the best hazard descriptor of metal-NPs and to identify the most relevant endpoints. The necessity for assessing the risk of NPs on case-to-case basis is often proposed in order to take the unique features of NPs of interest. The environmental risk assessment framework on chemical basis has been the standard approach to assess the environmental risks of NPs thus far. There are little advances in the field of nano-ecotoxicology, due to data gaps and significant uncertainties, which is resulting in troubleshooting for successful achievement of environmental risk assessments of released of metal-NPs in particular or NPs in general. A scientifically valued understanding of the hazardous properties of NPs requires a level of multidisciplinary research that has not yet been performed globally, thus an effective interdisciplinary collaboration is needed between nanotechnologists, ecotoxicologists and material scientists for developing a scientifically defensible fact profile for the purpose of risk assessment.