Chapter 1.

Introduction
1. INTRODUCTION

To date the awareness of toxic metal related pollution has gained the focus of general interest due to the technological evolution in recent years that made it possible to detect them even at low level. Among various contaminants and hazardous compounds, release of metal and the metal containing waste generated by various sources represents a major fraction of waste materials globally (Abdelouas, 2006; Borch et al., 2010). Heavy metals are ubiquitous natural elements and can be found in the earth crust (Malle, 1992; Nies, 1999; Mighall et al., 2002). Due to erosion processes like natural weathering and abrasion of rocks, soils and sediments, a significant fraction of natural metals are continuously being mobilized and released in the environment. There are three main sources of environmental toxic metal pollution: i) industry, ii) agricultural use, and iii) sewage sludge. Intense industrialization has intensified the problems relating to environmental pollution and the deterioration of several ecosystems with the accumulation of toxic metals. Sub sources of release of toxic metals include industries involved in electroplating, plastics manufacturing, agrochemical production, dye manufacturing and wastes left after mining and metallurgical processes (Zouboulis et al., 2004). Tailings from metal-mining operations are also a significant source of contamination, and can lead to contamination of the surrounding top soils and groundwater as well, because of significant leaching, resulting in high metal concentrations in the environment. The levels of metals in sewage sludge reflect the extent of industrialization of the area served by the local sewage system. Significant quantities may be added by metal-contaminated wastewater runoff derived from sources including atmospherically deposited metals, residues from pesticide usage, phosphate detergents and industrial effluent, particularly from the metal-processing industry (Zhu and Tabatabai, 1995). Growing attention is being given to health hazards presented by the existence of heavy metals in the environment; their accumulation in living tissues through food chain, thus posing a serious public health problem (Zouboulis et al., 2004).

Among the different heavy metal pollutions encountered in soil Cd ranked second and Cd pollution needs special attention because it has been identified as a significant pollutant due to its high toxicity and solubility in water (Pinto et al., 2004). Cadmium or its compounds are released in to the environment by several ways which have been categorized by United Nations Environment Programme, (http://www.unep.org/pdf/annualreport/UNEP_AR_2006) under the following heads:

a. Natural mobilization of Cd from the Earth’s crust and mantle through volcanic activity and weathering processes.

b. Release of Cd by anthropogenic activities due to the mobilization of contaminant Cd in raw material such as phosphate minerals.

c. Release of Cd by anthropogenic activities due to intentional use of Cd in products and processes.
d. Releasing of Cd due to re-mobilization of historic anthropogenic components having remnant that was previously deposited in soils, sediments, landfills and waste/tailings piles. Naturally occurring Cd is normally found to be associated with other elements such as oxygen (CdO), chlorine (CdCl$_2$), or sulfur (CdSO$_4$ or CdS). Cadmium may also occur as common constituents in the form of oxides, sulfides, and carbonates in zinc, lead, and copper ores. Thus industries involved in ore mining results significant release of Cd in the environment. Cadmium is of enormous use in manufacturing batteries, dyes, metal-coats and plastic stabilizers, as it could stand against corrosion. It is a non-essential element and several agencies like Environmental Protection Agency (EPA) and Occupational Safety and Health Administration (OSHA) have advocated its permissible levels as less than 5 ppb in drinking water and less than 100 μg/m$^3$ and 200 μg/m$^3$ respectively, as Cd in fumes and dust in air of workplace.

Cadmium can exert toxic effects both in direct and indirect ways on biota. Even though Cd does not participate in any biological process, but on accumulation inside the cells or organisms it may affect severely depending on the dose. Some toxic effects exerted by Cd in microbes include, (i) enzyme dysfunction, (ii) inhibition of DNA-mediated functions, (iii) affecting ecological interactions, and (iv) changing pathogenicity (Kabata-Pendias and Pendias, 2001; Mohanpuria et al., 2007). In addition, Cd has been reported to affect the viability of bacterial cells and could convert the cells to non-culturable form (Paton et al., 1997; Khan and Scullion 2002; Lakzian et al., 2002; Shi et al., 2002; Paudyal et al., 2007; Bondarenko et al., 2010).

Currently nanotechnology is a fast-growing, interdisciplinary field of science, diminishing the traditional boundaries between the classical science subjects (Ray et al., 2009). The massive use of nanoparticle (~1 to 100 nm in size) has been singled out by industry and governments to become the world’s largest industrial revolution, and it carries the potential to substantially benefit environmental quality through pollution prevention, treatment, and remediation and hence nanotechnology could also lead to serious environmental problems since the environmental behaviour and fate of synthesized nanoparticles (NPs) are not predictable from that of chemically similar but larger bulk counterparts. Being smaller size, NPs reveal unique properties having substantially increased specific surface and Gibbs free energy, which enhance its chemical reactivity. Such specific reactivity of NPs allows us to understand that its biological behaviour and effects on living organisms can become significantly different when particle size decreases. The results of toxicity studies have also led to a reconsideration of the traditional concepts of toxic risk assessment, which is normally based on the dose-response ratio, with the dose expressed in terms of mass or concentration. Indeed, the results obtained so far clearly indicated that on the nano-scale, factors such as specific surfaces, surface modifications, particle number, concentration, dimensions and surface features (stereochemistry, ionization status, redox
potential, mobilization, intermolecular force etc.), all those factors must be considered in toxicological studies.

Release of nanoparticles to the environment is likely inevitable with the increasing production and demand. Routes of such release to the environment are multiple and could be summarized as follows (Biswas and Wu, 2005; Boxall et al., 2008):

a) cleaning of production chambers;
b) spills from production, transport, and disposal of NPs or products;
c) disposal of waste containing NPs including incomplete waste incineration and landfills;
d) insufficient treatment sewage containing NPs;
e) leaching of NPs from waste containing nanomaterials.

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. In the environment, some biocatalysts can change the surface properties of NPs, so they are converted to colloids. In colloidal forms NPs remain mobile because they do not form conglomeration and are not settled down as immobilized form.

In the course of time it is a possibility for a wide exposure of the entire ecosystem to synthesized metal-NPs through the water and soil. If engineered NPs applications develop as projected globally right now, the increasing concentrations of NPs in groundwater and soil may present the most significant exposure avenues for assessing environmental risk, as some nanomaterials can form a stable colloidal species in water from both a powder and an organic solution and hence enter groundwater (Colvin, 2003). Further studies that can evaluate the potential toxicity of synthesized NPs are needed.

Few data are available regarding the consequences of the release of NPs into the environment and their effect on microorganisms (Klaine et al., 2008). 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 (Weisner et al., 2006). Different biogeochemical cycles may be affected due to the exposure to metal-NPs at a hazardous concentration. Metal-NPs cause toxicity to planktonic bacteria (Nowack and Bucheli, 2007). However, most studies advocate that NPs and bacterial surfaces will be in close contact, similar to artificial laboratory conditions. In nature, however, NPs can be greatly diluted and interact with organic matter and soil surfaces that will reduce the chance of NPs-bacteria interactions. 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. Thus, many different mechanisms of damage have been hypothesized, depending on features of NPs, experimental conditions, dose and test microorganisms.
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Bacteria exist in aquatic environments as planktonic cells or as biofilms attached to substratum (Brown et al., 1998; Dunne, 2002; Ciston et al., 2008) and contribute a pivotal role in ecological sustenance (Iwamoto and Nasu, 2001; Dean-Ross et al., 2002; Bellinaso et al., 2003). Upcoming concern over the potentially negative impact of synthesized metal-NPs on living organisms and our habitat has motivated scientists to analyze the toxicology of metal-NPs on cells and to identify NP toxicity mechanisms. Thus considering the overwhelm release of NPs in the environment it is necessary to determine not only the benefits from the application of NPs, but also to evaluate the possible negative impacts on the environment from the accumulation and transformation of deposited NPs. Release of nanosized Cd particles from the industries involved in manufacturing quantum dots for both medical diagnostic imaging and targeted therapeutics is creating a global concern (Bentolila et al., 2005; Blum et al., 2012). In majority cases CdO and CdS NPs are used a raw materials for manufacturing quantum dots. Thus toxicological issues of CdO and CdS NPs have been considered in this study.

Considering this background the present work was aimed to find the effect of Cd and Cd-NPs toxicity on *E. coli* K-12 MG1655 as a model organism.

The major objectives are:

1. To find the effect of Cd-salt (CdCl$_2$) and synthesized Cd-NPs (CdO and CdS NPs) on the growth and cell morphology.
2. To find the effect of Cd-salt and Cd-NPs (CdO and CdS NPs) on cell division and on the expression of selected cell division proteins (FtsZ and FtsQ) both at transcriptional and translational level.
3. To find the effect of such treatments on septum formation and nuclei segregation pattern in *E. coli*.
4. To track the effect of such treatment on the intercellular state of oxidative balance.