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Full Length Research Paper

Comet assay and some biochemical studies on *Bacillus cereus* using heavy metals and ultraviolet (UV)

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Genotoxicity tests (comet assay) are used to detect the toxicity of the materials that causes damage to the deoxyribonucleic acid (DNA). *Bacillus cereus* strain used in this study was isolated and identified previously by 16S rRNA with accesion number AB599718.1. This study aimed to investigate the impact of ultraviolet (UV) and environmental pollutants (cadmium and lead) on *B. cereus* as a bacterial contaminant of food. *B. cereus* was grown on L.B medium supplement with lead or cadmium followed by exposure to UV radiation as combined effect with heavy metal. To study the cytogenetic shape of total DNA genomic in individual cells. DNA damage (genotoxicity) was analyzed by using comet assay. The results indicated that, the metabolic rate of *B. cereus* was affected by the metal adsorption rate. Changes in the concentration of DNA damage, glutathione (GSH) (record 13 mg/mg protein with 1 mM Cd, 13 mg/mg protein without Cd and lead and high value with lead at 10 mM) while lipid peroxidation values were (7 nM/mg protein with Cd and high value 5.5 nM/ mg with lead). This method could be used as a useful biomarker for the identification of the presence of environmental contamination. In the future, for heavy metals removal, immobilized on waste biomaterials (there are no sense in this phrase). Input of heavy metals imposes a selective pressure that may favor the growth and activity of resistant/tolerant microbes.

Key words: *Bacillus cereus*, comet assay, ultraviolet (UV), cadmium, lead, glutathione, lipid peroxidation.

INTRODUCTION

Human is surrounded by a tremendous number of hazardous chemicals in the environment that potentially threaten human health. Exposure to environmental chemicals is most correctly characterized as exposure to mixture of these agents. The metal cadmium (Cd) and lead (Pb) are among the leading toxic agents detected in the environment. With the increase in suspected hazardous environmental chemicals there should be an increase in cytotoxic tests. The present cytotoxic research tests are of high cost and labor demand (Watanabe and Suzuki, 2002). Heavy metal pollution is one of the current most troublesome environmental problems due to the widespread use of metals for industrial and agricultural purposes (Khan et al., 2000). Among the nonessential metals, cadmium (Cd) and lead (Pb) poses a most concerning threat due to their higher mobility and bioavailabilty (Peralta-Videaa et al., 2009). Its entry in the agricultural soils arises from commonly used practices such the application of phosphate fertilizers (Chen et al., 2007; Kabata, 2004) and industrial and domestic sludges which often exerts a negative impact in soil microbial communities (Goyal et al., 2008).

These heavy metals influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity. Heavy metals can damage the cell membranes, alter enzymes specificity, disrupt cellular functions and damage the structure of the DNA. Toxicity of these heavy metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions. Also, toxicity can occur as a result of alterations in the conformational structure of the nucleic acids and proteins and interference with oxidative phosphorylation and osmotic balance (Rathnayake et al.,

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2009).

UV radiation is composed of UVA (315 to 400 nm) and UVB (290 to 320 nm). It has a significant contribution to the biological effects of UV radiation since it is absorbed by cellular DNA. Sunlight, due to its content of UV rays, has an important role in the sterilization that occurs under natural conditions. Due to these properties, UV radiation has found applications in control of bacteria in indoor air, water supplies and many heat sensitive food products (Cadet et al., 2005; Mouret et al., 2006).

The comet assay is used for a wide range of applications, including DNA damage and repair studies, genetic toxicology testing, biomonitoring and nutritional research. DNA migration can be determined visually by the categorization of comets into different 'classes' of migration (Collins et al., 1993) or by using an eyepiece micrometer to estimate image or tail length. The advantages of the Scandinavian Center of Gynecological Endoscopy (SCGE) technique include: (1) the collection of data at the level of the individual cell, allowing for more robust types of statistical analyses; (2) the need for small numbers of cells per sample (<10,000); (3) its sensitivity for detecting DNA damage; and (4) that virtually any eukaryotic cell population is amenable to analysis (Błasiak and Trzeciak, 1998).

The aim of the present study is directed towards the evaluation of the oxidative stress resulting from physical UV exposure and chemical treatment (Cd and lead) loading to *B. cerus* would affect UV exposure. Cadmium and lead were chosen as a model for metal pollution due to their higher mobility and bioavailability (Bizily et al., 2000; Liu et al., 2000).

Comet and image analysis are recommended, with the measurements of parameters such as the percentage of DNA in the tail (percent migrated DNA), tail length and tail moment (fraction of migrated DNA multiplied by some measure of tail length). Of these, tail moment and/or tail length measurements are the most commonly reported, but there is much to recommend the use of per cent DNA in tail, as this gives a clear indication of the appearance of the comets and, in addition, is linearly related to the DNA break frequency over a wide range of levels of damage. The approach or parameter used must be clearly defined and, if not typical, be justified (Błasiak and Trzeciak, 1998).

Comet assay (genotoxicity tests) is used to detect if the materials causes damage to the DNA. DNA is an important substance of the human body which is responsible for the functioning of cells. DNA is a nucleic acid which contains genetic information (Ostling and Johanson, 1984; Ansari et al., 2004; Merk and Speit, 1999; Singh, 2000). New chemicals are being added each year to the existing burden of toxic substances in the environment. This has led to increased pollution of ecosystems as well as deterioration of the air, water, and soil quality. Excessive agricultural and industrial activities adversely affect biodiversity, threatening the survival of species in a particular habitat as well as posing disease risks to humans. Some of the chemicals, for example, pesticides and heavy metals, may be genotoxic to the sentinel species and/or to non-target species, causing deleterious effects in somatic or germ cells. Test systems which help in hazard prediction and risk assessment are important to assess the genotoxic potential of chemicals before their release into the environment or commercial use as well as DNA damage in flora and fauna affected by contaminated/polluted habitats. The comet assay has been widely accepted as a simple, sensitive, and rapid tool for assessing DNA damage and repair in individual eukaryotic as well as some prokaryotic cells, and has increasingly found application in diverse fields ranging from genetic toxicology to human epidemiology. Sentinel species are the first to be affected by adverse changes in their environment. Determination of DNA damage using the comet assay in these indicator organisms would thus provide information about the genotoxic potential of their habitat at an early stage. This would allow for intervention strategies to be implemented for prevention or reduction of deleterious health effects in the sentinel species as well as in humans (Valencia et al., 2011).

Techniques that permit the sensitive detection of DNA damage and repair are critically important in the field of environmental research. DNA damage caused by environmental agents is often tissue- and cell-type specific so the technique dealing with individual cells seems to be an optimal one. The single cell gel electrophoresis technique (comet assay) detects migration of DNA from individual cell nuclei following alkaline treatment (Singh et al., 1988,1990). This technique is especially sensitive in detecting DNA double- and single-strand breaks, alkali-labile damage, and excision repair sites in individual cells. It can be applied to virtually any eukaryotic cell population that can be obtained as a single-cell suspension; it only requires extremely small cell samples (from 1 to 10,000 cells) and results can be obtained in a single day. Additionally, the cost of performing the assay is reasonably economical. The basis of the method is that broken DNA molecules can migrate more readily in an electric field than intact molecules. When cells are embedded in agarose and subsequently lysed to remove proteins, smaller DNA molecules are able to migrate away from the residual nucleus. When DNA is subsequently stained with a fluorescent DNA-binding dye like ethidium bromide, propidium iodide or 4',6-Diamidino-2-phenylindole dihydrocloride (DAPI) and viewed using a fluorescence microscope, the observed objects resemble comets with a head region containing undamaged DNA and a tail containing broken DNA. The absolute and relative amounts of DNA damage vary according to the specific wavelength of radiation (Kielbassa et al., 1997) in addition to their doses; DNA is the main target for UV radiation-induced damage. Preliminarily, the DNA damage in the present work was confirmed by comet

assay technique, the increased DNA damage in the UV exposed cells may be due to the deficiencies of natural processes (Osman et al., 2008).

MATERIALS AND METHODS

Bacterial strain

B. cereus used in this study was isolated from food and identified by 16S rRNA previously on gene bank with accession number AB599718.1 (El-hadedy and Abu El-nour, 2012).

Cell culture

B. cereus pre-culture was carried out using LB medium (Luria-Bertani) (Bacto-tryptone 10 g, Bacto-yeast extract 5 g and NaCl 10g , pH was adjusted at 7.5 for LB agar medium 15 g agar was added (Sezonov et al., 2007).

To study the effect of cadmium and lead exposure, cells were grown in LB medium supplemented with the metal under study.

Bacillus cereus exposure to cadmium or lead:

B. cereus was inoculated in LB medium supplemented with cadmium as cadmium chloride of 1, 5 and 10 mM concentration or to LB medium supplemented with lead as lead acetate of 1, 5 and 10 mM. Experiments were done in 250 ml Erlenmeyer flasks containing 100 ml of LB medium.

Determination of the effect of metals on bacterial growth

Toxicity of the selected metals to *B. cereus* was determined using three concentrations of each metal either cadmium ($CdCl_2$) or lead ($Pb(CH3COO^-)_2$. These concentrations were 0, 1, 5 and 10 mM. L.B medium inoculated with bacterial inoculums and L.B medium supplemented served as controls. All the experiments were conducted in triplicate. Cultured media were incubated at 30°C on an orbital shaker at 150 rpm. Bacterial growth was measured in terms of optical density at 600 nm for 4 days at 24 h intervals.

Ultraviolet (UV) rays irradiation of samples

B. cereus cells were grown at (pH 7, 37°C and shaking 150 rpm), harvest and washed three times with physiological solution (NaCl (0.9 %), the pellet was resuspended in deionized water. *B. cereus* suspensions in saline solution with concentration (0.9 %) were exposed to short-wavelength UV-B rays (254 nm) in sterile Pyrex glass Petri dishes (the *B. cereus* cells was exposed to UV in deionized water or in saline solution. The time of exposure bacterial cells to UV radiation was 10, 20 min and 30 min. Lamp irradiance was quantified with a UV detector (IL1400A; International Light, Newburyport, Mass.) by placing the detector at the same height as the sample surface 100000 cell/ ml

Comet assay

Comet assay was described by Mahima et al. (2002) method modification from the procedure of (Singh et al., 1988).

Comet analysis

The objects were observed at 320x magnification in a Zeiss Axhiophot fluorescence microscope attached to a video camera (IMAC-CCD S30, Computer System GmbH, Boblingen, Germany) connected to a personal computer-based image analysis system ISIS3 v. 2.0 (Metasystem, Althussheim, Germany). Fifty images were randomly selected from each sample and the comet length (diameter of nucleus plus migrated DNA) was measured on the screen. Two parallel tests were performed per individual for a total of 100 cells, and mean comet length was calculated. Comet length is positively correlated with the level of DNA breakage in a cell (Singh et al., 1988). Because the distribution of the comets was heterogeneous, histograms were applied to display information. The mean value of the comet length in a particular sample was taken as an index of DNA damage in this sample.

Biochemical analysis

Cells preparation

The cells treated with cadmium or lead, were collected, and pellets obtained washed twice using deionized distilled water, and the centrifugation was carried out at (1400 g for 3 min at 4°C). For glutathione and lipid peroxidation determination, aliquots were taken from the whole homogenized lysate.

Oxidative stress parameters

Lipid peroxidation level

Analysis of lipid peroxidation was carried out according to the procedure of Yoshioka et al. (1979). The method is

based on the measurement of malondialdehyde (MDA) as the main end product of lipid peroxidation. The breakdown product of 1,1,3,3 tetramethoxypropane was used as standard.

Reduced glutathione level

Reduced glutathione (GSH) was estimated as yellow color which developed when 5, 5 dithiol-bis (2-nitrobenzoic acid) added to sulfhydryl compounds according to the method described by Beutler et al. (1963).

Protein concentration

Protein concentration in microbial cell lysate soluble fraction was determined according to Lowry et al. (1951) the method based on color reaction.

Statistical analysis

Statistical analysis was done using SPSS software programmed one-way ANOVA.

Determination of cadmium and lead concentrations

B. cereus pellets was harvested from broth medium, washed three times and digested in a boiling mixture of H_2NO_3 and H_2O_2 (1:1) until complete digestion of the organic materials. Cadmium and lead concentrations were measured in the prepared samples using atomic absorption spectrophotometer, UNICAM 939, USA (Central lab in National Center for Radiation Research and Technology, (NCCRT)

RESULTS AND DISCUSSION

From the result illustrated in Figure 1, the amount of DNA able to migrate and, to a lesser extent, the distance of migration, are indications of the number of strand breaks present in that cell. Cells with increased DNA damage display an increased migration of chromosomal DNA from the nucleus towards the anode. In the alkaline version of the comet assay, DNA single strand breaks and alkali labile sites become apparent, and the extent of DNA migration indicates the level of DNA breakage in the cell (Ashby et al., 1995). It has been shown that the comet assay is able to detect a broad spectrum of mutagens (Fairbairn et al., 1995). Because of the comet assay sensitivity, the need for only small numbers of cells was suggested as an ideal technique for assessing the possible environmental consequences of hazardous waste pollution by the assessment of genotoxic damage in sentinel organisms (Tice, 1995). Also data in Figure 1 showed that DNA damage shows examples of the comets resulted from the exposure to 0, 1, 5 and10 mM of Pb or Cd. The comets resulting from exposure to Cd showed no significant changes compared to control. Relatively undamaged cells (upper panel) give comets consisting of a compact head with or without a very short tail, indicating double-stranded DNA.

Comets originating from damaged cells (lower panel) have a distinct head with a tail. The mean comet lengths for the bacterial cells exposed to UV rays for 10, 20 and 30 min, as compared with appropriate controls, are represented in Tables 1 and 2 and Figures 2, 3 ,4 and 5 using 10 cells of each treatment. It can be seen from this Table 1 that Cd at the applied concentrations did not evoke a significant effect on DNA migration, while Pb evoked an increase in comet length at concentration of 10 mM the increase was 72% as compared with the control. The increase in comet length was dose-dependent. The most basic way of viewing the data from the comet assay is the distribution of cells according to the percentage of DNA in tail moment, which is positively correlated with comet length (Ashby et al., 1995). It could be observed from this Figure 1 that comets resulting from exposed to Pb contain more DNA in their tails than comet resulting from control cells and that exposed to UV with cd and lead. The obtained results indicated that lead causing more damage effect on DNA tail after treatment with Pb more than Cd, due to *B. cereus* adsorption rate as affected by metabolic rate. Continuing to analyze in order to determine a difference exists between metabolically active and inactive cells with respect to metal adsorption (Fein et al., 2003).

In the comet assay, the short time elapsed between treatments and measuring of the DNA damage, UV is essential agent more time of exposure resulted in DNA tail more (UV is mutagenic effect). Data of comet was supported by growth curves of *B. cereus* as illustrated in Figure 2. *B. cereus* growth curves showed a higher sensitivity to cadmium compared to that of lead and control. Growth inhibition was greatly affected by metal concentration.

The growth curves for *Bacillus cereus* in the presence of different metal concentrations are shown in Figure 2. It exhibited a growth curve similar to the typical bacterial growth curve over the experiment time period (72 h). *B. cereus* showed growth and reached its maximum at 24 h. A decrease in growth (measured in terms of optical density) was observed upon increasing metal concentration at any given time interval compared to the control without metal amendment. The lower optical density values revealed that the bacterial growth was affected due to the presence of metal in the growth medium. However, the reduction of the growth in the presence of increased concentration of the metals used in the study was evident throughout the experiment

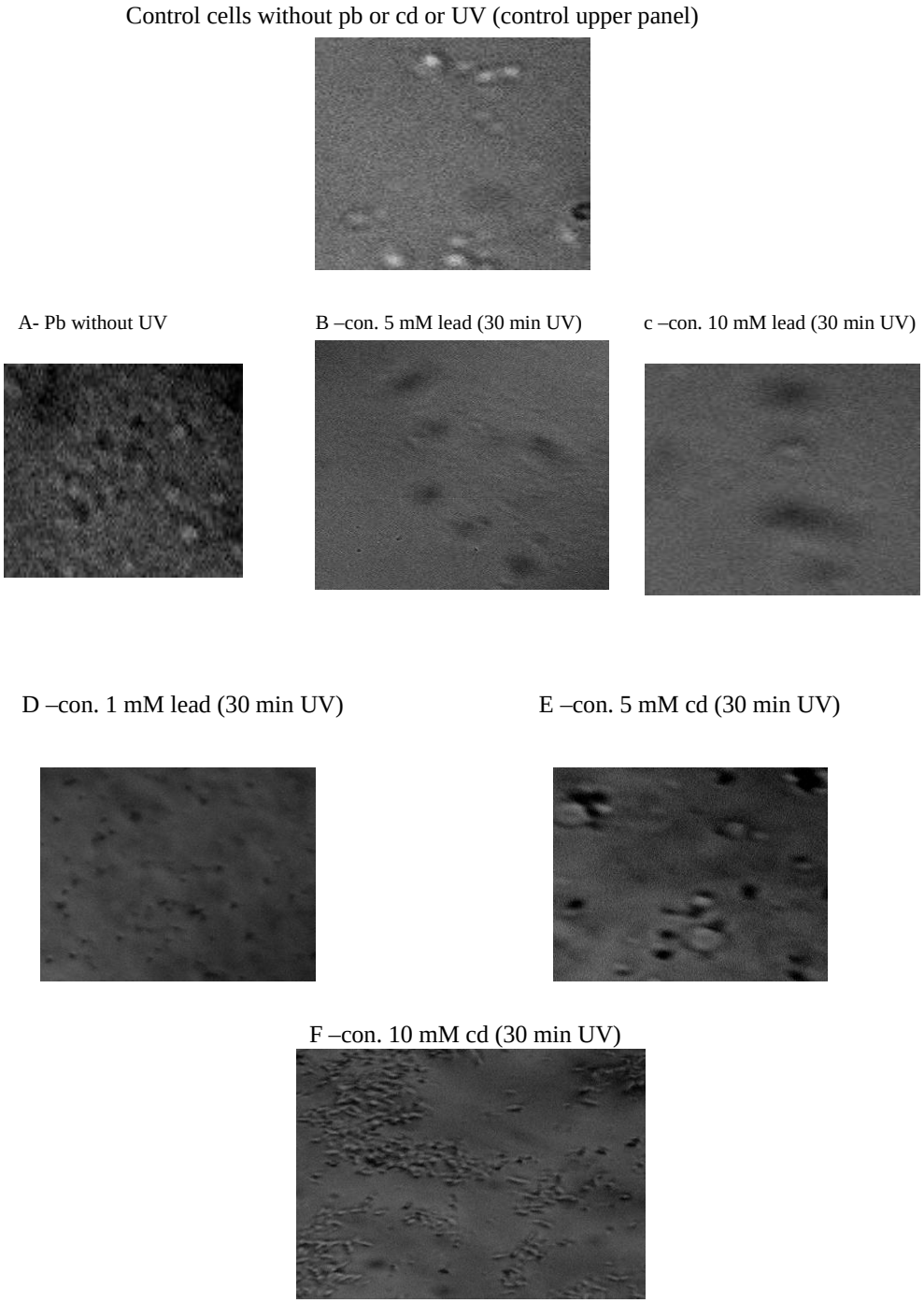

Figure 1. Typical fluorescence microscope images (comets) of the ethadium -stained DNA of Bacillus cereus cells exposed to uv with different times and pb and cd with conc 1, 5 and 10 mM isomalathion (lower panel) as compared with the control (upper panel).

compared to the control without metal.

Figure 3 shows quantitative bioaccumulation of cadmium and lead by *B. cereus*, organism has higher ability to bioaccumulation Cd more than lead. Bioaccumulation pattern can be observed for both metals with increasing metal concentration which means no saturation of biomass by concentration metal used in this study. Indicating that available sites probably exist. Physiological characterization of the isolate also indicates possible application of this strain for bioremediation of sites with Cd contamination (Mehrasbi et al., 2009).

The rationale for using *Bacillus* cells to study the

Table 1. Comet length *(µm)* of the *B. cereus* cells after treatment with cadmium or lead and exposure to UV rays.

UV Irradiation (min)	Treatment					
	Pb Mean ± st error			Cd Mean ± st error		
	10 mM	5 mM	1 mM	10 mM	5 mM	1 mM
10	35.4 ± 2.50	18.4 ± 0.40	10 ± 0.836	2 ± 1.0	2.6 ± 0.548	8.2 ± 0.583
20	37.6 ± 2.62	24.6 ± 2.50	15.6 ± 0.748	1.4 ± 0.547	2.4 ± 0.548	4.4 ± 0.548
30	37.4 ± 1.80	25.4 ± 0.68	18.2 ± 0.374	1.2 ± 0.447	1.8 ± 0.837	3.2 ± 1.483

Table 2. Head intensity length (µm) of the *B. cereus* cells after treatment with cadmium or lead and exposure to UV rays.

UV Irradiation (min)	Treatment					
	Pb Mean ± st error			Cd Mean ± st error		
	10 mM	5 mM	1 mM	10mM	5 mM	1 mM
10	5488.4 ± 39.04	6484.2 ± 128.99	7924.4 ± 1.88	8935.2 ± 208.82	6673.4 ± 460.45	6162.2 ± 88.64
20	5507.6 ± 39.22	6479.2 ± 128.75	7919.6 ± 2.31	10557.6 ± 190.39	7055.8 ± 637.52	4971.4 ± 253.24
30	5455 ± 72.33	6493.6 ± 116.91	7912.2 ± 4.77	12648.2 ± 121.05	8374.2 ± 195.41	6514.4 ± 630.68

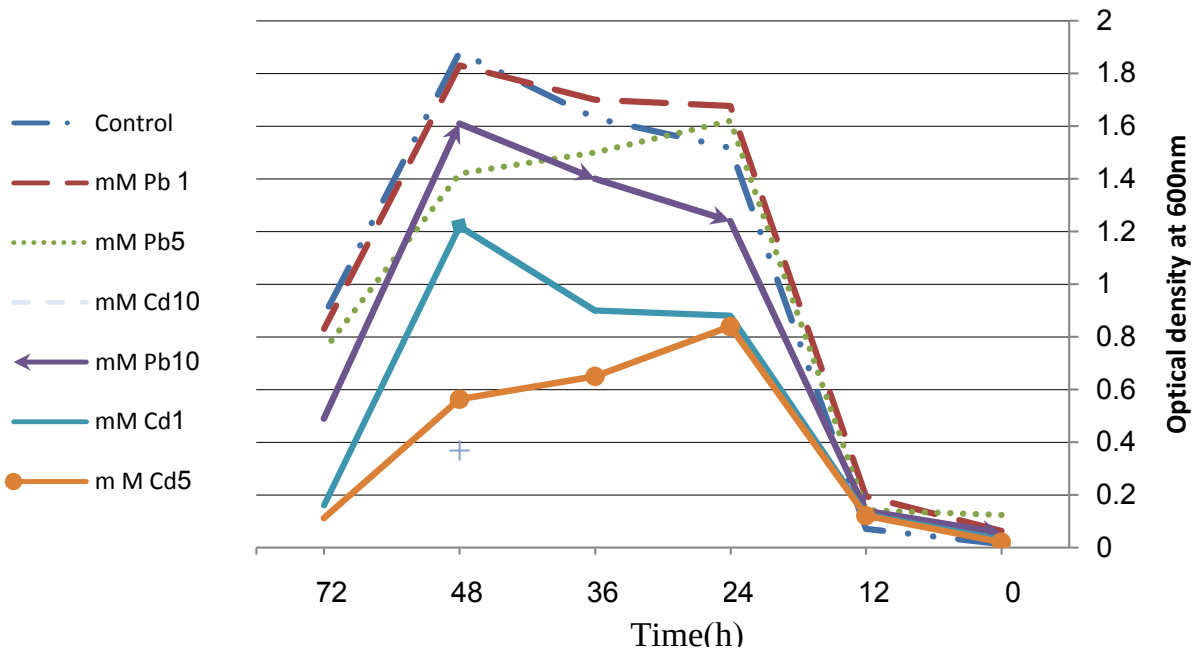

Figure 2. Growth curves of *B. cereus* in the presence of different concentrations of Cd or Pb.

uptake of heavy metal is related to the previous knowledge that gram-positive cells accumulate a much higher amount of heavy metals than gram-negative cells. Due to carboxyl groups and teichoic acids, associated to the peptidioglycan layers of the cell wall are the main agents in the uptake of heavy metals (Da Costa and de França, 1996). Da Costa (2001) found that *B. cereus* was the best cadmium accumulator and *B. subtilis*, the best lead biosorber in his study among many *Bacillus* strains.

Selenska-Pobell et al. (1999) stated that *B. cereus* is a highly resistant and accumulator for heavy metals Cd and Pb from an aqueous solutions.

Bioaccumulation of Cd and lead toxic heavy metals pollutants by *B. cereus* consider bioremediation process. Cadmium ions are taken into sensitive bacterial cells by the energy dependent manganese transport system, where they cause rapid cessation of respiration by binding to sulfahydryl group in protein. Resistance to

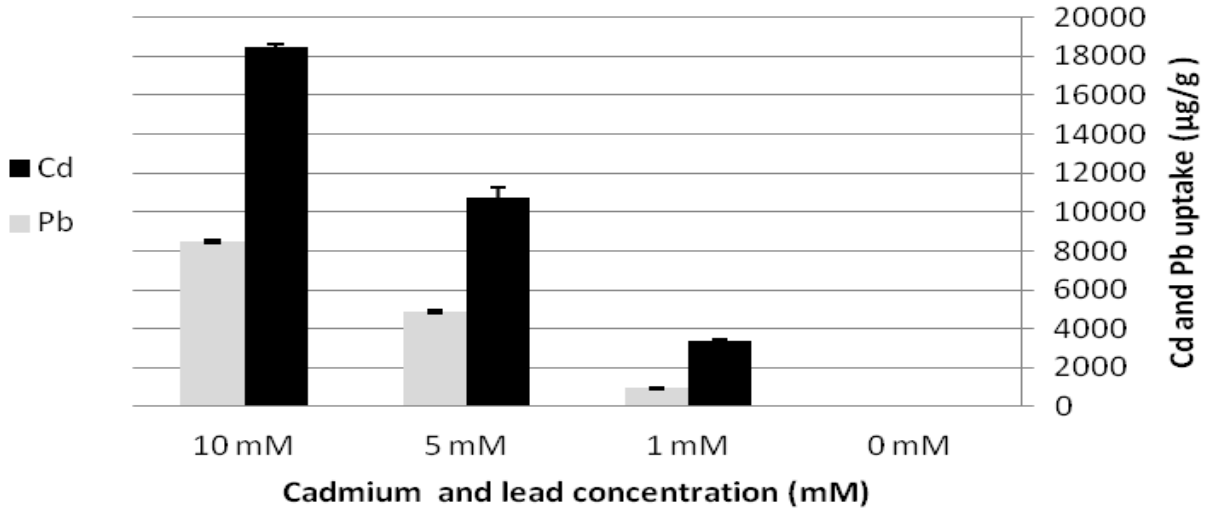

Figure 3. Cadmium and lead uptake by *B.cereus.*

cadmium is a common plasmid specified function in *S. aureus* (Beveridge, 1989).

Microorganisms have a high surface area to volume ratio because of their small size and therefore provide themselves with a large contact area that can interact with matter in the surrounding environment. The ability of the microorganisms to grow and survive under high metal concentrations is attributed to stress induced selection of these microbes in particular environments. Various mechanisms have been postulated for the development of metal resistance in microorganisms (Hughes and Poole, 1989; Gadd, 1990; Silver, 1998). However in general, all these strategies are found either to prevent the entry of metal ions into the cell or to actively pump out the metal ions from the cell (Roane et al., 1996).

The removal of lead by *B. cereus* revealed that, the percentage of metal removal by the bacteria decreased with increasing concentration of lead. The enhancement in metal sorption could be due an increase in electrostatic interactions involving sites of progressively lower affinity for metal ions (Al-Asheh and Duvnjak, 1995; Puranik and Pakniker, 1999).

Bioaccumulation is the enhanced active uptake and intracellular deposition of metals, which has been reported for many metals including lead (Sakurai and Haung, 1995). This process is dependent on the metabolic activity of the cell referred to its intrinsic biochemical and structural properties, physiological and genetic adaptation, environmental modification of metal specification, availability and toxicity (Cha and Cooksey, 1991; Cooksey, 1993).

Several studies have suggested that heavy metals as Cd and Pb causes oxidative stress and induces oxidative damage by disturbing the antioxidant defense systems (Waisberg et al., 2003). *B. cereus* is a highly resistant and accumulator for heavy metals Cd and Pb from an aqueous solutions. (Selenska-Pobell et al., 1999).

Recent studies showed the impact of secondary oxidative stresses and that it was suggested to act as a general mechanism in cellular death when bacteria are exposed to toxic agents and conditions (Kohanski et al., 2007; Mols et al., 2009, 2010).

In general, when *Bacillus sp.* cells are exposed to conditions that abruptly affect the electron transport chain an oxidative stress response can be observed. This oxidative stress encompasses the formation of reactive oxygen species (ROS), including superoxide (O^{2-}), hydroxyl ($OH^{\cdot}$) and peroxynitrite (ONOO-) (Mols and Abee, 2011).

Effect of cadmium exposure and UV irradiation on *B. cereus* cells showed enhancement of glutathione concentration after exposure to UV irradiation also 1 and 5 mM of Cd induced GSH concentration and UV irradiation at 10 min, with increasing time of irradiation was decreased GSH concentration. When cells exposed to 10 mM of cadmium showed decreased of GSH concentration which was slightly ameliorated with UV irradiation at 10 min. Figure 4(b) illustrated GSH concentration with lead exposure was greatly decreased.

In this study, *B cereus* parent cells showed strong induction of Glutathione (GSH) levels under the effect of UV irradiation. GSH level showed increase in GSH levels in cells exposed to 1 mM Cd about two folds compared to control, although GSH level was significantly decreased with higher doses of Cd 5 mM and 10 mM. In UV irradiated cells GSH level was strongly induced by irradiation at 10 min compared to non irradiated cells, whereas GSH levels was significantly decreased at 20 min of exposure to UV irradiation. *B cereus* name of bacteria must write etailc cells exposed to lead acetate showed no significant changes of GSH levels when exposed to different Pb concentrations. Whereas UV

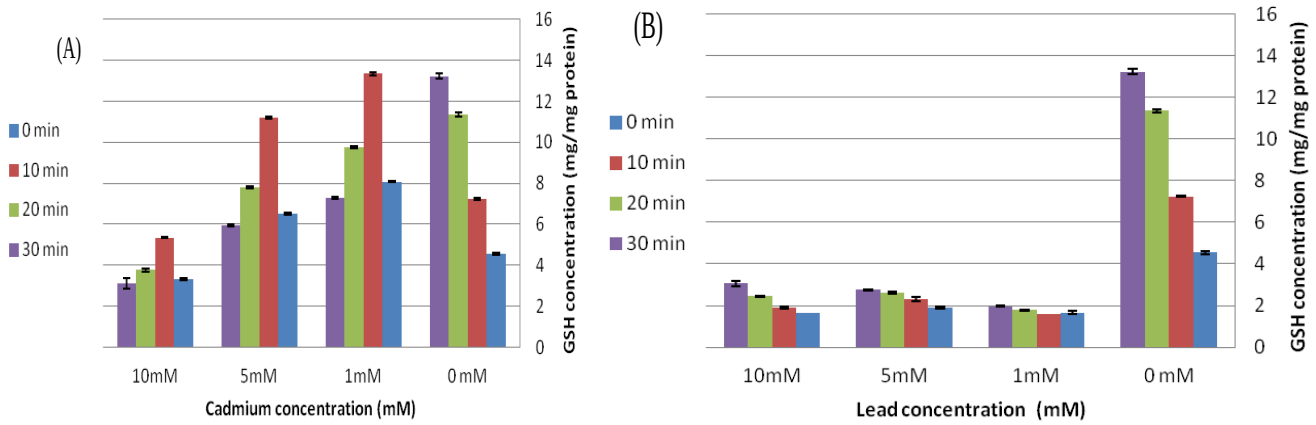

Figure 4. Comparison of reduced glutathione concentration from *B. cereus* exposed to: (A) 1,5 and 10 mM of Cd, (B) 1,5 and 10 mM of Pb , and irradiated by UV rays for 10 and 20 min.

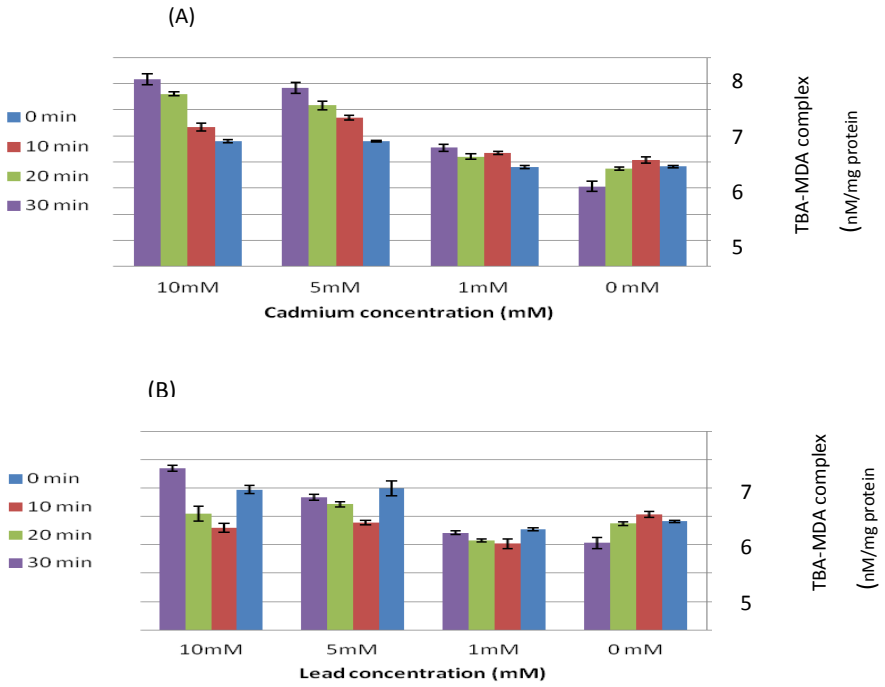

Figure 5. Comparison of TBA-MDA complex from *B. cereus* exposed to: (A) 1,5 and 10 mM of Cd, (B) 1,5 and 10 mM of Pb , then irradiated by UV rays 10 and 20 min.

irradiation effectively stimulated GSH production in cells without lead at 10 and 20 min of irradiation, although GSH level was significantly decreased in cells treated with and UV irradiated.

Figure 5 showed increased formation of malondialdehyde (MDA) complex with increasing metal concentration with cadmium and lead. UV irradiation with cadmium significantly increased MDA complex formation with time of irradiation, while with lead MDA complex formation was sig decreased with increasing time of irradiation. The results presented in Figure 5 confirm the increased oxidative stress in bacteria grown in the presence of Cd(II). Besides other stress factors, UV irradiation can generate oxidative stress through reactive oxygen species (ROS) leading to the production of oxidative damage. GSH protect cells against such stress (Dixon et al., 1998). This protection mechanism is often accelerated by increased amounts of GSH (May et al., 1998).

Lipid peroxidation level was estimated as illustrated on

Figure 5 (A and B) TBA- MDA complex level in *B. cereus* from cells exposed to low metal concentration (1mM cadmium or 1 mM lead) was lower than that of control, on the other hand UV irradiation caused no significant change. The complex level was significantly elevated in cells exposed to high concentrations of cadmium or lead (5 and 10 mM). Exposing *B. cereus* cells to UV rays significantly increased TBA –MDA complex. In cadmium treated cells thiobarbituric acid (TBA) – malondialdehyde (MDA) increased with increasing the time of UV exposure. On the other hand TBA- MDA complex from cells exposed to lead and UV was lower than that from cells without lead.

It is clear that bacteria possess all needed components to adapt to enhanced ROS levels and they include the adjustments of antioxidant potential, regulatory and metabolic needs. The systems of response are based on cysteine**,** which plays a prominent role in the cellular defense against ROS (ROS scavenging) in addition to using its thiols to protect cysteine groups from oxidative damage. GSH is an important cellular antioxidants, being involved in protection against Reactive oxygen species and reactive nitrogen species (ROS/RNS) mediated damage to cell components (Lushchak, 2011). Thus, inhibition or depletion of such antioxidant defenses by Cd or Pb exposure could increase lipid peroxidation. As expected, significantly enhanced MDA level was observed. UV irradiation resulted in a strong, fivefold increase, of intracellular GSH (l-gamma-glutamyl-l-cysteinylglycine) play an important role in protection against oxidative stress, detoxification, transport and enzymatic catalysis (Kim et al., 2005). Also, glutathione homeostasis plays a vital role in the maintenance of mtDNA and respiratory competency of cells. Its biological significance is mainly related to the free sulphydryl moiety of the cysteine residue, which confers unique redox for thiol-disulfide exchange and nucleophilic properties.

Despite the possibility to regulate ROS influx into the cell, the regulation of cellular antioxidant potential seems more critical for survival under continuous exposure to externally induced oxidative stress (Lushchak, 2011).

Previous research in Cd has indicated the involvement of reactive oxygen stress induced cellular damage (Yang et al., 1996a,b; Szuster-Ciesielska et al., 2000). One important aspect of Cd ions is that they covalently bind to sulfhydril groups (Zhu et al., 2011). Although this is partially the cause for its high toxicity, this feature is also used by several organisms to rend the metal harmless to the cell, through sequestration with metal-detoxifying ligands, which converts it into a more innocuous form. Metallothioneins are a vast group of small cysteine-rich peptides, which bind metal ions in their SH moieties, reducing their toxicity. Previous studies reported that glutathione (GSH) molecules can also sequester Cd (Gusmão et al., 2005; Lin et al., 2007), but this ability has been shown in yeast cells. GSH was dramatically increased in rhizobium tolerant strains after Cd exposure, suggesting its importance in metal stress coping (Sá-Pereira et al., 2007).

Conclusion

The obtained data indicate that single cell gel electrophoresis (comet assay) is a highly sensitive technique to detect DNA damage induced by heavy metals, so it can be used as a useful tool for environmental studies. Also glutathione concentration can be used as a biomarker for environmental stress and MDA (Malondialdehyde) is a widely accepted biomarker of lipid peroxidation.

REFERENCES

Al-Asheh S, Duvnjak Z (1995). Adsorption of copper and chromium by *Aspergillus carbonarius*. Biotechnol. Progress, 11: 638-642.

Ansari KM, Chauhan LKS, Dhawan A, Khanna SK, Das M (2004). Unequivocal evidence of genotoxicity of argemone oil in mouse. Int. J. Cancer, 112: 890-895.

Ashby JA, Tinwell H, Lefevre PA, Browne MA (1995). The single cell gel electrophoresis assay for induced DNA damage (comet assay): measurement of tail length and moment. Mutagenesis, 10: 85.

Beveridge TJ (1989). Role of cellular design in bacterial metal accumulation and mineralization. Ann. Rev. Microbiol., 43: 147-171.

Beutler E, Duron O, Kellin BM (1963). Improved method for the determination of blood glutathione. J. Lab. Clin. Med., 61: 882-888.

Bizily SP, Kim T, Kandasamy MK, Meagher RB (2003). Subcellular Targeting of Methylmercury Lyase Enhances Its Specific Activity for Organic Mercury Detoxification in Plants. Plant Physiol., 131(2): 463-471.

Błasiak J, Trzeciak A (1998). Single Cell Gel Electrophoresis (Comet Assay) as a Tool for Environmental Biomonitoring. An Example of Pesticides. Polish J. Environ. Stud., 7(4): 189-194

Cha JS, Cooksey DA (1991). Copper resistance in *Pseudomonas syringes mediated* by periplasmic and outer membrane proteins. Proceedings of the National Academy of Sciences, USA, 88: 8915-8919.

Chen W, Chang AC, Wu L (2007). Assessing long term environmental risks of trace elements in phosphate fertilizers. Ecotoxicol. Environ. Safe, 2007; 67: 48–58.

Collins AR, Duthie SJ, Dobson VL (1993). Direct enzymic detection of endogenous oxidative base damage in human lymphocyte DNA. Carcinogenesis, 14: 1733–735.

Cooksey DA (1993). Copper uptake and resistance in bacteria. Mol. Microbiol., 7: 1-5.

Da Costa ACA, Duta PF (2001). Bioaccumulation Of Copper, Zinc, Cadmium And Lead By *Bacillus* Sp.,

Bacillus Cereus, Bacillus Sphaericus And *Bacillus Subtilis* . Braz. J. Microbiol., 329: 1

Da Costa ACA (1999). Chemical interactions between mercurial species and surface biomolecules from structural components of some biological systems. *In:* Ebinghaus, W.; Lacerda, L.D. and Salomons, W. (eds.). Mercury Contaminated Sites: Risk Assessment and Solutions, Environmental Science Series, Chapter I-8, Springer-Verlag, Heildelberg, pp. 159-178.

Da Costa ACA, De França FP (1996). Cadmium uptake by biosorbent seaweeds:adsorption isotherms and process conditions. Sep. Sci. Technol., 31: 2373-2393. Adsorption of Lead and Cadmium from Aqueous Solution by Using Almond Shells.

Da Costa ACA, Duta FP (2001). Bioaccumulation of copper,zinc,cadmium and lead by Bacillus Sp. Bacillus cereus, Bacillus sphaericus and Bacillus subtilis. Braz. J. Microbiol., 32: 1-5.

Fairbairn DW, Olive PL, O'neill KL (1995). The comet assay: a comprehensive review. Mutat. Res., 339: 37.

Dixon VDW, Sallmen S, Hurwitz M (2001). Far Ultraviolet Spectroscopic Explorer Limits on Far-Ultraviolet Emission from Warm Gas in Clusters of Galaxies . The Astrophysical J., 550:L25-L29, 2001 March 20.

El-Hadedy D, Abu ES (2012). Identification of Staphylococcus aureus and Escherichia coli isolated from Egyptian food by conventional and molecular methods. J Gen. Eng. Biotech., in press.

Fein J, Borrok D, Schaefer J (2003). Cadmium and Lead Adsorption for *P. putida* and *B. subtilis*. Summer REU.

Gadd GM (1990). Metal tolerance. In: Microbiology of extreme environment. Edwards, Clive (Eds.). McGraw-Hill publications company, N.Y., pp. 178-210.

Goyal S, Meenu SW, Gera R, Kapoor KK, Kundu BS (2008). Impact of sewage sludge application on soil microbial biomass, microbial processes and plant growth- a review. Agric. Rev., 29(1): 1–10.

Gusmão LAI, Caçoilo CS, de Almeida EM, Figueira P (2005). Glutathione-mediated cadmium sequestration in *Rhizobium leguminosarum*. Enzyme and Microbial Technol., 39(4): 763-769.

Hughes MN, Poole RK (1989). Metals and microorganisms.Chapman and Hall, New York, NY.

Kabata PA (2004). Soil–plant transfer of trace elements-an environmental issue. Geoderma, 122: 143–149.

Kielbassa C, Roza L, Epe B (1997). Wavelength dependence of oxidative DNA damage induced by UV and visible light. *Carcinogenesis*, 18(4): 811-816, ISSN 0143-3334.

Kim, NR, Jung LIM, Soo HS (2005). In and KIM, Seung Wook. 2005. Optimization of feed conditions in a 2.5-l fed batch culture using rice oil to improve cephalosporin C production by *C. acremonium* M25. World J. Microbiol. Biotechnol., 21(5): 787-789.

Khan MS, Zaidi A, Oves M, Wani PA (2000). Heavy metal toxicity to legumes. Dept. of Agricultural Microbiology, Faculty of Agricultural Sciences, Aligarh Muslim Univ., Aligarh, India. pp. 197-225

Kohanski MA, Dwyer DJ, Hayete B, Lawrence CA, Collins JJ (2007). A common mechanism of cellular death induced by bactericidal antibiotics. Cell, 130: 781–783.

Liu Z, Steward R, Luo L (2000). Drosophila Lis1 is required for neuroblast proliferation, dendritic elaboration and axonal transport. Nat. Cell Biol., 2(11): 776-783.

Lin R, Wang X, Luo Y, Wenchao DW, Guo H, Yin D (2007). Effects of soil cadmium on growth, oxidative stress and antioxidant system in wheat seedlings (*Triticum aestivum* L.). Chemosphere, 69(1): 89-98.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin-Phenol reagents. J. Biol. Chem., 193: 265-275.

Lushchak VI (2011). Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. Comp. Biochem. Physiol. Toxicol. Pharmacol., 153: 175-190.

Mahima B, Alok D, Devendra P, Alok KP, Neeraj M, Prahlad KS (2002). Gender related differences in basal DNA damage in lymphocytes of healthy Indian population as revealed by the alkaline Comet assay. Mutat. Res., 520: 83-91.

May M, Vernoux T, Leaver C, van Montagu M, Inze D (1998). Glutathione homeostasis in plants: Implications for environmental sensing and plant development. J. Exp. Bot., 49: 649-667.

Mehrasbi MR, Farahmandkia Z, Taghibeigloo B, Taromi, A (2009). Adsorption of lead and cadmium from aqueous solution by using almond shells. Water Air Soil Poll., 199: 343–351.

Merk O, Speit G (1999). Detection of crosslinks with the comet assay in relationship to genotoxicity and cytotoxicity. Environ. Mol. Mutagen, 33(2): 167-172.

Mols M, Pier I, Zwietering MH, Abee T (2009). The impact of oxygen availability on stress survival and radical formation of *Bacillus cereus*. Int. J. Food Microbiol., 135: 303–311.

Mols M, van Kranenburg R, van Melis CC, Moezelaar R, Abee T (2010). Analysis of acid-stressed *Bacilluscereus* reveals a major oxidative response and inactivation-associated radical formation. Environ. Microbiol., 12: 873–885.

Mols M, Abee T (2011). Primary and secondary oxidative stress in *Bacillus*. Environ. Microbiol., 13(6): 1387-1394.

Osman AG, Mekkawy IAA, Verreth J, Wuertz S, Kloas W, Kirschbaum F (2008). Monitoring of DNA breakage in embryonic stages of the African catfish Clarias gariepinus (Burchell, 1822) after exposure to lead nitrate using alkaline comet assay. Environ. Toxicol., 23: 679–687.

Ostling O, Johanson KJ (1984). Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem. Biophys. Res. Commun., 123: 291-298.

Peralta-Videaa JR, Lopeza ML, Narayana M, Saupea GJ,

Torresdeya G (2009). The biochemistry of environmental heavy metal uptake by plants: Implications for the food chain. Int. J. Biochem. Cell Biol., 41: 1665–1677.

Puranik PR, Pakniker KM (1999). Biosorption of lead, cadmium and zinc by *Citrobacter*strain MCM B-181: Characterization studies. Biotechnol. Progress, 15(2): 228-237.

Rathnayake VN, Mallavarapu M, Nanthi B, Ravi N (2009). Tolerance of Heavy Metals by Gram Positive Soil Bacteria. World Acad. Sci. Eng. Technol. p. 53.

Roane TM, Pepper IL, Miller RM (1996). Microbial remediation of metals, *In* R.L. Crawford, and D.L. Crawford (Eds.), Bioremediation: principles and applications. Cambridge University Press, Cambridge, United Kingdom, pp. 312-340.

Sá-Pereira P, Rodrigues M, Castro IV, Simões F (2007). Identification of an arsenic resistance mechanism in rhizobial strains. World J. Microbiol. Biotechnol., 23(10): 1351-1356.

Sakurai R, Haung PM (1995). Cadmium adsorption on the Hydroxy aluminum- Montmorillonite complex as influenced by oxalate. In P.M. Huang, J. Berthelin, J.M. Bollag, W.B. McGill, A.L. Page (Eds.), Environment impact of soil component interactions: vol.2, Metals, Other Inorganics and Microbial Activities. CRC Press/Lewis publishers, Boca Raton, FL. pp. 39-46.

Selenska-Pobell S, Panak P, Miteva V, Boudakov I, Bernhard G, Nitsche H (1999). Selective accumulation of heavy metals by three indigenous *Bacillus strains, B. cereus, B. megaterium and B. sphaericus*, from drain waters of a uranium waste pile. FEMS Microbiol. Ecol. 29: 59–67.

Sezonov G, Joseleau-Petit D, D'Ari R (2007). *Escherichia coli* Physiology in Luria-Bertani Broth, J. Bacteriol. 2007 December, 189(23): 8746–8749.

Silver, S. (1998). Genes for all metals – a bacterial view of the Periodic Table. J. Indust. Microbiol. Biotechnol., 20: 1-12.

Singh NP (2000). Microgels for estimation of DNA strand breaks, DNA protein crosslinks and apoptosis. Mutation Res., 455: 111–127.

Singh NP, Danner DB, Tice RR, Pearson JB, Brantl LJ, Schneider EL (1990). DNA damage and repair with age in individual human lymphocytes. Mutat. Res. 237, 123.

Singh NP, Mccoy MT, Tice RR, Schneider EL (1988). A simple technique for quantification of low levels of DNA damage in individual cells. Exp. Cell Res., 175: 184.

Szuster-Ciesielska A, Stachura A, Slotwinska M, Kaminska T, Sniezko R, Paduch R, Abramczyk D, Filar J, Kandefer-Szerszen M (2000). The inhibitory effect of zinc on cadmium-induced cell apoptosis and reactive oxygen species (ROS) production in cell culture. Toxicology, 145: 159–171.

Tice RR (1995). The single cell gel/comet assay. *In* Environmental Mutagenesis (Philips D. H, Venitt S., eds.); BIOS Scientific Publishers: Oxford, pp. 315-339.

Valencia' LC, García A, Ramírez-Pinilla PM, Fuentes LJ (2011). Estimates of DNA damage by the comet assay in the direct-developing frog *Eleutherodactylus johnstonei* (Anura, Eleutherodactylidae). Genet. Mol. Biol. p. 34.

Waisberg M, Joseph P, Hale B, Beyersmann D (2003). Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology, 192: 95–117.

Watanabe M, Suzuki T (2002). Comparative biochemistry and physiology Toxicology pharmacology CBP, 131(4) 491-500.

Yang X, Baligar VC, Martens DC, Clark RB (1996a). Plant tolerance to nickel toxicity: I. Influx, transport, and accumulation of nickel in four species. J. Plant Nutr., 19: 73–85.

Yang X, Baligar VC, Martens DC, Clark RB (1996b). Plant tolerance to nickel toxicity: II. Nickel effects on influx and transport of mineral nutrients in four species. J. Plant Nutr., 19: 265–279.

Yoshioka t, kawada K, Shimada T (1979). Lipid peroxidation in maternal and cord blood and protective mechanism against activated oxygen toxicity in the blood. Am. J. Obstet. Gynecol., 135(3): 372-376.

Zhu, J.; Jeffrey, M.; Susan,K.; and David, P. (2011). Reactivity of Zn-, Cd-, and Apo-Metallothionein with Nitric Oxide Compounds: In Vitro and Cellular Comparison. Chem Res Toxicol., 23(2): 422–431.