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Comparative Genotoxicity Analysis of Heavy Metal Contamination in Higher Plants

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1. Introduction

Heavy metal pollution basically results from natural sources like volcanic eruptions, weathering of rocks and anthropogenic sources like mining. These activities are significantly increased in the past few decades as a result of burning of fossil fuels, industrial activities, automotive emissions, use of metal-enriched materials, mining, farm manures, wastewater irrigation, sewage sludge, pesticide usage, industrial and domestic wastes and many other factors. Heavy metals may enter the food chain as a result of their uptake by edible plants, thus, the determination of heavy metals in environmental samples is very important. For screening and monitoring the impacts of heavy metals, higher plants which provide useful genetic system, have been used as a biomonitor/bioindicator of cytogenetic and mutagenic effects (Constantin & Owens, 1982; Grant, 1994, Kachenko et al. 2004; Alirzaveya et al. 2006).

Plants are used as biomonitor / bioindicator of pollution and the major advantages of them are the following; they are eukaryotes and like animals, are able to process complex pollutant molecules (promutagen - mutagen), there is a positive correlation with mammalian cytogenetic assays for mutagenesis, easy to grow, resistant to environmental stresses, do not contaminate easily, allow assays of a range of environmental conditions, also with cultured cells; used for outdoor monitoring (Sandermann, 1994). Hence, the usage of plants as bioindicator in ecotoxicology have been reported in several studies (Grant, 1994; Knasmuller et al., 1998).

2. Effects of heavy metal on plants and defense mechanism

Some metals e.g. Mn, Cu, Zn, Mo and Ni are essential for normal growth and development of plants at appropriate concentrations as cofactors and or required for structural and catalytic components of proteins and enzymes (Moustakas et al., 1994; Nedelkoska & Doran, 2000). However, toxic levels of heavy metal ions induce several cellular stress responses and damages different cellular components such as membranes, proteins and DNA (Patra et al., 1998; Waisberg et al., 2003; Jimi et al., 2004). When heavy metals accumulate in plant tissues, they alterate in various vital growth processes, such as mineral nutrition (Greger & Lindberg, 1987, Ouzounidou et al., 1992) transpiration (Poschenrieder et. al., 1989), photosynthesis (Lidon & Henriques, 1991, enzyme activities-related to metabolism

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(Nussbaum et. al., 1988) and biosynthesis of chlorophyll (Lidon & Henriques,1991), nucleic acids (Shah & Dubey, 1995; Doncheva et. al., 1996) and seed germination (Ouzounidou et. al., 1992).

Lead (Pb) naturally occurs in uncontaminated soils are generally in the range of 20 to 50 mg kg^{-1} (Nriagu, 1978). In industrialized areas, Pb up to 1000 mg kg^{-1} or above has been recorded (Angelone & Bini, 1992). Although it is not an essential element for plants, it gets easily absorbed and accumulates in different parts of plants, causes anatomical changes by binding with essential enzymes and cellular components and inactivates them in primary leaves and decreases the number of epidermal cells/mm and growth parameters (Chaudhry & Qurat-ul-Ain, 2003). Toxicity of Pb in plants causes a number of toxicity symptoms as stunted growth, chlorosis, and blackening of root system and inhibits photosynthesis, upsets mineral nutrition and water balance, changes hormonal status and affects membrane structure and permeability (Sharma a& Dubey, 2005).

Copper (Cu) known to be an essential micronutrient for plant nutrition is generally occurs in the range of 20-30 ppm in uncontaminated areas and sediments and less than 2 ppb in natural waters (Nriagu, 1979; Salomons & Forstner, 1984; Moore & Ramamoorthy, 1984; Baccini, 1985). Cu ions play a significant role in cell metabolism and also catalyse the redox reactions in which 0_2 is the electron acceptor, being reduced to H_20_2 or H_20 (Gupta, 1979). However its deleterious effects usually arise toxic levels in mining areas (higher than 2000 ppm) (Freedman & Hutchinson, 1980; Humphreys & Nicholls, 1984). Excesses of Cu ions in plant tissues may induce a wide range of biochemical effects and metabolic disturbances which are responsible for a strong inhibition of growth, sometimes accompanied by anomalous development (Sommer, 1931; Lipman & McKinney 1931) and block photosynthetic electron transport at the reducing site of photosystem I and at the oxidizing site of photosystem II (Arnon & Stout 1939).

In natural soils cadmium (Cd) content is estimated to be about 0.06–0.50 mg/kg. Also, accession of Cd to environment and its several potentially toxic consequences in soil–plant–animal–human system have increased due to industrial, agricultural and municipal activities (Baker et al., 1979; Qadir et al., 2000). Cd is easily translocated from plant roots to stems and leaves (Yang et al., 1998), and interfere with physiological processes, resulting in declined productivity (Florijn & Van Beusichem, 1993) and harness photosynthetic activity, chlorophyll content, plant growth and induce oxidative stress (Zhou & Huang, 2001; Yi and Ching, 2003; Zhou et al., 2003). Cd stress leads to protein degradation through amino acid metabolism resulting in decreased plant growth (Rai & Raizada, 1988) and inhibits the activity of enzymes such as nitrate reductase and nitrite reductase is reported by Boussama et al. (1999a, 1999b). Previous reports indicated that Cd can cause significant reduction in the germination rate in *Triticum* and *Cucumis* (Munzuroglu & Geckil, 2002) or inhibit germination and the growth of *Arabidopsis* embryos (Li et al., 2005).

Zinc (Zn) is essential microelement that is indispensable for normal plant growth. The essentiality of Zn in low concentration for root and stem elongation was shown in previous researches (Mazé, 1915; Sommer & Lipman, 1926; Skoog, 1940). But at high concentrations, it is toxic for plants like cadmium, lead and copper. Zn toxicity occurs in plants by contaminated soils with mining and smelting activities (Chaney, 1993). Also genetic variations in sensitivity to Zn toxicity has been mapped in plants (Dong et al., 2006).

Plants could develop efficient and specific physio-biochemical mechanisms and overcome environmental stress (Sandalio et al., 2001). For instance, some of them store toxic metals in roots in order to prevent the dispersal of ions into the other parts of the plant (Fernandes & Henriques, 1991). Plants tolerate metallic stress by developing the following defence mechanisms.

- Excretion of complex compounds that reduce metal availability in the soil or in the water.
- Metal exclusion through selective uptake of elements.
- Metal retention in the roots, preventing its translocation to the shoot.
- Metal immobilization in the cell wall.
- Metal accumulation in vacuoles and inclusions.
- Increased production of intracellular metal-binding compounds.
- Development of metal-tolerant enzymes

Heavy metal toxicity effects biological molecules, for example, when metals binds to S group, blocks the active site of enzyme, and may cause conformational changes in enzymes, disrupts the cellular homeostasis and cause oxidative damage by generating reactive oxygen species (ROS) such as singlet oxygen, hydrogen peroxide, hydroxyl radical which cause lipid peroxidation, membrane defects and unstability of enzymes in higher plants (Webber, 1981; Freedman & Hutchinson, 1981; Aust et al., 1985). Chloroplast, mitochondrial and plasma membrane are linked to electron transport and generate ROS as by products (Becana et al., 2000). Their presence causes oxidative damage to the biomolecules such as lipids, proteins and nucleic acids (Kanazawa et al., 2000). A variety of abiotic stresses including drought, salinity, extreme temperatures, high irradiance, UV light, nutrient deficiency, air pollutants, metallic stress etc. lead to formation of ROS and result directly or indirectly in molecular damage (Lin & Kao, 1999). The regulation of ROS is a crucial process to avoid unwanted cellular cytotoxicity and oxidative damage (Halliwell & Gutteridge, 1989). Effects and results of ROS (Reactive Oxygen Species) are shown in Figure 1.

To scavenge ROS and avoid oxidative damage plants posses the antioxidative enzymes superoxide dismutase, peroxidase and catalase (Kanazawa et al., 2000). SOD plays a determinant role in protection against the toxic effects of oxidative stress by scavenging superoxide radicals and providing their conversion into oxygen and hydrogen peroxide (McCord & Fridovich, 1969). Four different classes of SOD have been distinguished depending on the metal at the active center, manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn) (Miller & Sorkin, 1997). Previous studies with plants have demonstrated that, most of the SODs are intracellular enzymes. A class of SODs consist with Cu (II) and Zn (II) at active site (Cu/Zn SOD), another Mn(III) at active site (MnSOD), and with Fe (III) or Ni (III) at the active site (FeSOD). Cu/Zn SODs are generally found in the cytosol of eukaryotic cells and chloroplasts; membrane associated MnSODs are found in mitochondria and also reported in chloroplasts and peroxisomes in some plants; the dimeric FeSODs which is lacking in animals have been reported in chloroplasts of some but not all, plants (Salin & Bridges 1980; Del Rio et al. 1983; Droillard & Paulin 1990; Van Camp 1994; Fridovich, 1995).

Peroxidases are heme-containing monomeric glycoproteins that utilize either H_2O_2 or O_2 to oxidaze a wide variety of molecules (Yoshida, 2002). They are located in cytosol, vacuole,

cell wall as well as in extra cellular space and use guaiacol as electron donor, utilise H_2O_2 in the oxidation of various inorganic and organic substrates (Asada et al., 2006).

Catalase is in age dependent manner and scavenge H_2O_2 generated during the photorespiration and β-oxidation of fatty acids (Lin & Kao, 2000) and one of the crucial antioxidant enzyme that scavenge ROS generated under stress conditions in plants. It catalyzes the conversion of H_2O_2 to O_2 and H_2O, in this way prevents longer H_2O_2 action which may lead to cell disturbances and DNA damages. The antioxidative responses of catalase to heavy metal stress found predominantly in peroxisomes. Hence, researchers have been investigating antioxidant responses for different plant species contaminated with heavy metals. The inhibition of catalase activity is essential for avoidance of heavy metal stress-related damage (Willekens et. al., 1995).
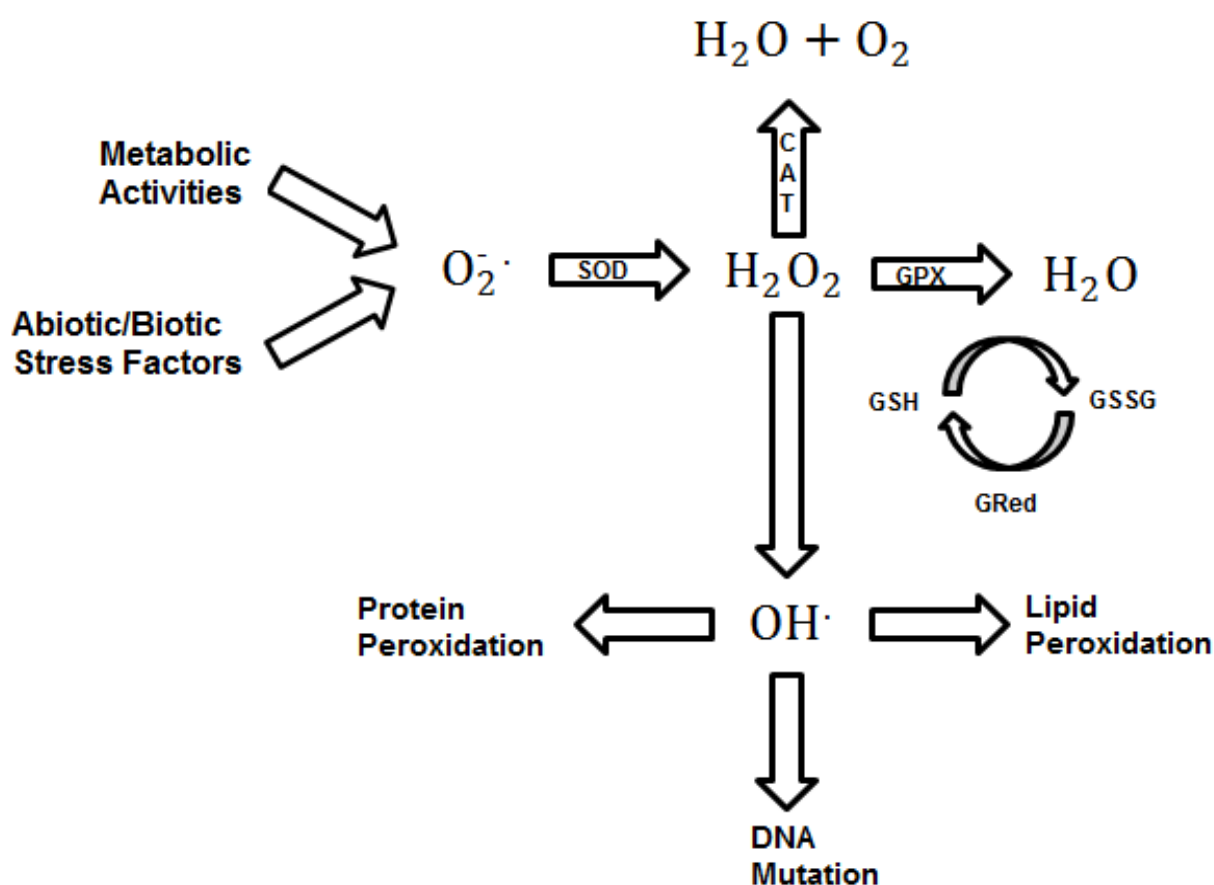

Fig. 1. Effects and results of ROS (Reactive Oxygen Species) (www.biozentrum.uni-frankfurt.de/Pharmakologie/index.html, 2008).

3. Detection of injury related to heavy metal accumulation in plants by molecular markers

With the invention of polymerase chain reaction (PCR) technology (Mullis & Faloona, 1987), PCR based molecular markers techniques were developed. Random amplified Polymorphic DNA (RAPD), one of the PCR based molecular marker techniques, is simple, rapid and low cost assay. The knowledge of genome is not required; in addition a single short random oligonucleotide primer is used. RAPD assay detects wide range of DNA damages (point mutations, inversions, deletions) and at the same time large number of samples can be studied. RAPD does not require radiolabelling for visualisation. In RAPD studies, similarities and diversities are described by appearance of new bands, disappearance of bands, and variation in band intensities. Despite its many advantages there are also some limitations. Generally it is claimed that RAPD profiles are not reproduciple but no evidence of such event is presented (Atienzar & Jha, 2006). After optimisation of RAPD, many studies have confirmed the reproducibility of the assay (Benter et. al., 1995; Atienzar et al., 2000).

Ecotoxicological literature displayed that RAPD assay is a fundamental tool to evaluate the effects of toxicants on organisms under optimized conditions. For genetoxicity studies, RAPD can be used as a diagnostic marker. The presence, absence and intensity of bands are related to DNA damages, mutations by genotoxicants. RAPD assay was successfully used to monitorize DNA changes induced by heavy metals such as lead, cadmium, copper (Enan, 2006; Liu et al., 2005; Körpe and Aras, 2011), UV and x-ray (Kuroda et al., 1999). DNA changes include damages and mutations that can be generated by toxicants directly and/or indirectly. According to RAPD profile, the genomic template stability (GTS, %) could be calculated as '100 – (100 (a/n))' where 'n' is the number of bands in control RAPD profile and 'a' the average number of changes in sample profiles. DNA damages and mutations may alter a primer binding site and thus genomic template stability changes and polymorphism occurs within dose-dependent treatments and non-treatment organisms.

The toxic effects of heavy metals on plants can be detected with various biomarkers. The use of both population and molecular marker is fundamental to determine clearly the effects of toxicants on organisms. Liu et al. (2005) used barley (*Hordeum vulgare* L.) seedlings as bioindicator of cadmium pollution, changes were observed by total soluble protein level, root growth as population markers and RAPD as molecular marker. In another study, rice (*Oryza sativa* L.) seedlings were exposed to Cd concentrations. To assess the effects of Cd on plants; growth parameters, levels of gluthatione and phytochelatins were measured and Amplified Fragment Length Polymorphism (AFLP) technique was used to determine the Cd induced genetic variation (Aina et al., 2007).

AFLP is a method generated by restriction digestion of genomic DNA, ligation of adapters (recognition sequences to restricted DNA), pre amplification reactions, selective amplification, gel electrophoresis (polyacrylamide gel), followed by visualization through autoradiography or by fluorescence methods. In AFLP assay, the number of selective nucleotides in AFLP primers, motif of selective nucleotide and genome size (Agarwall et al., 2008). AFLP assay does not require any prior knowledge of DNA sequence. AFLP assay is a successful tool for measuring genotoxic activity due to toxicants. In heavy metal polluted

and nonpolluted areas, Muller et al. (2004) described genetic variation of *Suillus luteus* population using AFLP In other study, *Arabidopsis thaliana* (L.) was used as bioindicators of two genotoxic substances (potassium dichromate and dihydrophenanthrene) (Labra et al., 2003). To evaluate the effects of organic and inorganic genotoxic substances, germination test and AFLP analysis were used.

Eggplant (*Solanum melongena* L.) seedlings as bioindicator of a range of copper concentrations were studied with population and molecular markers in our laboratory (Körpe and Aras, 2011). Treated and non-treated groups were analysed, changes in growth were detected with root lenght, dry weight, total soluble protein content and changes in DNA with RAPD assay. Root-biomass production was significantly decreased by increased Cu^{2+} concentrations (P < 0.05) after 21 days of exposure, compared with the control seedlings. The principal events observed following the exposure to Cu^{2+} were the loss of normal bands and appearance of new bands, compared with the normal control seedlings. We found that these changes were dose-dependent. The use of various biomarkers could help to detect the effects of toxicants at various levels of the organism's health status (Liu et al., 2007).

Cu^{2+} and Zn^{2+} participate in vital growth processes such as mineral nutrition, photosynthesis, mitochondrial respiration, cell wall metabolism and hormone signaling pathways (Nussbaum et al., 1988; Costa de et. al., 1994). Soydam Aydın et. al., (2011a in process); compared the effects of Cu^{2+}, Zn^{2+} and $Cu^{2+}+Zn^{2+}$ treatments on root elongation, dry weight, total protein and changes in RAPD band profiles of cucumber (*Cucumis sativus* L.). As a result, cumulative and antagonistic effect were observed between Cu^{2+} and Zn^{2+} contamination in terms of population parameters and RAPD band profiles. It was shown that root lengths of cucumber were decreased with the increased concentration (p<0.01) of Cu^{2+}, Zn^{2+} and $Cu^{2+}+Zn^{2+}$ treatments after 21d of exposure. Authors suggested that DNA damages and mutations might alter primer binding sites and thus genomic template stability changes due to metal exposure are shown in Table 1. GTS values belong to Cu contaminations were approximately conserved at 40-45 % levels. Generally, lower GTS values were observed at the lower concentrations of metals. Effects of combined solutions were higher than the effects Cu^{2+} alone on GTS values (Table 1). An extreme adverse affect was observed at all concentrations of Zn^{2+} treatments which the effects of Zn^{+2} treatment on DNA remained to be identified.

Tomato (*Lycopersicon esculentum* L.) seeds germinated in various concentrations of $Pb(NO_3)_2$ solution were used for measuring population parameters such as dry weight, total soluble protein content, radicula length and ultimately in IRs and also determining genotoxic effect of lead reflecting as appearance or disappearance of bands in RAPD profiles in our laboratory. Inhibition or activation of radicula elongation was considered to be the first evident effect of metal toxicity in the tested plants. The data obtained from RAPD band profiles and GTS revealed consistent results with the population parameters especially total soluble protein content. There was a positive correlation between GTS values and root growth results (dry weight, radicula length) at 40 ppm Pb^{+2} contaminated samples. On the other hand, 40 ppm was considered as the point of maximum appearance and disappearance of new bands in RAPD assay (Table 2).

Because of high reactivity of Cd^{+2}, it can directly influence growth, senescence and energy synthesis processes (Tiquia et. al., 1996; Turner et.al., 2002). Cadmium (Cd^{+2}) is a multitarget toxicant for most organisms studied, and it is a well established human carcinogen. DNA damage induced by Cd^{+2} contamination has been shown by changes in RAPD profile (disappearance of bands and appearance of new PCR products occured in the profiles) compared with root elongation, dry weight, total protein amount (Soydam Aydın et. al., 2011b in press). Changes in the soluble protein content of root tips in okra seedlings exhibited a significant decrease with the increased concentration of Cd^{+2} contamination. Most of the new band appearances and disappearances in RAPD assay were shown in 30 ppm contamination which maximum inhibition of total protein contenthas also occured. This research concluded that Cd has a genotoxic effect which may induce DNA mutation or structural changes and RAPD is a suitable molecular marker for screening DNA damage induced by non-lethal levels of Cd solutions. Effect of different heavy metal concentrations on RAPD profiles reflect as changes in GTS (%). According to comparison of GTS % between plant and heavy metal, the most stabile genomic template was determined in tomato seedlings exposed to 80 ppm and 240 ppm Pb^{+2} concentrations. The most significant reduction was seen at 640 ppm Zn^{2+} solution and a direct proportion was found in this metal concentration with GTS values, root length and dry weight in cucumber seedlings. It was remarkable that different concentrations of Zn^{2+} significantly decreased average GTS (%) values in cucumber, while GTS values of Pb exposed tomato seedlings were average at 85,55 %. We determined heavy metal toxicity on higher plants and on the basis of GTS % inhibition and they showed the following order: Zn > Cu >Cd Pb > (Figure 2.).

Heavy metal Concentration	Averange % GTS values of Cu exposed eggplant	Averange % GTS values of Pb exposed tomato	Average %GTS values of Cd exposed okra	Average %GTS values of Cu^{2+} exposed cucumber	Average %GTS values of Zn^{2+} exposed cucumber	Average %GTS values of combined solution of Cu^{2+} Zn^{2+} exposed cucumber
Control	100	100	100	100	100	100
30 ppm	90	-	59.0	-	-	-
40 ppm	-	78,14	-	40,85	2,56	15,98
60 ppm	77,5	-	76,4	-	-	-
80 ppm	-	90,62	-	41,54	7,62	15,98
120 ppm	53,75	81,81	72.5	-	-	-
160 ppm	-	87,10	-	45,76	7,62	30,69
240 ppm	46,25	90,08	-	45,90	4,44	42,05
320 ppm	-	-	-	51,27	1,58	35,02
640 ppm	-	-	-	29,40	1,95	38,69

(-) Not recorded

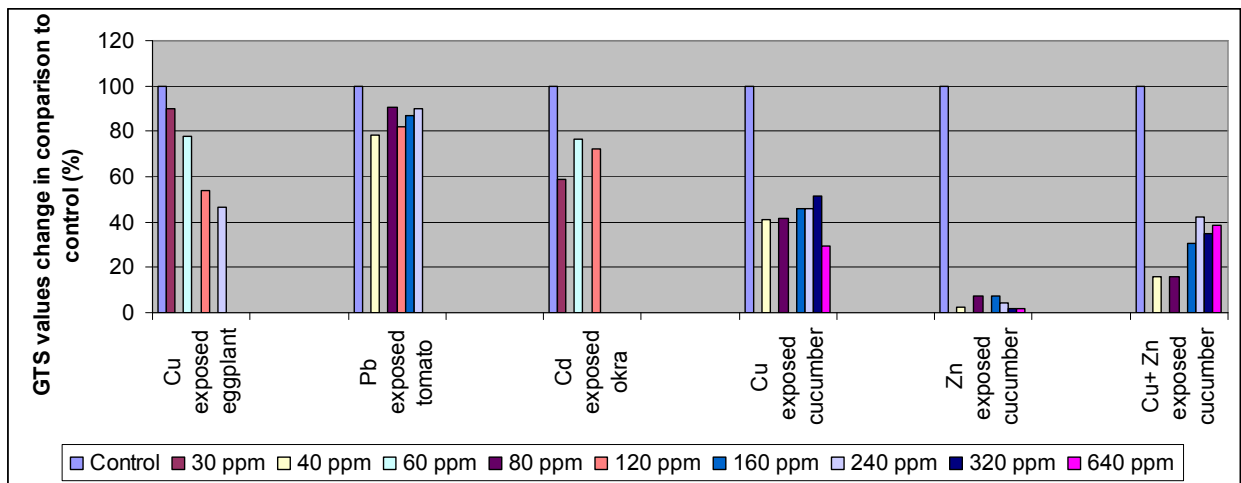Table 1. Effect of different heavy metals concentration on GTS of plant.

Fig. 2. % change in GTS values in comparison to control.

## 4. Detection of injury related with heavy metal accumulation in plants by real-time PCR

Before using real- time PCR to detect heavy metal injury in plants, the first question is what is the real time PCR? Some believe the growth of the amplification curves have to be able to watch during PCR on a computer monitor in order to be truly 'real-time'. This of course is not the case but is not the only reason for using real-time PCR. It has many advantages when compared with conventional PCR system. While conventional PCR systems have many disadvantages as labor intense, hazardous materials (e.g., 32P), low resolution & sensitivity, low dynamic range, poor discrimination among homologous genes or transcript sizes, results not expressed as numbers, not very quantitative, real-time PCR solve all these problems (Dorak, 2006). A PCR reaction has three phases, exponential phase, linear phase and plateau phase as conventional PCR and during the exponential phase PCR product will ideally double during each cycle if efficiency is perfect, i.e. 100% (Joshua et al., 2006).

The major disadvantages of real-time PCR are that it requires expensive equipment and reagents. In addition, due to its extremely high sensitivity, hard experimental design and an in-depth understanding of normalization techniques are imperative for accurate conclusions (Marisa et al., 2005). Data should be normalized absolutely or relatively. Absolute quantification employs an internal or external calibration curve to derive the input template copy number.The standard or calibration curve which we generated with Light Cycler 480 real-time PCR instrument is shown in Figure 3. Absolute quantification is important in case that the exact transcript copy number needs to be determined, while, relative quantification is sufficient for most physiological and pathological studies. Relative quantification relies on the comparison between expression of a target gene versus a reference gene and the expression of same gene in target sample versus reference samples (Pfaffl, 2001).

Attia et. al., 2009 used quantitaive real- time PCR to determine the regulation of superoxide dismutase gene expression under light conditions interacts with salt stress in *Arabidopsis thaliana* plants (Col, Columbia, and N1438). Plants were grown for 15 d under two light regimes provides different growth rates. The medium contained 0–85 mM NaCl. Shoot biomass and ion accumulation were measured. Superoxide dismutase (SOD) activity was assayed on gels, and the expressions of SOD genes were studied using real-time PCR.
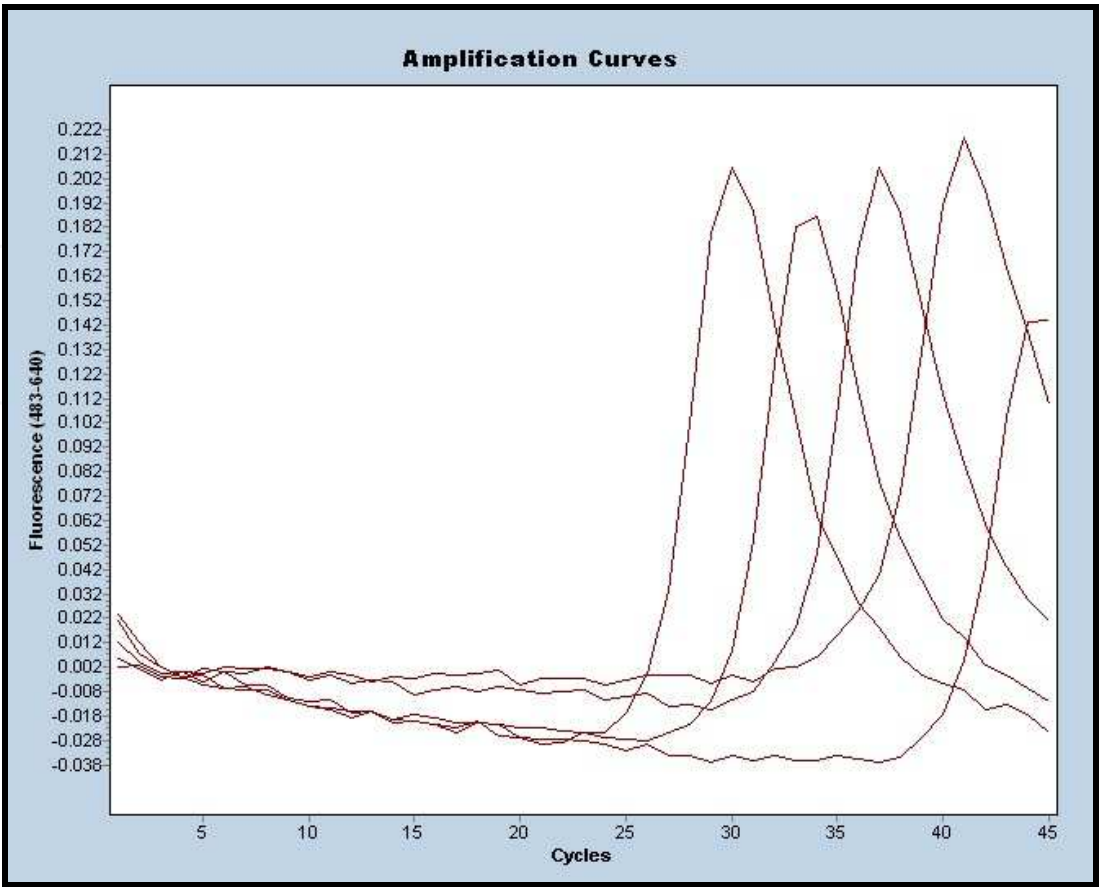
(A)
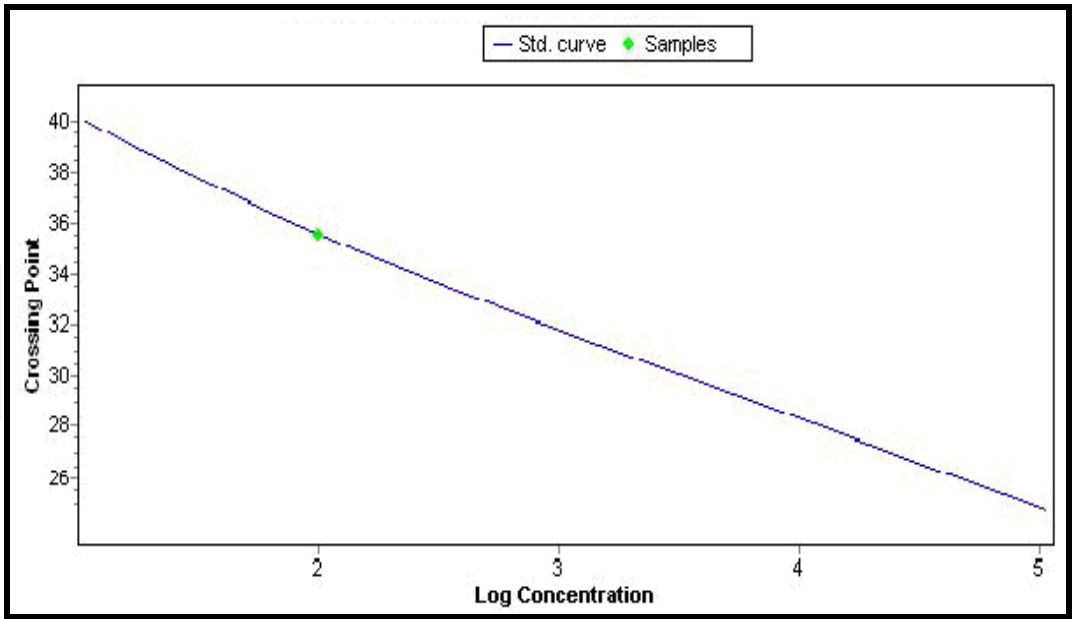
(B)

Fig. 3. Real-time PCR. (A) The PCR amplification curve charts the accumulation of fluorescent emission at each reaction cycles of standart curve.(B) is the output of a serial dilution experiment from an Light Cycler 480 real-time PCR instrument (Efficiency: 1.937 Error: 0.00769 Slope: -3.483 YIntercept: 43.43)

Research hypothesizing that oxidative stress occurred when light energy input exceeded energy utilization when salt inhibited growth, and that oxidative stress induced overexpression of some SOD genes.

The molecular responses of hydroponically cultivated tomato plants to As(V) or Cr(VI) were assessed by transcript accumulation analysis of genes coding for products potentially involved in heavy metal tolerance. A quantitative real-time PCR experiment was performed to determine the effects of As(V) or Cr(VI) at concentrations ranging from 80 to 640 mM on protein genes Hsp90-1, MT2- and GR1-like using RNA isolated from 24h treated tomato roots or shoots. As(V) increased MT2- and GR1-like transcripts in treated tomato roots but Cr(VI) treatment slightly affected the transcript levels (Goupila et. al., 2009).

A quantitative real-time PCR assay was used to determine transgene copy number in some plants and one of the most sensitive and reliably quantitative methods for gene expression analysis (Chiang et al., 1996; Ingham et al., 2001; Callaway et al., 2002; Song et al., 2002). Many researchers declare that, there is also need to know that what extent heavy metals can induce changes in major lipid components of the cell membranes. Most of these reports have been focused mainly on lipid peroxidation which induce production of ROS and represent the first targets for metal toxicity in plants (Somashekaraiah et. al., 1992; De Vos et al., 1993, Meharg, 1993).

Cd and Pb stress are shown to distrupt the cellular homeostasis and cause oxidative damage to plants due to increased level of reactive oxygen species (ROS) which cause lipid peroxidation, membrane defects and unstability of enzymes in higher plants. Based on the knowledge of ROS a study was conducted to determine the effects of lead (Pb) and cadmium (Cd) elements on lipid peroxidation, catalase enzyme activity and gene expression profile in tomato (*Lycopersicum esculentum* L.). 25 days-old plants grown in controlled media were used for stress treatments. For application of heavy metal stress Pb and Cd were added to the hydroponic solution for 24h at a concentration from 0 (control), 80, 160, 320, 640 and 1280 mM of Pb or Cd.

Estimation of lipid peroxidation analysis based malondialdehyde (MDA) which is a marker of oxidative lipid injury which changes in response to environmental factors lead to stress in plants (Hodges et.al., 1999) and the most significant increase in the MDA content were seen in the samples exposed to 320 μM concentration of Pb contamination, while the lowest degree of MDA content was determined at the samples exposed to 1280 μM concentration of Pb. Assay of catalase activity was performed by the method of Aebi et. al., (1988) based on 240 nm absorbance and quantitative real-time PCR was performed with Light Cycler ® 480 System (Roche), thermal cycler. Primers and probes sequences (presented in Table 2) of target gene catalase (CAT) and actin (ACT) used for normalization were designated on sequences of tomato genes available in the databank (http://www.ncbi.nlm. nih.gov/).

CAT gene expressions showed a complex pattern under heavy metal contamination and enzyme activity results were strongly up-regulated with this pattern at the same concentration ($p < 0.05$). Our results confirm that heavy metal contaminations are related to impairment of ETS (Electron transport system) of membranes that caused an increase of forms of reactive oxygen species (ROS) includes O^{2-}, H_2O_2, OH^- and HO^{2-}. Many genes play a crucial role for responding heavy metal stresses at transcriptional level and CAT is one of these genes that encode catalase enzyme. In this case, CAT as an antioxidant defense system

CAT2 F	CTTTCCTCTTCgACgATATTggTA
CAT2 S	TATTCCCCAAgATTACAggCAT
CAT2 A	CCgACTCggATTgCCTT
CAT2 R	gTgATTTgCTCCTCCgACTC
CAT2 FL	CAACAgggCTggAAAATCAACTTATgT-FL
CAT2 LC 640	AAgTTCCACTggAAgCCCACATgT p
ACT F	CATTgTCCACAgAAAgTgCTTCTA
ACT S	TCTgTTTCCCggTTTTgCTATTAT
ACT A	AACCACATTAAATggAAACATgAgAT
ACT R	TgCATCAggCACCTCTCAAg
Actin FL	ATTCATAgCCCCCACCACCAAAC-FL
Actin LC 640	TCTCCATCCCATCAAAAAAACAAATTgACT p

Table 2. Primer and Probe Sequences of CAT (catalase) and ACT (actin).

component, which can protect plants from cellular injury by removing excessively produced H_2O_2, is activated (Qilin et al., 2009).

5. Conclusion

The results of these studies have shown the advantages of using plants as bioindicator of heavy metal toxicity. Plants could develop different defense mechanisms against heavy metal stress such as storing toxic metals in roots in order to prevent the dispersal of ions into the other parts of the plant (Fernandes & Henriques, 1991). Also, alteration in total soluble protein content is one of the important effect which promote senescence or reduce protein synthesis by preventing the protein content of plants (Gupta, 1986). To measure of some parameters at the population level can facilitate the interpretation of the data at the molecular level. Though the plant genome is very stable, its DNA might be damaged due to the exposure to stress factors and it can be shown as differences in band profiles of molecular markers. Plants exposed to heavy metal stress also show rapid and temporary drops in growth rate and activate antioxidant defense system by producing ROS which alter MDA content and gene expression and enzyme activity patterns of CAT. We also suggest that; molecular markers such as RAPD, AFLP combined with population biomarker and quantitative real-time PCR technique can be used for determining the effects of heavy metal toxicity in plants and real –time PCR is the most reliable technique to determine the responses given by the plant against heavy metal toxicity at gene expression level.

6. Acknowledgement

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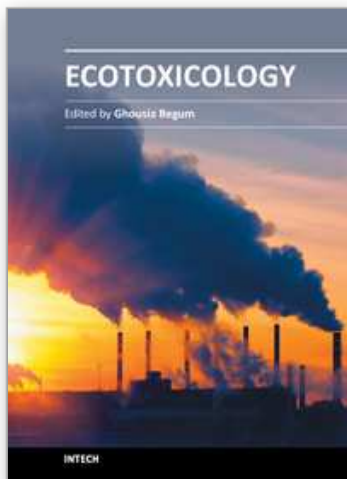
Ecotoxicology

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This is a good book on upcoming areas of Ecotoxicology. The first chapter describes genotoxicity of heavy metals in plants. The second chapter offer views on chromatographic methodologies for the estimation of mycotoxin. Chapter three is on effects of xenobiotics on benthic assemblages in different habitats of Australia. Laboratory findings of genotoxins on small mammals are presented in chapter four. The fifth chapter describes bioindicators of soil quality and assessment of pesticides used in chemical seed treatments. European regulation REACH in marine ecotoxicology is described in chapter six. X-ray spectroscopic analysis for trace metal in invertebrates is presented in chapter seven. The last chapter is on alternative animal model for toxicity testing. In conclusion, this book is an excellent and well organized collection of up dated information on Ecotoxicology. The data presented in it might be a good starting point to develop research in the field of ECOTOXICOLOGY.

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