(LUMO) of P3TAA estimated from the oxidation potential and the band gap (π–π^*) value are situated ~ -2.95 eV vs vacuum. As reported in the literature, the conduction band (CB) of TiO_2 and the valance band level of CuI are situated at ~ -4.2 eV and ~ -4.85 eV vs vacuum respectively[2,10,16]. Therefore, upon illumination a majority of the excited dye molecules attached covalently to the TiO_2 electrode transfer electrons to the conduction band of TiO_2, while the holes are transferred to the valence band of the CuI, thus producing a respectable photocurrent through an external circuit.

In conclusion, we have successfully constructed and demonstrated the possibilities of fabrication of volatile solvent-free polymer-sensitized solar cells composed of chemically attached thiophene polymer to an inorganic semiconductor TiO_2 with CuI as the hole transporting material. Even though these values are far from practical applications, we believe that the methodology demonstrated here would lead to the use of conducting polymers in solar cells more effectively.

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Induction of oxidative stress and ultrastructural changes in moss *Taxithelium nepalense* (Schwaegr.) Broth. under lead and arsenic phytotoxicity

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Changes in physiological, biochemical and ultrastructural level under lead (Pb) and arsenic (As) phytotoxicity were investigated in moss, *Taxithelium nepalense*. Decrease in dry matter and total chlorophyll was observed in moss under metal treatment. Increase in lipid peroxidation, hydrogen peroxide and superoxide anion radical were visible under Pb compared to As. Accumulation of Pb was found to be maximum compared to As. An increase in superoxide dismutase activity with a concomitant decrease in catalase, peroxidase and glutathione reductase activity was recorded. Both ascorbate and glutathione accumulated to a greater extent under Pb treatment in moss. A distortion in thylakoid ultrastructure in moss chloroplast was noticed under Pb compared to As and control.

THE living organism possesses the ability to withstand specific quantity of essential and non-essential elements present in the environment, and utilize them for their growth and development. These elements are toxic if taken up at a higher concentration than required[1]. In contrast to the essential elements which serve as metabolic precursors for plants, the non-essential elements do not have any known metabolic function. Non-essential elements are grouped together into one major category termed as heavy metals. Heavy metals are the integrated component

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of the biosphere and are released through industrial effluents and other human activities. With the advancement of science and technology, environmental pollution has become a global problem. Rapid industrialization has led to the accumulation of major atmospheric pollutants like SO_x, NO_x, CO_2, metal ions, etc. Plastic, pesticides, disinfectants, tanneries, electroplating, etc. have also added many metal ions into the ecosystem.

Heavy metals are known to cause oxidative damage and biochemical lesions in plant cells[2]. The heavy metal-induced toxicity results in proline accumulation and alteration of various enzymic activities[3,4]. In addition to these effects, heavy metal ions induce alterations in chlorophyll biosynthesis and electron transport activities, and other enzymes related to photosynthetic process. The *in vitro* uptake of heavy metals in plants results in interference of growth and metabolism by triggering secondary responses such as oxidative damage by producing highly reactive oxygen species (ROS). Disintegration of biomembranes by lipid peroxidation is a general mechanism of stress-induced responses in living systems with the generation of ROS like OH^- and O_2^- radicals[4]. These ROS are highly active in the hydrophobic environment rather than in hydrophilic aqueous systems. Lead (Pb) and Arsenic (As) are two toxic heavy metals that can cause oxidative damage in plants. Lead toxicity in many plants is known to be associated with inhibition of growth, changes in enzyme activities, leaf chlorosis[5–7], reduction in photosynthetic rate and also inhibition of root elongation[8,9]. Arsenic occurs predominantly in the soil and also in groundwater. Like lead, arsenic can also induce phytotoxic symptoms and can generate ROS. Likewise, other heavy metals like chromium were found to inhibit seed germination, seedling growth and also induce oxidative damage and various biochemical lesions[2].

Bryophytes are important contributors to the biomass in various ecosystems. The capacity of bryophytes to accumulate various metal ions, has opened the possibility of its use as a biomonitoring agent of environment quality in remote areas[10]. Bryophytes grow in almost all kinds of habitats like rocks, stones, hillsides, tree trunks, etc. Although most of the plant species are affected by the presence of metal ions in the environment, a few lower plants and higher plants have evolved populations with the ability to survive and thrive in metal-rich soil. These plants are referred to as hyperaccumulators. Hyperaccumulators are plant species that have the potential to survive uptake and sequester high levels of metal ions in their tissue without exhibiting any phytotoxic symptoms[11]. Bryophytes being hyperaccumulators of metals, can have the potential phytoremediation capacity. The ability of the bryophytes to accumulate metals is because of its high surface-to-surface ratio[12]. Hence they can be used as biosensors to monitor environment quality and as indicators of morphological and genomic changes induced by heavy metals[13–15]. However, little is known about the subsequent mechanism of oxidative stress and molecular damage in bryophytes under metal pollution[16]. *Taxithelium nepalense* (Schwaegr.) Broth is a subtropical genus belonging to the family Sematophyllaceae and occurs generally on tree barks of wet forests. In India, it is commonly called pleurocarpous moss and is widely distributed in the wet forests of the country. It is a terricolous moss highly sensitive to pollution and can be used as biomonitoring agent to monitor air quality. The present investigation was carried out to analyse phytotoxicity response of *T. nepalense* (Schwaegr.) Broth to lead and arsenic, with the induction of oxidative stress and ultrastructural changes in moss cells.

Fresh samples of *Taxithelium* sp. were collected during July 2003 from the Botanical Garden at Assam University, Silchar and brought to the laboratory in polythene bags. After thorough washing in running tap water and then in distilled water, the moss was transferred to plastic petri plates containing various concentrations (0, 10, 100 and 1000 μM) of lead acetate and sodium meta arsenate. The plates were transferred to a growth chamber under continuous white light provided with cool, fluorescent white tubes (Philips 20W TLD, India), with a photon flux density of 52 μ E m^{-2} s^{-1} (PAR) and kept at 22 ± 2°C. After 24 h of metal treatment, the plant material was taken for biochemical and enzymic estimation and transmission electron microscopy (TEM) observations.

To determine the dry matter, the plants were dried in an oven at 70°C for 2 days. The dried samples were then weighed to determine the plant dry matter. The total chlorophyll content was determined spectrophotometrically as described by Arnon[17]. To determine Pb^{2+} and As^{2+}, the plants were oven-dried at 70°C for 2 days. The dried samples were then digested, as described by Humpries[18], in glass tubes containing 5 ml concentrated HNO_3 and placed in a heat block at 100°C until the solution became clear. The sample volume was raised to 20 ml by adding distilled water. The concentration of total Pb^{2+} and As^{2+} in the tissue was measured by an atomic absorption spectrometer (Perkin Elmer-3110, Germany).

The total peroxide content was estimated by homogenizing the plant material in 5% trichloroacetic acid (TCA), as suggested by Sagisaka[19]. Lipid peroxidation was measured as the amount of MDA determined by thiobarbituric acid (TBA) reaction, as described by Heath and Packer[20]. Plant material (0.2 g) was homogenized in 2 ml of 0.1% (m/v) TCA. The homogenate was then centrifuged at 10,000 *g* for 20 min. To 1 ml of the supernatant, 1 ml of 20% TCA containing 0.5% TBA and 0.01 ml butylated hydroxyl toluene (BHT: 4% solution in ethanol) were added. The mixture was heated at 95°C for 30 min in a water-bath and then cooled in ice. The contents were centrifuged at 10,000 *g* for 15 min and absorbance was measured at 532 nm and corrected for 600 nm. The estimation of O_2^- was done as suggested by Elstner and Heupel[21] by monitoring the nitrate formation from hydroxyl amine with some modifications. The plant material was

homogenized in 3 ml of 65 mM phosphate buffer (pH 7.8) at 5000 *g* for 10 min. The reaction mixture contained 0.9 ml of 65 mM phosphate buffer, 0.1 ml of 10 mM hydroxyl amine hydrochloride and 1 ml of the supernatant plant extract. After incubation at room temperature (25°C) for 20 min, 1 ml of 17 mM sulphanilamide and 1 ml of 7 mM α-naphthyl were added. After reaction at 25°C, 1 ml of diethyl ether was added and centrifuged at 1500 *g* for 5 min and the absorbancy was read at 530 nm. A standard curve with NO_2^- was established to calculate the production rate of O_2^- from the chemical reaction of O_2^- and hydroxylamine.

Extraction and assay of enzymes were done by homogenizing the plant material in 0.1 M phosphate buffer (pH 6.8) in pre-chilled mortar and pestle under cold condition. The extract was centrifuged at 4°C for 15 min at 14,000 *g* in a cooling centrifuge. The supernatant was used for the assay of catalase (CAT), guaiacol peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD). CAT and GPx were assayed according to Chance and Maehly[22]. Five millilitres of the assay mixture of catalase comprised of 3 ml of 0.1 M phosphate buffer (pH 6.8), 1 ml of 30 mM H_2O_2 and 1 ml of the enzyme extract. The reaction was stopped by adding 10 ml of 2% H_2SO_4 after 1 min incubation at 20°C. The acidified reaction mixture was titrated against 0.01 M $KMnO_4$ to determine the quantity of H_2O_2 utilized by the enzyme. Three millilitres of the assay mixture of GPx comprised of 0.1 M phosphate buffer (pH 6.8), 30 mM guaiacol, 30 mM H_2O_2 and 0.3 ml enzyme extract. The rate of change in absorbance at 420 nm was measured using an UV-visible spectrophotometer (Systronics, Mumbai, India). The assay of SOD was done according to Giannopolitis and Ries[23]. Three millilitres of the assay mixture of SOD comprised 79.2 mM Tris-HCl buffer (pH 8.9) containing 0.12 mM EDTA and 10.8 mM tetramethyldiamine, bovine serum albumin (0.0033%), 6 mM nitroblue tetrazolium (NBT), 600 μM riboflavin in 5 mM KOH and 0.2 ml enzyme extract. The reaction mixture was illuminated by placing the glass tubes between two fluorescent tubes (Philips, 20W, India) and the reaction was terminated by switching the light-off. The increase in absorbance due to formation of formazen was recorded at 560 nm. GR was determined according to the method suggested by Smith *et al.*[24].

For determination of ascorbate and glutathione, 0.2 g of plant material was homogenized in 5% (m/v) sulphosalicylic acid and the homogenate was centrifuged at 10,000 *g* for 10 min. The supernatant was used for the estimation of ascorbate according to Oser[25] and glutathione according to Griffith[26].

For TEM study, the thalli of metal-treated moss and control samples were fixed overnight in 1.5% glutaraldehyde in 0.2 M phosphate buffer at room temperature overnight, as suggested by Makela *et al.*[27]. Post-fixation, cutting and staining were carried out at the Electron Microscopy Division of the Sophisticated Analytical Instrumentation Facility, Regional Sophisticated Instrumentation Centre, North-Eastern Hill University, Shillong. Three sections/treatments were examined and photographed with Jeol 1200X electron microscope (Jeol System Co., Akishima, Tokyo, Japan) for chloroplast ultrastructure analysis.

Data represent means of three separate experiments ± standard error. Data were analysed by Student's *t*-test at $P = 0.05$ significance level.

A significant growth inhibition measured in terms of dry matter of moss was visible with an increase in metal concentrations (Figure 1 *a*). At 1000 μM concentration, the dry mass showed a 41.3% decrease in case of Pb and 38.5% decrease in case of As compared to control. The inhibition of growth was accompanied by a decrease in chlorophyll content, which reduced by 41.2% in case of

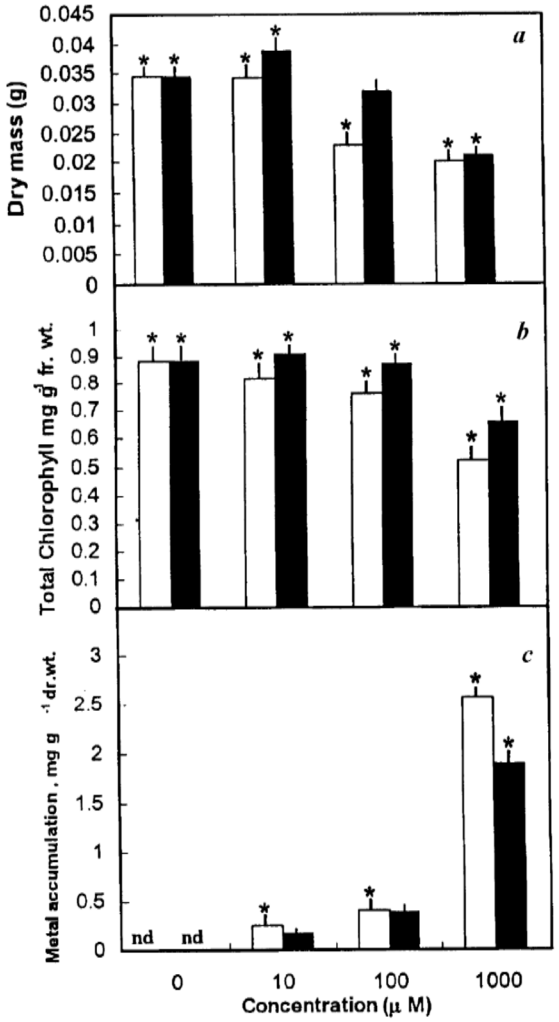

Figure 1. Changes in drymass (*a*), total chlorophyll (*b*) and metal accumulation (*c*) in *Taxithelium nepalense* under Pb (□ and As (● after 24 h. Data represented are the means of three separate experiments ± SE. Data were analysed by Student's *t*-test at $P = 0.05$ significance level (*). nd, Not detectable.

Pb and 25.5% in case of As compared to control plants (Figure 1 *b*). The accumulation of Pb in moss cells was found to be higher than that of As after 24 h of metal treatment (Figure 1 *c*).

An increase in total peroxide content was noticed under both the heavy metal treatments (Figure 2 *a*), which increased with increase in concentration of Pb and As. The increase was markedly significant at 100 µM with 172.5% and 151.2% increase for Pb and As respectively, and 213.5% for Pb and 177% for As at 1000 µM concentration.

As an indicator of lipid peroxidation, the content in thiobarbituric acid reactive substances (TBARS) was measured (Figure 2 *b*). With increase in the concentration of Pb and As, the TBARS content showed a significant increase. However, the increase was noticed at 100 µM concentration with 227.2% in case of Pb and 173.4% in case of As and the highest at 1000 µM with 258.3 and 181.1% in case of Pb and As respectively, compared to control plants. The change O_2^- is shown in Figure 2 *c*. With the increase in concentration of Pb and As, the superoxide anion production also increased. The increase was higher under Pb than As. Pb-treated plants showed 355.8% increase at 10 µM concentration, which increased by 669.6% and 784.3% at 100 and 1000 µM concentration respectively, compared to the control. In case of As, at 10 µM the production of O_2^- was decreased approximately by twofold compared with Pb. At 100 and 1000 µM concentration of As, production was enhanced only by 353.9 and 546% compared to control.

Changes in antioxidant enzymes are shown in Figure 3. Under metal treatment, the activity of SOD showed an initial increase with increase in metal concentration, but decreased at higher concentration. After 12 h of treatment, SOD activity gradually increased in case of both the metals; a decrease was noticed at 1000 µM concentration. In case of CAT, a decrease was noticed in moss cells under metal treatment. However, a decrease was noticed in the case of Pb (29.1%) compared to As (25.1%). The peroxidase (POX) activity showed a gradual decrease with higher inhibition of its activity under Pb (37.7%) compared to As (30.5%). The activity of GR was found to decrease with increase in concentration of Pb with that of As treated moss cells.

Changes in non-enzymic antioxidants, ascorbate and glutathione are shown in Figure 4. The ascorbate content showed an increase with increase in metal concentration. The increase was pronounced in case of Pb-treated plants. At 1000 µM, the ascorbate content increased by 296% in case of Pb, with only 164.4% in case of As compared to control. The glutathione activity also showed an increase with increase in concentration of Pb and As. The increase was highest at 1000 µM concentration for both the metals, but more pronounced effect was noticed in case of Pb where it increased by 173% compared to control. In case of As the increase was 149.4%, which was approximately 1.5 times lower than that of Pb.

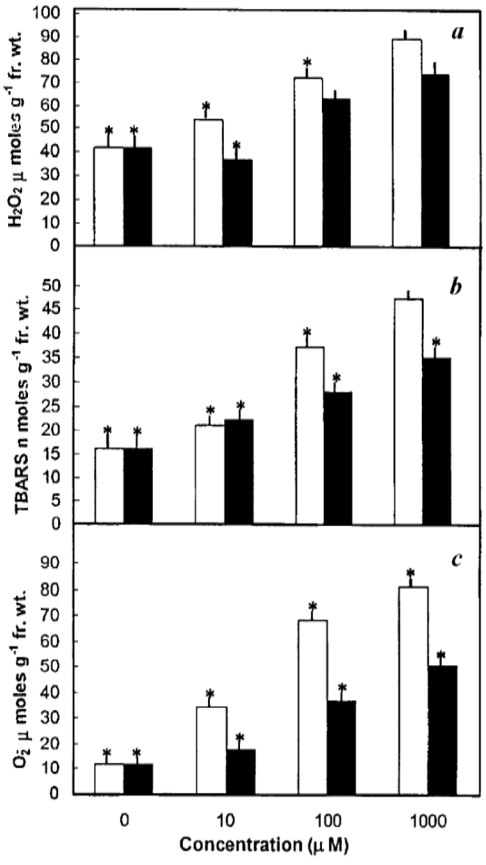

Figure 2. Changes in total peroxide (*a*), TBARS content (*b*) and superoxide anion radical production (*c*) in *T. nepalense* under Pb (□ and As (● treatment after 24 h. Others same as in Figure 1.

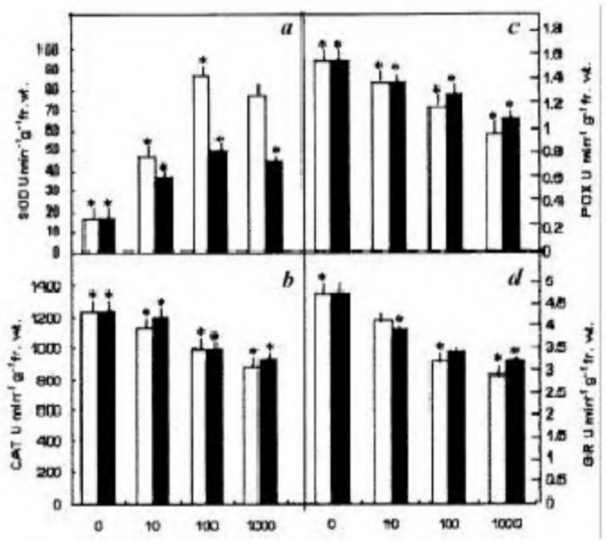

Figure 3. Changes in superoxide dismutase (*a*), catalase (*b*), peroxidase (*c*) and glutathione reductase (*d*) in *T. nepalense* under Pb (□) and As (●) treatment after 24 h. Others same as in Figure 1.

TEM analysis of chloroplast ultrastructure is shown in Figure 5. As a consequence of Pb and As treatment, distortion of the thylakoid membrane was observed under both the metals compared with the chloroplast of the control plants. Marked increase in degree of distortion of thylakoid and plastoglobule was noted at higher Pb concentration followed by As compared to control.

The decrease in dry biomass and chlorophyll content under Pb and As in moss cells were similar with that of other higher plants and moss studied, which may be a metal-specific response on chlorophyll biosynthesis as seen for other heavy metals like Cu, Cd, Cr, Zn, etc.[16,28–30]. A decrease in dry biomass as seen in results under metal treatment may be due to a degradation of chlorophyll resulting in inhibition of photosynthesis[31]. Plants are known to hyperaccumulate various metals and this helps in bioremediation of the environment[12,13,32]. Though at lower concentration of metal, accumulation was slow, at higher concentration both Pb and As showed higher accumulation as seen for higher plants and *Sphagnum*, suggesting that *Taxithellium* with an efficient bioconcentration mechanism can act as pollution indicator[12,13,33].

As a part of triggering of common defence pathways in plant cells to abiotic stress, cytotoxic hydrogen peroxide gets accumulated which acts as a secondary messenger[34]. Accumulation of peroxide is a general stress response, which has been observed in plants exposed to various biotic and abiotic stresses[35]. A similar increase in H_2O_2 level was marked in both Pb and As treatments as seen for moss under other heavy metals toxicity and for higher plants[4,16].

Lipid peroxidation is a process by which the functionality and integrity of the membrane is affected and can produce irreversible damage to cell function. Lipid peroxidation gets initiated by ROS such as O_2^-, $^{\cdot}OH$, and $^1O_2$

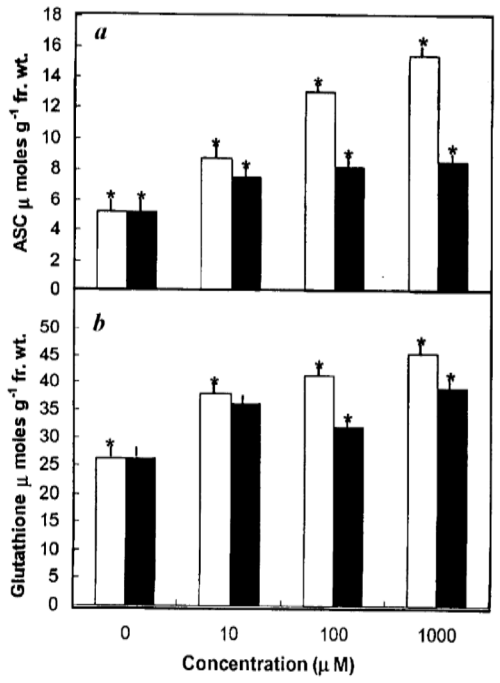

Figure 4. Changes in ascorbate (***a***) and glutathione (***b***) in *T. nepalense* under Pb (□) and As (●) treatment after 24 h. Others same as in Figure 1.

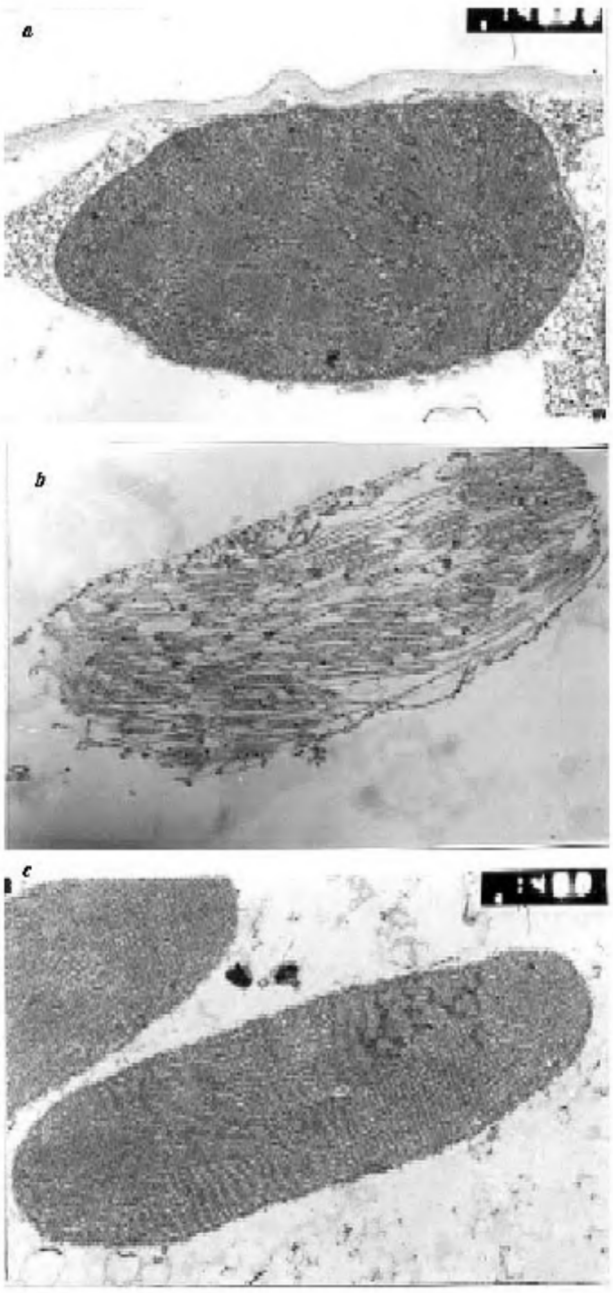

Figure 5. TEM analysis of chloroplast of *Taxithelium nepalense* with or without Pb and As. ***a***, Chloroplast ultrastructure after 24 h without Pb or As. ***b***, Micrograph obtained after 24 h of 1000 : M Pb treatment. Complete distortion in chloroplast membrane and disorganization of thylakoid are seen with change in shape. ***c***, Chloroplast after 24 h of 1000 : M of As. Change in chloroplast structure and disorganization of thylakoid can be seen.

or by lipooxygenases[36]. Both lead and arsenic enhanced the TBARS level in moss, which is an index of lipid peroxidation and oxidative stress[6,16]. Pb and As are not redox metals like Cu and Fe and therefore cannot catalyse Fenton-type reactions yielding hydroxyl radicals. However, this may indirectly cause oxidative stress by disturbing the photosynthetic electron transport that will lead to the production of ROS as substantiated by the degradation of chlorophyll and electron micrographs of chloroplast showing thylakoid disintegration.

An increase in superoxide radical at higher concentrations suggested a loss of normal reduction of oxygen to water and other electron carriers during mitochondrial electron transport. O_2^- is a moderately reactive, short-lived ROS with a half-life of 2–4 μs, which cannot cross biomembranes and is dismutated readily[37] to H_2O_2.

The activity of antioxidative enzymes determines the steady-state levels of ROS in the cell and augmentation of the antioxidative defence plays a pivotal role in regulating oxidative stress. SOD is a key enzyme in protecting the cell against oxidative stress. It catalyses the dismutation of superoxide radical to H_2O_2 and oxygen. An increase in SOD activity indicates higher production of H_2O_2, which has been seen in the case of an increase in endogenous peroxide accumulation[16,38]. Decrease in CAT, GPx and GR by heavy metal ions could result from the attack caused by metal ion-induced ROS, which may be possible in case of non-redox metals causing elevated lipid peroxidation, indirectly resulting in free radical production[4,7,25,29,36]. Ascorbate plays a role as a primary cellular antioxidant and also functions as secondary antioxidant because it represents a cellular reservoir to regenerate α-tocopherol, which scavenges lipid peroxide radicals[39,40]. Glutathione is a measure of non-protein thiol in plants and is involved in the detoxification of heavy metals and xenobiotic compounds that play an important role in gene activation for protection against oxidative stress[41]. Increase in ascorbate and glutathione content therefore, might restrict heavy metal-induced lipid peroxidation and oxidative stress[3,35].

TEM analysis showed disturbances in the cell structure of metal-treated plants with a disruption in chloroplast ultrastructure with a disorganized thylakoid system. The damage in thylakoid structure suggests important disturbances in the metabolic function of organelles affecting chlorophyll biosynthesis, photosynthesis and activities of redox enzymes[38,42], justifying a decrease in growth.

From the above study, it can be concluded that imposition of heavy metal toxicity induces a concentration-dependent oxidative stress as evidenced by biochemical changes, oxidative damage and antioxidant activity. Ultrastructural distortions suggest that the mechanism of heavy metal toxicity might be characterized by oxidative stress.

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Trichromatic sorting of *in vitro* regenerated plants of gladiolus using adaptive resonance theory

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A machine vision system is described to sort the regenerated plants of gladiolus into groups using trichromatic features of leaves. The machine vision system consisted of a scanner, image analysis software and an adaptive resonance theory neural network. Leaf attributes extracted from the image histograms and used for network classification are the mean brightness, grey-scale level and the maximum pixel count. The system was able to sort the regenerated plants into two distinct groups based on the photometric behaviour. Vigilance parameter had a significant effect on grouping. The approach may provide a means of selecting plants suitable for *ex vitro* transfer and also helps in quality control of commercial micropropagation.

THE primary goal of commercial micropropagation is to achieve a large number of genetically identical, physiologically uniform and developmentally normal plants with the ability to survive upon transfer to *ex vitro* conditions in a relatively short period of time. However, one of the major problems in commercialization of the micropropagation technique is the poor survival of regenerated plants upon *ex vitro* transfer. The intrinsic quality of the regenerated plants is largely responsible for its survival during the period of acclimatization. Various approaches such as photoautotrophic micropropagation[1], use of raft and immersion culture with or without growth retardants[2–4] and machine vision system have been adopted to reduce the costs and improve plant survival.

In plant tissue culture system, machine vision has found applications in growth determination of suspension cultures[5] and regenerated whole plants[6], somatic embryo sorting[7], automatic shoot separation[8] and selection of embryogenic cultures[9]. The purpose of this work is to test the hypothesis whether regenerated plants can be sorted out into groups based on their photometric behaviour using image analysis system coupled with neural network algorithm. It is well understood that the successful clustering of regenerated plants gives an opportunity to identify and select plants amenable for *ex vitro* survival.

In the present communication, we describe a method to project the trichromatic variations of regenerated plants and sort them out into groups using adaptive resonance theory (ART2). ART2 is a neural network algorithm deri-

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