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Studies on the Effects of Platinum Group Elements on *Lactuca sativa* L.

[1,2]Victor J. Odjegba, [2]Murray T. Brown and [3]Andrew Turner
[1]Department of Botany and Microbiology, University of Lagos,
Akoka-Yaba, Lagos, Nigeria
[2]School of Biological Sciences, University of Plymouth,
Plymouth PL 48 AA, United Kingdom
[3]School of Ocean, Earth and Environmental Sciences, University of Plymouth,
Lymouth PL 48 AA, United Kingdom

Abstract: The uptake, accumulation and effects of environmentally relevant platinum group elements (PGE; Rh, Pd and Pt) on *Lactuca sativa* were investigated. Seedlings were exposed to nutrient solution amended with mixed solutions of PGE (0, 0.3, 0.5, 0.7 and 1.0 ppm each) of PGE (Pt, Pd and Rh as a mixture) for a period of 4 weeks. The results showed a significant ($p<0.05$) reduction in plant dry weight, nitrate reductase activity, protein content and photosynthesis efficiency when treated with concentrations greater than 0.5 ppm. The treatments also induced oxidative stress and membrane damage, as indicated by catalase activity and lipid peroxidation assays, respectively. Metal analysis in plant tissues by ICP-MS showed that accumulation followed the order Rh ~ Pt >> Pd. Seedlings exposed to 1.0 ppm of PGE for a period of 4 weeks accumulated about 50 mg kg^{-1} of Rh and Pt and about 30 mg kg^{-1} of Pd. The results of this study have exemplified the potential effects of these elements, albeit at concentrations in excess of typical environmental levels.

Key words: Platinum group elements, accumulation, toxicity, environment, *Lactuca sativa*

INTRODUCTION

In recent years, there has been a requirement in many countries that all new motor vehicles must be equipped with catalytic converters in an effort to reduce air pollution. The modern three-way catalysts consist of a ceramic monolith in the form of honeycomb. On the monolith, a wash-coat of γ-alumina is applied so as to increase the active surface and PGE (platinum, palladium and rhodium) are then dispersed on the wash-coat. The catalytic converters can remove about 90% of CO, unburnt HC and NO_x from the exhaust gasses. Although the benefits of this technology to the environment are obvious, studies have indicated that due to thermal, mechanical and chemical impacts, particles containing PGE are emitted into the environment (Verstraete *et al*., 1998). As a result, a considerable increase in the concentrations of Pt, Pd and Rh in vegetation, soils and surface waters, especially at sites adjacent to motorways with high traffic, have been observed over the past few decades (Locatelli *et al*., 2005).

Despite growing concerns over the potential environmental and biological impacts of PGE, there is limited quantitative and mechanistic information about their uptake and behaviour in natural systems. In particular, their potential to enter the human food chain deserves attention because it has been reported that compounds of Pt (IV) and Pd (II) are nephrotoxic and gastrointestinal irritants, while Pt (II) compounds may have mutagenic, genotoxic or carcinogenic properties (Vlasankova, 2001). The practice of cultivating leafy vegetables for both human and livestock consumption in gardens

Corresponding Author: Victor J. Odjegba, Department of Botany and Microbiology, University of Lagos, Akoka-Yaba, Lagos, Nigeria

adjacent to roads carrying heavy traffic therefore represents a significant vector for the exposure of PGE to humans. To this end, the study reported here aimed to investigate the uptake, accumulation and effects of aqueous PGE on hydroponically cultivated *Lactuca sativa* (lettuce), a leafy vegetable of economic importance.

MATERIALS AND METHODS

Germination Study

Individual plasma emission standards of Rh (III), Pd (II) and Pt (IV) in 1.2 M HCl were obtained from BDH/Merck. Lettuce seeds were purchased from a local seed store in the city of Plymouth, UK in a single batch and enough for the study. The nutrient solution was a quarter-strength Hoagland's solution (pH 6.5) as modified by Punshon and Dickinson (1997) containing (mM): 2.5 NH_4 $(NO_3)_2$, 1.25 Ca $(NO_3)_2$, 1.5 KNO_3, 0.5 KH_2PO_4, 1.5 $MgSO_4$ and 0.25 NaCl and (μM): 10.0 H_3BO_3, 2.3 $MnCl_2$ and 0.025 $H_2M_0O_4$. Each Petri dish contained 10 seeds and received 2 mL of solution every 2 days for 10 days; all dishes were placed in a temperature controlled growth cabinet at 23°C.

Seedling Growth and Treatments

Lettuce seedlings of similar height (10 cm) were selected for experimental use. Nutrient solution devoid of the PGE served as control. Seedlings were grown in 15 mL glass test-tube, one per tube and exposed to one of five concentrations of PGE (0, 0.3, 0.5, 0.7 and 1.0 ppm) dissolved in the nutrient solution described above. Both the control and treated solutions were adjusted to pH 6.5 using potassium phosphate buffer. The experiment was carried out in a growth chamber maintained at 23°C, a photoperiod of 16:8 h (L:D), relative humidity of 61.3% and irradiance of 70 μmol m^{-2} s^{-1} for 6 weeks. Solutions were replaced every 5 days to avoid depletion of nutrient and metals. There were 20 replicates per treatment.

Growth Measurements and Chemical Analyses

Lipid Peroxidation

Lipid peroxidation in the leaves was estimated by measuring the content of thiobarbituric acid reactive compounds (TBAR), mainly malondialdehyde according to a modified procedure of Wang and Jin (2005). Fresh leaves (0.2 g) were ground in liquid N_2 and extracted in 1 mL 20% trichloroacetic acid. The extract was centrifuged (10000 g) for 5 min at 4°C. Supernatant (0.5 mL) was mixed with 0.5 mL of 0.6% (w/v) thiobarbituric acid solution comprising 10% TCA. The mixture was incubated for 30 min in a boiling water bath and allowed to cool; measurements were made at 450, 532 and 600 nm. Malondialdehyde (μM/g f. wt) was calculated as 6.45 $(A_{532}-A_{600})$-0.56 A_{450}.

Nitrate Reductase Activity

The extraction and assay of nitrate reductase was estimated by a modified method of Fan *et al.* (2002). Fresh leaves (0.2 g) was ground and extracted in 2 mL of distilled water, centrifuged (10000 g) for 5 min at 4°C. One milliliter of the crude extract was incubated at 25°C in the dark for 1 h in 3 mL of the substrate assay solution in a test tube. The substrate assay solution contained 1 mL each of 0.1 M KNO_3, 15 mL L^{-1} propan-1-ol and 0.1 M potassium phosphate buffer (pH 7.5). One milliliter of the solution was transferred using a pipette after the incubation period into a clean test tube. One milliliter each of 1% sulphanilic acid and 0.02% naphthylenediamine (NED) was added, the mixture was thoroughly shaken and left to stand for 1 h to allow full colour development. A blank was prepared by mixing 1 mL of substrate assay solution, 1 mL of 1% sulphanilic acid and 1 mL of 0.02% naphthylenediamine in a test tube. The mixture was allowed to stand for 1 h for colour development. Colour development due to nitrite was then measured spectrophotometrically at 540 nm. The values were compared to a standard curve generated using solutions of $NaNO_2$.

Protein Estimation

This was achieved according to the method outlined by Bradford (1976). Thus, 100 mg of Coomassie Brilliant Blue G-250 were dissolved in 50 mL of 95% ethanol. To this, 100 mL of 85% potassium phosphate buffer (pH 7.5) were added. The mixture was made to 1000 mL with distilled water and this is the protein reagent. Five mL of the reagent were mixed with 0.1 mL of extract and read after 2 min at 595 nm. Bovine serum albumin (BSA) standard solutions (10-100 μg mL^{-1}) protein in 0.1 mL was mixed with 5 mL of reagent and absorbance read at 595 nm. The blank was 0.1 mL of buffer and 5 mL of reagent. Protein content was determined from a standard curve generated from solutions of BSA.

Chlorophyll Fluorescence

Chlorophyll fluorescence of photosystem II of the plant was measured with a PAM fluorometer (FMS 1, Hansatech Instruments Ltd., Norfolk, UK). The measurements were performed at room temperature (24°C). The time of measuring was 5 sec and irradiance was set at 75% of maximum. The efficiency of photosystem II photochemistry (ΦPSII), maximum quantum yield (F_v/F_m), the efficiency of water splitting apparatus (F_o/F_v), the plastoquinone pool (Fv/2), initial fluorescence (F_0) and the variable fluorescence (F_v) were recorded.

Metal Analysis in Plant Tissues

About 0.5 g of oven dried tissue samples were weighed individually in to quartz digestion vessels. Ten milliliter of concentrated HNO_3 and 2 mL of 30% HCl were added and the contents heated to near-dryness. Ten milliliter of 20% HNO_3 were then added and the contents refluxed. After cooling, the digests were filtered through Whatman No. 1 filter papers into individual 25 mL volumetric flasks and adjusted to volume with 2% HNO_3. Metal concentrations were determined in the digests inductively coupled plasma-mass spectrometry (ICP-MS) using a Thermo elemental Plasma Quad 2+ fitted with a Meinhard dissolved solids nebuliser. 115-In and 193-Iridium were added to all samples and mixed standards (prepared over the working range in 10% HNO_3) at a concentration of 100 ppb each to act as internal standards over the mass/charge range studied.

Data Analysis

All measurements in triplicates were analysed using one way analysis of variance and differences in means were determined by Tukey's pairwise comparisons.

RESULTS

Effect of PGM on Germination of Lactuca Sativa Seeds

PGE concentrations of < 0.7 ppm had no effect on the germination efficiency of the seeds, but above this concentration, there was a significant ($p = 0.05$) reduction in the number of germinated seeds with corresponding increase in PGE concentration (Table 1).

Table 1: Effects of PGE on the germination of *Lactuca sativa*

PGE concentration (ppm)	Percentage germination
0	100a
0.3	100a
0.5	100a
0.7	90ab
1.0	80b

Different letter(s) indicate significant difference at $p = 0.05$ according to Tukey's pairwise comparison

Table 2: The dry weight (g) of *Lactuca sativa* after 4 weeks of treatment with PGE

PGE concentration (ppm)	Dry weight (x 10^{-2}g)
0	4.94±0.04a
0.3	4.88±0.04a
0.5	4.22±0.05b
0.7	3.99±0.07c
1.0	3.36±0.05d

Different letter(s) indicate significant difference at p = 0.05 according to Tukey's pairwise comparison

Table 3: Leaf area (cm^2) of *Lactuca sativa* treated with various concentrations (ppm) of PGE

PGE concentration (ppm)	Leaf area (cm^2)
0	3.91±0.18a
0.3	3.69±0.32a
0.5	3.46±0.12a
0.7	2.71±0.08b
1.0	2.33±0.02b

Different letter(s) indicate significant difference at p = 0.05 according to Tukey's pairwise comparison

Plants' Dry Weight

There was no significant (p = 0.05) difference in the dry weight of the control and those exposed to 0.3 ppm of PGE. Plants treated with 7.0 and 1.0 ppm had dry weights of 0.0399±0.0007 and 0.0336±0.0005 g, respectively. These values were significantly (p = 0.05) lower than 0.0494±0.0004 g observed for the control plants (Table 2).

Effect on Leaf Area

The leaf area of the plant decreased with increase in PGM concentrations. The control plants had a mean value of 3.91±0.16 cm^2, while plants treated with 0.5, 0.7 and 1.0 ppm had 3.46±0.12, 2.71±0.08 and 2.33±0.02 cm^2, respectively (Table 3).

Effect of PGM on Catalase Activity

We examined the effect of PGM on catalase activity of the plant. Catalase activity at 25°C for the control plants was 0.283±0.006 mmol mg^{-1} protein min^{-1}. There was a significant (p = 0.05) increase in the enzyme activity of plants treated with PGE (Fig. 1). The increase in the enzyme activity was proportional to the concentration of the PGE to which they were exposed.

Effect on Lipid Peroxidation

The malondialdehyde content measured spectrometrically was found to be significantly (p = 0.05) more in PGE treated plants than the control (Fig. 2). A mean value of 2.827±0.199 µM g^{-1} f.wt was recorded for the control plant while 3.787±0.163, 5.217±0.199 and 8.850±0.060 µM g^{-1} f.wt were the mean values for plants treated with 0.3, 0.7 and 1.0 ppm of PGE, respectively.

Nitrate Reductase Activity

The effect of PGE on nitrate reductase activity (NRA) in the crude extract of *L. sativa* was examined. The result showed a reduction in NRA when exposed to PGE. The control plants had a mean NRA value of 0.209±0.012 µmol h^{-1} g^{-1} f.wt. The value was significantly (p = 0.05) lower than 0.117±0.003 µmol h^{-1} g^{-1} f.wt observed for plants treated with 1.0 ppm of PGE (Fig. 3).

Effect of PGM on Total Protein Content

A decrease in total protein content was observed in all the PGE-treated plants (Fig. 4). The lowest value (0.389±0.003 mg g^{-1} f. wt) was observed in plants treated with 1.0 ppm of PGE.

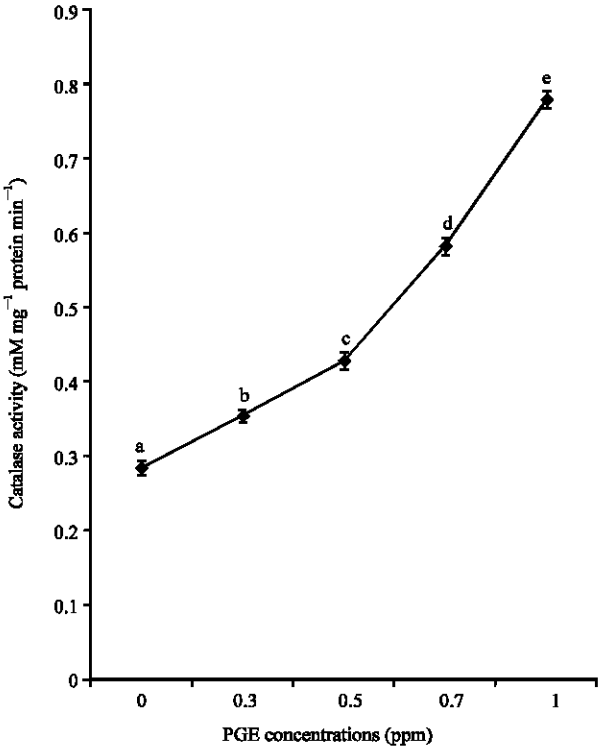

Fig. 1: Catalase activity of *Lactuca sativa* treated with PGE. Different letter(s) indicate significant difference at p = 0.05 according to Tukey's pairwise comparison

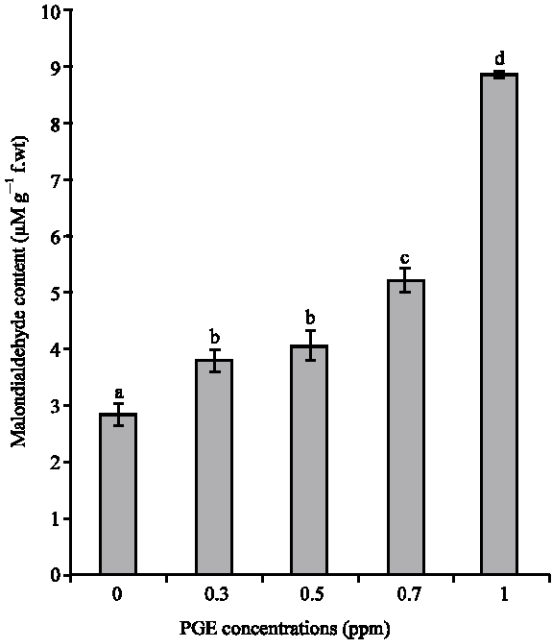

Fig. 2: Lipid peroxidation in leaves of *Lactuca sativa* treated with PGE. Different letter(s) on columns indicate significant difference at p = 0.05 according to Tukey's pairwise comparison

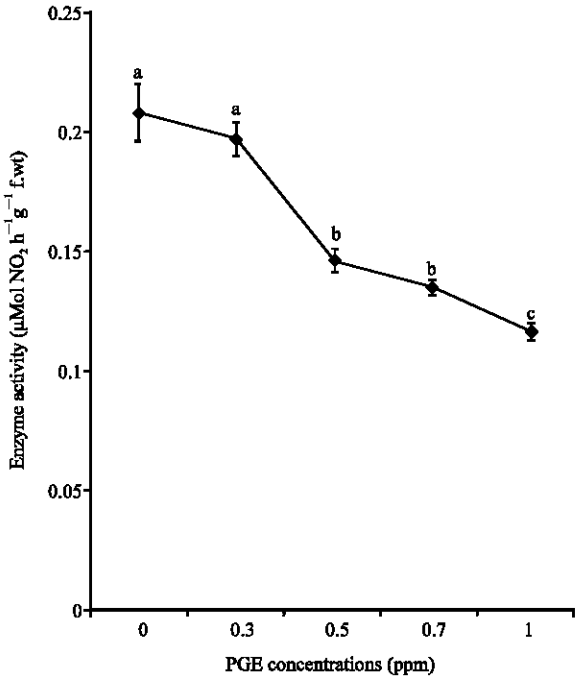

Fig. 3: Nitrate reducatase activity of *Lactuca sativa* exposed to various concentrations of PGE. Different letter(s) indicate significant difference at p = 0.05 according to Tukey's pairwise comparison

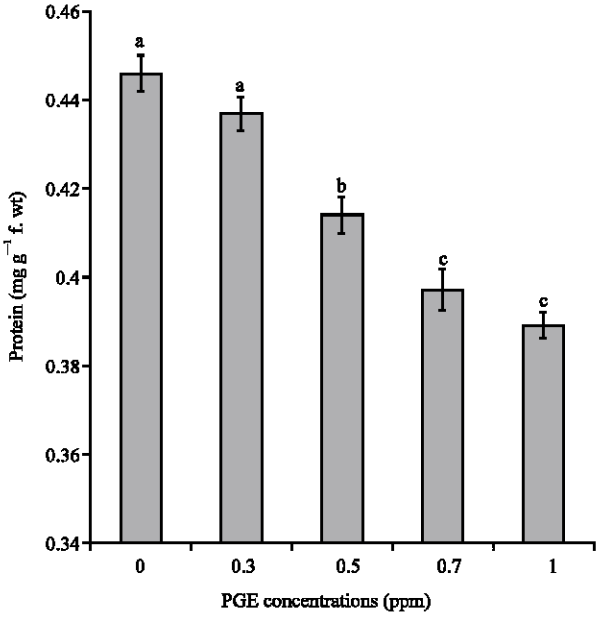

Fig. 4: Effect of various concentrations of PGE on the protein content of *Lactuca sativa*. Different letter(s) indicate significant difference at p = 0.05 according to Tukey's pairwise comparison

Chlorophyll Fluorescence Parameters

Chlorophyll fluorescence parameters of leaves are presented in Fig. 5-10. The efficiency of photosystem II photochemistry (ΦPSII) in leaves of PGE-treated plants was lower than those of the control. The effect was significant (p = 0.05) when PGE concentration was 1.0 ppm. Similar trend was

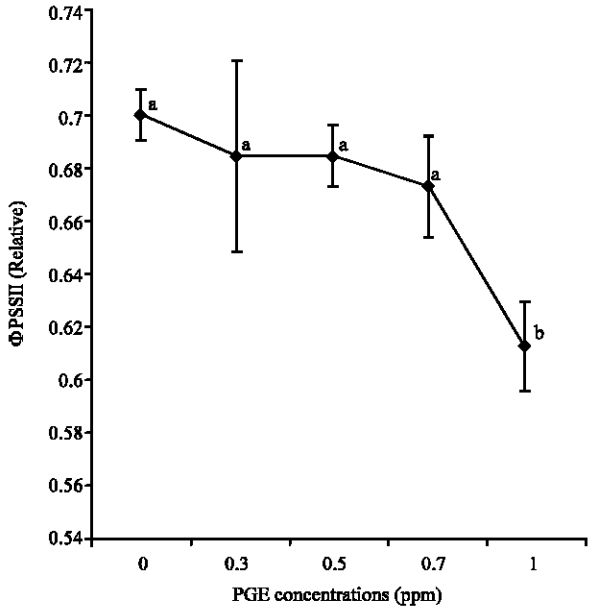

Fig. 5: Toxicity of PGE on the photosytem II of *Lactuca sativa*. Different letter(s) indicate significant difference at p = 0.05 according to Tukey's pairwise comparison

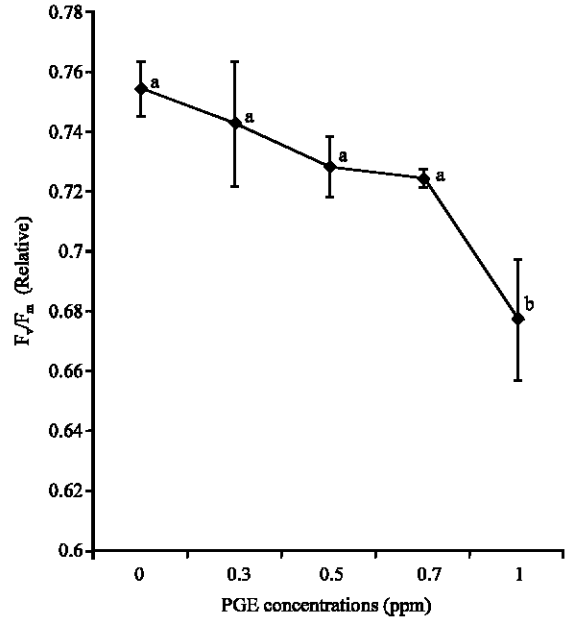

Fig. 6: PGE-induced reduction in maximum quantum yield of *Lactuca sativa*. Different letter(s) indicate significant difference at p = 0.05 according to Tukey's pairwise comparison

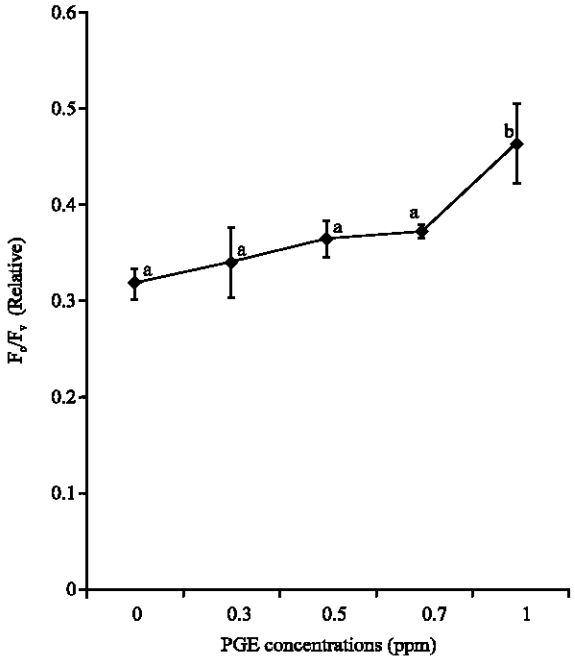

Fig. 7: Effects of PGE on the efficiency of the water-splitting apparatus (F_o/F_v) of *Lactuca sativa*. Different letter(s) indicate significant difference at p = 0.05 according to Tukey's pairwise comparison

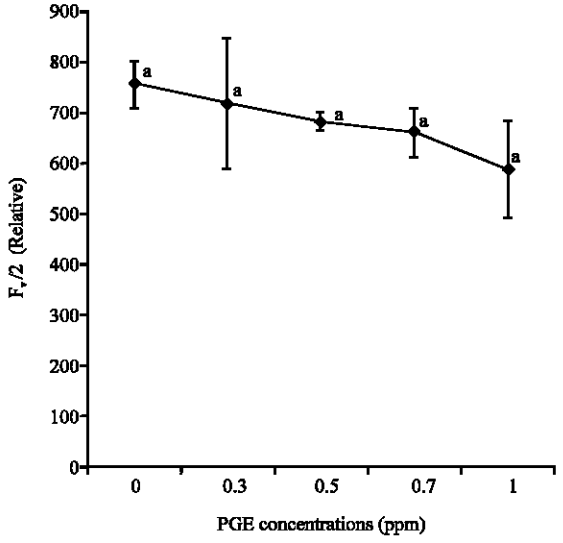

Fig. 8: Effects of PGE on the plastquinone pool of *Lactuca sativa*. The same letter(s) indicate no significant difference at p = 0.05 according to Tukey's pairwise comparison

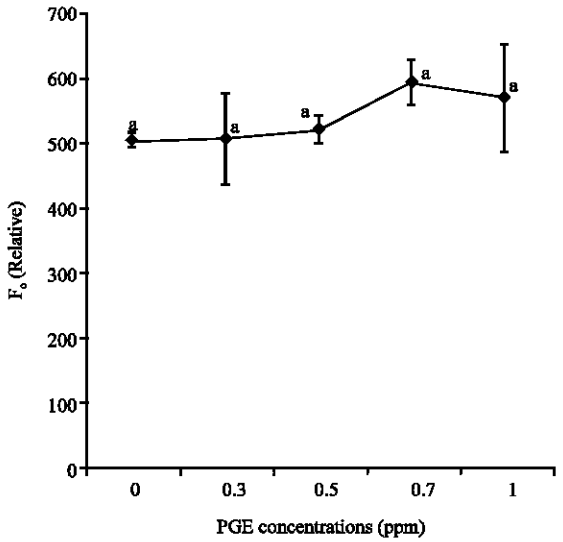

Fig. 9: Initial fluorescence (Fo) of *Lactuca sativa* as affected by different concentrations of PGE. The same letter(s) indicate no significant difference at p = 0.05 according to Tukey's pairwise comparison

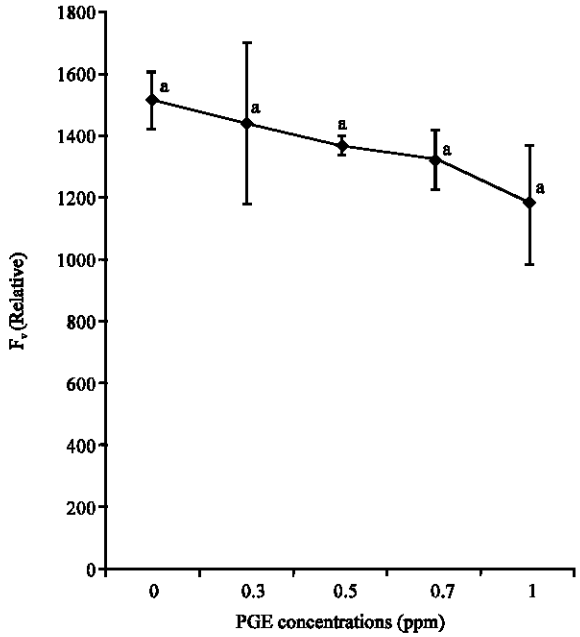

Fig. 10: Effect of PGE on the variable fluorescence (F_v) of *Lactuca sativa*. The same letter(s) indicate no significant difference at p = 0.05 according to Tukey's pairwise comparison

found in F_v/F_m, a parameter that measures the intrinsic (or maximum) efficiency of photosystem II. The PGE reduced this parameter in a concentration-dependent manner, which was more pronounced in plants treated with 1.0 ppm of PGE. The PGE-induced changes in chlorophyll fluorescence kinetics also included a progressive rise in values of F_0/F_v which was significant (p = 0.05) when treatment

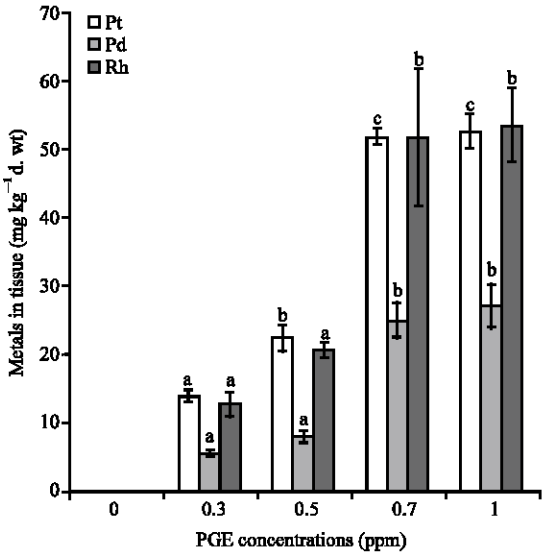

Fig. 11: Metal concentrations (mg kg^{-1} d.wt) in *Lactuca sativa* following exposure to concentrations (ppm) of PGE. Different letter(s) on identical columns indicate significant difference at p = 0.05 according to Tukey's pairwise comparison

concentration was 1.0 ppm (Fig. 7). Figure 8 presents the impact of the PGE on plastoquinone pool ($F_v/2$) of *L. sativa*. The result showed that plastoquinone pool values were lowered by PGE in a concentration-dependent manner. Data showing the effects of PGE on initial fluorescence (F_0) and variable fluorescence (F_v) of *L. sativa* are as shown in Fig. 9 and 10. The treatment increased the F_0 fluorescence level. In contrast, the variable fluorescence yield (F_v) was decreased by the treatment. The severity of the effects as indicated by both parameters was concentration dependent.

Metal Accumulation in Plants

Data showing the accumulation of Pt, Pd and Rh by the experimental plant is as shown in Fig. 11. The accumulation was concentration dependent. Accumulation of Pd was the least while Pt and Rh were at pa (Fig. 11). Plants exposed to 1.0 ppm of PGE for the experimental period had mean values of 52.65±2.46, 27.01±3.29 and 53.51±5.24 of Pt, Pd and Rh, respectively.

DISCUSSION

The changes in parameters measured due to PGE treatments revealed that these metals are toxic to plants. The inhibition of growth as indicated by dry weight and leaf area could be a result of direct interference with enzymes of physiological importance. Similar effects have been reported from studies involving metals like Cd, Cu, Pb, Hg and Ni (Clijsters *et al*., 1999; Chatterjee and Chatterjee, 2000; Odjegba and Fasidi, 2004). Catalase is one of the scavenging enzymes for reactive oxygen species (ROS) found in plants and it is primarily involved in the decomposition of H_2O_2 to water and molecular oxygen. ROS are produced as a consequence of electron transport processes in photosynthesis and aerobic respiration (Foyer *et al*., 1994; Mano *et al*., 2002). ROS are reactive and potentially harmful to cells, causing oxidation of lipids, proteins and nucleic acids. Its generation by the chloroplast can be increased by factors such as excess light, drought, salt and heavy metal stress and in some cases conditions of limiting carbon dioxide by activation of the photorespiratory pathway

resulting in H_2O_2 production in the peroxisomes. The result from this study indicated increased activity of catalase which was concentration dependent. The corresponding increase in catalase activity was necessary to meet up with the detoxification process in order to keep the cells alive.

The result of our study indicated higher contents of malondiadehyde in PGE-treated plants, indicating that exposure to PGE induced cell membrane damage through lipid peroxidation. The membrane damage may be related to the excessive generation of ROS as a result of PGE treatments. Similar results have been reported that a high level of copper induced lipid peroxidation in *Silene cucubalus* roots (De Vos *et al*., 1991) and *Triticum aestivum* leaves (Luna *et al*., 1997). A significant decrease in nitrate reductase activity was observed at PGE level as low as 0.5 ppm. The interaction of the PGE with the enzyme could be as a result of the metals binding to functional ligands of the enzyme involved in the catalytic action. Nitrate reductase contains essential-SH Cys residues (Campbell, 1996) which could be modified by PGE. The low protein content in PGE-treated plants could be related to disruption of nitrogen metabolism in the affected plants (Solomonson and Barber, 1990).

Chlorophyll fluorescence analysis has become one of the most widely used techniques available to plant physiologists and ecophysiologists. Light energy absorbed by chlorophyll molecules in a leaf undergo one of three processes: (I) It can be used in photosynthesis, (ii) excess energy can be dissipated as heat or (iii) It can be re-emitted as light-chlorophyll fluorescence. These three processes occur in competition and an increase in the efficiency of one results in a decrease in the yield of other two (Maxwell and Johnson, 2000). Therefore, by measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photosynthesis and heat dissipation can be gained. Our study showed that PGE reduced the efficiency of photosystem II photochemistry (ΦPSII) as well as the maximum quantum yield of PSII (F_v/F_m) indicating the phenomenon of photoinhibition which may be due to partial blocking of electron transport from PSII to PSI by the stress factor. The results agreed with the findings of Bjorkman and Demmig (1987) and Johnson *et al*. (1993). There was an increase in the values of F_0/F_v at high PGE concentrations, indicating that the test metals had effect on the water-splitting apparatus. The reduction in the plastoquinone pool as represented by the $F_v/2$ ratio (Fig. 8) could be one of the possible causes for the reduced quantum yield under metal stress. The result from this study showed that minimal fluorescence (F_0) increased under PGE stress. The F_0 is an emission from the excited chlorophylls in PSII antenna in competition to excitation energy transfer to the PSII reaction center, which takes place before the excitons reach the reaction centre (Mallick and Mohn, 2003). Since F_0 originates from chlorophyll a antennae associated with the PSII light-harvesting complex (Karukstis, 1991), it is evident from this study that the efficiency of energy transfer from this complex to the PSII reaction center was reduced under PGE stress.

The study showed that *Lactuca sativa* accumulated substantial amounts of the metals capable of altering the physiological status and inhibiting the normal growth of the plant. In conclusion, the study reported here established the status of PGE as potent environmental pollutants based on the results presented. Plants could accumulate these metals to significant concentrations enough to cause harmful effects to the plant itself, livestock and humans. Therefore, the release of these metals into the environment requires serious attention.

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