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AGRONOMY

# Perchlorate Uptake and its Effect on Physiological, Biochemical and Growth Parameters of Eucalyptusplant under Ammonium Perchlorate Stress

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**Abstract**

Perchlorate is a significant environmental pollutant affecting public health. When entered in ecosystem it is highly toxic. Present investigation focused on the influence of perchlorate on physiological and biochemical parameters of *Eucalyptus citriodora* and also its accumulation in plant tissue and depletion from soil. *Eucalyptus* plants were treated with varying concentration of ammonium perchlorate from 1000ppm, 2000ppm, 5000ppm and 10000 ppm. Perchlorate inside the plant tissue and in soil was quantified at regular intervals. Amount of perchlorate inside plant tissue observed to increase with number of days after treatment and is proportional to concentration of perchlorate in soil. Morphological characters like shoot length, root length, number of leaf were observed to decrease with increasing concentration of perchlorate. The present study revealed decrease in chlorophyll and reducing sugar content in leaf tissue under varying concentration of perchlorate and is indirectly proportional to amount of perchlorate. While total protein, proline, and phenol content showed a gradual increase with increase in concentration of perchlorate and in different experimental set up, suggesting increase in non-enzymatic stress parameters with increased level of toxicity. An increased activity of peroxidase enzyme was observed with increased dose of ammonium perchlorate. It can be concluded that perchlorate related influence on plant are both qualitative and quantitative and depends upon its concentration and duration of exposure.

**Highlights**

- Perchlorate in the soil is a potential abiotic stress to Eucalyptus plants affecting its physiological and biochemical parameters.
- Presence of perchlorate inside the Eucalyptus plant tissue enhances the activity of stress related enzyme and amount of protein, phenol and proline.

**Keywords:** Ammonium perchlorate, *Eucalyptus*, guaiacol peroxidase

Perchlorate in the soil and ground water is a potential health hazard and a contaminant of concern. Perchlorate salt is used as an oxidizing agent in rocket propellants and explosives (Bradford *et al.* 2006). The U.S. Environmental Protection Agency (USA EPA) reported perchlorate contamination in ground waters of south- western United States, used for municipal drinking water supplies [Urbansky 2002]. Perchlorate salts are soluble in water. These ions are risk factors for human health. They substitute iodine and interrupt thyroid iodine uptake in human beings resulting in abnormal growth and development by

reducing thyroid hormone production. Since first discovered in drinking water in the USA, it has been detected in several parts of the world including India. A recent study shows the presence of perchlorate in different regions of India ranging from 0.009- 7700 µg/l near Sivakasi followed by Chennai (0.005-24µg/l) during fireworks (Isobe *et al.* 2012). The U.S. EPA and a number of researchers investigated the status of perchlorate accumulation in vegetation, its uptake and metabolism by higher plants (Seyfferth and Parker, 2008). Studies suggest role of higher plants, both as dietary components, and as a key entry point into the food chain (Parker 2009). In the year 1999, Nzengung, V. A. showed that rooted cuttings of woody plants like *Eucalyptus spp.* is able to remove perchlorate from solutions (Nzengung, Wang and Harvey). Although earlier research has been conducted on uptake of perchlorate in plants but data regarding its growth, physiological and biochemical effect on plant is very less. Physiological and molecular aspects of *Arabidopsis thaliana* and *Nicotiana tobacum* plants were shown to be affected by perchlorate (Hamissou 2011).

Phytoremediation is an emerging technology that promises effective removal and degradation of toxic contaminant from environment. The present investigation was under taken to study the role of plant in uptake and accumulation of perchlorate in plant tissue and depletion from the soil. Efforts were also undertaken to find out effect on growth, physiological and biochemical parameters of *Eucalyptus* plant in presence of ammonium perchlorate stress. Studies on enzymatic and non-enzymatic parameters under stress condition were also conducted. Antioxidative enzymes get activated in presence of reactive oxygen species which ultimately scavenge the oxygen radicals and to make the plant tolerant against oxidative damage.

## Materials and Methods

Present study was conducted in the Department of Biotechnology, Modern College, Ganeshkhind, Pune, India during 2011-2013. Plants of *Eucalyptus citriodora* were collected from local nursery of Pune, (Maharashtra, India) and were maintained in green house under controlled environmental conditions. Plants were established in well insulated plastic pots (15cm ×15cm×12 cm) containing 2.5 kg garden soil mixed with biofertilizers (cowdung manure).

### *Experimental set up*

Perchlorate treatment consisted of irrigating the experimental plants once in 15 days with 100ml solution of ammonium perchlorate. Four sets of experimental plant were maintained with varying concentration of perchlorate ranging from 1000ppm (0.1%), 2000ppm (0.2%), 5000ppm (0.5%), 10000 ppm (1.0%). Each treatment was replicated three times (ten plants per treatment). The control plants without perchlorate treatment were also maintained and irrigated with distilled water. Treated plants were also irrigated with distilled water thrice a week. Data is represented as an average of three replicates for each experimental set up.

### *Perchlorate analysis from plant tissue*

Perchlorate amount was analyzed spectro-photometrically by method given by Pourreza, N.; Mousavi, H.Z. (2005) with modification [8]. Leaf tissue was homogenized in distilled water and kept overnight on shaker at room temperature. The homogenate was centrifuged at 10,000 X g and supernatant was treated with activated charcoal to remove chlorophyll pigments. The extract was reacted with thionine chloride in presence of formate buffer ($pH_3$) which form blue colour complex with perchlorate. Perchlorate was calculated by measuring absorbance at 603nmon UV- Visible spectrophotometer (ChemitoSpectra scan, UV2600) and amount taken from standard curve of ammonium perchlorate. Perchlorate amount was expressed in µg/g fresh weight.

### *Perchlorate analysis from soil*

Perchlorate amount was analysed spectrophotometrically by method given by Pourreza, N.; Mousavi, H.Z.. Soil solution was prepared by dissolving soil in distilled water in 1:2

ratio and kept overnight on shaker. Soil solution was then filtered through whatmann no.1 filter paper. The filtrate was reacted with thionine chloride in presence of formate buffer ($pH_3$) which form blue colour complex with perchlorate. Perchlorate was calculated by measuring absorbance at 603nm on UV-Visible spectrophotometer (Chemito Spectra scan, UV2600) and amount taken from standard curve of ammonium perchlorate. Perchlorate amount was expressed in µg/g.

***Determination of plant growth***

Plant growth was analysed by determining parameters like shoot length, root length, number of leaves, percentage of chlorosis of leaf and fresh weight is also estimated at regular interval of time.

***Estimation of chlorophyll content***

Chlorophyll estimation was performed spectrophotmetrically by method given by Arnon, D.I.. Fresh leaf was homogenized in 80% acetone and homogenate was centrifuged for 5000 X g for 5 minutes and step was repeated until a clear solution was obtained, and absorbance of solution was taken at 645 nm, 652 nm and 663 nm on UV- Visible spectrophotometer (ChemitoSpectrascan, UV2600). The amount of chlorophyll a, chlorophyll b and total chlorophyll was calculated and expressed in mg/gm fresh weight of leaf tissue.

***Estimation of reducing sugar***

Reducing sugar investigation was done by dinitrosalicylic acid method, which involved extraction of leaf in 80% ethanol and heating at 80°C. 3ml of residue dissolved in distilled water was incubated with 3 ml of DNS (Dinitrosalicylic Acid) reagent in boiling water bath for 5 minutes, then 1 ml of 40% Rochelle salt (Potassium sodium tartrate) solution was added and absorbance was measured at 510nm on UV- Visible spectrophotometer (ChemitoSpectrascan, UV2600). Reducing sugar was calculated from standard curve of glucose and is expressed in mg/g fresh weight of plant tissue (Somogyi, 1952; Krishnaveni *et al.* 1984).

***Estimation of total protein***

Total protein content was determined by Bradford method. Leaf sample homogenized in 0.1M phosphate buffer (pH 7.0) with dye binding solution (Commassie Brilliant Blue G-250) for 15mins and absorbance at 595 nm on UV- Visible spectrophotometer (ChemitoSpectrascan, UV2600) was taken after the blue colour was developed. Total protein was calculated from the standard graph of bovine serum albumin protein and expressed in mg/g fresh weight of plant tissue.

***Estimation of proline content***

Total proline content was determined by the technique of Bates *et al.*. Homogenized leaf extract was prepared in 3% aqueous sulphosalicylic acid in mortar and pestle and filtered through Whatman No. 2 filter paper. The filtrate was incubated with glacial acetic acid and acid ninhydrin in boiling water bath for 1hr; reaction was terminated by keeping the reaction mixture on ice for 1min. 4ml toluene was added to mixture for separation of proline. Amount was calculated by measuring absorbance at 520nmon UV- Visible spectrophotometer (ChemitoSpectrascan, UV2600). Proline was taken as standard for estimation and amount expressed in mg/g fresh weight of plant tissue.

***Estimation of total phenol***

Total phenol content under stress conditions as well as in control was estimated following the method of Malik and Singh (1980). Leaf tissue was homogenized in 80% ethanol and the homogenate centrifuged at 10,000X g for 20 minutes. The supernatant was evaporated at 80°C. The residue was dissolved in distilled water; 3ml extract was incubated with 0.5 ml Folin Ciocaltuae reagent (FCR) for 30mins. To the reaction mixture, 20% $Na_2CO_3$ was added and kept on boiling water bath for 1min. Absorbance was measured at 650nmon UV- Visible spectrophotometer (ChemitoSpectrascan, UV2600) for calculating the amount of total proline content. Catechol was used as standard for calculations.

*Estimation of Guaiacol-Peroxidase Enzyme*

Level of peroxidase activity was analysed spectrophotometrically by the technique given by Putter in 1974. Leaf tissue was homogenized in chilled 0.1M phosphate buffer (pH-7) and homogenate centrifuged at 18,000X g under low temperature(5°C) for 15 min. Supernatant was used as enzyme extract. The reaction mixture included: buffer solution (3 ml), guaiacol solution (0.05 ml), enzyme extract (0.1 ml), $H_2O_2$ solution (0.03 ml). The activity of enzyme was measured spectrophotometrically by taking absorbance at 436 nm on UV- Visible spectrophotometer (ChemitoSpectrascan, UV2600). Enzyme Activity was calculated by formula= $(3.18 \times 0.1 \times 1000)/ (6.39 \times 1 \times \Delta t \times 0.1) = 500/ \Delta t$, and expressed as Units/Litre.

**Results and Discussion**

The present study was conducted with the primary aim to establish whether *Eucalyptus citriodora* plant absorbs perchlorate from the soil and the quantity which the plant absorbs may be directly correlated with the depletion of perchlorate in the soil. Further objective were to determine its effect on plant growth, physiological and biochemical aspects. The data generated clearly reveals a correlation of perchlorate with *Plant Physiology*ogy and growth. Ammonium perchlorate used for treatment is found to have profound effect on growth of plant and at the same time plants are able to uptake and accumulate perchlorate in their tissue basically in leaves. The uptake and accumulation of perchlorate inside plant tissue and depletion from soil was estimated regularly every week after treatment. Amount of perchlorate evaluated in plant tissue and also in soil is depicted in Figure 1. The amount of perchlorate estimated inside the leaf tissue was found to increase with number of days after treatment and also with increasing concentration of perchlorate. Results showed that perchlorate amount was 0.22µg/ gm fresh weight in plant irrigated with 1000 ppm (0.1%) of ammonium perchlorate treatment on 1st week which increased to 2.21µg/gm fresh weight by 4th week. Similarly with increasing concentration of perchlorate in different treatment taken during the present study, amount of perchlorate increased in leaf tissue from 2.21µg/gm fresh weight in 1000 ppm (0.1%) of ammonium perchlorate treatment to 7.54µg/gm fresh weight in10000ppm (1.0%) of ammonium perchlorate treated plant on 4th week [Figure 1 (A.)].

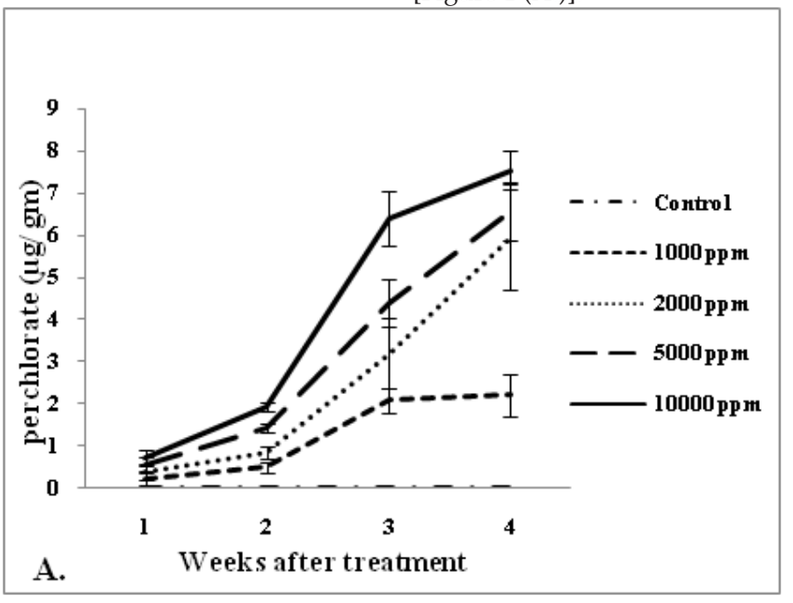


**Figure 1. A.** Perchlorate uptake in plant tissue

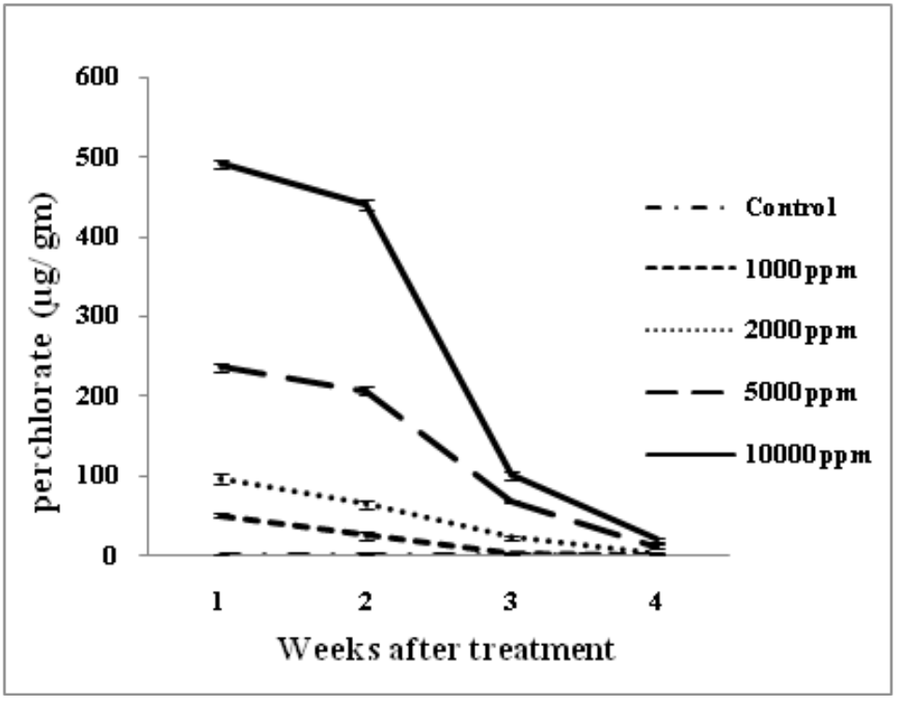


**Figure B.** Amount of perchlorate in soil

**The represented values are average of triplicates ± Standard Deviations**

The concentration of perchlorate in the leaf tissue of *E. citriodora* increased gradually with days after treatment as well as with the perchlorate concentration in the soil. At the same time amount of perchlorate decreased in soil with days after treatment as it was absorbed by the plant. The bioconcentration factor (BCF), provides an index of ability of the plant to absorb and accumulate perchlorate with respect to its concentration in the contaminated soil. This is defined as the ratio of the perchlorate concentration in the plants to that in the soil. Table 1 gives the BCF values with increasing concentration of perchlorate calculated after $4^{th}$ week of treatment. There is estimated to be a gradual decrease in BCF with increase in concentration of perchlorate in soil.

Few Previous studies reported that some plants are capable of absorbing perchlorate from water and soil (Seyfferth and Parker 2007; Voogt and Jackson 2010 and Yang and Her 2011). The results of present study indicate that plants of *E. citriodora* have ability to absorb and accumulate perchlorate in their tissue from soil and also play important role in depleting toxic perchlorate ions from soil. Perchlorate inside the plant shows a profound effect on its growth. The analysis of growth of *E. citriodora* plant under perchlorate stress revealed that perchlorate gradually reduces the overall growth of plant as well affects its various physiological and biochemical parameters studied during present investigation. Fresh weight of the plant under treatment showed significant reduction with increase in amount of perchlorate.

**Table 1. Amount of perchlorate in soil, leaf tissue of *Eucalyptus citriodora* plant and Bioconcentration Factors calculated after 4th week of treatment**

**All the values are average of triplicates (30 plants) ±** Standard Deviations

| Perchlorate Treatment (ppm) | Amount of Perchlorate in Soil (µg/gm) | Amount of Perchlorate in leaf tissue (µg/gm) | BCF |
|---|---|---|---|
| Control | O. | ND | NC |
| 1000 (0.1%) | 1.29±0.3 | 2.21±0.3 | 1.71±0.5 |
| 2000 (0.2%) | 3.56±1.2 | 5.95±0.8 | 1.67±0.1 |
| 5000 (0.5%) | 11.90±2.8 | 6.57±0.5 | 0.55±0.08 |
| 10000 (1.0%) | 20.20±3.3 | 7.54±0.6 | 0.37±0.06 |

Control = without perchlorate, ND = Not detected, NC = Not calculated.

Morphological parameters (Table-2) like shoot length, root length and number of leaves/shoot were also found to be decreasing with increasing concentration of perchlorate in soil. The length of shoot was observed to be affected much more whereas difference in root length under various treatments was comparatively less (Table-2). Similarly number of leaves per shoot measured was almost half at higher concentration (10000ppm) of ammonium perchlorate as compared to the plants growing without ammonium perchlorate. It was observed that there was curling and necrosis of shoot apices and falling of leaves in plants growing in presence of ammonium perchlorate. While the percentage of chlorosis / plant estimated to be increased under perchlorate treatment as compared to control plant without treatment. However curling of leaves per plant was much more in the plants under experimental setup of treatment 10000ppm (1%) ammonium perchlorate in comparison to plants under 1000 ppm treatment.

**Table 2. Effect of perchlorate on morphology, biomass production and chlorosis of leaves of *E. citriodora* plant. All the quantitative data is represented as mean of three replicates (30 plants) ± Standard deviation (S.D.)**

| Perchlorate concentration (ppm) | Shoot length (cm) | Root length (cm) | No.of leaves/ shoot | % chlorosis | Fresh weight (gm) |
|---|---|---|---|---|---|
| 0 | 42.6±2.0 | 7.5±2.6 | 11±1.3 | 1±0.04 | 28.3±2.06 |
| 1000 | 38.8±2.7 | 7.4±1.1 | 10±1.2 | 9±0.2 | 22.0±1.05 |
| 2000 | 32.0±0.35 | 7.4±1.7 | 8±1.6 | 17±0.05 | 20.3±1.1 |
| 5000 | 28.0±1.3 | 6.1±2.9 | 4±2.7 | 25±0.01 | 16.0±1.7 |
| 10000 | 25.4±2.5 | 5.2±1.2 | 4±2.3 | 35±0.03 | 12.9±2.02 |

**Table 3. Effect on chlorophyll, total protein and proline content of plant (3 weeks after treatment) with increasing concentration of perchlorate treatment. All the results are mean ± S.D. of three replicates (FW – fresh weight)**

| Perchlorate Treatment (ppm) | Chl a (mg/ gFW) | Chl b (mg/g FW) | Total Chl (mg/ gFW) | Protein Content (mg/g FW) | Proline Content (mg/g FW) |
|---|---|---|---|---|---|
| 0 | 1.05±0.05 | 1.16±0.01 | 2.21±0.01 | 1.57±0.05 | 0.18±0.05 |
| 1000 | 0.89±0.02 | 1.00±0.01 | 1.78±0.01 | 2.02±0.3 | 0.43±0.03 |
| 2000 | 0.65±0.03 | 0.82±0.005 | 1.48±0.005 | 2.42±0.5 | 0.56±0.6 |
| 5000 | 0.60±0.1 | 0.45±0.005 | 1.06±0.01 | 2.81±0.2 | 0.66±0.3 |
| 10000 | 0.40±0.5 | 0.35±0.01 | 0.76±0.01 | 3.30±0.04 | 0.73±0.02 |

Hamissou, M. in 2011 reported chlorosis and necrosis in tobacco and *Arabidopsis* plants irrigated with perchlorate solutions. Three weeks after treatment, falling of leaves was also observed in 10000 ppm condition. Hence the present studies clearly indicate that under perchlorate treatment, morphological parameters and growth in terms of fresh weight of the *E. citriodora* plant gets affected negatively though plants are able to survive and grow under perchlorate treatment.

Present investigation was also carried out to find the effect of perchlorate on different physiological and biochemical parameters under experimental conditions.Perchlorate was observed to cause physiological and biochemical stresses to plant that may be due to interference with the metabolic processes of plant. Earlier studies on stress conditions showed that abiotic and heavy metal stress affects photosynthetic activity of plants (Xie *et al.* 2009).

**Table 4. Amount of reducing sugar, total phenol and activity of peroxidase enzyme in plant tissue under perchlorate stress (3 weeks after treatment). Results are mean ± S.D. of three replicates**

| Perchlorate Treatment (ppm) | Reducing Sugar (mg/ gFW) | Total Phenol (mg/g FW) | Enzyme activity (units/g FW) |
|---|---|---|---|
| 0 | 51.47±1.3 | 0.80±0.07 | 152.7±3.0 |
| 1000 | 50.97±3.6 | 1.16±0.02 | 176.3±1.5 |
| 2000 | 38.13±4.0 | 1.39±0.04 | 205.5±2.3 |
| 5000 | 20.34±2.7 | 1.58±0.06 | 249.2±1.8 |
| 10000 | 12.92±3.5 | 1.79±0.06 | 254.0±3.9 |

FW: Fresh weight

During present studies, the amount of Chlorophyll was estimated as a measure of photosynthetic activity of plant both under stress and control conditions. The result reveals that total chlorophyll content (Chla+b) has decreased with increase in perchlorate stress concentration as compared to the control plant [Table -3, Figure 2(A.)].

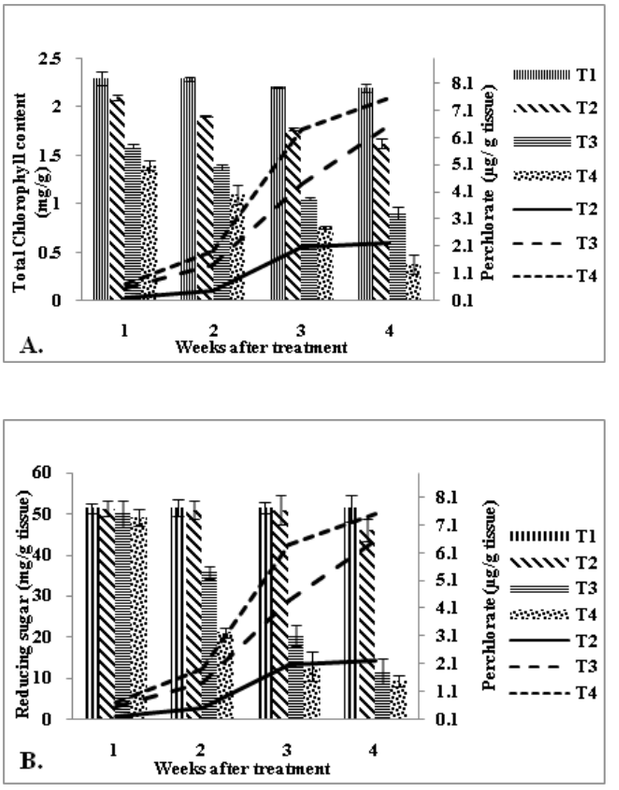


**Figure 2.** Effect of perchlorate on A. - total chlorophyll B. - Effect of perchlorate on reducing sugar Line depicts the amount of perchlorate inside plant tissue

The represented values are average of triplicates ± Standard Deviations

Treatments: T1- without ammonium perchlorate; T2- 1000 ppm ammonium perchlorate;

T3- 5000 ppm ammonium perchlorate; T4- 10000 ppm ammonium perchlorate

Amount of total chlorophyll, calculated to be 1.89 mg/g fresh weight of leaf tissue in the experimental set up having 1000 ppm ammonium perchlorate whereas 0.76mg/g of chlorophyll under10000 ppm of ammonium perchlorate (Table 3). Figure 2(A) clearly shows there is a gradual decrease in chlorophyll content with the increasing amount of perchlorate in leaf tissue of *E. citriodora* time after treatment. The decrease in total chlorophyll inside plant tissue was estimated to be 1.3 times in fourth week after treatment as compared to first week in the set of experiment having 1000 ppm (0.1%) ammonium

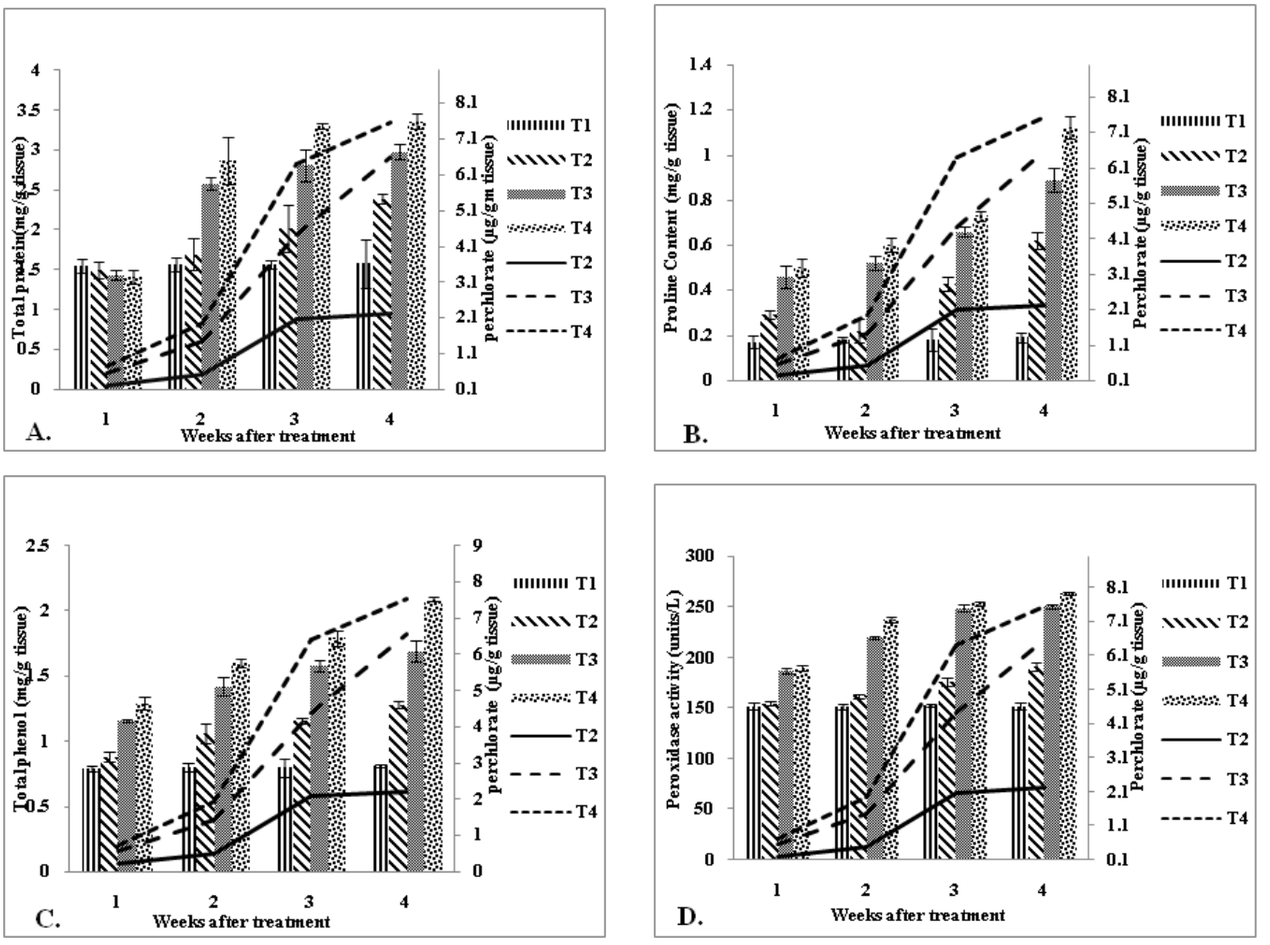


**Figure 3:** Effect of ammonium perchlorate on A- Total protein; B- Total proline, C- Total phenol; D- Peroxidase activity.
The represented values are average of triplicates ± Standard Deviations
Line depicts the amount of perchlorate inside plant tissue
Treatments: T1- without ammonium perchlorate; T2- 1000 ppm ammonium perchlorate;
T3- 5000 ppm ammonium perchlorate; T4- 10000 ppm ammonium perchlorate

perchlorate, whereas there observed to be almost 3 times decrease in the total amount of chlorophyll on fourth week after treatment as compared to first week in plants given 10000 ppm (1.0%) treatment of ammonium perchlorate. Similarly the amount of perchlorate accumulated in plants was found to be indirectly proportional to the presence of chlorophyll in leaf tissue [Figure 2(A.)].

Total protein content of plant tissue is an important biochemical parameter depicting abiotic and biotic stress conditions in plants. There was observed to be approximately two fold increase in total protein content in plants treated with ammonium perchlorate concentration (10000ppm) as compared to control plants without perchlorate treatment (Table 2). Total protein content calculated during present study was 1.57mg/g fresh weight of leaf tissue in plant without perchlorate treatmentand3.30 mg/g fresh weight of leaf tissue in plant with 10000ppm concentration of ammonium perchlorate (Table 3). The data represented in table-3 also reveals that

the total protein content gradually increases with increasing amount of ammonium perchlorate in soil. The protein content of plant is directly correlated with the amount of perchlorate in tissue, as it is apparent from Figure 3(A). Initially the amount of total protein was comparatively less (first week after treatment) but later it increases with increasing amount of perchlorate in the leaf tissue with time after treatment. For 10000 ppm (T4) treatment, total protein content was found to be almost twice the value calculated on first week. This indicates that plants under abiotic stress will experience an increase in protein expression so as to provide the necessary defense and tolerance mechanism against stress conditions. Hamissou, M. in 2011 reported increase in total protein content in response to perchlorate application in *Arabidopsis thaliana* and *Nicotiana tobacum* as the concentration of perchlorate increased. Previous reports on heavy metal stress showed an increase in quantity of total protein content in plant tissue (Patel *et al.* 2005).

Proline is a basic amino acid found in plants. Free proline is known to play a role in plants under stress conditions. The amount of proline is reported to be increased under physiological and pathological stress conditions (Kuznetsov and Shevyakova 1997). Hence during present study, analysis of proline was performed to find out effect of perchlorate on it. Results of present study also showed an increase of total proline content with increase in ammonium perchlorate treatment (Table 3). The concentration of perchlorate as low as 1000ppm (0.1%) was enough to stimulate approximately 2.5 folds of proline inside plant tissue as compared to the control plant (without any perchlorate treatment).As per the table-3, the proline content was 0.43mg/g fresh leaf tissue (with 2000ppm of perchlorate), whereas for 10000ppm, it was 0.73 mg/g fresh leaf tissue. With increasing days after treatment the total proline content was found to be proportional to amount of perchlorate in the tissue of *E. citriodora* plant as it is increased with increasing amount of perchlorate in the plant tissue. Figure – 3(B.) gives the relation of proline content with perchlorate amount in tissue, the amount of proline is increased with concentration of perchlorate as well as days after treatment. The amount of proline in plant tissue was estimated to be 0.29 mg/g fresh weight in Ist week and 0.62 mg/g fresh weight after fourth week in 1000 ppm (T2) treatment whereas 0.46 mg/g fresh weight in Ist week & 0.89 mg/g fresh weight in 5000 ppm (T3) treatment. Similar trend was observed in accumulation of perchlorate in plant tissue that also found to be increased with days after treatment as well as with increase in amount of perchlorate in soil. Suggesting thereby that ammonium perchlorate is giving a physiological stress to *Eucalyptus* plant and in its response, proline content is altered.

Similar trend was observed in amount of total phenol content in plant tissue estimated during present investigation. Phenols are aromatic compound present in all parts of the plant. Phenols are known to accumulate inside plant tissue under different abiotic and heavy metal stress. These compounds are known to offer resistance to various abiotic and biotic stresses in plants (Dixon and Paiva 1995). Esteban, *et al.* (2008), studied effect of Hg stress on phenolic contents inside white lupin plants and reported an increase in total phenols with stress. During present study, total phenol content was found to be increased with increase in ammonium perchlorate treatment (Table 4), as well as with increasing amount of perchlorate in leaf tissue [Figure 3 (C.)]. The measured values of total phenol ranged from 0.80 mg/g fresh weight of leaf tissue under control conditions to 1.79 mg/g fresh weight of leaf tissue (10000ppm). Total phenol content was known to be increased from1.16 mg/g fresh weight on first week to 1.69 mg/g fresh weight of leaf tissue on fourth week in plants given 5000 ppm (0.5%) treatment [Figure 3 (C.)].

Attempt was also made to find out the amount of reducing sugar in *Eucalyptus* plant tissue under different treatment of perchlorate, as a parameter of growth and development of plant. Table 4 depicts the amount of reducing sugar in control as well as plants irrigated with different amount of ammonium perchlorate. There was observed to be reduction in reducing sugar content inside plant tissue with

increasing concentration of ammonium perchlorate. Similar trend is apparent from Figure -2(B.) which shows a gradual decrease in amount of reducing sugar with increase in amount of perchlorate in plant tissue with time after treatment. Previous reports showed decrease in amount of reducing sugar in *Saccharum officinarum* plant under heavy metal (Ni, Pb) stress conditions (Misra *et al.* 2010) and in maize plant (Narwal and Singh 1993) under cadmium stress. Present studies showed almost 5 fold reductions in content of reducing sugar by fourth week after treatment [Figure 2(B.)] in plants with10000 ppm (1.0%) and 5000 ppm (0.5%) of ammonium perchlorate and similarly the amount of perchlorate accumulated in plant tissue was also increased drastically with time and increasing perchlorate in soil.

**The represented values are average of triplicates ± Standard Deviations**

Under different oxidative environmental stress conditions antioxidative enzymes are known to get activated in plants which ultimately help in enhancing defence mechanism of plant against oxidative damage. Perchlorate is a known strong oxidizer; it may also cause oxidative stress to plant as a result of building up of reactive oxygen species Present investigations were carried out to find activity of guaiacol peroxidase enzyme. Guaiacol peroxidase is an important group of peroxidase which oxidizes guaiacol as a commonly used reducing substrate. They are present in cytoplasm of cell and involved in various metabolic process of growth and development of plant. The present study showed an increase in the activity of this enzyme under various perchlorate

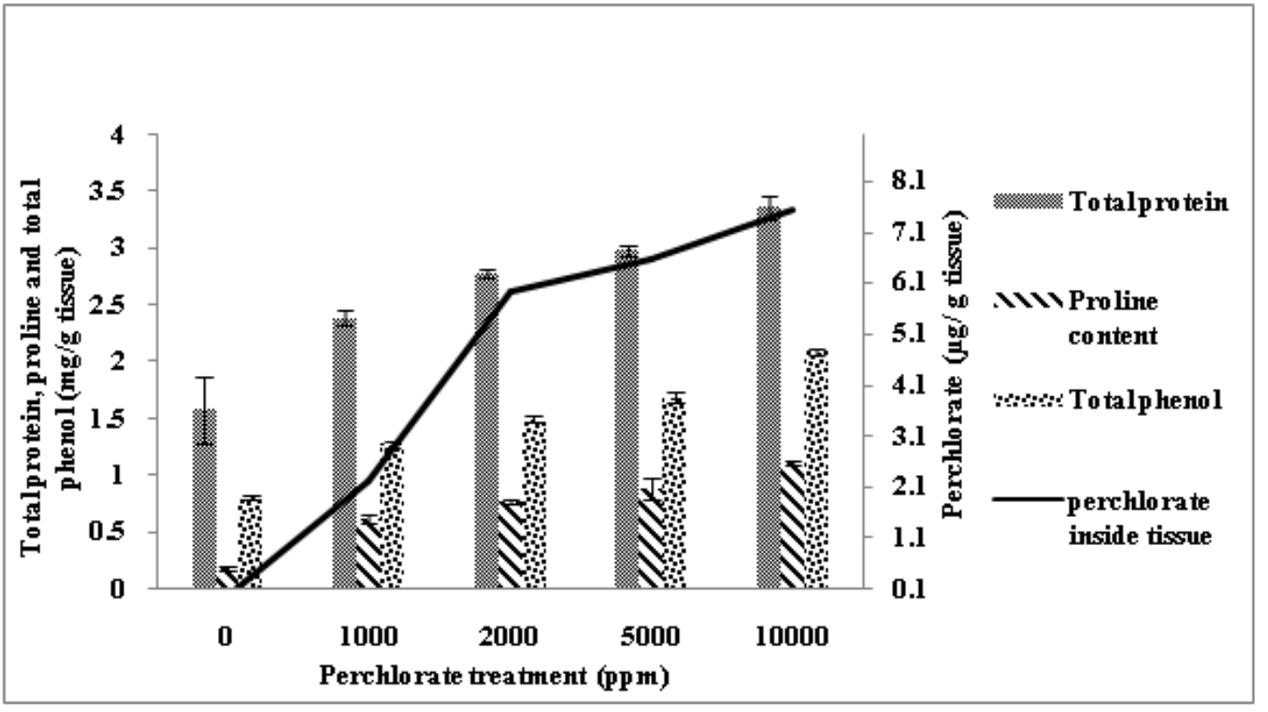


**Figure 4.** Effect of ammonium perchlorate on amount of total protein, proline, total phenol after 4th weeks of treatment. Line depicts amount of perchlorate inside leaf tissue

treatments. The activity of peroxidase was calculated to be 152.74 units/litre in control plant (without treatment of perchlorate) and 176.29 units/litre in plants with10000 ppm perchlorate stress condition (Table 4).The activity of peroxidase enzyme was 155.1 units/litre after Ist week of treatment of 1000 ppm (0.1%), which increased to 190.9 units/litre in plants by fourth week after treatment [Figure 3 (D)]. The activity of peroxidase enzyme was also found to be directly correlated with the amount of perchlorate in plant tissue as well as with time after treatment. As the amount of perchlorate increased in leaf tissue, the activity of enzyme increased gradually with time in the plants having varying concentrations of ammonium perchlorate [Figure 3 (D)]. Earlier reports showed increase activity of peroxidase (SOD and Ascorbate) enzyme in *Arabidopsis thaliana* and *Nicotiana tobacum* under perchlorate treatment (Hamissou 2011). Activity of this peroxidase enzyme was earlier shown to be increased in the leaves of *Carthamus tinctorius* plant under salt stress condition (Tayefi-Nasrabadi *et al.* 2011).

The stress related parameters of plant estimated during present preliminary investigations like content of total protein, proline and phenol shows a linear relationship with time after treatment and directly correlated with perchlorate concentration as they were found to be increased with increasing amount of perchlorate in plant tissue and also in soil (Figure 4).

## Conclusion

Present investigation clearly indicates that perchlorate in the soil is a potential abiotic stress to *Eucalyptus* plants affecting its physiological and biochemical parameters. Though the growth of the Eucalyptus plants is getting affected adversely in presence of perchlorate, plants are able to absorb and accumulate perchlorate in their leaf tissue. Suggesting thereby plants can be used as source of phytoremediation for depletion of environmental perchlorate. The different biochemical and physiological parameters were found to be correlated with amount of perchlorate in plant and soil and with time after treatment. Presence of perchlorate inside the Eucalyptus plant tissue enhances the activity of stress related enzyme and amount of protein, phenol and proline.

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