International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X, Impact Factor: RJIF 5.12
Received: 17-02-2021, Accepted: 24-03-2021, Published: 03-04-2021
Volume 6, Issue 2, 2021, Page No. 374-381

Implementation of arbuscular mycorrhizal fungi on lead nitrate PB $(no_3)_2$ tolerance in green gram (*Vigna Radiata.* (l*)* R. Wilczek

T Thiyagarajan, K Prakash*, S Karuthamma P Munnaji, T Ravimycin
Ecology Environmental Biotech Lab, Department of Botany Annamalai University, Tamil Nadu, India

Abstract
The present investigation was carried out to determine the effect of lead nitrate $Pb(NO_3)_2$ on bio chemical analysis of green gram **(***Vigna radiata.*(L*)* R. Wilczek. The green gram seeds were treated under control, 2.5, 5, 7.5, 10, and 12.5 mg of pb $(No3)_2$. Every treatments was replicated thrice in a randomized block design. Observations were complete on bio chemical analysis of chlorophyll 'a', chlorophyll 'b', total chlorophyll, carotenoid, reducing sugar, non-reducing sugar, total sugar, protein, proline, amino acid. of green gram. all result when compared with control show that lead nitrate metal adversely affects the growth of green gram by reducing bio chemical activity. The present research work was to carried out the effect of different concentrations of lead nitrate with AMF on, bio chemical activity, of green gram grown under pot culture experiment.

Keywords: green gram, bio chemical, chloroyall'a', chlorophll 'b', carotenoid, reducing sugar, non-reducing sugar, protein, proline

Introduction
Environmental pollution has been converted into an explanation focus of distress for all the nations worldwide, as not only the developing countries but developed nations as well are affected by and suffer from it. Pollution has many forms, the air we breath, the water we drink, the ground where we cultivate our food crops and even the increasing noise we hear everyday-all contribute to health problems and lesser quality of life Heavy metals are defined as metallic elements that have a relatively high density compared to water. With the assumption that heaviness and toxicity are inter-related, heavy metals also include metalloids, such as arsenic, that are able to induce toxicity at low level of exposure. Environmental pollution is increasing with each passing year and inflicting serious and permanent injury to the world. Environmental contamination is different types, namely water, soil, noise, air, and light-weight. These cause damage to the living system. The most important effluent dischanging industries are daneryes, textiles, paper milIs, iron and steel industreies, fertilizers units etc. industries effluents containing organic and inorganic compounds various forms of heavy metals suspended solids and other materials which naturally affect the water quality as well as ecosystem. Several methods are already being used to clean up the environment from these kinds of contaminants, but most of them are costly and far away from their optimum performance. Mycophytoremedial is a term functional to a group of technology that use plants to reduce, remove, or immobilize environmental toxins, primarily those of anthropogenic origin, with the aspire of restoring area sites to a condition useable for public applications. Arbuscular mycorrhizal fungi (AMF) are amongst the most common soil fungi and the majority of plant species have associations with AM fungal species.

Materials and Methods
The seeds green gram (co-8) was obtained from Tamilnadu Agricultural University (TNAU), Coimbatore and Tamilnadu. The uniform seeds are selected for the experimental purpose. Source of lead nitrate $Pb(NO_3)_2$ stock solution prepared by dissolving the molecular weight of $pb(NO_3)_2$ and different concentrations *viz.*, (garden soil - Control,T1-2.5mg,T2-2.5mg+AMF,T3-5mg,T4-5mg+AMF,T5-7.5mg,T6-7.5mg+AMF,T7-10mg,T8-10mg+AMF,T9-12.5mg,T10-12.5mg+AMF) of $pb(NO_3)_2$ the solution were prepared freshly at the time of experiments. The pods were filed with 5 Kg of garden soil, selected green gram seeds were sown in the pods irrigated with normal tap water was maintained as the control.

Am Fungi
The AM Fungi (*Glomus fasciculatum*) were collected from Department of Microbiology Tamil Nadu ANNAMALAI UNIVERSITY,CHIDAMBARAM, Tamil Nadu, India.

Biochemical Analyses
Photosynthetic pigments
The photosynthetic pigments such as chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoid and biochemical contents such as reducing sugar, non-reducing sugar, total sugar, amino acid, proline and protein were estimated in the fifteenth day old seedling grown in the laboratory conditions.

Chlorophyll (Arnon, 1949)
Five hundred mg of fresh leaf material was ground with 10 ml of 80 per cent acetone with a mortar and pestle. The homogenate was centrifuged at 800 rpm for 15 min. The supernatant was saved. The residue was re-extracted with 10 ml of 80 per cent acetone. The supernatant was saved and the absorbance values were read at 645 nm and 663 nm in a UV-spectrophotometer (Hitachi). The chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents were estimated and expressed in mg/g fresh weight basis.
Chlorophyll 'a' = (0.0127) ×(O.D 663) – (0.00269) × (O.D 645)

Chlorophyll 'b' = (0.0229) × (O.D 645) - (0.00488) × (O.D 663)
Total chlorophyll = (0.0202) × (O.D 645) + (0.00802) × (O.D 663)

Carotenoid (Kirk and Allen, 1965)
The extract of the same plant used for chlorophyll estimation was used for carotenoid estimation. The acetone extract was read at 480 nm in spectrophotometer. The carotenoid contents were expressed in mg/g fresh weight basis. The carotenoid content was calculated by using the following formula:
Carotenoid

Carotenoid = (O.D 480) – (0.114) × (O.D 663) – (0.638) × (O.D 645)

Estimation of sugars (Nelson, 1944)

Extraction
Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10 ml of 80 per cent ethanol. The homogenate was centrifuged for 10 min at 800 rpm. The supernatant was saved. Then the ethanol is evaporated in a water bath at 50C. The net content was made upto 20 ml with distilled water and the extract was used for the estimation of reducing sugar.

Estimation
One ml of extract was taken in a 25 ml marked test tube. 1 ml of reagent 'C' was added. Then, the mixture was heated for 20 min at 100 ℃ in a boiling water bath, cooled and 1 ml of arsenomolybdate reagent was added. The solution was thoroughly mixed and diluted to 25 ml with distilled water. The sample was read in a UV-Spectrophotometer at 520 nm. The sugar contents were expressed in mg/g fresh weight basis.

Preparation of reagents
Reagent A: Twenty five gram of anhydrous sodium carbonate, 25 g of sodium potassium tartarate, 20 g of sodium bicarbonate and 200g of anhydrous sodium sulphate were dissolved in 800 ml of distilled water and made upto 1000 ml. Then it was filtered and stored in a glass stoppered brown bottle.

Reagent B: Fifteen per cent copper sulphate containing 1 or 2 drops of concentrated sulphuric acid.

Reagent C: Fifty ml of reagent A and one ml of reagent B were mixed well and it was prepared freshly at the time of experiment.
Arsenomolybdate reagent: To 450 ml of distilled water, 25 g of ammonium molybdate, 21 ml of concentrated sulphuric acid were added and 3 g of sodium arsenate was dissolved in 25 ml of distilled water. The mixture was kept in a water bath at 37 □C for 24 to 48 hrs. The reagent was stored in a glass stoppered brown bottle.

Non-reducing sugars (Nelson, 1944)
Non-reducing sugars present in the ethanol extracts (extraction as in reducing sugars) were hydrolysed with sulphuric acid to reducing sugars. Reducing sugars present in the hydrolysates were estimated following Nelson's method. The difference between the total sugars and the reducing sugars correspond to the non-reducing sugars.

Hydrolysis
One ml of extract was taken in a test tube and evaporated to dryness in a water bath for 15 min. To the residue, 1 ml of distilled water and 1 ml of 0.1 N sulphuric acid were added. The mixture was hydrolysed by incubating at 49 □C for 30 min in a thermostat. The solution wasneutralized with 0.1 N NaOH (5 ml) and the methyl redas indicator. To this, 1 ml of reagent C (copper reagent) was added and heated for 20 min, cooled and 1 ml of arsenomolybdate reagent was added. The content was made upto 25 ml and the absorbance was read at 495 nm in a UV-spectrophotometer. The reducing sugar contents were expressed in mg/g fresh weight basis. Blank was prepared with 1 ml of distilled water.

Estimation of amino acid (Moore and Stein, 1948)

Preparation of reagents

Ninhydrin reagent
80 mg of stannous chloride was taken and dissolved 50 ml of citrate buffer at pH 5.0 was added to 2 g of ninhydrin in 50 ml of methyl cellulose. Both solutions were mixed thoroughly.

Diluting reagent
Distilled water and n-propanol were mixed in equal volume.

Extraction
0.5 grams of plant material were ground well with 10 ml of 80 per cent ethanol in a pestle and mortar. The homogenate was centrifuged at 800 rpm for 10 minutes and the supernatant was saved. The supernatant was made upto 10 ml with 80 per centethanol.

Estimation
1 ml of extract and 1 ml of ninhydrin reagent were added, mixed thoroughly in a Folin-Wu tube and the content was heated for 20 minutes in a boiling water bath at 100□C. After 20 minutes, the content was removed from the water bath and cooled under tap running water. The content was mixed thoroughly made upto 10 ml with diluting solution. Then, the solution was read at 570 nm in a UV-Spectrophotometer.

Proline (Bates *et al.*, 1973)

Extraction
Five hundred mg of plant material was taken in a pestle and mortar and homogenized with 10 ml of 3 per cent aqueous sulfosalicylic acid. Then, the homogenate was filtered through whatman No. 2 filter paper. The residue was re-extracted two times with 3 per cent sulfosalicylic acid and pooled. The filtrates were made upto 20 ml with 3 per cent sulfosalicylic acid and used for the estimation of proline.

Estimation
Two ml of extract was taken in a test tube and 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid were added to it. Themixture was incubated for an hour at 100`C in a water bath. The tubes were transferred to an ice bath to terminate the reaction. Then, to each test tube, 4 ml of toluene was added and mixed vigorously using a test tube and stirred for 10-20 seconds. The toluene containing the chromophore was separated from the aqueous phase with the help of separating funnel and the absorbance was measured at 520 nm in a UV- Spectrophotometer (Hitachi

U-2900) using an appropriate blank. The proline content was determined from a standard curve prepared with proline and the results are expressed in mg g-1 fr. wt. basis.

Estimation of protein (Lowry *et al*., 1951)
Preparation of reagents
Reagent A
0.4 g of sodium hydroxide was dissolved in distilled water and made upto 100 ml. To this solution, 2 g of sodium carbonate was added.

Reagent B
One per cent of copper sulphate mixed with equal volume of 2 per cent for sodium potassium tartarate.

Reagent C
Fifty ml of reagent A and 1 ml of reagent B was mixed. That is reagent C. Reagent C prepared at the time of experiment.
Chapter-3 Materials and Methods - 66
Folin-phenol reagent
One ml of Folin-phenol reagent was diluted with 2 ml of distilled water**.**

Extraction
0.5 g of plant materials were weighed and ground well in a pestle and mortar with 10 ml of 20 per cent TCA (trichloroacetic acid). The homogenate was centrifuged for 15 minutes at 800 rpm. The supernatant was discarded. To the pellet, 5 ml of 0.1 N NaOH was added and centrifuged for 5 minutes. The supernatant was saved and made upto 10 ml of 0.1 N NaOH. This extract was used for the estimation of protein.

Estimation
1 ml of the extract was taken in a 10 ml test tube and 5 ml of reagent C was added. This solution was mixed thoroughly and kept in darkness for 10 minutes. After that 0.5 ml Folin-phenol reagent was added. It was kept in dark for 30 minutes. The sample was read at 660 nm in UV Spectrophotometer.

Result and Discution
Exprimental Results
The present investigation deals with the effects of various concentrations of LEADNITRATE with AMF on biochemical content variation and yield components of GREEN GRAM (Vigna radiata.(L) R. Wilczek. In addition, some bioremediation treatments were given to LEAD NITRATE and polluted soil and their response on the growth and yield of GREEN GRAM (Vigna radiata.(L) R. Wilczek was studied and the results are presented in this PAPER.

Biochemical Analyses
Photosynthetic pigments
The effect of different concentrations of lead nitrate with AMF on chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll content of green gram is shown in table (1, 2, 3) The highest content of chlorophyll ‘a’ recorded at AMF treated plant(1.635),and The highest content of chlorophyll ‘b’ recorded at AMF treated plant (0.992), total chlorophyll and carotenoid recorded at AMF treated plant (2.627). Were recorded in different concentration of lead nitrate treated plants at 15, 30, 45, 60, 75 DAS respectively. The lowest values of chlorophyll ‘a’ (0.021 mg g-1 fr. wt.), chlorophyll ‘b’ (0.622 mg g-1 fr. wt.), total chlorophyll and carotenoid (0.087 mg g-1 fr. wt.) were recorded 12.5 lead nitrate treated plant of cow pea.

Reducing sugar
The impact of different concentrations of lead nitrate with AMF on reducing sugar contents (mg g-1 fr. wt.) in leaf of green gram at 15, 30, 45, 60 and 75 DAS is given in Figure 4. The highest reducing sugar content of leaf (2.42, 4.20 and 3.89 mg g-1 fr. wt.), was recorded in AMF treated plants at 15, 30, 45, 60 and 75 DAS respectively. The lowest reducing sugar content of leaf (1.75, 2.69 and 2.26 mg g-1 fr. wt.) was observed in 12.5 concentration of lead nitrate treated plants at 15, 30, 45, 60 and 75 DAS respectively.

Non-reducing sugar
The impact of different concentrations of lead nitrate with AMF on Non reducing sugar contents (mg g-1 fr. wt.) in leaf of green gram at 15, 30, 45, 60 and 75 DAS is given in Figure 5. The highest Non reducing sugar content of leaf (2.42, 4.20 and 3.89 mg g-1 fr. wt.), was recorded in AMF treated plants at 15, 30, 45, 60 and 75 DAS respectively. The lowest Non reducing sugar content of leaf (1.75, 2.69 and 2.26 mg g-1 fr. wt.) was observed in 12.5 concentration of lead nitrate treated plants at 15, 30, 45, 60 and 75 DAS respectively.

Total sugar
The impact of different concentrations of lead nitrate with AMF on total sugar contents (mg g-1 fr. wt.) in leaf of green gram at 15, 30, 45, 60 and 75 DAS is given in Figure 6. The highest total sugar content of leaf (2.42, 4.20 and 3.89 mg g-1 fr. wt.), was recorded in AMF treated plants at 15, 30, 45, 60 and 75 DAS respectively. The lowest total sugar content of leaf (1.75, 2.69 and 2.26 mg g-1 fr. wt.) was observed in 12.5 concentration of lead nitrate treated plants at 15, 30, 45, 60 and 75 DAS respectively.

Proline
The effect of different concentrations of lead nitrate with AMF on proline contents (mg g-1 fr. wt.).) in leaf of green gram at 15,30,45, 60 and 75 DAS is given in Figure 7. The lowest proline content of leaf (1.994, 2.859, 4.155, 4.934 and 2.336 mg g-1 fr. wt.) was recorded in the plants grown in control at 15, 30, 45, 60 and 75 DAS respectively. The highest proline content of leaf (7.218, 8.934, 10.636, 11.116 and 8.432 mg g-1 fr. wt.) was observed in 12.5 concentration of lead nitrate treated plants at 30, 60 and 90 DAS respectively.

Protein
The impact of different concentrations of lead nitrate with AMF on protein contents ((mg g-1 fr. wt.)).) in leaf of green gram at 15,30,45, 60 and 75 DAS is given in Figure 8. The highest protein content of leaf (2.42, 4.20 and 3.89 mg g-1 fr. wt.), was recorded in AMF treated plants at 15, 30, 45, 60 and 75 DAS respectively.
The lowest protein content of leaf (1.75, 2.69 and 2.26 mg g-1 fr. wt.) was observed in 12.5 concentration of lead nitrate treated plants at 15, 30, 45, 60 and 75 DAS respectively.

Biochemical Parameters

Photosynthetic pigments

The biochemical aspects such as chlorophyll 'a', chlorophyll 'b', total chlorophyll, protein, reducing sugars, non reducing sugars, total sugar, were high at AMF as compare control. But, the higher level of lead nitrate inhibited all the morphological and biochemical parameters. The mineral and enzyme contents also showed the same trend. But proline, amino acid and enzymes like catalase, were low in control. Then it was found increase with increasing heavy metal concentrations.

The decrease in chlorophyll content was also reported in sunflower (Zengin and Munzuroglu, 2006) and Almond (Elloumi *et al*., 2007) Solanum rycopericum Hediji *et al*. (2010). Under heavy metal stress the photosynthetic pigment were degraded, it might be damage of the photosynthetic phase II reaction centre in the leaf (Chugh and Sawhney, 1999; Deniz *et al*., 2007; Li *et al*., 2008). Shakya *et al*. (2008) reported that the chlorophyll 'a', chlorophyll 'b' and total chlorophyll were reduced with the treatment of copper in Thuidium delicatulum, T. sparsifolium and Ptychanthus striatus.

Zhang *et al*., 2014 studied that the destruction of the chlorophyll structure and decrease the number of chloroplasts and even disappeared under the Cd stress in Populus deltoides and P. nigra leaves. Nwugo and Huerta, 2008 concluded that the Cd treatment suppress the photosynthesis and also inhibit the activity of key enzymes of the Calvin cycle and the photosynthetic electron transport chain. Moreover many researchers supported that the reduction of chlorophyll pigments due to cadmium application Chen *et al*. (2011), Liu *et al*. (2014) and Abd_Allah *et al*. (2015) for mustard; cotton and sunflower, respectively. In contrast, the chlorophyll contents were enhanced with the addition AMF inoculation (Malekzadeh *et al*. 2012). Siedlecka and Krupa, 1996 mention that the cadmium treatment estroyed the structure of the chloroplasts, influenced chlorophyll synthesis or accelerated its degradation rate in Phaseolusvul garis.

The chlorophyll content and proteins declined with increasing Cr concentration Rai *et al*. (1992). The study mentions that the AM fungi protect degradation of both chlorophyll and proteins against heavy metal treatment, even higher concentration of Cr Abdul Razak (1985). Moreover, the study (Shamsi *et al*., 2007) reveled that The decrease in chlorophyll might be mediated through the reduced uptake of Mg, that Cd toxicity reduced the uptake of Mg, which is the integral part of the chlorophyll.

The chlorophyll contents were increased with the association mycorrhizae, it might the mycorrhizae enhance nutrient uptake by plants reported previously by Andrade *et al*. (2008). Hashem *et al*., 2016 [11, 13] revealed that the Cd stress reduced chlorophyll content, a negative impact that was mitigated by AMF. Enhanced chlorophyll content in AMF-inoculated plants and subsequent recovery of Cd-stressed plants may be due to the effect of AMF on magnesium uptake in tomato. Sheng *et al*. (2008) also showed a close relationship between chlorophyll content and magnesium uptake as it forms an important part of chlorophyll molecule.

Protein, sugar, Amino acid and Proline

The reduction of proteins content and enzyme activity was inhibited due to heavy metal treatment Schützendübel and Polle, 2002. Kanwal *et al*., 2016 [12] indicate that AMF inoculated wheat plants with different Zn concentrations showed increased plant growth than non metal treated plants. Some studies (Li *et al*., 2011) reported that mycorrhizal colonization do not decrease in plants growing with high metal contents. Ling-Zhi *et al*., 2011 reveled that the AMF enhances the resilience of crop plants through its active participation in nutrient uptake and maintaining cell water content. Tripathi and Tripathi, 1999 concluded the protein content in Albizia lebbak has been interpreted either due to reduced de novo synthesis of proteins or increased decomposition of proteins into amino acids.

Moreover, cadmium stress, decrease in protein control was related with increased protease activity in soybean (Balestrasse *et al*. 2003). Verma *et al*. (2012) showed that the soluble protein content decreased in seedlings with increasing concentration of cadmium chloride over the control seedlings of Sesbania sesban. The amino acids can directly or indirectly influence the physiological activities of the plant (Sharma, 1985; Shafiq and Iqbal, 2005; Street *et al*., 2007). Total free amino acid was increased with the increasing concentration of heavy metals as reported by Bhardwaj *et al*. (2009). The heavy metals have decreased the content soluble sugar with increasing concentration, which are more important constituent is manufactured during photosynthesis and broken down during respiration by plants (Hemalatha *et al*., 1997). Dhir *et al*. 2004 demonstrated proline content were accumulated in shoots of *B. juncea, Triticum aestivum* and *Vigna radiate* with the influence of cadmium. Zengin and Munzuroglu (2006) found that proline accumulation increased with the exposure to cadmium in hydrophytes of Ceratophyllum, Wolffia, and Hydrilla. Recent study (Ferrol *et al*. 2016) reported that the plants inoculated with AMF under heavy metal stress may result in the expression of specific genes, which are responsible for the production of proteins (including metallothioneins) that increase the resistance of plants to stress.

Shaaban *et al*., 2015 reported that the total soluble protein content was significantly decreased with increasing the heavy metal (Cd or Pb) concentrations as compare to VAM inoculation increased significantly the total soluble protein content with influence of heavy metal levels. Additionally, VAM fungi may be can detoxify the heavy metals via exudation of metal-binding proteins (Howe *et al*., 1997).

Hayat *et al*., 2011 reveled that the Increase in proline accumulation in Cd-stressed plants has earlier been demonstrated in tomato. Hashem *et al*., 2016 [11, 13] suggested that the proline content were enhanced under stress conditions. Further enhancement of proline in AMF-inoculated plants supports the potential role of AMF and proline in plants. Shekoofeh *et al*. (2012) reported that the AMF-induced enhancement of proline content and subsequent mitigation of salt stress in Ocimum basilicum. Proline accumulation in response to heavy metal stress, osmotic stress, drought, and high levels of salinity has been one of the indicator physiological factors (Ashraf and Foolad 2007). Although metal-induced proline accumulation in plant tissues has been observed (Andrade *et al*., 2009; Fariduddin *et al*., 2009), reports on the effects of mycorrhizal symbiosis in proline or soluble amino acid contents are scarce or null under metal stress conditions.

Conclusion

The biochemical aspects such as chlorophyll 'a', chlorophyll 'b', total chlorophyll, protein, reducing sugars, non-reducing sugars, total sugar, were high at AMF as compare control. But, the higher level of lead nitrate inhibited all the morphological and biochemical parameters. The mineral nutrients like nitrogen, phosphorus, potassium, sodium, calcium, magnesium, copper, iron and zinc significantly declined with increase in heavy metal concentration. However, it was increased with treatment of AMF treatment as compare to control

But proline, amino acid and enzymes like catalase, and peroxidase were low in AMF then the control. Then it was found increase with increasing heavy metal concentrations. Then it was found increase with increasing heavy metal concentrations.

The lead nitrate uptake by green gram plants at different stages, maximum lead nitrate accumulation in plants at T10 concentration of lead nitrate plant and the minimum accumulation in plant T1 treated plants.

Table 1: Effects of Lead nitrate and AMF on the Chlorophyll 'a' content of GREEN GRAM (Vigna radiata.(L) R. Wilczek

Treatments with Lead nitrate + AMF	Chlorophyll 'a' (mg/g^{-1} fresh weight)				
	Day After Sowing (DAS)				
	15	30	45	60	75
Control	1.93±0.05	3.220±0.09	4.73±0.14	5.40±0.15	5.32±0.15
2.5 mg pb(no3)2	1.40±0.04	2.98±0.08	4.25±0.12	4.21±0.12	4.80±0.14
2.5 mg pb(no3)2 + AMF	1.98±0.05	3.41±0.10	5.36±0.16	5.63±0.16	5.45±0.17
5 mg pb(no3)2	1.00±0.03	2.29±0.06	3.40±0.10	3.82±0.10	3.58±0.11
5 mg pb(no3)2 + AMF	1.63±0.04	2.96±0.08	4.21±0.12	4.73±0.13	4.49±0.14
7.5 mg pb(no3)2	1.72±0.02	1.88±0.04	2.07±0.06	2.52±0.07	2.20±0.08
7.5 mg pb(no3)2 + AMF	1.39±0.04	2.40±0.07	3.64±0.10	3.76±0.11	3.00±0.12
10 mg pb(no3)2	0.72±0.01	1.01±0.03	1.93±0.05	1.94±0.05	1.11±0.06
10 mg pb(no3)2 + AMF	0.80±0.03	1.72±0.05	2.41±0.07	2.54±0.07	1.83±0.08
12.5 mg pb(no3)2	0.41±0.00	1.79±0.02	1.33±0.03	1.10±0.03	0.21±0.00
12.5 mg pb(no3)2 + AMF	0.63±0.01	1.83±0.03	1.94±0.05	1.98±0.04	0.33±0.06

Table 2: Effects of Lead nitrate and AMF on the Chlorophyll 'b' content of GREEN GRAM (Vigna radiata.(L) R. Wilczek

Treatments with Lead nitrate + AMF	Chlorophyll 'b' mg/g^{-1} fresh weight)				
	Day After Sowing (DAS)				
	15	30	45	60	75
Control	0.97±0.02	1.72±0.05	2.00±0.06	2.30±0.06	0.682±0.02
2.5 mg pb(no3)2	0.87±0.02	1.31±0.03	1.47±0.04	1.92±0.05	0.32±0.009
2.5 mg pb(no3)2 + AMF	1.015±0.03	1.68±0.05	1.93±0.05	2.24±0.06	0.79±0.02
5 mg pb(no3)2	0.54±0.01	0.95±0.02	0.96±0.02	1.01±0.03	0.21±0.006
5 mg pb(no3)2 + AMF	1.96±0.05	1.28±0.03	1.42±0.04	1.97±0.05	0..60±0.01
7.5 mg pb(no3)2	0.38±0.01	0.62±0.01	0.72±0.02	0.86±0.02	0.17±0.005
7.5 mg pb(no3)2 + AMF	1.10±0.03	1.01±0.03	1.11±0.03	1.52±0.04	0.46±0.01
10 mg pb(no3)2	0.27±0.008	0.42±0.01	0.42±0.01	0.52±0.01	0.12±0.003
10 mg pb(no3)2+ AMF	0.80±0.02	0.95±0.02	1.00±0.03	1.13±0.03	0.37±0.01
12.5 mg pb(no3)2	0.16±0.00	0.26±0.01	0.25±0.01	0.31±0.01	0.09±0.00
12.5 mg pb(no3)2 + AMF	0.52±0.01	0.60±0.018	0.63±0.01	0.92±0.02	0.15±0.004

Table 3: Effects of Lead nitrate and AMF on the Total Chlorophyll content of GREEN GRAM (Vigna radiata.(L) R. Wilczek

Treatments with Lead nitrate + AMF	Total Chlorophyll (mg/g^{-1} fresh weight)				
	Day After Sowing (DAS)				
	15	30	45	60	75
Control	2.61±0.07	4.19±0.12	6.45±0.19	7.63±0.22	7.21±0.21
2.5 mg pb(no3)2	1.73±0.05	3.85±0.11	5.56±0.16	6.73±0.20	5.69±0.17
2.5 mg pb(no3)2 + AMF	2.77±0.08	4.42±0.13	7.04±0.21	8.09±0.24	7.56±0.22
5 mg pb(no3)2	1.21±0.03	2.84±0.08	4.35±0.13	4.89±0.14	4.39±0.13
5 mg pb(no3)2 + AMF	2.23±0.06	4.92±0.14	5.49±0.16	6.76±0.20	6.05±0.18
7.5 mg pb(no3)2	1.00±0.03	1.97±0.05	2.69±0.08	3.66±0.11	3.24±0.09
7.5 mg pb(no3)2 + AMF	1.85±0.05	3.50±0.10	4.66±0.13	5.52±0.16	4.87±0.14
10 mg pb(no3)2	0.65±0.01	1.29±0.03	2.35±0.07	2.84±0.08	2.36±0.07
10 mg pb(no3)2 + AMF	1.48±0.04	2.52±0.07	3.36±0.10	3.96±0.11	3.55±0.10
12.5 mg pb(no3)2	0.30±0.01	0.96±0.02	1.59±0.04	2.12±0.06	1.35±0.04
12.5 mg pb(no3)2 + AMF	0.78±0.02	1.65±0.04	2.5±0.07	3.15±0.09	2.16±0.06

Table 4: Effects of Lead nitrate and AMF on the reducing sugar content of GREEN GRAM (Vigna radiata.(L) R. Wilczek

Treatments with Lead nitrate + AMF	reducing sugar (mg/g^{-1} fresh weight)				
	Day After Sowing (DAS)				
	15	30	45	60	75

Control	2.42±0.07	2.83±0.08	3.43±0.10	3.98±0.11	3.10±0.09
2.5 mg pb(no3)3	2.31±0.06	2.62±0.07	2.96±0.08	3.14±0.09	2.73±0.08
2.5 mg pb(no3 + AMF	2.89±0.08	3.15±0.09	3.87±0.11	4.13±0.12	3.24±0.09
5 mg pb(no3)	2.14±0.06	2.98±0.08	3.17±0.09	3.94±0.11	2.15±0.06
5 mg pb(no3)2 + AMF	2.74±0.08	3.02±0.09	3.58±0.10	4.02±0.12	2.89±0.08
7.5 mg pb(no3)2	1.69±0.05	2.36±0.07	2.97±0.08	3.23±0.09	2.13±0.06
7.5 mg pb(no3)2 + AMF	1.97±0.05	2.16±0.06	2.34±0.07	3.98±0.11	2.76±0.08
10 mg pb(no3)2	1.34±0.04	1.77±0.05	1.94±0.05	2.34±0.07	1.38±0.04
10 mg pb(no3)2 + AMF	1.92±0.05	1.98±0.05	2.20±0.06	3.17±0.09	1.93±0.05
12.5 mg pb(no3)2	0.94±0.02	1.38±0.04	1.23±0.03	1.18±0.03	0.98±0.02
12.5 mg pb(no3)2 + AMF	1.24±0.03	1.78±0.05	1.82±0.05	2.13±0.06	1.31±0.03

Table 5: Effects of Lead nitrate and AMF on the non-reducing sugar content of GREEN GRAM (Vigna radiata.(L) R. Wilczek

Treatments with Lead nitrate + AMF	**Non reducing sugar (mg/g^{-1} fresh weight)**				
	Day After Sowing (DAS)				
	15	**30**	**45**	**60**	**75**
Control	3.84±0.11	4.23±0.12	4.93±0.14	5.18±0.15	4.34±0.13
2.5 mg pb(no3)2	3.54±0.10	3.89±0.11	4.13±0.12	4.97±0.14	4.16±0.12
2.5 mg pb(no3)2 + AMF	4.12±0.12	4.23±0.12	4.96±0.14	5.19±0.15	4.64±0.13
5 mg pb(no3)2	3.14±0.09	3.26±0.09	3.84±0.11	4.73±0.14	3.39±0.10
5 mg pb(no3)2 + AMF	3.38±0.10	3.84±0.11	4.17±0.12	4.98±0.14	3.78±0.11
7.5 mg pb(no3)2	2.73±0.08	2.96±0.08	3.39±0.10	3.86±0.11	2.86±0.08
7.5 mg pb(no3)2 + AMF	3.16±0.09	3.24±0.09	3.95±0.11	4.14±0.12	3.10±0.09
10 mg pb(no3)2	2.24±0.06	2.45±0.07	2.97±0.08	3.23±0.09	2.18±0.06
10 mg pb(no3)2 + AMF	2.83±0.08	2.98±0.08	3.16±0.09	3.94±0.11	2.97±0.08
12.5 mg pb(no3)2	1.43±0.04	1.72±0.05	2.16±0.06	2.88±0.08	1.52±0.04
12.5 mg pb(no3)2 + AMF	1.91±0.05	2.31±0.06	2.83±0.08	3.18±0.09	1.96±0.05

Table 6: Effects of Lead nitrate and AMF on the total sugar of GREEN GRAM (Vigna radiata.(L) R. Wilczek

Treatments with Lead nitrate + AMF	**Total sugar (mg/g^{-1} fresh weight)**				
	Day After Sowing (DAS)				
	15	**30**	**45**	**60**	**75**
Control	6.26±0.18	7.06±0.21	8.36±0.25	9.16±0.27	8.44±0.25
2.5 mg pb(no3)2	5.85±0.17	6.51±0.19	7.09±0.21	8.11±0.24	6.89±0.20
2.5 mg pb(no3)2 + AMF	7.01±0.21	7.38±0.22	8.83±0.26	9.32±0.27	7.88±0.23
5 mg pb(no3)2	5.28±0.15	6.24±0.18	7.01±0.21	8.77±0.26	5.54±0.16
5 mg pb(no3)2 + AMF	6.12±0.18	6.86±0.20	7.75±0.23	9.00±0.27	6.67±0.20
7.5 mg pb(no3)2	4.42±0.13	5.32±0.159	6.36±0.19	7.09±0.21	4.99±0.14
7.5 mg pb(no3)2 + AMF	5.13±0.15	5.40±0.16	6.29±0.18	8.12±0.24	5.86±0.17
10 mg pb(no3)2	3.58±0.10	4.22±0.12	4.91±0.14	5.57±0.16	4.56±0.13
10 mg pb(no3)2 + AMF	4.75±0.14	4.96±0.14	5.36±0.16	7.11±0.21	4.90±0.14
12.5 mg pb(no3)2	2.37±0.07	3.10±0.09	3.39±0.10	4.06±0.12	2.50±0.07
12.5 mg pb(no3)2 + AMF	3.15±0.09	4.09±0.12	4.65±0.13	5.31±0.15	3.27±0.09

Table 7: Effects of Lead nitrate and AMF on the proline of GREEN GRAM (Vigna radiata.(L) R. Wilczek

Treatments with Lead nitrate + AMF	**Proline (mg/g^{-1} fresh weight)**				
	Day After Sowing (DAS)(ppm)				
	15	**30**	**45**	**60**	**75**
Control	1.99±0.05	2.85±0.08	4.15±0.12	4.93±0.14	2.33±0.06
2.5 mg pb(no3)2	3.25±0.09	4.55±0.13	5.36±0.16	6.18±0.18	3.23±0.09
2.5 mg pb(no3)2 + AMF	1.39±0.04	2.34±0.07	3.18±0.09	3.83±0.11	1.34±0.04
5 mg pb(no3)2	4.13±0.12	5.14±0.15	6.23±0.18	7.34±0.22	3.93±0.11
5 mg pb(no3)2 + AMF	3.84±0.11	4.83±0.14	5.93±0.17	6.34±0.19	3.14±0.09
7.5 mg pb(no3)2	4.74±0.14	6.75±0.20	8.73±0.26	8.93±0.26	4.14±0.12
7.5 mg pb(no3)2 + AMF	4.34±0.13	5.75±0.17	6.75±0.20	7.84±0.23	3.98±0.11
10 mg pb(no3)2	6.95±0.20	8.13±0.24	9.96±0.29	10.35±0.31	6.18±0.18
10 mg pb(no3)2 + AMF	6.15±0.18	7.63±0.22	9.05±0.27	9.95±0.29	5.99±0.17
12.5 mg pb(no3)2	7.21±0.21	8.93±0.26	10.63±0.31	11.11±0.33	8.43±0.25
12.5 mg pb(no3)2 + AMF	6.85±0.20	7.95±0.23	9.83±0.29	9.31±0.27	7.15±0.21

Conclusion

Lead Nitate pb(no3)2 is one of several heavy metals that cause severe environmental contamination in soil, sediments and groundwater. Several methods are already being used to clean up the environment from these kinds of contaminants, but most of them are costly and far away from their optimum performance. Mycophytoremedial is a term functional to a group of technologies that use plants to reduce, remove, degrade, or immobilize environmental toxins, primarily those of anthropogenic origin, with the aspire of restoring area sites to a condition useable for private or public applications. Arbuscular mycorrhizal fungi

(AMF) are amongst the most common soil fungi and the majority of plant species have associations with AM fungal species.ir The present investigation has been carried out to find out the effect of Lead Nitrate an am fungi on seed enzyme activities of green gram plants the co-7 varieties of Greengram seeds were obtained from the Tamil Nadu agricultural university Coimbatore. The Lead Nitrate salts were used for the treatment purpose The enzyme activity aspects such as catalyse, peracidase were high at AMF as compare control. But, the higher level of lead nitrate inhibited all enzymes like catalase, and peroxidase were low in AMF then the control. Then it was found increase with increasing heavy metal concentrations. Then it was found increase with increasing heavy metal concentrations. The present investication have enhanced on tremendous increase in the productivity by using AM fungi which is an symbiotic fungal association with the higher plants tried to reduce the toxic nature of the industrial waste specifically on the lead nitrate an life scavenging mechanisms to protect the agricultural field in to from the antagonistic action of lead nitrate.

References

1. Chandlee JM, Scandalios JG. Regulation of catl gene expression in the scutellum of maize during early sporophytic development. proc. natl acad sci,1984:81:4903-4907.
2. Kumar KB, Khan PA. Peroxidase and polyphenol oxidase in excised ragi (eleusine coracana cv.pr 202) leaves during senescence. Int J Exp. Bot,1982:20:412-416.
3. Foyer CH. Noctor oxidant and antioxidant signalling in plands are evalution of the concept of oxidative stress in a physiological context
4. Chaoui AS, Mazhoudis MH, E El-Ferjani. Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (phaseolus vulgaris l) plant sci,1997:127:139-47.
5. Rabie GH, Shaltout A, Shaaban M, LD, Metwally RA. Evaluation of vesicular mycorrhizal fungi effects on growth and heavy metals uptake by maize plants. Egyt. J. Bot.3rh,2013:321-335.
6. Tong Y, Kneer R, Zhu Y. Vacuolar compartmentalization: a second-generation approach to engineering plants for phytoremediation. Trends Plant Sci,2004:9:7-9.
7. Qu YP, Fang YL, Liu YL, Song SR, Zhang A, Zhou GR *et al*. Effects of AM fungal on the secondary metabolites of grape under cadmium stress. J. Northwest For. Univ,2009:24:101-105.
8. Azcón R, Perálvarez MDC, Roldán A, Barea JM. Arbuscular mycorrhizal fungi, Bacillus cereus, and Candida parapsilosis from a multicontaminated soil alleviate metal toxicity in plants. Microb Ecol,2010:59:668-677.
9. Garg N, Aggarwal N. Effect of mycorrhizal inoculations on heavy metal uptake and stress alleviation of Cajanus cajan (L.) Millsp. genotypes grown in cadmium and lead contaminated soils. Plant Growth Regul,2012:66:9-26.
10. Bhaduri AM, Fulekar MH. Assessment of arbuscular mycorrhizal fungi on the phytoremediation potential of Ipomoea aquatic on cadmium uptake, 3 Biotech,2012:2:193-198.
11. Abeer Hashem A, Abd Allah EF, Alqarawi AA, Dilfuza Egamberdieva. Bioremediation of adverse impact of cadmium toxicity on Cassia italica Mill by arbuscular mycorrhizal fungi, Saudi Journal of Biological Sciences,2016:23:39-47.
12. Kanwal Sadia, Asma Bano, Riffat Naseem Malik. Role of arbuscular mycorrhizal fungi in phytoremediation of heavy metals and effects on growth and biochemical activities of wheat (Triticum aestivum L.) plants in Zn contaminated soils, African Journal of Biotechnology,2016:15(20):872-883.
13. Abeer Hashem, Abd_Allah EF, Alqarawi AA, Asma A, Al Huqail, Egamberdieva D *et al*. Alleviation of cadmium stress in Solanum lycopersicum L. by arbuscular mycorrhizal fungi via induction of acquired systemic tolerance, Saudi Journal of Biological Sciences,2016:23:272-281.
14. Fergusson JE, editor. The Heavy Elements: Chemistry, Environmental Impact and Health Effects. Oxford: Pergamon Press,1990.
15. Duffus JH. Heavy metals-a meaningless term? Pure ApplChem,2002:74(5):793-807.
16. Abdul-Baki AA, Anderson JO. Vigour determination in soybean application of dairy manure on germination and emergence of some selected crops. J. Environ. Qual, 1973:3:396-399.
17. Chou CH, Chiang YC, Khan CI. Impact of water pollution on crop growth in Taiwan. *Bot. Bull. Acad. Sinica*,1978:19:107-124.
18. Arnon DI. Copper enzymes in isolated chloroplasts phenol oxidase in *Beta vulgaris*. Plant Physiol,1949:24:1-5.
19. Kirk JTO, RL Allen. Dependence of chloroplast pigments synthesis on protein synthetic effects of acitilione. *Biochem. Biophys. Res. Commun*,1965:27:523-530.
20. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with Folin-phenol reagent. *J. Biol. Chem*,1951:193:265-275.
21. Nelson N. A photometric adaptation of the Somogyis method for the determination of reducing sugar. *Ann. Chem*,1944:3:426-428.
22. Moore S, Stein WH. Photometric method for use in the chromatography of amino acids. J Biol Chem,1948:176-388.
23. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies, Plant and Soil,1973:39:205-208.
24. Chandlee JM, Scandalios JG. Regulation of *Catl* gene expression in the scutellum of maize during early sporophytic development. *Proc. Natl. Acad. Sci*., USA,1984:81:4903-4907.
25. Kumar KB, Khan PA. Peroxidase and polyphenol oxidase in excised ragi (*Eleusine coracana* cv. PR 202) leaves during senescence. Ind. J. Exp. Bot,1982:20:412-416.
26. Jackson ML. Soil chemical analysis, Prentice Hall of India Private Limited, New Delhi,1958:22-31.
27. Black CA. Methods of soil analysis Part 2. In Chemical and Microbiological properties. American Society of agronomy, Inc. Madison, Wiscosin,1965:242.
28. Yoshida S, Fordo V, Cork J, Gomez K. Laboratory manual for physiological studies of rice 3rd Ed., The

International Rice Research Institute, Philippines,1972:11-23.
29. Williams CH, Twine V. In: Modern Methods of Plant Analysis, Vol. 5, (Ed.) Peach, K and M.V. Tracey. Springer Verlag, Berlin,1960:3-5.