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Analysis of Abiotic Stress Induced Metabolomic Changes in *Vigna radiata*

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Abstract:

The present study involves the use of Phenol, Toluene, Xylene and Hexane as xenobiotics, to induce abiotic stress in mung seedlings under *in vitro* conditions and to evaluate their impact on seedling's physiological parameters besides alteration in metabolomic profile with respect to control. The toxicity level was found to be high in case of Phenol followed by Hexane, Xylene and Toluene. Metabolomic analysis revealed that, at low concentration of these compounds, there was an elevation in the contents of the metabolomic parameters such as Total Soluble Sugar, Total Reducing Sugar, Total Phenolic Contents and Total Protein Contents, followed by a sharp decline at higher concentrations. Protein profiling revealed a band of97.4KDaproteins in all the stress induced plant which was not detected in untreated control. This protein is most probably a part of the stress machinery in the plant.

Keywords: Abiotic stress, Chaperones, Heavy metals, Heat shock proteins (Hsps) Metabolomic analysis, *Vignaradiata,*Virat HY45, Xenobiotic

1.0 Introduction:

Plants face numerous abiotic stresses such as drought, salinity, xenobiotics and toxic heavy metals, nutrients imbalance and a broad spectrum of temperature. These factors severely impair the normal physiological process and productivity of a plant. Plants have adapted various strategies to cope up with this problems. Response of a plant cell to these stresses is very crucial and largely achieved by changes in gene expression pattern, which in turn modulates certain metabolic and defensive pathways through changes in proteins, both qualitatively and quantitatively (Timperio, 2008). Protein dysfunction/misfolding is commonly associated with abiotic stresses. Heat Shock Proteins (Hsps)/ Chaperones re-establishes the protein conformation, thus maintaining cellular homeostasis(Wang, 2004). Hsp 60, Hsp 70 and Hsp 90 plays a significant role in this process by interacting with various co chaperones (Hartl, 1996; Horwich, 1998). Osmolytes, which are low molecular weight sugars, organic acids, polyols and nitrogen containing compounds, generally found in higher plants, helps in osmotic adjustments for plants subjected to salt stress (Ashraf, 2004). Glycine betaine is an important quaternary ammonium compound that accumulates to a significant level in salt tolerant plants, serving to protect cellular enzymes by interacting with it (Chen, 2011). Plant secondary metabolites such as methyl jasmonate, jasmonic acid and salicylic acid, accumulates in plants subjected to abiotic stress and thus helps plants to adapt the stress environment (Ravishankar, 2011).

Environmental pollution and accumulation of xenobiotic is widespread, due to rapid industrialization and current agricultural practices. Existing pattern of industrial activity is introducing new organic compounds and novel chemicals into the environment, exerting negative influences on man and environment leading to toxicity in plants (Ravichandran, 2011). Another major category of abiotic stress involves heavy metals that have been absorbed by the plants results in growth inhibition, increasing senescence which leads to decreasing crop yield. Heavy metals such as Zn, Fe, Ni, Co, Cu, and Mn are required in minute amounts in plants but excessive amount can have detrimental effects on plants. Other heavy metals such as Pb, Cd, Hg, and As are regarded as major threat to plants and other organisms as they do not have any beneficial utility (Chibuike, 2014). V. radiata (mung) is a plant of the family Fabaceae. It is one of the most widely used pulse crop in India. It has great value as food and is a cheap source of protein for direct human consumption (Mubarak, 2005).

Up to best of our knowledge it is the first report on green gram for abiotic stress in xenobiotic stressed seedlings with respect to their control to describe the common physiological tolerance. We made an attempt to investigate the effect of these xenobiotics stresses (phenol, toluene, xylene and hexane) on some important metabolomics processes of *Vigna radiata* (mung beans, Variety- Virat). We wish to bring into limelight the physiological response of the crop under simulated environmental stress factors.

2.0 Materials and Methods:

2.1 Sample Source for the Study Plant:

Certified Moong seeds (*Vignaradiata*) (Virat HY45) were brought from a certified shop. The seed were of research grade. The study was conducted at Dr. B. Lal Institute of Biotechnology, Jaipur in 2014.

2.2 InvitroDevelopment of Plantlets Under Abiotic Stress Condition.

2.2.1 Standard Blotter Method:

Circular discs of blotting paper equal to a diameter of Petri plate (90 cm) were cut and moistened with sterile distilled water and then placed onto the Petri plates(3 blotter papers per plate). These were autoclaved at 15 PSI for 30 minutes. Surface sterilized seeds with 0.1% mercuric chloride solution for 3-5 minutes and washed for about 8-10 times with sterilized distilled water to remove traces of $HgCl_2$. The blotter papers were moistened with xenobiotics(10 ppm, 100 ppm, 300 ppm, 500 ppm, and 1000 ppm)and with sterile distilled water as control, than incubated asrecommended by International Seed Testing Association (ISTA).The seeds (10 seeds/plate)were aseptically transferred on the surface of moistened blotting paper and incubated at 28^{o}C for 5-6 days for germination; symptoms and root-shoot length in xenobiotic induced seeds with respect to the control, were recorded(Ghoonem, 2002; Doyer, 1938; De Temp, 1953).

2.2.2 Water Agar Seedling Symptom Test:

Xenobiotics supplemented MS media of different concentrations (10 ppm, 100 ppm, 300 ppm, 500 ppm, 1000 ppm) were prepared. Surface sterilized seeds were inoculatedin test tubes containing media supplemented with xenobiotics(1 seed/test tube). Observations were made to record germination percent, seedling symptom and seedling mortality with respect to the control were observed. The test plants were incubatedat26^{O}C± 2 ^{O}C with 2000 lux illumination and 16 hours of photoperiod (Ghoonem,2002; Muskettand Malone, 1941).

2.2.3 Metabolomic Analysis of Xenobiotic Stress Induced Plants:

The root shoot length of the plants were recorded and proceeded for analysis of metabolomic parameters such as Total Protein Content(Randall & Lewis, 1951),Total Phenolic Content estimation(Chanda & Dave, 2009),Total Soluble Sugar Content(DuBois, Gilles, Hamilton, Rebers, & Smith, 1956), Total Reducing Sugar Content (Miller, Gail Lorenz ,1959).

2.2.4 Protein Profiling of Stress Induced Plants:

Isolation of crude protein by TCA method(Caruso et al., 2009)and SDS PAGE of protein sample was performed(Schagger and von Jagow, 1987).

3.0 Results and Discussion:

The exploration of the xenobiotics compounds (Phenol, Toluene, Hexane and Xylene) for induction of abiotic stress on *Vigna radiata* and to evaluate its effect on the plant growth parameters under *in vitro* conditions is a pioneering approach. The result of the study conduct is justified by comparing it with the outcome with respect to other abiotic stress on *Vigna radiata*

3.1 Standard Blotter Method (SBM):

SBM was used to evaluate the impact of xenobiotic on germination and root shoot length (Fig 1, 4). Data represents that in treated seeds root shoot length decreases with increase in concentration till 100 ppm (phenol); 500 pm (toluene), 1000 ppm (hexane) and beyond that the seeds fail to germinate with respect to the control (Fig 1).If we rate the overall level of toxicity of the compounds at decreasing order based on SBM method, then, Phenol> Toluene >Hexane > Xylene.

(Fig 1: Recovered in vitro grown seedlings from the SBM method)

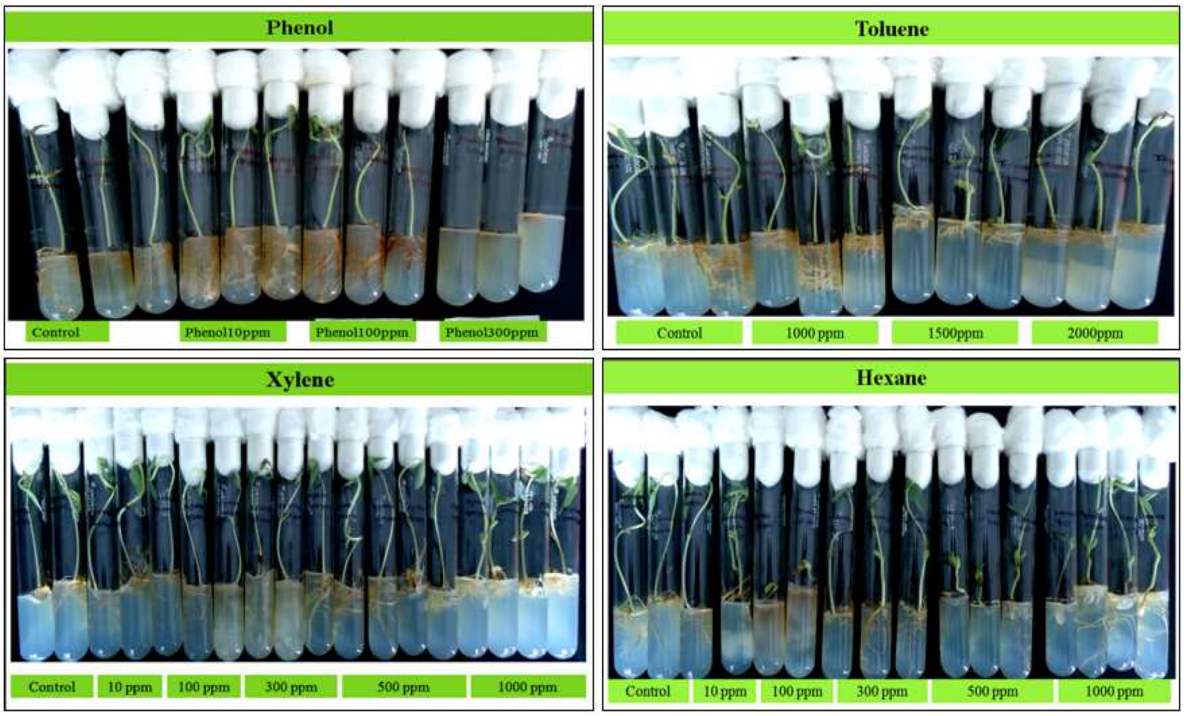
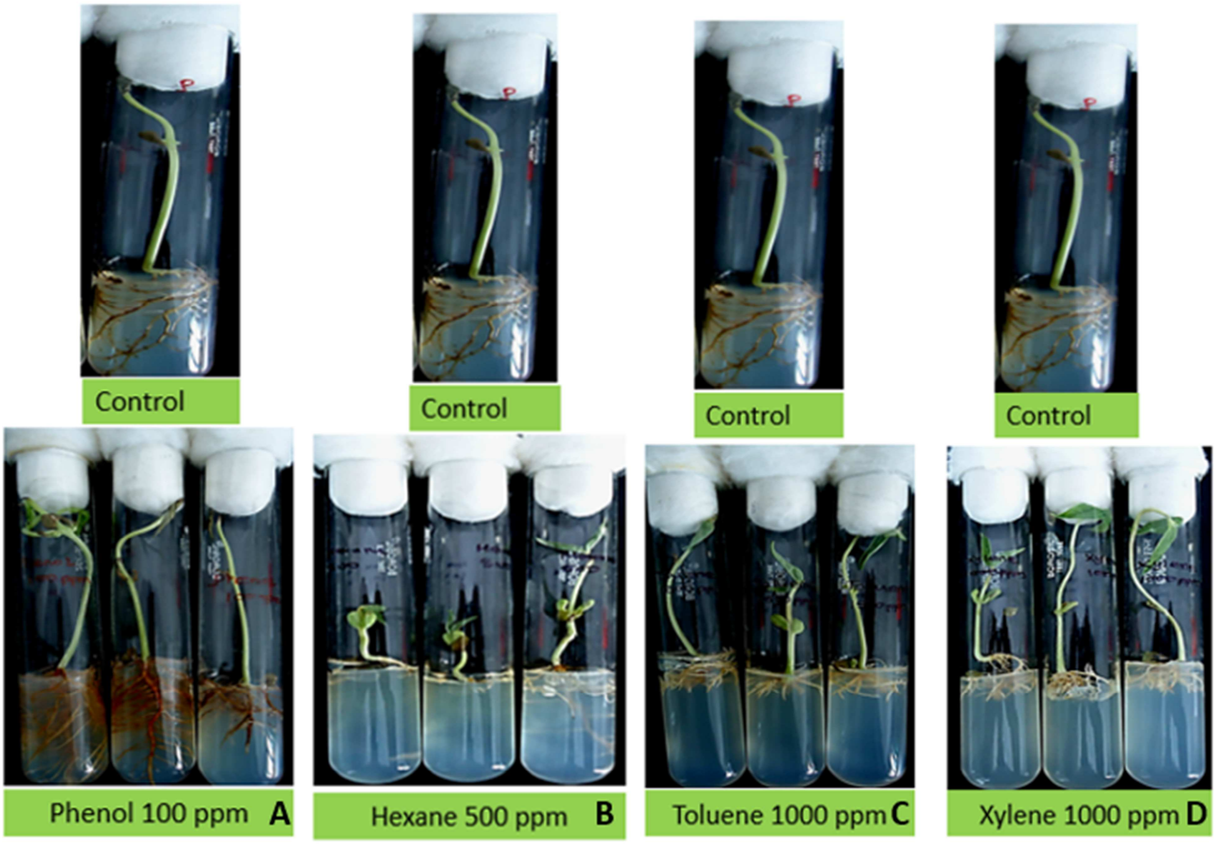


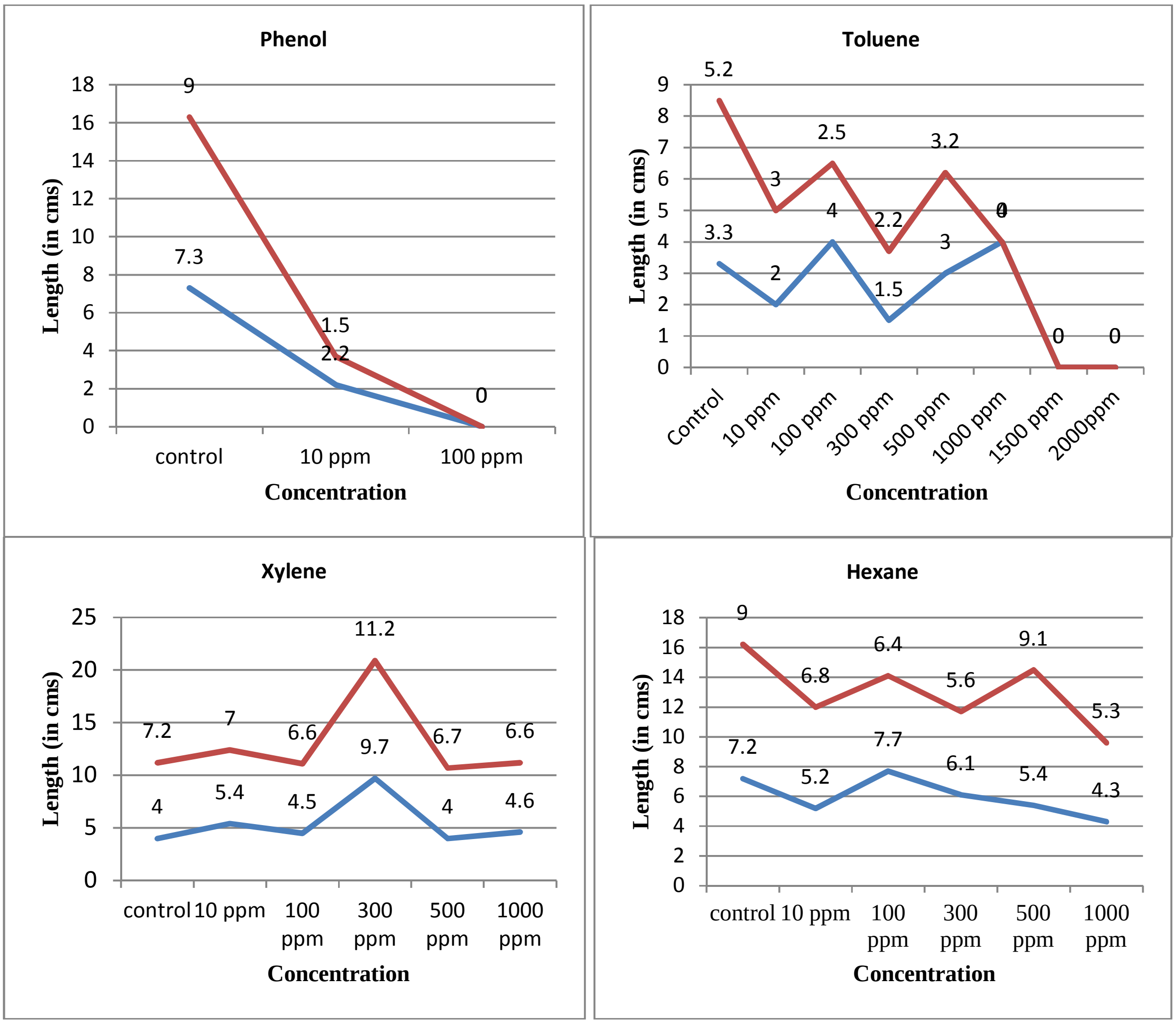
Fig 2: Induction of xenobiotic stress to the seeds at various concentrations (10 ppm, 100 ppm, 300 ppm, 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm) by WAST method and the phenotypic symptoms observed



(Fig 3: Symptoms observed due to stress induction obtained in WAST methodA Phenol, B Hexane, CToulene and D Xylene)

3.2 Water Agar Seedling Symptom Test (WASST):
During WASST similar trend was visualized along with the well noted symptoms (Fig 3). Phenol at 100 ppm concentration showed thicker browner roots with increase in density in comparison with the control (Fig 3 a). The rooting density in Hexane 500 ppm reduced significantly as compared to the control (Fig 3 b). Toluene at higher concentrations (1000 ppm, 1500 ppm, 2000 ppm) (Fig 3c) and xylene at 1000 ppm showed negative geotropism (Fig 3 d).Another study suggests that the root length decreased significantly due to Cadmium Chloride(Gill, Sharma, Singh, & Bhullar, 2001;Hirve M and Bafna A 2013)Arsenic and manganese(Mumthas, Chidambaram, & Sundaramoorthy, 2010); Chromium(Suthar, Pansuriya, Kher, Patel, & Nataraj, 2014);Zinc Sulphate (Nag Pratima, and Mukherhji 1989) and Silver nanoparticles but the root growth is totally terminated at 1600 µg/ml(Mazumdar& Ahmed, 2011)as compared to untreated seedlings in all varieties of in*Vignaradiata.*

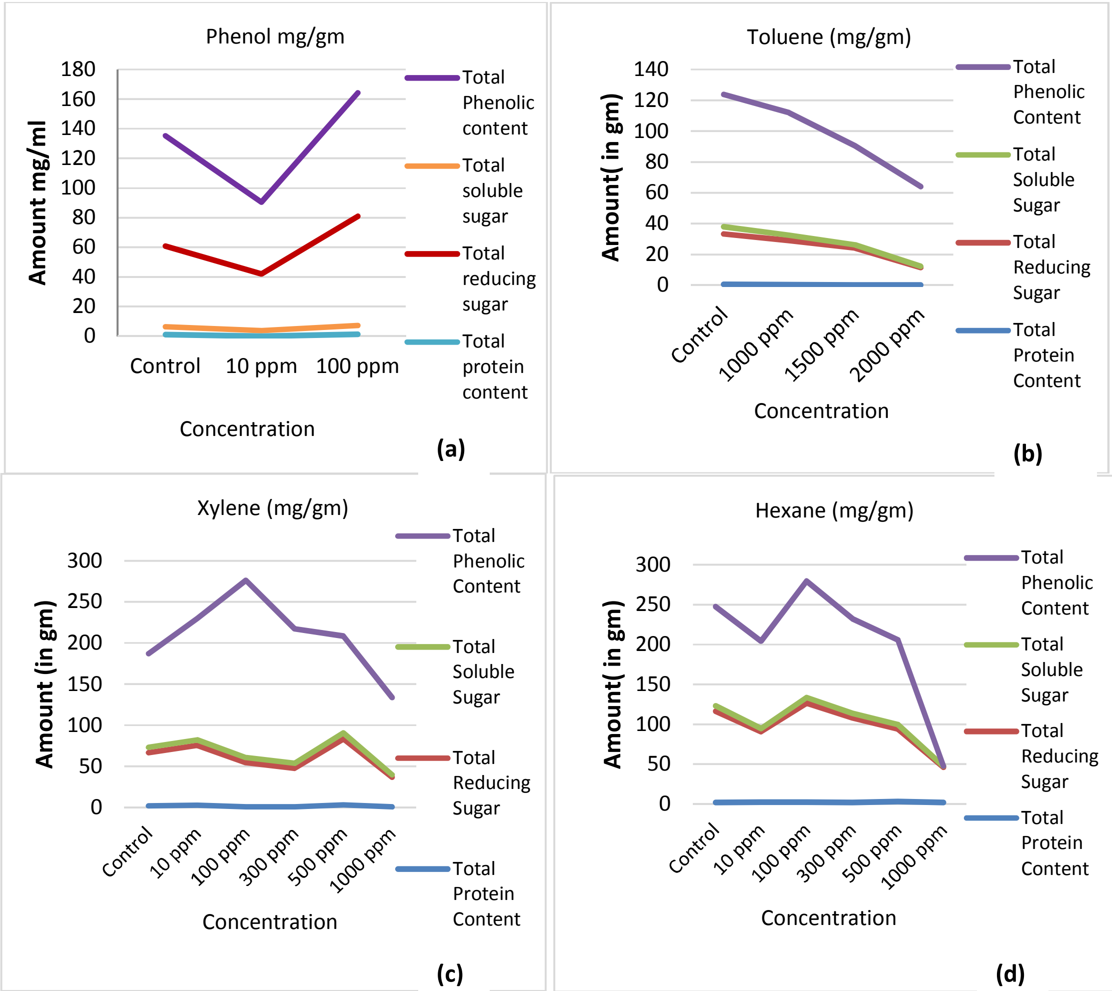
3.3 Metabolomic Analysis of the Stress Induced Seedlings:
Assessment of the phenotypic parameter (metabolomic analysis) of a plant indicates the assimilation of xenobiotic. During metabolomic analysis, changes in parameters such as Total Protein Content, Total Soluble Sugar, Total Reducing Sugar and Total Phenolic Content were evaluated from recovered stress induced seedlings with respect to control group seedlings.



(Fig 4: Histogram of the root (blue) shoot (red) length (in cm) observed due to stress compounds for comparative analysis)

3.4 Total Protein Content:

Evaluation of the level of protein content is important as they are the key players in plant cellular metabolism and results in the onset of stress protein mechanism. Data from the present study revealed, the total protein content of seedling decreased after10 ppm Phenol and xylene, in hexane 500 ppm but there is a rise beyond these concentration as compared to the control. In case of toluene, the level of protein content of the seedlings decreased gradually with increasing concentration as compared to the control(Fig 5).Similarly in another study, seedlings treated with Cadmium Chloride showed a significant decrease in protein content at all its concentrations(Hirve and Bafna ,2013)and in chromium (Nag Pratima, and Mukherhji,1989) at 50 µM an increasing amount of protein followed by a rapid decrease as compared to untreated seedlings in all studied varieties of *Vignaradiata*.



(Fig 5: Comparative analysis of changes in Total Protein Content, Total Soluble Sugar Content, Total Reducing Sugar Content, Total Phenolic Content due to (a) Phenol, (b)Toulene, (c) Xylene and (d)Hexane on stress induction at various concentrations of xenobiotics)

3.5 Total Soluble Sugar Content:

Soluble Sugar is an important candidate involved in providing protection of plants from various forms of stress. They serves to protect proteins on stress conditions and also functions as an osmoprotectant. Data revealed that,in phenol and xylene treatedseedlings, there is a decrease in total soluble sugar content at 10 ppm concentration followed by an increase when compared with the control (Fig 5).Toluene and hexane treated seedlings showed a decline in the total soluble sugar content with increasing concentration of with respect to control. Similar report was reportedin a study using $HgCl_2$ and $ZnSO_4$ treated seeds, showed a significant decrease in total soluble sugar content at highest concentration of mercuric chloride and zinc (80% and 11%) reduction in total soluble sugar content respectively (Nag Pratima, and Mukherhji, 1989).

3.6 Total Reducing Sugar Content:

The role of reducing sugar shares almost similar function as that of soluble sugar. Reducing sugar serves as an important indicator as there is a decrease in their content under stress condition. They also serves to protect proteins on stress conditions and act as an osmoprotectant. Present study indicates that on treatment of seedlings with phenol and xylene (10 ppm) there is a decrease in total reducing sugar content followed by an increase with high concentration when compared with the control. Whereas in toluene and hexane treated seedlings, showed a decrease in the total reducing sugar content with increasing concentration of xenobiotic (Fig 5). Similar results were quoted in another study sorghum(Gill et al., 2001)seedlings treated with NaCl showed a dramatic increase in total reducing sugar content in shoot, root and endosperm whereas Triticale seeds (Samad and Karmokar,2013)increase in the total soluble sugar content 1.6-6.5 fold in as compared to control.

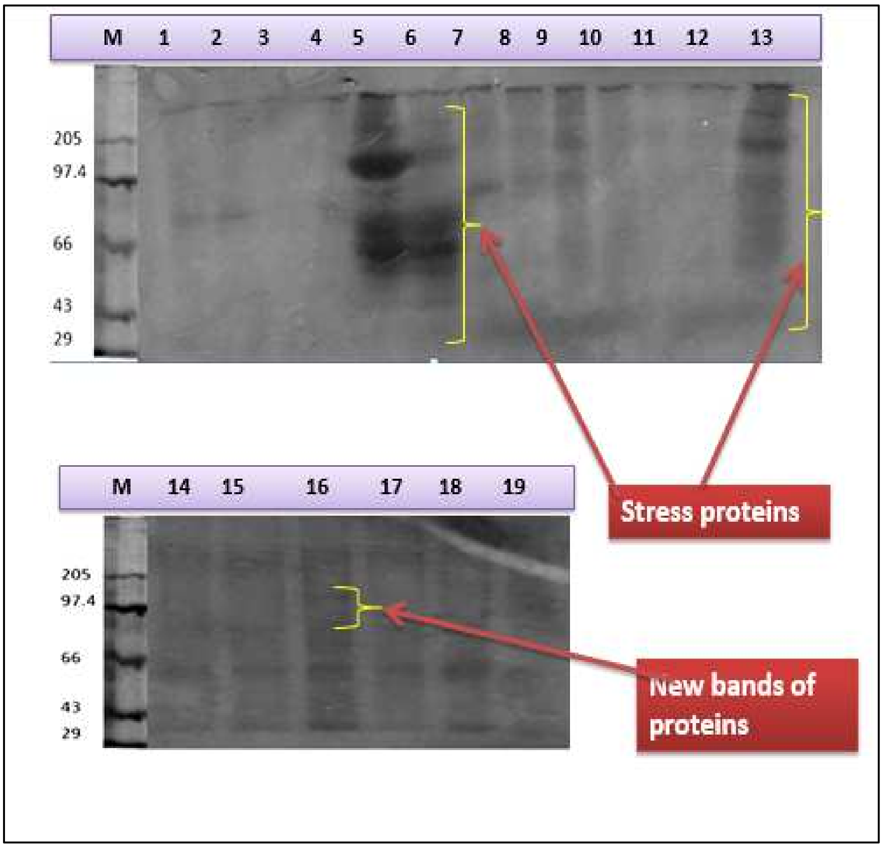
3.7 Total Phenolic Content:

Assessment of phenolic content in plant is important as it acts as the precursor for the synthesis of many biomolecules. On stress condition there is a significant decrease in the content in the phenolic contents of the plants. On treatment of seedlings with phenol (10 ppm) and Xylene (100 ppm)there is a decrease in total phenolic content at concentration followed by an increase when compared with the control (Fig 5).Toluene and hexane treated seedlings showed a decrease in the total phenolic content with increasing concentration of xenobiotic. In a study seedlings treated with silver nanoparticles showed an increase in total phenolic contents at 50ppm concentrations while lead nitrate treated seeds showed a decrease in total phenolic contents at 120 ppm concentrations(Najafi, Jamei, Najafi, & Jamei, 2014).

3.8 Protein Profiling of the Xenobiotically Stressed Plants:

Protein profiling serves to be an important tool for understanding the proteomics of a stress induced plant. Proteins are the key players of any cell as they are responsible for maintaining normal homeostasis in the cell. When a plant is exposed to any kind of stress whether abiotic or biotic it synthesizes various stress machinery proteins that are responsible for safe guarding the plants up to a certain range of the stress. Identification of the unique proteins that are generated during the stress condition could serve as beneficial as we can engineer plants with better stress tolerance.

On comparison of the protein bands of control with the test plants (Fig 6), we can see in toluene 1000 ppm and 1500 ppm there is an over expression of the proteins when compared to the control. These proteins may be a part of the stress machinery of the cell. In hexane 1000 ppm there is an overexpression of the protein of molecular weight 97.4 KDa and also two new bands appeared in comparison with the control. In xylene also some new band of protein of molecular weight appeared at 97.4 KDa with respect to the control. Similarly in other concentrations of xenobiotically stressed seedling showed an elevated protein expression. This differential protein expression is a part of adaptive response of the plant in stressed condition. Hence a protein of 97.4 KDa may be most probably involved in stress machinery or it may be a stress protein as its expression can clearly be seen only in the xenobiotically stressed seedlings with reference to the control. In a different study of salt stress on *Vigna species*, increased protein bands intensity and induced some new bands. Salinity stress induced two new bands between 45 and 22 kDa, respectively, in salt tolerant genotypes. Furthermore, band intensity of the salt treated genotypes was higher than the control plants(Win, Oo, Hirasawa, Ookawa, & Yutaka, 2011).Iron stress in *Withaniasomnifera,* found that several new protein bands like 98.14, 38.16, 23.27, 21.25 and 17.49 kDa are newly synthesized and some (87.53 and 66.70 kDa) are expressed more, in leaf samples of treated plants(Rout & Sahoo, 2012).Water stress deficit in *Vigna radiate* led to the overexpression of Late Embryogenesis abundant (LEA) protein, emv2.This protein is suspected to protect the plants in water deficit stress conditions (Rajesh et al., 2008).



(Fig 6: Protein Profiling of the xenobiotically stressed *invitro* recovered seedlings: M- Marker (Merck 29-205KDa, lane 1-3 phenol treated (1-control, 2-100 ppm, 3- 300 ppm); 4-7 toluene treated (4- control, 5-1000 ppm, 6- 1500 ppm, 7-2000 ppm); 8-13 hexane treated (8- control, 9- 10 ppm, 10- 100 ppm, 11- 300 pm, 12- 500 ppm, 13- 1000 ppm); 14-19 xylene treated (14- control, 15- 10 ppm, 16- 300 ppm, 17- 300 ppm, 18- 500 ppm, 19- 1000 ppm)

4.0 Conclusion:

Modernization has led to the betterment of human life. Life is becoming easier with rapidity in comparison to the last few decades. This advancement in human life has come due to the industrial revolution. Today the scale for the development of a country depends on its industries and natural resources it is having. As more industries and factories are being set up there is an exponential rise in the level of the pollutants released by these industries. Major contributors to this pollution are the textile, paints and petro chemical industries. They are polluting the natural bodies at an alarming rate. The worst thing happens when these factories are setup in the agricultural belt. Stress induced the mung seedlings under *in vitro* conditions with the xenobiotics to study the effects of those compounds on the seedlings and to assess the changes in the metabolomics parameter in the plant due to the stress condition. The result obtained from the SBM Method was further validated from the Water Agar Seedling Symptom Test the severity of the effect on the seedling physiological parameters were in the order as Phenol followed by Hexane, Xylene and Toluene. In all the metabolomic parameters, initially there was an increase in the level of the phytochemicals at low concentration of the compounds followed by a final decrease in the contents of the phytochemicals at high concentration of the compound. Protein profiling of the stressed induced plant revealed band of 97.4 KDa protein was found common in all the stress induced plant compared with the control. So this might be the protein that is most probably a part of the stress machinery in the plant.

5.0 Acknowledgement:
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References:
1) Timperio, A. M., Egidi, M. G., &Zolla, L. (2008). Proteomics applied on plant abiotic stresses: role of heat shock proteins (HSP). Journal of proteomics,71(4), 391-411.
2) Wang, W., Vinocur, B., Shoseyov, O., & Altman, A. (2004). Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response.Trends in plant science, 9(5), 244-252.
3) Hartl, F. U. (1996). Molecular chaperones in cellular protein folding.
4) Bukau, B., &Horwich, A. L. (1998). The Hsp70 and Hsp60 chaperone machines. Cell, 92(3), 351-366.
5) Ashraf, M. P. J. C., & Harris, P. J. C. (2004). Potential biochemical indicators of salinity tolerance in plants. Plant Science, 166(1), 3-16.
6) Chen, T. H., & Murata, N. (2011). Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. Plant, cell & environment, 34(1), 1-20.
7) Akula, R., &Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. Plant signaling & behavior, 6(11), 1720-1731.
8) Ravichandran, S. (2011). Possible Natural ways to eliminate toxic heavy metals. International Journal of Chemtech Research CODEN (USA), IJCRCC, 3(4), 1886-1890.
9) Chibuike, G. U., &Obiora, S. C. (2014). Heavy metal polluted soils: effect on plants and bioremediation methods. Applied and Environmental Soil Science, 2014.
10) Mubarak, A. E. (2005). Nutritional composition and antinutritional factors of mung bean seeds (Phaseolus*aurous*) as affected by some home traditional processes. Food Chemistry, 89(4), 489-495.
11) Anonymous (1990). International rules of seed testing, International seed testing association. Seed science and Technology, 18, 299-513.
12) Ghoonem K., Al Sahli, An Improved Method of Seed Health Testing, Plant Pathology Journal. DOI: 10.3923/ppj.2002.11.13
13) Chanda, S., & Dave, R. (2009). In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. Afr J Microbiol Res, 3(13), 981-996.
14) Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances.Analytical chemistry, 28(3), 350-356.
15) Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar.Analytical chemistry, 31(3), 426-428..
16) Caruso, G., Cavaliere, C., Foglia, P., Gubbiotti, R., Samperi, R., &Laganà, A. (2009). Analysis of drought responsive proteins in wheat (Triticum durum) by 2D-PAGE and MALDI-TOF mass spectrometry. Plant Science, 177(6), 570-576..
17) Schägger, H., & Von Jagow, G. (1987). Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Analytical biochemistry, 166(2), 368-379..
18) Hirve, M., &Bafna, A. (2013). Effect of Cadmium exposures on growth and biochemical parameters of Vigna radiata seedlings. International Journal of Environmental Sciences, 4(3), 315-322.
19) Nag, P., Nag, P., Paul, A. K., &Mukherji, S. (1989). The effects of heavy metals, Zn and Hg, on the growth and biochemical constituents of mungbean (Vigna radiata) seedlings. Botanical Bulletin of Academia Sinica, 30, 241-250.
20) Mazumdar, H., & Ahmed, G. U. (2011). Synthesis of silver nanoparticles and its adverse effect on seed germinations in Oryza sativa, Vigna radiate and Brassica campestris. Int. J. Adv. Biotechnol. Res,2(4), 404-413.
21) JayaRambabu N, Siva Kumari B., K.VenkateswarRao (2014), Germination and growth characteristics of Mung bean seeds affected by Synthesized Zinc oxide nanoparticles. International Journal of Current Engineering and Technology, 4: 321-328.
22) Mumthas, S., Chidambaram, A. A., Sundaramoorthy, P., & Ganesh, K. S. (2010). Effect of arsenic and manganese on root growth and cell division in root tip cells of green gram

(Vigna radiata L.). Emirates Journal of Food and Agriculture, 22(4), 285.

23) Ashok, K., Bisht, B. S., Manish, K., &Lalit, K. (2010). Effects of Ni and Zn on growth of Vigna mungo, Vigna radiata and Glycine max. International Journal of Pharma and Bio Sciences, 1(2).
24) Ikram, N., Dawar, S., Zaki, M. J., &Abass, Z. (2010). Effect of (/sup 60/cobalt) gamma rays on growth and root rot diseases in mungbean (vignaradiata L.).
25) Suthar, B., Pansuriya, J., Mafatlal, M. K., PATEL, V. R., & Nataraj, M. (2014). Biochemical Changes under Chromium Stress on Germinating Seedlings of Vigna radiata. NotulaeScientiaBiologicae, 6(1), 77-81.
26) SAEIDEH, N., & RASHID, J. (2014). Effect of silver nanoparticles and Pb (NO 3) 2 on the yield and chemical composition of mung bean (Vigna radiata).Journal of Stress Physiology & Biochemistry, 10(1).
27) Gill, P. K., Sharma, A. D., Singh, P., &Bhullar, S. S. (2001). Effect of various abiotic stresses on the growth, soluble sugars and water relations of sorghum seedlings grown in light and darkness. Bulg. J. Plant Physiol, 27(1-2), 72-84.
28) Samad, R., &Karmoker, J. L. (2014). Effects of NaCl stress on accumulation of K+, Na+, Cl-, NO3-, sugar and proline contents in the seedlings of Triticale-I. Bangladesh Journal of Botany, 42(2), 189-194.
29) Win, K. T., Aung, Z. O., Hirasawa, T., Ookawa, T., & Yutaka, H. (2013). Genetic analysis of Myanmar Vigna species in responses to salt stress at the seedling stage. African Journal of Biotechnology,10(9), 1615-1624.
30) Rout JyotiRajan and SahooSantiLata (2012), Morphological and protein profile alteration in Withaniasomniferawith response to iron stress. Indian Journal of Life science. 2(3):21-25.
31) Rajesh S and Raveendra M (2008). Over expression analysis of **emv2** gene coding for late embryogenesis protein from *Vigna radiata.* Journal of Stress Physiology & Biochemistry,4 (7): 9-16.
32) DOYER, L.C., 1938, Manual for the determination of seed-borne diseases. Wageningen, Netherland: International Seed Testing Association, p.59. 7.
33) De Temp J. (1953). The blotter method of seed health testing programme. ISTA 28: 133-151.
34) Muskett, A. E., & Malone, J. P. (1941). The Ulster Method for the Examination of Flax Seed for the Presence of Seed-Borne Parasites. Annals of Applied Biology,28(1), 8-13.