

# TRICHODERMA MEDIATED MITIGATION OF LEAD TOXICITY IN MUSTARD

Yaman<sup>1</sup>, Prasann Kumar<sup>1, 2\*</sup>, Chandramohan Mehta

<sup>1</sup>Department of Agronomy, School of Agriculture

Lovely Professional University, Jalandhar, Punjab, India, 144411

<sup>1, 2</sup> Division of Research and Development

Lovely Professional University, Jalandhar, Punjab, India, 144411

\*Email: prasann0659@gmail.com

## Abstract

Soils polluted with heavy metals have become common all around the world because of increase in geologic and anthropogenic activities. There is very adverse effect on the production of the crops, soil health, soil fertility and also very hazardous for the human health due to the accumulation of heavy metals in the soil and contaminate the environment. Among all the heavy metals lead (Pb) is one the major concerning metal which impose very disturbing effect on the physiological activities of the plants like electron transport, enzymatic activities, membrane structure, photosynthetic activities and hormonal status. It is also a potent factor for the pollution of environment. Pb pollutants are indispensable for modern human life, soil contamination with Pb is not likely to decrease in the near future. To check the effect of lead and Trichoderma on mustard various treatments are provided. Lead toxicity is produced by using lead acetate (90 ppm/kg of soil) and application of Trichoderma (5g/10 kg of soil) to the soil before sowing and estimate different biochemical parameters like total soluble protein, total soluble sugar, total soluble phenol, membrane injury index (MII) and membrane stability index (MSI) to know the physiological condition of the plant and how application of Trichoderma helps to mitigate the lead toxicity.

**Keywords:** Anthropogenic, Environment, Enzymatic, Geologic, Hormonal, Lead, Mitigate, Photosynthetic, Soil health, Trichoderma

## Introduction

Problem of heavy metals contamination is increasing day by day in India due to mining activities, coal burning power plants, thermal power plants and excess use of fertilizers in agriculture for crop production. Lead, cadmium, chromium, copper and mercury are major heavy metals which cause various environmental pollution specially in areas with very high anthropogenic pressure. Due to the accumulation

of heavy metals in crop land soils highly affects the agricultural production by decreasing the growth of the crops and soil health. Soil microbes play a very important role for maintaining the soil health and fertility but in heavy metals contaminated soils their population is very low. Metals toxicity impose a very negative impact on the plants and also on the ecosystem.

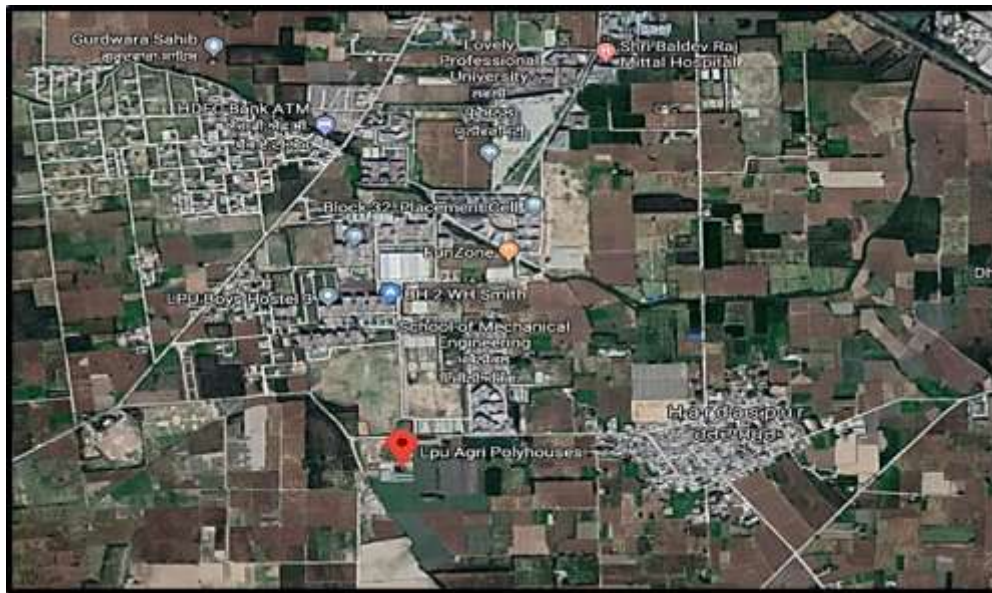
Lead (Pb) is one of the major heavy metal and has greater potential to cause environmental pollution. Apart from the natural weathering processes, Pb contamination of the environment has resulted from mining and smelting activities, Pb containing paints, gasoline and explosives as well as from the disposal of municipal sewage sludges enriched in lead (Chaney and Ryan, 1994). Major sources of lead are chimneys of factories using Pb, exhaust fumes of automobiles, effluents from storage batteries, industry, mining and smelting of Pb ores, metal plating, pesticides and fertilizers use. Lead contamination in soil and environment has become most serious global problem which has also had a bad effect on the human health. As many of the Pb pollutants are indispensable for modern human life, soil contamination with Pb is not likely to decrease in the near future (Yang et al., 2000). It has been observed that most of the lead contaminated soils are present near the industrial areas. Soils which are affected with lead contain 400-800 mg Pb/kg of soil while its range is 1000 mg/kg of soil near the industrial area (Angelon and Bini, 1992).

Lead adversely affects the production of the crop because it inhibits the growth of roots, stunted growth of the plant and chlorosis (Burton et al., 1984). Lead even in very small quantity disturbs various physiological processes inside the plants like hormonal status, membrane structure, water potential, electron transport and enzymatic activities. At the cellular level lead inhibits the enzymatic activities containing sulphhydryl (-SH) groups necessary for their activity (van Assche and Clijsters, 1990). Heavy metals affected areas are poor in organic matter and microbial activity is low. Therefore, application of *Trichoderma* helps to improve the performance of plants and protect them from any kind of plant stress. To check the effect of lead toxicity and *Trichoderma* application on mustard we select one genotype of mustard (PBR-357) and grown in the pots having dimensions (30 cm in diameter and 25cm in height) at Lovely Professional University, Jalandhar. The main objective behind our study is to check the effect of lead on the biochemicals of mustard and how *Trichoderma* helps to mitigate the effect of lead toxicity in soil.

### Methodology

The experiment was conducted in the pots with all the natural conditions at the School of Agriculture, Lovely professional University (LPU), Phagwara, Jalandhar (Punjab). Jalandhar is located on the irrigated plain between Sutlej and Beas rivers. Jalandhar is surrounded by Ludhiana district in East, Kapurthala in West, Hoshiarpur in North and Ferozepur in South. The experimental area is situated at an altitude of 232 m above mean sea level, having latitude and longitude 31.244604 and 75.701022 respectively (Figure 1).

Fig.1: Google photo of the experiment site



(Source: Google Earth, 2020)

### Climatic Conditions:

Climate of Jalandhar is characterized by hot and dry summers and wet and humid monsoons. In winter, there is much less rainfall in Jalandhar than in summer. Phagwara's climate is a local steppe climate. The average annual temperature is 24.1 degree Celsius. June is the hottest month of the year with average temperature of 33.6 degree Celsius and with the average temperature of 12.7-degree Celsius January is the coldest month. The average annual rainfall is 686 mm.

### Treatments Details:

The pot experiment was conducted with one genotype of Mustard PBR-357 taken from the Punjab Agricultural University, Ludhiana (Punjab). This experiment was conducted on the agricultural farm located at Lovely Professional University, Jalandhar. Pots which are used for sowing filled with well pulverized and porous soil and mix with vermicompost. We have use 12 pots having dimensions like diameter is 30cm and height is 25cm. For this experiment four treatments (T0, T1, T2 and T3) and three replications (R1, R2 and R3) were taken. There is soil application of all the treatments before sowing of the mustard. There is treatments of heavy metal i.e. Lead in the form of lead acetate (90 ppm/ kg of soil) and *Trichoderma atroviridae* (5g/10 kg of soil).



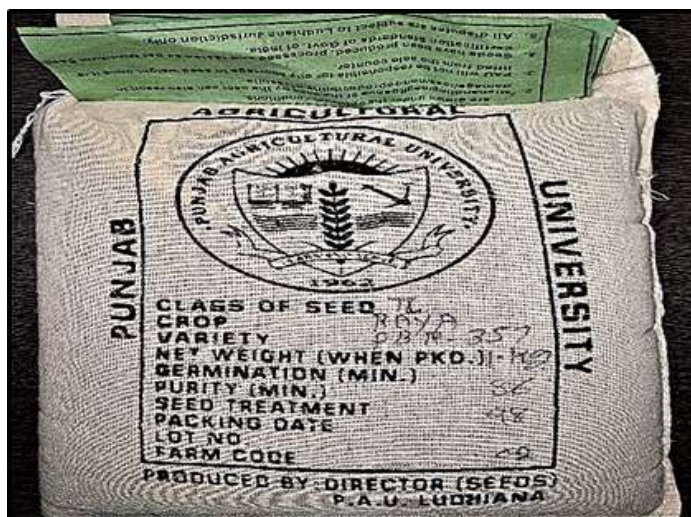


Fig. Mustard Genotype (PBR-357)

Table 1: Treatments Details

Treatments	Details of the treatments	Time of application
T0	Control	Before sowing
T1	Trichoderma (5g/10 kg of soil)	Before sowing
T2	Pb (90 ppm/kg of soil)	Before sowing
T3	Pb + Trichoderma (90ppm + 5g)	Before sowing

Table 2: Layout Details

S.No.	Particulars	Details
1.	Design	CRD
2.	Treatments	4
3.	Replication	3
4.	Total Pots	12
5.	Soil/pot	10 kg
6.	Genotype	PBR-357

**Observations to be recorded:**

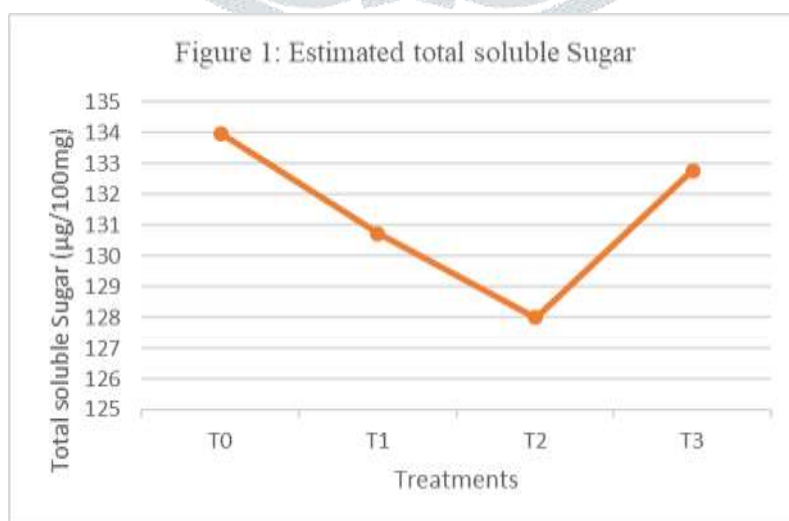
The various observations were recorded from the plant samples which were taken 15 days after sowing (DAS) by following the Standard procedure for estimation of biochemical's:

- i. **Total Soluble Sugar:** It is estimated by the method proposed by Sadasuvam and Manickam (1992).
- ii. **Total Soluble Protein:** It is estimated by the method proposed by Bradford (1976).
- iii. **Total Phenols:** For the estimation of total phenol the protocol of Mahadevan and Sridhar (1982) is followed.
- iv. **Chlorophyll Index:** SPAD meter is used for the measurement of chlorophyll.
- v. **Membrane injury index (MII) and Membrane stability index (MSI):** Sullivan, C. Y., 1971

### Results and Discussion

#### ➤ Total Soluble Sugar (TSS)

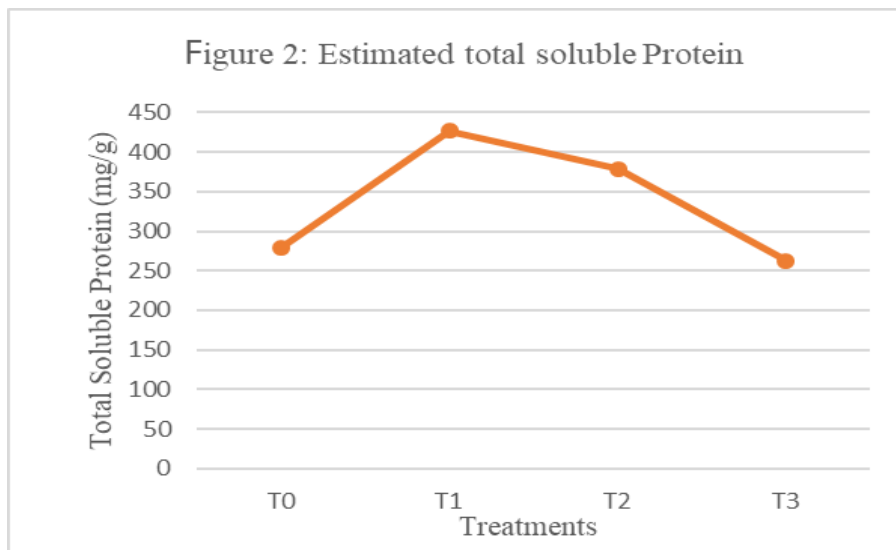
TSS is estimated with the help of Sadasuvam and Manickam (1992). Results have been shows that there is 4.46 % decrease in production of total soluble sugar in T2 as compare to T0. There is no significant difference between T0 and T3 for production of total soluble sugar. Hence, we can say that application of Trichoderma in lead affected soil maintain the production of total soluble sugar in mustard crop.



Where, T0: Control, T1: Trichoderma, T2: Lead & T3: Lead + Trichoderma

### ➤ Total Soluble Protein (TSP)

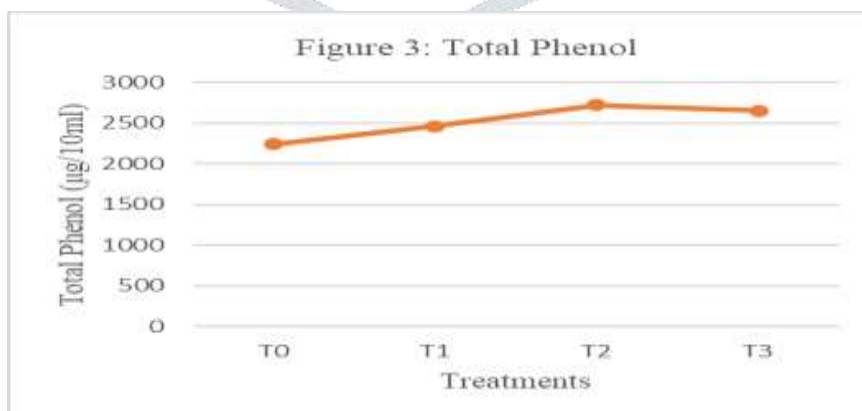
Bradford method is use for the estimation of total soluble protein. There is maximum amount of total soluble protein in the T1 as compare to the all treatments. According to the results we can say that application of Trichoderma can increase the total soluble protein in the mustard crop.



Where, T0: Control, T1: Trichoderma, T2: Lead & T3: Lead + Trichoderma

### ➤ Total phenols

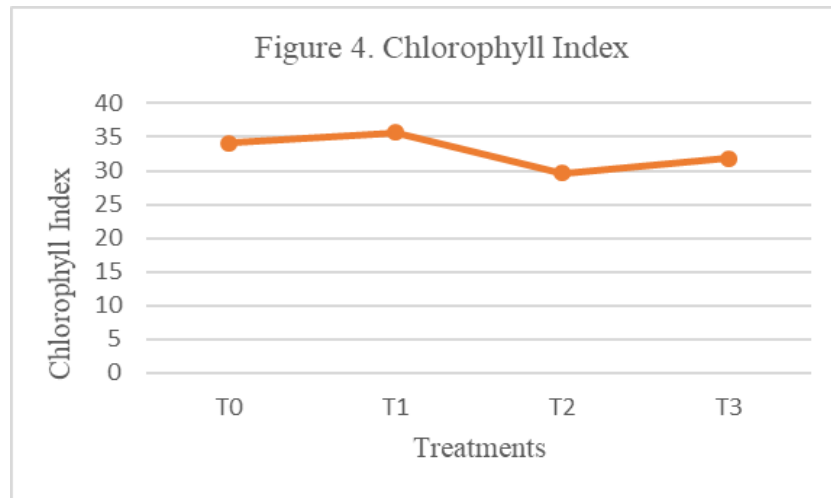
For the estimation of total phenol Mahadevan and Sridhar's (1982) method is widely followed. Phenolic compounds play very significant physiological role in the plant life cycle. Phenol production is increased when plants are under abiotic stress conditions. Results also shows increased amount of phenol production when mustard crop is provided with lead stress (T2). According to the results we can say that application of Trichoderma to lead affected soils can reduce the production of phenolic compounds to a certain level.



Where, T0: Control, T1: Trichoderma, T2: Lead & T3: Lead + Trichoderma

### ➤ Chlorophyll Index

SPAD (Soil Plant Analysis Development) meter is use for the measurement of chlorophyll index. There is 15.32% decrease in chlorophyll index in T2 as compare to T0. Maximum amount of chlorophyll index is found in Trichoderma treated pots (T1) as compare to the all treatments.



Where, T0: Control, T1: Trichoderma, T2: Lead & T3: Lead + Trichoderma

### ➤ Membrane injury index (MII) and Membrane stability index (MSI):

Results shows that membrane injury index (MII) is higher for the treatment where we apply heavy metal (T2) as compare to all treatments. T3 has the lowest MII among all the treatments i.e. 12.36%, 21.93% and 24.21% low as compare to T0, T1 and T2 respectively.

On the other hand, membrane stability index (MSI) is lowest for T2 among all the treatments. When we compare T3 with T2 then there is 15.98% increase in membrane stability index. Application of Trichoderma shows very positive results. Hence, according to the results we can say that if we apply Trichoderma in lead affected soil then it can help to reduce membrane injury and can maintain the membrane stability.



Where, T0: Control, T1: Trichoderma, T2: Lead & T3: Lead + Trichoderma

**Conclusion:**

Lead is the potential pollutants which readily accumulates in the soil, sediments and even in very small quantity in plants it highly disturbs the metabolic activities. However, lead has no useful effect on the plants but it is easily absorbed and uptake by the plants. Its uptake highly depends upon the soil pH, cation exchange capacity and particle size of the soil. Lead inhibits activities of many enzymes, reduce mineral uptake, disturb water balance and damage membrane structure. Lead toxicity causes synthesis of ATP synthesis, lipid peroxidation and DNA damage due to increase in ROS production. As a result, affects the growth and development of the crops. Hence, to reduce the impact of lead toxicity in soil application of Trichoderma plays very significant role which helps to improve physiological condition and performance of the plants.

**Acknowledgments**

Yaman and Prasann Kumar gratefully acknowledge the support provided by Lovely Professional University.

**Author Contributions**

The study was designed by Prasann Kumar & Yaman and the biochemical protocolizations were established, the experiment was carried out and the data analyzed and interpreted were collected. The paper has been written by Prasann Kumar and Yaman.

**Conflict of Interest Statement**

The authors state that they have no interest in conflicts.

**References**

- Kumar, P. 2013o. "Cultivation of traditional crops: an overlooked answer. Agriculture Update, vol.8 (3), pp.504-508.
- Kumar, P., Dwivedi, P. 2015p. "Role of polyamines for mitigation of cadmium toxicity in sorghum crop" Journal of Scientific Research, B.H.U., 59, pp.121-148.
- Gogia, N., Kumar, P., Singh, J., Rani, A. Sirohi, Kumar, P. 2014q. "Cloning and molecular characterization of an active gene from garlic (*Allium sativum* L.)" International Journal of Agriculture, Environment and Biotechnology, vol.7 (1), pp.1-10.
- Kumar, P., 2014r. "Studies on cadmium, lead, chromium, and nickel scavenging capacity by in-vivo grown *Musa paradisiacal*. using atomic absorption spectroscopy" Journal of Functional and Environmental Botany, vol.4(1), pp.22-25.
- Kumar, P., Dwivedi, P., Singh, P., 2012s. "Role of polyamine in combating heavy metal stress in *stevia rebaudiana* Bertoni plants under in vitro condition" International Journal of Agriculture, Environment and Biotechnology, 5(3) pp.185-187.
- Mishra, P.K., Maurya, B.R., Kumar, P. 2012t. "Studies on the biochemical composition of *Parthenium hysterophorus* L. in different season" Journal of Functional and Environmental Botany, 2(2): 1-6.



- Kumar, P., Mandal, B., Dwivedi, P. 2011u. "Heavy metal scavenging capacity of *Mentha spicata* and *Allium cepa*" Medicinal Plant-International Journal of Phytomedicines and Related Industries vol. 3(4) pp. 315-318.
- Kumar, P., Mandal, B., Dwivedi, P. 2011v. "Screening plant species for their capacity of scavenging heavy metals from soils and sludges. Journal of Applied Horticulture, 13 (2), 144-146.
- Kumar, P., Pathak, S. 2016w. "Heavy metal contagion in seed: its delivery, distribution, and uptake" Journal of the Kalash Sciences, An International Journal, 4(2), 65-66.
- Pathak, S., Kumar, P., Mishra, P.K., Kumar, M. 2016x. "Plant-based remediation of arsenic-contaminated soil with special reference to sorghum- a sustainable approach for a cure". Journal of the Kalash Sciences, An International Journal, 4(2): 61-65.
- Kumar, P., Harsavardhn, M. et al., 2018y. "Effect of Chlorophyll a/b ratio in Cadmium Contaminated Maize Leaves Treated with Putrescine and mycorrhiza" Annals of Biology 34(3)-281-283.
- Kumar, P., Yumnam, J. et al., 2018z. "Cadmium Induced Changes in Germination of Maize Seed Treated with Mycorrhiza" Annals of Agri-Bio Research, 23(2); 169-170.
- Kumar, P., Pandey, A.K., et al., 2018aa. "Phytoextraction of Lead, Chromium, Cadmium, and Nickel by *Tagetes* Plant Grown at Hazardous Waste site" Annals of Biology, 34(3): 287-289.
- Kumar, P., Kumar, S. et al., 2018bb. "Evaluation of Plant Height and Leaf Length of Sorghum Grown Under Different Sources of Nutrition" Annals of Biology, 34(3): 284-286.
- Kumar, P., Krishna, V., et al., 2018cc. "Assessment of Scavenging Competence for Cadmium, Lead, Chromium and Nickel Metals by in vivo Grown *Zea mays* L. using Atomic Absorption Spectrophotometer, Annals of Ari-Bio Research, 23(2): 166-168.
- Singh, S., Kumar, V., Datta, S., Wani, A.B., Dhanjal, D.S., Romero, R. and Singh, J. (2020). Glyphosate uptake, translocation, resistance emergence in crops, analytical monitoring, toxicity, and degradation: a review. Environmental Chemistry
- Kumar, P. Purnima et al., 2018e. "Impact of Polyamines and Mycorrhiza on Chlorophyll Substance of Maize Grown under Cadmium Toxicity" International Journal of Current Microbiology and Applied Sciences, vol. 7(10), pp. 1635-1639.
- Kumar, P. Pathak, S. 2019f. "Responsiveness index of sorghum (*Sorghum bicolor* (L.) Moench) grown under cadmium contaminated soil treated with putrescine and mycorrhiza" Bangladesh J. Bot. vol.48 (1).
- Kumar, P. Siddique, A. et al., 2019g. "Role of Polyamines and Endo-mycorrhiza on Leaf Morphology of Sorghum Grown under Cadmium Toxicity" Biological Forum – An International Journal. vol.11 (1) pp. 01-05.
- Siddique, A. Kumar, P. 2018h. "Physiological and Biochemical basis of Pre-sowing soaking seed treatments-An overview" Plant Archive, 18(2), pp. 1933-1937.
- Siddique, A., Kandpal, G., Kumar P. 2018i. "Proline accumulation and its defensive role under Diverse Stress condition in Plants: An Overview" Journal of Pure and Applied Microbiology, vol.12 (3) pp.1655-1659.
- Pathak, S., Kumar, P., P.K Mishra, M. Kumar, 2017j. "Mycorrhiza assisted approach for bioremediation with special reference to biosorption", Pollution Research, Vol. 36(2).
- Prakash, A., P. Kumar, 2017k. "Evaluation of heavy metal scavenging competence by in-vivo grown *Ricinus communis* L. using atomic absorption spectrophotometer" Pollution Research, vol.37(2), pp.148-151.
- Kumar, P., Mandal, B., 2014L Dwivedi, "Combating heavy metals toxicity from hazardous waste sites by harnessing scavenging activity of some vegetable plants" vegetos, vol.26(2), pp. 416-425.
- Kumar, P., Mandal, B., Dwivedi P., 2014m. "Phytoremediation for defending heavy metal stress in weed flora" International Journal of Agriculture, Environment & Biotechnology, 6(4), pp. 587-595.

- Kumar, P., Kumar, P.K., Singh, S. 2014n. "Heavy metal analysis in the root, shoot and a leaf of psidium guajava l. by using atomic absorption spectrophotometer" *Pollution Research*, .33 (4) pp.135-138.
- Kumar P. 2018iii. Herbicide selectivity and Resistance with special reference Agriculture crops. In: *Stress Tolerance and Plant Productivity* [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-86841-56-8][p. 58-70]
- Kumar P.2018iv. Role of Rhizobium in Enhancing the yield and Yield attributes of crops with special references to chickpea [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-86841-56-8][p. 86-104].
- Kumar P. 2018v. Arsenic Induced toxicity in Plants with Special Reference to their Oxidative Damage. [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-86841-56-8][p. 86-104].
- Sharma, P., Dubey, R.S., Lead toxicity in plants. Department of Biochemistry, Faculty of Science, Banaras Hindu University, Varanasi-221005, India
- Kumar P. 2018vi. Metals and Micronutrients-Food Safety Issue. [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-86841-56-8][p. 126-147].
- Kumar P. 2018vii. Role of Farming Practices on Environment. [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-86841-56-8][p. 148-158].
- Kumar P. 2018viii. Irrigation with Special Reference to Crop Production. [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-86841-56-8][p. 159-177].
- Kumar P., Pathak S. 2018ix. Cultivation of Fodder and Forage Crops: with Special Reference to Berseem, Oat, Lucern, and Maize. In: *Cultivation Techniques in Modern Agriculture* [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-88854-03-0][p. 6]
- Kumar P., Pathak S. 2018x. Sugarcane Cultivation: A Significance Way for Sustainability. In: *Cultivation Techniques in Modern Agriculture* [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-88854-03-0][p. 31]
- Kumar P., Pathak S. 2018xi. Potato and Sugar beet Cultivation: A Sugar Crop with Poor Man's Friends. In: *Cultivation Techniques in Modern Agriculture* [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-88854-03-0][p. 55]
- Kumar P., Pathak S. 2018xii. Tobacco Cultivation: A crop of Economic Value. In: *Cultivation Techniques in Modern Agriculture* [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-88854-03-0][p. 76]
- Kumar P., Pathak S. 2018xiii. Use of Robotics for Agricultural Innovation. In: *Plant Physiology: Stress, Disease, and Management* [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-88854-03-0][p. 130]
- Kumar P., Pathak S. 2018xiv. Absorption of Water by Plants with Special Reference to the physiology of Cells. In: *Plant Physiology: Stress, Disease, and Management* [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-88854-00-9][p. 9]
- Kumar P., Pathak S. 2018xv. Seed Dormancy with Special Reference to Crop Growth Physiology – Functional Relationship. In: *Plant Physiology: Stress, Disease, and Management* [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-88854-00-9][p. 27]
- Kumar P., Pathak S. 2018xvi. Role of Polyamines and mycorrhiza for the mitigation of salinity stress in Sorghum. In: *Plant Physiology: Stress, Disease, and Management* [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-88854-00-9][p. 51]

- Kumar P., Pathak S. 2018xvii. Crop Production: Concepts and Practices. In: Crop Plants: Issues and Management [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-88854-02-3][p. 6]
- Kumar P., Pathak S. 2018xviii. Maize: the Queen of Cereals. In: Crop Plants: Issues and Management [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-88854-02-3][p. 29]
- Singh, S., Kumar, V., Singla, S., Sharma, M., Singh, D.P., Prasad, R., Thakur, V.K. and Singh, J. (2020). Kinetic Study of the Biodegradation of Acephate by Indigenous Soil Bacterial Isolates in the Presence of Humic Acid and Metal Ions. *Biomolecules*, 10, 433
- Sood, M., Sharma, S.S., Singh, J, Prasad, R., and Kapoor, D. (2020). Stress Ameliorative Effects of Indole Acetic Acid on *Hordeum vulgare* L. Seedlings Subjected to Zinc Toxicity. *Phyton – International Journal of Experimental Botany*, 89(1), 71-86
- Bhadrecha, P., Bala, M., Khasa, Y.P., Arshi, A., Singh, J. and Kumar, M. (2020). *Hippophae rhamnoides* L. rhizobacteria exhibit diversified cellulase and pectinase activities. *Physiology and Molecular Biology of Plants*.
- Singh, S., Kumar, V., Datta, S., Dhanjal, D.S., Sharma, K., Samuel, J. and Singh, J. (2020). Current advancement and future prospect of biosorbents for bioremediation. *Science of the Total Environment*, 709, 135895.
- Sharma, R., Jasrotia, K., Singh, N., Ghosh, P., Sharma, N.R., Singh, J., Kanwar, R. and Kumar, A. (2020). A Comprehensive Review on Hydrothermal Carbonization of Biomass and its Applications. *Chemistry Africa*, 3(1):1-19
- Singh, S., Kumar, V., Kapoor, D., Kumar, S., Singh, S., Dhanjal, D.S., Datta, S., Samuel, J., Dey, P., Wang, S., Prasad, R. and Singh, J. (2020). Revealing on hydrogen sulfide and nitric oxide signals co-ordination for plant growth under stress conditions. *Physiologia Plantarum*, 168(2): 301-317.
- Bhati, S., Kumar, V., Singh, S. and Singh, J. (2020). Synthesis, Characterization, Antimicrobial, Anti-tubercular, Antioxidant Activities and Docking Simulations of Derivatives of 2-(pyridine-3-yl)-1H-benzo[d]imidazole and 1,3,4-Oxadiazole Analogy. *Letters in Drug Design & Discovery*.
- Singh, S., Kumar, V. and Singh, J. (2019). The effects of Fe(II), Cu(II) and Humic Acid on biodegradation of atrazine. *Journal of Environmental Chemical Engineering*, 8: 103539.
- Sharma, M., Singh, J., Chinnappan, P., and Kumar, A. (2019). A comprehensive review of renewable energy production from biomass-derived bio-oil. *Biotechnologia* 100(2):179-194.
- Kumar P. 2018i. Role of Paclobutrazol for the mitigation of water logged stress in *Cicer arietinum* L. In: Stress Tolerance and Plant Productivity [Ed. Kumar P and Bharti P.K, Discovery Publication, New Delhi][ISBN: 978-93-86841-56-8][p. 1-14]