

Quantitative illustration of Aluminium-toxicity on *Oryza sativa* L. treated with Silk Fibroin Nanoparticle and KNO_3^+ Protein Nanoparticle in the level of Anthocyanin and Carotenoids

Prasann Kumar^{1, 2}, Purnima¹, Tapan Kumar²

¹Department of Agronomy, School of Agriculture

Lovely Professional University, Jalandhar, Punjab, 144411, India

²Divisions of Research and Development

Lovely Professional University, Jalandhar, Punjab, 144411, India

Abstract

To verify the responses of silk fibroin nanoparticle and KNO_3^+ Protein Nanoparticle on *Oryza sativa* L. to Al toxicity in relation to Anthocyanin and Carotenoids Contents, the present investigation was carried out. A uniform decrease in pigmentation was marked as the primary signs of aluminium injury. At the same time the application of these nanoparticles leads to reversal of the primary results. It was noticed that the Anthocyanin content was enhanced by 44.04% in T5 in the leaf sample. The treatments T4, when compared with T1, showed that Fibroin NPs reduced the total carotenoids content by only 3.80% whereas KNO_3 NPS in T5 reduced the same by 28.37% when applied along with Aluminium stress.

Keywords: Agriculture, Biotic, Crops, Density, Economy, Food, Silk, Nanoparticles

INTRODUCTION

Aluminium (Al) is the third largest metallic element in the Earth's crust following oxygen and silicon. A large quantity of aluminosilicate soil minerals is incorporated and very small amounts are found in soluble form, able to influence biotechnology systems [1]. The bioavailability is restricted mainly to the acid environment and consequently to the toxicity. The most significant limitations of agriculture production are acidic soils (with a pH of 5.5 or lower). Acidic soils have an adverse effect on the production of staple food crops, in particular grain crops [2]. Further acidification of agricultural soils is the result of certain agricultural practices such as the removal of products, the liquidation of nitrogen below the root zone, improper use in nitrogen fertilizers and organic build-up.

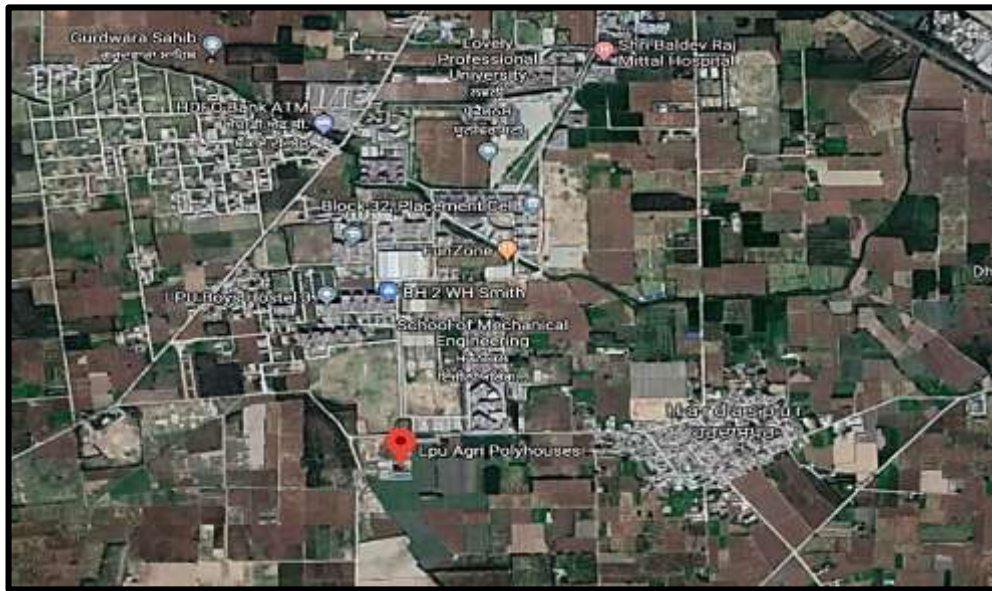
Ample of detoxification methodologies have been adopted by the plants in order to fight back with the metal toxicity and their accumulation such as a cellular antioxidant system which constitutes Superoxide dismutase (SOD), Ascorbate peroxidase (APX), Glutathione reductase (GR) and Catalase (CAT). To cope with Aluminium toxicity plants have developed certain adaptive mechanisms to survive in even toxic conditions which are as follows. (1) Exclusion or Resistance to Aluminium to exclude the entry of metal into the cell by secreting certain Organic acids or phenolic compounds which further bind Al^{+3} and prevents its uptake into the cytosol. (2)

Exhibiting of Internal tolerance by compartmentalizing Al in vacuoles, the formation of Al complexes with organic substances in the cytosol and improved scavenging via ROS, to avoid toxicity [1, 2, 3 and 5]. They help in the detoxification of oxyradical which further inhibits the oxidation of biomolecules [1, 2, 3, 4 and 5]. In India, it has been mainly grown in the Gangetic plains and coastal areas [6, 7, 8, 9 and 10]. Amongst the wild species, seven are tetraploid ($2n=48$) and rest are all diploids ($2n=24$). It is a semi-aquatic crop which is grown in standing water with very high water requirement [11, 12, 13, 14 and 15]. The word rice is derived from the French word *ris* or the Italian word *riso* which came out to be a modification of the Sanskrit word *vrihi* [16, 17, 18 19 and 20]. World paddy production was 759.6 million tonnes in 2017 where China leads India by producing 210.3 million tonnes as against 166.5 million tonnes by the latter (www.fao.org). Paddy yielded 3.85 tonnes per hectare and was harvested over 42.9 million hectares (ricestat.irri.org). Among the Indian states West Bengal bags first position(2015-2016) in rice production (15.10%) followed by Uttar Pradesh (11.99 %), Punjab (11.33%), Tamil Nadu (7.65%), Andhra Pradesh (7.18%), Bihar (6.22%), Chattisgarh (5.84%), Orissa (5.64), Assam (4.93), Haryana (3.98) (www.apeda.com). Rice protein content differs from 6% to 14% based upon rice variety and culture environment [21, 22, 23, 24, 25 and 26]. It was thought that cultivated rice was originated in South India by De Candolle (1886) and Watt (1892). Vavilov, on the other hand, insisted on India and Burma to be the center of origin of cultivated rice [27, 28 29 and 30]. Aluminum is the most abundant element in the earth's crust after Oxygen and silicon (atomic number: 13; atomic mass: 26.982; melting point: 660.3°C) [31, 32, 33, 34 and 35]. It was in the 19th century that a Danish physicist Christian Oersted first discovered Al by electrolytic reduction. Aluminum exposure on humans is said to have many deleterious effects. (1) Colon Inflammation and Inflammatory Bowel Disease (IBD) [36, 37, 38, 39 and 40].

METHODOLOGY

The experiment was conducted at Natural Ventilated Poly house, School of Agriculture, Lovely Professional University (LPU), Phagwara, Punjab. The farm situated at attitude 232 meters above sea level, latitude 31.244604 N and longitude 75.701022 E as per Google map (Figure 1).

Figure 1. Google map of the experimental site



(Source: Google Earth, 2019)

CLIMATE CONDITION

Punjab Trans-Gangetic Plains Region Phagwara falls in the Central Plain Zone of Punjab. Generally, in June the hottest month of the year with a maximum temperature of 45°C and a minimum of 27°C, the annual average temperature is 24°C. In January during winters the temperature falls down up to 4 to 6°C. Monsoon starts in the last of June / early of July having a normal annual rainfall of 686mm.

TREATMENTS DETAILS

The pot experiment was conducted on the farm of the School of Agriculture, Lovely Professional University, Jalandhar Punjab with one genotype Pusa Basmati 1121 of Rice. Genotype took from Punjab Agriculture University, Punjab. The pot size for the experiment will be diameter: 30 cm and height 25 cm. Heavy metal stress was created by foliar application of aluminium (100ppm) at the flowering stage. KNO₃ protein nanoparticle (1%) and Fibroin Nanoparticle (1%) were applied through a foliar application at the flowering stage. The various measurements were taken at 90 DAT (Table 1 and 2).

Table 1: Treatments Detail

Treatments	Details of the treatments
T-0	Control
T-1	Al (100ppm)
T-2	Fibroin nanoparticle (1%)
T-3	KNO ₃ protein nanoparticle (1%)
T-4	Al (100ppm) + Fibroin nanoparticle (1%)
T-5	Al (100ppm) + KNO ₃ protein nanoparticle (1%)
T-6	Al (100ppm) + KNO ₃ protein nanoparticle (1%) + Fibroin Nanoparticle (1%)

Table 2: Layout Details

S. No.	Particulars	Details
1.	Layout	CRD
2.	Treatment	7
3.	Replications	3
4.	Total Number of pots	7*3=21
5.	Soil per pot	7 kg
6.	Genotype	Pusa Basmati 1121

OBSERVATION RECORDED

The observations were recorded at 90 days after treatment (DAT). The recorded observations of biochemical and the standard procedure adopted during the course of study are given below:

BIOCHEMICAL PARAMETERS**Anthocyanin content**

The anthocyanin content in the treated sample was measured by adopting the protocol given by Swain and Hills in the year 1959.

Total Carotenoids content

The total carotenoids content in the treated sample was measured by adopting the protocol given by Jensen A in the year 1978.

RESULTS and DISCUSSION**Anthocyanin content (mg g⁻¹ fresh weight)**

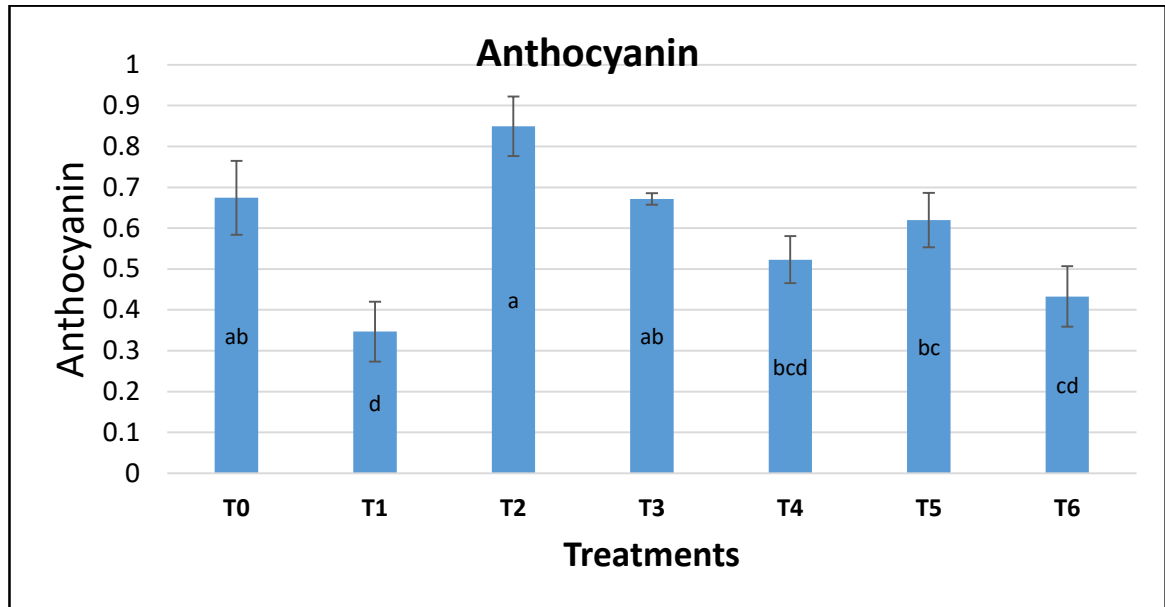
In the rice variety Pusa Basmati 1121, under aluminum toxicity stress, Silk-Fibroin Nanoparticle (NP) and potassium Nitrate (KNO₃) effect and their combination were studied. Data were recorded at 90 days after transplanting (DAT) (Table 3 and Fig. 2). It is evident that, compared to control (T₀) at 90 DAT interval, the average amount of anthocyanine was significantly reduced when exposed to high metal stress (T₁) by 48.58%. Exogenous application of KNO₃ particles on the leaves (T₃) enhanced the average anthocyanin by 48.37% as compared to (T₁) at 90 DAT. In comparison to T₁, the exogenous application of Fibroin Nanoparticle (T₂) showed enhancement in the average anthocyanin by 59.18%, on proposed DAT. The treatments T₄, when compared with T₁, showed that Fibroin NPs enhanced the average anthocyanin content by only 33.70% whereas KNO₃ NPS in T₅ enhanced the same by 44.0% when applied along with Aluminium stress. The average anthocyanin was significantly enhanced by about 19.90% with respect to T₁ when treated with Fibroin NPs upon Aluminium stress whereas only sole Fibroin NPs were applied (T₆). KNO₃ Nanoparticles when applied upon Aluminium stress (T₆). Many scientist discussed how Al stress on plants immediately suppresses the respiration process and produces

Reactive Oxygen Species (ROS) [41, 42, 43, 44 and 45]. Mitochondrial Alternative Oxidase (AOX) was found to suppress Al stress by inhibiting ROS accumulation thereby reducing mitochondrial oxidative stress and enhancement in the growth capability of tobacco cells [46, 47, 48, 49, 50 and 51]. Investigators discussed the Al hyperaccumulator plants especially the Symplocaceae family which includes many tropical and evergreen plant species. These species (seedlings and saplings) were grown in a hydroponic system with and without Al. It was seen that these seedlings were able to absorb the Al from the solution if provided with so and they showed a comparatively less mortality rate as compared to control treatment. Al as one of the major limiting factor affecting the plant productivity especially in the acidic soils and is said to pose around 25-80% of yield losses depending upon the plant's cultivar [22, 29, 45 and 53]. The wild species and flowering species of *Camellia japonica* L. Wild species possess red colour whereas the flowering species possess purple colour which may revert back to red colour of the wild species the next year. This purple colouration in the plants was said to be the outcome of Al chelation with the anthocyanin [38, 45, 49 and 53].

Table 3. Anthocyanin of rice during Kharif

Treatments	Anthocyanin content in rice leaves at 90 DAT
T0	0.6743 ^{ab} ± 0.090523
T1	0.34667 ^d ± 0.073106
T2	0.84937 ^a ± 0.072921
T3	0.6715 ^{ab} ± 0.013895
T4	0.52292 ^{bcd} ± 0.057718
T5	0.61958 ^{bc} ± 0.066616
T6	0.43283 ^{cd} ± 0.074351

where, Data are in the form mean ± SEM. Significance at $P \leq 0.05$ using SPSS ver. 22. T0= Control; T1: Aluminium chloride (100ppm); T2: Fibroin nanoparticle (1%); T3: KNO₃ nanoparticle (1%); T4: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%); T5: Aluminium chloride (100ppm) + KNO₃ Nanoparticle (1%); T6: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%) + KNO₃ Nanoparticle (1%).

Figure 2. Anthocyanin of rice during *Kharif*

where, Data are in the form mean \pm SEM. Significance at $P\leq 0.05$ using SPSS ver. 22. T0= Control; T1: Aluminium chloride (100ppm); T2: Fibroin nanoparticle (1%); T3: KNO₃ nanoparticle (1%); T4: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%); T5: Aluminium chloride (100ppm) + KNO₃ Nanoparticle (1%); T6: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%) + KNO₃ Nanoparticle (1%).

Total carotenoids content (mg g⁻¹ fresh weight)

The effects of Silk Fibroin Nanoparticle (np) and Potassium Nitrate (KNO₃) and the combination of these effects was studied on the total level of carotenoids of Pusa Basmati 1121 under Aluminium stress. It is obvious that, when exposed to heavy metal stress (T1), the mean total carotenoid content was decreased significantly by 22.77% in comparison with Control (T0) at an interval of 90 DAT. Exogenous application of KNO₃ particles on the leaves (T3) reduced the total carotenoids content by 12.48% as compared to (T1) at 90 DAT. In comparison to T1, the exogenous application of Fibroin Nanoparticle (T2) showed enhancement in the total carotenoids content by 19.6%, on proposed DAT. The treatments T4, when compared with T1, showed that Fibroin NPs reduced the total carotenoids content by only 3.80% whereas KNO₃ NPS in T5 reduced the same by 28.37% when applied along with Aluminium stress. The total carotenoids content was significantly reduced by about 7.86% with respect to T1 when treated with Fibroin NPs upon Aluminium stress whereas only sole Fibroin NPs were applied (T6). KNO₃ Nanoparticles when applied upon Aluminium stress (T6). The recent current world investigation concluded that various soil enzymes, as well as microbial fauna, get influenced by the activity of nano particles [24, 28, 34, 43 and 51]. Four different metal oxide engineered nano particles (Zinc oxide nanoparticles, Titanium dioxide nanoparticles, cerium dioxide nanoparticles, and magnetite nanoparticles) had experimented as amendments, three replicates each among two different soils i.e, black soil and alkaline soils in China. MO-engineered nanoparticles had been given under four concentration (0.5, 1.0 and 2.0 mg g⁻¹) for 15 and 30 days). Soil enzymatic activities (invertase, catalase, urease, and phosphatase) under Zinc oxide nano particles visualized a greater significant effect in comparison to others and even that black soil was less vulnerable to nano particles as that in saline soils [34, 37

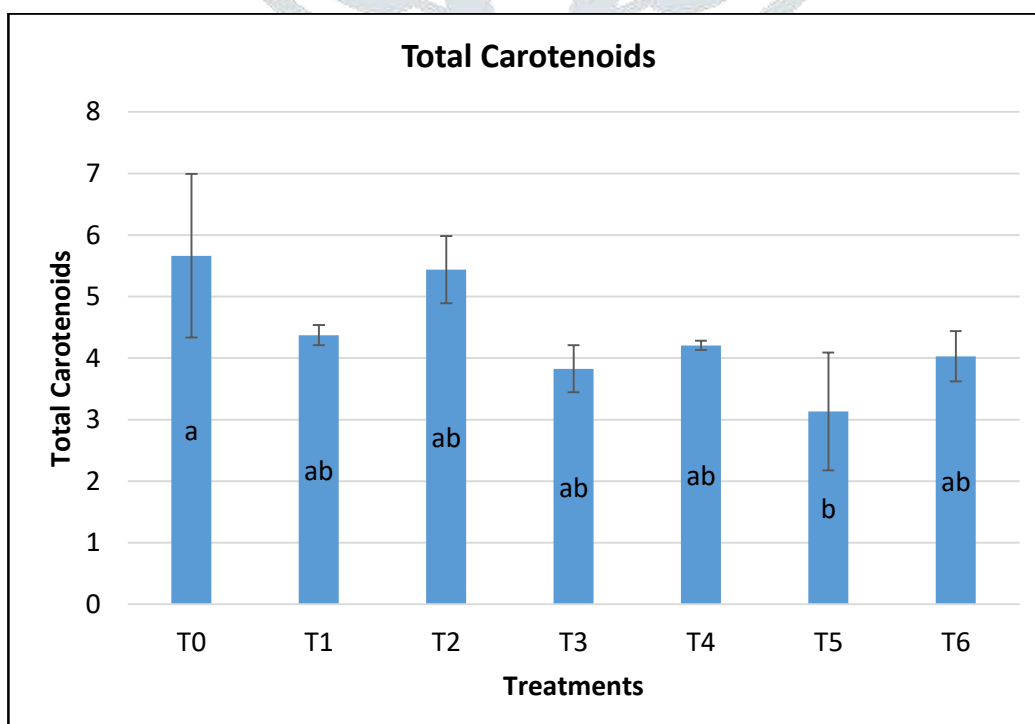
38, 39 and 40]. Saline alkaline soils testing results showed a drop in its microbial community as a consequence of the treatments [21, 24, 26, 27 and 29]. Investigators discussed on the threats that can be posed upon by the surging use of nano particles or nano-based products upon the various living and nonliving forms on earth. Mass research has been already performed on its construction and application but its contamination has still not been evaluated much [34, 37, 39 and 45].

Table 4. Total Carotenoids in rice leaf during *Kharif*

Treatments	Total carotenoids content in rice leaf at 90 DAT
T0	5.66287 ^a ± 1.330365
T1	4.37295 ^{ab} ± 0.162803
T2	5.43957 ^{ab} ± 0.546144
T3	3.82709 ^{ab} ± 0.382674
T4	4.20664 ^{ab} ± 0.076069
T5	3.13199 ^b ± 0.958194
T6	4.02899 ^{ab} ± 0.408348

where, Data are in the form mean ± SEM. Significance at $P \leq 0.05$ using SPSS ver. 22. T0= Control; T1: Aluminium chloride (100ppm); T2: Fibroin nanoparticle (1%); T3: KNO₃ nanoparticle (1%); T4: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%); T5: Aluminium chloride (100ppm) + KNO₃ Nanoparticle (1%); T6: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%) + KNO₃ Nanoparticle (1%).

Figure 3. Total Carotenoids in rice leaf during *Kharif*



where, Data are in the form mean \pm SEM. Significance at $P\leq 0.05$ using SPSS ver. 22. T0= Control; T1: Aluminium chloride (100ppm); T2: Fibroin nanoparticle (1%); T3: KNO₃ nanoparticle (1%); T4: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%); T5: Aluminium chloride (100ppm) + KNO₃ Nanoparticle (1%); T6: Aluminium chloride (100ppm) + Fibroin nanoparticle (1%) + KNO₃ Nanoparticle (1%).

Conclusion

Based on the above study it is clear that, the influence of metal and metal oxide nanoparticles on various crops at several diagnostic levels. Where growth and yield of crops were seen to be highly constrained, the concentration of nano particle was seen to rise in different plant parts such as grains, which could lead an easy entry to the food and humans. The provided treated nano particles diagnose as the suitable mitigation for the remediation of Al toxicity in the *Oryza sativa* L. with special references to anthocyanin and carotenoids contents.

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Author Contributions

The study was designed by P.K. and Purnima, the biochemical protocolizations were established, experiments were carried out and the data analyzed and interpreted were collected. The paper has been written by P.K and T.K.

Conflict of Interest Statement

The authors state that they have no interest conflict.

References

1. P. Kumar, P. Dwivedi, "Cadmium-induced alteration in leaf length, leaf width and their ratio of glomus treated sorghum seed" *Journal of Pharmacognosy and Phytochemistry*, vol.7 (6), pp. 138-141, 2018.
2. P. Kumar, S. Kumar et al. "Glomus and putrescine based mitigation of cadmium-induced toxicity in maize" *Journal of Pharmacognosy and Phytochemistry*. vol.7 (5), pp.2384—2386, 2018.
3. P. Kumar, L. Misao, et al., "Polyamines and Mycorrhiza based mitigation of cadmium-induced toxicity for plant height and leaf number in maize" *International Journal of Chemical Studies*, vol.6 (5), pp.2491-2494, 2018.
4. P. Kumar, P. Dwivedi, "Putrescine and Glomus Mycorrhiza moderate cadmium actuated stress reaction in *Zea mays* L. by means of extraordinary reference to sugar and protein" *Vegetos*. vol.31 (3), pp.74-77, 2018.
5. P. Kumar, Purnima et al., "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, 2018.
6. P. Kumar, S. Pathak, "Responsiveness index of sorghum (*Sorghum bicolor* (L.) Moench) grown under cadmium contaminated soil treated with putrescine and mycorrhiza" *Bangladesh J. Bot.* vol.48 (1), 2019.

7. P. Kumar, A. Siddique, et al., "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, 2019.
8. A. Siddique, P. Kumar, "Physiological and Biochemical basis of Pre-sowing soaking seed treatments-An overview" Plant Archive, vol.18(2), pp. 1933-1937, 2018.
9. A. Siddique, G. Kandpal, P. Kumar "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, 2018.
10. S. Pathak, P. Kumar, P.K Mishra, M. Kumar, "Mycorrhiza assisted approach for bioremediation with special reference to biosorption", Pollution Research, vol. 36(2), 2017
11. A. Prakash, P. Kumar, "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, 2017.
12. P. Kumar, B. Mandal, P. Dwivedi, "Combating heavy metals toxicity from hazardous waste sites by harnessing scavenging activity of some vegetable plants" vegetos, vol.26(2), pp. 416-425, 2014.
13. P. Kumar, B. Mandal, P. Dwivedi, "Phytoremediation for defending heavy metal stress in weed flora" International Journal of Agriculture, Environment & Biotechnology, vol.6(4), pp. 587-595, 2014]
14. P. Kumar, P.K. Kumar, S. Singh, "Heavy metal analysis in the root, shoot and leaf of psidium guajava l. by using atomic absorption spectrophotometer" Pollution Research, vol.33 (4) pp.135-138, 2014.
15. P. Kumar, "Cultivation of traditional crops: an overlooked answer. Agriculture Update, vol.8 (3), pp.504-508, 2013.
16. P. Kumar, P. Dwivedi, "Role of polyamines for mitigation of cadmium toxicity in sorghum crop" Journal of Scientific Research, B.H.U., vol.59, pp.121-148, 2015.
17. N. Gogia, P. Kumar, J. Singh, A. Rani, A. Sirohi, P. Kumar, "Cloning and molecular characterization of lactine gene from garlic (*Allium sativum* L.)" International Journal of Agriculture, Environment and Biotechnology, vol.7 (1), pp.1-10, 2014.
18. P. Kumar, "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, 2014.
19. P. Kumar, P. Dwivedi, P. Singh, "Role of polyamine in combating heavy metal stress in stevia Rebaudiana Bertoni plants under in vitro condition" International Journal of Agriculture, Environment and Biotechnology, vol.5(3) pp.185-187, 2012.
20. P.K. Mishra, B.R. Maurya, P. Kumar, "Studies on the biochemical composition of *Parthenium hysterophorus* L. in different season" Journal of Functional and Environmental Botany, vol. 2(2) pp.1-6, 2012
21. P. Kumar, B. Mandal, P. Dwivedi, "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, 2011.

22. P. Kumar, B. Mandal, P. Dwivedi, "Screening plant species for their capacity of scavenging heavy metals from soils and sludges. Journal of Applied Horticulture vol.13 (2) pp.144-146, 2011.
23. P. Kumar, S. Pathak, "Heavy metal contagion in seed: its delivery, distribution, and uptake" Journal of the Kalash Sciences, An International Journal, vol. 4(2), pp. 65-66, 2016.
24. S. Pathak, P. Kumar, P.K. Mishra, M. Kumar, "Plant-based remediation of arsenic-contaminated soil with special reference to sorghum- a sustainable approach for the cure". Journal of the Kalash Sciences, An International Journal, 4(2): 61-65, 2016.
25. P. Kumar, P. Dwivedi P, "Role of polyamines for mitigation of cadmium toxicity in sorghum crop" Journal of Scientific Research, B.H.U., vol. 59, pp.121-148, 2015.
26. P. Kumar, "Signal transduction in the plant with respect to heavy metal toxicity: An overview" CRC Press, Taylor & Francis Group, Pp. 394, 2018.
27. P. Kumar, P. Dwivedi, "Phytoremediation of cadmium through Sorghum" Daya Publishing House. Pp. 311-342, 2014.
28. P. Kumar, A.K. Pandey, V. Krishna, "Phytoextraction of lead, chromium, cadmium, and nickel by tagetes plant grown at the hazardous waste site" Annals of biology, vol.34(3), pp. 287-289, 2018.
29. P. Kumar, S. R. Dey, Komal, et al., "Effect of Polyamines and endo-mycorrhiza on chlorophyll a, b ratio and total carotenoids in leaves of Sorghum grown under cadmium toxicity" International Journal of Chemical Studies. vol.7 (1), pp. 2402-2406, 2019.
30. P. Kumar, P. Dwivedi, "Ameliorative Effects of Polyamines for Combating Heavy Metal Toxicity in Plants Growing in Contaminated Sites with Special Reference to Cadmium" CRC Press, Taylor & Francis Group, UK. Pp. 404, 2018.
31. P. Kumar, A. Siddique, Thakur Vishal, Singh Manpreet, "Effect of putrescine and glomus on total reducing sugar in cadmium treated sorghum crop" Journal of Pharmacognosy and Phytochemistry, vol.8(2), pp.313-316, 2019.
32. P. Kumar, A. Siddique, Thongbam Satyajyoti, Chopra Prafful, Kumar Sunil, "Cadmium-induced changes in total starch, total amylose and amylopectin content in putrescine and mycorrhiza treated sorghum crop" Nature, Environment and Pollution Technology, vol.18(2), 2019.
33. P. Kumar, S. R. Dey, "Carbon Pools: Key for Global Climate Models" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
34. P. Kumar, S. R. Dey, "Three Dimensional Approach for the Mitigation of Cadmium and Lead Toxicity in Legumes" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
35. P. Kumar, S. R. Dey, "Apple of Tropics: Its Production and Quality Assurance with Special Reference to Nutrition" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
36. P. Kumar, S. R. Dey, "Wheat Cultivation: Strategic Cereal Crops for Majority" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
37. P. Kumar, S. R. Dey, "Sorghum [*Sorghum bicolor* (L.) Moench]: A Major Cereal of the World" Accepted in: EnV Book Series, Discovery Publication, 2020-21.

38. P. Kumar, S. R. Dey, "Cadmium Induced Toxicity in Legumes with special reference to *Pisum sativum* L." Accepted in: EnV Book Series, Discovery Publication, 2020-21.
39. P. Kumar, S. R. Dey, "Aluminum Induced Oxidative Stress in Rice (*Oryza sativa* L.)" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
40. P. Kumar, S. R. Dey, "Isolation of Secondary Metabolites: An Overview" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
41. P. Kumar, S. R. Dey, "Leaf Area Index: Process Based Model" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
42. P. Kumar, S. R. Dey, "Effect of Nitrogen and Phosphorus on Pearl Millet with Respect to Its Growth and Development" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
43. P. Kumar, S. R. Dey, "Fluorine and Its Effect on Crops" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
44. P. Kumar, S. R. Dey, "Polyamine and plant stress response: Potential mechanism of action" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
45. P. Kumar, S. R. Dey, "Physiological Attributes Of Sorghum" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
46. P. Kumar, S. R. Dey, "Plant Morphology: An Overview" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
47. P. Kumar, S. R. Dey, "Metal Extraction from Soil by Microorganism-Assisted Plant" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
48. P. Kumar, S. R. Dey, "Photosynthesis it's Significance in Plant Growth, Development and Bio productivity Gaseous Fluxes in Atmosphere" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
49. P. Kumar, S. R. Dey, "Plant Hormones, Senescence and Abscission In Crops" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
50. P. Kumar, S. R. Dey, "Degradation of Natural Resources: Perhaps the Gravest Lapses of Mankind" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
51. P. Kumar, S. R. Dey, "Cultivation of Black Rice: Remunerative Advantage for Farmers" Accepted in: EnV Book Series, Discovery Publication, 2020-21.
52. Malta PG, Arcanjo-Silva S, Ribeiro C, Campos NV, and Alves-Azevedo A. (2016). *Rudgea viburnoides* (Rubiaceae) overcomes the low soil fertility of the Brazilian Cerrado and hyperaccumulates aluminum in cell walls and chloroplast. *Plant Soil*, 408, 369–384.
53. Moreno-Alvarado M, García-Morales S, Trejo-Téllez LI, Hidalgo-Contreras JV, and Gómez-Merino FC. (2017). Aluminum enhances growth and sugar concentration, alters macronutrient status and regulates the expression of NAC transcription factors in rice. *Frontiers in plant science*, 8, 73.