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2 **AMELIORATIVE ROLE OF BIOCHAR AND NANODAP IN MITIGATING SEWAGE**  
3 **SLUDGE INDUCED CADMIUM STRESS IN *PRAECITRULLUS FISTULOSUS***

4  
5 **Abstract**

6 Sewage sludge (SS) is valued for its nutrient-rich properties but often contains cadmium  
7 (Cd), posing risks to plant growth and human health. Cadmium stress significantly affects  
8 plant growth. It interferes with critical biochemical and physiological processes. This tension  
9 results in a reduction in biomass and a delay in nutrient intake, which in turn slows down  
10 development. Cadmium also generates reactive oxygen species that induce oxidative stress,  
11 which results in the destruction of cell components, including DNA, proteins, and lipids. This  
12 injury reduces the levels of chlorophyll, which in turn hinders growth and complicates  
13 photosynthesis. Cadmium's toxicity disrupts hormone balance, enzyme activity, and cell  
14 structure, leading to reduced agricultural output and subpar plant growth. Acidified biochar  
15 can effectively resolve this issue. Biochar is characterised by a high cation exchange capacity  
16 and oxygen-rich functional groups that facilitate the immobilisation of heavy metals in the  
17 soil through surface complexation and precipitation. Treating biochar with acid enhances  
18 cadmium immobilization by creating additional adsorption sites. Acidified biochar can  
19 significantly improve plant growth by increasing water retention, improving soil structure,  
20 and stimulating microbial activity as a slow-release nutrient source. Cadmium (Cd)  
21 contamination from sewage sludge (SS) is a critical challenge for sustainable agriculture, as  
22 it suppresses plant growth, disrupts nutrient absorption, and reduces crop yield. This study  
23 evaluated the ameliorative role of **rice husk biochar (RHB)** and **Nano-diammonium**  
24 **phosphate (Nano-DAP)** in mitigating sewage sludge-induced cadmium stress in  
25 *Praecitrullus fistulosus* (Tinda). The experiment included eight treatments: control, SS alone,  
26 SS + Cd, SS + Cd + biochar, SS + Cd + Nano-DAP, and their combinations. Results showed  
27 that cadmium stress (SS + Cd) significantly reduced plant height (36%), leaf area (50%),  
28 biomass, photosynthetic pigments, and fruit weight (45%) compared to control. Physiological  
29 parameters such as chlorophylls, carotenoids, and nutrient uptake (P and K) also declined  
30 sharply under Cd stress. Application of biochar improved soil properties by immobilizing Cd,  
31 while Nano-DAP alleviated nutrient deficiencies, both contributing to partial recovery of  
32 plant growth and yield. The combined treatment (SS + Cd + RHB + Nano-DAP) was most  
33 effective, improving plant growth and fruit weight by ~45% and reducing Cd accumulation in  
34 shoots by ~50%. Two-way ANOVA confirmed highly significant effects of treatments on  
35 both morphological and physiological parameters ( $p < 0.0001$ ). These findings underscore

36 the potential of integrated biochar–nanofertilizer strategies to sustainably manage heavy  
37 metal–contaminated soils and ensure secure crop production.

38 **Keywords:** Cadmium toxicity, sewage sludge, *Praecitrullus fistulosus*, Biochar, nanoDAP,  
39 phytoremediation

40

## 41 **1. Introduction**

42 Sewage sludge provides essential organic matter and nutrients for soils, but its heavy metal  
43 content, particularly cadmium, limits its use due to potential ecological and food safety risks.  
44 Research has shown that SS typically contains over 50% organic matter (OM) by dry weight  
45 (Carabassa et al., 2018; DelīBacaket al., 2020; Kominkoet al., 2017; Zuo et al., 2019). SS is an  
46 organic waste that serves as a soil conditioner due to its exceptional source of organic matter  
47 (OM), macronutrients (N, P, K, Ca, Mg, etc.), and micronutrients (Zn, Mn, Cu, etc.), including  
48 nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and iron (Fe)  
49 (Kolodziej et al., 2023). Additionally, they help to enhance the soil's microbial activity, plant  
50 yield, and physical characteristics (Tejada & Gonzalez, 2007; Yazdanpanah, 2016). SS is widely  
51 employed as a natural fertiliser on agricultural land (Hechmietal., 2021; Lamastra et al., 2018;  
52 Nogueira et al., 2013). SS is widely employed as a natural fertiliser on agricultural land  
53 (Hechmietal., 2021; Lamastra et al., 2018; Nogueira et al., 2013). Industrial effluent and sewage  
54 are combined, resulting in the accumulation of hazardous heavy metals such as lead (Pb),  
55 cadmium (Cd), chromium (Cr), nickel (Ni), mercury (Hg), and arsenic (As) in sewage sediment  
56 (Singh and Agrawal 2007; Latareet al., 2014). In addition to supplying nutrients to plants, SS  
57 also contains a substantial amount of detrimental elements, including arsenic (As), nickel (Ni),  
58 chromium (Cr), cadmium (Cd), lead (Pb), and others (Rastetter and Gerhardt 2017). One major  
59 reason preventing sewage sludge from being used as fertilizer is heavy metals' existence in it  
60 (Camargo *et al.*, 2016; Chen 2019). Because certain metals cannot break down naturally,  
61 applying SS to crops may cause a slow build-up of those metals in the soil (Charlton *et al.*,  
62 2016; Hasnine*et al.*, 2017). These metals have the potential to enter the food chain or aquifers,  
63 which would present a significant threat to public health and the environment (Liang et al.,  
64 2011). Because heavy metals are hydrophobic, they are typically found in sewage sludge.  
65 According to Tiruneh *et al.*, (2014), these components are connected to the solid portion of  
66 wastewater. There have been reports of the beneficial effects of SS spraying on a variety of  
67 crops, including wheat (Latareet *al.*,2017), spinach (Goluiet *al.*,2014), and rice (Latareet  
68 *al.*,2017). The use of sewage sludge as a substrate for microbiological activity enhances the  
69 activity of soil enzymes (Stark et al., 2008; Fernandez et al., 2009; Sciubba et al., 2013).

70 According to Singh and Agrawal (2009), okra (*Abelmoschus esculentus* L.) specimens  
71 contained levels of Cd, Pb, and Ni that exceeded the permissible limits of Indian norms 65 days  
72 after sowing and 40% (w/w) SS application. In the following order, these elements accumulate  
73 at a lower rate: Cd > Zn > Cu > Pb.

74 Photosynthetic activity, chlorophyll content, and carbon-fixing capacity are all decreased in  
75 plants exposed to Cd toxicity. Osmotic stress decreases transpiration, reduces stomatal  
76 conductance, and lowers leaf relative water content, thereby impairing plant physiological  
77 processes (Gallego et al., 2012; Rizwan et al., 2016). Keeping soil and plant system free of the  
78 potential for heavy metal contamination, its agricultural application necessitates monitoring  
79 (Jatav S. *et al.*, 2022). The extremely toxic element cadmium (Cd) is bad for plants, animals, and  
80 people. Increased Cd concentration in agricultural soil is the result of advancement in both  
81 agriculture and industry (Bojorquez *et al.*, 2016). Such as soils, where it is usually found in trace  
82 amounts (Zhao et al., 2020). In both aquatic and terrestrial organisms, it exhibits biological  
83 activity. (Chellaiah, 2018). Cadmium poses serious health risks to humans and animals when  
84 plants absorb and accumulate it from contaminated soils because it moves rapidly through  
85 polluted soil systems (Chen et al., 2016). Cadmium is a non-essential metal that negatively  
86 affects plant development and proliferation. According to Annu et al. (2016), the primary  
87 sources of Cd in soil are direct application techniques such as phosphate fertilizer use and sewage  
88 sludge disposal, as well as atmospheric deposition.

89 Through cadmium inhibition of iron uptake and movement to key parts, plants face growth  
90 issues. The chloroplast creates iron-based protein ferredoxin which regulates the photosystem and  
91 this process needs iron. The chloroplast faces problems since iron delivery to the site experiences  
92 obstruction and thus chlorophyll levels drop. The lower chlorophyll levels endanger the photosynthesis  
93 process because plants struggle to grow. While cadmium toxicity affects hormone balance  
94 and enzyme activity and cell structure, plants show poor development. The toxicity of cadmium  
95 leads to reduced growth and impacts crop production. The cadmium promotes reactive oxygen species  
96 like hydrogen peroxide and superoxide radicals thus damaging cell parts. Because cadmium affects  
97 zinc and iron absorption, plants experience chlorosis and this appears in leaf color change.  
98 (Xu *et al.*, 2017). Indian standards permit 3–6 mg/kg of cadmium (Cd) in soil, whereas  
99 European standards limit Cd to 1 mg/kg (Mawari G. et al., 2023). Exposure to Cd reduces the  
100 germination of soybean, lettuce, and sugarbeet (*Beta vulgaris* L.) (Li et al., 2013; Guilherme et  
101 al., 2015). Elevated Cd levels also decrease the concentrations of essential nutrients such as  
102 magnesium (Mg), calcium (Ca), and potassium (K) in soils cultivated with cucumber (*Cucumis*  
103 *sativus* L.), maize (*Zea mays* L.), tomato (*Lycopersicon esculentum* L.), and lettuce (*Lactuca*  
104 *sativa* L.) (Nazar et al., 2012). Plants exposed to Cd commonly exhibit chlorosis and abnormal  
105 or redirected growth as early visible symptoms (Jali et al., 2016). Higher toxicity levels lead to

106 necrosis and severe growth inhibition (Hermans et al., 2011). Yadav et al. (2020) reported that  
107 sewage sludge collected from districts such as Karnal, Panipat, and Sonapat contained total Cd  
108 concentrations ranging from 0.2 to 6.5 mg/kg, while dissolved Cd levels varied between 0.024  
109 and 0.451 mg/L depending on sludge source and industrial input. Despite these documented  
110 risks, researchers have conducted relatively limited studies on cadmium toxicity in plants and on  
111 effective remediation strategies to restrict Cd mobilization in the soil rhizosphere. Recently,  
112 researchers have shown interest in activated carbon biochar as a possible remedy for this  
113 issue (Rehman, M. Z. et al., 2020).

114  
115 Biochar is a carbon-rich, porous solid that develops when biomass is pyrolyzed anaerobically  
116 (Zakaria, M. R., et al., 2023). It has drawn interest due to its potential to increase crop output,  
117 improve soil health, and sequester carbon to slow down climate change.

118 Biochar produced via biomass pyrolysis effectively immobilizes heavy metals through  
119 adsorption and soil property improvements. Biochar improves soil structure, increases water  
120 retention, and enhances nutrient availability by boosting the soil's cation exchange capacity.  
121 These properties make it particularly beneficial for degraded or nutrient-deficient soils  
122 (Shahzad, A. S. et al., 2023). In addition, the oxygen-containing functional groups present on  
123 biochar surfaces help immobilize heavy metals in soil through surface complexation and  
124 precipitation mechanisms. Treating biochar with acid further increases cadmium immobilization  
125 by exposing additional adsorption sites and enhancing its metal-binding capacity. Biochar is  
126 added to soil to enhance its nutrient retention capacity, microbial populations, pH, organic  
127 matter (OM), fertility, moisture retention, soil aggregate stability, and carbon  
128 sequestration which supports soil biodiversity, provides a home for helpful microorganisms, and  
129 increases plant resistance to stressors like drought and heavy metal toxicity (Fuke, 2021).  
130 Recent field studies underline its success in reducing Cd uptake in crops while enhancing yield  
131 and soil health. It has the ability to clean up metal-contaminated soil due to its beneficial  
132 properties, which comprise a sizable surface area, a high capacity for cation exchange, a porous  
133 structure, a negatively charged surface, and functional groups that contain oxygen. Additionally,  
134 it lessens the leaching of heavy metals and nutrients, minimizing environmental damage.  
135 Biochar application can prevent heavy metals (HMs) from entering the food chain and posing  
136 health risks to humans (Zhao et al., 2021). Researchers have recently shown great interest in  
137 using biochar to remediate cadmium (Cd)-contaminated agricultural soils (El-Naggar et al.,  
138 2019; Zhang et al., 2019). Yang et al. (2023) demonstrated that phosphorus-enriched biochar  
139 reduced heavy metal accumulation in rice by more than 40%. Similarly, Ren et al. (2020)  
140 reported that phosphate-modified biochar decreased DTPA-extractable Cd in soil and reduced  
141 its uptake in *Brassica rapa*. Biochar application in a metal contaminated soil can effectively

142 lessen the buildup of metal in several rice portions (Mukherjee *et al.*, 2023). RHB can  
143 successfully enhance the mechanical and physical characteristics of soils (Qu *et al.*, 2014).  
144 Biochar made from agricultural waste has strong  $Pb^{2+}$  and  $Cd^{2+}$  adsorption capabilities (Amen *et*  
145 *al.*, 2020). Bian *et al.*, (2022) discovered that cadmium levels in cabbage leaves were lowered  
146 when RHB was added to contaminated soil. The addition of RHB boosted plants' absorption of  
147 heavy metals (Karam *et al.*, 2022).

148  
149 Nano fertilizers are characterized primarily by their microscopic size. By reducing losses and  
150 facilitating nutrient entry into plants, their greater contact area and response increase nutrient  
151 usage efficiency. Vegetable production has extensively explored the use of nanotechnology,  
152 which includes enhanced seed germination, seedling growth, detection and management of  
153 biotic and abiotic stressors, and improved yield and quality. Nanofertilizers can decrease  
154 nutrient loss through leaching and improve the nutrition delivery of fertilizers applied to the  
155 soil (Sheikh, L., et al. (2025). Nanoparticle-enabled fertilizers like nanoDAP, benefit from  
156 increased nutrient efficiency and may bolster plant tolerance to heavy metal stress and also used  
157 as soil amendments by farmers. Such integrated interventions are emerging as sustainable  
158 strategies in modern remediation science. Nanofertilizer, which increases plants capacity to  
159 absorb nutrients, is one of the most significant applications of nanotechnology (Mousavi and  
160 Rezai; Srilatha, 2011 and Ditta 2012). Nanofertilizers provide a more effective and economical  
161 alternative to conventional fertilizers because they regulate nutrient release through  
162 nanoparticle-based delivery systems. These fertilizers contain one or more essential plant  
163 nutrients in nanoparticle form, and at least half of their particles measure less than 100  
164 nanometers in diameter (Yadav, A. et al., 2023). They give plants more surface area for an  
165 assortment of metabolic processes, which accelerates photosynthesis, increases the amount of  
166 dry matter generated, and raises crop output (Charu Gupta, 2020). Phosphorus nanofertilizer is a  
167 relatively new type of fertilizer that has the ability to drastically alter how food is grown.  
168 Diammonium phosphate (DAP) is the most commonly used phosphatic fertilizer because of its  
169 advantageous physical characteristics and high composition (18% N and 46%  $P_2O_5$ ) in the  
170 overall constitution. Therefore, using this fertilizer in nano form will be very beneficial  
171 (Chamuahe *et al.*, 2023). NanoDAP serves as an effective alternative to conventional fertilizers  
172 because it releases nutrients gradually and in a controlled manner after application, thereby  
173 reducing water pollution and nutrient runoff. In agricultural settings, its use greatly boosts yield  
174 and fertility (Kushwaha *et al.*, 2023).

175  
176 The herbaceous plant locally known as "tinda," or Indian baby pumpkin  
177 (*Praecitrullus fistulosus*), is a member of the Cucurbitaceae family (Tindall 1983; Tyagi 2012).

178 Its fruit and seeds are used as food, fuel, and diabetic therapy(Mukesh 2010).The fruit is  
179 consumed prepared as a vegetable. It is a great plant among therapeutic plants because it has all  
180 the necessary elements in the optimal amounts for good health (Kirtikar 1998). It is high in  
181 lipids, proteins, fiber, and carbs. he crop thrives in warm, sunny climates with daytime  
182 temperatures between 25 and 30°C and nighttime temperatures of 18°C or higher, but it  
183 performs poorly in cold and humid conditions. Farmers in India cultivate it during two main  
184 seasons: the rainy season from mid-June to the end of July and the dry season from February to  
185 the end of April. Sandy soils with easy-to-pierce roots are preferred by tinda with pH 6.5-7.5.  
186 During the dry season, it is advised to water two to three times a week (Tyagi Nidhi *et al.*,  
187 2012). The most likely place of origin is northwest India. Farmers grow this crop extensively in  
188 northwestern India and cultivate smaller areas in Bihar, Gujarat, Maharashtra, and the western  
189 regions of Uttar Pradesh, Punjab, Haryana, Rajasthan, and Delhi. So, aresearch is planned to  
190 track how sewage sludge affects Indian baby pumpkin (*Praecitrullus fistulosus*)  
191 agriculture.*Praecitrullus fistulosus* (tinda) is nutritionally significant in India, yet its growth  
192 can be compromised by Cd contamination.

#### 196 **Taxonomic Hierarchy:**

<ul style="list-style-type: none"><li>• <b>Kingdom:</b> Plantae</li><li>• <b>Order:</b>Cucurbitales</li><li>• <b>Family:</b> Cucurbitaceae</li><li>• <b>Subfamily:</b>Cucurbitoideae</li><li>• <b>Genus:</b><i>Praecitrullus</i></li><li>• <b>Species:</b><i>Praecitrullus fistulosus</i></li></ul>

197  
198 Researchers frequently apply both synthetic and natural amendments to immobilize heavy  
199 metals in contaminated soils. Among these, biochar serves as an effective soil conditioner  
200 because it reduces the bioavailability of heavy metals and improves food safety through its  
201 strong metal-binding capacity. Panghal et al. (2021) assessed heavy metal contamination at  
202 major traffic intersections in Rohtak City, Haryana, and within industrial clusters developed  
203 by the Haryana State Industrial and Infrastructure Development Corporation (HSIIDC). They  
204 reported mean soil concentrations of cadmium (Cd) at  $7.54 \pm 5.89$  mg/kg, iron (Fe) at  $209.80$   
205  $\pm 137.44$  mg/kg, zinc (Zn) at  $127.39 \pm 80.43$  mg/kg, nickel (Ni) at  $21.57 \pm 24.02$  mg/kg, and

206 chromium (Cr) at  $17.05 \pm 10.73$  mg/kg. The researchers evaluated soil contamination using  
207 the prospective ecological risk index (RI), contamination factor (CF), and pollution load  
208 index (PLI). Their analysis identified cadmium as the metal posing the highest ecological  
209 risk. The findings clearly indicate elevated cadmium levels in the metropolitan soils of  
210 Rohtak. For this reason, a study will be done to examine how *Praecitrullus fistulosus* is  
211 affected by cadmium toxicity in soil incorporated sewage sludge and to ascertain how  
212 nanoDAP and biochar affects tinda growth related parameters and production.

213

#### 214 **4. Materials and Methods**

215 Experimental Location and Design:

216 We conducted a pot experiment using sandy soil, which allowed easy root penetration, had a  
217 pH of 6.5–7.5, was rich in inorganic matter, and provided good drainage. We collected the  
218 soil from a depth of 0–15 cm and added sewage sludge obtained from the 10-megalitre-per-  
219 day (MLD) sewage treatment plant in Sector 25, Rohtak, Haryana ( $28^{\circ}30'–29^{\circ}05' N$  latitude  
220 and  $76^{\circ}03'–76^{\circ}51' E$  longitude). We carried out the experiment at the Department of Botany,  
221 Baba Mastnath University, Rohtak, Haryana.

222

223

224

Material	Source
Seeds- Ludhiana Special	Doctors Seeds India, Ludhiana, Punjab
Nano DAP	IFFCO
Cadmium ( $CdCl_2$ )- SRL	Haryana scientific, Rohtak
Rice husk biochar	Shraddha Agro Zone, Pune
Sewage sludge	STP sector 25, Rohtak
Soil	Nearby farm

225

226



Figure.1 Collection of Sewage sludge and sandy soil

227



228

229

230

**Experimental design:** The experimental layout utilized a completely randomized design (CRD) comprising a total of 24 individual pots, each with a capacity of 30 kg, that were filled with a mixture of soil, sewage sludge, biochar, and nano DAP. Eight groups in three replicas will be planted in plastic pots with 2 seeds per pot. In this study eight treatment groups, with three replicates per group, were planted in 30 kg pots with agriculture soil (organic matter 15 g/kg) and two *Praecitrullus fistulosus* (Ludhiana special variety) seeds were sown per pot, each were assessed at 60 days after sowing (DAS).The treatments were as follows:

239 **1.**Control(soil without any amendments),

240 **2.**30%SS+ 70%Soil,

241 3.30%SS+ 50mg/kg Cd,

242 4.30%SS+ 5% biochar(RHB),

243 5.30%SS+ 50mg/kg Cd+ 5% biochar (RHB),

244 6.30% SS+5ml/l nanoDAP,

245 7.30% SS+ 50mg/kg Cd+5ml/l nanoDAP

246 8.30% SS+ 50mg/kg Cd+ 5ml/l nanoDAP+5% biochar(RHB)

247

248 **Treatment plan:** Plant leftovers and unwanted materials were removed after the pre-plant  
249 soil and sewage sludge were gathered and allowed to sun-dry for a few days. We filled each  
250 pot with 20 kg of soil amended with sewage sludge and labeled it according to the specific  
251 treatment percentage.

252 **Seed Collection, Screening, Sowing, and Irrigation:**

253 We collected seeds of *Praecitrullus fistulosus*, soaked them in water for 24 hours, and sowed  
254 them in May 2024. We carried out regular irrigation throughout the growth period, and we  
255 completed the final harvest in August 2024. For instant availability, cadmium and biochar  
256 were combined during the sowing process. Six hours prior to seeding, seeds were primed  
257 with 5ml/l nano-DAP. Two seeds were planted two to three cm deep in each pot with the  
258 seeds properly spaced apart. It took ten to twelve days for the seeds to germinate. Watering  
259 was done immediately after seeding and then two to three times a week after that.

260 **Harvesting and data collection:** After 60 days from the day they were planted, the fruits  
261 were ripe and ready to be picked. When required, intercultural duties like irrigation, weeding,  
262 and thinning were done by hand. The statistical analysis was done using SPSS.

263

### 264 3.6. Morphological traits

265 Sixty days after sowing, the chosen growth parameters for *Praecitrullus fistulosus* plants  
266 grown in different treatments were measured. These included plant height, fresh weight, dry  
267 weight, leaf area, fruit weight, and the number of leaves. To measure plant height, a metre  
268 scale was used to measure from the ground to the tip of the plant. We counted how many  
269 leaves each plant had. A metre scale was used to measure the area of the leaves. Weight of  
270 leaves and roots, both fresh and dry (kg): After taking fresh weight (kg)(leaves and roots)  
271 plants were put in oven at 60°C until constant weight. The final weight was then logged, and  
272 the average weight was used to calculate the plant's dry weight. The weight of the fruits was  
273 measured using an electronic scale.

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### 3.7. Physiochemical traits.

*Praecitrullus fistulosus* leaves contain carotenoids, total chlorophyll, chlorophyll a, and chlorophyll b. The total nitrogen, phosphorus, and potassium content were measured. It was estimated that the leaves were fully opened from the top 60 DAS from intact leaf tissue. At 664, 647, and 480 nm, pigments are measured spectrophotometrically, and their concentrations are computed using formulas developed empirically by Moran.

**The nitrogen content was calculated using the volume of acid consumed and expressed as percentage nitrogen (% N) on a dry weight basis using the formula:**

$$\text{Nitrogen (\%)} = \frac{(V_s - V_b) \times N \times 1.4007}{W}$$

Where,

- $V_s$  = Titration value for the sample (mL)
- $V_b$  = Titration value for the blank (mL)
- $N$  = Normality of standard acid
- $W$  = Weight of sample (g)
- **1.4007** = Constant (atomic weight of nitrogen  $\times 100 / 1000$ )

**Total Phosphorus(P)**-The phosphorus content in plant samples was determined following the method of Jackson (1973). For plant samples, the results were reported as mg/g of dry weight.

$$\text{Phosphorus (mg/g)} = (C \times V \times D) / (W \times 1000)$$

Where:

- $C$  = Concentration of phosphorus from the standard curve ( $\mu\text{g/mL}$  or ppm)
- $V$  = Final volume of the digest or extract (mL)
- $D$  = Dilution factor (if the aliquot was further diluted)
- $W$  = Weight of the plant/soil sample taken for digestion (g)
- **1000** = Conversion factor from  $\mu\text{g}$  to mg (if needed)

**Total Potassium(K)**-Total potassium method was used to determine the levels in plant samples of Hanway and Heidel (1952), as outlined in Jackson (1973). The potassium concentration in mg/kg dry weight of the plant sample was obtained by comparing the

sample readings to the calibration curve. Calculation:

$$\text{Potassium (mg/kg)} = \frac{C (\text{mg L}^{-1}) \times V (\text{L})}{W (\text{kg})}$$

W (kg)

Where

- C = concentration from the photometer (mg L<sup>-1</sup>)
- V = final volume of the extract (L)
- W = weight of the dry sample (kg)

### **Results and discussion:**

In the present study, sewage sludge was sourced from the municipal treatment facility in Rohtak, Haryana, to evaluate cadmium (Cd) stress on *Praecitrullus fistulosus* and assess the impact of various remediation treatments. Therefore, for experimental consistency, cadmium treatment (CdCl<sub>2</sub>) was applied at 50 ppm concentration. The experiment utilized sewage sludge (SS) collected from the Rohtak sewage treatment plant, which was analyzed to contain Cd concentration of 3.5 mg/kg. Studies from Rohtak and nearby districts (like Delhi NCR and Haryana) have reported cadmium concentrations in sewage sludge ranging from 3 to 10 mg/kg dry weight, depending on the source and industrial load. 3.4 – 7.6 mg/kg Cd (reported in sludge from municipal and industrial combined treatment facilities in Haryana). Actual concentration in Rohtak's sewage sludge may vary based on industrial discharge, treatment process, and sampling time. To induce cadmium toxicity and study its remediation, different treatments are used. Sewage sludge (SS, 6 kg/pot, 50 mg/kg Cd) and remediation using rice husk biochar (RHB, 1kg/pot) and Nano DAP (5ml/l) are used to measure the toxicity of Cd. These amendments were applied at the time of sowing to ensure uniform exposure from the initial growth phase. **Sewage Sludge (SS)**, 30% w/w (6 kg in 20 kg soil) mixed thoroughly in soil 7 days before sowing. **Cadmium (Cd)**, 50 ppm (as CdCl<sub>2</sub>) applied in solution form and mixed into the soil just before sowing. Seeds were primed with **Nano DAP**(5ml/l) six hours prior to seeding for immediate nutrient availability. **Biochar (RHB)**, 5% w/w (1 kg in 20 kg soil) mixed before sowing to allow stabilization.

SS significantly reduced growth plant height by ~25%, leaf area by ~30%, biomass by ~40%—consistent with known effects of Cd toxicity. Biochar-amended treatments improved growth and biomass—likely via Cd immobilization through increased soil pH, cation exchange capacity, and transformation of Cd into more stable soil fractions. The combined treatment yielded the best

outcomes: 30–45% increases in growth parameters over SS alone, and a ~50% reduction in Cd accumulation in shoots. This aligns with emerging insights that biochar-NP combinations can synergistically enhance Cd phytoremediation through improved soil stabilization and nutrient delivery.

### **Morphological traits of *Praecitrullus fistulosus* under Cadmium Stress and Remediation**

**treatments:** Young vines with initial leaves emerging from runners. This early stage is when treatments like sewage sludge (SS) and amendments (RHB, Nano DAP, Cd) are applied at sowing. A fuller green canopy of palmate-lobed leaves and developing tendrils, representing a mid-vegetative phase before flowering—ideal to observe physiological effects of treatments like biochar or nano DAP on leaf area and vigor. Flowering typically begins ~30 days after sowing. The plant develops distinctive yellow unisexual flowers prior to fruit set. Round, apple-gourd fruits begin forming ~40 DAS, appearing as green spherical 5–8 cm fruits. This study evaluated the effects of cadmium (Cd) toxicity and the potential ameliorative effects of rice husk biochar (RHB) and Nano DAP by evaluating the morphological traits of *Praecitrullus fistulosus* at 60 DAS. In 20 kg soil-filled pots, each group was planted with varying amounts of Cd, RHB, and Nano DAP in addition to 6 kg of sewage sludge.

Plant height in the control group (Group 1: Soil only) achieved the **highest plant height (72.00 ± 7.21 cm)**, indicating ideal development circumstances without any treatment stress and minimum in Group 3: SS + Cd) - (46.00 ± 7.00 cm; \*\*\*p < 0.001). **The number of leaves** per plant was in the control group produced the most leaves (15.33 ± 2.52) and in Group 3 had a significant drop in leaf number (7.00 ± 1.00) indicating the negative influence of cadmium on shoot development. **Leaf area** is a fundamental characteristic that influences photosynthetic efficiency and biomass buildup in plants. Under control conditions, Group 1 had the highest mean leaf area (111.33 ± 12.01 cm<sup>2</sup> per plant), indicating healthy growth and robust foliage development. Exposure to Cd (Group 3) resulted in a significant reduction in leaf area to 58.33 ± 27.57 cm<sup>2</sup> (\*\*\*p < 0.001), indicating cadmium's harmful effect on leaf expansion and cell division. The highest Leaf biomass **fresh leaf weight** was recorded in the control group (Group 1: 0.08 ± 0.02 kg/plant), which served as the baseline for healthy plant growth. Cadmium exposure in Group 3 (SS + Cd) drastically reduced fresh leaf weight to 0.02 ± 0.01 kg/plant, reflecting the severe phytotoxic effects of Cd on leaf development and turgidity. Similarly, **dry leaf weight** followed the same trend. Group 1 showed the highest dry weight (0.03 ± 0.01 kg/plant), while Group 3 experienced a drastic decline (0.004 ± 0.00 kg/plant). The control group had the largest **fresh root weight** (0.03 ± 0.001 kg per plant). Cd-stressed plants (Group 3) showed a significant

decrease ( $0.01 \pm 0.011$  kg/plant), suggesting toxic inhibition of root elongation and development. In terms of **dry root weight**, the same pattern emerged. Control plants had the maximum dry root weight ( $0.008 \pm 0.0002$  kg), whereas Cd exposure (Group 3) significantly decreased this characteristic ( $0.002 \pm 0.0005$  kg). The control group had the maximum **fruit weight** ( $53.13 \pm 7.71$  kg), indicating excellent plant development in non-stressed conditions. Cadmium poisoning considerably decreased fruit weight (Group 3:  $29.00 \pm 2.00$  kg;  $***p < 0.001$ ), indicating a deleterious influence of heavy metals on reproductive production.

### **Physiochemical Traits of *Praecitrullus fistulosus***

Plant physiological status is strongly linked to pigment content, which has a direct impact on photosynthetic efficiency and stress tolerance. In the current study, the concentrations of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and macronutrient content (total nitrogen, phosphorus, and potassium) were analyzed to assess the impact of cadmium (Cd) toxicity and the ameliorative effects of rice husk biochar (RHB) and Nano DAP. Chlorophyll a, exhibited the highest concentration in the control group ( $1.27 \pm 0.25$  mg/g FW), indicating healthy photosynthetic activity and exposure to cadmium (Group 3: SS + Cd) significantly reduced chlorophyll a content to  $0.57 \pm 0.15$  mg/g FW ( $*p < 0.05$ ), highlighting the photosynthetic impairment caused by Cd stress. The control group had a chlorophyll b concentration of  $0.77 \pm 0.15$  mg/g FW, which reduced to  $0.33 \pm 0.15$  mg/g FW in Group 3 (SS + Cd). The greatest content was noted in the control group ( $2.03 \pm 0.040$  mg/g FW), while a substantial reduction was seen in the cadmium-stressed group (Group 3:  $0.90 \pm 0.26$  mg/g FW;  $***p < 0.001$ ). The control group's carotenoid content was  $0.47 \pm 0.15$  mg/g FW, but Cd-stressed plants (Group 3) experienced a reduction to  $0.30 \pm 0.10$  mg/g FW. The control group showed the greatest total nitrogen level ( $2.28 \pm 0.046\%$ ), indicating adequate nutrient uptake in unstressed plants. Under Cd stress (Group 3), nitrogen concentration decreased to  $1.70 \pm 0.063\%$ , while the difference was not statistically significant (ns). Phosphorus is necessary for energy metabolism and root growth. The control plants exhibited a phosphorus level of  $0.67 \pm 0.064\%$ . Cadmium stress resulted in a pronounced decrease (Group 3:  $0.21 \pm 0.06\%$ ), suggesting impaired phosphorus assimilation. Additionally, the control plants showed the highest potassium content at  $1.89 \pm 0.24\%$  the Cd-stressed group (Group 3) had a lower level of  $1.24 \pm 0.38\%$ .

**Table 1: Impact on Morphological and Physiochemical Traits (60 DAS)**

Trait	Group 1: Control (Soil only)	Group 2: Soil + SS	Group 3: SS + Cd	Group 4: SS+ biochar (RHB)	Group 5: SS+ Cd + biochar (RHB)	Group 6: SS+ nanoD AP	Group 7: SS+ Cd + nanoD AP	Group 8: SS+ Cd+ nanoD AP + biochar (RHB)
<b>A. Morphological Traits</b>								
Plant Height (cm)	72.00 ± 7.21	60.33± 7.09 <sup>ns</sup>	46.00 ± 7.00 <sup>***</sup>	60.00 ± 4.00 <sup>ns</sup>	51.67 ± 3.21 <sup>***</sup>	63.67 ± 10.50 <sup>ns</sup>	53.67 ± 4.04 <sup>**</sup>	56.33 ± 9.45 <sup>*</sup>
Number of Leaves (per plant)	15.33 ± 2.52	11.00 ± 1.00 <sup>ns</sup>	7.00 ± 1.00 <sup>ns</sup>	11.00 ± 2.00 <sup>ns</sup>	8.00 ± 1.00 <sup>ns</sup>	12.00 ± 3.00 <sup>ns</sup>	9.00 ± 2.65 <sup>ns</sup>	10.67 ± 3.06 <sup>ns</sup>
Leaf Area (cm <sup>2</sup> /plant)	111.33 ± 12.01	79.00 ± 13.53 <sup>**</sup> *	58.33 ± 27.57 <sup>**</sup> *	78.00 ± 9.00 <sup>***</sup>	63.33 ± 3.79 <sup>***</sup>	85.00 ± 8.19 <sup>***</sup>	67.67 ± 4.16 <sup>***</sup>	72.00 ± 10.15 <sup>**</sup> *
Fresh Weight - Leaves (kg/plant)	0.08 ± 0.02 <sup>ns</sup>	0.06 ± 0.02 <sup>ns</sup>	0.02 ± 0.01 <sup>ns</sup>	0.06 ± 0.02 <sup>ns</sup>	0.03 ± 0.01 <sup>ns</sup>	0.07 ± 0.02 <sup>ns</sup>	0.04 ± 0.02 <sup>ns</sup>	0.05 ± 0.02 <sup>ns</sup>
Dry Weight - Leaves (kg/plant)	0.03 ± 0.01 <sup>ns</sup>	0.007 ± 0.00 <sup>ns</sup>	0.004 ± 0.00 <sup>ns</sup>	0.007±.03 <sup>ns</sup>	0.005±.001 <sup>ns</sup>	0.009±.001 <sup>ns</sup>	0.006±.001 <sup>ns</sup>	0.007±.001 <sup>ns</sup>

<b>Fresh Weight - Roots (kg/plan t)</b>	<b>0.03±.001</b>	<b>0.019±.005<sup>ns</sup></b>	<b>0.01±.011<sup>ns</sup></b>	<b>0.019±.001<sup>ns</sup></b>	<b>0.014±.001<sup>ns</sup></b>	<b>0.022±.001<sup>ns</sup></b>	<b>0.016±.003<sup>ns</sup></b>	<b>0.018±.001<sup>ns</sup></b>
<b>Dry Weight - Roots (kg/plan t)</b>	<b>0.008±.002</b>	<b>0.005±.001<sup>ns</sup></b>	<b>0.002±.005<sup>ns</sup></b>	<b>0.005±.009<sup>ns</sup></b>	<b>0.003±.001<sup>ns</sup></b>	<b>0.006±.001<sup>ns</sup></b>	<b>0.004±.001<sup>ns</sup></b>	<b>0.005±.001<sup>ns</sup></b>
<b>Fruit Weight (kg/fruit )</b>	<b>53.13 ± 7.71</b>	<b>41.33 ± 5.51<sup>ns</sup></b>	<b>29.00 ± 2.00***</b>	<b>41.62 ± 3.36<sup>ns</sup></b>	<b>34.42 ± 6.03**</b>	<b>45.47 ± 7.04<sup>ns</sup></b>	<b>37.24 ± 7.40*</b>	<b>40.42 ± 2.82<sup>ns</sup></b>
<b>B. Physiochemical Traits</b>								
<b>Chlorophyll a (mg/g FW)</b>	<b>1.27 ± 0.25</b>	<b>0.80 ± 0.10<sup>ns</sup></b>	<b>0.57 ± 0.15*</b>	<b>0.83 ± 0.15<sup>ns</sup></b>	<b>0.65 ± 0.12<sup>ns</sup></b>	<b>0.88 ± 0.04<sup>ns</sup></b>	<b>0.70 ± 0.08<sup>ns</sup></b>	<b>0.76 ± 0.11<sup>ns</sup></b>
<b>Chlorophyll b (mg/g FW)</b>	<b>0.77 ± 0.15</b>	<b>0.53 ± 0.15<sup>ns</sup></b>	<b>0.33 ± 0.15<sup>ns</sup></b>	<b>0.46 ± 0.10<sup>ns</sup></b>	<b>0.44 ± 0.07<sup>ns</sup></b>	<b>0.58 ± 0.11<sup>ns</sup></b>	<b>0.45 ± 0.08<sup>ns</sup></b>	<b>0.50 ± 0.18<sup>ns</sup></b>
<b>Total Chlorophyll (mg/g FW)</b>	<b>2.03 ± 0.040</b>	<b>1.33 ± 0.12*</b>	<b>0.90 ± 0.26***</b>	<b>1.29 ± 0.23*</b>	<b>1.09 ± 0.19**</b>	<b>1.47 ± 0.14<sup>ns</sup></b>	<b>1.15 ± 0.11**</b>	<b>1.26 ± 0.25*</b>
<b>Carotenoids (mg/g FW)</b>	<b>0.47 ± 0.15</b>	<b>0.38 ± 0.11<sup>ns</sup></b>	<b>0.30 ± 0.10<sup>ns</sup></b>	<b>0.39 ± 0.12<sup>ns</sup></b>	<b>0.33 ± 0.09<sup>ns</sup></b>	<b>0.42 ± 0.04<sup>ns</sup></b>	<b>0.35 ± 0.03<sup>ns</sup></b>	<b>0.38 ± 0.03<sup>ns</sup></b>
<b>Total</b>	<b>2.28 ±</b>	<b>1.98 ±</b>	<b>1.70 ±</b>	<b>2.06 ±</b>	<b>1.83 ±</b>	<b>2.10 ±</b>	<b>1.86 ±</b>	<b>1.96 ±</b>

<b>Nitrogen</b> (% DW)	<b>0.046</b>	<b>0.033<sup>ns</sup></b>	<b>0.063<sup>ns</sup></b>	<b>0.053<sup>ns</sup></b>	<b>0.023<sup>ns</sup></b>	<b>0.027<sup>ns</sup></b>	<b>0.056<sup>ns</sup></b>	<b>0.059<sup>ns</sup></b>
<b>Phosphorus</b> (%) DW)	<b>0.67 ±</b> <b>0.064</b>	<b>0.28 ±</b> <b>0.04<sup>ns</sup></b>	<b>0.21 ±</b> <b>0.06<sup>ns</sup></b>	<b>0.30 ±</b> <b>0.10<sup>ns</sup></b>	<b>0.25 ±</b> <b>0.03<sup>ns</sup></b>	<b>0.32 ±</b> <b>0.10<sup>ns</sup></b>	<b>0.26 ±</b> <b>0.03<sup>ns</sup></b>	<b>0.28 ±</b> <b>0.08<sup>ns</sup></b>
<b>Potassium</b> (%) DW)	<b>1.89 ±</b> <b>0.24</b>	<b>1.63 ±</b> <b>0.44<sup>ns</sup></b>	<b>1.24 ±</b> <b>0.38<sup>ns</sup></b>	<b>1.65 ±</b> <b>0.20<sup>ns</sup></b>	<b>1.40 ±</b> <b>0.47<sup>ns</sup></b>	<b>1.71 ±</b> <b>0.48<sup>ns</sup></b>	<b>1.50 ±</b> <b>0.32<sup>ns</sup></b>	<b>1.61 ±</b> <b>0.33<sup>ns</sup></b>

Note: Data as mean ± standard deviation (SD). We indicate statistical significance as follows:

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , and ns = non-significant.

A two-way ANOVA to evaluate how different treatments (row factor) and morphological traits (column factor) affected the growth of *Praecitrullus fistulosus*. The analysis showed that the treatments significantly influenced overall morphological performance, with the row factor producing a highly significant effect:  $F(7, 128) = 713.7$ ,  $P < 0.0001$ .

The column factor (morphological traits) also showed a significant effect  $F(7, 128) = 13.49$ ,  $P < 0.0001$ , suggesting variability in the response of different traits to the treatments. The residual variance was relatively low ( $MS = 32.13$ ), reflecting consistency in the dataset and supporting the robustness of the treatment effects. These results confirm that both treatment type and the specific morphological traits significantly influenced plant growth responses under the experimental conditions.

**Table 4: Two-Way ANOVA Summary for Morphological Trait Variation under Different Treatments and Conditions**

	SS	DF	MS	F (DFn, DFd)	P value
Row Factor	160523	7	22932	F (7, 128) = 713.7	$P < 0.0001$
Column Factor	3033	7	433.3	F (7, 128) = 13.49	$P < 0.0001$
Residual	4113	128	32.13		

**Note: DF: Degrees of Freedom; SS: Sum of Squares; MS: Mean Square; F: F-ratio (between-group df, within-group df); P value: Statistical significance ( $\alpha = 0.05$ ).**

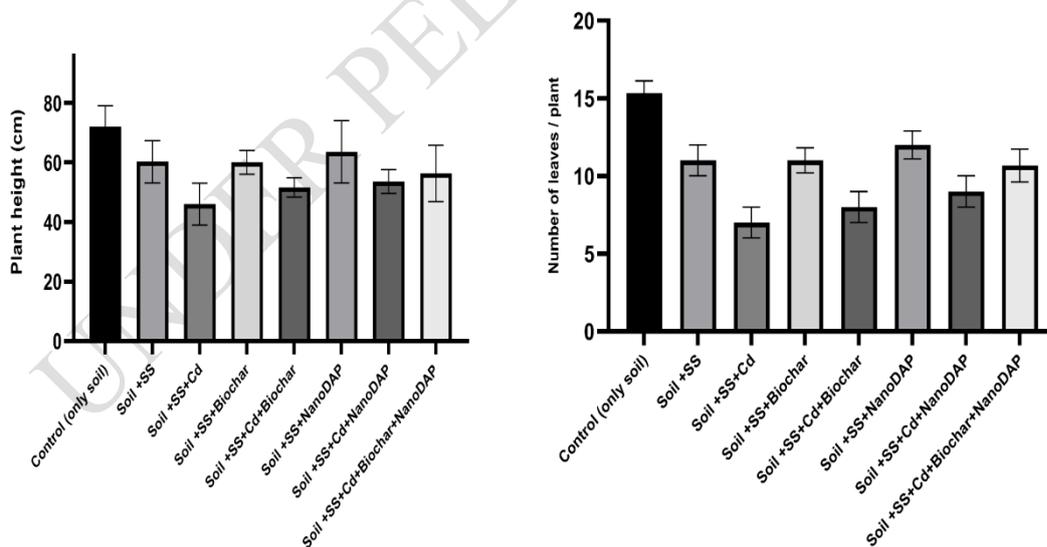
A two-way ANOVA was performed to evaluate the effects of different treatments and physiological traits on *Praecitrullus fistulosus*. The results revealed that the row factor (treatments) had a highly significant effect, with an F-value of  $F(6, 112) = 136.9$  and  $P < 0.0001$ ,

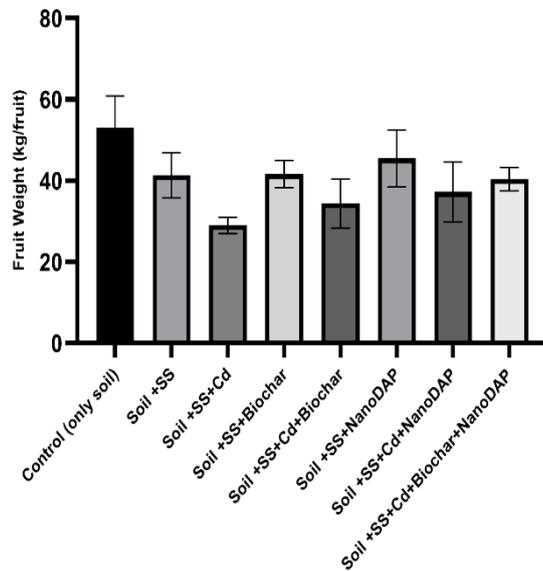
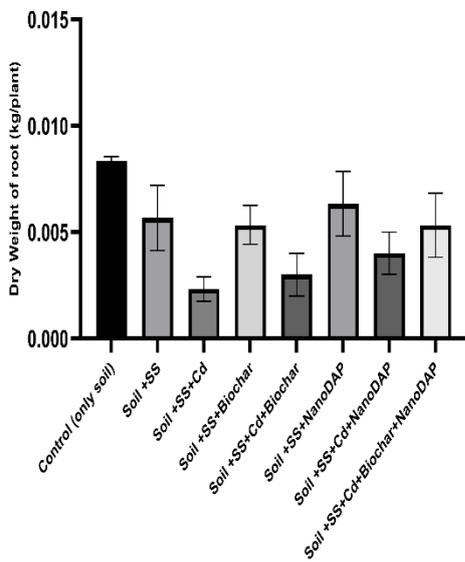
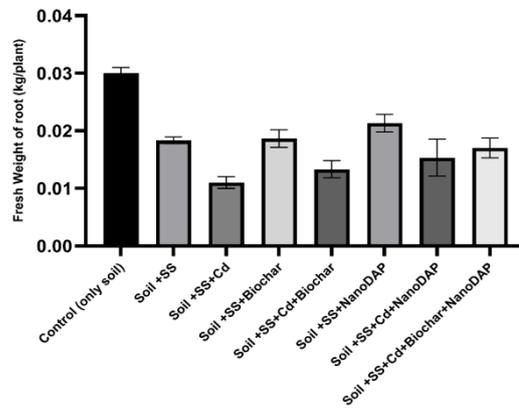
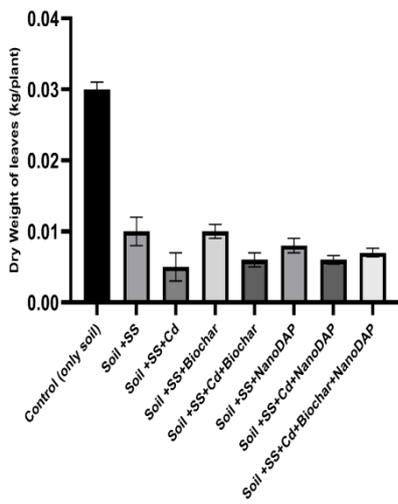
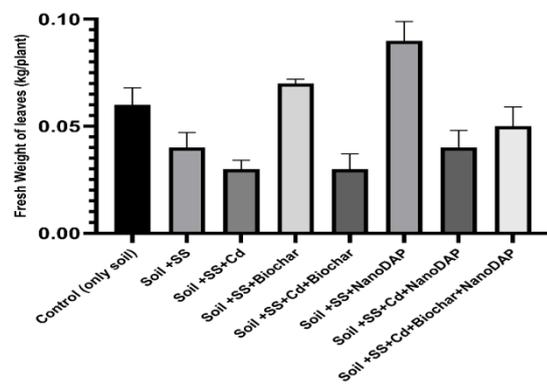
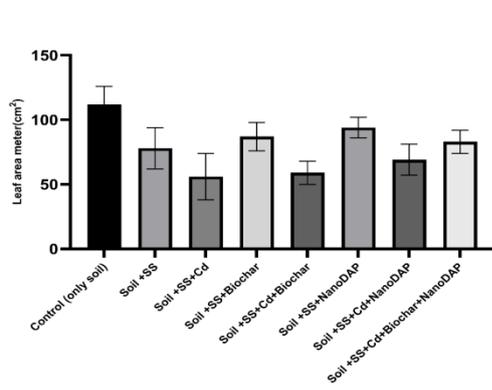
indicating that the various soil amendments and cadmium exposures significantly influenced physiological performance. The column factor (type of physiological trait) also exhibited a significant impact  $F(7, 112) = 8.715, P < 0.0001$ , reflecting that the physiological parameters responded differently to the treatments. The control plants exhibited a phosphorus level of  $0.67 \pm 0.064\%$ . Cadmium stress resulted in a pronounced decrease (Group 3:  $0.21 \pm 0.06\%$ ), suggesting impaired phosphorus assimilation. Additionally, the control plants showed the highest potassium content at  $1.89 \pm 0.24\%$  specific physiological traits significantly contributed to the variation observed in plant physiological responses.

**Table 5: Two-Way ANOVA Summary for Physiological Trait Variation under Different Treatments and Conditions**

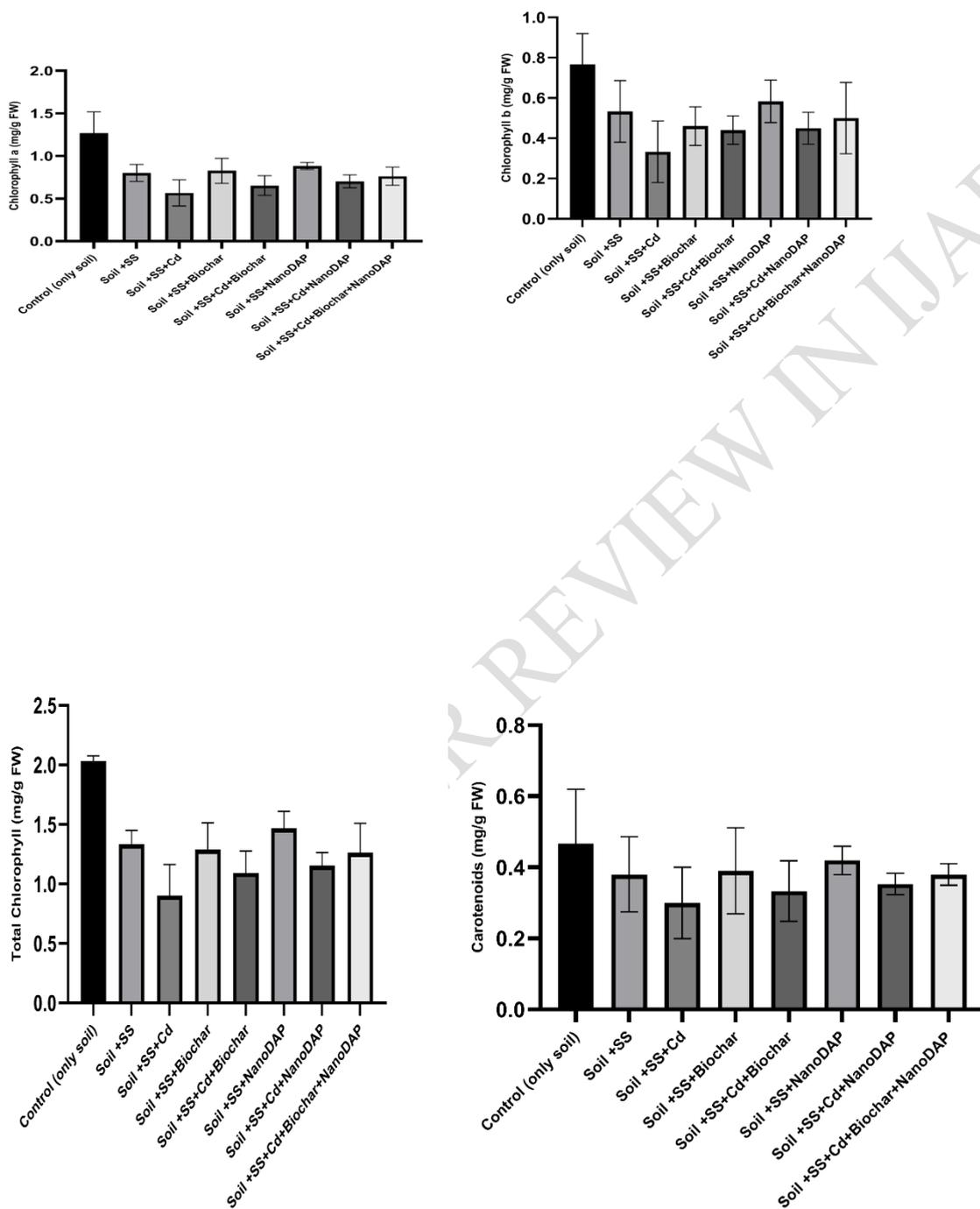
	SS	DF	MS	F (DFn, DFd)	P value
Row Factor	60.15	6	10.03	F (6, 112) = 136.9	P<0.0001
Column Factor	4.468	7	0.6382	F (7, 112) = 8.715	P<0.0001
Residual	8.202	112	0.07323		

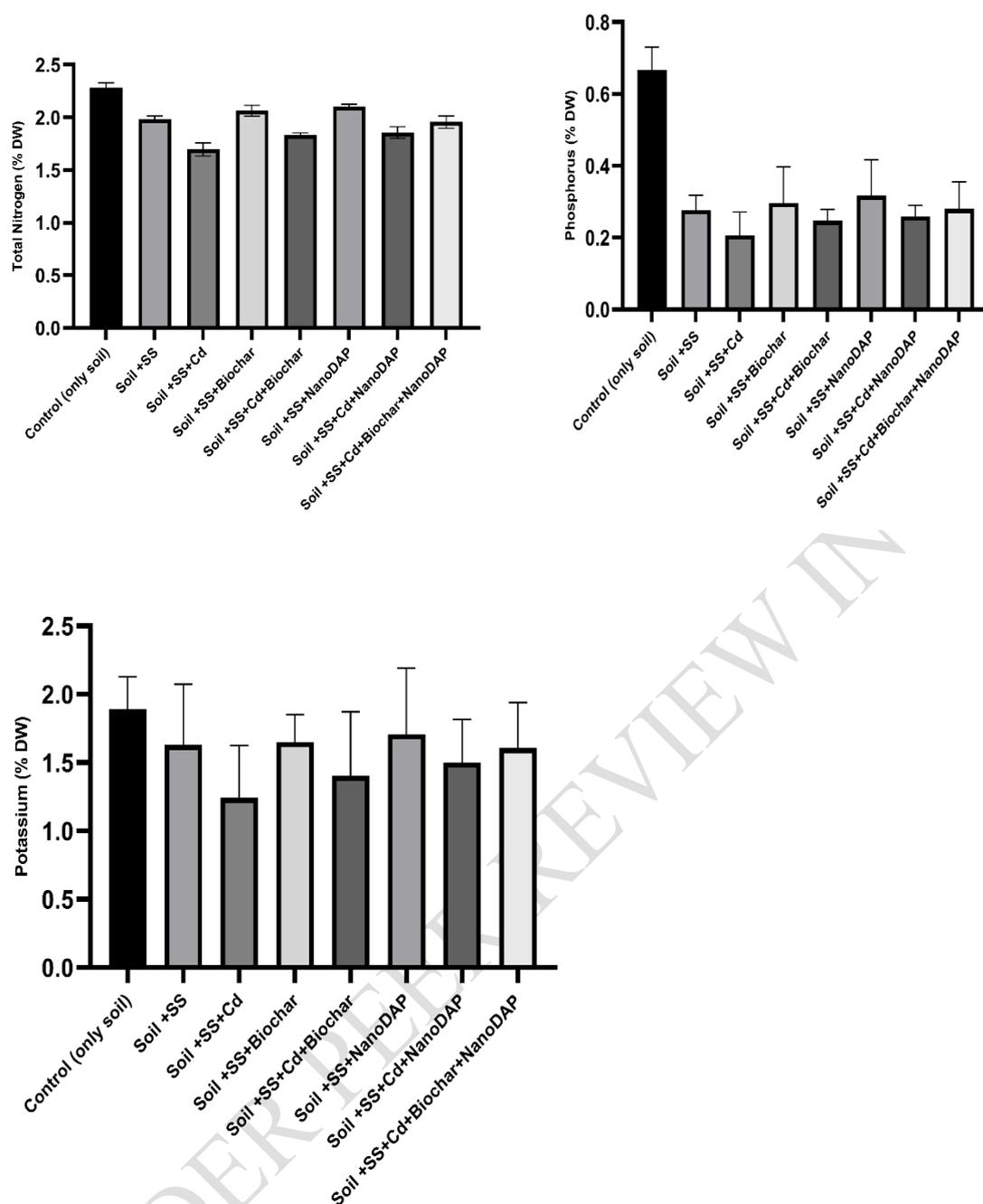
**Note:** DF: Degrees of Freedom; SS: Sum of Squares; MS: Mean Square; F: F-ratio (between-group df, within-group df); P value: Statistical significance ( $\alpha = 0.05$ ).





**Figure 1. Effect of Treatments on Morphological Traits of *Praecitrullus fistulosus*, Including (a) Plant Height, (b) Number of Leaves, (c) Leaf Area, (d–g) Biomass Parameters (Fresh and Dry Weight of Leaves and Roots), and (h) Fruit Weight.**





**Figure 2. Influence of Treatments on Physiochemical Parameters of *Praecitrullus fistulosus*, Including (a–c) Chlorophyll Content (Chlorophyll a, b, and Total), (d) Carotenoids, and (e–g) Macronutrient Composition (Nitrogen, Phosphorus, and Potassium).**

#### 4. Conclusion

Sewage sludge amendments, while beneficial for soil fertility, pose a clear risk via cadmium contamination. Biochar and nanoDAP, especially used together, effectively reduce Cd bioavailability and uptake while supporting plant growth. The synergy between immobilizing

and nutrient-enhancing effects offers a practical strategy for the sustainable and safe utilization of sewage sludge in agriculture. Cadmium contamination from sewage sludge poses a serious risk to crop productivity and food safety. The present study demonstrated that *Praecitrullus fistulosus* exhibited substantial growth inhibition, nutrient imbalance, and pigment loss under Cd stress. However, the application of rice husk biochar and Nano-DAP—particularly in combination—significantly ameliorated Cd toxicity by immobilizing cadmium in soil, enhancing nutrient uptake, restoring photosynthetic efficiency, and improving plant biomass and fruit yield. Integrating biochar with nanofertilizers provides a promising and eco-friendly strategy to manage heavy metal pollution in agricultural soils. Future work should focus on field-scale validation, long-term soil health monitoring, and optimization of amendment dosages to ensure sustainable and safe crop production under sewage sludge application.

#### References:

1. Banu, Narasimhan. (2015). Extraction and estimation of chlorophyll from medicinal plants. *International Journal of Science and Research (IJSR)*. 4. 209-212.
2. Abreu-Junior, C.H.; Brossi, M.J.D.; Monteiro, R.T.; Cardoso, P.H.S.; Mandu, T.D.; Nogueira, T.A.R.; Ganga, A.; Filzmoser, P.; de Oliveira, F.C.; Firme, L.P.; et al., 2019, Effects of sewage sludge application on unfertile tropical soils evaluated by multiple approaches: A field experiment in a commercial Eucalyptus plantation. *Sci. Total Environ.* 655, 1457–1467.
3. Alvarenga, P.; Mourinha, C.; Farto, M.; Santos, T.; Palma, P.; Sengo, J.; Morais, M.C.; Cunha-Queda, C. 2015, Sewage sludge, compost and other representative organic wastes as agricultural soil amendments: Benefits versus limiting factors. *Waste Manag.* 40, 44–52.
4. Awasthi SK. Prevention of Food Adulteration Act No 37 of 1954. Central and State Rules as Amended for 1999. Ashoka Law House; 2000.
5. Azadi, N., and Raiesi, F. (2021). Salinity-induced changes in cadmium availability affect soil microbial and biochemical functions: Mitigating role of biochar. *Chemosphere* 274, 129924.
6. Bai, J.; Sun, X.; Xu, C.; Ma, X.; Huang, Y.; Fan, Z.; Cao, X. 2022, Effects of Sewage Sludge Application on Plant Growth and Soil Characteristics at a *Pinus sylvestris* var. *mongolica* Plantation in Horqin Sandy Land. *Forests* 13, 984.
7. Bamagoos, A. A., Alharby, H. F., and Abbas, G. (2022). Differential uptake and translocation of cadmium and lead by quinoa: A multivariate comparison of

- physiological and oxidative stress responses. *Toxics* 10 (2), 68.
8. Baysal, A., Ozbek, N. and Akman, S. (2013) Determination of Trace Metals in Wastewater and Their Removal Processes. INTECH, London.
  9. Chao, Y., Yuan, J., Li, S., Jia, S., Han, J., and Xu, L. (2018). Analysis of transcripts and splice isoforms in red clover (*Trifolium pratense* L.) by single-molecule long-read sequencing. *BMC Plant Biol.* 18 (1), 300.
  10. Chen, X.B., Hu, Y.J., Qin, H.L., Zhang, X.F., Su, Y.R., Li, H.X., 2020c. Characteristics of soil nitrogen cycle and mechanisms underlying the increase in rice yield with partial substitution of mineral fertilizers with organic manure in a paddy ecosystem: a review. *Ying Yong Sheng tai xue bao J. Appl. Ecol.* 31 (3), 1033–1042.
  11. Cucina, M.; Ricci, A.; Zadra, C.; Pezzolla, D.; Tacconi, C.; Sordi, S.; Gigliotti, G. 2019, Benefits and risks of long-term recycling of pharmaceutical sewage sludge on agricultural soil. *Sci. Total Environ.* 695, 133762
  12. Cuypers, A. et al. (2023) Cadmium-induced oxidative stress responses and acclimation in plants require fine-tuning of redox biology at subcellular level. *Free Radic Biol. Med.* 199, 81–96.
  13. Dawar, K. et al. (2023) Evaluating the efficacy of activated Carbon in minimizing the risk of Heavy metals Contamination in Spinach for Safe Consumption. *ACS Omega.* 8, 24323–24331.
  14. Fewtrell, L. J., Prüss-Üstün, A., Landrigan, P., and Ayuso-Mateos, J. L. (2004). Estimating the global burden of disease of mild mental retardation and cardiovascular diseases from environmental lead exposure. *Environ. Res.* 94 (2), 120–133.
  15. Gu, W., Wang, Y., Feng, Z., Wu, D., Zhang, H., Yuan, H., Sun, Y., Xiu, L., Chen, W., Zhang, W., 2022. Long-term effects of biochar application with reduced chemical fertilizer on paddy soil properties and japonica rice production system. *Front. Environ. Sci.* 742.
  16. Han, J., et al. (2024). A biochar-based amendment improved cadmium immobilization, reduced its bioaccumulation, and increased rice yield. *Frontiers in Environmental Science* Frontiers.
  17. Islam, F. et al. (2024) Dynamic interplay of silica-coated iron oxide nanocomposite on soil-plant system to mitigate cd toxicity in rice. *J. Clean. Prod.* 471, 1–9.
  18. Islam, R., Al Foisal, J., Rahman, M., Lisa, L.A. and Paul, D.K. (2016) Pollution Assessment and Heavy Metal Determination by AAS in Wastewater Collected from Kushtia Industrial Zone in Bangladesh. *African Journal of Environmental Science*

and Technology, 10, 9-17.

19. Jamil, W., Chandio, N., Rafiq, M. *et al.*,(2023). Improving industrial wastewater irrigation: the effects on Indian baby pumpkin (*Praecitrullus fistulosus*) cultivation. *Appl Water Sci* **13**, 217.
20. Jones, C.G., Daniel Hare, J. & Compton, S.J. (1989) Measuring plant protein with the Bradford assay. *J Chem Ecol* **15**, 979–992.
21. K.L. Prakash, Harmeet Singh, Santhosh Kumar T.M and R.K. Somashekar (2018) Assessment of Heavy Metals in Municipal Sewage Sludge: A case study of Kadubeesanahalli Sewage Treatment Plant, Bangalore, India. *International Journal of Advanced Research in Engineering and Technology*, 9(3), pp 266–275.
22. K.L. Prakash, Harmeet Singh, Santhosh Kumar T.M and R.K. Somashekar,2018, Assessment of Heavy Metals in Municipal Sewage Sludge: A case study of Kadubeesanahalli Sewage Treatment Plant, Bangalore, India. *International Journal of Advanced Research in Engineering and Technology*, 9(3), pp 266–275.
23. Kang, X., Geng, N., Li, X., Yu, J., Wang, H., Pan, H., et al.,(2022). Biochar alleviates phytotoxicity by minimizing bioavailability and oxidative stress in foxtail millet (*Setaria italica*) cultivated in Cd- and Zn-contaminated soil. *Front. Plant Sci.* 13.
24. Kominko, H.; Gorazda, K.; Wzorek, Z.; Wojtas, K. 2018, Sustainable Management of Sewage Sludge for the Production of OrganoMineral Fertilizers. *Waste Biomass Valorization* 9, 1817–1826
25. Kumar, A., Kumar, A., Cabral-Pinto, M. M. S., Chaturvedi, A. K., Shabnam, A. A., Subrahmanyam, G., et al., (2020). Lead toxicity: Health hazards, influence on food chain, and sustainable remediation approaches. *Int. J. Environ. Res. Public Health* 17 (7), 2179.
26. Kumar, V.; Eid, E.M.; Al-Bakre, D.A.; Abdallah, S.M.; Širi'c, I.; Andabaka, Ž.; Kumar, P.; Goala, M.; Adelodun, B.; Singh, J.; et al., 2022 Combined Use of Sewage Sludge and Plant Growth-Promoting Rhizobia Improves Germination, Biochemical Response and Yield of Ridge Gourd (*Luffa acutangula* (L.) Roxb.) under Field Conditions. *Agriculture*12, 173.
27. Kyayesimira, Juliet & Ssemaganda, Abbey & Muhwezi, Godfrey & Andama, Morgan.(2019). Assessment of Cadmium and Lead in Dried Sewage Sludge from Lubigi Feecal Sludge and Wastewater Treatment Plant in Uganda. *Journal of Water Resource and Protection.* 11. 690-699.
28. Lazdina, D., Lazdins, A., Bebre, I. and Gusarevs, I. (2017) Technological Process of

- Sewage Sludge De-Watering by Impulse Method and Assessment of Options to Use Dried Material. *Engineering for Rural Development*, 16, 217-221.
29. Mañas, P.; Castro, E.; de las Heras, J. 2014, Application of treated wastewater and digested sewage sludge to obtain biomass from *Cynara cardunculus* L. *J. Clean. Prod.* 67, 72–78.
30. Massri, M. and Labban, L. (2014) Comparison of Different Types of Fertilizers on Growth, Yield and Quality Properties of Watermelon (*Citrillus lanatus*). *Agricultural Sciences*, 5, 475-482.
31. Mawari G, Kumar N, Sarkar S, Daga MK, Singh MM, Joshi TK, Khan NA. (2022) Heavy Metal Accumulation in Fruits and Vegetables and Human Health Risk Assessment: Findings From Maharashtra, India. *Environ Health Insights*.16:11786302221119151. PMID: 36061256; PMCID: PMC9434655.
32. Mghweno, L.R., Makokha, A.O., Magoha, H.S. and Wekesa, J.M. (2008) Environmental Lead Pollution and Food Safety around Kampala City in Uganda. *Journal of Applied Biosciences*, 12, 642-649.
33. Mourad, A. M. I., Amin, A.E.-E.A.Z., and Dawood, M. F. A. (2021). Genetic variation in kernel traits under lead and tin stresses in spring wheat diverse collection. *Environ. Exp. Bot.* 192.
34. Mukherjee, S., Singh, S. K., Jatav, S. S., Patra, A., & Reddy, G. P. (2023). Effect of Biochar Application on Heavy Metal accumulation in Different Parts of Paddy Plant. *International Journal of Environment and Climate Change*, 13(11), 4491–4500.
35. Muthusarayanan, S., Sivarajasekar, N., Vivek, J., Paramasivan, T., Naushad, M., Prakashmaran, J., et al.,(2018). Phytoremediation of heavy metals: Mechanisms, methods and enhancements. *Environ. Chem. Lett.* 16 (4), 1339–1359.
36. Namdari, M., et al. (2024). Effect of biological sewage sludge and its derived biochar on accumulation of potentially toxic elements by corn. *Scientific Reports*.
37. Nan, Q., Xin, L., Qin, Y., Waqas, M., Wu, W., 2021. Exploring long-term effects of biochar on mitigating methane emissions from paddy soil: a review. *Biochar* 3 (2), 125–134.
38. Nano-enabled agrochemicals: mitigating heavy metal toxicity and enhancing crop adaptability. *Journal of Nanobiotechnology* (2024) BioMed Central.
39. Nunes N, Ragonezi C, Gouveia CSS, Pinheiro de Carvalho MÂA.2021; Review of Sewage Sludge as a Soil Amendment in Relation to Current International Guidelines: A Heavy Metal Perspective. *Sustainability*. 13(4):2317.

40. Omidi, A. H., Cheraghi, M., Lorestani, B., Sobhanardakani, S., and Jafari, A. (2019). Biochar obtained from cinnamon and cannabis as effective adsorbents for removal of lead ions from water. *Environ. Sci. pollut. Res. Int.* 26 (27), 27905–27914.
41. Qiu, B., Tao, X., Wang, H., Li, W., Ding, X., and Chu, H. (2021). Biochar as a low-cost adsorbent for aqueous heavy metal removal: A review. *J. Anal. Appl. Pyrolysis* 155, 105081.
42. Radulescu, et al., (2014) Determination of Heavy Metal Levels in Water and Therapeutic Mud by Atomic Absorption Spectrometry. *Romanian Journal of Physics*, 59, 1057-1066.
43. Rahi, A. A. et al. (2022) Toxicity of Cadmium and nickel in the context of applied activated carbon biochar for improvement in soil fertility. *Saudi J. Biol. Sci.* 29, 743–750.
44. Rashid, I.; Murtaza, G.; Zahir, Z.A.; Farooq, M. 2018, Effect of humic and fulvic acid transformation on cadmium availability to wheat cultivars in sewage sludge amended soil. *Environ. Sci. Pollut. Res.* 25, 16071–16079.
45. Rehman, M. Z. et al. (2020) Effect of acidified biochar on bioaccumulation of cadmium (Cd) and rice growth in contaminated soil. *Environ. Technol. Innov.* 19.
46. Review: Synergistic potential of biochar and nanoparticles in phytoremediation and enhancing cadmium tolerance in plants. *Elsevier* 2024.
47. Salam, M. M. A., Kaipainen, E., Mohsin, M., Villa, A., Kuittinen, S., Pulkkinen, P., et al.,(2016). Effects of contaminated soil on the growth performance of young salix(*salixschwerinii* e. l. wolf) and the potential for phytoremediation of heavy metals. *J. Environ. Manage.* 183 (Pt 3), 467–477.
48. Seal, Tapan & Chaudhuri, Kausik. (2017). High Performance Liquid Chromatography Method for the Estimation of Water Soluble Vitamin in Five Wild Edible Fruits Consumed by the Tribal People of North-Eastern Region in India. *International Journal of Current Microbiology and Applied Sciences.* 6. 2900-2913.
49. Shahzad, A. S. et al. (2023) Acidified biochar improves lead tolerance and enhances morphological and biochemical attributes of mint in saline soil. *Sci. Rep.* 13, 8720.
50. Sheikh, L. et al. (2023) Evaluating the effects of cadmium under saline conditions on leafy vegetables by using acidified biochar. *Pakistan J. Bot.* 55, 33–39.
51. Sheikh, L., Naz, N., Oranab, S. *et al.* (2025) Minimization of cadmium toxicity and improvement in growth and biochemical attributes of spinach by using acidified biochar. *Sci Rep* 15, 5880.

52. Shweta Gautam, Priya Singh, Yogesh Shivhare. 2011 *Praecitrullusfistulosus*: A Miraculous Plant. *Asian J. Pharm. Tech.* 1(1): Jan.-Mar. Page 9-12.
53. Singh, KM and Singh, Pushpa. 2020. Challenges of Ensuring Food and Nutritional Security in Bihar. *Development Economics: Macroeconomic Issues in Developing Economies eJournal*,9 (23).
54. Speciation study: effect of biochar addition to sewage sludge on cadmium, copper, and lead in soil. *Elsevier* 2019.
55. Steele, J.C.; Meng, X.Z.; Venkatesan, A.K.; Halden, R.U. 2022, Comparative meta-analysis of organic contaminants in sewage sludge from the United States and China. *Sci. Total Environ.* 821, 153423.
56. Swain, Adyasha & Singh, Satish & Mohapatra, Kiran Kumar & Patra, Abhik. (2021). Sewage sludge amendment affects spinach yield, heavy metal bioaccumulation, and soil pollution indexes. *Arabian Journal of Geosciences.* 14. 717.
57. Tomczyk A, Boguta P, Sokołowska Z (2019) Biochar efficiency in copper removal from Haplic soils. *Int J Environ Sci Technol.*
58. Uchimiya, M., Lima, I. M., Klasson, K. T., and Wartelle, L. H. (2010). Contaminant immobilization and nutrient release by biochar soil amendment: Roles of natural organic matter. *Chemosphere* 80 (8), 935–940.
59. Uchimiya, M., Lima, I. M., Klasson, K. T., and Wartelle, L. H. (2010). Contaminant immobilization and nutrient release by biochar soil amendment: Roles of natural organic matter. *Chemosphere* 80 (8), 935–940.
60. Union E. *Heavy Metals in Wastes*. European Commission on Environment; 2002.
61. Xinde, C., Lena, M., Yuan, L., Bin, G., and Willie, H. (2011). Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. *Environ. Sci. Technol.* 45 (11), 4884–9.
62. Yadav, A.; Yadav, K.; Abd-Elsalam, K.A. 2023, Nanofertilizers: Types, Delivery and Advantages in Agricultural Sustainability. *Agrochemicals* 2, 296–336.
63. Yang, K., Wang, X., Cheng, H., and Tao, S. (2022). Enhanced immobilization of cadmium and lead adsorbed on crop straw biochars by simulated aging processes. *Environ. pollut.* 302, 119064.
64. Yanru, W., Xinxin, M., Muhammad, S., Yong, Y., and Qingming, Z. (2022). Effects of corn stalk biochar and pyrolysis temperature on wheat seedlings growth and soil properties stressed by herbicide sulfentrazone. *Environ. Technol. Innovation* 25.
65. Ye, S., Zeng, G., Wu, H., Liang, J., Zhang, C., Dai, J., Xiong, W., Song, B., Wu, S., Yu, J., 2019. The effects of activated biochar addition on remediation efficiency of

- co- composting with contaminated wetland soil. *Resour. Conserv. Recycl.* 140, 278–285.
66. Yu, X., Lu, S., 2020. Double effects of biochar in affecting the macropore system of paddy soils identified by high-resolution X-ray tomography. *Sci. Total Environ.* 720, 137690.
67. Zakaria, M. R., Farid, M. A. A., Andou, Y., Ramli, I. & Hassan, M. A. (2023) Production of biochar and activated carbon from oil palm biomass: current status, prospects, and challenges. *Ind. Crops Prod.* 199, 116767.
68. Zhang, J., Zhang, Q., Xing, J., Li, H., Miao, J., and Xu, B. (2021). Acetic acid mitigated salt stress by alleviating ionic and oxidative damages and regulating hormone metabolism in perennial ryegrass (*Lolium perenne* L.). *Grass Res.* 1 (1), 1–10.
69. Zhao, Y., Deng, Q., Lin, Q., Zeng, C. & Zhong, C. (2020). Cadmium source identification in soils and high-risk regions predicted by geographical detector method. *Environ. Pollut.* 263, 114338.
70. Zhou, F., et al. (2022). Biochar amendment reduces cadmium uptake by stimulating cadmium-resistant PGPR in tomato rhizosphere. *Ecotoxicology and Environmental Safety*.