

Application of Zinc Modified Biochars to Enhance Zinc Availability, Speciation and Pearl Millet Growth in Zn-Deficient Soil

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Abstract: Zinc deficiency poses a serious threat to numerous crops and global human population. Recently, soil biochar amendment has been promoted as a sustainable farming method. However, its consequence on the bioavailability of Zn in cereal crop has not been fully addressed. In the present study, 0.01% Zn-SO₄ (w/w) was loaded in fruit waste, farmyard manure and coconut shell pyrolysis at 400°C, for 2 h to make fruit waste Zn-modified biochar (FWZn-MBC), farmyard manure Zn-modified biochar (FYZn-MBC) and coconut shell Zn-modified biochar (CSZn-MBC). Except control treatment, all the modified biochars were applied at 1, 2 and 4% doses on zinc availability, speciation, pearl millet growth and chemical properties in Zn-deficient soil. The obtained results show that Zn mobility in soil was 70.04% with application of FYZn-MBC 4% as compared to other treatments. The maximum uptake of Zn in shoot and root by pearl millet plant was observed by 74.02 and 53% with addition of FYZn-MBC 4% as compared to control soil, due to increase of soil organic matter. The chlorophyll SPAD values in the pearl millet aerial surface increased from 30.23 to 39.24% with application of FYZn-MBC 4% than control soil. The exchangeable fraction of Zn increased from 7.34 to 14.71% with application of FYZn-MBC 4%. The correlation matrix results indicated that CaCO₃ positively correlated with soil pH, and soil organic matter had strong correlation with chlorophyll. Overall, future studies must be carried out to examine the long-term impact of FYZn-MBC 4% on Zn phyto-availability in Zn-deficient soil.

Keywords: Modified amendments, zinc phytoavailability, fractionation, pearl millet growth, soil chemical properties.

Introduction

Zinc (Zn) is a micro trace element, it has several biological processes for proper plant growth, development, human and animal health (Gondal et al., 2021; Patil et al., 2023; Youssef et al., 2024). Low and toxic level of Zn in soil-plant system may affect plant development, soil and human health (Noulas et al., 2018; Van Eynde et al., 2023). Several studies reported that the Zn deficiency in human body is associated with diet quality intensified by Zn deficient soils (Vivas et al., 2006). Zn is partially mobile in soil and mostly deficient in sandy, low organic matter content and compacted soil (Alloway, 2008). Zn is an important micronutrient for both plants and animals. Approximately 1.1 billion individuals, or 17% of the global population, may be at risk for the zinc deficiency. It has been observed that the Zn in normal soils ranges from 10 to 300 mg/kg, while 50-55 mg/kg on the average basis (Malle, 1992; Barber, 1995; Kiekens, 1995). Soils of Pakistan, especially in rice track areas are deficient in Zn by approximately 70% (Younas et al., 2023). Chen et al. (2017) reported that nearly 50% of population in Asia (around 2 billion people) is at the risk of Zn-deficit. Furthermore, Wessells & Brown (2012) stated that around 17.3% of people globally are at risk of insufficient Zn intake. Noulas et al. (2018) suggested that the main areas of Zn-deficient soils and food crops should be treated with Zn additives, mostly fertilizers in order to increase Zn

phyto-availability, and Zn use efficiency to crops. To sort out Zn deficiency problem in soil-plant system, farmers are normally applying Zn sulfate and Zn chelated sources, Zn foliar and seed Zn coating for improving Zn bioavailability and crop yield (Zulfiqar et al., 2023). Li et al. (2013) observed that Zn deficiency decreased the plant height, biomass and Zn uptake in the root and shoot by sorghum. Maharajan et al. (2023) stated that Zn deficiency affects some plants growth and development including sorghum. Zn deficiency in rice track areas has been increasing continuously resulting in reduced rice growth and yield parameters (Nawaz et al., 2021; Sher et al., 2022). The data were collected from the Science direct publications by using key words "Zinc deficiency in the soils of Pakistan" from 2005 to 2024 (Fig. 1).

However, mobility and availability of Zn from soil-plants system depends on many factors such as soil pH, total Zn proportion, quantity, temperature, soil mineralogy and quality of soil organic matter (Mousavi, 2011). There are two concepts of Zn immobilization/phytoremediation such as a) immobilization of Zn polluted sites and reduce their uptake by plant amended with different additives (Yuan et al., 2019; Han et al., 2020; Yang et al., 2021; El-Naggar et al., 2024), and b) concept is phytoremediation of Zn in soil system through hyper-accumulator plants

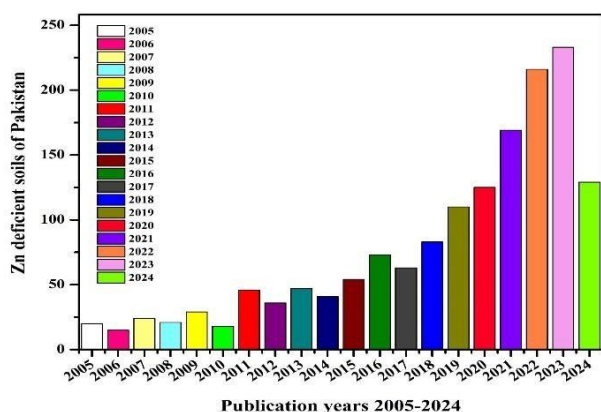


Fig. 1 Published literature showing zinc deficiency in the soils of Pakistan from 2005-2024.

(Belouchrani et al., 2016; Soares et al., 2021), and organic acids (Garrido et al., 2018; Guo et al., 2019; Hu et al., 2021; Zhang et al., 2023). Noulas et al. (2018) address the issue of zinc (Zn) shortage in crops and people, it is crucial to have a fundamental understanding of the dynamics of zinc (Zn) in soils, water, and plants. Recena et al. (2021) assessed the Zn availability in soil-plant system and Zn fertilizers use efficiency amended in soil medium, as a result found that poorly crystalline Fe-oxides and P availability showed a positive role on Zn accessibility to plants in soil. In the earlier study Khan et al. (2004) applied ZnSO_4 at 0, 5, 10 and 15 kg dosage to enhance the Zn availability in the leaf and roots by paddy crop in eight different calcareous soils. Liu et al. (2018) assessed that chemical fractions and availability of zinc in winter wheat amended with N application rates 0.05, 0.2, and 0.4 g kg^{-1} and Zn application rates 0 and 10 mg kg^{-1} , as a result found positive response on plant growth and yield. Shahgoli et al. (2021) compared the impact of different biochars on DTPA-Zn in acidic and alkaline soils. Kuziemska et al. (2022) applied Zn at 200, 400 and 600 mg Zn/kg doses along with cattle manure, chicken manure and spent mushroom substrate for enhancing yield, Zn bioaccumulation factor and tolerance indices by cocksfoot plants. Chattha et al. (2023) evaluated the impact of foliar zinc application on different growth stages to increase the growth, yield, quality, and kernel bio-fortification of fine rice. Novelty point of view, the impact of Zn-modified biochars at different doses on Zn availability, fractions, uptake by pearl millet plant and soil chemical properties has not been documented. To fill the above gaps, the aim of present study was to assess the impact of fruit waste Zn-modified biochar (FWZn-MBC), farmyard manure Zn-modified biochar (FYZn-MBC) and coconut shell Zn-modified biochar (CSZn-MBC) applied at 1, 2 and 4% doses on zinc availability, speciation, pearl millet growth and chemical properties in Zn-deficient soil. It was hypothesized that different Zn-modified biochars at 1, 2 and 4% doses may enhance the Zn availability in soil and increase their uptake by plant in Zn-deficient soil.

Materials and Methods

Soil Collection and Characterization

The soil of the present study was collected from District Larkana. The studied soil was deficient in Zn due to continuous growing of native and/or hybrid cereal and vegetables crops, as a result this area had become deficient in trace elements especially in Zn. In addition, farmers normally applied Zn sulphate and Zn-chelated sources to sort out Zn deficiency problem in the soil-plant system. In the present study, randomly composite soil samples were collected from surface layer from different points at 0-15 cm. The composite soil samples were air dried for 4-5 days at room temperature. All the debris and non-soil material was removed manually and then soil samples were ground to pass 2mm sieve size. The selected basic properties of soil samples are given in Table 1.

Table 1. Basic parameters of soil and Zn-modified biochars.

Parameter s	Soil	FWZn-MBC	FYZn-MBC	CSZn-MBC	*Target value of soil
Sand %	15.18	-	-	-	-
Silt %	24.10	-	-	-	-
Clay	60.72	-	-	-	-
Textural class	Clay	-	-	-	-
EC dS m^{-1}	0.39±0.4	0.24±0.2	0.98±0.3	0.69±0.2	-
pH	7.71±0.2	8.09±0.7	7.98±0.5	8.20±0.1	-
OM %	0.85±0.9	13.87±0.6	21.06±0.9	15.54±1.3	-
CEC	11.8±0.3	23.5±0.4	29.7±0.2	31.6±1	-
Lime CaCO_3 (%)	5.41±0.7	16.12±0.6	11.5±0.5	17.9±0.8	-
DOC mg/kg	14.5±0.1	23.6±0.3	21.3±0.2	29.6±1	-
Total Zn mg/kg	33.8±0.6	7.4±0.4	11.3±0.8	6.5±0.7	50
Biochar yield (g/100g dry matter)	-	30.27±0.2	31.12±0.8	33.1±0.6	-

Note: FWZn-MBC= Fruit waste Zn-modified biochar, FYZn-MBC= Farmyard manure Zn- modified biochar, and CSZn-MBC= Coconut shell Zn- modified biochar. *Denneman, Robberse, 1990), Source. WHO (1996).

Material Collection and Biochar Preparation

In the present work, fruit waste was collected from fruit market at Karachi. Farmyard manure was collected from Cattle colony, Karachi, and coconut shells were collected from local fruit market of Karachi. In addition, Zn- SO_4 in the form of solid was purchased from scientific store at Urdu Bazar Karachi. Approximately, 0.01% Zn- SO_4 (w/w) was loaded in fruit waste, farmyard manure and coconut shell and then pyrolyzed in the muffle furnace at 400 °C, for 2 h aiming to make modified biochars such as fruit waste Zn-modified biochar (FWZn-MBC), farmyard manure Zn-modified biochar (FYZn-MBC) and coconut shell Zn-modified biochar (CSZn-MBC) (Kołodnyńska et al., 2012; Ding et al., 2014; Saletnik et al., 2016). The dry matter yield of all Zn-modified biochars was noted after pyrolysis process and muffle furnace cooled. The chemical properties of Zn-modified

biochars was mentioned in Table 1. All the Zn-modified biochars were amended in Zn-deficient soil at 1, 2 and 4% doses. The treatment design and doses are indicated in Table 2.

Table 2. Treatment code and dose.

Treatments	Code	Dose
T1	Ck	0 %
T2	FWZn-MBC	1%
T3	FWZn-MBC	2%
T4	FWZn-MBC	4%
T5	FYZn-MBC	1%
T6	FYZn-MBC	2%
T7	FYZn-MBC	4%
T8	CSZn-MBC	1%
T9	CSZn-MBC	2%
T10	CSZn-MBC	4%

Pot experiment

A pot experiment was carried out under laboratory conditions to assess the impact of Zn-modified biochars on pearl millet plant growth, Zn solubility in soil, soil chemical properties and uptake of Zn by pearl millet plant. Approximately, 1 kg dried sieved soil along with 1, 2 and 4% of each doses of Zn-modified biochars were carefully mixed together with soil except control treatment and placed in the plastic bottles. The soil was moistened 70% with distilled water and kept all the pots for 1 week incubation period aiming to react Zn-modified biochars with soil particles for modification in soil properties and enhancing Zn content. Approximately, 10 seeds of pearl millet plant were sown in each pot. The trial was setup including (1 control + 9 treatments + 3 replications + 1 soil + 1 crop pearl millet), total 40 pots. During the germination process about 80% soil moisture was kept. After germination of 1 week 5 healthy plants were left in each pot. Everyday plants were watered with distilled water, and the evaporated water was maintained on the daily basis as per plant need. No any chemical fertilizer was added in the pots throughout the experiment duration.

Soil and biochars analysis

Soil texture was determined by Bouyoucos Hydrometer method (Bouyoucos, 1962). The soil texture of studied soil is shown in Figure 2. The pH and EC was determined in a 1:2 (soil + water) ratio for soil and 1:10 ratio was used for Zn-modified biochars by using a digital pH/EC meter (USEPA, 2004). Organic matter in soil and Zn-modified biochars was determined by Walkley-Black method (Jackson, 1969). Calcium carbonate CaCO_3 in soil and Zn-modified biochars was measured by using acid neutralization method (Kanwar & Chopra, 1959). The CEC in soil and Zn-modified biochars was detected following the USEPA Method 9080 (USEPA, 1986). The dissolved organic carbon (DOC) in soil and Zn-modified biochars was measured in a 1:10 ratio (W/V) soil/material and ultra-pure water by using an automated TOC analyzer Shimadzu TOC-

V (Antoniadis & Alloway, 2002). The total Zn content in soil and Zn-modified biochars were determined via digesting a mixture of H_2SO_4 and HClO_4 (1:1 v/v) by PerkinElmer Atomic Adsorption Spectrophotometer (Pin AAcle 900F, China), followed by (Burt 2004; Ure 1990).

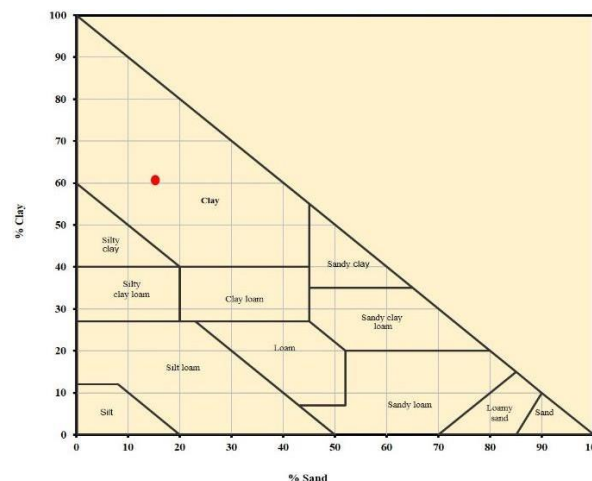


Fig. 2. Textural class of studied soil.

Zn fractions in soil

The Zn speciation such as residual, organic matter bonded, Fe, Mg oxide bounded and exchangeable in soil were tested by using the BCR-sequential extraction method through AAS (Z-2000, Japan) using ICP-OES (Lo & Yang, 1998).

Plant analysis

The plants were harvested 30 days after sowing. All the plants were carefully uprooted from each pot and washed with distilled water and then cleaned with tissue papers for removing any contamination. All the plants were dried in oven for 3-4 days at 65°C . After that dry biomass was noted, and all the plants as per treatment were separately grinded by electric mill. After that, all the grinded plant samples were kept in polyethylene bags for testing the total Zn in pearl millet plant. About, 0.5g of dried pearl millet root and shoot biomass and 0.2g of Zn-modified biochars were digested on a hot plate by adding a mixture of acids (HNO_3 : HClO_4 at ratio 2:1), after digestion, Zn concentration in plant biomass was tested by using atomic absorption spectrometry AAS, PerkinElmer Analyst™ 800 according to Jones Jr & Case (1990), and Zhu et al. (2018). Chlorophyll SPAD values in leaves by pearl millet plant were detected by Chlorophyll SPAD 502 meter (Lahori et al., 2020).

Statistical analysis

The data of three replicated was statistically analysed for one-way analysis of variance at $p < 0.05$ HSD test by using Statistics 8.1 software. The mean value and standard deviation was performed Excel 2016. All the

mean data and standard error were used for make graphs through Origin Pro. 8.5v. The correlogram layout was performed among the studied parameters by using Statistical Tools for High-Throughput Data Analysis (STHDA).

Results and Discussion

The dry biomass of pearl millet plants increased with application of studied biochars in Zn-deficient soil. The maximum increase of pearl millet dry biomass by 43.02% was observed with application of FYZn-MBC 4% as compared with other treatments (Fig. 3a). The highest chlorophyll SPAD values in the pearl millet leaves increased ranging from 30.23 to 39.24 with addition of FYZn-MBC 4% as compared with control soil (Fig. 3b). The highest electrical conductivity (EC) was noted ranging from 0.41 to 0.55 ds/m¹ with addition of CSZn-MBC 4% than control soil (Fig. 3c). Compared with control treatments, the highest soil pH value increased from 7.74 to 9.05 amended with CSZn-MBC 4% as compared with control soil (Fig. 3d). Farooq et al. (2020) stated that zinc and mango pruning wood made biochar as amendment improved the wheat production as compared with control soil.

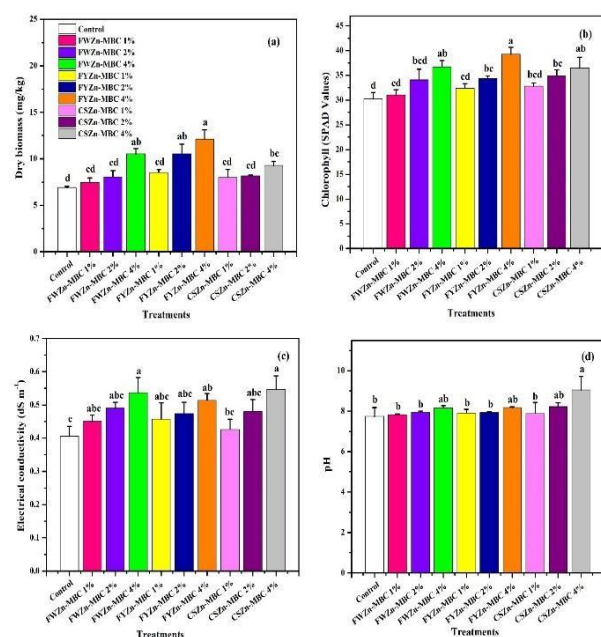


Fig. 3. Impact of Zn-modified biochars on pearl millet plant dry biomass (a), chlorophyll (b), EC (c), and pH (d).

The application of FYZn-MBC 4% in soil significantly increased the soil organic matter (SOM) up to 46.25% than control soil (Fig. 4a). The cation exchange capacity (CEC) in soil was promoted up to 50.16% amended with CSZn-MBC 4% than control treatment (Fig. 4b). Compared with control soil, the maximum lime (CaCO₃) proportion in soil was observed by 53.44% with application of CSZn-MBC 4% (Fig. 4c). The dissolved organic carbon (DOC) in soil increased by 43.26% with addition of CSZn-MBC 4% as compared with other treatments (Fig. 4d). Nejad et al. (2021)

found that EC, pH and CEC in soil were increased with addition of rice biochar and maple biochar at 1 and 2% application rate as compared to control treatment. Liu et al. (2015) observed an increase of DOC in soil with application of mixed crop straw-derived biochar. Lahori et al. (2023) reported that an increase of CaCO₃ was observed in soil with application of organic press mud compost. Our results are in-line with Saleem et al. (2024) who found an increase of maize shoot dry weight, soil EC, pH and soil organic matter (SOM) in alkaline calcareous soil with application of maize-straw biochar as compared with control soil.

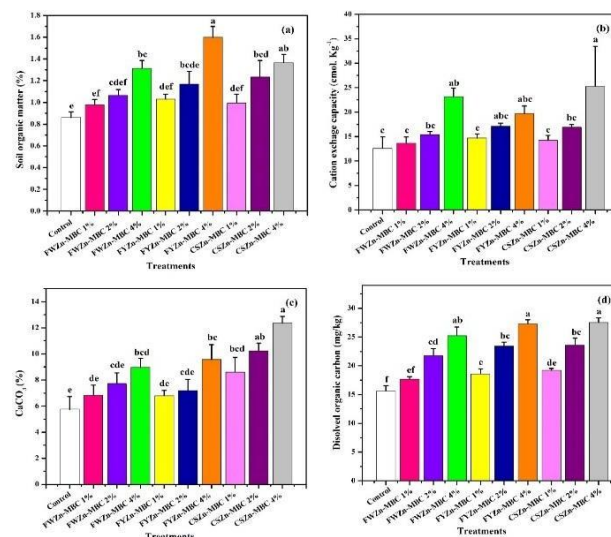


Fig. 4. Impact of Zn-modified biochars on soil O.M (a), CEC (b), CaCO₃ (c), and DOC (d).

The maximum Zn content in soil increased by 70.04% with application of FYZn-MBC 4% followed by other treatments (Fig. 5a). The exchangeable fraction of Zn increased from 7.34 to 14.71% with application of FYZn-MBC 4%, whereas CSZn-MBC 4% amendment reduced it from 7.34 to 2.10% than control treatment. It was observed that the Fe, Mg oxide-bonded fraction of Zn increased from 3.7 to 6.21% with application of FWZn-MBC 4%, but the application of CSZn-MBC 4% reduced it from 3.7 to 1.12% as compared with control soil. The organic matter-bonded speciation of Zn increased from 2.87 to 6.1% with application of FYZn-MBC 4% amendment. However CSZn-MBC 4% amendment evidently reduced the organic matter-bonded fraction from 2.87 to 1.76% than control. The residual fraction of Zn in soil increased from 4.76 to 6.13% with addition of FWZn-MBC 4%, whereas the maximum reduction of Zn residual fraction was observed from 4.76 to 1.76% with application of CSZn-MBC 4% as compared with control treatment (Fig. 5b). The above results reveal that the higher dose of FWZn-MBC 4% had increased the Fe, Mg oxide-bonded and residual fractions of Zn in soil. Similarly, the application of FYZn-MBC 4% as amendment evidently enhanced the exchangeable and organic matter-bonded fractions of Zn in soil, which could be the main reason of maximum enhanced Zn uptake in the shoot and root by pearl millet plant. However, the addition of CSZn-

MBC 4% at higher dose potentially reduced the exchangeable, Fe, Mg oxide-bonded, organic matter-bonded and residual fractions of Zn in soil as compared with control treatment. Overall, it was noted that the behavior of Zn-modified biochars was dissimilar on the Zn fractions in the soil medium, it could be due to changes in soil properties, type of biochar, application rate and soil type. Blake & Goulding (2002) examined that plants have a natural mechanism for accumulating and storage of the nutrients based on their bioaccumulation in the soil system as per plant need. Fan et al. (2016) reported that soil organic matter is the main mechanism underlying the Zn sorption in soil. Kuziemska et al. (2022) stated that Zn mobility in soil depends on organic matter content in the soil. Hussain et al. (2022) found that rock phosphate-vegetable modified biochar at 0.5% and green coconut-modified biochar at 2% enhanced the mobility of Zn in agriculture polluted soil. Nejad et al. (2021) observed that exchangeable and carbonate fraction of Zn was reduced in soil with application of rice biochar and maple biochar by 1 and 2% as compared with control soil.

The Zn accumulation in the shoot and root by pearl millet plant evidently increased with application of Zn-modified biochars than control soil. It was found the maximum Zn absorption in the shoot and root by pearl millet plant was observed by 74.02% and 53% with addition of FYZn-MBC 4% than control treatment (Fig. 5c, d). It can be assumed that FYZn-MBC 4% was highly effective for enhancing Zn uptake by pearl millet plant due to increase of exchangeable and organic matter-bonded fractions of Zn in the soil.

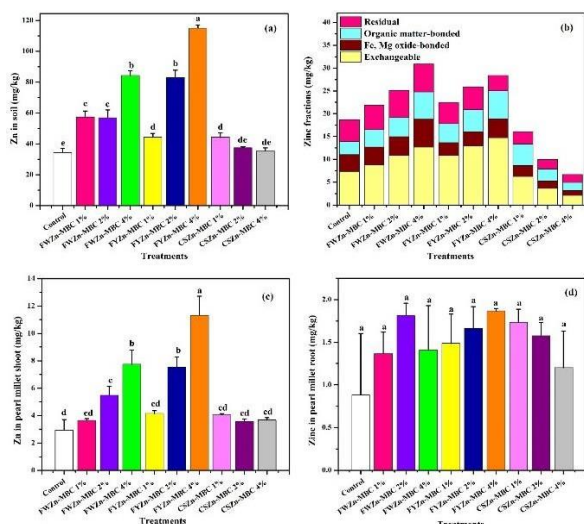


Fig. 5. Impact of Zn-modified biochars on Zn in soil (a), Zn fractions (b), Zn in shoot (c), and Zn in root (d).

It was found that farmyard manures are enriched in Zn source, as a result can release Zn proportion in soil system at favorable soil conditions, application rate and aging factor of amendments. Gupta et al. (2016) stated the accumulation of Zn^{2+} in divalent ions in the root by plant through mass flow and diffusion mechanisms. Moreno-Lora et al. (2019) examined that the mobility

and availability of Zn from soil-plant system as a result depends on changes in soil properties and biotic factors in the rhizosphere. However, Nejad et al. (2021) revealed that accumulation of Zn in the root and shoot by lettuce was reduced with application of rice biochar and maple biochar at 1 and 2% dosage than control soil. Hussain et al. (2022) observed that the Zn uptake in the root and shoot by mustard plant with application of green coconut shell modified biochar, chicken manure modified biochar and vegetable waste modified biochar as compared with control treatment.

Correlation matrix of studied parameters

The data in Figure 6 indicated the correlation matrix among electrical conductivity (EC), pH, cation exchange capacity (CEC), dissolved organic carbon (DOC), lime ($CaCO_3$), soil organic matter (OM), chlorophyll (CL), Zn in soil, Zn in pearl millet shoot and Zn in pearl millet root. The results showed that lime ($CaCO_3$) was positively and significantly correlated with soil pH. Furthermore, soil organic matter (OM) had highly significant correlation with Cl content by pearl millet plant. Soil CEC was found to be positively and significantly correlated with soil EC. The Zn in pearl millet shoot was significantly correlated with Zn in soil. Fang et al. (2017) revealed that soil pH had a negative correlation with Zn in soil medium. Lahori et al. (2023) stated that soil organic matter (OM) was positively correlated with plant dry biomass.

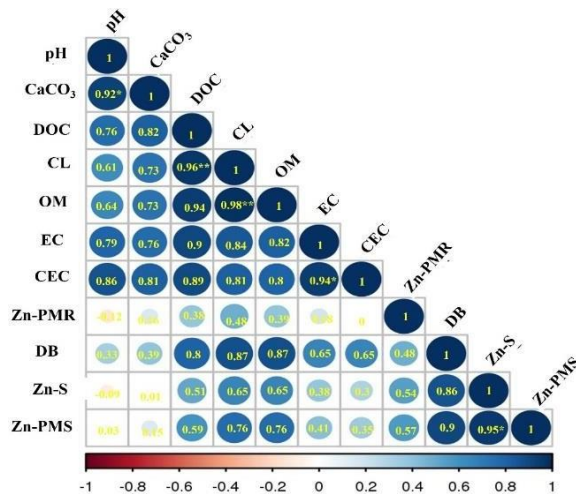


Fig. 6. Corrologram layout between pH, $CaCO_3$, dissolved organic carbon (DOC), chlorophyll (CL), organic matter (OM), electrical conductivity (EC), cation exchange capacity (CEC), zinc in pearl millet shoot (Zn-PMS), dry biomass (DB), zinc in soil (Zn-S), and Zn in pearl millet root (Zn-PMR).

Conclusion

It was concluded that, the pearl millet plant dry biomass increased with addition of Zn-modified biochars. It was observed that FYZn-MBC 4% showed high potential to enhance plant biomass as compared with other treatments. Soil chemical properties increased at FWZn-MBC 4%, FYZn-MBC 4%, and CSZn-MBC 4% than control treatment. All the Zn-modified biochars

evidently increased the total Zn in soil as compared with control treatment. It was noted that Zn availability in soil, pearl millet shoot and root evidently increased with application of FYZn-MBC 4%. The application of FWZn-MBC 4% had increased the Fe, Mg oxide-bonded and residual fractions of Zn in soil. Moreover, FYZn-MBC 4% as amendment had increased the exchangeable and organic matter-bonded fractions in soil. Similarly, the addition of CSZn-MBC 4% as amendment reduced the exchangeable, Fe, Mg oxide-bonded, organic matter-bonded and residual fractions of Zn in soil due to increase of soil pH and CaCO₃. Overall, the application of Zn along with farmyard manure made biochar highly effective to enhance Zn availability in soil and increase their uptake by plant in Zn-deficient soil. Future studies must be carried out to assess the long-term impact of these studied amendments on Zn bio-fortification, Zn and plant interaction, soil biological properties, Zn mechanism, enzymatic activities and hybrid rice cultivation under field conditions.

Conflicts of Interest: The authors declare no conflicts of interest in this research.

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