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Synthesized activated-biochar and modified-fly ash curbed Cd and Pb uptake in polluted soils and wastewater-irrigated wheat (*Triticum aestivum*)

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ABSTRACT

Wastewater-irrigation for crops becomes inevitable due to freshwater scarcity, which tends to develop lead (Pb) and cadmium (Cd) toxicity in wastewater-irrigated crops and soils. Therefore, for effective removal of Pb and Cd, an ecological strategy is needed. The present study was carried out during winter (*rabi*) season of 2023–2024 at ICAR-Indian Institute of Soil Science, Bhopal, Madhya Pradesh aimed to prepare steam-activated wheat straw biochar (SABC), and microwave-assisted alkali-infused fly ash (MMFA); to assess their effect on the Pb and Cd uptake by wheat (*Triticum aestivum* L.) in polluted- and wastewater-irrigated soils. Results revealed that SABC and MMFA amendments significantly improved plant growth compared to control and unamended soils. The MMFA and SABC reduced the Cd and Pb uptake, respectively by the above- and below-ground biomass of wheat. The addition of SABC considerably raised the *p*H and SOC of the soil by 0.18 units and 26%, respectively over control, polluted- and wastewater-irrigated soils. The SABC and MMFA amended soils, reduced the DTPA-extractable Pb and Cd by 58–72% and 35–61%, respectively. Furthermore, assessment of the translocation factor (TF) and bioconcentration factor (BCF) also support the capacity of SABC and MMFA to immobilize Pb and Cd in soil and lower their phytoavailability in contaminated soil, thereby reducing concerns related to food security.

Keywords: Cadmium, Lead, Modified fly ash, Steam-activated biochar, Wastewater irrigation, Wheat

By 2025, it's predicted that two-thirds of the world's population will experience water scarcity; hence, recycling of wastewater becomes essential. Every year, about 5500 billion cubic meters (BCM) of fresh water and subsequently global-food chain is contaminated with heavy metals like lead (Pb) and cadmium (Cd) (Minhas *et al.* 2022). Both Pb and Cd are fatal to humans. According to World Health Organization (WHO) and United States Environmental Protection Agency (USEPA), Pb above 0.01 mg/L in wastewater and 5.0 mg/L in water reclaimed from effluent for irrigation; whereas, Cd above 0.003 mg/L in wastewater and 0.01 mg/L in water reclaimed from effluent for irrigation is considered unsafe (Kinuthia *et al.* 2020). Therefore, to reduce the levels of Pb (II) and Cd (II), wastewater must be appropriately treated before being used. Physical, chemical, and biological techniques such as flocculation, electrodialysis, adsorption, membrane separation, filtration, ion exchange, and precipitation can all be used to get rid of metal ions (Shrestha *et al.* 2021, Juve *et al.* 2022, Minhas *et al.* 2022). Among several techniques, the adsorption technique is comparatively cheaper, having high reusability of adsorbents, design flexibility, low energy consumption, and stable operating conditions (Shrestha *et al.* 2021). The problem of cost involvement and source accessibility incurred during the synthesis of adsorbents may be resolved by using agricultural residues like wheat straw and industrial wastes like fly ash as adsorbents. However, due to a variety of issues, such as low metal removal efficacy, limited surface area, fewer active sites for adsorption, and fewer surface functional groups, wheat straw, and fly ash are unable to function as an effective adsorbent directly. Production of steam-activated wheat straw biochar (SABC) and microwave-assisted modified fly ash (MMFA) could expose more surface functional groups, thereby increasing the metal adsorption capability (Chen *et al.* 2022, Ding *et al.* 2022). However, very few information is available on this topic. To adsorb two heavy metals (Pb and Cd) efficiently from wastewater, the present work presents the preparation and comparison of one organic material, such as physically modified agro-waste (steam-activated wheat straw biochar), with an industrial waste, such as microwave-

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assisted modified fly ash. Synthesized adsorbents were applied to produce wheat (*Triticum aestivum* L.) under wastewater irrigation.

MATERIALS AND METHODS

The present study was carried out during winter (*rabi*) season of 2023–2024 at ICAR-Indian Institute of Soil Science, Bhopal, Madhya Pradesh. Steam-activated wheat-straw biochar (SABC) was synthesized using wheat straw feedstock (40.1% C and 0.3% N) at ICAR-Central Institute of Agricultural Engineering, Bhopal, Madhya Pradesh at a temperature of 700°C in a batch-style tubular stainless-steel reactor (length 600 mm, internal diameter 100 mm). A steam generator producing steam at a pressure of two bars was used to create SABC. Microwave-assisted modified-fly ash (MMFA) was synthesized by reacting fly ash with 10% NaOH, followed by microwave-irradiation at a power of 700 W (temperature 60 °C) for 10 min, finally washing with distilled water and drying. Synthesized SABC and MMFA were characterized physiochemically and microscopically. Detailed characteristics of SABC and MMFA is presented in Table 1. Scanning electron microscopic (SEM) images of SABC and MMFA are depicted in Fig. 1, which indicates the surface morphology of SABC and MMFA.

A pot-culture experiment with wheat var. HI-1544 was conducted at ICAR-Indian Institute of Soil Science, Bhopal (23.3078° N, 77.4069° E), Madhya Pradesh at the net house using 3 kg of soil/pot (Vertisol type of soil). The experimental soil had 7.93 *p*H, 0.19 dS/m electrical conductivity, 0.52% soil organic C, 78 mg/ kg available-N, 5.6 mg/kg available P, 174 mg/kg available K, 0.17 mg/kg total Pb and 0.09 mg/kg total Cd. A pot experiment was conducted with 80:40:40 kg/ha equivalent doses of N: P_2O_5: K_2O. Total seven treatments were implemented, viz. T_1, Control; T_2, Polluted soil; T_3, Polluted Soil + SABC (1%, w/w); T_4, Polluted soil + MMFA (1%, w/w); T_5, Fresh soil + Wastewater application; T_6, Fresh soil + Wastewater application + SABC (1%, w/w); T_7, Fresh soil + Wastewater application + MMFA (1%, w/w). The experiment was laid out in a completely randomized design (CRD) replicated thrice. In these treatments, polluted soil was created by blending one level of each Pb (II) (200 mg/kg of soil) and Cd (II) (10 mg/kg of soil). The necessary quantities of Pb (II) and Cd (II) were introduced in solution form as $Pb(NO_3)_2$ and $Cd(NO_3)_2.4H_2O$, and both were mixed thoroughly with the soil, allowing the soils to equilibrate for a month to achieve polluted soil. In other treatments, Pb (II) and Cd (II) were applied through synthetic wastewater (sewage water enhanced with $Pb(NO_3)_2$ and $Cd(NO_3)_2.4H_2O$, respectively) to the fresh soil during irrigation. The

Table 1 Detailed characteristics of steam-activated wheat-straw biochar and microwave-assisted modified fly ash

Parameters	SABC	MMFA	Methods
*p*H (1:5)	10.86 ± 0.15	9.56 ± 0.12	*p*H and EC meter
EC (1:5) (dS/m)	4.31 ± 0.26	0.67 ± 0.02	
Total C (%)	69.50 ± 1.05	0.02 ± 0.01	Energy-dispersive X-ray spectroscopy (EDS) Spectra analysis
Total N (%)	0.58 ± 0.04	Traces	
Total O (%)	20.85 ± 0.53	53.62 ± 2.60	
Total Si (%)	2.69 ± 0.21	13.77 ± 1.11	
Total P (%)	ND	0.08 ± 0.02	
Total- K (%)	4.29 ± 0.27	0.55 ± 0.05	
Total Ca (%)	0.65 ± 0.03	239.30 ± 6.65*	
Total Mg (%)	0.07 ± 0.00	83.80 ± 2.69*	
Total Fe (%)	0.13 ± 0.02	1.10 ± 0.04	ICP-OES analysis
Total Mn (μg/g)	119.93 ± 4.72	97.72 ± 5.63	
Total Cu (μg/g)	42.93 ± 4.31	63.76 ± 3.77	
Total Zn (μg/g)	36.06 ± 2.72	44.83 ± 3.59	
Total Ni (μg/g)	24.80 ± 2.40	34.96 ± 1.98	
Total Cr (μg/g)	16.33 ± 2.47	9.63 ± 0.42	
Total Co (μg/g)	3.06 ± 0.23	29.63 ± 2.64	
Total Pb (μg/g)	0.17 ± 0.02	9.44 ± 1.85	
Total Cd (μg/g)	ND	ND	
Specific surface area (m^2/g)	326 ± 6.30	29.3 ± 2.51	EGME

SABC, Steam-activated wheat-straw biochar; MMFA, Microwave-assisted modified fly ash.

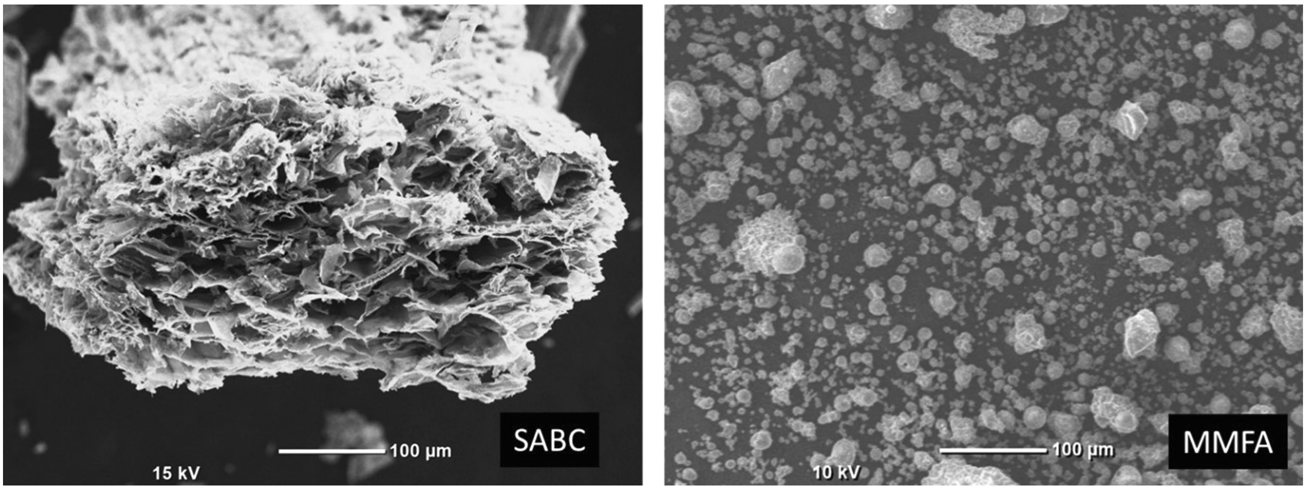

Fig. 1 Scanning electron microscopic (SEM) images of synthesized steam activated wheat-straw biochar (SABC) and microwave-assisted modified fly ash (MMFA).

polluted soil contained the same quantities of metals, with fresh soil receiving precisely the same amounts of metal through the synthetic wastewater. As stated by the World Health Organization (WHO), the acceptable thresholds for lead (Pb) and cadmium (Cd) in soil are regarded to be 85 mg/kg for Pb and 0.8 mg/kg for Cd, respectively. Polluted soil had comparatively higher Cd and Pb concentration than the safe permissible limit. In each pot, treatments were implemented following standard protocols (Sarkar *et al.* 2018) and moisture was maintained 60% of water holding capacity. Treated pots were kept for a week with continuous monitoring of soil moisture. Initially 8 wheat seeds (soaked in water for 6 h) were hand dipped at ~5 cm soil depth, but 4 germinated plants were kept finally. Water (both fresh and wastewater) was applied at regular intervals. Total water quantity was calculated following the total water requirement of wheat for first 60 days after sowing (DAS). After 60 DAS, wheat plants from each treatment were uprooted carefully and washed with tap water before measuring shoot and root fresh weight. Leaf area and chlorophyll contents were measured in fresh leaves following protocols elaborated in Saha *et al.* (2021). Dry weight of shoot and root was taken after oven drying at 65 ± 2°C for two days (until constant weight achieved). The Cd and Pb concentrations in wheat shoot and root was determined under diacid wet-digestion (HNO_3: $HClO_4$ at 9: 3 ratio) on a sand-bath hotplate. Cd and Pb concentrations were determined using ICP-OES (Perkin Elmer® Avio® 560 Max ICP-Optical Emission Spectrometer, Shelton, USA). Post-harvest fresh soil samples were used to assess dehydrogenase (DHA) and fluorescence di-acetate (FDA) enzymatic assay following protocols listed in Sarkar *et al.* (2022) and rest of the soil samples were dried and processed for soil chemical analysis. Chemical analysis for soil and plant samples were performed following the protocols listed in Jackson (1973).

$$\text{Bioconcentration factor (BCF)} = \frac{\text{Heavy metal conc.in harvested leaves}}{\text{Same metal conc.in soil}}$$

$$\text{Translocation factor (TF)} = \frac{\text{Heavy metal conc.in harvested leaves}}{\text{Same metal conc.in the root}}$$

Pot experiment data were processed through SPSS 22.0 (SPSS Inc., Chicago, IL, USA) to assess the Analysis of Variance (ANOVA) for a completely randomized design (CRD). Post hoc mean separation ($p \leq 0.05$) was determined by Tukey's honestly significant difference (HSD) test. All the figures were drawn using a Microsoft Office Excel 2019 worksheet.

RESULTS AND DISCUSSION

Application of SABC and MMFA both in polluted- (T_3, T_6) and fresh- (T_4, T_7) soils correspondingly had significant ($p<0.05$) and positive impact on shoot-, root- weight (both fresh and dry), leaf area and total chlorophyll content of wheat over the T_1 (control) and the treatments (T_2 and T_5) without any amendments (Table 2). In addition, the best positive response in terms of shoot-, root- weight, leaf area, and total chlorophyll content was observed in the treatments received SABC (T_3 and T_6) over MMFA (T_4 and T_7) which may be due to the presence of more structural pores provided better adsorption properties to SABC over MMFA. According to Abbas *et al.* (2018), biochar had a positive impact on the growth, biomass, and reduction of Cd and Na uptake in wheat cultivated in salty soil contaminated with Cd in ambient conditions. Biochar application might have improved the overall sorption capacity of soil and hence, reduced metal mobility in soil (Sharma and Nagpal 2018). Polluted soils (T_2) without any amendments (no SABC and MMFA) decreased the fresh shoot-, dry shoot-, fresh root-, dry root- weight, leaf area, and total chlorophyll content of wheat by ~18.5, 21.0, 18.9, 31.4, 37.3 and 23.1% than the control (T_1), respectively. Whereas, treatments including fresh soil with wastewater application (T_5) had recoded ~9.7, 8.2, 15.2, 27.5, 34.8, and 11.5% lesser fresh shoot-, dry shoot-, fresh root-, dry root- weight, leaf area, and total chlorophyll content of wheat. Metals like Pb and Cd negatively affect the plants through increased leaf chlorosis, cell death, suppression of root development, obstruction of pigment formation, and plant metabolism (Hussain *et al.* 2023). Moreover, growing wheat on metal-polluted soil with freshwater irrigation has a comparatively higher negative impact than that of grown on fresh soil with

Table 2 Effects on shoot weight, root weight, leaf area, and chlorophyll content of wheat

Treatments	Shoot weight (g/pot)		Root weight (g/pot)		Leaf area (cm^2)	Total chlorophyll (mg/g)
	Fresh	Dry	Fresh	Dry		
T_1	13.37 ± 0.99bc	4.14 ± 0.18bc	3.22 ± 0.20a	1.02 ± 0.17b	16.1 ± 2.10b	1.04 ± 0.06bc
T_2	10.90 ± 1.30d	3.27 ± 0.26d	2.61 ± 0.10b	0.70 ± 0.10c	10.1 ± 1.38c	0.80 ± 0.02d
T_3	16.89 ± 1.19a	5.49 ± 0.50a	3.27 ± 0.30a	1.30 ± 0.04a	21.3 ± 1.93a	1.37 ± 0.09a
T_4	13.42 ± 1.07bc	4.24 ± 0.53bc	2.67 ± 0.36ab	1.09 ± 0.16ab	15.3 ± 0.58b	1.05 ± 0.06bc
T_5	12.08 ± 1.45cd	3.80 ± 0.49cd	2.73 ± 0.11ab	0.74 ± 0.07c	10.5 ± 0.93c	0.92 ± 0.06cd
T_6	17.26 ± 1.00a	5.60 ± 0.27a	3.04 ± 0.25a	1.32 ± 0.05a	22.2 ± 2.24a	1.42 ± 0.07a
T_7	14.78 ± 0.30b	4.66 ± 0.30b	2.64 ± 0.15b	0.94 ± 0.14bc	14.6 ± 1.54b	1.12 ± 0.04b

Means with different lowercase letters are significantly different at $p<0.05$ according to Tukey's HSD test. Results are presented in Mean ± SD format. Treatment details are given under Materials and Methods.

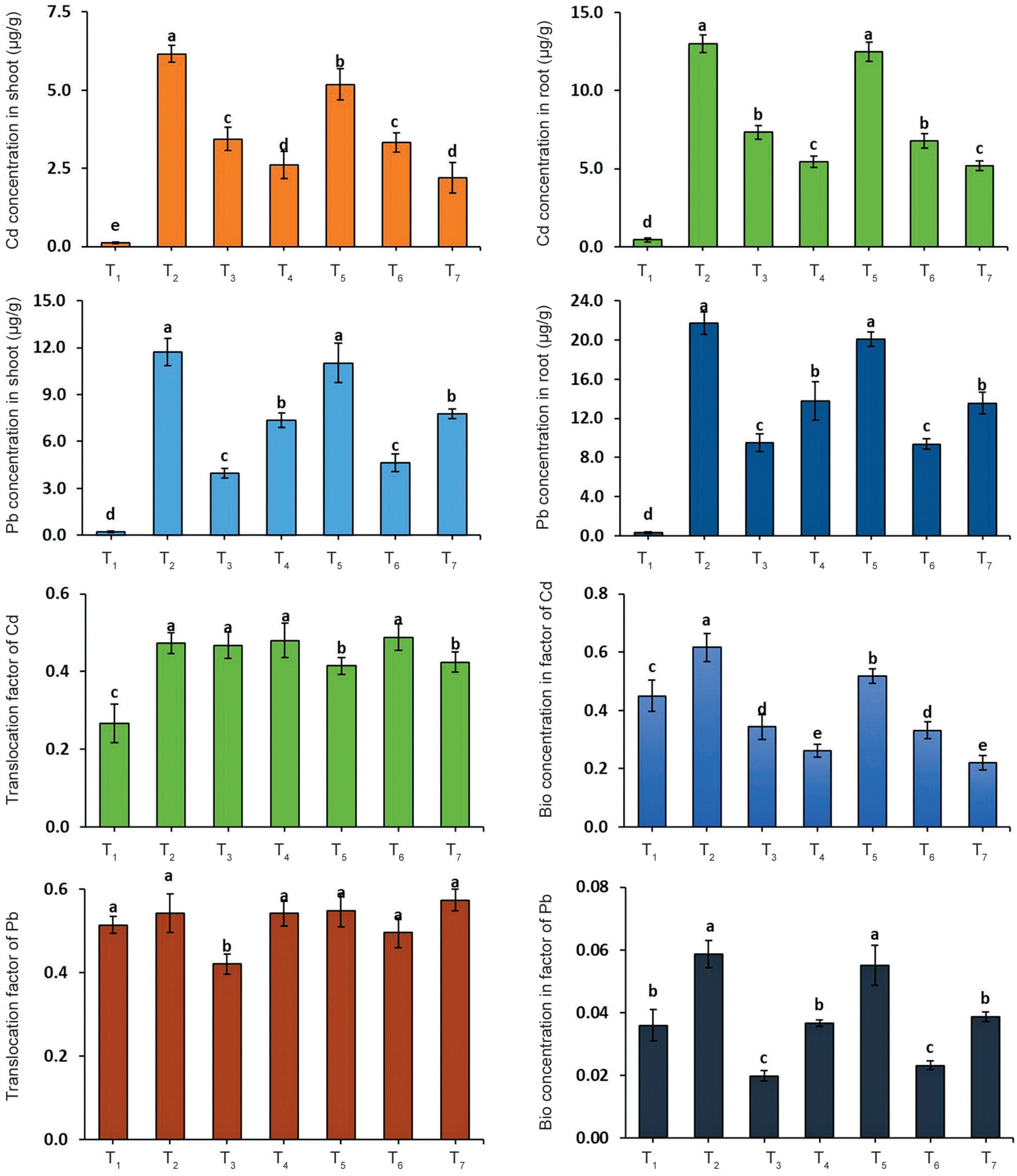

Fig. 2 Effects on Cd and Pb concentrations in wheat shoot and root, translocation factor, and bioaccumulation factor. Means with different lowercase letters are significantly different at $p<0.05$ according to Tukey's HSD test. Error bars indicate standard deviation (SD). Treatment details are given under Materials and Methods.

wastewater irrigation which might result from the supply of dissolved organic carbon, inorganic nutrients (NPK), and microorganisms from wastewater to support plant growth. However, the impact of both the synthesized amendments (SABC and MMFA) had comparatively better response in crop acclimatization and establishment on polluted soils over fresh soils.

On the other hand, both Cd and Pb concentrations in the shoot and root of wheat were significantly increased in T_2 and T_5 treatments, where amendments were not applied (Fig. 2). Cd and Pb concentrations in roots were considerably higher than in leaves. However, SABC and

MMFA application in soil significantly reduced both the metal ions concentrations in wheat plants as compared to T_2 and T_5 treatments. But, the maximum reduction of Pb concentration in the shoot (65.2%) and root (54%) were found in T_3 as compared to T_2, respectively under polluted soils; whereas, the highest reduction of Pb concentration in the shoot (54.5%) and root (50%) were found in T_6 as compared to T_5, respectively under wastewater applied fresh soils. Surprisingly, in T_4 compared to T_2, the maximum reduction of Cd concentration in the shoot (58%) and root (53.8%) was observed under polluted soils; in T_7 compared to T_5, the greatest reduction of Cd concentration in the shoot (70%) and root (61.5%) were observed under fresh soils treated with wastewater.

The translocation factor (TF) of any specific metal is the ratio of its concentration in the shoot to that in the root. Since, it mostly depends on the plant system, non-significant effect in TF of Pb and Cd was observed among the treatments (Fig. 2). However, bioconcentration factor (BCF) is the most significant indicator, which is the ratio of the concentration of metal in the harvested tissue to the corresponding soil. A plant is said to be bioaccumulating metal when its BCF for metal ions is greater than 1, but if it is less than 1, the plant is only absorbing the metal. The BCF for Cd is ten times greater than that of Pb is possibly due to Cd being mobile in both soil and plant, whereas Pb is immobile in soil (Yamada and Katoh 2020, Hussain *et al.* 2021). The application of MMFA (T_4 and T_7) reduced the BCF of Cd to 0.3 while the application of SABC (T_3 and T_6) reduced the BCF of Pb to 0.02.

SABC application resulted in an increase in soil *p*H from 7.87 to 8.05 in both T_3 and T_6 treatments (Table 3). SABC, a carbon-rich material can raise the soil *p*H because of its high *p*H (10.86) and the presence of exchangeable bases (Ca, Mg, K, etc.) easily released into the soil solution resulting increase in soil *p*H (Geng *et al.* 2022). Similarly, in SABC-amended soil, organic carbon content (SOC) significantly increased from ~0.5 to 0.63 % and ~0.53 to 0.67% under post-harvest polluted soil and wastewater-treated fresh soil, respectively, which might be due to its higher carbon content (~69.5%) added through SABC. According to Zheng *et al.* (2022), the recalcitrant nature of majority of the carbon present in SABC indicates that carbon mineralization during the wheat growth period was almost insignificant. No significant changes in EC were found due to the application of SABC and MMFA, however, wastewater-treated fresh soil showed significantly higher (50%) EC than polluted soil due to the presence of dissolved ions in the wastewater (Khashei Siuki *et al.* 2023). Results denoted that SABC-treated soils (T_3 and T_6) have higher Pb adsorption capacity than Cd, whereas inverse conditions were observed in MMFA-treated soils (T_4 and T_7). Soil amendments such as SABC and MMFA reported a positive impact by reducing the DTPA-extracted Pb and Cd concentration in the range of ~58–72% and ~35-61%, respectively, in the treated soils as compared to polluted and wastewater-applied soils without any amendment (Table 3). Pb has a higher affinity for most functional groups, such as carboxylic and phenolic hydroxyl groups in SABC, as evidenced by its smaller hydrated radius (0.401 nm) and higher electronegativity (2.33) compared to Cd's hydrated radius (0.426 nm) and lower electronegativity (1.69) (Mehrali-Afjani and Nezamzadeh-Ejhieh 2020). T_3 and T_6 have demonstrated better immobilization potential to decrease Pb concentration due to the raised *p*H of soil solution by SABC, which resulted increased precipitation and sorption of Pb in the soil. Besides, because of its amorphous structure, MMFA has a higher specific surface area and is rich in Al and Si elements and Cd forms a mononuclear complex that is adsorbed on alumina, and the release of Si elements strengthens the complex's stability. Furthermore, MMFA contains Na ions that can exchange with Cd and stabilize them more readily than Pb. (Zhou *et al.* 2020). The reduced concentration of Cd in T_4 and T_7 could be attributed to the strong Cd sorption on the exchange sites of MMFA through physical attachment and precipitation of Cd.

In addition, compared to the control, both DHA and FDA activity in the post-harvest soils were significantly decreased in the range of 17–33% and 29–38%, respectively in the

Table 3 Effects on chemical and enzymatic activities in the wheat post-harvested soil

Treatment	Soil *p*H	EC (dS/m)	SOC (%)	DTPA-Cd (µg/g)	DTPA-Pb (µg/g)	DHA (µg TPF/g soil/ day)	FDA (µg fluorescein/g soil/day)
T_1	7.87 ± 0.07b	0.21 ± 0.01c	0.50 ± 0.01d	0.04 ± 0.01d	0.09 ± 0.01e	36.6 ± 1.36c	16.7 ± 1.07b
T_2	7.90 ± 0.03b	0.23 ± 0.01bc	0.50 ± 0.01d	4.64 ± 0.30a	18.9 ± 1.49a	24.3 ± 1.90e	10.3 ± 0.48c
T_3	8.05 ± 0.04a	0.25 ± 0.02b	0.63 ± 0.02b	2.89 ± 0.12b	7.99 ± 1.43d	52.2 ± 1.84a	24.3 ± 1.31a
T_4	7.91 ± 0.01b	0.26 ± 0.03b	0.51 ± 0.01cd	1.78 ± 0.41c	11.1 ± 1.47c	37.0 ± 1.40c	12.9 ± 0.64c
T_5	7.88 ± 0.04b	0.41 ± 0.02a	0.53 ± 0.02cd	4.89 ± 0.20a	20.3 ± 1.64a	30.6 ± 1.04d	11.8 ± 0.87c
T_6	8.06 ± 0.02a	0.43 ± 0.02a	0.67 ± 0.02a	2.64 ± 0.21b	5.51 ± 1.04d	51.3 ± 2.49a	24.1 ± 1.95a
T_7	7.94 ± 0.02b	0.39 ± 0.02a	0.54 ± 0.01c	1.69 ± 0.22c	14.6 ± 0.31b	41.5 ± 1.16b	15.8 ± 0.81b

EC, Electrical conductivity; SOC, Soil organic C; DTPA, Diethylenetriaminepentaacetic acid; DHA, Dehydrogenase enzyme; FDA, Fluorescein diacetate hydrolysis. Means with different lowercase letters are significantly different at $p<0.05$ according to Tukey's HSD test. Results are presented in Mean ± SD format. Treatment details are given under Materials and Methods.

contaminated soils, where amendments were not applied (Table 2). As the spatial structure and activity of enzymes can be destroyed by heavy metals (Cd and Pb) through direct binding with their protein active groups and they can also prevent soil-living microorganisms from proliferating and growing, which can decrease the number of enzymes released from those microorganisms (Yeboah *et al.* 2021, Yang *et al.* 2022). However, the addition of SABC in the contaminated soil increased the DHA and FDA activities up to 2 times. Biochar can raise the root biomass and the carbon content in the soil which can lead to a higher C/N ratio, and stimulate microbial and enzymatic activities in the soil through a priming effect (Jiang *et al.* 2021).

A decreased level of Cd and Pb in wastewater irrigated and polluted soils, as well as their accumulation in wheat plants, may be achieved by incorporating MMFA and SABC, respectively. Additionally, the aboveground biomass of wheat was increased by both amendments. The addition of SABC caused an increment in *p*H, SOC, and enzymatic activities in soils. This study implies that the unsuitable Cd and Pb polluted soils can be used for crop production after being restored by applying MMFA and SABC. To properly understand the roles of biochar and modified fly ash, more field-scale experiments in naturally occurring multi-contaminated soils are needed.

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