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Comparative assessment of phytoavailable cadmium by using various extractants in texturally different contaminated soils during the growth of maize crop

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Phytoavailable cadmium (Cd) refers to the fraction of total cadmium in soil that is easily taken up by plants, which degrades the environmental quality and can cause contamination in the food chain. Cd contamination causes high carcinogenicity and toxicities, which harm both the environment and human health, including damage to the kidneys, bones, and respiratory system. In this study, seven extracting agents like ammonium bicarbonate diethylene triamine penta acetic acid (ABDTPA) and neutral salts having low and higher concentrations were used for the determination of phytoavailable Cd in sandy clay loam (SCL) and clay loam (CL) soils contaminated with Cd at naturally-Cd, Cd-20, Cd-40, and Cd-60 mg kg^{-1} and correlate it with Cd contents in roots, shoots, and grains of maize crop. Extraction efficiency of extractants remains in order: 10 M NH_4AOc > ABDTPA > 1 M NH_4AOc > 0.1 M $CaCl_2$ > 0.01 M $CaCl_2$ > 1 M $NaNO_3$ > 0.1 M $NaNO_3$. ABDTPA showed significantly the highest positive correlation with maize roots, shoots, and grains Cd contents in naturally Cd contaminated SCL and CL soils. While for artificially contaminated SCL and CL soils contaminated with Cd at Cd-20, Cd-40, and Cd-60 contamination levels, 0.1 M $CaCl_2$ extractable Cd showed a stronger positive significant correlation with Cd contents in roots, shoots, and grains of maize crop. Overall results concluded that 10 M NH_4AOc has the highest efficiency to extract Cd and 0.1 M $NaNO_3$ has the lowest Cd extraction efficiency. Correlation results showed that ABDTPA and 0.1 M $CaCl_2$ were found to be the most suitable extractants for predicting the bioavailability of Cd. Highly concentrated extractants give a stronger positive correlation as compared to low concentrated extractants. SCL soil showed stronger positive correlations as compared to CL soil.
Keywords: Phytoavailable Cd, various extractants, Cd contamination levels, various textured soils, maize crop.

INTRODUCTION

Agricultural soils are contaminated with cadmium (Cd), which is becoming a major concern these days and has received global attention in recent decades (Usman *et al*., 2023; Zia-ur-rehman *et al*., 2023). Anthropogenic activities such as mining, smelting, phosphate fertilizers, contaminated manures including biosolids, industrial effluents, waste discharges, and raw sewage water for irrigation activities are the major sources of Cd buildup in soils (Ahmed *et al*., 2021; NaziaTahir *et al*., 2021). The toxic concentration of Cd deteriorates the soil productivity leading to diminishing availability of arable lands, interfering with the normal functioning of plants which is expressed as decreased growth rate and disturbed metabolic activity, and becoming a risk to human health (Azhar *et al*., 2019; Chen *et al.*, 2019), causing cancer and serious cardiovascular disorders (Ebrahimi *et al.*, 2020; NaziaTahir *et al*., 2021). The Cd is found in both free and complex forms in soil, and the chemical speciation and varying distribution of Cd (adsorbed or free) in soil determine its phytoavailability (Ondrasek *et al.*, 2020; Riaz *et al.*, 2021). Cd is mostly present in soil that is bound by solid phases, and it is rapidly released into the soil solution, where it is then available to uptake by plants. Instead of existing as Cd^{2+} ions, Cd is also present in the soil as organic and inorganic complexes and is bound to organic matter, organic ligands, metal ions, and Mn and Fe oxides (Imoto and Yasutaka, 2020; Yuan *et al.*, 2021). Adsorption, desorption, dissolution, precipitation, and the complex formation of Cd-ligand are factors involved in the partitioning of Cd in soils and its bioavailability to plants (Bali *et al.*, 2020; Zhang *et al.*, 2022). The toxic effects of Cd on plants are strongly influenced by the mobility and bioavailability of Cd in the soil and its uptake by plants (Hamid *et al.*, 2019a,b; Yuan *et al.*, 2021). Determination of Cd mobility, toxicity, and bioavailability in soil is essential for soil quality contamination and environmental assessments (Kumpiene *et al*., 2017). Furthermore, these assessments are helpful in the selection of

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suitable remediation techniques to reclaim such metal-contaminated soils (Xiao *et al.*, 2017). Since total metal concentration has poor accuracy for determining trace metal toxicity and mobility in soil, it is essential to assess bioavailable metals in soil, particularly agricultural soil, and their chemical forms to determine the trace metal mobility and toxicity in soil (Wu *et al.*, 2019; Sánchez-Donoso *et al.*, 2021). More precisely, the bioavailable fraction of metals is the portion of the total metal contents of soil that remains available for plant roots uptake and accumulation in different plant tissues grown on contaminated soil (Rong *et al.*, 2020; Sánchez-Donoso *et al.*, 2021).

Single and sequential extraction techniques in soils and sediments are widely used for analyzing the metal fractions and predicting their mobility and bioavailability (Jalali and Hurseresht, 2020; Kumkrong *et al*., 2022; Mbodji *et al*., 2022). The single extraction approach aims to dissolve a phase whose elemental content is readily mobilizable and, ideally, correlates with the bioavailability in plants. In the sequential extraction methods, a sample is subjected to a series of progressively harsher chemicals to dissolve forms that are becoming more and more resistant to dissolution (Kumkrong *et al*., 2022; Mbodji *et al*., 2022). Single extraction technique can be used to provide useful information on bioavailability and toxicity fractions of potentially harmful Cd metal that is present in the soil for plant uptake (Milicevic *et al.*, 2017; Jalali and Hurseresht, 2020). Single extraction technique is cost-effective approach for extracting the trace elements fractionation from soils according to their different mobility and potential bioavailability (Sihlahla *et al.*, 2020). The lack of consistency among the various procedures used, which prevents the standardization of these methods and makes data comparison difficult is the fundamental drawback of single extraction methods (Pueyo *et al.*, 2004; Zhu *et al.*, 2019). Sequential extraction methods can potentially extract a significant fraction of bioavailable Cd from the solid phase and are considered suitable for predicting the fraction of Cd available to uptake by plants from soil (Kamila, 2018; Tytła *et al.*, 2022). Environmental studies frequently use sequential extraction techniques to determine the mineralogical and/or chemical forms of contaminants that are hazardous in soils and sediments. Metals bound to various mineral or organic phases can potentially be estimated more accurately using sequential extraction techniques (Abedin *et al.*, 2012; Álvarez-Quintana *et al.*, 2022). Sequential extraction methods are time-consuming and laborious methods as compared to single extraction method which uses a single extractant to extract the trace metal (Kumkrong *et al*., 2022; Mbodji *et al*., 2022). Several single extraction techniques which are using single extracting solvents such as neutral salts $NaNO_3$, NH_4AOc, and $CaCl_2$ are used to evaluate the mobility and availability of Cd metal fractions in soil (Jalali and Hurseresht, 2020; Ma *et al*., 2020; Sihlahla *et al*., 2020). Several sequential extraction procedures and extractants like DTPA, ABDTPA, EDTA, etc. are being used to find out the bioavailable metal fraction in contaminated soils (Luo *et al.*, 2019; Jalali and Hurseresht, 2020; Jalali *et al.*, 2022).

Linear correlation between Cd concentration extracted by ammonium bicarbonate diethylene triamine penta acetic acid (ABDTPA) and neutral salts under low concentration and that in plant parts is often used in previous literature. Extractants concentrations were chosen on the basis of concentrations of these extractants used in the previous literature and then increased the concentration and made the higher concentrated extractants solutions. The reason for choosing these higher concentrations was, under low concentrated neutral salts solutions, few experiments have been done which have not given promising results but in the current experiment concentration of the neutral salt solution is increased and their linear correlation was found with Cd contents in different tissues of maize crop for the identification of the most suited extraction method in alkaline calcareous soils to extract the Cd. In the present study, seven extractants ABDTPA and neutral salts with low and higher concentrations were used to extract the Cd from soil and correlate it with Cd concentrations in maize roots, shoots, and grains. The bioavailability of Cd from soils is not estimated by a single extractant method that is globally accepted up till now. The main objectives of the present study were (i) To compare the efficiency of ABDTPA and low and highly concentrated extractants to extract the bioavailable portion of Cd from texturally different soils (ii) to correlate the extracted Cd by ABDTPA and low and highly concentrated extractants with maize roots, shoots, and grains Cd contents grown in texturally different soils (iii) To investigate the extractability and correlation pattern between two texturally different calcareous soils.

MATERIALS AND METHODS

***Collection and preparation of the soil*:** Soil samples were taken from agricultural fields of villages 236/R.B., Kajlay (31°18'53.2"N 73°06'59.7" E), and Basti Sandela (30°14'19.3"N 71°26'24.5" E) situated near Faisalabad and Multan cities respectively, irrigated with untreated raw city effluents. Soil samples were collected from 20 cm below the soil's surface. The collected soil samples were air dried, and after air drying soil samples were further grounded by using the wooden pestle. These grounded soil samples were stored in plastic zipper bags for analysis after being passed through a 2 mm sieve. Hydrometer was used to measure the particle size classes of soil by analyzing the soil samples (Bouyoucos, 1962). A conductivity meter (Sensodirect Con 200s-Lovibond, England), calibrated with a 0.01 N KCL solution was used to measure the electrical conductivity (EC) of soil saturated extract and to measure the pH of the soil, pH meter (HM-12P, Korea) was used (Richards, 1954). Standard protocols determined by (US Salinity Laboratory Staff, 1954; Richards, 1954) were used to measure the sodium adsorption ratio (SAR) and soluble salts. Soil's Physicochemical parameters are given in supplementary Tables 1-2.

Table Supplementary 1. Physicochemical properties of soils used for the experiment.

Soil characteristics	Units	Faisalabad soil	Multan soil
Soil texture	-	Sandy clay loam	Clay loam
Sand	%	56.15	40.15
Silt	%	21.70	29.15
Clay	%	22.15	30.70
Saturation percentage	%	37.00	40.00
pHs	-	7.37	7.80
ECe	dS m^{-1}	1.75	2.70
TSS	$mmol_c\ L^{-1}$	17.50	27.00
CO_3^{-2}	$mmol_c\ L^{-1}$	0.25	0.80
HCO_3^-	$mmol_c\ L^{-1}$	3.20	3.10
Cl^-	$mmol_c\ L^{-1}$	12.05	12.70
$Ca^{2+} + Mg^{2+}$	$mmol_c\ L^{-1}$	10.75	8.10
SO_4^{2-}	$mmol_c\ L^{-1}$	2.00	10.40
Na^+	$mmol_c\ L^{-1}$	6.75	18.90
SAR	$(mmol\ L^{-1})^{1/2}$	2.91	9.39

Table Supplementary 2. ABDTPA extractable Cd concentration of different textured soils.

Soil texture	ABDTPA-Cd	Units	N-Cd	20-Cd	40-Cd	60-Cd
Sandy	Before spiking	mg kg^{-1}	0.34	-	-	-
clay	After spiking	mg kg^{-1}	0.34	10.32	21.03	30.57
loam	After experim.	mg kg^{-1}	0.32	7.47	16.38	24.12
Clay	Before spiking	mg kg^{-1}	0.27	-	-	-
loam	After spiking	mg kg^{-1}	0.27	9.23	19.28	28.75
	After experim.	mg kg^{-1}	0.24	6.49	14.48	22.52

*N-Cd (naturally Cd contaminated), 20-Cd, 40-Cd, and 60-Cd (Cd contamination levels 20, 40, and 60 mg kg^{-1}).

***Spiking of soil with Cd*:** The calculated Cd concentration as $CdSO_4.8/3H_2O$ at rates of 20, 40, and 60 mg Cd kg^{-1} soil was dissolved in water equal to the soil saturation percentage. This Cd-containing solution was then evenly sprinkled and properly mixed with all the soil layers. Polyethylene-lined plastic containers were used to keep this Cd contaminated soil for 90 days to establish Cd equilibrium in the soil. The humidity level was regulated at saturation% by maintaining the weight of the soil containers every two weeks. After the completion of the incubation period, this contaminated soil was thoroughly air-dried, ground, and then subjected to a 2-mm sized sieve.

***Experimentation and treatment plan*:** A pot experiment was conducted in the well-aerated glasshouse at the ISES, UAF, Pakistan. In the glass house, relative humidity during the experiment remained around 58-65% and the mean average temperature remained around 28±5°C. Air circulation and natural sunlight were available in the glasshouse throughout the entire cycle of crop growth. For SCL and CL soils, the designed treatments were naturally Cd contaminated N-Cd, Cd-spiked soil @ 20 (20-Cd), Cd-spiked soil @ 40 (40-Cd), and Cd-spiked soil @ 60 (mg Kg^{-1}) 60-Cd. Treatments were applied according to a completely randomized design. Each pot was initially sown with five hybrid maize (*Zea mays* L. Hybrid Monsanto DK-6789) seeds, however, only two seedlings were kept in a pot after eight days of germination. NPK (nitrogen, phosphorus, potassium) fertilizers were applied to each pot. Each pot received 125 mg kg^{-1} of urea, 60 mg kg^{-1} of di-ammonium phosphate, and 65 mg kg^{-1} of potassium sulphate, respectively. To maintain the required level of nitrogen in the soil, the nitrogen (N) added using di-ammonium phosphate was subtracted from urea, of which half dose was applied at the time of sowing and the remaining half was applied in two splits after 30 and 60 days of germination. Weeds were pulled as soon as they arose, and the soil moisture content was consistently kept at roughly 70% of its water-holding capacity during the entire period of the experiment. The crop was harvested when it reached the maturity stage after 90 days of sowing.

***Harvesting and data recording of maize*:** Shoot dry weight, root dry weight, and grain weight measurements were made after the maize crop was harvested when it reached physiological maturity. After being harvested, collected samples of roots and shoots were rinsed in distilled water and then kept in the oven and maintained the temperature at 65°C until a constant dry weight was attained.

***Plant sample preparation and analysis*:** Samples of plant roots, shoots, and grains were digested using a 2:1 di-acid mixture (HNO_3:$HClO_4$) (Estefan *et al*., 2013). Plant tissues were ground into powder form and placed in flasks. A di-acid mixture solution was then added to the flasks, and the flasks were kept undisturbed for 24 hours before being placed on a hot plate to digest until a colorless solution was produced. Colorless samples were cooled, distilled water was used to dilute the solution, and then by using distilled water a suitable volume was made and kept in firmly wrapped bottles for analysis of Cd metal (Ryan *et al*., 2001). Atomic absorption spectrophotometer (AAS) (Solar S-100, Thermo Electron, USA) was used for measuring the Cd concentration in digested samples.

***Soil samples preparation and analysis*:** A sampling core was used for taking the composite soil samples from each pot. Samples of soil were air-dried before passing through a 2 mm sieve. (Soltanpour, 1985), proposed extraction method was used for measuring the bioavailable Cd. Take 10 g soil sample in the falcon tube and add 20 mL ABDTPA solution. Shaken the tubes on a mechanical shaker for 30 minutes at 200 rpm, then centrifuged the falcon tubes for 20 minutes at 1000 rpm. Whatman filter paper 42, was used to filtrate the centrifuged mixture, then the supernatant solution was analyzed by AAS (Solar S-100, Thermo Electron, USA).

***Preparation of extractants to measure phytoavailable Cd in soils*:** Process of extraction of different extractants was given as:

I. ABDTPA: 1 N NH_4HCO_3 + 0.005 M DTPA: Ten gram of soil was taken in 20 mL newly prepared ABDTPA solution, shaken for 30 minutes, filtered, and Cd concentration was determined using the filtrate (Soltanpour, 1985).

II. $CaCl_2$: Two gram of soil was taken in 20 mL of newly prepared 0.01 M $CaCl_2$ and 0.1 M $CaCl_2$ solution separately, shaken for three hours, filtered, and Cd concentration was determined using the filtrate (Novozamsky *et al.*, 1993).

III. $NaNO_3$: Eight gram of soil was taken in 20 mL of newly prepared 0.1 M $NaNO_3$ and 1 M $NaNO_3$ solution separately, shaken for two hours, filtered, and Cd concentration was determined using the filtrate (Gupta and Aten, 1993).

IV. NH_4AOc. Two gram of soil was taken in 20 mL of newly prepared 1 M NH_4AOc and 10 M NH_4AOc solution separately and shaken for 2 hours, filtered and Cd concentration was determined using the filtrate (Symeonides and Mcrae, 1977).

***Statistical analysis*:** Pearson correlation analysis was performed between the Cd concentrations extracted from the soil with different extractants and maize roots, shoots, and grains Cd contents using Statistix 8.1 software for Windows. The figures were plotted using Microsoft Excel 2016.

RESULTS

***Crop yields and biomass of maize crop at different Cd contamination levels*:** The results of the present study showed that by increasing the Cd concentration in clay loam (CL) and sandy clay loam (SCL) soils from naturally contaminated to Cd-20, Cd-40, and Cd-60 mg kg^{-1} contamination levels caused a significant ($P < 0.05$) decrease in maize growth and yield. Maximum roots, shoots, and grains dry weight (g pot^{-1}) were recorded in naturally contaminated CL soil which was 8.36, 26.25, and 10.73 respectively, and the minimum dry weight (3.32, 14.79, and 5.34) respectively, was recorded in Cd-60 contaminated soil as shown in Fig. Supplementary 1a-c. Roots, shoots, and grains dry weight in Cd-20 and Cd-40 contaminated soils remained as 5.91, 20.87, 8.59 and 4.55, 16.95, 7.24 respectively. For SCL soil, maximum roots, shoots, and grains dry weight (g pot^{-1}) was recorded in naturally contaminated soil which was 6.52, 21.97, 8.60 and the minimum dry weight (2.65, 11.03, 4.36) respectively, was recorded in Cd-60 contaminated soil as shown in Fig. Supplementary1a-c. Roots, shoots, and grains dry weight remained as 4.39, 15.51, 7.08 and 3.58, 12.67, 6.24 respectively, at other contamination levels such as Cd-20, and Cd-40.

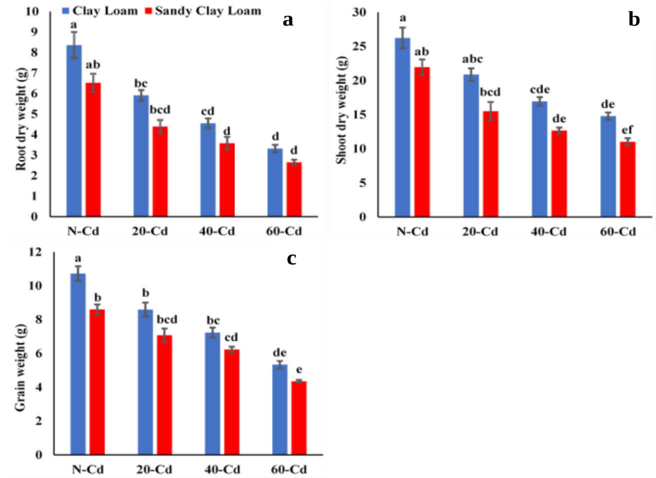

Figure Supplementary 1. Effect of different Cd contamination levels on the root dry weight (a), shoot dry weight (b), and grain weight (c), of maize crop grown in different Cd, contaminated clay loam (CL), and sandy clay loam (SCL) soils. Standard errors are indicated by the error bars, and means are highlighted by the bars. By using Tukey's Honest Significant Difference (HSD) as the post hock test, different letters on the bars indicate that the treatments are statistically significant ($p < 0.05$). On X-Axis different concentrations of Cd contamination levels of both textured soils were shown as *N-Cd (naturally Cd contaminated), 20-Cd, 40-Cd, and 60-Cd (Cd contamination levels 20, 40, and 60 mg kg^{-1}).

***Concentration of Cd in various tissues of maize crop at different Cd contamination levels*:** Cd concentration in different tissues of maize crop increased significantly ($P < 0.05$) with increasing the Cd contamination levels in SCL and CL soils (Fig. 1a-c). Maximum roots, shoots, and grains Cd concentration (mg kg^{-1}) was recorded in SCL soil at Cd-60 contamination level, which was 135.30, 27.63, and 3.70 respectively, and minimum roots, shoots, and grains Cd concentration (7.78, 2.27, and 0.21) respectively, was recorded in naturally Cd contaminated soil. The roots, shoots and grains Cd concentration remained as 92.62, 20.38, 2.04 and 59.32, 10.30, 0.99 respectively, in Cd-40 and Cd-20 contaminated SCL soil. For CL soil, maximum roots, shoots, and grains Cd concentration (mg kg^{-1}) was recorded in Cd-60 contaminated soil, which was 123.30, 23.03, and 3.50 respectively, and minimum roots, shoots, and grains Cd concentration (6.72, 2.18, and 0.20) respectively, was recorded in naturally Cd contaminated soil. Roots, shoots, and grains Cd concentration at Cd-40 and Cd-20 contamination levels remained as 82.28, 16.79, 1.92 and 52.99, 7.90, 0.94 respectively.

Uptake of Cd by plant tissues of maize crop at different Cd contamination levels: The Cd uptake by various tissues of maize crop increased significantly ($P < 0.05$) with enhancing the Cd contamination levels in contaminated CL and SCL soils (Fig. 1d-f). The Cd uptake (µg pot^{-1}) in maize roots, shoots, and grains was recorded maximum such as 408.62, 335.75, and 18.64 respectively, for Cd-60 contaminated CL soil, and roots, shoots, and grains Cd uptake was recorded minimum (54.86, 56.86, and 2.11) respectively, for naturally Cd contaminated soil. The Cd uptake for roots, shoots, and grains in Cd-40 and Cd-20 contaminated soil remained as 372.39, 267.79, 13.91 and 312.05, 162.62, 8.05 respectively. For SCL soil, maximum roots, shoots, and grains Cd uptake (µg pot^{-1}) was recorded in Cd-60 contaminated soil which was 357.71, 303.25, and 16.11 respectively, and minimum roots, shoots and grains Cd uptake (50.17, 49.51, and 1.77) respectively, was recorded in naturally contaminated soil. Similarly, the Cd uptake in roots, shoots and grains of Cd-40 and Cd-20 contaminated soils remained as 330.22, 256.93, 12.69 and 257.75, 157.40, 6.95 respectively.

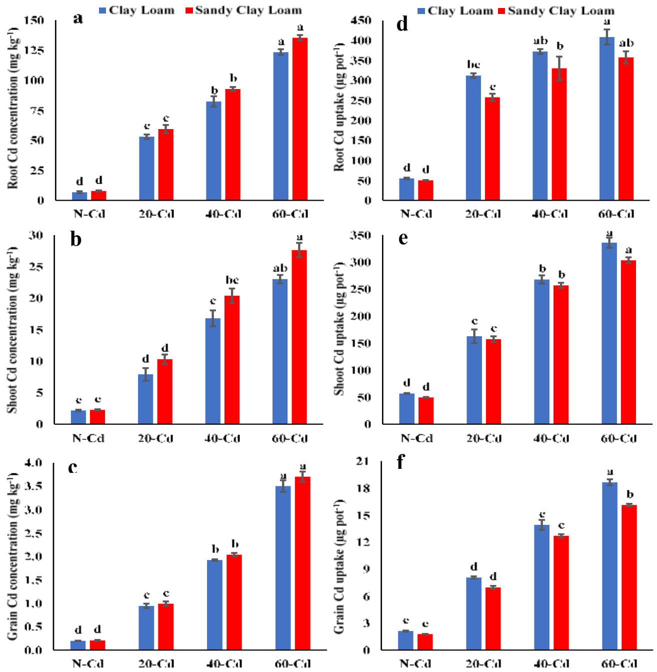

Figure 1. Effect of different Cd contamination levels on the roots Cd concentration (a), shoots Cd concentration (b), grains Cd concentration (c), roots Cd uptake (d), shoots Cd uptake (e), and grains Cd uptake (f) of maize crop grown in different Cd contaminated clay loam (CL) and sandy clay loam (SCL) soils. Standard errors are indicated by the error bars, and means are highlighted by the bars. By using Tukey's Honest Significant Difference (HSD) as the post hock test, different letters on the bars indicate that the treatments are statistically significant ($p < 0.05$). On X-Axis different concentrations of Cd contamination levels of both textured soils were shown as *N-Cd (naturally Cd contaminated), 20-Cd, 40-Cd, and 60-Cd (Cd contamination levels 20, 40, and 60 mg kg^{-1}).

***Comparative extractability of different extractants to extract Cd at different contamination levels*:** Extractants differed significantly ($P < 0.05$) to extract Cd from CL and SCL soils contaminated with Cd at different contamination levels after harvest of maize crop (Fig. 2a-b). In naturally contaminated CL soil maximum Cd (mg kg^{-1}) was extracted by 10 M NH_4AOc (0.58) followed by 1 M NH_4AOc (0.32) > 0.1 M $CaCl_2$ (0.31) > ABDTPA (0.24) > 1 M $NaNO_3$ (0.15) > 0.01 M $CaCl_2$ (0.11) > 0.1 M $NaNO_3$ (0.07). Cd-20 contamination level showed that maximum Cd (mg kg^{-1}) was extracted by 10 M NH_4AOc (9.23) followed by ABDTPA (6.49) > 1 M NH_4AOc (4.50) > 0.1 M $CaCl_2$ (3.88) > 0.01 M $CaCl_2$ (2.10) > 1 M $NaNO_3$ (2.09) > 0.1 M $NaNO_3$ (0.82). Cd-40 contamination level showed that maximum Cd (mg kg^{-1}) was extracted by 10 M NH_4AOc (22.37) followed by ABDTPA (14.48) > 1 M NH_4AOc (9.30) > 0.1 M $CaCl_2$ (7.75) > 0.01 M $CaCl_2$ (4.07) > 1 M $NaNO_3$ (3.40) > 0.1 M $NaNO_3$ (2.19). Cd-60 contamination level showed that maximum Cd (mg kg^{-1}) was extracted by 10 M NH_4AOc (33.81) followed by ABDTPA (22.52) > 1 M NH_4AOc (13.18) > 0.1 M $CaCl_2$ (10.18) > 0.01 M $CaCl_2$ (5.51) > 1 M $NaNO_3$ (5.23) > 0.1 M $NaNO_3$ (3.16).

For naturally contaminated SCL soil maximum Cd (mg kg^{-1}) was extracted by 10 M NH_4AOc (0.64) followed by 1 M NH_4AOc (0.36) = 0.1 M $CaCl_2$ (0.36) > ABDTPA (0.32) > 1 M $NaNO_3$ (0.19) > 0.01 M $CaCl_2$ (0.13) > 0.1 M $NaNO_3$ (0.09). Cd-20 contamination level showed that maximum Cd (mg kg^{-1}) was extracted by 10 M NH_4AOc (10.68) followed by ABDTPA (7.47) > 1 M NH_4AOc (5.27) > 0.1 M $CaCl_2$ (4.54) > 0.01 M $CaCl_2$ (2.58) > 1 M $NaNO_3$ (2.23) > 0.1 M $NaNO_3$ (0.90). Cd-40 contamination level showed that maximum Cd (mg kg^{-1}) was extracted by 10 M NH_4AOc (23.07) followed by ABDTPA (16.38) > 1 M NH_4AOc (10.97) > 0.1 M $CaCl_2$ (9.22) > 0.01 M $CaCl_2$ (4.57), 1 M $NaNO_3$ (3.83) > 0.1 M $NaNO_3$ (2.36). Cd-60 contamination level showed that maximum Cd (mg kg^{-1}) was extracted by 10 M NH_4AOc (34.77) followed by ABDTPA (24.12) > 1 M NH_4AOc (14.62) > 0.1 M $CaCl_2$ (11.48) > 0.01 M $CaCl_2$ (6.25), 1 M $NaNO_3$ (6.07) > 0.1 M $NaNO_3$ (3.60).

Correlation analysis between Cd concentration in maize tissues and Cd concentration extracted with different extractants at different Cd contamination levels*:** Extractants extractable Cd ***concentration positively and significantly ($P < 0.05, 0.01, 0.001$) correlates with Cd concentration in maize roots, shoots, and grains grown in CL and SCL soils at naturally Cd contaminated, Cd-20, Cd-40, and Cd-60 contamination levels (Tables 1-3). In naturally contaminated CL soil, maximum positive correlation was found for ABDTPA followed by > 0.1 M $CaCl_2$ > 10 M NH_4AOc > 0.01 M $CaCl_2$ > 1 M $NaNO_3$ > 1 M NH_4AOc > 0.1 M $NaNO_3$. In soil contaminated with Cd-20 contamination level maximum positive correlation was found for 0.1 M $CaCl_2$ followed by ABDTPA > 1 M $NaNO_3$ > 0.01 M $CaCl_2$ > 10 M NH_4AOc > 0.1 M $NaNO_3$ > 1 M NH_4AOc.

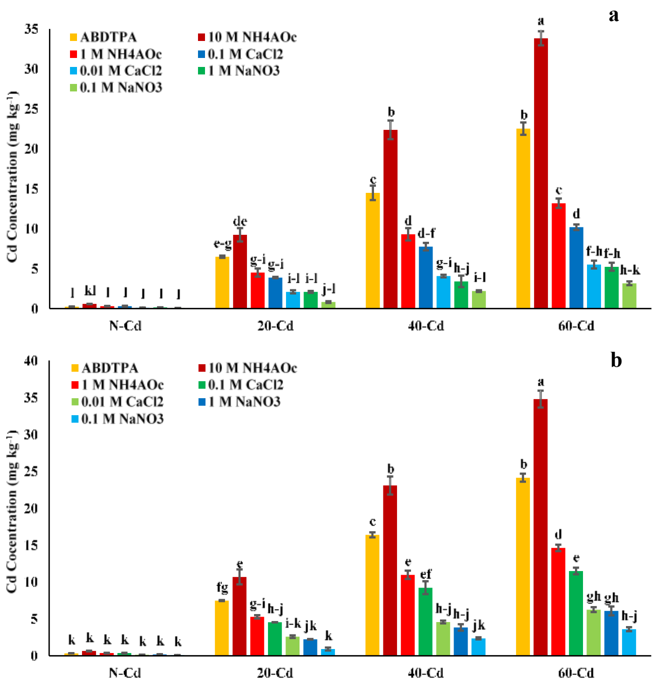

Figure 2. The extractability of ABDTPA (ammonium bicarbonate diethylene triamine penta acetic acid) 0.01 M $CaCl_2$, 0.1 M $CaCl_2$ (calcium chloride), 0.1 M $NaNO_3$, 1 M $NaNO_3$ (sodium nitrate), 1 M NH_4AOc, 10 M NH_4AOc (ammonium acetate) extractants to extract Cd from clay loam soil (a), and sandy clay loam soil (b). Standard errors are indicated by the error bars, and means are highlighted by the bars. By using Tukey's Honest Significant Difference (HSD) as the post hock test, different letters on the bars indicate that the treatments are statistically significant ($p < 0.05$). On X-Axis different concentrations of Cd contamination levels of both textured soils were shown as *N-Cd (naturally Cd contaminated), 20-Cd, 40-Cd, and 60-Cd (Cd contamination levels 20, 40, and 60 mg kg^{-1}).

In soil contaminated with Cd-40 contamination level maximum positive correlation was found for 0.1 M $CaCl_2$ followed by 1 M $NaNO_3$ > ABDTPA > 10 M NH_4AOc > 0.01 M $CaCl_2$ > 1 M NH_4AOc > 0.1 M $NaNO_3$. In soil contaminated with Cd-60 contamination level maximum positive correlation was found for 0.1 M $CaCl_2$ followed by ABDTPA > 1 M $NaNO_3$ > 0.01 M $CaCl_2$ > 10 M NH_4AOc > 0.1 M $NaNO_3$ > 1 M NH_4AOc.

In naturally contaminated SCL soil, maximum positive correlation was found for ABDTPA followed by 0.1 M $CaCl_2$ > 1 M $NaNO_3$ > 10 M NH_4AOc > 0.01 M $CaCl_2$ > 0.1 M $NaNO_3$ > 1 M NH_4AOc. Cd-20 contaminated soil showed maximum positive correlation for 0.1 M $CaCl_2$ followed by 1 M $NaNO_3$ > ABDTPA > 0.1 M $NaNO_3$ > 10 M NH_4AOc > 0.01 M $CaCl_2$ > 1 M NH_4AOc. Cd-40 contaminated soil showed maximum positive correlation for 0.1 M $CaCl_2$ followed by 1 M $NaNO_3$ > 0.1 M $NaNO_3$ > 0.1 M $CaCl_2$ > ABDTPA > 10 M NH_4AOc > 1 M NH_4AOc. Cd-60 contaminated soil showed maximum positive correlation for 0.1 M $CaCl_2$ followed by 1 M $NaNO_3$ > ABDTPA > 0.1 M $NaNO_3$ > 0.01 M $CaCl_2$ > 10 M NH_4AOc > 1 M NH_4AOc.

***Correlation analysis between Cd uptake in maize tissues and Cd* concentration *extracted with different extractants at different Cd contamination levels*:** Pearson correlation analysis was performed to evaluate the Cd metal uptake in maize tissues (roots, shoots, and grains) and concentrations of Cd extracted with different extraction methods from CL and SCL soils contaminated with Cd as shown in Tables 4-6. In naturally contaminated CL soil, maximum positive correlation was found for ABDTPA followed by 0.1 M $CaCl_2$ > 10 M NH_4AOc > 0.01 M $CaCl_2$ > 1 M $NaNO_3$ > 1 M NH_4AOc > 0.1 M $NaNO_3$. In soil contaminated with Cd-20, the maximum positive correlation was found for 0.1 M $CaCl_2$ followed by ABDTPA > 1 M $NaNO_3$ > 0.01 M $CaCl_2$ > 10 M NH_4AOc > 0.1 M $NaNO_3$ > 1 M NH_4AOc. In soil contaminated with Cd-40 the maximum positive correlation was found for 0.1 M $CaCl_2$ followed by 1 M $NaNO_3$ > ABDTPA > 10 M NH_4AOc > 0.01 M $CaCl_2$ > 1 M NH_4AOc > 0.1 M $NaNO_3$. In soil contaminated with Cd-60 contamination level maximum positive correlation was found for 0.1 M $CaCl_2$ followed by ABDTPA > 1 M $NaNO_3$ > 0.01 M $CaCl_2$ > 10 M NH_4AOc > 0.1 M $NaNO_3$ > 1 M NH_4AOc.

Table 1. Pearson correlation between Cd concentration in maize roots and Cd extracted with different extractants at different Cd contamination levels

Soil types	Cd level	ABDTPA	0.01 M $CaCl_2$	0.1 M $NaNO_3$	1 M NH_4AOC	0.1 M $CaCl_2$	1 M $NaNO_3$	10 M NH_4AOc
Clay loam	N-Cd	0.665*	0.431	0.256	0.352	0.585*	0.397	0.511
	20-Cd	0.579*	0.458	0.360	0.259	0.661*	0.530	0.408
	40-Cd	0.493	0.447	0.388	0.419	0.640*	0.604*	0.489
	60-Cd	0.523	0.435	0.314	0.259	0.596*	0.438	0.334
Sandy clay	N-Cd	0.761**	0.466	0.424	0.263	0.624*	0.586*	0.489
loam	20-Cd	0.659*	0.516	0.569*	0.251	0.764**	0.762**	0.550
	40-Cd	0.518	0.522	0.559*	0.170	0.727**	0.706*	0.465
	60-Cd	0.538	0.442	0.474	0.158	0.698*	0.639*	0.351

*, **Indicate significant relationship at probability level, $P < 0.05$, and $P < 0.01$, respectively (n=12).

*N-Cd (naturally Cd contaminated), 20-Cd, 40-Cd, 60-Cd (Cd contamination levels 20, 40, and 60 mg kg^{-1}).

Table 2. Pearson correlation between Cd concentration in maize shoots and Cd extracted with different extractants at different Cd contamination levels.

Soil types	Cd level	ABDTPA	0.01 M $CaCl_2$	0.1 M $NaNO_3$	1 M NH_4AOC	0.1 M $CaCl_2$	1 M $NaNO_3$	10 M NH_4AOc
Clay loam	N-Cd	0.773**	0.566*	0.403	0.493	0.704*	0.535	0.639*
	20-Cd	0.680*	0.571*	0.479	0.383	0.753**	0.636*	0.523
	40-Cd	0.555	0.642*	0.454	0.483	0.694*	0.661*	0.551
	60-Cd	0.622*	0.541	0.426	0.374	0.689*	0.543	0.445
Sandy clay loam	N-Cd	0.820**	0.549	0.510	0.355	0.697*	0.662*	0.571*
	20-Cd	0.723**	0.590*	0.639*	0.336	0.818**	0.816**	0.622*
	40-Cd	0.580*	0.583*	0.619*	0.242	0.776**	0.756**	0.529
	60-Cd	0.567*	0.474	0.505	0.193	0.723**	0.666**	0.385

*, **Indicate significant relationship at probability level, $P < 0.05$, and $P < 0.01$, respectively (n=12).
*N-Cd (naturally Cd contaminated), 20-Cd, 40-Cd, 60-Cd (Cd contamination levels 20, 40, and 60 mg kg^{-1}).

Table 3. Pearson correlation between Cd concentration in maize grains and Cd extracted with different extractants at different Cd contamination levels.

Soil types	Cd level	ABDTPA	0.01 M $CaCl_2$	0.1 M $NaNO_3$	1 M NH_4AOC	0.1 M $CaCl_2$	1 M $NaNO_3$	10 M NH_4AOc
Clay loam	N-Cd	0.924***	0.782**	0.653*	0.726**	0.881***	0.758**	0.836***
	20-Cd	0.857***	0.776**	0.704*	0.624*	0.906***	0.825***	0.739**
	40-Cd	0.734**	0.803**	0.649*	0.675*	0.843***	0.818**	0.731**
	60-Cd	0.722**	0.649*	0.544	0.495	0.780**	0.651*	0.562*
Sandy clay loam	N-Cd	0.962***	0.791**	0.762**	0.640*	0.893***	0.870***	0.807**
	20-Cd	0.830***	0.719**	0.761**	0.492	0.904***	0.903***	0.740**
	40-Cd	0.743**	0.745**	0.774**	0.447	0.894***	0.880***	0.701*
	60-Cd	0.744**	0.667*	0.693*	0.417	0.865**	0.821**	0.590*

*, **, ***Indicate significant relationship at probability level, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively (n=12). *N-Cd (naturally Cd contaminated), 20-Cd, 40-Cd, 60-Cd (Cd contamination levels 20, 40, and 60 mg kg^{-1}).

Table 4. Pearson correlation between Cd uptake in maize roots and Cd extracted with different extractants at different Cd contamination levels

Soil types	Cd level	ABDTPA	0.01 M $CaCl_2$	0.1 M $NaNO_3$	1 M NH_4AOC	0.1 M $CaCl_2$	1 M $NaNO_3$	10 M NH_4AOc
Clay loam	N-Cd	0.831***	0.645*	0.492	0.576*	0.771**	0.615*	0.711**
	20-Cd	0.648*	0.535	0.441	0.343	0.724**	0.602*	0.487
	40-Cd	0.516	0.471	0.412	0.443	0.660*	0.625*	0.512
	60-Cd	0.468	0.377	0.253	0.197	0.564*	0.380	0.273
Sandy clay loam	N-Cd	0.876***	0.635*	0.598*	0.452	0.769**	0.738**	0.655*
	20-Cd	0.650*	0.506	0.559	0.240	0.756*	0.754*	0.540
	40-Cd	0.504	0.507	0.545	0.154	0.716**	0.693*	0.450
	60-Cd	0.630*	0.541	0.571*	0.269	0.775**	0.722**	0.455

*, **, ***Indicate significant relationship at probability level, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively (n=12). *N-Cd (naturally Cd contaminated), 20-Cd, 40-Cd, 60-Cd (Cd contamination levels 20, 40, and 60 mg kg^{-1}).

Table 5. Pearson correlation between Cd uptake in maize shoots and Cd extracted with different extractants at different Cd contamination levels.

Soil types	Cd level	ABDTPA	0.01 M $CaCl_2$	0.1 M $NaNO_3$	1 M NH_4AOC	0.1 M $CaCl_2$	1 M $NaNO_3$	10 M NH_4AOc
Clay loam	N-Cd	0.668*	0.436	0.261	0.356	0.589*	0.402	0.516
	20-Cd	0.688*	0.579*	0.488	0.392	0.760**	0.644*	0.532
	40-Cd	0.484	0.576*	0.378	0.409	0.632*	0.596*	0.480
	60-Cd	0.527	0.439	0.319	0.264	0.600*	0.443	0.339
Sandy clay loam	N-Cd	0.789**	0.505	0.464	0.305	0.659*	0.622*	0.527
	20-Cd	0.723**	0.590*	0.639*	0.336	0.818**	0.816**	0.621*
	40-Cd	0.484	0.487	0.526	0.131	0.700*	0.677*	0.430
	60-Cd	0.689*	0.606*	0.634*	0.344	0.822**	0.774**	0.524

*, **, ***Indicate significant relationship at probability level, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively (n=12). *N-Cd (naturally Cd contaminated), 20-Cd, 40-Cd, 60-Cd (Cd contamination levels 20, 40, and 60 mg kg^{-1}).

In naturally contaminated SCL soil, maximum positive correlation was found for ABDTPA followed by 0.1 M $CaCl_2$ > 1 M $NaNO_3$ > 10 M NH_4AOc > 0.01 M $CaCl_2$ > 0.1 M $NaNO_3$ > 1 M NH_4AOc. The Cd-20 contaminated soil showed maximum positive correlation for 0.1 M $CaCl_2$ followed by 1 M $NaNO_3$ > ABDTPA > 0.1 M $NaNO_3$ > 10 M NH_4AOc > 0.01 M $CaCl_2$ > 1 M NH_4AOc. Cd-40 contaminated soil showed maximum positive correlation for 0.1 M $CaCl_2$

Table 6. Pearson correlation between Cd uptake in maize grains and Cd extracted with different extractants at different Cd contamination levels.

Soil types	Cd level	ABDTPA	0.01 M $CaCl_2$	0.1 M $NaNO_3$	1 M NH_4AOC	0.1 M $CaCl_2$	1 M $NaNO_3$	10 M NH_4AOc
Clay loam	N-Cd	0.796**	0.597*	0.438	0.526	0.731**	0.566*	0.667*
	20-Cd	0.724**	0.620*	0.532	0.439	0.792**	0.682*	0.575*
	40-Cd	0.658*	0.735**	0.565*	0.592*	0.781**	0.752**	0.654*
	60-Cd	0.768**	0.700*	0.601*	0.554	0.822**	0.703*	0.618*
Sandy clay loam	N-Cd	0.854***	0.600*	0.562*	0.412	0.740**	0.707*	0.621*
	20-Cd	0.748**	0.620*	0.667*	0.371	0.838***	0.837***	0.650*
	40-Cd	0.638*	0.641*	0.675*	0.313	0.820**	0.802**	0.590*
	60-Cd	0.717**	0.637*	0.664*	0.380	0.844***	0.798**	0.557

*, **, ***Indicate significant relationship at probability level, $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively (n=12). *N-Cd (naturally Cd contaminated), 20-Cd, 40-Cd, 60-Cd (Cd contamination levels 20, 40, and 60 mg kg^{-1}).

followed by 1 M $NaNO_3$ > 0.1 M $NaNO_3$ > 0.01 M $CaCl_2$ > ABDTPA > 10 M NH_4AOc > 1 M NH_4AOc. Cd-60 contaminated soil showed maximum positive correlation for 0.1 M $CaCl_2$ followed by 1 M $NaNO_3$ > ABDTPA > 0.1 M $NaNO_3$ > 0.01 M $CaCl_2$ > 10 M NH_4AOc > 1 M NH_4AOc.

DISCUSSION

***Comparative efficiency of different extractants to extract Cd at different Cd contamination levels*:** Our study showed that 10 M NH_4AOc has the highest efficiency to extract the bioavailable Cd from SCL and CL soils contaminated with Cd at naturally Cd, Cd-20, Cd-40, and Cd-60 contamination levels while 0.1 M $NaNO_3$ have the minimum efficiency to extract the bioavailable Cd (Fig. 2a-b). Neutral salt solutions having higher concentration is relatively a new technique to extract Cd metal from various textured soils and in literature, very limited studies have been found to report such data. However, literature is available about low concentrations of extractants (0.01 M $CaCl_2$, 1 M NH_4AOc, and 0.1 M $NaNO_3$) efficiency to extract Cd and correlation with Cd contents in different tissues of plants. Neutral salt solutions are useful in determining the availability of trace elements in soil because they stimulate interactions between trace elements and the soil, much like natural soil solutions (Peijnenburg *et al*., 2007). Similar results were reported by, Jalali and Hurseresht, (2020) who revealed that NH_4AOc was more effective for extracting metal ions from the soil than other methods. NH_4AOc is a particularly effective extractant for Cd because it has a high affinity for Cd ions. NH_4AOc forms a complex with Cd ions, making them more soluble and easier to separate from other metals through chelation (López *et al.*, 2022; Xu and Fu, 2022). The cationic metals are removed from soil exchange sites by ammonium-based chelators such as ammonium acetate, where complex metals in the soil solution are chelated by the counter ion acetate, as a result, it makes the ammonium acetate extractant one of the most aggressive extractant (Meers *et al*., 2007a,b).

Results demonstrate that following 10 M NH_4AOc, ABDTPA showed the highest efficiency to extract the Cd as compared to $CaCl_2$ and $NaNO_3$ (Fig. 2a-b). Results support previous studies that have shown that complexing agent ABDTPA extracts soil-available Cd better than cation-exchange extractants, such as NH_4NO_3, $CaCl_2$, and $NaNO_3$ (Zbíral, 2016; Luo *et al.*, 2019; Jalali *et al.*, 2022). Jalali *et al*., (2022) found that in calcareous soil chelating agent DTPA was most effective for extracting Cd. Luo *et al*., (2019), demonstrate that ABDTPA extractant has the highest capacity to extract the Cd metal. Ramtahal *et al*., (2015) showed that ABDTPA has higher efficiency to extract the Cd metal as compared to neutral salts. Extraction efficiency remained as ABDTPA (37.4%) > $CaCl_2$ (12.7%) > NH_4NO_3 (7.7%). This high efficiency may be due to the reason that ABDTPA complexing agents have the capacity to exchange both carbonate-bound and organic fractions (Lou *et al* 2019; Jalali *et al*., 2022). ABDTPA and DTPA metals extraction procedures involve the formation of strong complexes with metals by consuming organic ligands. Additionally, the formation of DTPA-Cd chelates is determined by the characteristics of the soil, particularly soil pH, organic matter content, source, amount, and metal contaminants form, as well as contaminants aging in the soil (Zia-ur-Rehman *et al.*, 2008; Jalali *et al.*, 2022).

Sodium nitrate and calcium chloride are both considered weak extractants, and the results determined that extraction with these two neutral salt solutions resulted in very low detection of Cd (Fig. 2a-b). However, extractants having higher concentrations (0.1 M $CaCl_2$, and 1 M $NaNO_3$) extracted higher amounts of Cd as compared to their respective low concentrated extractants (0.01 M $CaCl_2$, and 0.1 M $NaNO_3$). Pueyo *et al*., (2004) found that 0.1 M $NaNO_3$ removed less Cd than 1 M $NaNO_3$ or 0.01 M $CaCl_2$. The enhanced extractability of $CaCl_2$ can be attributed to the fact that Ca has a significantly higher competition level for cation sorption to the soil matrix. $CaCl_2$ was considered to be a background electrolyte solution for soil that was capable of readily extracting exchangeable metal pools (Houben *et al.*, 2013). Calcium chloride and magnesium chloride, are divalent metal chloride salts that are suspected to mobilize greater quantities of metal (Abedin *et al.*, 2012). $NaNO_3$ extraction agent (0.1 mol L^{-1}) does not form complexes or dissociate in exchange processes, it does not influence the equilibrium between the solid and solution phases of the reaction (Gupta and Sinha, 2007). According to de Santiago-Martín *et al*., (2013), trace elements' less extractability with neutral salts may be attributable to their specific adsorption to soils instead of retention on exchange sites. $NaNO_3$ would mostly remove exchangeable elements due to its similar ionic strength to soil solution, hence its addition has no impact on the equilibrium between soil solid and soil solution phases (Gupta and Sinha, 2007). Similarly, Cd extracted by 0.1 M $NaNO_3$ was much lower as compared to $CaCl_2$ and ABDTPA extractants (Zia-ur-rehman *et al*., 2008). $NaNO_3$ extraction solution has lower exchangeable content as compared to the $CaCl_2$ extraction solution because organic matter adsorption sites have weak competition with monovalent cations (Zhu *et al.*, 2012; Jalali and Hurshmeit, 2020).

***Pearson correlation between extracted Cd and plant Cd concentration at different Cd contamination levels*:** Pearson correlation analysis showed that in naturally Cd-contaminated SCL and CL soils ABDTPA showed the highest positive significant correlation with roots, shoots, and grains Cd concentration and uptake as compared to other extractants (Tables 1-6). Luo *et al*., (2019) demonstrate that ABDTPA extraction has the highest positive correlation with Cd uptake in wheat roots, shoots, and grains. Similarly, according to the findings of Zia-ur-rehman *et al*., (2008), ABDTPA extractable Cd showed the significantly highest positive correlation with Cd contents in grains and straws of rice harvested from SCL soil as compared to other extractants. The high correlation may be due to the presence of a high concentration of $CaCl_2$ in the ABDTPA extractant, Ca^{2+} may influence the desorption of other divalent cations from soils, particularly Cd^{2+} and Zn^{2+} (Zia-ur-rehman *et al*., 2008). Ramtahal *et al*., (2015) found that Cd concentration in tissues of cacao plant such as shells, nibs, leaves, and pods showed the highest significant positive correlation with ABDTPA and DTPA-extracted Cd.

In artificially contaminated SCL and CL soils with Cd at Cd-20, Cd-40, and Cd-60 contamination levels the correlation coefficient obtained by 0.1 M $CaCl_2$-extractable Cd was significantly stronger than other extractants (Tables 1-6). Calcium (Ca^{+2}) is the predominant cation in calcareous soils, and its solution's ionic strength is similar to that of the soil solution, which may be the possible reason for its high suitability to extract trace metals from the soil solution (Rezaei *et al*., 2021). Ma *et al*., (2020) found that $CaCl_2$ extracted Cd concentration showed the best correlation with Cd contents in rice grains. Similarly, Walker *et al*., (2003) found that 0.1 M $CaCl_2$ had a significantly positive correlation (R^2 =0.56) with Cu contents in tissues of radish plants. According to the findings of Meers *et al*., (2007a) $NaNO_3$ and $CaCl_2$ solutions showed the best correlation with shoot Cd contents. According to Zhang *et al*., (2022) the $CaCl_2$ extraction procedure provided the most accurate predictions of the Cd and Zn concentrations in soil solutions. Similarly, Abderrazzak and Fethi., (2021) determined that $CaCl_2$ extraction is the most effective extraction procedure for heavy metals, as compared to NH_4NO_3 and $NaNO_3$ extraction methods. Zhang *et al*., (2010) also found that Cd bioavailability from zinc-lead mine tailing paddy soils was best predicted by $CaCl_2$ and NH_4AOc extraction methods, Cd extracted with these extractants has a higher positive correlation with Cd contents in rice tissues.

***Conclusions*:** The investigations of this study concluded that 10 M NH_4AOc has the highest efficiency to extract the bioavailable Cd from SCL and CL soils, while 0.1 M $NaNO_3$ has the minimum efficiency to extract the bioavailable Cd. Pearson correlation results showed that ABDTPA and $CaCl_2$ were found to be the most suitable extractants for predicting the bioavailability of Cd metal. ABDTPA showed the significantly highest positive correlation with maize roots, shoots, and grains Cd concentration and uptake in naturally Cd contaminated SCL and CL soils. While for artificially contaminated SCL and CL soils results remained more consistent with higher concentrated 0.1 M $CaCl_2$ extractable Cd which has a stronger positive significant correlation with Cd contents in the roots, shoots, and grains of maize crop. SCL soil showed stronger positive correlations as compared to CL soil. The results also demonstrate that grains-Cd had a stronger positive correlation as compared to roots-Cd, and shoots-Cd. Overall, ABDTPA and 0.1 M $CaCl_2$ consistently performed as compared to other extractants. Neutral salts, having higher concentrations particularly $CaCl_2$, performed well especially under alkaline conditions at higher contamination levels. Soil conditions and different contamination levels may have a non-significant or significant impact on the effectiveness of any particular extraction technique. So future research will focus on field-level implications of these extractants to investigate the correlation between Cd extracted by these extractants with Cd concentration in different plant tissues.

***Conflict of Interest*:** All the authors declare no conflict of interest regarding this paper.

***Authors' Contribution Statements*:** MA conceptualized and designed, material preparation, data collection, and analysis, prediction of data, and first drafting. MZR supervised, conceptualized, designed, and critically reviewed and evaluated the manuscript. GM and SH commented on the manuscript.

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