Predicting chemical speciation of metals in soil using Visual Minteq

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ABSTRACT

• Soil processes affect metal chemical speciation and their biogeochemical activity.
• The current study predicted chemical speciation of eight metals in two soil layers.
• Divalent forms of metals predominated in both soil layers (79.9%).
• Chromium showed a chemical speciation that varied from that of the other metals (95.8% as CrOH$^+$).
• Mean percentage ages of all metal ions were similar for all 15 field locations investigated.

From soil contamination and risk assessment perspectives, it is imperative to understand the ecological processes occurring in soils. Certain soil processes greatly affect chemical speciation of potentially toxic metals (PTMs), and thus also influence their biogeochemical activity. The current study analyzed chemical speciation of eight PTMs (Cd, Cr, Fe, Cu, Mn, Ni, Zn, and Pb) in upper and lower soil layers for 15 agronomic fields of Vehari-Pakistan using Visual Minteq software. The divalent forms of most PTMs (PTM^{2+}) generally predominated in both soil layers (79.9% overall occurrence). However, chromium revealed a different pattern of chemical speciation (95.8% as CrOH$^+$) compared to other PTMs. The mean percentage of all the PTMs^{2+} was slightly higher for the lower soil layer (81.3%) than in the upper layer (78.4%), the trend being same for all the PTMs, except Cr. This higher PTMs^{2+} percentage in lower soil layers than upper layers was due to lower content of organic matter and other anions such as Cl$^-$ and HCO$_3^-$. The mean percentage ages of all the PTMs^{2+} was similar among all the 15 agronomic fields, which was confirmed by strong Pearson correlation values ($R^2 > 0.95$). The PCA graph grouped all the agronomic fields and PTM^{2+} closely, except Cr^{2+} and Cu^{2+}. This grouping confirmed the similar chemical speciation of PTMs, except Cu and Cr in studied fields.

Keywords heavy metals, free ionic forms, agricultural soils, multivariate analysis, PCA
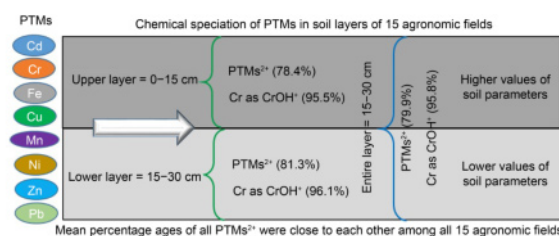
1 Introduction

Soil pollution by potentially toxic metals (PTMs) generates severe health and safety concerns for plant, animal, and human (Shahid et al., 2020; Wang et al., 2022; Khalid et al., 2022). There exist numerous natural and human-driven sources of PTMs in soils (Bing et al., 2021; Szukalska et al., 2021). Key PTM sources include mining, industrial activities, unwise use of agrochemicals, untreated municipal wastewater, contaminated groundwater irrigations, automobiles, coal-burning power plants etc. Consequently, numerous reports have revealed PTM build-up in soils above their optimal and

even toxic levels (Table 1 and Table S1). Approximately, more than 10 million sites globally are considered to be highly polluted, spanning an area of about 50 million acres (Khalid et al., 2017). Among these sites, more than half of the sites are contaminated with PTMs.

Despite considerable research and availability of abundant data on other aspects of soil chemistry, there is comparatively scarce data regarding chemical speciation of PTMs in soils. In fact, majority of the literature studies concerning soil-plant transfers (remediation or risks assessment) have measured and revealed only total PTM soil contents without further detail (Liu et al., 2021; Natasha et al., 2022a). Recent literature shows that total PTM soil levels may not accurately govern and express their bio-physio-chemical activity in soil-

Table 1 Mean and range values of PTMs (mg kg⁻¹) reported in published literature data (Table S1).

PTM	Soil level		Times higher than limit	
	Mean	Range	Mean	Range
Lead	7798	88.6−39800	130	1.5−663
Cadmium	45	3.6−300	45	3.6−300
Chromium	241	131−417	2	1.3−4.2
Copper	1493	1106−3200	15	1.8−32
Manganese	17919	NA	9	NA
Nickel	156	67−475	3	1.3−9.5
Arsenic	398	5−1540	80	1−308

plant interactions (soil adsorption and desorption, phyto-availability, soil-root-shoot transfers, compartmentation inside plants, toxicity and detoxification). Rather, the chemical speciation of a PTM in soil better correlates with its bio-physio-chemical activity (Shahid et al., 2012; Zhang et al., 2018). Hence, it is highly relevant to measure the chemical speciation of PTMs in studies reporting their bio-physio-chemical activity in soil-plant interactions.

Chemical speciation refers to existence of a PTM in various oxidation/chemical forms. Numerous soil physico-chemical attributes are capable of altering PTM speciation in soil. For example, soil redox potential, pH, organic carbon type and percent, soil ligands, microorganisms, plant species, and root excretions have been reported to modify PTM soil speciation (Shahid et al., 2018; Mir et al., 2022). Soil, especially rhizosphere soil represents a complicated ecosystem involving a great number and various types of microorganisms, organic matters, solid components and minerals (Qu, 2022). A soil system also comprises numerous fine organic/inorganic particles of varied quantity, compositions, and surface attributes, which eventually govern PTM soil activities and cycling.

The interactions of PTMs with different soil constituents under varied conditions of pH, redox potential, organic carbon and microbes ultimately determine the chemical speciation. For example, the chemical forms of PTMs vary with their binding/interaction with soil solids, their ionic form occurrence in soil solution, adsorption on soil mineral surfaces and organic matter (Shahid, 2021).

Additionally, the chemical form of a PTM depends on chelation or precipitation with numerous organic and mineral compounds (Liu et al., 2021). The interaction of PTMs with soil biotic/abiotic constituents takes place via various biological, chemical, and physical processes, thereby forming different types of PTM-mineral or PTM-organic complexes/chelates. Formation of these complexes alters the intrinsic characteristics of PTMs (Huang et al., 2017; Liu et al., 2021). These PTM interactions and their resultant chemical forms finally govern their bio-physio-chemical activity in the soil-plant interface. Hence, understanding soil PTM speciation is

of great importance in studies dealing with risk assessments and remediation. This is due to the known fact that varied chemical forms of a PTM have different bio-physio-chemical activities.

Consequently, some studies have evaluated chemical speciation of PTMs in different growth media under varied conditions (Nolan et al., 2003; Huang et al., 2017; Zhang et al., 2018). There are various methods of PTM speciation determination. Primarily, methods involving PTM extraction from soil have been widely used to predict PTM speciation (Li et al., 2022). Moreover, PTM soil speciation analysis using different types of instruments has not followed a coherent pattern (Li et al., 2022). Unfortunately, the majority of instruments and extraction methods have low PTM detection limits. In addition, the percentages of PTMs in different chemical forms are also very varied. Moreover, these instrumental/extraction methods require use of expensive chemicals. In short, these methods are expensive, complicated and time-consuming and so they are not ideal, especially for large-scale practical application.

Recent development in computer-based simulation modeling has provided some rapid and alternative techniques to predict PTM speciation in a specified medium. Numerous computer-based speciation models have been established which have successfully predicted PTM speciation in soils and waters. These computer-based simulation models/software include Visual Minteq, CHESS, ECOSAT, MINTEQ A2, CHEAQS, SOLTEQ, PHREEQC, PHREEQC and WHAM VI (Shahid et al., 2011a). These models are less demanding of data and time compared to conventional analytical methods. The programming of these models involves different equations, and considers different reactions and interactions of PTM with complexants, in order to predict speciation.

Based on the above-mentioned facts, the current study aims to predict the chemical speciation of various PTMs in upper and lower soil layers of different agronomic fields using Visual Minteq. The purpose was to assess the relative percent occurrence of various forms of PTMs in natural soil systems.

2 Methodology

2.1 Study area description

In this study, soil samples were collected from 15 different agronomic fields (F1−F15) of Vehari-Pakistan. These 15 agronomic fields are located in the sub-urban areas of three Tehsils of District Vehari. This district is located in central Punjab (30.044° N, 72.344° E), and represents an agricultural area with > 90% land used for crop cultivation. Therefore, the environmental issues of the study area are mainly related to local agricultural activities.

One major environmental issue, although not very severe, of the study area is the occurrence of PTMs in different environmental compartments (soil and water). Previous studies have shown the existence of different types of PTMs at varying levels in the groundwater and soil of the area (Niazi et al., 2018; Natasha et al., 2022b). Sampling fields mainly used for the cultivation of different types of crops and vegetables were selected. These fields are routinely irrigated with different types of irrigation waters such as tube well water, canal water and city wastewater. Some previous studies have reported the occurrence of different PTMs, especially arsenic (As) in these irrigation waters (Shah et al., 2020). Owing to the varied chemical composition of these irrigation waters, it was believed that these fields may contain PTMs in different chemical forms, which could affect their soil-plant transfers as well as the allied toxicity and risk hazards.

The samples were collected from 0 to 15 cm (upper layer) and 15−30 cm (lower layer) soil. After proper soil processing and digestions, total soil contents of eight PTMs (Cd, Cr, Fe, Cu, Mn, Ni, Zn and Pb) were analyzed using an atomic absorption spectrophotometer (Sarwar et al., 2020). Moreover, basic soil attributes such as electrical conductivity (EC), pH, carbonate, ionic contents, and soil organic matter were also determined in 1:1 soil:water extracts following the methods of the ICARDA manual.

The detailed analytical procedures and the total soil contents of PTMs were reported in our previous study (Sarwar et al., 2020). The mean concentrations of these PTMs were as following: 1.6 mg kg^{-1} of Cd, 40 mg kg^{-1} of Cu, 60 mg kg^{-1} of Cr, 8598 mg kg^{-1} of Fe, 268 mg kg^{-1} of Mn, 1.9 mg kg^{-1} of Ni, 31 mg kg^{-1} of Pb and 102 mg kg^{-1} of Zn in upper soil layer. The respective PTM concentrations in lower soil layer were 1.6 mg kg^{-1} of Cd, 35 mg kg^{-1} of Cu, 61 mg kg^{-1} of Cr, 14570 mg kg^{-1} of Fe, 278 mg kg^{-1} of Mn, 2.0 mg kg^{-1} of Ni, 30 mg kg^{-1} of Pb and 103 mg kg^{-1} of Zn. Overall, both soil layers revealed similar PTM contents.

2.2 Trace elements chemical speciation in soil

To predict the chemical speciation of selected eight PTMs in the sampled soils (both soil-layers), we used Visual Minteq speciation software. This model uses the well-established thermodynamic-relationships among different chemical species of a PTM to calculate the equilibrium distributions. The Minteq database contains all the equilibrium constants for different chelating ligands to predict PTM ion speciation (Table 2). This speciation model requires certain soil characteristics as input variables to predict PTM soil speciation. The required soil characteristics include: PTM concentrations, pH, EC, major cations and anions (organic and inorganic ligands). All these soil parameters were determined for all the 15 agronomic fields. The major cations and anions used in this study to predict chemical speciation of PTMs

Table 2 Values of equilibrium constants (from Minteq database) used for Pb speciation prediction (Shahid et al., 2011a).

Reactions	log$K$
$Pb^{2+} + H_2O = PbOH^+ + H^+$	−7.6
$Pb^{2+} + 2H_2O = Pb(OH)_2 + 2H^+$	−17.09
$Pb^{2+} + 3H_2O = Pb(OH)_3^- + 3H^+$	−28.09
$Pb^{2+} + Cl^- = PbCl^+$	1.55
$Pb^{2+} + NO_3^- = PbNO_3^+$	1.17
$Pb^{2+} + SO_4^{2-} = PbSO_4$	2.69
$Pb^{2+} + 2SO_4^{2-} = Pb(SO_4)_2^{2-}$	3.47
$Pb^{2+} + PO_4^{3-} + 2H^{+1} = PbH_2PO_4^+$	21.07

Minteq database contains such equilibrium constants for all the PTMs.

include sodium, potassium, calcium, magnesium, chlorides, carbonates and bicarbonates. The concentration of PTMs and other soil characteristics were used as input data for Visual Minteq software.

2.3 Statistical analysis

The relative % values of different chemical forms of all the PTMs in soil were calculated using Microsoft Excel 2019. Similarly, XLSTAT (2018) was used to perform multivariate analyses.

3 Results and discussion

3.1 Chemical speciation of PTMs in soil

Table 3 describes the mean relative abundance (%) of different chemical forms of each PTM in upper and lower layers of 15 agronomic fields. To keep the results simple and easily understandable, we have presented here the two major/dominant species of each PTM. In fact, the relative abundance (%) of other species of each PTM were very low (generally < 0.1%), and therefore these species are not presented here.

The major predicted species of selected PTMs in agronomic fields were Pb^{2+} and $PbOH^+$ for Pb, Cd^{2+} and $CdCl^+$ for Cd, Zn^{2+} and $ZnOH^+$ for Zn, Mn^{2+} and $MnCO_3$ for Mn, Fe^{2+} and $FeOH^+$ for Fe, Cu^{2+} and $CuOH^+$ for Cu, Cr^{2+} and $CrOH^+$ for Cr, and Ni^{2+} and $NiOH^+$ for Ni. Among these chemical forms of PTMs, the free ionic forms (PTM^{2+}) were more dominant compared to other species such as CO_3, HCO_3, OH, etc. Overall, all the studied PTMs showed an average 79.9% occurrence in PTM^{2+} form in the entire soil layer (0 to 30 cm). These relative percentage ages were 78.4% and 81.3%, respectively for upper and lower soil layers. Previously, studies have also revealed the presence of PTMs in different chemical states in soils as well as the predominance of PTM^{2+} (Zhang et al., 2018).

Table 3   Mean predicted values of different chemical forms of PTMs, averaged for both layers combined, and also upper and lower soil layers individually.

PTM	Speciation	0−30 cm	0−15 cm		15−30 cm		% variation
			Mean	SD	Mean	SD	
Pb	Pb^{2+}	86.5	83.5	11.6	89.5	5.5	4.3
	$PbOH^+$	12.5	15.1	10.5	9.8	5.3	−120
Cd	Cd^{2+}	99.1	98.8	0.9	99.4	0.5	0.3
	$CdCl^+$	0.8	1.1	0.9	0.6	0.5	−86
Zn	Zn^{2+}	99.2	98.9	1.0	99.5	0.3	0.3
	$ZnOH^+$	0.6	0.8	0.7	0.3	0.3	−645
Mn	Mn^{2+}	100	100	0.0	100	0.0	0.01
	$MnCO_3$	0.0	0.0	0.0	0.0	0.0	−1166
Fe	Fe^{2+}	99.8	99.7	0.3	99.9	0.1	0.1
	$FeOH^+$	0.2	0.3	0.3	0.1	0.1	−647
Cu	Cu^{2+}	50.2	41.8	31.2	58.7	24.6	38
	$CuOH^+$	5.6	5.8	2.2	5.5	2.0	−123
Cr	Cr^{2+}	4.2	4.5	10.4	3.9	7.5	−5
	$CrOH^+$	95.8	95.5	10.4	96.1	7.5	0.2
Ni	Ni^{2+}	99.9	99.9	0.1	99.9	0.0	0.04
	$NiOH^+$	0.1	0.1	0.1	0.0	0.0	−545
Average	PTM^{2+}	79.9	78.4	6.9	81.3	4.8	4.7
Minimum		–	4.5	0.0	3.9	0.0	−4.8
Maximum		–	100	31	100	25	38
Average	*Others except* PTM^{2+}	14.4	14.8	3.1	14.1	2.0	−416
Minimum		–	0.0	0.0	0.0	0.0	−1166
Maximum		–	95.5	10.5	96.1	7.5	0.2
Average	*All*	47.2	46.6	5.0	47.7	3.4	−206

SD represent standard deviation. % variation shows variations between upper and lower soil layers.

3.2 Comparison of chemical speciation of each PTM in soil

Results showed varied trends when different PTMs were compared for relative abundance of their different chemical forms. For example, Pb^{2+} was the predominant form (94.3%) in the entire soil layer (0−30 cm) as well as for upper (92.3%) and lower (96.4%) soil layers (Table 3). The trend was same for Cd^{2+} (99.5%, 99.3%, and 99.6%), Zn^{2+} (99.8%, 99.6%, and 99.9%), Mn^{2+} (100%, 100%, and 100%), Fe^{2+} (99.9%, 99.9%, and 100%) and Ni^{2+} (100%, 99.9%, and 100%), respectively for both layers combined, and for upper and lower soil layers (Table 3). Previously, several studies have also reported the dominant existence of PTM^{2+} in soil solutions. For example, Nolan et al. (2003) showed the predominance of Zn^{2+} (97%), Pb^{2+} (66%), Cu^{2+} (46%), and Cd^{2+} in various contaminated and agricultural sites in Australia. They also revealed the efficiency of different computer-based models to accurately predict chemical speciation of PTMs in soil. Similarly, Huang et al. (2017) used Visual Minteq software and reported > 95% dominance of Cd^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+}, relative to other species, in aqueous solution under acidic conditions.

Overall, Cr and Cu revealed a somewhat different chemical speciation compared to other PTMs (Table 3). Copper showed 50.2% presence in combined soil layers, 41.8% in upper and 58.7% in lower soil layers. Under alkaline conditions Cu quickly converts to its OH forms. In this study, 12% of $Cu_2(OH)_2^{2+}$, $Cu_3(OH)_4^{2+}$ also showed presence in the soil solution.

Cr^{2+} showed an entirely opposite trend compared to other PTMs (Table 3). Results showed only 4.2%, 4.5% and 3.9% presence of Cr^{2+}, respectively in combined, upper and lower soil layers. Chromium also exists in different oxidation forms (−2 to +6), but trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) forms are the most stable and common. Hence, Cr^{2+} is not the prominent form of Cr.

In addition to PTM^{2+}, the other commonly found forms of each PTM were also compared for both the soil layers. Except Cu and Cr, all other PTMs showed very low relative percentage ages in the entire soil layer for other chemical species: 12.5% of $PbOH^+$, 0.8% of $CdCl^+$, 0.6% of $ZnOH^+$,

minute traces of $MnCO_3$, 0.2% of $FeOH^+$, 5.6% of $CuOH^+$, and 0.1% of $NiOH^+$ (Table 3). The relative percentage agens of these PTM species were similar for upper and lower soil layers. In contrast to other PTMs, $CrOH^+$ showed 95.8% dominance in the entire soil layer. The trend was similar for upper (95.5%) and lower (96.1%) soil layers.

It is well-reported that in addition to free ionic forms, PTMs also exist combined with different ligands. Generally, soil solution pH is a key variable controlling existence of a PTM in different chemical forms. PTMs also exist combined with SO_4, PO_4 or CO_3 under acidic conditions and with HCO_3 and OH under alkaline conditions. In the current study, the pH in the majority of agronomic fields was slightly alkaline (up to pH8.1), and therefore PTMs were also present, although in low concentration, in their OH forms.

3.3 Comparison of chemical speciation of PTMs in upper and lower soil layers

When the relative abundance of different species of PTMs was compared for upper and lower soil layers, the mean percentage age of all the $PTMs^{2+}$ was slightly higher for lower layer (81.3%) than upper layer (78.4%) (Table 3). Overall, $PTMs^{2+}$ were 5% more dominant in the lower layer than in the upper layer, and this trend was almost same for all the PTMs. The respective values were 4.3% for Pb, 0.3% for Cd, 0.3% for Zn, 0.01% for Mn, 0.1% for Fe, 38% for Cu and 0.04% for Ni. In the case of other chemical species of PTMs, the trend was the opposite to that of $PTMs^{2+}$. Overall, the other PTM chemical forms ($PbOH^+$, $CdCl^+$, $ZnOH^+$, $MnCO_3$, $FeOH^+$, $CuOH^+$, and $NiOH^+$), showed a higher presence in the upper layer than in the lower layer. $CrOH^+$ was the exception to this.

This higher PTM^{2+} percentages in lower soil can be due to lower contents of organic matter and other cation/anions such as Cl^- and HCO_3^- (Table S2). Overall, the upper soil layer has higher values of OM, EC, Cl, and HCO_3 by 36%, 16%, 38% and 9%, respectively, compared to lower soil layer. These soil parameters resulted in $PTMs^{2+}$ conversion to other PTM forms due to complexation with different ligands ($PbOH^+$, $CdCl^+$, $ZnOH^+$, $MnCO_3$, $FeOH^+$, $CuOH^+$, and $NiOH^+$) in upper soil layers. Hence, the variation in the levels of cationic/anionic ligands in upper and lower soil layers can affect PTM speciation.

3.4 Comparison of chemical speciation of PTMs among 15 agronomic fields

When the relative abundance of different chemical forms of PTMs was compared among 15 agronomic fields, the mean percentage ages of all the $PTMs^{2+}$ were similar (Tables 4−5, Tables S3−S4). Overall, there were 5% and 3.4% variations, respectively for upper and lower soil layers, among the S.D.

Table 4 Chemical speciation of PTMs in different agronomic fields (F1−F4) in upper soil layer

PTM	Speciation	F1	F2	F3	F4
Pb	Pb^{2+}	63.3	69.8	70.4	88.1
	$PbOH^+$	32.1	27.4	27.2	11.2
Cd	Cd^{2+}	99.1	97.7	99.2	98.6
	$CdCl^+$	0.7	2.2	0.1	1.4
Zn	Zn^{2+}	97.2	97.9	98.0	99.4
	$ZnOH^+$	2.0	1.5	1.5	0.5
Mn	Mn^{2+}	99.9	99.9	99.9	100
	$MnCO_3$	0.1	0.0	0.0	0.0
Fe	Fe^{2+}	99.2	99.4	99.4	99.8
	$FeOH^+$	0.8	0.6	0.6	0.2
Cu	Cu^{2+}	9.3	11.8	11.4	35.6
	$CuOH^+$	6.0	5.8	5.5	5.7
Cr	Cr^{2+}	0.6	0.8	0.8	2.4
	$CrOH^+$	99.4	99.2	99.2	97.6
Ni	Ni^{2+}	99.7	99.8	99.8	99.9
	$NiOH^+$	0.3	0.2	0.2	0.1
Average	*M^{2+}*	*71*	*72*	*72*	*78*
Average	*Others*	*18*	*17*	*17*	*15*

The data of remaining fields (F5 to F15) for upper soil layer is presented in Table S2.

Table 5 Chemical speciation of PTMs in different agronomic fields (F1−F4) in lower soil layer.

PTM	Speciation	F1	F2	F3	F4
Pb	Pb^{2+}	87.0	86.7	84.3	97.0
	$PbOH^+$	11.9	12.2	14.4	2.7
Cd	Cd^{2+}	98.9	99.4	99.5	99.5
	$CdCl^+$	1.1	0.6	0.0	0.5
Zn	Zn^{2+}	99.5	99.5	99.2	99.9
	$ZnOH^+$	0.2	0.2	0.2	0.0
Mn	Mn^{2+}	100	100	100	100
	$MnCO_3$	0.0	0.0	0.0	0.0
Fe	Fe^{2+}	99.9	99.9	99.9	100
	$FeOH^+$	0.1	0.1	0.1	0.0
Cu	Cu^{2+}	52.3	39.3	36.8	91.9
	$CuOH^+$	5.8	4.5	5.1	2.1
Cr	Cr^{2+}	0.9	0.9	0.7	4.4
	$CrOH^+$	99.1	99.1	99.3	95.6
Ni	Ni^{2+}	100	100	99.9	100
	$NiOH^+$	0.0	0.0	0.0	0.0
Average	*M^{2+}*	*80*	*78*	*78*	*87*
Average	*Others*	*15*	*15*	*15*	*13*

The data of remaining fields (F5 to F15) for lower soil layer is presented in Table S3.

values of all the PTMs among all the fields (Table 3). These small S.D. variations show an overall similarity in the chemical speciation of different PTMs among the 15 fields. The possible reason may be similar soil characteristics of all the 15 fields.

Cultivation of different types of crops may affect the chemical speciation of PTMs by various processes such as (i) releasing organic ligands, via roots, into rhizosphere soil, (ii) altering soil chemical properties (such as rhizosphere pH), and influencing microbial community/abundance (Jiang et al., 2023). Generally, hyper-accumulator and tolerant plants secrete high quantities of root exudates, thereby greatly affecting the chemical speciation of PTMs in soil (Shahid et al., 2011b; Gavrilescu, 2022). In fact, the plant root exudates are capable of resulting in complexation of PTMs in soil and thereby decreasing the concentration of free PTM ions. Numerous studies dealing with the phytoremediation of PTMs from contaminated soils using hyper-accumulator plants have revealed the secretion and interaction mechanisms of root exudates with PTMs (Khalid et al., 2017). However, under the current cultivation system of the study area, most of the factors such as crop/vegetable type and environmental conditions were similar. Therefore, different soils did not show great variations in chemical speciation of PTMs.

The Pearson correlation matrixes were calculated to assess the correlations among the 15 agronomic fields (Tables S5-S6). Pearson correlation matrix confirmed the similarity among all the fields by showing strong correlation ($R^2 > 0.95$) values. These results revealed that until there are significant changes in soil chemical characteristics (pH, Eh, EC, cations, anions), the extent of chemical speciation does not vary greatly.

3.5 Principal component analysis

To trace general trends among different PTMs (Cd, Cr, Fe, Cu, Mn, Ni, Zn and Pb) and different agronomic fields (F1−F15), multivariate analysis was carried out both for upper and lower soil layers. Overall, both the soil layers showed similar trends.

For both soil layers, multivariate analyses divided the data into several component factors (CFs). However, the first of these, CF1, mainly contributed to covariance/correlation of the data (Table S7). There were 97% contributions of CF1 to total variance for each of upper and lower soil layer (Table S7). The respective contribution was very small (3%) for CF2. All the agronomic fields have almost similar contributions to CF1 (5.7% to 6.9%). This shows an overall similarity in chemical speciation of PTMs for different agronomic fields.

The PCA graphs comparing all the agronomic fields (F1−F15) also showed an overall similarity among all the fields for both soil layers. PCA graph grouped all the fields overlapping each other in upper (Fig. 1) and lower layers (Fig. S1), thus showing high similarity among them. This confirmed that the chemical speciation of all the PTMs did not differ significantly among 15 agronomic fields.

When different chemical forms of PTMs were compared, PCA graph grouped all the PTM^{2+} closely. The grouping of these PTM^{2+} was due to their predominance (> 95%) in soil solutions. However, Cu^{2+} and Cr^{2+} were exceptions and were not dominant forms but were grouped separately. Thus, PCA confirmed the varied speciation of Cu and Cr compared to other PTMs. The trend was same for both the layers (Fig. 2 for upper and Fig. S2 for lower layers).

The other forms of PTMs (PbOH$^+$, CdCl$^+$, ZnOH$^+$, MnCO$_3$, FeOH$^+$, CuOH$^+$, and NiOH$^+$) also showed grouping in both soil layers. The CrOH$^+$ was an exception to this behavior (Figs. 2 and S2) due to its high presence (> 95%) in both soil layers compared to relevant form of others PTMs.
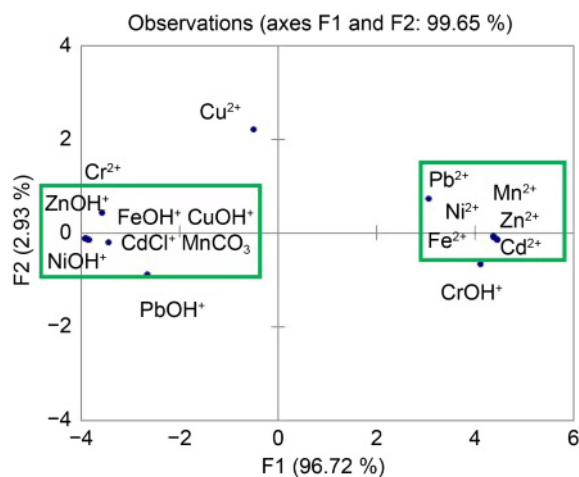

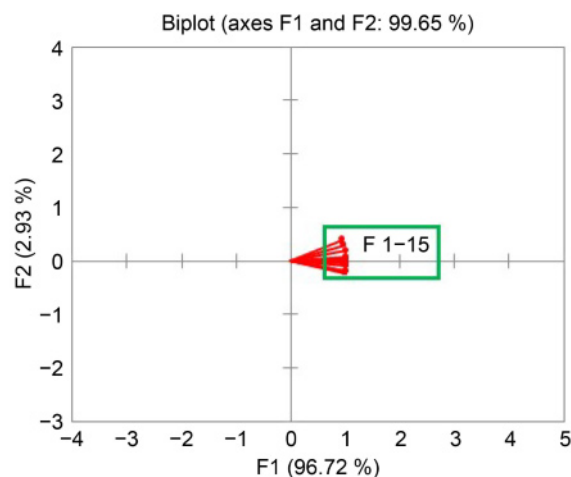**Fig. 1** PCA graph of different species of all the PTMs for upper soil layers.

Fig. 2 PCA graph of all the agronomic fields (F1−F15) for upper soil layers.

3.6 Environmental application of data

The above data regarding the occurrence of PTMs in different chemical forms among 15 agronomic fields as well as their covariance/correlation reveal the importance of chemical speciation of PTMs in soil-plant interaction. Results demonstrated that different PTMs may exist in different chemical forms in soil. Moreover, there is some variation, between PTMs, of dominant chemical forms under prevailing soil conditions. In particular, Cu and Cr dominant forms differed significantly from other PTMs such as Pb, Cd, Zn, Mn, Fe and Ni. These variations in the occurrence of PTMs in different chemical forms can ultimately alter their phyto-uptake and phyto-toxicity. Studies have revealed significant variations in plant physiological responses under varied applied forms of PTMs (Abbasi et al., 2022). The activation of plant tolerance and scavenging mechanisms (such as through the actions enzymes, hormones and ligands) also varies with the applied form of PTMs (Shahid, 2021; Abbasi et al., 2022).

Some studies have reported that the accumulation and distribution of PTMs between edible and non-edible plant tissues vary greatly with differences in the chemical speciation of PTMs. Hence, the build-up of PTMs in edible plant tissues and the associated potential health risks may differ for different chemical forms of PTMs (Shahid et al., 2020). The phenomenon has been explained in detail by numerous previous risk assessment studies, which highlighted that chemical forms of PTMs in soil govern their phyto-uptake, build-up in comestible parts, and allied health hazards (Rafiq et al., 2017; Abbasi et al., 2022).

Moreover, the data of the current study compared chemical speciation of PTMs for upper and lower soil layers. It was observed that PTMs may exist in similar chemical forms when there is no major difference in the physico-chemical characteristics of different soil layers. Hence, the downward PTM leaching may not differ greatly for the upper soil layers up to 30 cm due to their similar characteristics. However, this may not apply for different soil types with varying physico-chemical characteristics.

4 Conclusions

Current study showed that the free ionic forms of PTMs (PTM^{2+}) predominate in soil. However, Cr^{2+} did not follow this trend, possibly because of its general existence as Cr^{3+} and Cr^{6+}. The occurrences of $PTMs^{2+}$ were slightly lower for upper soil layer than lower soil layer, the trend almost being same for all the PTMs except Cr. In fact, higher levels of organic matter and other anions such as Cl^- and HCO_3^- present in upper soil layers resulted in interactions with PTM^{2+}, and hence decreased $PTMs^{2+}$ ratios in upper soil

than lower soil layers. Moreover, all the 15 agronomic fields showed almost similar chemical speciation of PTMs. This was also confirmed by strong Pearson correlation ($R^2 > 0.95$) values between the 15 fields and this similarity was also evidenced by co-grouping in PCA graph. However, Cr^{2+} and Cu^{2+} were grouped separately due to their more varied chemical speciation than that seen in other PTMs. Prediction of chemical speciation of PTMs in soil solutions can have a better correlation with their biochemical activity (phyto-uptake, accumulation and toxicity) in soil-plant interfaces than can be achieved with less detailed analysis, such as of total PTM concentrations only.

Conflict of interest

Authors declare no-known competing conflict of interests.

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Electronic supplementary material

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