

Accumulation, risk assessment of trace elements in soil-herbage systems and effects of elevation on subalpine grassland in the northeast of Tibet Plateau

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Abstract

Ecological environment of remote grassland has become increasingly serious in many countries due to mining, tourism, grazing and other human activities. In this study, a total of 15 pairs of soil-herbage samples were collected in the northeast of the Tibet Plateau to study the relationship between physicochemical properties and content of trace elements in soils and elevation, and to examine the accumulation and fractionation of heavy metals in soil-herbage systems. In addition, the ecological risk of the subalpine grassland was also assessed. The average concentrations of Hg, As, Cu, Zn, Pb, Cd, Cr and Mn in soil were higher than their background values of Gansu soil, but the average concentrations of these heavy metals in herbage satisfied Hygienical Standard for Feeds. The speciation analysis of heavy metals in soil indicated that the exchangeable content of heavy metal was very low, except Pb, Cd, Mn. There was a linear relationship between pH, CaCO₃, total phosphorus (TP), organic matter (OM), concentrations of Hg, As, Zn, Pb, Cr and Mn in soils, dry weight of herbage and elevation, while there was a quadratic curve trend between Cu, Cd in soils and elevation. The results of risk assessment showed that there was no obvious ecological risk in the study area.

1. Introduction

Excessive heavy metals in soils are a potential threat to the ecological system and have inspired serious concern due to their persistence and toxicity (Luo et al., 2012; Yang et al., 2014). Contamination of heavy metals in soil leads to their bioaccumulation through the food chain which poses risks to human health (Peralta-Videa et al., 2009; McLaughlin et al., 2000). Soil heavy metal contamination can also cause groundwater contamination through leaching (Mulligan et al., 2001). The analysis of surface soil (0-20cm) is the most valuable for studying heavy metal contamination in grassland (Martin et al., 2017), because anthropogenic heavy metal input usually accumulates in topsoil. Grasses also take up elements predominantly from surface soil (Hou et al., 2014; Kismányoky and Tóth, 2010).

The pollution level of heavy metals in soil mainly depends on its total amount, chemical forms, sources and some soil physical or chemical factors (Chai et al., 2015). Therefore, in the monitoring and assessment of soil pollution, the possible sources of heavy metals in soils should also be distinguished except for the total concentration and chemical speciation of heavy metals as well as some physicochemical parameters (Zhao et al., 2014; Oliva and Espinosa, 2007). The major natural source of heavy metals in soil usually comes from the parent materials (Blaser et al., 2000; Alloway, 1995). It is generally believed that some human activities had a high contribution of heavy metals in soil such as industrial gas emissions (Hovmand et al., 2013), agricultural and industrial waste discharges (Khan et al., 2008; Boularbah et al., 2006), fertilizers and pesticides (Nziguheba and Smolders, 2007).

The elevation gradient influences soil properties and climate (Gaston, 2000), and these factors causes changes of plant community structure and biomass (Dong et al., 2004). On the elevation gradient, the soil properties in different locations may be different due to the differences in temperature, precipitation and parent material. In alpine areas, elevation is one of the most important factors for the micro-climate

conditions (temperature and precipitation) which determine the growth and distribution of plants (Parmesan, 2006). Even a short geographical distance can lead to significant changes in climate variables such as temperature, precipitation and radiation (Zhang et al., 2013). Elevation not only affects the content of heavy metals in soil during soil formation, but also through long-distance transportation, such as precipitation and atmospheric deposition. However, little work has been done in subalpine grasslands of the Tibet Plateau on the effects of elevation on plant biomass, distribution of soil properties and the contents of trace elements.

According to the national pollution survey bulletin, the over standard rates of cultivated land, forestland, grassland of pollutants in China are 19.4%, 10.0% and 10.4%, respectively, and the over standard rates of Cd, As and Hg are 7%, 2.7% and 1.6%, respectively. China's grassland accounts for 40% of the total land area, three times that of cultivated land. Grassland is not only a necessary place for grazing, but also a paradise for wildlife. There have been many mining activities and other human activities in the Qilian Mountain (in the northeast of Tibet Plateau), northwest China in recent years. Therefore, it is worth examining the effects of human activities on the soils and herbage in this area.

Whether the environmental quality and herbage quality are at a safe level or affected by human activities has been widely concerned. In order to evaluate the soil and herbage quality of grassland, a study was carried out since August 2018. The aims of this paper were to analyze the relationship between soil physicochemical properties, trace elements content and elevation, to investigate the chemical fractionations of trace elements in soils of a subalpine grassland in the northeast of Tibet Plateau, and to examine the ecological risk of the grassland system.

2. Materials And Methods

2.1 Study area

The subalpine grassland is located in the middle section of the Qilian Mountains in Sunan county, Gansu, China, which is a remote mountain grassland. And the study area is a part of Qilian Mountain Grassland which is one of the major nature pastures and now is a national tourist attraction. The climate in this area is alpine and semi-arid, and the elevation ranges between 2300 and 5500 m asl (the snow line is about 4600 m asl). The average annual precipitation is 433.6 mm, and the annual evaporation is 1488 mm. The average annual sunshine duration is 1893 hours, and the average annual temperature is 5.4°C (Cao et al., 2020). In this region, grasslands dominate the south-facing slope aspects, while Qinghai spruce (*Picea crassifolia* Kom.) forests was on north-facing slope aspects (Cao et al., 2020). Samples were collected in the south-facing slope aspects. The soils on the south-facing and semi-sunny slopes were predominantly sandy textured chestnut soils, and silty-sand textured subalpine meadow soil predominantly developed on the semi-shady slopes, while the north-facing slopes are predominantly silty-sand textured grey cinnamon soils (Qin et al., 2016). The parent materials of soil are various, mainly Aeolian Loess (mainly silt) and rock weathering. The national tourist attraction was divided into three parts, i.e. core area, buffer area and test area. The test area can receive tourists, and the buffer zone has strict restrictions on human

activities, while the core area does not allow any human activities that are harmful to the environment. The samples in this paper were collected from buffer area and test area. Sampling point locations were shown in Figure 1.

2.2 Samples collection

A total of 15 pairs of representative samples were collected, representing different types of human activity areas, different elevation (ranges between 2600 and 3200 m asl), different terrain, and various special sites. Detailed description of the sampling points was shown in supplementary materials. Soil samples were collected at a depth of 0-20 cm, and the aboveground part of herbage were collected at the same sampling sites in units of one square meter. The soil samples were stored in a polyethylene bag, and plant samples were stored in a cloth bag until transported to the laboratory.

2.3 Samples analysis

The soil samples were air-dried at room temperature for two weeks, with one part was ground and sieved through <2 mm mesh to measure pH and electrical conductivity (EC), and the other part was ground and sieved through <0.149 mm mesh to measure organic matter, CaCO₃ and heavy metal content. The plant samples were classified before analyzing, and then baked at 105°C for an hour, baked at 75°C to constant weight.

Soil pH and EC were measured in a suspension of 1:2.5 at room temperature using pH meter (PHS-3C, REX, Shanghai, China) and EC meter (DDS-307, REX, Shanghai, China) respectively. OM was determined by Potassium dichromate volumetric method-dilute heating method and the carbonate content (CaCO₃) in the soil was determined by titration (Lu, 2000).

Soil and plant samples were digested by using microwave digestion system (Anton Paar, Multiwave PRO 3000) (Li et al., 2018). Soil samples of 0.2g were digested with the mixture of HNO₃, HCl, HF and H₂O₂ (v/v, 3:1:1:1) and plant samples of 0.5g were digested with the mixture of HNO₃ and H₂O₂ (v/v, 4:1) to determine the total Cu, Zn, Pb, Cd, Cr, Mn (Li et al., 2019), while 0.2g soil samples or 0.5g plant samples were digested with the mixture of HCl and HNO₃ (v/v, 3:1) to determine the total Hg and As (Zhang et al., 2018). The Chemical speciation of heavy metals of the soil was obtained using the Tessier sequential extraction procedure (Tessier et al., 1979). Hg and As in soil and plant samples were analyzed by Atomic fluorescence morphology analyzer (AFS-8220, Beijing Jitian, China) and Cu, Zn, Pb, Cd, Cr, Mn were analyzed by AAS (ICE-3500, Thermo, USA).

2.4 Quality control and assurance

The reagents and chemicals were of the guaranteed reagent. Reagent blanks, the standard reference soil samples (GSS-8) and plant samples (GSB-24) from the Center of National Standard Reference Material of China were measured in order to monitor the analytical accuracy and precision. Each sample was performed in three replicates. The recoveries of total metals content in standard reference samples were

within 85% to 115%. The recovery in Tessier sequential extraction procedure were 88.7%-100.3% for Hg, 90.8%-97.9% for As, 90.8%-97.3% for Cu, 87.6%-98.9% for Zn, 93.8%-100% for Pb, 73.1%-96.6% for Cd, 92.0%-99.7% for Cr, 97.6%-99.8% for Mn.

2.5 Bioaccumulation factor (BCF)

The BCF referred to the ratio of metal concentration in plants to that in soil (Yao et al., 2019) (BCF > 1 indicates heavy metal accumulation). And it was calculated as follows:

$$BCF = \sigma / C$$

where σ was the concentration of heavy metals in aboveground parts of plants, and C was the concentration of heavy metals in soils.

2.6 Geo-accumulation index (I_{geo})

The I_{geo} can show an idea of the degree of heavy metals contamination in soil. I_{geo} was calculated using the regional background values (Damiani et al., 1987). The background values were obtained from the Backgrounds of Soil Environment in China (CNEMC, 1990). The I_{geo} values were calculated as follows:

$$I_{geo} = \log_2 \left[\frac{C_n}{1.5B_n} \right]$$

where C_n was the concentration of elements, B_n was the geochemical background values of the elements, and 1.5 was used to correct possible variations of the background values for the given elements.

The geological accumulation index was divided into seven levels, $I_{geo} \leq 0$, indicating no pollution; $0 < I_{geo} < 1$, indicating no pollution to moderate pollution; $1 < I_{geo} < 2$, indicating moderate pollution; $2 < I_{geo} < 3$, indicating moderate pollution to strong pollution; $3 < I_{geo} < 4$, indicating strong pollution; $4 < I_{geo} < 5$, indicating strong pollution to extreme pollution; $I_{geo} > 5$, indicating extreme pollution.

2.7 Ecological risk assessment

The contamination factor (CF) was used to evaluate the contamination status of heavy metals in soil or sediment as an individual index (Williams and Antoine, 2020). Tomlinson et al. (1980) divided the pollution degree into four grades according to the CF value. CF value < 1 indicates low contamination, and $1 \leq CF < 3$ and $3 \leq CF < 6$ indicate moderate and considerable contamination, respectively. $CF \geq 6$ indicates high contamination. The CF values were calculated as following formula:

$$CF = C_i / S_i$$

where C_i was the concentration of each element, S_i was the geochemical background value of the element.

Potential ecological risk index (RI) was developed by Hakanson (1980) to assess the potential risk of heavy metals in soil to the ecosystem. RI was calculated as follows:

$$RI = \sum Er = \sum CF \times T_i$$

where T_i was the toxicity coefficient, and the coefficients for each metal were Hg = 40, As = 10, Cu = 5, Zn = 1, Pb = 5, Cd = 30 and Cr = 2.

It was considered low ecological risk if RI less than 150, and $150 \leq RI < 300$ suggested moderate ecological risk, $300 \leq RI < 600$ suggested considerable ecological risk, $RI \geq 600$ suggested very high ecological risk.

Nemerow index (P_N) reflects the integrated pollution degree of different heavy metals in the same region (Vu et al., 2017). The P_N were calculated by following equations:

$$P_N = \sqrt{\frac{CF_{avg}^2 + CF_{max}^2}{2}}$$

where CF_{avg} and CF_{max} were the average and the maximum of CF. Standards for grading metals contamination were as follows: uncontaminated ($P_N \leq 1$), slightly contaminated ($1 < P_N \leq 2.5$), moderately contaminated ($2.5 < P_N \leq 7$), and heavily contaminated ($P_N > 7$) (Yuan et al., 2020).

3. Results And Discussion

3.1 Multivariate statistical analysis

3.1.1 Physicochemical properties and heavy metal concentrations.

The average contents of heavy metals in plants were compared with the Hygienical standard for feeds (CNSBQTS, 2017). As shown in the Table 1, the concentrations of heavy metals in herbage were far lower than the standard contents, indicating that the concentrations of heavy metals in herbage in this area were at a safe level, which was suitable for grazing.

The pH of soils ranged from 7.9 to 8.2 with an average value of 8.1 (Table 2). EC in soils was within the range of 165 - 282 $\mu\text{s cm}^{-1}$ and with an average of 215 $\mu\text{s cm}^{-1}$. The EC in soil reflects the content of soluble salt directly. High EC value could affect microbial community and herbage growing. CaCO_3 contents in soils ranged from 43.39 to 183.45 g kg^{-1} , with a mean value of 68.48 g kg^{-1} . Calcium carbonate in soil was one of the main factors of alkalinity ($\text{pH} > 7$). OM in soils ranged within 48.77 - 160.79 g kg^{-1} with a mean value of 88.68 g kg^{-1} which was higher than the background.

The mean values of Hg, As, Cu, Mn, Cd, Zn, Pb, Cr were $40.9 \pm 10.8 \mu\text{g kg}^{-1}$, $13.2 \pm 1.3 \text{mg kg}^{-1}$, $34.0 \pm 2.1 \text{mg kg}^{-1}$, $876 \pm 159 \text{mg kg}^{-1}$, $0.232 \pm 0.057 \text{mg kg}^{-1}$, $81 \pm 16 \text{mg kg}^{-1}$, $34.8 \pm 7.3 \text{mg kg}^{-1}$, $136.6 \pm 31.6 \text{mg kg}^{-1}$ respectively. The mean values of heavy metals were lower than the Environmental Quality Standard for Soil (CNEPA,1995) except Cd and Cr. All of the mean values of metals in soils were higher than their background values of Gansu Province soil (CNEMC, 1990), suggesting that heavy metal concentrations in the studied soils were affected by human activities, but not leading to a significant contamination of soils. The coefficient variations (CV) of heavy metals were comparatively low, suggesting that the concentrations levels of these heavy metals in soils were less variable, and the extrinsic inputs of the metals into the soils were limited.

3.1.2 Correlation analysis

The results of correlation analysis were shown in Table 3. For soil physicochemical properties, pH showed a positive correlation with Pb, indicating that neutral-alkaline soil was good for the accumulation of lead in soil. EC showed a significant positive correlation with mercury ($r = 0.528$, $p < 0.05$) but negative with arsenic ($r = -0.600$, $p < 0.05$). The reason might be that the properties of mercury and arsenic were different. Hg was positively charged and As was negatively charged. It was also revealed that soluble salts might reduce arsenic concentration. OM showed a negative correlation with arsenic ($r = -0.546$, $p < 0.05$), contrary to the results of some other researchers (Quenea et al., 2009), which might be caused by regional differences. CaCO_3 content correlated positively with Cd ($r = 0.656$, $p < 0.01$), indicating that calcium carbonate promotes the accumulation of Cd in soil, which also confirmed that cadmium and calcium may have similar properties. Hg, As, Cu, Mn, Cd, Zn, Pb and Cr were not significantly correlated with each other, implying that the sources of these heavy metals were different.

3.1.3 Relationship between elevation and soil physicochemical properties

Elevation was the most important factor affecting the abiotic environment by changing climatic and topography (Holechek et al., 2010, Roukos et al., 2017). Most subalpine grasslands were on steep slopes, both elevation and degree of slope influenced plant diversity and soil properties (Hadjigeorgiou et al., 2005, Roukos et al., 2011).

Linear regression analysis (Figure 2) showed that the contents of CaCO_3 and TP decreased significantly with the increase in elevation, while the contents of soil organic matter increased significantly with the

increase in elevation. EC and herbage had a positive correlation with elevation, while pH had a negative correlation with elevation, but this trend was not very obvious.

The decrease in CaCO_3 content with the increase of elevation could be due to the increase in soil organic matter and water content (Ali et al., 2019). The difference in soil parent material and small area climate might also be the reasons. The change of pH, temperature and precipitation also affected the change of CaCO_3 with elevation (Ali et al., 2017), because acidity, temperature and precipitation affected the solubility of CaCO_3 .

The change of OM with elevation was mainly related to temperature and plant density. Lower temperature delays decomposition of OM (Charan et al., 2012), because low temperature reduces soil enzyme activity. As the main source of organic matter, the vegetation material also had a positive relationship with vegetation density (Williams et al., 2003). The plant yield in the study area increased with the increase in elevation. However, the content of soil organic matter at low elevation was still higher than that at high elevation in this study, which could be because of grazing in the study area. Grazing was more frequent at higher elevation (more than 3000 m asl) in this area, and as a result, the enrichment efficiency of organic matter in soil was greatly reduced. Total Kjeldahl nitrogen (TKN) had almost no linear correlation with elevation, which was consistent with Cao et al (2020). But there was a significant negative correlation between TP and elevation. This could be caused by the dissolution of soil soluble phosphorus into rainwater and its downward migration along the slope (Roberts and Bettany, 1985). In addition, soil water content, phosphorus adsorption capacity, pH value and the interaction among microclimate, topography and vegetation might also cause the change of soil total phosphorus with elevation (He et al., 2016).

3.1.4 Relationship between elevation and soil trace element

Elevation was one of the factors affecting the distribution of heavy metals in subalpine grassland soil. Affected by elevation, the precipitation increased with the increase in elevation. When the elevation reached a certain level, the precipitation decreased and the total deposition might be changed (Salerno et al., 2015; Reiners et al., 1975). The Figure 3 showed that except for Cu and Cd, the content of other trace elements was linear with elevation.

The concentration of Hg, Zn and Cr increased with elevation, while that of As, Pb, Mn decreased. Hg, in the forms of Hg^0 , gaseous oxidized Hg, and particulate phase mercury (Grigal, 2002), can undergo long-range atmospheric transport, due to its volatilization and persistence in the environment. Then, Hg entered the soil through precipitation or atmospheric deposition, which might be one of the reasons for the positive correlation between Hg and elevation. The change trend of Zn and Cr were similar to that in Magnani et al. (2018), but Mn and Pb were not the same to some studies (Magnani et al., 2018; McGee and Vallejo, 1996; Reiners et al., 1975). As a matter of fact, in addition to air pollution, topographic conditions and microclimate also affect the distribution of heavy metals (Bergamasch et al., 2002; Cong et al., 2015). Several studies in Alps showed that deposition was increased with elevation (Camarero et

al., 2009), but it was not found in other studies (Kang et al., 2007). The non-linear changes of Cu and Cd indicated that these two elements might be affected by many factors, such as human activities, atmospheric deposition, topographic conditions and parent materials. As a metalloid, the concentration of arsenic was affected by many factors, such as pH, speciation (arsenate, arsenite), organic matter, etc (Cai et al., 2002; González et al., 2006). In this study, arsenic concentration and organic matter showed a significant negative correlation, indicating that arsenic was more affected by organic matter concentration, which was different from the results of González et al. (2006).

3.1.5 BCFs in herbage

As shown in Table 2, Hg, Cd and Zn were the most translocated metals. BCFs were in the decreasing order of Hg (0.417) > Cd (0.393) > Zn (0.302) > Cu (0.181) > Mn (0.087) > Pb (0.021) > As (0.019) > Cr (0.004). There were many factors to control the accumulation and bioavailability of heavy metals, including: sequestration and speciation, active/passive transfer processes, redox states and the response of plants to elements in relation to seasonal cycles (Badr et al., 2012). Soil structure and texture also affected the absorption of heavy metals by herbage. Unlike other elements, Zn occurs in the soil frequently in easily soluble forms (Gawryluk et al., 2020) and Zn is usually accumulated the most in the aboveground tissues of plants in an ecosystem where this element occurs in the air (Kabata-Pendias and Pendias, 2001).

All BCFs in this study were less than 1, indicating that heavy metals were not accumulated excessively in plants. It was worth noting that the BCF of mercury, cadmium and zinc in herbage in the study area reached 0.3. Although the concentration of heavy metals in herbage was at a safe level, the concentration of these three metals in soil should be monitored.

3.2 Assessment of ecological risk

3.2.1 Geo-Accumulation Index

The I_{geo} of Hg, Cd and Cr in most samples reached level 1 to 2 (Table 4), indicating that Hg, Cd and Cr in the soil of the study area were more obviously affected by human activities compared with other metals. And 93% samples reached level 1 of Pb, 33% samples reached level 1 of Mn, and the other samples were level 0, implying that Pb, Mn in the soil slightly affected by human activities. As, Zn and Cu in soil were almost not affected by human beings, because almost all samples were evaluated at level 0.

Qilian Mountain was rich in mineral resources (Qin et al., 2016). According to the survey, there were some mining sites around the study area. Although some of the mining sites were closed at the request of the government, the impact on the soil quality directly or through atmospheric subsidence was long-term. The behavior of herdsman in the process of grazing, other human activities such as traffic in the scenic area may be the main reason that the metal content in the soil in the study area was higher than the background value.

3.2.2 Potential ecological risk index and Nemerow pollution index

The contamination level of single element was generally low, and most CF values were ranged from 1 to 3 (Table 5), which indicated that they were at moderate contamination and the impact of human activities was limited (Williams and Antoine, 2020). Arsenic, zinc, copper and manganese in most sampling sites reached moderate contamination. The sites with high zinc ecological risk were concentrated in areas with high elevation, which were mainly affected by grazing activities, while the sites with high ecological risk of arsenic were concentrated in the areas with low altitude, and the human impact was relatively less frequent. The higher ecological risk of mercury in high elevation areas might be related to the migration characteristics of mercury. It was well known that mercury could migrate with the atmosphere and enter the soil with precipitation and atmospheric deposition. The higher precipitation in high elevation in study area made the soil more likely to enrich mercury. As mentioned above, plant biomass in the study area increased with elevation, and higher plant density might have a positive effect on mercury interception in precipitation.

In terms of RI (Table 6), 5 sampling sites had RI values lower than 150, indicating low ecological risk, and other sampling points were in the range of 150-300, which implying that the ecological risk was moderate. The maximum RI value was 227.31 (S8 sampling site), which was still far below the upper limit value of moderate risk. The results showed that most of the study area was affected by human activities, but it had no serious impact on the ecological environment.

The calculated P_N values of trace elements were presented in Table 5. The data indicated that most of the study area were slightly contaminated by trace elements. It was worth noting that S6 and S12 sampling sites were moderately contaminated. The S6 sampling points was located in the buffer zone of the reserve, which was a pasture in autumn and winter. Therefore, grazing was a factor affecting the environmental quality.

From the analysis results of risk assessment, it could be seen that most of the soils in the study area were at or below the moderate risk. Only a few sampling sites reached high risk for Hg or Cr or Cd, which was due to serious human activities in these sites.

3.3 Chemical speciation of heavy metals

The chemical activity, mobility, bioavailability of heavy metals in the environment and their impact on ecosystem or organism couldn't be well explained only based on the total amount of elements, therefore, the present study examined the different chemical forms of heavy metals in soil samples through the Tessier extraction procedure aside from determining the total concentration. The results were shown in the Figure 4.

Exchangeable state referred to the part of metal that was not specifically adsorbed on the surface of soil colloid, but also easily absorbed by plant roots. Among the eight metals, the largest proportion of exchangeable state to total value was Pb, and they were in the decreasing order of Pb (20.58%) > Mn (9.15%) > Cd (6.59%) > Cu (3.96%) > Cr (3.49%) > Zn (2.50%) > As (0.05%) > Hg (0.03%). The activity of Pb was obviously higher than that of other metals, indicating that Pb had a strong contribution from

anthropogenic source in soils and a high probability transferring from soil to plants and underground water (Ma and Rao, 1997; Kaasalainen and Yli-Halla, 2003). The activity of As and Hg was very low, indicating that the impact of the two heavy metals on the environment was limited. The results were different from the BCFs analyzed above, which might be due to the low content of heavy metals in the soil and the limited absorption of plants, so even though the exchangeable metal content was high, the metal content absorbed by plants was still small due to the low enrichment ability of plants. For example, the exchangeable state content of Pb accounted for 20.58% of the total value, but the BCF value was 0.021, while the BCF value of Hg was the largest (0.471), but the exchangeable state content of Hg only accounted for 0.03% of the total value. It made the quality of herbage in the study area not at risk. However, it was still necessary to detect the exogenous introduction of heavy metals, especially Pb and Hg.

As shown in the Figure 4, in terms of chemical form distribution, the metals studied could be divided into three categories. Hg and As were mainly in the form of residue, organic matter and iron manganese oxide (94.75% and 92.63%, respectively), indicating that the bioavailability and mobility of these two heavy metals were limited. The residue, organic matter and iron manganese oxide of Cu, Zn and Cr accounted for 86.57%, 88.8% and 87.01% of the total value, respectively. These three metals mainly existed in stable form, but the proportion of this form was significantly lower than Hg and As. Pb, Cd and Mn were the most environmentally risky metals, and their exchangeable and carbonate bound states accounted for 35.65%, 44.59% and 25.09% of the total values, respectively, and it confirmed the strong contribution of anthropogenic pollution to their accumulation.

4. Conclusions

Due to the differences in microclimatic and terrain factors and human activities, some physicochemical properties of the grassland soils in the northeast of Tibet Plateau had a significant linear relationship with elevation, and Hg, As, Zn, Pb, Cr, Mn in soils also showed a linear relationship with elevation, but the relationship between Cu, Cd and elevation tends to be quadratic. Trace elements content in soil were affected by certain human activities, but they did not cause serious ecological problems risk, and heavy metals in herbage were still at a safe level. The analysis of soil trace element speciation showed that the active chemical form of Hg, As, Cu, Zn, Cr was low, which did not pose obvious ecological risk, but the activity of Pb, Cd, Mn was higher.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Qianfang Yang completed sample processing, analyzed and interpreted the data, and was a major contributor in writing the manuscript. Shengli Wang and Zhongren Nan provided the experimental conditions and funds, and directed the experiment and designed the framework of the paper. Cuicui Zhao gave guidance on grammar and content. All authors read and approved the final manuscript.

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Tables

Table 1

Descriptive statistics of heavy metals of plant samples

Variable	Unit	Min	Max	Mean	SD ^a	CV ^b	Hygienic standard ^c
Hg	μg kg ⁻¹	12.471	21.684	17.085	2.759	16%	100
As	μg kg ⁻¹	134.524	541.966	250.394	120.189	48%	4000
Cu	mg kg ⁻¹	4.864	8.902	6.156	1.242	20%	
Zn	mg kg ⁻¹	4.815	49.805	24.574	11.372	46%	
Pb	mg kg ⁻¹	0.164	1.625	0.737	0.395	54%	30
Cd	mg kg ⁻¹	0.057	0.159	0.091	0.030	33%	1
Cr	mg kg ⁻¹	0.232	1.140	0.567	0.257	45%	5
Mn	mg kg ⁻¹	48.113	124.313	76.561	21.123	28%	

^a Coefficient of Variation.

^b Standard deviation.

^c Hygienical Standard for Feeds (CNSBQTS, 2017) (in Chinese).

Table 2

Descriptive statistics of heavy metals and physicochemical properties of surface soils.

Variable	Unit	Min	Max	Mean	SD	CV	Soil environmental quality ^a	Soil background ^b	BCF ^c
pH		7.9	8.2	8.1	0.1	0.9%	8.5		
EC	$\mu\text{S cm}^{-1}$	165.00	282.00	215.15	36.57	17%			
CaCO ₃	g kg^{-1}	43.4	183.5	70.6	34.3	49%			
OM	g kg^{-1}	48.8	160.8	88.7	38.1	43%	26.5		
TKN	g kg^{-1}	1.29	9.61	4.78	2.65	55%			
TP	g kg^{-1}	0.32	0.80	0.58	0.13	22%			
Hg	$\mu\text{g kg}^{-1}$	28.1	60.8	40.9	10.8	26%	150	20.00	0.417
As	mg kg^{-1}	11.0	15.4	13.2	1.3	10%	15	12.60	0.019
Cu	mg kg^{-1}	30.5	39.0	34.0	2.1	6%	35	24.10	0.181
Zn	mg kg^{-1}	56	116	81	16	19%	100	68.50	0.302
Pb	mg kg^{-1}	15.6	50.2	34.8	7.3	21%	35	18.80	0.021
Cd	mg kg^{-1}	0.150	0.376	0.232	0.057	25%	0.2	0.12	0.393
Cr	mg kg^{-1}	100.0	218.0	136.6	31.6	23%	90	70.20	0.004
Mn	mg kg^{-1}	585	1071	876	159	18%		653.00	0.087

^a Grade I of environmental quality standard for soils (CNEPA, 1995) (in Chinese).

^b The Backgrounds of Gansu Province soil (CNEMC, 1990) (in Chinese).

^c Bioaccumulation Factor.

Table 3

Pearson correlations between heavy metal concentrations and physiochemical properties of surface soils.

Variable	pH	EC	CaCO ₃	OM	TKN	TP	Hg	As	Cu	Zn	Pb	Cd	Cr	Mn
pH	1													
EC	-0.295	1												
CaCO ₃	0.539*	-0.238	1											
OC	-0.443	0.482	-0.254	1										
TKN	-0.589*	0.151	-0.267	0.210	1									
TP	-0.285	0.318	0.058	0.047	0.612*	1								
Hg	-0.236	0.528*	-0.214	0.351	0.242	0.224	1							
As	0.364	-0.600*	0.366	-0.546*	-0.182	-0.320	-0.266	1						
Cu	0.059	0.255	-0.296	-0.426	-0.049	0.121	0.294	-0.228	1					
Zn	-0.229	-0.281	0.103	-0.194	-0.062	-0.034	-0.137	-0.029	-0.212	1				
Pb	0.522*	-0.354	0.268	-0.742**	-0.154	-0.116	-0.260	0.426	0.097	0.147	1			
Cd	0.342	-0.031	0.656**	-0.110	0.108	-0.001	0.150	0.247	-0.087	-0.076	0.283	1		
Cr	-0.093	0.295	-0.341	0.164	0.399	0.158	0.101	-0.341	0.125	-0.197	0.185	0.216	1	
Mn	0.125	0.403	-0.202	-0.353	0.265	0.335	0.086	-0.230	0.462	-0.077	0.425	0.074	0.485	1

* Correlation is significant at the 0.05 level (2 tailed).

** Correlation is significant at the 0.01 level (2 tailed).

Table 4

Geoaccumulation indexes (I_{geo}) of the heavy metals in the soils.

Sample Sites	Hg		As		Cu		Zn		Pb		Cd		Cr		Mn	
	I_{geo}	Level														
S1	0.10	1	-0.37	0	-0.09	0	-0.19	0	0.16	1	-0.18	0	0.18	1	-0.50	0
S2	0.51	1	-0.52	0	-0.24	0	0.17	1	0.03	1	0.37	1	0.15	1	-0.64	0
S3	0.02	1	-0.66	0	0.02	1	-0.30	0	0.46	1	0.48	1	0.56	1	-0.06	0
S4	0.60	1	-0.64	0	-0.14	0	-0.48	0	-0.85	0	0.03	1	0.00	1	-0.74	0
S5	-0.09	0	-0.42	0	-0.07	0	-0.35	0	0.32	1	0.40	1	0.63	1	0.13	1
S6	0.57	1	-0.78	0	-0.20	0	-0.29	0	0.25	1	0.71	1	1.05	2	0.03	1
S7	1.02	2	-0.76	0	0.11	1	-0.27	0	0.14	1	0.33	1	0.50	1	-0.09	0
S8	0.99	1	-0.41	0	-0.02	0	-0.65	0	0.29	1	0.74	1	0.51	1	0.09	1
S9	0.94	1	-0.53	0	-0.08	0	-0.58	0	0.31	1	0.21	1	0.21	1	-0.02	0
S10	0.37	1	-0.33	0	-0.11	0	-0.89	0	0.83	1	0.38	1	0.60	1	-0.22	0
S11	0.09	1	-0.53	0	-0.09	0	-0.02	0	0.21	1	0.14	1	0.51	1	-0.18	0
S12	0.29	1	-0.30	0	-0.14	0	-0.76	0	0.51	1	1.06	2	-0.07	0	-0.47	0
S13	0.37	1	-0.61	0	-0.08	0	-0.23	0	0.42	1	-0.26	0	0.30	1	0.05	1
S14	0.20	1	-0.50	0	-0.18	0	-0.48	0	0.47	1	0.26	1	0.08	1	-0.25	0
S15	0.09	1	-0.58	0	-0.07	0	-0.16	0	0.42	1	0.26	1	-0.07	0	0.10	1

Table 5

Contamination factors (CF) of the heavy metals in the soils.

Sample Sites	CF									P_N
	Hg	As	Cu	Cd	Pb	Cr	Zn	Mn		
S1	1.61	1.16	1.41	1.32	1.68	1.70	1.32	1.06	1.56	
S2	2.13	1.05	1.28	1.94	1.53	1.67	1.69	0.97	1.86	
S3	1.53	0.95	1.52	2.09	2.06	2.22	1.22	1.43	1.94	
S4	2.28	0.96	1.36	1.54	0.83	1.50	1.07	0.90	1.85	
S5	1.41	1.12	1.43	1.98	1.87	2.32	1.18	1.64	2.00	
S6	2.23	0.87	1.31	2.46	1.79	3.12	1.23	1.53	2.54	
S7	3.04	0.89	1.62	1.89	1.66	2.12	1.24	1.41	2.47	
S8	2.98	1.13	1.48	2.51	1.84	2.14	0.96	1.60	2.47	
S9	2.89	1.04	1.42	1.74	1.86	1.73	1.01	1.48	2.35	
S10	1.94	1.19	1.39	1.95	2.67	2.27	0.81	1.29	2.24	
S11	1.59	1.04	1.41	1.66	1.74	2.14	1.48	1.32	1.87	
S12	1.84	1.22	1.36	3.13	2.14	1.43	0.89	1.09	2.50	
S13	1.94	0.98	1.42	1.25	2.00	1.84	1.28	1.55	1.79	
S14	1.72	1.06	1.32	1.80	2.07	1.58	1.07	1.26	1.80	
S15	1.59	1.01	1.43	1.80	2.00	1.42	1.35	1.61	1.78	

Table 6

Potential ecological risk index (RI) of the heavy metals in the soils.

Sampling Sites	Er							RI
	Hg	As	Cu	Cd	Pb	Cr	Zn	
S1	64.25	11.64	7.04	39.65	8.39	3.40	1.32	135.69
S2	85.25	10.49	6.34	58.33	7.64	3.33	1.69	173.06
S3	61.00	9.51	7.60	62.63	10.31	4.43	1.22	156.70
S4	91.00	9.61	6.79	46.05	4.15	3.01	1.07	161.68
S5	56.25	11.18	7.17	59.35	9.36	4.63	1.18	149.12
S6	89.25	8.72	6.54	73.73	8.95	6.21	1.23	194.62
S7	121.50	8.86	8.09	56.70	8.28	4.24	1.24	208.92
S8	119.00	11.30	7.38	75.23	9.18	4.27	0.96	227.31
S9	115.50	10.42	7.12	52.13	9.32	3.46	1.01	198.95
S10	77.75	11.93	6.97	58.53	13.35	4.54	0.81	173.87
S11	63.75	10.41	7.05	49.68	8.68	4.28	1.48	145.33
S12	73.50	12.22	6.79	93.90	10.67	2.85	0.89	200.82
S13	77.50	9.84	7.10	37.60	10.02	3.69	1.28	147.04
S14	68.75	10.59	6.62	54.05	10.36	3.16	1.07	154.61
S15	63.75	10.07	7.17	53.90	10.02	2.85	1.35	149.09

Figures

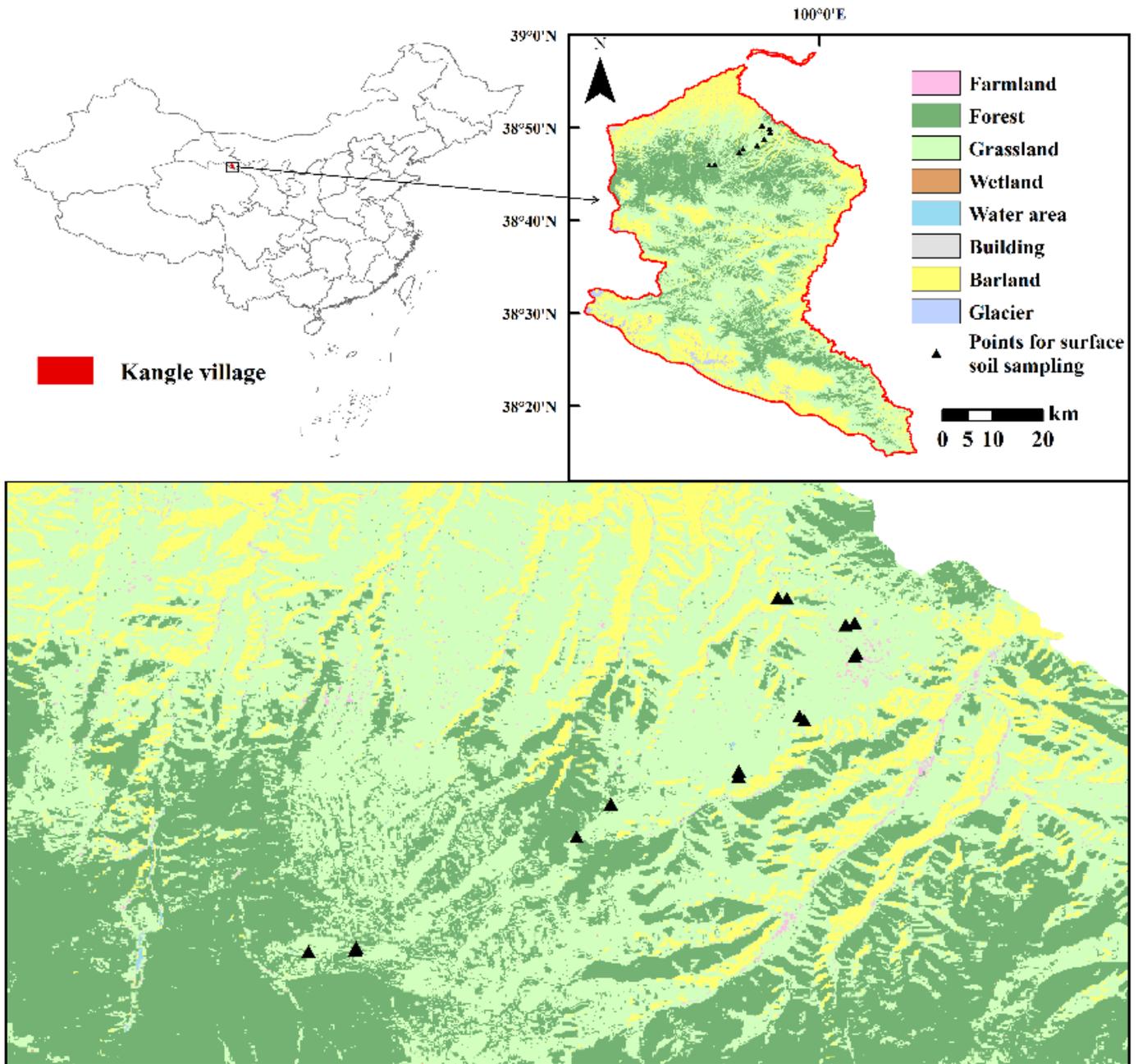


Figure 1

Soil sampling locations of the study area. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

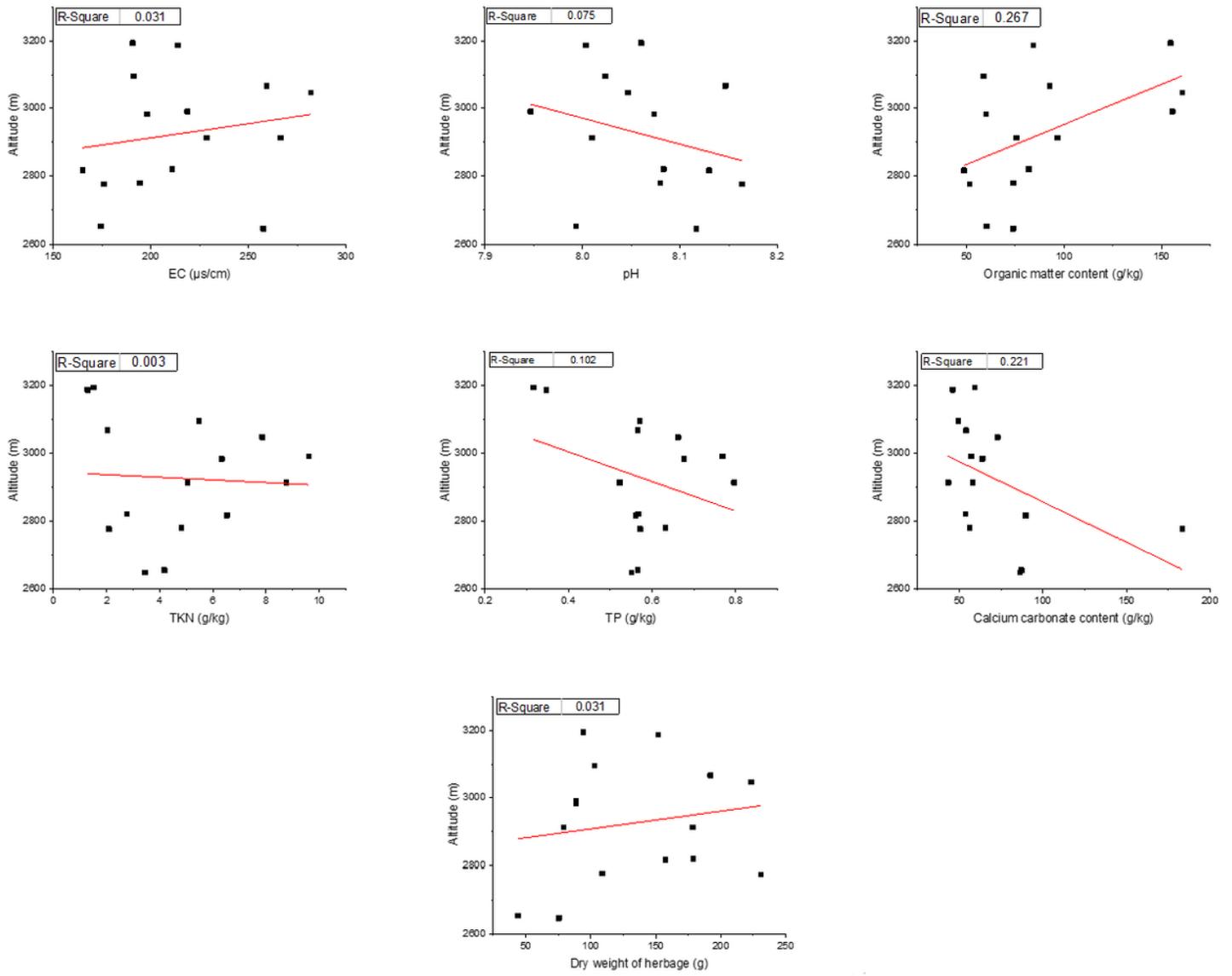


Figure 2

Physicochemical properties of topsoil and biomass of herbage along the altitudinal gradient.

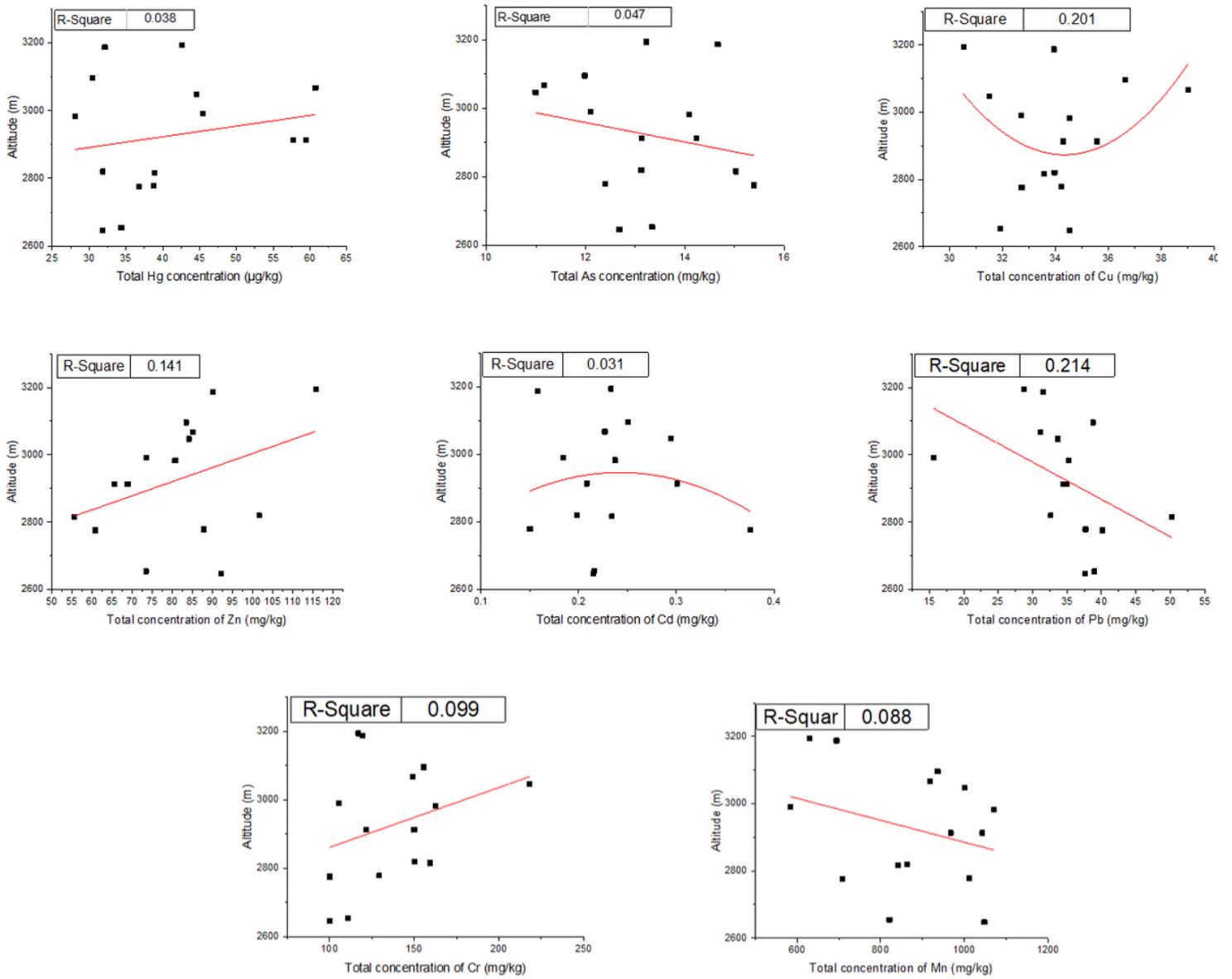


Figure 3

Concentrations of trace elements along the altitudinal gradient.

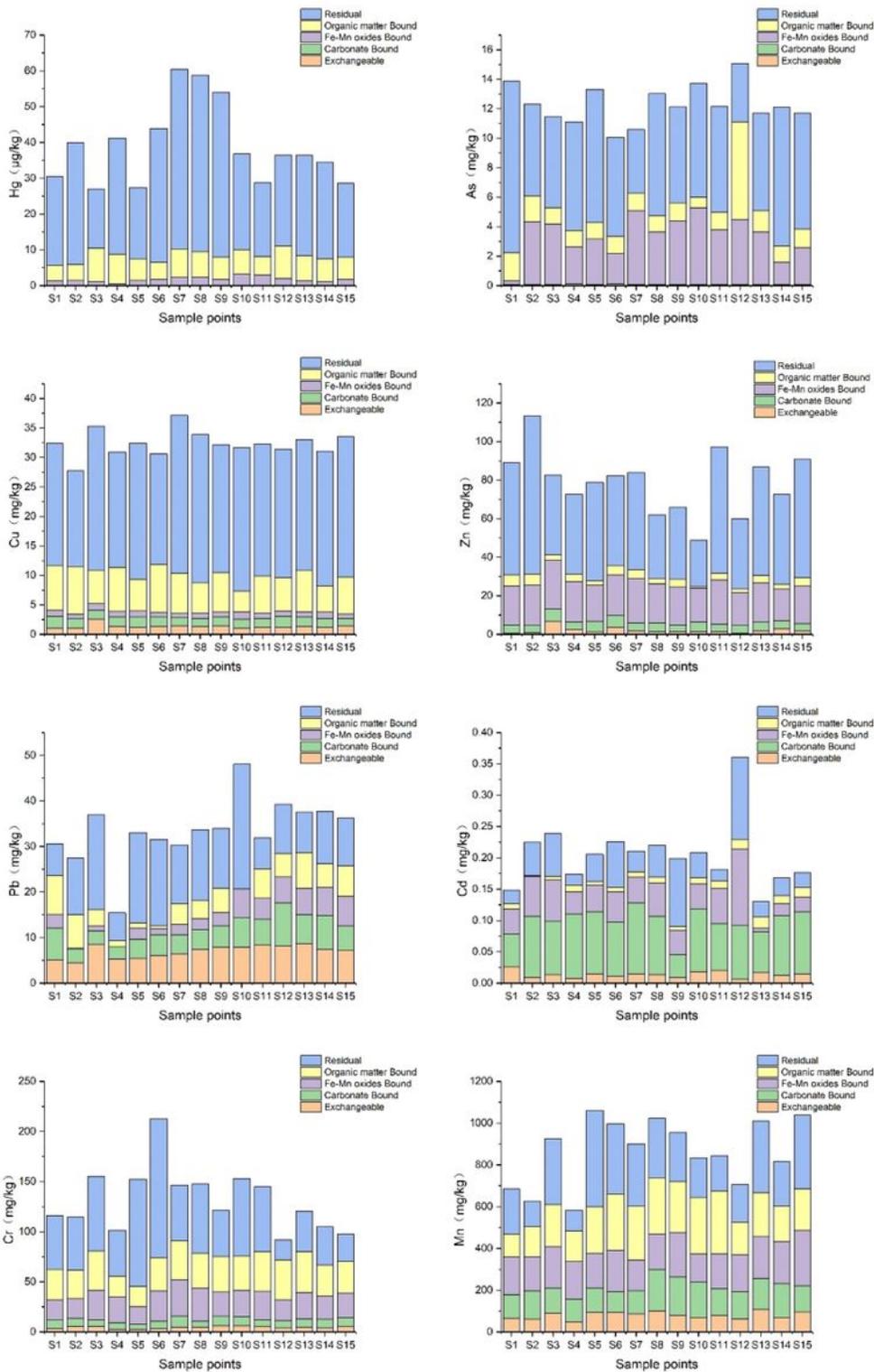


Figure 4

Chemical fractionation of heavy metals in surface soils of the study area.

Supplementary Files

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