

Article

Ecological Safety and Spatial Distribution of Mercury and Arsenic in Qinghai Spruce Ecosystems in Remote Plateau Mountains, Northwest China

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Abstract: The long-distance transport of toxic elements is a crucial external source of metals accumulated in remote forest ecosystems. Due to the harsh environmental conditions and the complexity and diversity of influencing factors in remote alpine ecosystems, research on toxic elements is very limited, especially in the dry zone. In this regard, mercury (Hg) and arsenic (As) in soil and plant samples were investigated in a Qinghai spruce forest ecosystem at Sidalong Forest Farm (SDL), in the Qilian Mountains, northwest China. The results of the study showed a significant variation in the spatial distribution of Hg and As in soils, but a highly similar spatial distribution trend was found in mosses. High Hg and As concentrations in plants were found in moss, bark, and litterfall; the concentration of Hg was particularly high (BCF > 1). The Qilian Mountain spruce forest ecosystem was contaminated with exogenous Hg and As, with Hg, in particular, being the most serious form of contamination. The study results can provide baseline data for future forest management and research in the spruce forest ecosystem in Qinghai, northwest China.

Keywords: Hg and As; moss; Qinghai spruce; risk assessment; Qilian Mountains; arid area



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1. Introduction

Toxic elements (TEs) emitted into the atmosphere by various human activities, including coal and petroleum combustion, chemical manufacturing, mining, and smelting due to the development of industrialization and urbanization, are a threat to human health and ecological safety [1,2]. TEs adsorbed on fine particles can be precipitated on remote alpine ecosystems by long-distance transport under the effects of mountain interception and condensation, and coupled cloud water deposition—vegetation scavenging [3–5]. Previous research also confirmed that climate conditions (temperature and precipitation) in high mountains govern the distribution and accumulation of TEs [5–7]. Zhang et al. (2013) concluded that high altitude areas had more TEs than low altitude areas due to higher precipitation and lower temperature [8]. The elevated accumulation of TEs in the mountain ecosystems may greatly affect the local biodiversity and ecological security [4]. Despite the tremendous progress in pollution detection technology in recent years, pollution monitoring in remote high mountains is still lacking due to the relatively harsh external environment.

Mercury (Hg) and As are highly TE pollutants of widespread concern in the global academic community and have a significant harmful effect on human health and ecological security due to their bioaccumulation and persistence [9,10]. As a global pollutant, mercury can be deposited in many remote regions through complex atmospheric transport processes [11,12]. Some researchers have found that many remote ecosystems, including mountains, glaciers, and polar regions, are affected by atmospheric Hg deposition [5,13,14].

As is a ubiquitous TE in nature, having an average concentration of 1.8 mg kg^{-1} in the Earth's crust and a global mean content of 6.83 mg kg^{-1} in soil [1]. Quantities of 2320 t of Hg and 11,000 t of As are released into the biosphere due to human activities each year [15,16]. Outridge et al. (2018) also estimated that atmospheric Hg from anthropogenic emissions was about $2.5 \pm 0.5 \text{ kt yr}^{-1}$, and that $3.6 \times 10^3 \text{ kt yr}^{-1}$ of atmospheric Hg was deposited on terrestrial surfaces, based on a global Hg model [17]. Coal-fired power plants were considered a major anthropogenic source of atmospheric Hg emissions in China [18]. Forests are the mainstay of the terrestrial ecosystem on Earth, and play a crucial role in maintaining ecological balance and improving environmental quality, including in the functions of carbon and nitrogen sequestration, oxygen release, protection against soil erosion, and water reserve and biodiversity conservation [19]. The forest has also been identified as an important sink for atmospheric pollution due to depositions and plant uptake [19–21]. Therefore, it is important to understand the distribution and accumulation of Hg and As in the remote pristine natural forest ecosystem.

The Qilian Mountains, a typical arid and semi-arid virgin forest, are located at the junction of the Qinghai-Tibet Plateau, the Mengxin Plateau, and the Loess Plateau, with elevation ranging from 4000 to 6000 m (Figure 1). The Qilian Mountains are rich in glaciers, frozen soils, and wetland resources, and are the birthplace of many inland rivers such as the Heihe River. These mountains also play a crucial role in water conservation and biodiversity conservation [19]. TEs transported long distances by the atmosphere may significantly affect the ecological quality of protected areas in southwest China and the Tibetan Plateau [22,23]. Previous studies found that accumulation of TEs such as Hg and As had reached a certain level in the soil around the Qilian Mountains due to transportation, mining, atmospheric deposition, etc. [1,24–26], whereas few studies have been conducted on the ecological environment of the Qilian Mountains. The surrounding cities in Tianlaochi catchment include Zhangye City, Sunan County, Minle County, and Qilian County, where there are a large number of artificial facilities, industrial and mining facilities, waste disposal areas, and hydropower stations. The location of Tianlaochi catchment and its distance from cities and industrial facilities has been mapped in a previous article [19]. The concentrations of Hg and As in atmospheric wet deposition fluxes were higher in Qinghai spruce forest in Tianlaochi catchment than those in surrounding areas [19], and present a great danger to the ecological safety of the Qinghai spruce forest ecosystem. Therefore, the three primary aims of this work were to: (1) characterize the spatial distribution of Hg and As content in soil and moss; (2) understand the accumulation of Hg and As in forest vegetation tissue; and (3) assess the pollution level and ecological safety of Hg and As in remote alpine forest ecosystem. The results of this experiment can improve the understanding of forest ecological security and provide guidance for the management of remote forest ecosystems.

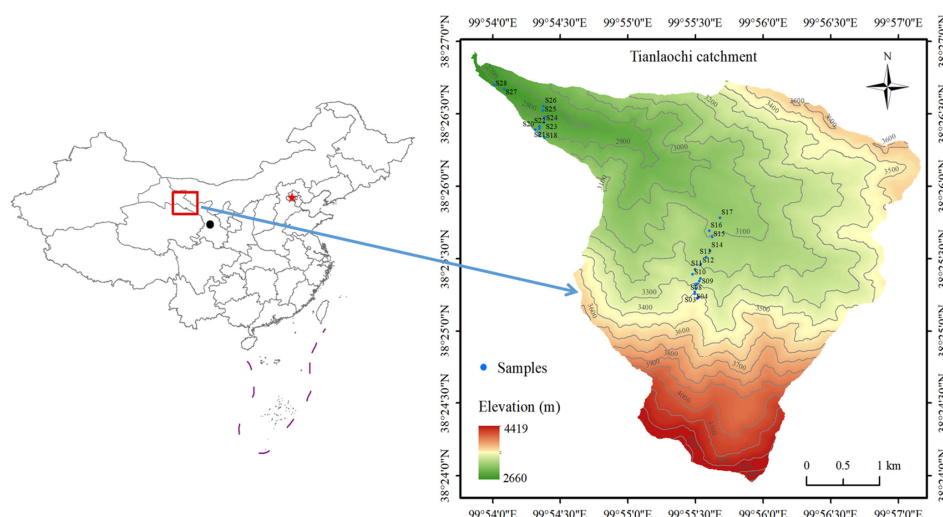


Figure 1. Sampling sites in the SDL from the Qilian Mountains, Gansu province, China.

2. Materials and Methods

2.1. Site Description

The study area is located in the Tianlaochi catchment of Sidalong Forest Farm (SDL) in the Qilian mountains ($99^{\circ}53'50''$ – $99^{\circ}57'10''$ E, $38^{\circ}23'58''$ – $38^{\circ}26'56''$ N, Figure 1) [19]. The altitude range of the study area is 2600–4450 m, in which the ecological environment is natural and subject to negligible disturbances from natural disasters and human activities. Qinghai spruce (*Picea crassifolia* Kom.) and Qilian sabina (*Juniperus przewalskii* Kom.) are two endemic arbor species that inhabit a wide range of habitats in the catchment. Qinghai spruce forest grows on the north slopes at an elevation from 2600 to 3540 m, and Qilian sabina forest is distributed on the south slopes at altitudes from 2700 to 3250 m. Various subalpine shrubs (*Caragana jubata* Poir. and *Salix gilashanica* C. Wang & P.Y. Fu) are distributed at high altitude areas from 3250 to 3750 m [27]. Moreover, moss (*Abietinella abietina*) is widely distributed in spruce forests due to its unique habitat. A section of the Qinghai spruce forest on a shady slope was chosen as the study object to eliminate the effects of topography and other factors. The predominant soil type in spruce forest is mountain grey cinnamon forest soil. The climate of the area is mainly controlled by the East Asian monsoon and westerly winds [28]. The area is characterized by a typical continental climate, with a yearly average temperature of 0.6 °C and an annual rainfall of 326–539 mm [19].

2.2. Sample Collection

Twenty-eight pairs of soil and moss samples were collected from 2687–3450 m in the summer of 2018, and the altitude interval of the two sampling points was approximately 25 m (Figure 1). For soil samples (S1–S28), the topsoil represented the soil layer of 0–20 cm, including the litter layer, and each soil sample was formed from five different locations at the same altitude. The depth of the litter layer was about 2 to 5 cm, depending on the age and density of the tree. The nine profile soil samples (S02, S04, S06, S08, S10, S12, S14, S16, and S18) were collected by a profiler at an altitude of 3060–3450 m with an interval of approximately 50 m. For each vertical profile, soil samples were collected at depth intervals every 10 cm until the bedrock layer or the 0–100 cm depth range. For moss samples, S8–S28 were primarily scratched from the ground under canopies of spruce forest and S1–S7 were taken from the ground above the timber line. Each moss site covered an area of 1 m² including 5 sub-sampling sites. In addition, Qinghai spruce tissues (bark, litterfall, foliage, and cone), and the above-ground parts of *Salix gilashanica* and *Caragana jubata* were collected corresponding to the sampling points 21, 3, and 5, respectively. The bark and foliage were collected from trunk and old branches (1–1.5 m above the ground) of five individual spruce trees, and the litterfall and cone were also picked up on the surface of the moss. The above-ground parts of *Salix gilashanica* and *Caragana jubata* were also collected. All samples were stored in polyethylene plastic bags and cloth bags, and immediately returned to the laboratory. For Hg and As analysis, all soil samples were air-dried for 2 weeks at 25 °C; after removal of coarse debris, samples were lightly ground and sieved through a 0.149 mm nylon mesh. Plant samples were then carefully cleaned with tap and de-ionized water, dried at 60 °C, ground and homogenized, and sieved through a 2 mm nylon mesh.

2.3. Chemical Analysis

Soil physicochemical properties including pH, electrical conductivity (EC), carbonate content (CaCO₃), and organic matter content (OM) were determined according to the methods described in Lu Rukun's book *Agronomic Analysis* [29]. Total nitrogen (TN) and total phosphorus (TP) in soil were analyzed by flow injection analysis (FIAstar5000 Analyzer, FOSS, Hillerød, Denmark). For Hg, As, and Fe analysis, 0.3 g of soil samples or 0.5 g of plant samples mixed with 10 mL of fresh mixture of HCl and HNO₃ (3:1, v/v) were digested in a microwave digestion apparatus (Anton Paar, Multiwave PRO) and diluted to 50 mL with 5% HNO₃ solution. The Hg and As concentrations were analyzed using

atomic fluorescence spectrometry (AFS-8220, Beijing Jitian Instrument Co., Ltd., Beijing, China). The total Fe in digestion was determined by atomic absorption spectrophotometry (Thermo Fisher, ICE 3000, Waltham, MA, USA). The detection limits were $0.01 \mu\text{g L}^{-1}$ for Hg, $0.01 \mu\text{g L}^{-1}$ for As, and 0.005mg L^{-1} for Fe.

For quality control and assurance, analytical or excellent grade reagents and chemicals, reagent blanks, triplicate samples, standard reference soil samples (GSS-8), and plant samples (GSB-24) from the Chinese National Standard Reference Material Center were applied in the chemical analysis process. All glassware and plastic containers were soaked in 10% HNO_3 (*v/v*) for more than 24 h and then cleaned with deionized water. The measured Hg and As in GSS-8 and GSB-24 were highly consistent with the certified values and their recoveries were around 90–105%. The relative standard deviation of replicate samples was below 10%.

2.4. Pollution Level Assessment

2.4.1. Bioconcentration Factor (BCF)

The bioconcentration factor (BCF) can reflect the ability of plants to accumulate heavy metal(loid)s from the soil [30]. The calculation formula is as follows:

$$BCF = C_p / C_s \quad (1)$$

where C_p and C_s are the concentrations of the *i*th element in the plants or plant organs and the corresponding soil samples (mg kg^{-1}), respectively.

2.4.2. Enrichment Factor

The soil element enrichment factor (EF) was used to evaluate the soil quality and the possible impact of human activities for the soil in the study area [31,32]. According to Gujre et al. (2021) [32], EF is defined as:

$$EF = (C_i / C_{ref})_{sample} / (B_i / B_{ref})_{background} \quad (2)$$

where C_i and C_{ref} are the concentrations of the *i*th element and the corresponding reference element in the soil samples (mg kg^{-1}), respectively. B_i and B_{ref} represent the mean concentrations of the *i*th element and the reference element in the soil background environment (mg kg^{-1}), respectively. Fe is considered as a reference element. A finer scaling of EF is given in Table S1. The EF classes were given by Gujre et al. (2021) [32].

2.4.3. Potential Ecological Risk

The ecological risk factor index of individual metal and multi-metal(loid)s was used as an indicator to evaluate the ecological security of Hg and As in the topsoil. The formula is:

$$E_r = T_r^i P_i \quad (3)$$

$$RI = \sum_{i=1}^m E_r^i = \sum_{i=1}^m T_r^i P_i \quad (4)$$

where E_r represents the single element ecological risk factor index; *m* represents the number of studied toxic elements; T_r^i is the toxicity response coefficient of toxic elements, which was 10 for As and 40 for Hg [33]; P_i is the ratio of the measured value of Hg and As in the sample to the local soil background value. *RI* represents the sum of the single ecological risks of the studied toxic elements. The classifications for E_r and *RI* [33] are given in Table S1.

2.5. Statistical Analysis

The statistical analysis of soil and plant measurement data was accomplished using SPSS 22.0 and Microsoft Excel 2010. Pearson correlation coefficient analysis (2-tailed) and one-way analysis of variance (Tukey's test) were performed using SPSS 22.0. The figures of this study were prepared using ArcGis10 and Origin 2017.

3. Results and Discussion

3.1. The Physicochemical Properties and Hg and As Concentrations in Topsoil

The results of statistical analysis of Hg and As, and the physicochemical properties in topsoil (0–20 cm), are shown in Table 1. The soil pH range was within 6.5–7.7 with a mean of 7.1, well below the local soil pH background value (8.4). Due to the decomposition of plentiful litterfall and the metabolites of the active rhizosphere microbiome [34], forest soil often contains rich organic matter. In this study, the OM ranged from 4.5% to 30.9%, with a mean of 18.9%, which was consistent with a previous study [18]. The CaCO_3 contents in topsoil were below the background values (Table 1). The Pearson correlation coefficient analysis indicated that soil pH and OM percentage was significantly negative ($p < 0.01$), whereas the Pearson correlation coefficient between soil pH and CaCO_3 percentage was significantly positive ($p < 0.05$). These phenomena were also observed in other mountains, such as Gongga Mountains [35] and Changbai Mountains [20]. The input and decomposition of litterfall leads to an increase in SOM and a decrease in pH, which contributes to the dissolution of CaCO_3 in forest soils. The EC represents the level of soil salt to a certain extent, and a high EC is not conducive to the growth of the local plant [36]. Soil EC ranged from 80 to $587 \mu\text{S cm}^{-1}$, with a mean value of $208 \mu\text{S cm}^{-1}$ in this study. The mean values of TN and TP in the sampled topsoil were 456 and 344 mg kg^{-1} , respectively.

Table 1. Descriptive statistics of Hg and As concentrations and physicochemical properties in surface soils.

	Min	Mean	Median	Max	SD ^a	CV ^b (%)	Skewness	K–S Test	Soil Background ^c
pH	6.5	7.1	7.1	7.7	0.3	4.4	0.04	0.200	8.5
EC ($\mu\text{S cm}^{-1}$)	80	208	187	587	119	57.0	1.33	0.168	—
CaCO_3 (%)	1.4	3.9	4.1	6.6	1.3	34.0	0.10	0.200	11.8
OM (%)	4.5	18.9	21.8	30.9	8.5	45.1	−0.31	0.037	2.7
TN (mg kg^{-1})	106	456	561	815	239	52.4	−0.17	0.002	—
TP (mg kg^{-1})	83	344	330	648	131	38.2	0.30	0.200	—
Hg ($\mu\text{g kg}^{-1}$)	14.4	39.3	41.0	69.9	13.9	35.3	0.23	0.200	20
As (mg kg^{-1})	2.9	5.5	5.4	10.7	1.67	30.1	1.17	0.007	12.6
Fe (%)	2.65	3.28	3.21	4.46	0.32	9.76	1.76	0.039	3.09

^a Standard deviation; ^b Coefficient of variation; ^c The Soil Environment Backgrounds of Gansu Province in China [37].

The Hg and As concentrations in SDL forest farm ranged from 14.4 to $69.9 \mu\text{g kg}^{-1}$ and 2.9 to 10.7 mg kg^{-1} , with mean values of $39.3 \mu\text{g kg}^{-1}$ and 5.5 mg kg^{-1} , respectively. The mean values of Hg and As in the topsoil were contrasted to the background value of Gansu Province [37] (Table 1); the mean Hg concentration in this area surpassed its soil background value, and the maximum concentration of As was lower than its non-polluted soil value. Results suggest that the As concentrations in the topsoil were at a safe level but there was a certain degree of accumulation of Hg. The CV for Hg (35.3%) and As (30.1%) indicates the concentration variations of Hg and As in topsoil were less in the study area, which also suggests that there was limited external input of Hg and As in remote alpine mountains [3,4,38]. The concentrations of Hg and As in alpine soil were lower than those in other land-use types, including agricultural land [2,9], wetland [12]; grassland [39]; and woodland soils [7]. This is mainly due to the fact that remote alpine areas are less affected by human activities. Moreover, we found that the Hg and As concentrations in topsoil were in the same order of magnitude as in other remote alpine regions affected by atmospheric Hg deposition [1,14,18], implying Hg and As accumulation of atmospheric depositions in topsoil of the study area. The study area is close to cities such as Qilian County and Zhangye City. Hg and As from traffic emissions, coal combustion, and biomass burning may be deposited in the region through long-range transport by atmospheric circulation [19].

3.2. Soil Hg and As Spatial Distribution

The Hg and As spatial distribution in topsoil along an elevational gradient is presented in Figure 2. The Hg concentrations in the topsoil were slightly decreased from 2680 to 2800 m, increased significantly to the forest staggered zone (approximately 3200 m), then decreased slightly with altitude. In contrast, a different finding was observed in the As content, which gradually decreased from the low altitude to the forest interlaced zone, and then showed an upward trend with the elevation gradient. The high Hg concentrations in topsoil were found to be in the vicinity of the forest staggered zone, which is consistent with Stankwitz et al. (2012), and is probably related to the enhanced deposition due to vegetation succession [5]. The As hotspots were distributed at the lower and higher altitudes. Mercury and As derived from human activities can be introduced to the topsoil from atmospheric deposition (precipitation and airborne particulate matters) or plant intake and succeeding decomposition [2,5]. The deposition fluxes (sum of fluxes in rainwater and particulate) of Hg and As in the Qinghai spruce forest were 8.7 and 46.3 $\mu\text{g m}^{-2}$, respectively, where the wet deposition fluxes of Hg mainly originated from rainwater, but those of As mainly existed in particulates [19]. These findings indicate differences in the sources of atmospheric input of Hg and As to the soils in the study area. The Hg and As hotspots at low altitudes may be related to the discharge of local point source pollutants, such as coal and biomass combustion, and mining activity [4,7]. The increasing canopy coverage of forests can enhance the deposition fluxes of Hg and As in particulates, and reduce the deposition fluxes of Hg and As in rainwater [19], which would affect the distribution pattern of Hg and As with the elevation gradient. In addition, the Hg adsorbed by low-altitude forest canopies can be quickly released into the topsoil or atmosphere due to the easier litterfall decomposition at the higher ground temperature [3]. The increase in Hg between 2800 m and the forest interlaced zone (approximately 3200 m) was attributed to the forest filtering effect and litterfall deposition. Some studies found that Hg accumulated in plants came mainly from the atmosphere, and a negligible amount was sourced from the soil [40]. Furthermore, the Hg accumulation ability of coniferous forests was found to be significantly higher than that of the broadleaf and broadleaf-coniferous forests due to the huge specific surface area of leaves [41]. Therefore, the degradation of the litterfall of coniferous forests may be a means for Hg to enter the soil. In general, the forest filtering effect may reduce the accumulation of toxic metal(loid)s in the soil [3], whereas the density of plants will decrease with increasing altitude, which also causes an increase in the Hg content of the soil with increasing altitudes. The decreasing trend of As from low elevation areas to the forested interspersed zone was ascribed to the interception effect of forests on particulate matter transported over a short distance. Evans and Hutchinson (1996) noted that soil received higher Hg at higher altitude due to higher precipitation [42]. Soils at high altitudes are directly exposed to more Hg and As in dry depositions and snowfall, but large amounts of Hg can be re-emitted to the atmosphere as vapor, resulting in limited Hg entering the soil reservoir [43]. In addition, sparse vegetation based on shrubs is not conducive to the capture of atmospheric Hg at high altitudes. This is in agreement with previous observations that Hg concentrations in surface soil were higher in the coniferous forest zone than in the alpine shrub vegetation zone [5]. Total Hg was significantly positively correlated with OM and TN and negatively correlated with soil pH values (Table S2), indicating that Hg concentrations in soil increased with the increase in soil TN and OM and the decrease in soil pH [20,36], probably due to the complexation or sorption of Hg by soil OM. This also supports our previous speculation that litterfall is an important input source of soil Hg. Soil As concentrations presented a significant positive correlation with soil TP and Fe (Table S2), which may be due to the adsorption of the portion of As in Fe-(hydr)oxides from weathering of the soil parent rock [36]. Although atmospheric depositions led to the enrichment of As in the topsoil, the main source of As in the topsoil of the study area was the soil parent material.

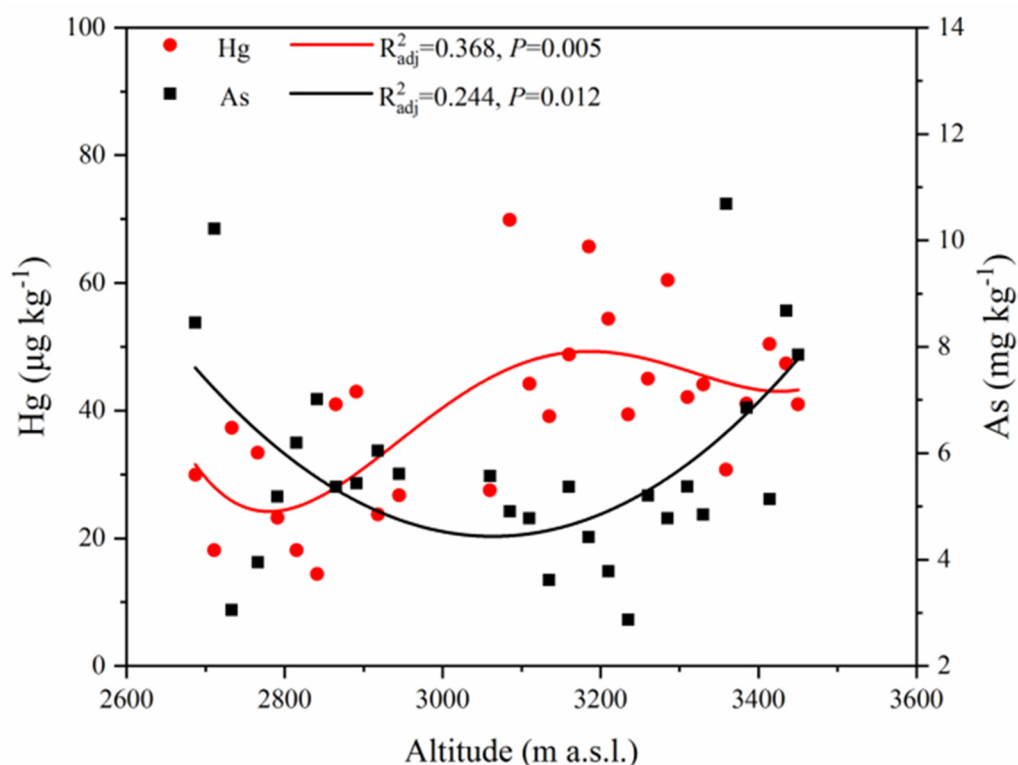


Figure 2. The altitudinal distributions of Hg and As in the topsoil.

The vertical distribution patterns of Hg and As concentrations in the nine profile soil samples are shown in Figure 3. Compared with the relatively uniform As vertical distribution, Hg displayed a certain accumulation degree in different soil depths, which exceeded the soil background values of Gansu province in China (Hg: 20 mg kg^{-1} ; As: 12.6 mg kg^{-1}). Mercury concentrations were generally significantly higher in surface soils than those in other layers, and were probably delivered to the soil as litterfall and atmospheric depositions [5]. Du et al. (2019) found that the highest Hg was observed in surface soils (0–10 cm) and its concentrations decreased significantly in the deeper layers, which was attributed to the high atmospheric Hg concentrations, resulting in elevated Hg loadings in surface soil [44]. Gruba et al. (2019) also observed that Hg concentrations decreased significantly from organic layers to mineral layers [6]. Significant positive correlations between Hg and SOM in the soil profile found in this study indicate that Hg was bound with SOM (Figure 4). This result is in accordance with previous studies [6,44]. However, no significant negative correlation between soil pH and Hg concentrations was observed in this study, which is different from the finding of Du et al. (2019) [44]. Therefore, SOM may play an important role in the migration and transformation of Hg in forest soils. Additionally, the Hg concentrations in deeper soil layers (>45 cm) were relatively stable and close to soil background values [37] in S10, S12, and S14, indicating that Hg was mainly accumulated in the surface soils. This can be explained by the fact that atmospheric Hg was mainly accumulated in surface soils and had little effect on the concentration of Hg in deeper soils [44]. The As concentrations in all samples from the soil profiles were below the soil background values (12.6 mg kg^{-1}) and slightly increased with increasing soil depth, which may be related to the soil parent material and its susceptibility to leaching. The soil in spruce forest is characterized by neutral pH and high organic matter (Figure 4), which is beneficial for As leaching. Tang et al. (2015) showed that As easily migrated from the surface soil to lower soil [1]. Brun et al. (2010) noted that As was mainly associated with the mineral horizons in Norway spruce forest soil [45]. The concentrations of As were negatively correlated with SOM in soil profiles (Figure 4), which is aligned with the finding

of Liu et al. (2018) [12]. Therefore, soil As may be primarily derived from rock minerals rather than litterfall input in the study area.

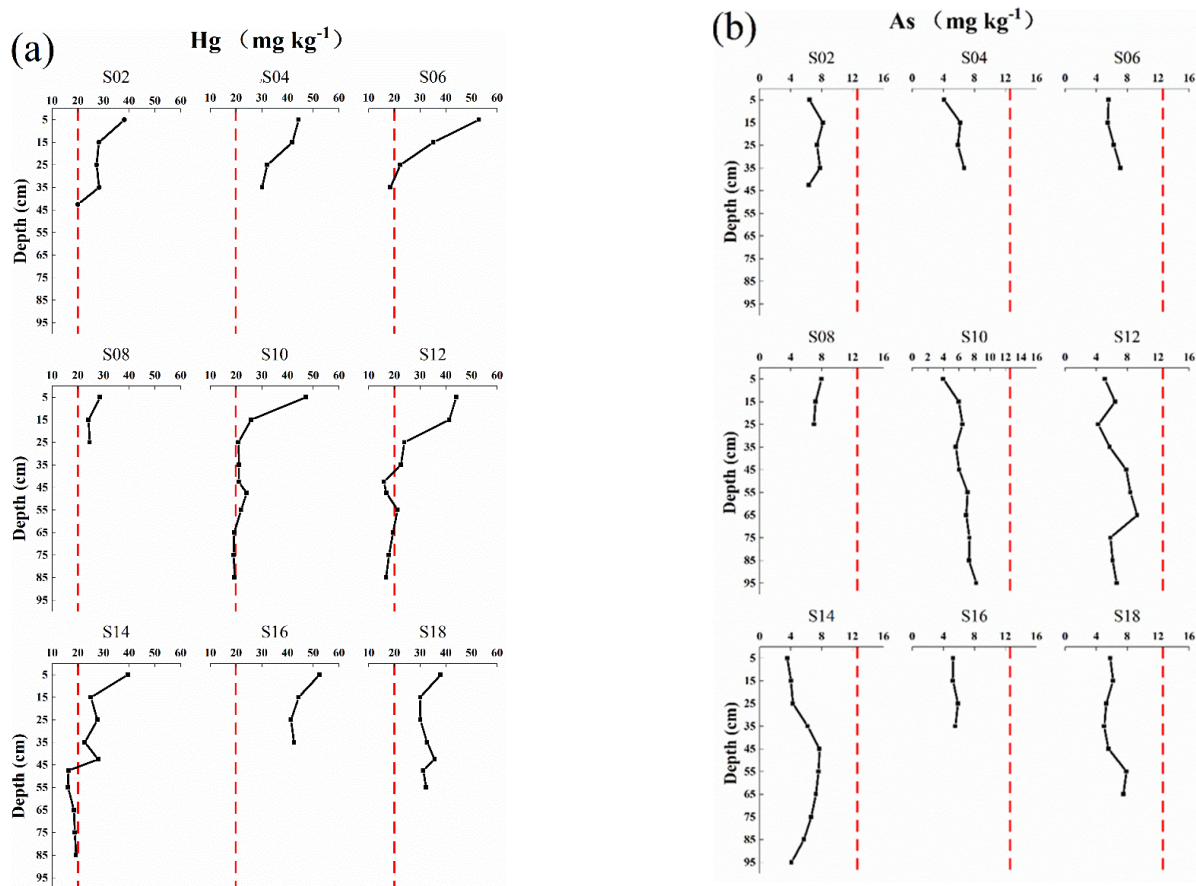


Figure 3. The vertical distributions of Hg (a) and As (b) in the soils (S02, S04, S06, S08, S10, S12, S14, S16, and S18 were from 3060 to 3450 m of SDL, respectively).

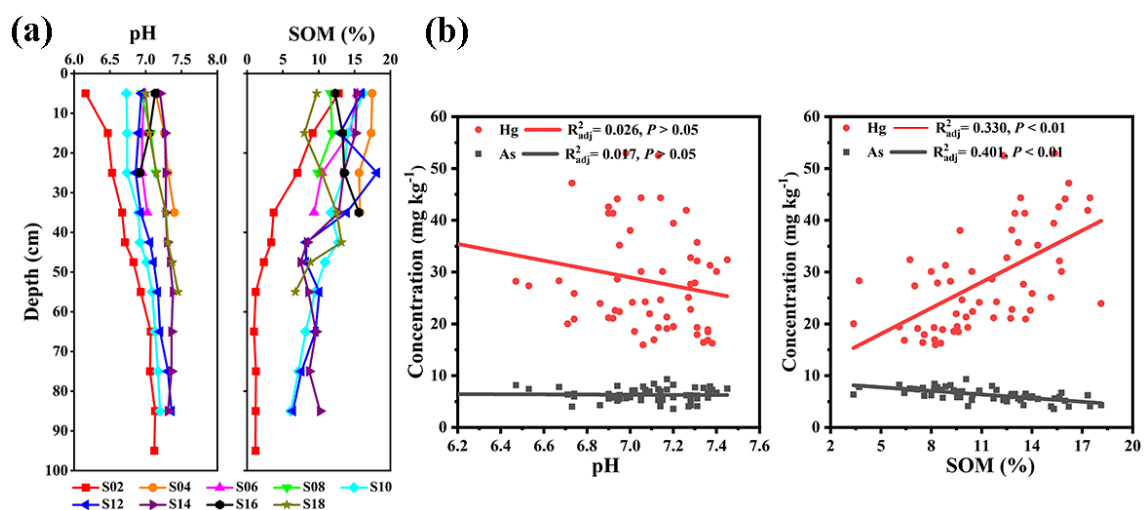


Figure 4. Vertical distribution of soil pH and SOM (a) and the correlation between Hg and As concentrations, and pH and SOM content, in the soil profile (b).

3.3. Mercury (Hg) and As Concentrations in Plants

Table 2 presents the range, average, median, and standard deviation (SD) of Hg and As concentrations in plants (moss, *Salix gilashanica*, *Caragana jubata*, and the different spruce

tissues). The average Hg concentration in plants was highest in spruce bark, followed by moss, litterfall, foliage, *Salix gilashanica*, *Caragana jubata*, and cones. Mercury tended to accumulate in the bark and moss, which may be related to its specific morpho-physiological characteristics and large specific surface area [46,47]. The average Hg concentrations in the bark were 1.91, 3.15, and 5.25 times higher than those in litterfall, foliage, and cones, respectively. Yang et al. (2018) thought that atmospheric Hg could be captured and retained in the bark through surface sorption [38]. Sen et al. (2015) noted that bark showed a high adsorption capacity of Hg by ion-exchange or complex formation mechanisms [46]. Mercury can also be solidified and accumulated in the bark by combining with the thiol-containing molecules or tannins [48]. The foliage of coniferous plants can absorb atmosphere Hg^0 through the stomata and accumulate Hg over a period of years due to the huge specific surface area [49]. Moreover, Hg can also migrate to different tissues of plants, also leading to differences in the distribution of Hg in plants [1,2,18]. Therefore, the high Hg concentrations in the bark indicate a marked input from atmospheric sources. Previous studies have found that alpine mosses have a very high capacity to accumulate Hg from atmospheric depositions [14,50]. Litterfall of spruce had relatively high Hg concentrations compared to those of foliage and cones, further supporting the atmospheric origin [45]. The average accumulation order of As in plants was found to be moss >> bark > litterfall > foliage > cone > *Caragana jubata* > *Salix gilashanica*, indicating that moss and bark may have a significant ability to accumulate As. It was found that the concentrations of Hg and As in the litterfall were two times greater than those of foliage, indicating litterfall increases the metal(loid) input in topsoil [2,14]. In addition, we found that the concentrations of Hg and As in moss were significantly higher than those in other plant tissues ($p < 0.05$) (Table 2). Therefore, we will further explore the variation in Hg and As in moss with altitude.

Table 2. The Hg and As concentrations in plants.

	N	Min	Mean	Median	Max	Standard Deviation
Hg Concentration ($\mu g\ kg^{-1}$)						
Moss	28	32.4	58.4	58.7	75.8	10.7a
<i>Caragana jubata</i>	5	7.52	13.7	13.9	18.3	4.04b
<i>Salix gilashanica</i>	3	8.83	16.4	15.6	24.8	8.01b
Bark	23	15.9	62.9	62.0	109	21.6b
Litterfall	23	12.1	32.6	28.1	132	23.9b
Foliage	23	2.64	20.1	20.7	42.1	8.13b
Cone	23	1.17	11.8	11.8	22.0	5.78b
As Concentration ($mg\ kg^{-1}$)						
Moss	28	1.42	2.83	2.57	8.63	1.37a
<i>Caragana jubata</i>	5	0.05	0.23	0.20	0.55	1.93bd
<i>Salix gilashanica</i>	3	0.14	0.19	0.18	0.25	0.53bd
Bark	23	0.77	1.47	1.43	2.08	0.36c
Litterfall	23	0.32	1.00	1.01	1.85	0.41bc
Foliage	23	0.19	0.39	0.34	0.74	0.15d
Cone	23	0.17	0.31	0.29	0.50	0.11d

Different lowercase letters after the standard deviation indicate significant statistically differences among Hg and As contents in plants ($p < 0.05$, Tukey test).

Wet and dry depositions were a direct and primary source of nutrients/pollutants for mosses, particularly for the carpet-forming species, with only insignificant elements taken from the soil [47]. Wang et al. (2019) also noted that moss can serve as a bioindicator of heavy metal(loid)s in alpine regions [50]. The changes in Hg and As concentrations in moss with altitude are presented in Figure 5. The results show that the Hg and As concentration in moss present a slowly increasing trend with altitude, which may be related to the increase in precipitation with increasing altitude [42]. Total Hg- and As-absorbed moss did not exhibit significant correlations with soil properties, but total Hg-absorbed moss exhibited significant positive correlations with Hg concentration in topsoil and the total As concentrations in moss (Table S3). These results also support the idea that

the Hg and As concentrations in the moss were primarily sourced from atmospheric depositions [14,51], and that the input of dead moss residues enhances the accumulation of Hg and As in soils [50].

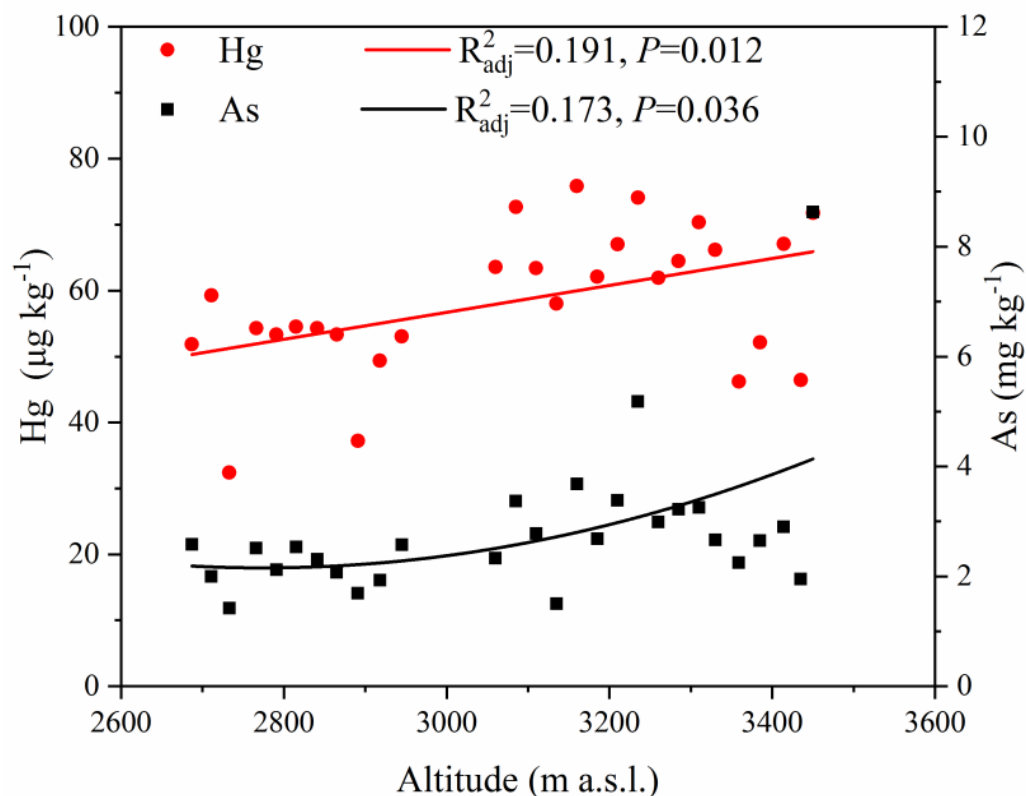


Figure 5. The altitudinal distributions of Hg and As in the moss.

The bio-concentration factor (BCF) is defined as the ratio of metal concentration in plant tissue to that in the corresponding topsoil sample, and is an important parameter for quantifying the plant uptake of Hg and As; this is displayed in Figure 6. The results show that the BCF of Hg in moss was mostly greater than 1 in the sampling sites, demonstrating that the accumulation of Hg concentrations in moss were higher than those in soil. For the different tissues of spruce, it was found that the proportions of samples having BCF values of Hg greater than 1 in bark, litterfall, and foliage were 64.3%, 32.1%, and 10.1%, respectively. However, the BCF values of Hg in shrubs and cones were <1 in all sampling sites. These results indicate that bark and moss (Table 2) and alpine shrubs are less enriched in Hg. This may be due to the high adsorption capacity of bark and moss for Hg [46,47]. The altitude was significantly negatively correlated with the BCF of moss, foliage, and bark ($p < 0.05$) (Table S4), which indicates that the bio-concentration of Hg decreased with increasing altitude. The negative correlations between BCF of Hg in plants and altitude might be related to the increase in atmospheric Hg^0 [14]. Except for the moss at sampling points S01 and S10, the BCF values of As in plant tissues at most of the sampling points were <1 , indicating that the As concentrations in plants were below those in soil. The high BCF of As in S01 moss was related to the maximum As accumulation in moss (8.64 mg kg^{-1}), whereas it could be attributed to a lower topsoil As concentration (2.87 mg kg^{-1}) in S10. The average BCF of As in moss was higher than that in other plants and tissues, which may be related to the fact that moss has a greater ability to enrich metal(loid)s from atmospheric particulates [19,50]. The altitude was significantly negatively correlated with the BCF of foliage and bark ($p < 0.05$), but was significantly positively correlated with the BCF of cones and *Caragana jubata* (Table S4).

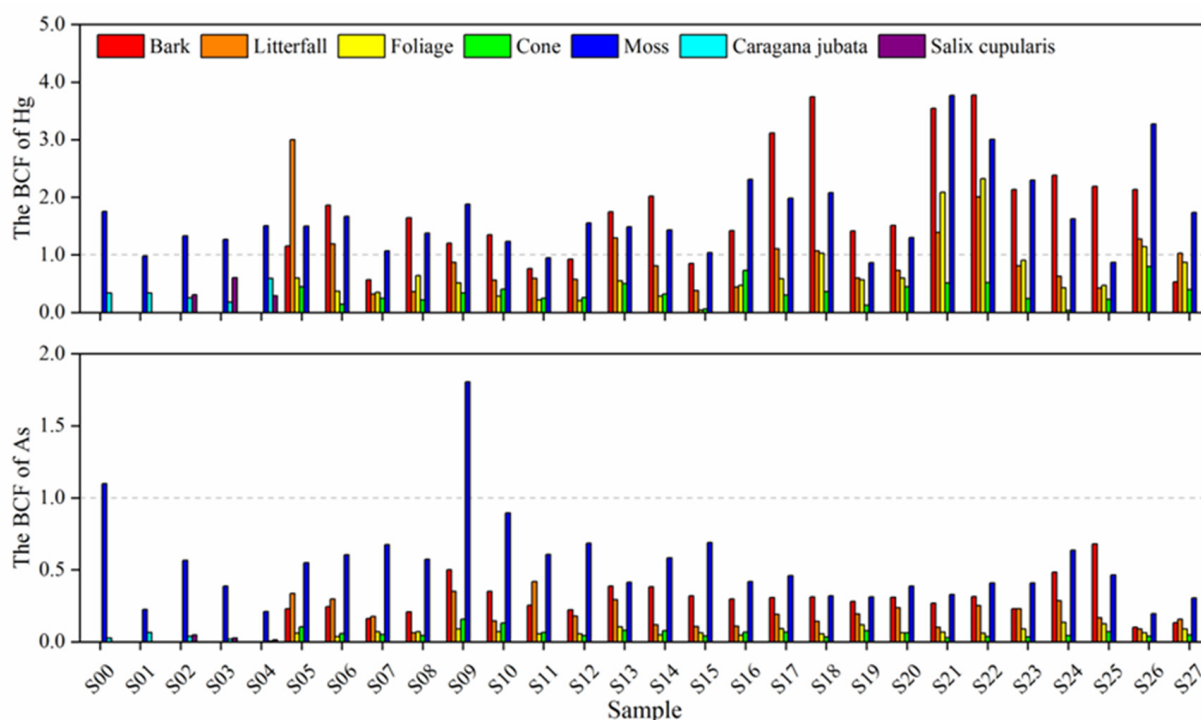


Figure 6. BCF of Hg and As from different plants.

3.4. Risk Assessment

The enrichment factor (EF) and potential ecological risk of Hg and As in topsoil of SDL are illustrated in Table S5. The EF of Hg and As in topsoil was calculated based on the soil background values of Gansu Province in China [37]. EF and E_r for soil Hg were much higher, ranging from 0.62 to 3.46 and 28.8 to 140, with mean values of 1.89 and 78.6, respectively. In contrast, the EF and E_r values of soil As were lower, with maximum values of 0.83 and 8.48, respectively. Overall, the enrichment and ecological risks of Hg were relatively higher than those of As. EF not only reflects the degree of soil heavy metal enrichment [35], but also assesses anthropogenic contribution in soil [4]. EF values between 0.5 and 1.5 indicate that the metal is sourced from natural processes, whereas values > 1.5 indicate that the metallic origin comprises anthropogenic sources [32]. Therefore, the main source of As in the surface soils of the study area was natural sources, whereas Hg was influenced by anthropogenic activities, which also validates the above discussion. The potential ecological risks of Hg and As are dependent on their concentrations and biological toxicity [12]. All soil samples were considered to have a low ecological risk for As, but over 90% of the soils had a moderate or strong ecological risk for Hg. Thus, Hg is considered to be a primary contributor to the potential ecological risk in the study area. This is related to the increased amount of Hg available in the environment [1] and the high potential toxicity of Hg to animals [12,33]. Overall, although the RI of Hg and As indicates that the pollution in the spruce forest ecosystem we investigated can be considered to be low, more focus should be given to the prevention and control of Hg pollution in this area.

4. Conclusions

The levels of Hg in the studied soils were higher than the soil background values of Gansu province. Results show that the spatial distribution of Hg and As in soil is varied and uneven throughout the Qinghai spruce ecosystem, whereas the trends in the spatial distribution of Hg and As in moss were similar. Atmospheric deposition may be one of the core causes of Hg pollution, but soil As is mainly derived from natural sources. High Hg and As concentrations in plants were found in moss, bark, and litterfall, and most BCF values of Hg in moss, bark, and litterfall were >1 , which demonstrates that moss, bark,

and litterfall had the ability to accumulate Hg. It is clear that the topsoil from SDL was moderately contaminated by Hg and subject to a low level of contamination of As. The results of this work can offer a reference for local authorities to understand the distribution and pollution level of Hg and As in the remote pristine natural forest ecosystem. Future research needs to study the storage of Hg, including in different soil layers of the forest floor.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f13081269/s1>, Table S1: Contamination categories based on enrichment factor and potential ecological risk. Table S2: Pearson correlation coefficients between Hg, As and other parameters in surface soils. Table S3: The Pearson correlation coefficient between Hg and As in moss (M-Hg and M-As), total soil Hg and As (S-Hg and S-As) and soil properties. Table S4: The Pearson correlation coefficient between altitude and the BCF of Hg and As in plant tissues. Table S5: Enrichment factor (EF) and potential ecological risk (Er and RI) of Hg and As in topsoil.

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