Potential correlation of heavy metals in surface soils with infestation of *Viscum album* in poplar trees

Prasesh Sharma*, Heike Steckel**, Andrea Koschinsky* and Ewald Schnug**

Abstract

Bioavailable heavy metal fractions in soil and *Viscum album* (mistletoe) samples from three areas of different contamination levels were analysed with ICP-OES to study the variability of heavy metal contamination in surface soils in Goslar, Germany, an area influenced by acid mine drainages from the Harz Mountains and long-term mining activities. The representativeness of single sampling points, and a potential correlation between soil contamination and mistletoe growth on trees were investigated in specific locations in which mistletoe numbers on trees seemed to be related to the contamination level of the soil. Significant variation was found in the concentration of heavy metals in the three locations with metals such as Pb, Cd and Cu showing average values of almost an order of magnitude higher (75 mg/kg, 0.40 mg/kg, and 10.5 mg/kg, respectively) in high contamination area compared to low metal contamination area (15 mg/kg, 0.09 mg/kg, and 1.2 mg/kg, respectively). Apart from the high contamination area, the concentrations of metals in context decrease with depth. Also, Pb and Fe show the highest, Zn and Cu show medium, and Cd and Ni show the lowest concentrations of all heavy metals measured in all areas. Mistletoe leaves samples from the low contamination area show interestingly high concentrations of heavy metals (12 mg/kg Cd, 13 mg/kg Cu, 5 mg/kg Ni, 0.9 mg/kg Pb, and 675 mg/kg Zn) compared to the high contamination area (<0.1 mg/kg Cd,10 mg/kg Cu, 0.4 mg/kg Cu, 1.1 mg/kg Pb, and 123 mg/kg Zn), indicating a possible threshold of metal contamination for the effective exclusion of metals by the plants. The results of this micro-ecosystem study elucidate the high variability between single sampling points within a contamination area and need to be considered in a large-scale geostatistical analysis of correlation of a mistletoe-heavy metal contamination.

*Keywords: heavy metals, soil contamination, mistletoe (*Viscum album*)*

Zusammenfassung

Mögliche Korrelation von Schwermetallen in Oberflächen-Böden mit dem Befall von Pappeln durch die Laubholzmistel (*Viscum album*)

Um die Variabilität der Schwermetallkontamination in Oberflächenböden in Goslar zu untersuchen, wurde der Anteil bioverfügbarer Schwermetalle in Böden und Misteln (*Viscum album*) mit ICP-OES analysiert. Das Gebiet ist stark durch lange Bergbauaktivitäten beeinflusst. Ziel war es festzustellen, wie repräsentativ einzelne Bodenproben sind für drei spezifische Gebiete, in denen die Zahl der Misteln auf den Bäumen einen Bezug zum Kontaminationsgrad der Böden zu zeigen schienen, und ob eine mögliche Korrelation zwischen dem Grad der Bodenkontamination und dem Bewuchs von Bäumen mit Misteln besteht. Die Metallgehalte in dem stark kontaminierten Gebiet lagen fast eine Größenordnung über den Gehalten im gering kontaminierten Gebiet (75 mg/kg Pb, 0,40 mg/kg Cd und 10,5 mg/kg Cu gegenüber 15 mg/kg Pb, 0,09 mg/kg Cd und 1,2 mg/kg Cu). Außer im stark kontaminierten Gebiet wurde eine Abnahme der Metalle mit der Tiefe im Boden festgestellt. Insgesamt waren von allen untersuchten Schwermetallen die Konzentrationen von Pb und Fe am höchsten und für Cd und Ni am niedrigsten. Interessanterweise zeigten die Mistelblätter höhere Metallgehalte im gering kontaminierten Gebiet im Vergleich zum hoch kontaminierten Gebiet, was möglicherweise auf einen Mechanismus zur Verhinderung von Metallaufnahmen durch die Pflanze zurückzuführen ist, der erst bei gewissen höheren Metallbelastungen wirksam ist. Die Ergebnisse dieser Mikro-Ökosystemstudie zeigen eine hohe Variabilität zwischen einzelnen Beprobungspunkten innerhalb eines untersuchten Gebietes, die bei einer großskaligen geostatistischen Analyse von Korrelation zwischen Schwermetallkontaminationen und Mistelbefall berücksichtigt werden müssen.

*Schlüsselwörter: Schwermetalle, Bodenkontamination, Misteln (*Viscum album*)*

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Introduction

High heavy metal contamination in the Harz Mountains (northern central Germany, Figure 1) has been known for a long time. Mining in the area has been carried out at least since the year 968 AD (Liessmann, 1992) but may have lasted for more than 2000 years. Evidence from 3rd and 4th centuries from slag and ore dating in addition to geochemical investigations show that mining activities took place in the region which exists till today (Klappauf, 1985; Matschullat et al., 1997). A quarter of the lead in the ore lost during processing reached the floodplains during flooding by rivers. Periodical contamination deposits can be distinguished in the region (Dreschoff, 1974). In addition to Pb, other heavy metals involved contaminating the soil are Zn, Cu, Cd, Ni etc. The heavy metal contamination in the area varies in the range of 0.2 to 200 mg//kg for Cd, 10 to 30,000 mg/kg for Pb, 7 to 10,000 mg/kg for Cu and 50 to 55,000 mg/kg for Zn in the soil (Gäbler, 2000). Mines are located in the heavily mined Rammelsberg area south of Goslar, and the smelting sites are found northwest of Goslar (Innerste reservoir) and on the east (Oker reservoir). Rivers (Innerste, Grane and Oker) flowing around the Goslar area have created respective floodplains in the north-west, west and east of the Goslar county respectively depositing the tailings from the mines and causing high heavy metal contamination in the region. Storage areas and slag heaps were sources of direct contamination and through time, the pollution was also spread out by wind. The study area of the project falls in the floodplains of the Innerste and Oker rivers in the northern forelands of the Harz Mountains.

The mistletoe (*Viscum album* ssp. *album*) is a parasitic flowering plant with functional chlorophyll which lives on a wide range of deciduous woody plant species (Zuber, 2004). Low to severe infestations by mistletoes have been observed in the deciduous trees in the Harz leading to a speculation that high infestation level of mistletoes in the area is assisted by high concentration of heavy metals in the surface soils of Goslar. The idea behind this hypothesis is that heavy metal contamination in the soil weakens the tree making it more susceptible to parasitic infection. No detailed study of this potential correlation has been carried out before. Preliminary investigation of information retrieval techniques for mistletoes using infrared techniques were carried out at the Julius Kuehn-Institute, Federal Research Centre for Cultivated Plants (JKI) (by Heike Steckel, Braunschweig). Simultaneously, a terrestrial mapping campaign was conducted in March 2004 to record the mistletoe distribution. A soil and plant sampling (mistletoe sampling) campaign was conducted in July and September 2005 for the micro-ecosystem study presented here.

Results from heavy metal analyses in soil and plant samples (mistletoe leaf and stem, and tree leaf) are presented here to investigate the representativeness of single samples in the three different contamination areas (high, medium and low contamination areas were chosen by Heike Steckel (PhD thesis) based on pre-existing data at JKI) and the occurrence of potential correlation between ground contamination and mistletoe growth in a 'micro-ecosystem' level.

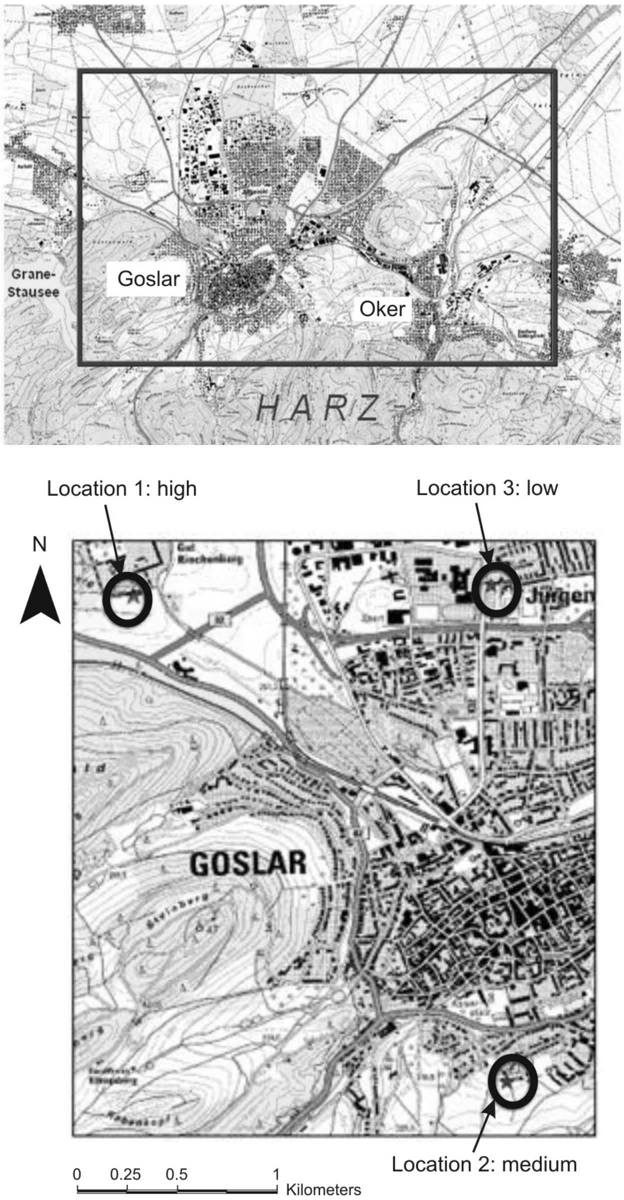

Figure 1:

Overview of the Harz mountains and Goslar county with the study sites (high, medium and low contamination)

Materials and methods

Site description:

The study was carried out in three different contamination areas (high, medium and low) in the city of Goslar, in the Federal state of Lower Saxony, Germany. The different contamination areas for the project were selected by evaluating soil contamination data from "Bodenplanungsgebiet" (Landkreis Goslar) and terrestrial mapping of mistletoes conducted by FAL, Braunschweig. Goslar lies in the northern part of the Harz Mountains at about 10° 25' E, 51° 54' N at an altitude of 260 to 360 m above sea level (Figure 1). The majority of the area is contaminated with heavy metals due to the mining activities carried out for centuries.

Soil and plant sampling and soil description:

The sampling campaign was carried out in the summer months of July (high contamination site) and August 2005 (medium and low contamination sites). Summer months were chosen because growth due to transport of nutrients (along with heavy metals) is maximum in the season and will give a good account of a quantitative measure of bioavailability of heavy metals. The sampling scheme was correlated with the large-scale investigation carried out by Heike Steckel (PhD thesis at JKI) where e.g. no separate investigation of the upper humus layer was done, but a general separation scheme in 30 cm depth steps was followed. In the high and medium contamination areas, sampling was carried out at depth intervals of 0 to 30 cm, 30 to 60 cm and 60 to 90 cm. In the low contamination area, due to the hard bedrock, sampling depth of 60 to 90 cm had to be excluded.

Soil and mistletoe samples were taken in each location. For soil sampling, buffer zones of 1 m, 3 m and 7 m were calculated on one half of the tree and of 1 m, 5 m, and 10 m in the other half. Eight spots were sampled in each concentric ring in an octagonal fashion. A soil corer 1.20 m long and 1 inch in diameter was used for coring. A total of 185 samples were taken from the three different contaminations areas (high-72, medium-65, low-48), all three depth layers (0 to 30 cm, 30 to 60 cm, 60 to 90 cm) included.

Soil profiles were taken in each area for characterization. Gleysol was predominant in the high contamination area (an open field in a farming area) with considerable amount of water content in the topsoil (0 to 30 cm) and the soil horizons below could not be properly distinguished. Profiles taken closer to the tree were relatively drier than that from the farming field. Soil components were a mixture of coarse sand and silt material with schists embedded in the column. The medium contamination area consisted of relatively dry brown earth (cambisol) with components ranging between medium silt to fine sand, a significant amount of schists present and no clear distinction between the horizons. The area of low contamination consists of parabrown earth (luvisol) with composition including schists similar to that of the medium contamination area but comparatively more water content than in the medium contamination area. The soil types mentioned in the three contamination sites are only representative for the specific sites chosen for sampling.

Mistletoe samples (leaf and stem) and tree leaf samples were taken in the three contamination areas (one poplar tree was chosen in each location) from two heights (3 m and 4.5 m) above ground level. A total of 21 plant samples were taken from the three contamination sites. For the high contamination area, the average number of mistletoes per tree was 12 for 16 trees where as for medium and low contamination areas it was 8 (for 6 trees) and 6 (for 12 trees), respectively.

Sample pre-treatment and analysis:

Soil samples were initially air dried for 7 days in a dust-free room after which the organic materials (twigs and leaves) and visible stone particles were removed from the dried sample and then gently crushed using a mortar and pestle. Sieving was then carried out with a 2 mm sieve and 8 g of each sample was weighed for further steps.

An ammonium acetate/di-Na-EDTA (AAAc-EDTA) (Lakanen and Erviö, 1972; Silanpää, 1982) extraction method was used for extraction of the bioavailable trace and heavy metals from the soil samples. 1 l of extract solution was prepared with 7.44 g di-Na-EDTA, 37.4 ml NH_3 (25 %) and 57.2 ml of acetic acid (96 %). 8 g of each sieved fraction were added to 80 ml (solid suspension density of 1:10) of extract solution in a 100 ml polyethylene bottle for shaking. After shaking for 1.5 hours in an automatic horizontal shaker (150/min), the mixture was filtered through folded filters. 25 ml of each filtrate was collected in porcelain cups and fumed off in a sandbath at 170 °C for 3 hours. The samples were then cooled at room temperature and then ashed in a muffle furnace for 4 hours at 490 °C for incineration. The samples were cooled for 2 hours after which 5 ml of 10 % HNO_3 was added and left for 2 hours. 20 ml of deionized water was then added to the sample and the mixture filtered with folded filter (size 593.5). An inductively coupled plasma – optical emission spectrometer (ICP-OES, Spectro Ciros) was used to analyse the concentrations of metals in soil in the filtrates (Al, Ca, Cd, Cu, Fe, K, Li, Na, Ni, P, Pb, Mn, Mg, S, Si, Ti, V, Zn).

Plant samples were dried until weight constancy was achieved at 50 °C and gently crushed. 0.5 g of sample material was weighed into the tubes in a liner. 6.0 ml HNO_3 and 1.5 ml H_2O_2 were pipetted into the tubes (4:1 ratio), and allowed for 10 minutes of reaction after which it was placed in a microwave oven. Samples were sequentially heated at 120 °C for 5 minutes and again at 200 °C for 5 minutes allowing 2 minutes of waiting time before raising the heating temperature. After cooling the samples for over 30 minutes they were filled with deionized water, shaken and the extracts were then filtered. Analysis was carried out with ICP-OES.

The chemical data obtained from ICP-OES were analysed using the statistics package SPSS (Version 12.0.1). Uni/multivariate and factor analyses were performed on the data sets where variation in concentration of heavy metals within one contamination area and in between different contamination levels were tested at $P<0.05$. Concentration data for the elements were interpolated using the ArcGIS 9.0 to visualize the lateral distribution of heavy metals around the trees in context.

Results

Descriptive statistics was used to illustrate the variation in concentration of different elements in various contamination and depth levels. Data for Cd, Cu, Fe, Ni, Pb and Zn, which are the environmentally most relevant metals of the elements analysed, are presented in Table 1, 2 and 3 for all three depth layers for all contamination areas. In all cases, concentrations for Pb and Fe are highest followed by Cu and Zn and lastly by Cd and Ni. Most metals show high surface values and the concentrations decrease with depth, except for some instances. In the high contamination area, in contrast, Fe, Cu, and Pb values increase with depth. This may be related to a stronger input of mining contaminants during former times and covering with younger, less contaminated material. Results from the high and medium contamination areas were comparable for some elements, and in general show values of almost an order of magnitude higher compared to samples from the low contamination area. Highest concentrations as well as dispersion are shown by Pb and Fe, followed by Cu and Zn and then by Cd and Ni. Heavy metals, when considered individually, show a large variability in concentration values as shown by the range (minimum and maximum values) and also the standard deviation.

Table 1:

Heavy metal statistics (AAAc-EDTA extraction) in 0 to 30 cm layer for high, medium and low contamination areas. Index: min-minimum, max-maximum, S.D.-standard deviation

	High contamination area (mg/kg)				Med. contamination area (mg/kg)				Low contamination area (mg/kg)			
	Mean	Min	Max	S.D.	Mean	Min	Max	S.D.	Mean	Min	Max	S.D.
Cd	4.01	0.37	8.98	2.24	3.87	0.31	6.89	2.16	0.87	0.23	1.18	0.2
Cu	105	23	238	55	44	6	71	22	12	1	21	4
Fe	806	233	3524	698	194	75	387	89	100	35	172	37
Ni	1.67	0.42	6.14	1.31	0.82	0.24	1.41	0.34	0.43	0.13	0.72	0.14
Pb	745	183	1107	273	698	97	2298	424	159	10	203	37
Zn	203	89	442	105	85	10	159	43	26	11	48	10

Table 2:

Heavy metal statistics (AAAc-EDTA extraction) in 30 to 60 cm layer for high, medium and low contamination areas. Index: min-minimum, max-maximum, S.D.-standard deviation

	High contamination area (mg/kg)				Med. contamination area (mg/kg)				Low contamination area (mg/kg)			
	Mean	Min	Max	S.D.	Mean	Min	Max	S.D.	Mean	Min	Max	S.D.
Cd	3.33	0.72	6.62	1.46	1.33	0.24	2.68	0.72	0.45	0.21	2.3	0.43
Cu	120	15	219	59	35	2	67	19	8	2	13	3
Fe	877	194	2482	520	186	39	553	121	85	35	166	36
Ni	1.19	0.26	2.19	0.65	0.55	0.09	1.04	0.28	0.32	0.11	0.61	0.12
Pb	811	292	1257	220	446	38	869	231	101	24	462	82
Zn	174	42	325	86	46	8	122	31	15	6	51	9

Table 3:

Heavy metal statistics (AAAc-EDTA extraction) in 60 to 90 cm layer for high and medium contamination areas (no samples from this layer for the low contamination area). Index: min-minimum, max-maximum, S.D.-standard deviation, bdl: below detection limit = <0.1 mg/kg

	High contamination area (mg/kg)				Med. contamination area (mg/kg)			
	Mean	Min	Max	S.D.	Mean	Min	Max	S.D.
Cd	3.04	0.44	7.83	1.67	0.44	0.08	1.56	0.36
Cu	148	12	237	55	12	bdl	36	11
Fe	1016	131	3041	715	107	28	534	104
Ni	1.71	0.18	5.81	1.66	0.29	0.03	0.92	0.18
Pb	859	265	1367	329	205	12	558	171
Zn	171	29	592	129	17	0	119	25

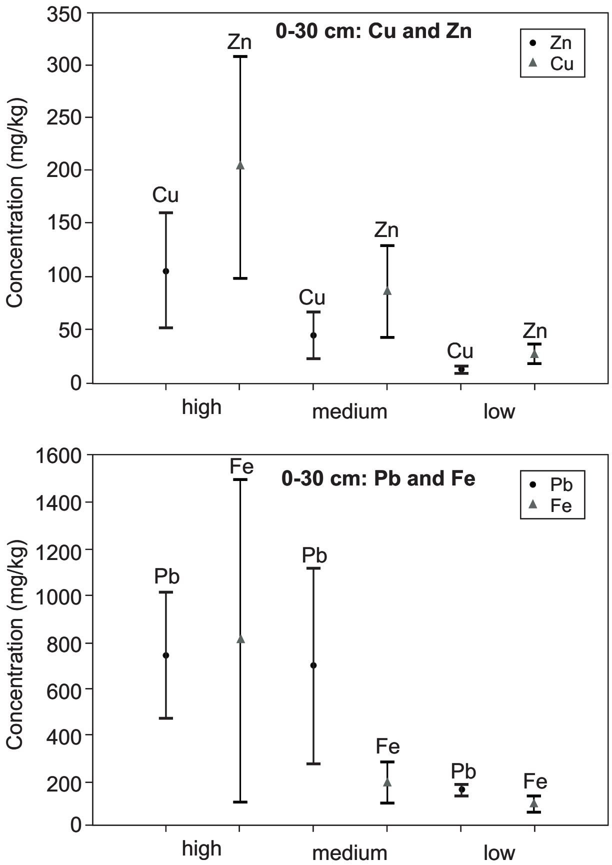

Figure 2:

Variability in concentrations of Cu, Zn, Pb and Fe in the 0 to 30 cm depth layer in different contamination areas

The results from the statistical analysis were plotted with Sigma plot 9.01. to depict the variability of metals with the standard deviations plot for 0 to 30 cm layer for Pb, Fe, Zn and Cu for all contamination areas (Figure 2). The standard deviation is plotted for the estimated marginal means obtained from SPSS. The large absolute standard deviation of the elements shows the variability of heavy metals within a single contamination site as well as between different contamination sites.

Factor analysis was conducted on the chemical data from soil samples. Principal component analysis (PCA) was chosen to extract the factors for analysis to investigate the depth-wise distribution of heavy and trace metals analysed (Figure 3). The general trend of the major heavy metals (Fe, Pb, Zn, Cu etc.) can be seen in the first component and represents a contaminant-bearing oxide phase. The second component consists of elements associated with sulphur (Ni, Co) probably from the mined ores. The last two components consist of elements present in the detrital fractions and ultimately the bed rock (Al, Ca, Mn, Si, Li etc). Degrees of factor loading of most elements do not vary significantly in different depth layers.

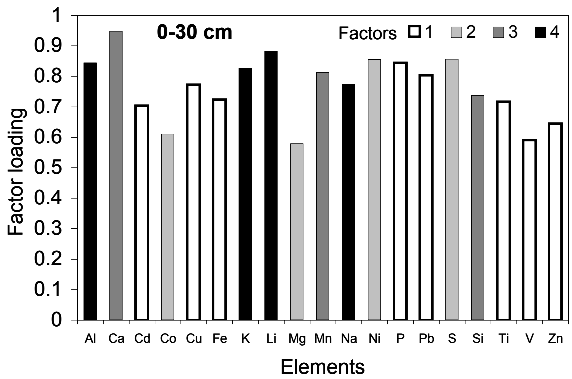

Figure 3:

Results from factor analysis for depth layer 0 to 30 cm

Plant samples showed a high degree of variability in the three sites with Fe and Zn showing the highest and Pb, Cd, Ni and Cu showing lowest concentrations. Table 4 and Table 5 list the enrichment factors in plant samples (above 3 m height from ground level) compared to soil samples (ratio of plant/soil concentrations) in the high and low contamination areas (0 to 30 cm) for samples within 2 m distance from the tree. Cd, Ni, Pb and Zn generally show higher concentrations in plant samples from the lower contamination area than in the high concentration area, however, Cu shows almost equal concentrations in both the contamination areas. Enrichment factors in plants from the low contamination area show values much higher compared to medium contamination area.

Table 4:

Concentrations and enrichment of heavy metals in plant samples (above 3 m in height from the ground level) compared to soil samples (AAAc-EDTA extraction) within 2 m distance from the tree in the high contamination area (0 to 30 cm) (T-L: tree leaf, Mis-St: Mistletoe stem, Mis-L: mistletoe leaf, EF: L - Enrichment factor in tree leaf, EF: M-St - Enrichment factor in mistletoe, bdl: below detection limit = <0.1 mg/kg).

Elements	Concentrations in soil (mg/kg)	Concentrations in plants (mg/kg)			Enrichment factors		
		T-L	Mis-St	Mis-L	EF:L	EF:M-St	EF:M-L
Cd	4.1	bdl	0.1	bdl	bdl	bdl	bdl
Cu	120	8.5	14.2	10.5	0.1	0.1	0.1
Ni	1.51	0.13	0.21	0.42	0.1	0.1	0.3
Pb	907	1.73	1.12	1.10	bdl	bdl	bdl
Zn	197	198	140	123	1	0.7	0.6

Table 5:

Concentrations and enrichment of heavy metals in plant samples (above 3 m in height from the ground level) compared to soil samples (AAAc-EDTA extraction) within 2 m distance from the tree in low contamination area (0 to 30 cm). For abbreviations, see Table 4.

Elements	Concentrations in soil (mg/kg)	Concentrations in plants (mg/kg)			Enrichment factors		
		T-L	Mis-St	Mis-L	EF:L	EF:M-St	EF:M-L
Cd	0.92	16.1	10.7	11.6	17.5	11.6	12.6
Cu	12.4	9.2	14.7	13.3	0.7	1.2	1.1
Ni	0.53	1.16	1.84	4.8	2.4	3.8	10.3
Pb	167	1.17	bdl	0.9	bdl	bdl	bdl
Zn	26.2	1065	557	675	40.6	21.3	25.8

Discussion

The variability in concentrations of the heavy metals tells us how representative the single samples from one area are with respect to the specific contamination level, which is an important result for the large-scale study carried out at JKI. The research at JKI focused on the relationship between heavy metals in soil, tree and mistletoe, and the correlation between the occurrence of mistletoes and the contamination levels at the in the vicinity of the tree. This was conducted by grid mapping and sampling in the Goslar area, analysing the soil and mistletoe samples as well as comparing and appraising the found data. However, a possible statistical relationship between mistletoe infestation and contamination level will depend upon the representativeness of single point samples analysed for different heavy metals. Results from all contamination levels show that the concentrations of heavy metals within each contamination area are highly variable. Thus, since the data for geostatistical analysis of the large scale study is dependent upon representativeness of single sampling points in the three different contamination sites it makes a significant difference where the sample is taken from. The top soil (0 to 30 cm), from which the tree takes up most of the nutrients shows highest variability in heavy metal concentrations in the high and medium contamination areas. Therefore, to compare mistletoe infestation with heavy metal contamination, it is important to understand the heavy metal distribution in a local scale as well and take representativeness of samples into account.

Heavy metal concentrations analysed from the collected samples in the 0 to 30 cm (surface) layer are comparable to values in literature with the average values for the top soil falling in the BW III (BW: Bodenwert) in the classification scheme of Eikmann and Kloke (1993) (BW is based on the classification scheme that classifies total heavy metal content in soils according to land use, vegetation and groundwater). A high variability in concentrations of different heavy metals with respect to contamination level in a single contamination site as well as in between different areas is well illustrated by Tables 1 to 3 and Figure 2. Concentrations of heavy metals in the medium and high contamination areas are comparable, with mean bioavailable values of some elements within 5 % of each other. Absolute variability of soil samples is highest in the high contamination area compared to that of the low contamination area. Concentrations of heavy metals in plant samples show extreme variability within a single tree as well and branches at different heights (3.0 and 4.5 m) showed different concentrations.

The idea behind a potential correlation of heavy metal contamination with mistletoe infestation of trees is as follows. It is known that heavy metal contamination causes biotic and abiotic stress in plants and above toxic levels it can cause symptoms such as chlorosis and necrosis, stunting, leaf discoloration and inhibition of root growth (William et al., 2000; Greger, 2004). Deciduous plants are susceptible to such stress which weakens their resistance and growth physiology further (Greger, 2004). As a result, parasites and hemiparasites such as *Viscum album* could find a perfect host for living as a parasite. Some correlation has been found between mistletoe infection and damaged host trees in deciduous trees; in a study conducted with pine trees, infected chlorotic pines hosted more mistletoes than healthy pines (Hartmann, 1990).

The average number of mistletoes on the trees was highest in the high-contamination area and lowest in the low-contamination area. Still, interpretation of mistletoe growth as a result of heavy metal contamination in surface soils is not straightforward because of various reasons. Firstly, studies of direct correlation between heavy metal contamination in surface soils and mistletoe growth have not been carried out before and therefore results from this study cannot be compared with those from others. Secondly, large variability of heavy metal concentration in soil and plant samples in a single contamination site make it difficult to interpret any association between soil contamination and the parasitic growth of mistletoes. Moreover, plant samples from low contamination area have the highest amount of heavy metals, and samples from high and medium contamination area show opposite results, i.e. the concentration in soil is significantly higher but the concentration of metals in the plant samples is significantly lower in comparison to the low contamination area. In addition, the concentrations obtained from the extraction procedure represent only a specific fraction of heavy metals in soil which gives an estimation of availability and not the amount which the plant really uptakes. Studies have shown that high level of heavy metals in soil do not always indicate similar high concentration in plants (Mulgrew and Williams, 2000). A study carried out by a specific kind of lichen illustrated that Cu, Pb and Zn concentrations in plants often correlate with aerial deposition but not with soil concentrations where as Ni and Cd correlated with deposition and soil concentration (Pilegard, 1984). Another possible explanation could be that the defence mechanisms of the trees against toxic metal uptake only become active when a certain threshold triggering this mechanism is reached. In our case, the low contamination site would be characterized by concentration below and in the high contamination area above that threshold. The complexity of mechanisms in uptake of heavy metals from soil makes it difficult to study a direct association of heavy metals and mistletoe infestation.

Moreover, the distribution of mistletoes in all contaminated sites is patchy. In the high contamination area the average number of mistletoes per tree was 12.6 (no. of mistletoes ranged from 4 to 17 per tree for 16 trees). For the medium concentration area the number of mistletoes ranged from 7.9 per tree for (number of mistletoes from 6 to 13 for 6 trees) and 5.7 per tree (number of mistletoes range from 2 to 8 for 8 trees) in the low contamination area. The average number of mistletoes per tree holds only during the time the soil was sampled for the project. The number of mistletoes definitely seems to follow contamination pattern numberwise, but interpretation in terms of a correlation between heavy metal contamination and mistletoe infestation has to be done with care.

Mistletoe distribution depends upon available hosts and migration routes of birds as well, as they spread the seed from the mistletoe flowers (Zuber, 2004; Wangerin, 1937). Heavy metal contamination might not have a direct influence on mistletoe distribution, however, it can have an indirect effect that once a mistletoe seed is dropped into one branch, contamination and mistletoe could have an interacting factor in weakening the tree. On the other hand, old and weak trees are more susceptible to mistletoe attack (Nierhaus-Wunderwald and Lawrenz, 1997). This could include trees which have already been weakened by heavy metal contamination. Mistletoe infestation causes reduced transport of nutrients and water to the host. Thus, there is a possibility that trees that have already had some stress due to high concentration of heavy metals could be prone to mistletoe attack (Tsopelas et al., 2003). Hence, high heavy metal concentration areas might host deciduous trees that are more susceptible to mistletoe infestation.

The work carried out in this study demonstrates the variability of heavy metals in a single location within a sampling area and the importance of single data points as the range of heavy metal contamination in any contamination site is very large, especially in the topsoil where the plants get their nutrients from. Therefore, single data points from the catchment area of the tree might not be representative of the average contamination influence on the tree. The correlation between mistletoe infestation and contamination of surface soils cannot be explained simply by measuring the concentration levels of heavy metals and number of mistletoe per tree because of various factors such as bird migration, bioavailability and susceptibility of hosts come into play. If these factors can be considered constant within the investigated area, a relationship might become obvious. Due to the lack of time in the project, the number of trees sampled was restricted to three (one tree per contamination area). For better results, more trees per contamination area should be considered. Possibly a correlation can be established based on statistical data from a larger number of trees within an area. Also, mistletoe ratings in different areas can be used to quantify the infestation levels. Sampling should be carried out in more than a single site for each of the contamination areas to quantify the variability of heavy metals. Lastly, proper soils profiling and better understanding of heavy metal uptake by deciduous trees and mistletoes would give a better understanding of the potential correlation of soil contamination and occurrence of mistletoes. If a statistical relationship between metal contamination of surface soil and mistletoe infestation could be established, this would offer a simple and cheap opportunity to provide a rough assess of potential metal contamination of soils in areas where no data on the soil contamination are available.

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