Soil-to-plant transfer of arsenic and phosphorus along a contamination gradient in the mining-impacted Ogosta River floodplain

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Graphical abstract:

Abstract

Riverine floodplains downstream of active or former metal sulfide mines are in many cases contaminated with trace metals and metalloids, including arsenic (As). Since decontamination of such floodplains on a large scale is unfeasible, management of contaminated land must focus on providing land use guidelines or even restrictions. This should be based on knowledge on how contaminants enter the food chain. For As, uptake by plants may be an important pathway, but the As soil-to-plant transfer under field conditions is poorly understood. Here, we investigated the soil-to-shoot transfer of As and P in wild populations of herbaceous species growing along an As contamination gradient across an extensive pasture in the mining-impacted Ogosta River floodplain. The As concentrations in the shoots of *Trifolium repens* and *Holcus lanatus* reflected the soil contamination gradient. However, the soil-to-plant transfer factors were fairly low, with TF values mostly below 0.07 ($TF = As_{shoot}/As_{soil}$). We found no evidence for interference of arsenic with phosphorus uptake by plants, despite extremely high molar As:P ratios (up to 1.2) in Olsen soil extracts of the most contaminated topsoils (0–20 cm). Considering the restricted soil-to-plant transfer, we estimated that for grazing livestock As intake via soil ingestion is likely more important than intake via pasture herbage.

Keywords: pollution, plant uptake, *Holcus lanatus*, *Trifolium repens*, *Apera spica-venti*, food chain

1. Introduction

Many riverine floodplains in industrialized countries accumulated high loads of potentially toxic trace elements which were carried along with the river from upstream sources such as mining and ore processing facilities (Byrne et al., 2012). The mining industry annually produces several Gt of tailings, a mixture of crushed rock and processing fluids (Kossoff et al., 2014). Tailings generated during processing of sulfidic ores are of environmental concern due to their elevated concentrations of potentially harmful metals (e.g. Cd, Cu, Pb, Hg, Zn) and metalloids (e.g. As, Sb). Furthermore, large amounts of acidity are generated when sulfidic tailings are oxidatively weathered. In the absence of carbonate minerals this often results in low pH drainage water which is commonly referred to as acid mine drainage (Lindsay et al., 2015). The most common approach to handle mine tailings is to store them behind dammed impoundments, the so called 'tailing dams' (Kossoff et al., 2014). They are typically constructed from locally-derived soil, waste rock and the tailings themselves. Ideally the impoundments should isolate the tailing to prevent their potentially harmful constituents from entering ground and surface waters, and to protect them from wind erosion. Unfortunately, isolation is often not sufficient, allowing the tailing tips to be eroded and drainage to leave the impoundments. Moreover, around 200 tailing dam failures were reported over the last century with an unknown number remaining unreported (Azam and Li, 2010). These incidents often release huge quantities of tailings into river systems and contaminate downstream floodplains (Kossoff et al., 2014). In some cases, mine tailings or slurries have been disposed directly into rivers or wetlands, causing severe pollution (Vogt, 2013).

Arsenic (As) is one of the most concerning hazardous elements entering the environment with or from mine tailings; the non-essential and carcinogenic metalloid is frequently elevated in mining-impacted riverine floodplain soils, as reported e.g. by Marron (1992), Hudson-Edwards et al. (2003), Tighe et al. (2005), and Jordanova et al. (2013). Since soil decontamination in river floodplains on a large scale is usually unfeasible, management of As contaminated floodplains must focus on providing land use guidelines and implementing usage restrictions in order to reduce human exposure and to foster natural attenuation processes (Wang and Mulligan, 2006). Therefore, knowledge about the distribution, chemical forms, mobility and availability of As in the soils is required. Uptake by plants may represent an important pathway for As to enter the food chain. However, the relationship between soil As and uptake by plants is still poorly understood, especially under field conditions and for plants other than commodity crops.

Numerous factors can affect the soil-to-plant transfer of As, including plant properties as well as environmental and soil chemical conditions, e.g., the chemical speciation of As in the rhizosphere (Moreno-Jiménez et al., 2012; Zhao et al., 2010). Under non-flooded, well-aerated conditions, As in soil is typically present as arsenate, which is the thermodynamically favorable form at high redox potentials. The oxyanion arsenate has similar sorption behavior as phosphate and the two molecules compete at various binding sites; the presence of phosphate can increase arsenate solubility in soil by competing for sorption sites on soil solid-phases (Bolan et al., 2013; Violante and Pigna, 2002). The two molecules also compete for uptake into plant roots. Both phosphate and arsenate enter the plant's root symplast via phosphate transporters (Zhao et al., 2009). Under flooded, anoxic conditions, arsenite dominates

the soil solution phase (Parsons et al., 2013; Weber et al., 2010). In contrast to arsenate, arsenite enters plants as neutral $\text{As}(\text{OH})_3$ via transporters belonging to the aquaglyceroporin membrane channel proteins which are involved in uptake of boric acid ($\text{B}(\text{OH})_3$) and orthosilicic acid ($\text{Si}(\text{OH})_4$) (Mukhopadhyay et al., 2014; Zhao et al., 2009).

Detoxification of arsenite in plant cells includes complexation by –SH groups of glutathione or glutathione-derived phytochelatins, and active transport into vacuoles where it is stored. Moreover, arsenite efflux to the external medium – an important and well researched detoxification strategy in microbes (Bhattacharjee and Rosen, 2007) – was observed for the roots of several plant species (Zhao et al., 2009). In case of arsenate uptake, the reduction to arsenite by specific glutathione-dependent reductase enzymes precedes those detoxification mechanisms (Zhao et al., 2009). In most plants, the site of detoxification is largely the roots and the root-to-shoot transport is restricted. However, a fraction of the As entering the root symplast will be translocated via the xylem to the shoot (Mendoza-Cózatl et al., 2011; Zhao et al., 2009); in the shoot it can be taken in by grazing livestock or wild animals.

Soil-to-shoot transfer factors (TF) from below 0.001, e.g. for *Rubus idaeus* L. (Otones et al., 2011), to higher than 10, e.g. for the As hyperaccumulating fern *Pteris vitata* L. (Ma et al., 2001), were reported ($\text{TF} = \text{As}_{\text{shoot}} / \text{As}_{\text{soil}}$, both in mg kg^{-1} dry mass). Vegetation surveys revealed that most terrestrial plants show TFs below 0.1 and that As (hyper)accumulation is a rare phenomenon (Baroni et al., 2004; Bergqvist and Greger, 2012; Tremlová et al., 2016).

Intraspecies variation in soil-to-shoot transfer factors can be considerable since, as mentioned earlier, many external factors determine how efficient plants transfer As from the soil to the shoot. Williams et al. (2007), e.g., surveyed commercially farmed temperate rice, wheat, and barley in Europe and the United States and found more than one order of magnitude variation in TF for these crops (0.05–3.84, 0.002–0.142, and 0.002–0.095, respectively). Today's knowledge does not allow satisfactory prediction of As TF for a given species and set of edaphic and environmental factors. In fact, we are just beginning to understand the mechanisms and factors controlling As soil-to-plant transfer. Pot experiments will be important to narrow the current gap of knowledge, but they must be accompanied by field studies in order to guide research towards ecologically relevant questions and experimental designs. A better understanding of As soil-to-plant transfer will allow to quantify the threat posed by the As burden of mining-affected floodplain soils to the ecosystem and the food chain via uptake by plants.

In this study, we investigated the soil-to-plant transfer of As for one leguminous (*Trifolium repens* L.) and two graminaceous plant species (*Holcus lanatus* L., *Apera spica-venti* L.) growing in an extensive pasture along a transect following a contamination gradient in the mining-impacted Ogosta River floodplain (Bulgaria). We aimed to elucidate whether As contamination gradients in the soils were reflected in the concentrations of As in the shoots of the three herbaceous species. In addition to total element concentration measurements, chemical soil extractions were conducted to obtain information on As and P availability along the transect. Furthermore, the As soil-to-plant transfer factors were calculated for the wild plant populations investigated in this study and compared to findings from field surveys as well as pot, lysimeter, and field experiments reported in the literature.

2. Materials and Methods

2.1. Study site

The study site is located in the floodplain of the Ogosta River, northwestern Bulgaria. The Ogosta River has its source in the West Balkan Mountains and drains into the Danube River. Large parts of its floodplain are used as pasture and arable fields. Due to mining activities the soils of the Ogosta River floodplain received large amounts of As and potentially hazardous metals. Historic gold mining in the Ogosta catchment began during Roman times, recent industrial metal exploration (Fe, Au, Pb, Ag) started in the early 1950s and ceased in the late 1990s. Over long periods, solid-loaded wastewater from two ore-dressing facilities was discharged into the river channels, contaminating the river sediments and the floodplain soils. Furthermore, after a tailing dam failure in 1964, large volumes of arsenopyrite-bearing, carbonate-rich sulfidic tailings sedimented in the floodplain and substantially contributed to the soil's contaminant burden (Jordanova et al., 2013; Stoyanova and Traykov, 2014). At present, the distributed arsenopyrite is, for the most part, oxidatively weathered and As is largely associated with the weathering products such as Fe(III) (hydr)oxides (Mandaliev et al., 2013).

We established a 700 m long transect which crosses the Ogosta River and cuts through regularly flooded grassland mainly used as extensive pasture (Figure 1). The site is located close to the inflow of the Ogosta River into the Ogosta Reservoir, one of the largest artificial lakes in Bulgaria created by a dam completed in 1986. Flood events in this area primarily result from raising water table of the Ogosta Reservoir. The overall topography of the grassland is flat. Digital elevation model based on LIDAR scanning revealed natural levees on both sides of the river. The maximum elevation difference along the transect is 1 m, with the levees and both endpoints of the transect being the most elevated points. Based on earlier studies (Mandaliev et al., 2013; Mikutta et al., 2014) we expected to find strong As contamination gradients in the soils along this transect.

2.2. Soil sampling and characterization

Soil samples were collected in June 2013 at 18 locations along the transect (Figure 1). At each location, two soil depths (0–20 and 20–40 cm) were sampled using a HUMAX core sampler. In detail, 4 to 6 cores were taken from an approximate area of 1 m² and homogenized by thorough mixing. Additionally, soil profiles (P1, P3, P4) were sampled at three locations (Figure 1). At the profiles, the topsoil (0–20 cm) was sampled in 5 cm intervals and the subsoil (below 20 cm depth) in 10 cm intervals. Soil from the entire intervals was representatively sampled and homogenized by mixing. The composite samples were transported to the laboratory and dried in an oven at 25 °C. The dry samples were passed through a Nylon sieve (<2 mm) and a representative subsample was additionally finely ground with a tungsten carbide disc swing mill (RS1, Retsch). The elemental composition (elements $z \geq 11$, except Hg) was determined using X-ray fluorescence spectrometry (XRF; XEPOS, Spectro). For XRF analyses, pellets were prepared containing 4 g of ground soil and 0.9 g of Licowax C (Clariant, Switzerland). Total Hg was determined on the ground samples by combustion atomic absorption spectrometry (CAAS; AMA 254 Advanced Mercury Analyzer, LECO). Two EnviroMAT contaminated soil standard reference materials (SS-1 and SS-2, SCP Science) were analyzed for quality control of XRF and four stream sediment reference materials (NCS DC 73307 to DC 73310, NACIS) for

quality control of CAAS (results in Supplementary Information (SI) Table S1 and S2). Also on the ground samples, total C and N contents were determined with a CHNS analyzer (CHNS-932, LECO) and total inorganic C was obtained with a Solid Sample Module (SSM-5000A, Shimadzu) connected to a C analyzer (TOC-L, Shimadzu). Total organic carbon was calculated as the difference between total C and inorganic C. The soil pH of the sieved soil was measured in 0.01 M CaCl_2 at a soil-to-solution ratio of 1:2.5 (g:mL) using a glass electrode (6.0234.100, Metrohm). Size distribution of mineral soil particles, i.e. percentages of clay (<2 μm), silt (2–63 μm), and sand (63–2000 μm), was determined using a laser diffraction particle size analyzer (LS 13 320, Beckman Coulter) after removal of soil organic matter with H_2O_2 . Detailed procedure and analysis of Wageningen ring analysis samples for method validation are given in the SI.

Figure 1: Schematic map of the soil transect. Sampling location at $x = 0$ is located at $43^{\circ}23.578' \text{ N} / 23^{\circ}6.753' \text{ E}$

2.3. Soil extractions

The soil extraction procedures included the Olsen method (Olsen et al., 1954) and a modified ascorbate-citrate extraction (Kostka and Luther III, 1994). The Olsen method is a commonly used method to assess the phytoavailability of phosphorus in soils and has also been positively evaluated for assessing the phytoavailability of As (Martinez-Sanchez et al., 2011). The procedure by Kostka and Luther III (1994) dissolves amorphous Fe(III) phases which are considered the reactive Fe pool with regard to microbial reduction under anoxic conditions (Hyacinthe et al., 2006). Furthermore, the ascorbate-citrate-extractable As was found to correlate well with As released during flooding of a contaminated soil (Weber et al., 2010). Throughout this study we used chemicals of at least analytical grade, and, if not otherwise specified, 18 M Ω cm Milli-Q water. Olsen-extractable concentrations of As and P were determined by extraction with 0.5 M NaHCO_3 solution. The pH of the extractant was adjusted to 8.5 using NaOH. In 50 mL tubes, 1 g of sieved soil and 20 mL extractant were shaken at 25 $^{\circ}\text{C}$ for 30 min on an end-over-end shaker.

Ascorbate-citrate-extractable concentrations of As and P were determined by extraction with 0.1 M ascorbate / 0.2 M citrate / 0.6 M HCO_3^- solution. In 50 mL tubes, 1 g of sieved soil and 40 mL extractant were shaken at 25 °C in the dark for 120 min on an end-over-end shaker. The extractant was prepared as follows. First, 52.62 g of disodium hydrogen citrate sesquihydrate ($\text{HOC}(\text{COOH})(\text{CH}_2\text{COONa})_2 \cdot 1.5\text{H}_2\text{O}$) and 50.41 g NaHCO_3 were dissolved in ca. 0.8 L H_2O . The solution was subsequently purged with N_2 gas for 1 h prior to the addition of 17.61 g ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$). Finally, the solution was made up to 1 L with H_2O and the pH was adjusted to 7.5 with NaOH. The extractant was kept in a plastic bottle wrapped in aluminum foil. In case it was not used on the day of preparation the solution was stored for a maximum of one day at 4 °C. After Olsen and ascorbate-citrate extraction, respectively, the tubes were centrifuged (15 min, 3300 g) and an aliquot of the supernatant was passed through a 0.22 μm Nylon membrane filter (WIC 8032, WICON). Total element concentrations in the filtered solutions were analyzed by ICP-OES (Vista-MPX, Varian). Additionally, in the Olsen extracts we measured inorganic monomeric orthophosphate spectrophotometrically (Cary 50, Varian) employing the molybdenum blue method (ISO 6878:2004). Interferences by arsenate were eliminated by prior reduction of arsenate to arsenite as detailed in the ISO method. The standards used for calibration in both, ICP-OES and spectrophotometry, were prepared with the corresponding extractant as the background solution. Extraction blanks were subtracted from all measurements.

2.4. Plant sampling and analysis

Whole shoots of *Trifolium repens*, *Holcus lanatus*, and *Apera spica-venti* were sampled in two consecutive summers, in the first weeks of June 2012 and 2013. These species were chosen because they are abundant along the transect as well as in other areas in the floodplain. The plant samples were collected at a maximum distance of 15 m from the soil transect. The sampling locations were recorded using a GPS receiver. Orthogonal projection onto the transect line depicted in Figure 1 was employed to obtain the position (x in m) along the soil transect. The plant shoots were frozen in the field using dry ice. The frozen samples were transported on dry ice to the laboratory where they were thawed and thoroughly washed with deionized H_2O . The samples collected in 2012 were freeze-dried, cut in smaller pieces with ceramic scissors and milled with a zirconium oxide ball mill (MM 200, Retsch; 4 min at 24 s^{-1}). The samples collected in 2013 were milled with liquid N_2 and a mortar and pestle and subsequently freeze-dried. The C and N concentrations of the milled samples were determined with a CHNS analyzer (CHNS-932, LECO). The concentrations of elements $z > 11$ were determined with ICP-OES and ICP-MS (7500ce, Agilent) after digestion. Triplicate 0.2 g milled subsamples were digested with 2.5 mL 67% HNO_3 , 1.5 mL 30% H_2O_2 and 1.5 mL H_2O in a microwave high-pressure autoclave system (TurboWave, MLS). Digestion blanks were subtracted from all measurements. Two plant standard reference materials (BCR-62, IRMM, and NCS DC 73349, NACIS) were analyzed for quality control. The results are listed in SI Table S3.

2.5. Data analysis

2.5.1. Exclusion of plant samples containing soil-derived impurities

Titanium (Ti), an element abundant in all soils but not in plants, was used as an indicator to identify plant samples containing soil particles which were not removed by washing. Readers are referred to Cook et al. (2009) and references therein for validation of this approach. Plant samples with Ti concentrations which were identified as statistical outliers, defined as values above the upper fence (third quartile +1.5 interquartile range) were excluded from the dataset. In total, 1 of 23 *T. repens* and 3 of 26 *H. lanatus* samples were identified as putatively containing impurities and excluded from further data analysis. The upper fences were 11.6 and 13.0 mg kg⁻¹, respectively. These values are close to the limit of 10 mg kg⁻¹ dw Ti suggested by Cook et al. (2009). The *A. spica-venti* samples all had very low Ti concentrations (≤ 2.2 mg kg⁻¹) and no outlier was identified.

2.5.2. Statistical analysis

For statistical analysis we used R V.3.2.0 (R Core Team, 2015) and OriginPro V.9.1.0 (OriginLab Corporation). Pearson product-moment correlation and Spearman's rank correlations were calculated using the function 'rccor' from the R package 'Hmisc' V.3.16-0 (Harell, 2015). For evaluation of general relationships we used Spearman correlation, whereas for assessing the predictive power of a variable we used Pearson correlation. Pearson correlation results are denoted as 'log10' in case a decadic log-transformation was applied prior to data analysis. Single-linkage clustering based on distance defined as 1-R (Spearman) was performed with the core R function 'hclust'. 1D spatial interpolation of soil transect data to the plant sample's position along the transect was conducted using the Akima spline method (Akima, 1970) as implemented in the OriginPro function 'interp1'. The splines are depicted in Figures 2 and 4, and SI Figures S1, S2 and S5.

2.5.3 Estimation of the relative contribution of plant As to intake by dairy cows

We assessed the importance of plant As to intake by dairy cows assuming that the total daily As intake can be described by intake of pasture and involuntarily soil ingestion according to Equation 1 adopted from Rodrigues et al. (2012). Intake by air and water is thereby considered negligible compared to that of soil and pasture. The relative fraction of intake attributed to pasture as a function of soil-to-shoot transfer factor (TF) was calculate with Equation 2 which was derived by rewriting Equation 1 and substituting with the definition of TF. For intake of pasture and soil by dairy cows we used values from McKone and Ryan (1989). These values are listed in the caption of Figure 9.

$$DI_{total} = DI_{pasture} + DI_{soil} = I_{pasture} * C_{pasture} + I_{soil} * C_{soil} \quad Eq. 1$$

$$\frac{DI_{pasture}}{DI_{total}} = \frac{I_{pasture} * TF}{I_{pasture} * TF + I_{soil}} \quad Eq. 2$$

where

$DI_{pasture}$, DI_{soil} , and DI_{total} are the daily intake of As via pasture, via soil, and in total, respectively (mg kg⁻¹),

$I_{pasture}$ and I_{soil} , are the intake of pasture and soil, respectively (kg dw d^{-1}),

$C_{pasture}$ and C_{soil} , are the As concentrations in pasture and soil, respectively ($\text{mg kg}^{-1} \text{ dw}$), and

TF is the soil-to-shoot transfer factor for the pasture plants, representing the ratio of $C_{pasture}$ to C_{soil} .

3. Results and Discussion

3.1. Soil geochemistry along the transect

The 63 collected soil samples from the transect across the Ogosta floodplain had sandy to silty loam textures according to the *Guidelines for Soil Description* (FAO (2006), SI Figure S2). They had mostly a circumneutral pH (6.5–7.5) which is characteristic for the upper stretch of the Ogosta floodplain (Mandaliev et al., 2013; Mikutta et al., 2014). The soils developed from river sediments as indicated by stratification being evident to a varying degree in the soil profiles. According to the WRB classification the soils were classified as Fluvisols (IUSS Working Group WRB, 2014). In agreement with earlier work on Ogosta floodplain soils (Mandaliev et al., 2013), elemental analysis revealed massive contamination with As. The concentrations ranged over three orders of magnitude, from 15 to 15600 mg kg^{-1} soil, exceeding the Bulgarian Maximum Permissible Level (MPL) for grasslands of 30 mg kg^{-1} more than 500 times. The exceedance of the corresponding MPLs by metals and metalloids decreased along the sequence $\text{As} \gg \text{Pb} > \text{Zn}, \text{Cu}, \text{Cd} > \text{Hg}$ (MPLs in SI Table S5). With regard to As, 92% of samples exceeded the MPL. For Pb exceedance was given for 33% of samples (max. equal to 5 times the MPL), for Zn, Cu, and Cd for less than 10% of samples (max. in the range of the MPLs). No exceedance of the MPL was observed for Hg.

Figure 2 shows the distribution of As, Mn, Fe, S, P, N, organic C, K, Si, and the pH along the soil transect (large panels) and the vertical soil profiles (small panels). On both sides, the concentration of As strongly decreased with increasing distance from the river (Figure 2a). The river banks ($x = 100 \text{ m}$ and -48 m) were more strongly contaminated in the subsoil (20–40 cm, 2700 and 8900 mg kg^{-1}) than in the topsoil (0–20 cm, 770 and 940 mg kg^{-1}). In contrast, more than 50 m away from the river banks the contamination was consistently higher in the topsoil than in the subsoil. At the sampling site most distant from the river ($x = -439 \text{ m}$), the concentrations were 30 and 15 mg kg^{-1} in the topsoil and subsoil respectively, which is above the baseline level for As in soils often being cited at $\leq 10 \text{ mg kg}^{-1}$ but in the range of typical background values for soils with naturally elevated concentrations (Smedley and Kinniburgh, 2002). The vertical profile P1 is a naturally open soil profile in the southern river bank of the intermittent channel developed by riverbed erosion. The arsenic concentration in this profile peaked at 15600 mg kg^{-1} in 35 cm depth. With greater depth the As concentration steadily decreased reaching concentrations below 100 mg kg^{-1} at depths greater than 75 cm. In the profiles P3 and P4, located 170 and 259 m away from the river banks, the concentration of As was highest at the soil surface (0–5 cm, 830 and 290 mg kg^{-1} respectively) and approached concentrations below 100 mg kg^{-1} at depths greater than 40 cm and 5 cm for P3 and P4, respectively. The pattern that the layer of highest contamination as well as the layer with levels below 100 mg kg^{-1} were located closer to the surface with increasing distance to the river might be explained by decreasing extent of sediment deposition with increasing distance to the river bank. Since the river-proximate parts of the transect are flooded more frequently, more sediments accumulate on top,

repeatedly burying the previous topsoil layers. Similar horizontal and vertical distribution patterns to the one described for As were observed for Mn, Fe, and S (Figure 2b–d) as well as for the trace metals Pb, Cu, Zn, and Hg (SI Figure S1a–d, Cd not shown as most values were below the limit of detection of 0.3 mg kg^{-1}). The similar distribution of these elements is due to the common source, the sulfidic mine waste.

The pH value of most samples was carbonate buffered at pH 7.1–7.5 (Figure 2e). Both, the natural soil material and the sulfidic material distributed during the major contamination events are rich in carbonates resulting in effective buffering of the acidity generated during the oxidative weathering of the sulfide minerals. The circumneutral pH is fortunate with regard to the risk associated with the main co-contaminants of concern, Pb, Cu, and Zn. Their pH-adsorption edge to major soil solid phases is below pH 7 and their solubility and, presumably, their plant availability are therefore low (Sparks, 2003). In contrast, the adsorption of arsenate is maximum at low pH and decreases with increasing pH above pH 4 for poorly crystalline Fe (hydr)oxides (Pierce and Moore, 1982), which were found to be the most important sorbent for arsenate in Ogosta soils (Mandaliev et al., 2013). The decreased pH values at locations between $x = -200 \text{ m}$ and -400 m to a minimum of pH 5.9 and 5.1 for top- and subsoils, respectively, coincide with decreased inorganic carbon and calcium contents (SI Figure S1e, f), consistent with the absence of the calcium carbonate buffer system in these soils. Apart from inorganic C and Ca, neither changes in the soil's composition nor changes in the particle size distribution reflect the decrease in pH observed for this part of the transect (Figure 2, SI Figure S1 and S2a).

The concentration of total P showed low variability along the transect (Figure 2f), but the concentrations on the north side of the river were slightly higher, presumably due to fertilizer application during historical usage as vegetable gardens. Along the soil profiles, P and even more pronounced N and organic C were enriched in the top soil layers and strongly decreased with depth (Figure 2f–h). This is a common pattern in vegetated soils as plants concentrate nutrients in the topsoil and deplete soil-born nutrients in the root zone (Jobbágy and Jackson, 2001). Almost all N was present in organic forms as we detected only traces of extractable ammonia and nitrate (<1% of total N) using spectrophotometry and ion-chromatography (data not shown). This explains the close resemblance of the distributions of N and of organic C. Overall, the concentrations of K and Si were fairly uniform across the transect and with soil depth, but they tended to be lowest in the most highly contaminated samples ($> 1000 \text{ mg kg}^{-1} \text{ As}$).

Figure 2: Total soil concentrations of As (a), Mn (b), Fe (c), S (d), P (f), N (g), organic C (h), K (i), Si (j), and the pH (e) at sampling locations x along the transect (large panels) and the vertical soil profiles (small panels). The locations x in meters refer to the transect axes depicted in Figure 1. The filled black diamonds ($\blacklozenge$) represent topsoil (0–20 cm) concentrations and the open black diamonds ($\lozenge$) subsoil (20–40 cm) concentrations, respectively. Lines connecting the data points are Akima spline interpolations. The positions of the soil profiles (P1, P3, P4) along the transect are marked with vertical dashed lines with the symbols at the end corresponding to the small panels. The blue shaded areas indicate the approximate position of the river channels.

Figure 3 shows the result of a cluster analysis including soil pH, particle size distribution (clay, silt, sand) and the total element concentrations. Positively correlated parameters are assigned to the same or proximate cluster, respectively. Negatively correlated parameters group into different or distant clusters, respectively (SI Table S6 lists the correlation coefficients and p-values). The distributions along the transect and the soil profiles of all these soil parameters are depicted in Figure 2 and SI Figures S1 and S2. Cluster C1 includes As, the trace metals Pb, Cu, Zn, and Hg, as well as Fe, Mn, S, Ca, and inorganic C. These elements are associated with the carbonate-rich, sulfidic contamination. Cluster C2 includes Al, Si, and K, which are associated with natural sediments, non-sulfidic sediments which are not related to mining activities. Cluster C3 includes P, N, and organic C of which the spatial distribution is mainly governed by cycling of these elements between biosphere and pedosphere. Consistent with the negative reflection of the contamination peaks in K and Si (Figure 2), the clusters C1 and C2 are negatively correlated. This can likely be explained by a dilution effect as the contamination material considerably contributed to the total mass of soil, diluting the natural sediments in place. The clustering in Figure 3 indicates that the contamination material is of courser texture (higher sand content) as compared to the natural sediment-associated material (see also the spatial distribution of particle sizes in SI Figure S2 as compared to the distribution of contaminants in Figure 2).

Figure 3: Scatterplot matrix and correlation/cluster analysis of soil pH, the particle size distribution (clay, silt, sand), and the total concentrations of Al, Si, K, Ca, inorganic C, S, Hg, Fe, Mn, As, Pb, Cu, Zn, P, N organic C, Na, and Mg. The data set includes all soil samples from this study ($n = 63$). The scatterplot coloring follows Spearman's Rank correlation results ($\alpha = 0.001$, significant $|R| > 0.40$). The hierarchical clustering is based on dissimilarity defined as $1 - R$ (Spearman). The correlation coefficients and p-values can be found in supplementary Table S6.

3.2. Extractability of As and P

In terms of absolute concentrations, the extractable As and P show a very similar spatial distribution as found for the total As and P concentrations. Figure 4 shows the distribution of the extractable As and P in relative terms, i.e. as fraction of the total element concentration of the corresponding element. Considering all 63 soil samples of this study, the relative extractability of As was higher than the extractability of P irrespective of the extraction protocol. On average, $6.1 \pm 2.9\%$ of total As but only $2.1 \pm 1.0\%$ of total P was Olsen-extractable (mean $\pm$ SD). The average ascorbate-citrate-extractable fractions amounted to $35.5 \pm 14.8\%$ and $12.1 \pm 5.5\%$ of the total for As and P, respectively. Only four samples, the subsoils with close-to-background As concentrations located at $x > 300$ m (≤ 33 mg kg⁻¹), deviated from this general observation. For these soils the ascorbate-citrate extractability for As was lower than for P. Interestingly, these four samples also deviated with respect to their Olsen extractability, with the Olsen extractability of As being higher than the corresponding ascorbate-citrate extractability (possible reasons will be discussed later).

On average, only $4.7 \pm 2.8\%$ of total Fe was dissolved by ascorbate-citrate extraction, indicating a high proportion of total Fe in crystalline phases (SI Figure S5a). With regard to the much greater fraction of As extracted, this implies that the poorly crystalline Fe (hydr)oxides are strongly enriched in As as compared to the bulk Fe (hydr)oxides. Similar to the observations reported in Mandaliev et al. (2013), we found for the ascorbate-citrate-extractable fractions that the molar As:Fe ratio in the soil profile P1 in depth between 20 and 46 cm exceeded 0.25, the maximum theoretical As loading proposed for ferrihydrite (Waychunas et al. (1993), SI Figure S5b). This indicates the presence of other poorly crystalline As-bearing Fe phases at such contamination hotspots, found to be located in or in close proximity of the river banks of the Ogosta River (Mandaliev et al., 2013).

Figure 4: Olsen-extractable As (a), ascorbate-citrate-extractable As (b), Olsen-extractable P (c), and ascorbate-citrate-extractable P (d) in soil at sampling locations x along the transect (large panels) and the vertical soil profiles (small panels). All concentrations expressed as percentage of the total concentration of the respective element in the soils. The locations x in meters refer to the transect axes depicted in Figure 1. The filled black diamonds ($\blacklozenge$) represent topsoil (0–20 cm) concentrations and the open black diamonds ($\diamond$) subsoil (20–40 cm) concentrations, respectively. Lines connecting the data points are Akima spline interpolations. The positions of the soil profiles (P1, P3, P4) along the transect are marked with vertical dashed lines with the symbols at the end corresponding to the small panels. The blue shaded areas indicate the approximate position of the river channels.

The highly contaminated soils close to the river banks generally showed low Olsen but high ascorbate-citrate extractability of As (Figure 4a–b and Figure 2a). In Figure 5a–b, the correlations between the extractability of As and the total As contents are shown. For the subsoils, the Olsen extractability decreased with increasing total As. For the topsoils, the trend seems also negative but no significant correlation was found (Figure 5a). The ascorbate-citrate extractability of As increased with increasing total As, both for the top- and the subsoils (Figure 5b). The Olsen and the ascorbate-citrate extractabilities of P were independent of total As (Figure 5c–d). The Olsen extraction is more efficient in desorbing arsenic and phosphorus than the ascorbate-citrate extraction (pH 8.5 versus 7.5), while, vice-versa, the ascorbate-citrate extraction is more effective in releasing the two elements from poorly crystalline Fe (hydr)oxides through dissolution of the mineral phase. Therefore, the trends observed for the extractabilities of As indicate that the relative fractions sorbed to surfaces decrease while the fractions associated with poorly crystalline iron mineral phases increase with increasing total As and degree of contamination, respectively. The low importance of desorption for As solubilization as compared to the release due to dissolution of poorly crystalline Fe (hydr)oxides becomes manifested in Olsen-extractable fractions being greater than the corresponding ascorbate-citrate-extractable fractions only in the four least contaminated subsoils sampled at $x > 300\text{m}$.

In both years, 2012 and 2013, the soils along the transect were not flooded and therefore presumably in oxic state for at least 5 months prior to sampling (SI Figure S3). We therefore assume that the chemical speciation of As was dominantly arsenate, to a large part associated with poorly crystalline iron minerals as documented by Mandaliev et al. (2013) for a similar transect located ca. 6 km upstream from the one in this study. A considerable fraction of P in soil can typically be present in organic forms (Blume et al., 2016). In the Olsen extracts inorganic monomeric orthophosphate was measured. This PO_4 -Olsen-P was positively correlated to the total Olsen-P (Spearman, $R = 0.51$, $p < 0.001$). The average share of PO_4 -P was $62 \pm 16\%$ of total Olsen-extractable P. The remaining fraction is organic P and inorganic polyphosphates, which might also contribute to potentially plant available P as they are usually hydrolyzed in soils over days to months depending on the compounds (Torres-Dorante et al., 2005). Compared to As, a greater fraction of P was not extractable with either method employed, i.e. was neither associated with the easily exchangeable fraction (Olsen), nor associated with poorly crystalline Fe hydr(oxides). We suggest that a large fraction of this remaining P might be present as sparingly soluble Ca phosphates. For carbonate buffered soils, this is supported by thermodynamic soil equilibria considerations (Lindsay, 1979) and experimental evidence for the presence of diverse Ca phosphates in such soils (e.g. Hinsinger (2001) and references therein). In contrast, solubility considerations (Magalhães, 2002) and spectroscopic studies on another Ogosta floodplain transect (Mandaliev et al., 2013) suggest that Ca arsenates are unlikely to be present in both non-calcareous and calcareous soils.

Figure 6 shows the molar As:P ratio for the total and the extractable concentrations as a function of total As in soil. Both, the ratio for the total as well as for the extractable concentrations clearly increased with total As in soil. The molar total As:P ratio observed in the topsoil samples ranged from 0.014 to 1.2 (Figure 6a, mean = 0.18, $n = 18$). The molar total As:P ratio observed in the subsoil samples ranged from 0.0084 to 3.8 (Figure 6b, mean = 0.42, $n = 18$). Because of the higher extractability of As compared to P, the molar As:P ratio in the extractable fractions were generally higher than the corresponding ratio of the totals. In the topsoils, the molar As:P ratio of the Olsen-extractable fractions was on average 0.43, ranging from 0.048 to 2.62. Considering that these Olsen-extractable

fractions were shown to represent the plant available fractions better than the total amounts (Anawar et al., 2008; Giri et al., 2012; Woolson et al., 1971), this finding implies that potential mutual interferences of As and P uptake by plants might be more pronounced than the total concentrations of As and P in soil suggest.

Figure 5: Olsen- and ascorbate-citrate-extractable fractions of As and P as percentage of total of the respective element in soil plotted against the total soil As concentration. The filled black diamonds (◆) correspond to the topsoils (0–20 cm) and the open black diamonds (◇) to the subsoils (20–40 cm), respectively. Significance code of the Spearman's rank correlation: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Figure 6: Molar As:P ratio for the total concentrations and the Olsen- and ascorbate-citrate-extractable concentrations plotted against the total soil As concentration. 'Olsen PO_4 ' refers to the ratio of Olsen-extractable monomeric orthophosphate to Olsen-extractable As. Lines serve as eye-guides, connecting the samples in the order of increasing soil As.

3.3. Soil-to-shoot transfer of As and P

Figure 7 shows the As and P concentrations in the shoots of the three investigated plant species along the transect. For *T. repens* and *H. lanatus*, the concentrations of As in the shoots varied over more than two orders of magnitude and median values were 3.2 and 1.6 mg kg⁻¹, respectively. Plants collected near the river channels generally exhibited higher As concentrations in their shoot than plants of the same species growing further away from the river. Hence, the contamination gradients in the soils were clearly reflected in the shoot As concentrations of *T. repens* and *H. lanatus*. For *A. spica-venti*, the three samples which were furthest from the river had the lowest As concentrations, consistent with less As in soil. Apart from that, there was no trend along the contamination gradient for *A. spica-venti*. Similar to total P in soil, the P concentrations in the plant shoots of all three species did not show trends along the transect (Figure 7d–f); median values were 3.0, 2.7, and 3.3 g kg⁻¹ for *T. repens*, *H. lanatus*, and *A. spica-venti*, respectively. Compared to As, intraspecies variation in shoot P was small (12%, 22%, and 8% relative SD for *T. repens*, *H. lanatus*, and *A. spica-venti*, respectively) and interspecies variation in shoot P concentrations was of similar magnitude as the intraspecies variation (relative SD of species medians and means of 10% and 11%, respectively). This is in line with Güsewell and Koerselman (2002) who reported interspecies variation being of similar magnitude than inter-site variation, i.e. variation in plant P explained by the site the plants were growing. Also, low phylogenetic variation in plant P concentration within the angiosperm was ascertained by White et al. (2012) and Broadley et al. (2004).

Plants sampled in different years but at spatially proximate sites had in most cases similar shoot As concentrations. SI Figure S4 shows the average monthly air temperature and the monthly precipitation from May to June for the two consecutive years. Apparently, the weather in the three months before the sampling was quite different between the years. Compared to 2012, the weather in 2013 was colder and wetter in March, comparably warm but slightly dryer in April, and warmer and much drier in May. The cumulative precipitation from March to May was 162 mm in 2013 while it was 207 mm in 2012. The apparent lack of differences in plant As between the years indicates that the difference in spring weather did not greatly affect the As transfer from soil to shoot. This might be different for years with spring flooding (e.g. year 2014, see SI Figure S3), when anoxic soil conditions occur and arsenite dominates the solution phase. For rice (*Oryza sativa* L.) it is well known that arsenite uptake is very efficient and that the As concentration in rice grown on flooded, anaerobic soil is significantly elevated as compared to the respective concentration when grown aerobically (Li et al., 2009; Xu et al., 2008). Mutation studies revealed that silicon transporters (aquaglyceroporins) are the major pathway of arsenite uptake into the root cells of rice (Ma et al., 2008). But, rice is a strong accumulator of Si, typically amounting to 5–10 wt% in shoots, and might therefore take up arsenite much more effectively than the plant species studied here, which exhibit Si shoot concentrations <1 wt% (SI Figure S8d–f). For *H. lanatus*, Wan et al. (2014) found no significant difference in As concentration in the shoots of plants grown on waterlogged and non-waterlogged soil from a mining area despite an increase in total dissolved As during waterlogging which was attributed to an increase in dissolved arsenite. It is noteworthy that the increase in arsenic solubility induced by water-saturation was small for the mining soils studied by Wan et al. (2014) as compared to contaminated riverine floodplain soils, e.g., as investigated by Weber et al. (2010) and Parsons et al.

(2013). Riverine floodplain soils are generally nutrient-rich as a result of deposition of nutrient-loaded sediments (Tockner and Stanford, 2002). The reported discrepancy in reductive release of As very likely resulted from differences in the soil's microbial activity determined by the nutrient availability. Therefore, the results reported by Wan et al. (2014) may not be generalized to the wild *H. lanatus* population growing along the floodplain soil transect studied here.

Figure 7: The concentrations of As (a–c) and P (d–f) in the shoots of *T. repens*, *H. lanatus*, and *A. spica-venti* at sampling locations x along the transect. The locations x in meters refer to the transect axes depicted in Figure 1. Orange circles (●) and green triangles (▲) represent samples collected in year 2012 and 2013, respectively. The error bars represent standard deviations of triplicate digests. The blue shaded areas indicate the approximate position of the river channels.

Figure 8 shows the As concentrations in the plant shoots as a function of spatially interpolated total As concentrations in the topsoils. Also indicated are lines of equal soil-to-plant transfer factor TF ($TF = As_{shoot}/As_{soil}$). Data compiled from the literature is plotted alongside with the data from this study. Tables S7 and S8 (see SI) summarize information on the type of study, the soils, the source of arsenic, and the major findings with regard to As soil-to-plant transfer. In this study, total soil As was significantly correlated with shoot As for *T. repens* and *H. lanatus* (Spearman, $R = 0.57$ and 0.68 , $p < 0.01$ and 0.001), whereas for *A. spica-venti* no significant correlation existed (Spearman, $p > 0.08$). Omitting the two extreme values at high soil As concentration, the TF observed in this study ranged from 0.004 to 0.07 for *T. repens* and from 0.002 to 0.03 for *H. lanatus*. The two extreme values with respect to TF, one with an exceptionally high TF at soil concentration of 921 mg kg^{-1} and one with an exceptionally low TF at soil concentration of 3566 mg kg^{-1} are present in both, the *T. repens* and the *H. lanatus* data. They correspond to sampling locations close to the river channels at $x = -53 \text{ m}$ (high value) and $x = 1 \text{ m}$ (low value), respectively. It might well be that the two exceptional values adequately describe the ecophysiological response of the plants at these

sites, but for the following reason these extreme TF values should be interpreted with caution. Close to the highly dynamic river channels the heterogeneity of the soil with respect to As distribution is particularly high. Therefore, the topsoil bulk sample taken in close proximity of the plants might not adequately represent the actual concentration in the root zone, leading to a calculated TF which does not adequately describe the ecophysiological response of the plants growing at those sites.

Baker (1981) introduced the categorization of plants into excluders, i.e. species maintaining the shoot concentration constant and low over a wide range of soil concentrations, indicators, i.e. species with regulated uptake and root-shoot translocation so that the shoot concentration reflects external levels, and accumulators, i.e. species concentrating the element in the shoot from low or high soil levels. Following this categorization, the overall soil-plant relationship of *T. repens* and *H. lanatus* may be described as indicator-like since the concentrations in the plants reflect the concentrations in the soils. But, as already pointed out by Baker (1981), when a wide range of soil concentrations are considered, the apparent overall soil-plant relationship might be spurious and not describe the actual ecophysiological response as the same species may act as excluder, indicator or accumulator over different ranges of soil metal concentrations. Another definition of excluder plants which is more convenient for field studies can be found in the reviews by Zhao et al. (2010) and Sharma et al. (2014); they call plants with a TF for As of below 0.1 excluders. According to this definition, all three plant species in this study behaved as excluders.

Figure 8 allows comparison of the soil-to-plant TFs observed in this study with data from field surveys as well as pot, lysimeter, and field experiments reported in the literature. For *T. repens*, 13 of 22 TFs observed in this study fall in the range of TFs collected from the literature, mostly into the lowest order of magnitude of values reported. The remaining TFs extend the range to lower values. For *H. lanatus* 21 of 23 TFs fall into the range of literature TFs, mostly into the lowest order of magnitude of values reported. All but two studies, Speir et al. (1992) and Lewińska and Karczewska (2013), were either field surveys or used uncontaminated or mining-affected soil for the experiments, without additional spiking of arsenic. In the experiments with *T. repens* conducted by Speir et al. (1992), uncontaminated soil mixed with sawdust, freshly treated with chromated copper arsenate, was used as the substrate. They observed exceptionally high soil-to-shoot transfer ($TF > 1$) in some treatments, but overall their results with respect to the plant uptake of As were ambiguous. Lewińska and Karczewska (2013) used uncontaminated soil spiked with arsenate or arsenite salt solution, and mine soil in their experiments. They observed higher As concentrations in the shoots of *H. lanatus* grown on the arsenite or arsenate spiked soils versus grown on mining soil, despite the 10 times higher As concentration in the mining soil. The soil-to-plant transfer for the plants grown on the spiked soil also stands out in the context of all data in Figure 8. This comparison highlights the limitations of experiments with spiked soils when studying phytoavailability or the natural ecophysiological response of plants to available As. It is known from isotope dilution studies that in soil, As is present in pools of different lability (e.g. Stroud et al., 2011; Tye et al., 2002). Typically the spiked element first loads in a pool of relative high lability and over time also partitions into less available pools. In freshly spiked soil the phytoavailability is thus elevated as compared to a non-spiked, aged soil with comparable composition and total As concentration. Unnaturally elevated phytoavailable As might even induce extreme plant phenotypes with disrupted homeostasis which would not be capable of maintaining a self-sustaining population (van der Ent et al., 2015; van der Ent et al.,

2013). In contrast, an ecophysiological response found in a present-day population is indicative for ecological relevance, underscoring the importance of field surveys to accomplish experiments with non-spiked soils.

Figure 8: Arsenic in plant shoots plotted against spatially interpolated values for total As in the topsoils (0–20 cm). The error bars represent standard deviations of triplicate digests. The spline used for interpolation is depicted in Figure 2a. Diagonal lines follow lines of equal soil-to-plant transfer factor (TF). Additional information on the data from the literature (references in the plot) is summarized in the SI in Tables S7 and S8. Red symbols indicate data from field surveys.

3.4. Nutritional status of plants

For all three plant species, the P concentration in the shoot (Figure 7a–c) was not related to the shoot As concentration (Figure 7d–f, Spearman, $p > 0.16$) despite the extreme As:P ratios in the total and extractable fractions in soil (Figure 6). Apparently, As uptake did not interfere with P uptake.

Under P-deficient conditions, plant roots often exude organic acids which increase the availability of phosphate (Rengel and Marschner, 2005) and likely also of arsenate. Furthermore, genes encoding phosphate/arsenate transporters are up-regulated, increasing the number of the corresponding proteins (Marschner, 2012). Arsenic uptake has been reported to be elevated under P-deficient conditions (Gulz et al., 2005) and improving the P status using fertilizer was suggested as a strategy to mitigate As uptake (e.g. Tripathi et al., 2007). Plant growth in anthropogenically little impacted temperate riverine floodplains were found to be (co-)limited by nutrients, typically by N, P, or K (e.g. Loeb et al. (2009) and references therein). In impacted temperate floodplains on the other hand, plant growth is apparently often no longer limited by nutrients, probably mainly as a result of manure and fertilizer application, and deposition of nutrient-loaded sediments (Antheunisse et al., 2006; Spink et al., 1998). Nutrient ratios in shoot biomass have been used to assess limitations of N, P, and K (Güsewell, 2004). N:P ratios below 10 might indicate N limitation for N concentrations below 13–14 g kg⁻¹ dw, and N:P ratios greater than 20 might indicate P limitation for P concentrations below 0.7–1 g kg⁻¹ dw (Güsewell (2004) and references therein). Limitation by K was proposed to be unlikely for N:K below 2.1 and K:P greater than 3.4 (Olde Venterink et al., 2003). Accordingly, no limitation by one of these three nutrient elements would be indicated in this study. The mean N:P ratios of the two grass species were below the critical value of 10, but the N levels of all three species were considerably higher than the 13–14 g kg⁻¹ suggested as additional criteria for N-limitation (see SI Figure S9 for an overview of NPK concentrations observed in this study). These findings are consistent with Spink et al. (1998) and Antheunisse et al. (2006) who found that the productivity of the herbaceous vegetation in many human-impacted European and North American riverine floodplains was not limited by nutrients. We acknowledge that the critical nutrient ratios and levels, on which we based our assessment, were found for the vegetation level and might therefore not hold when used, as here, on a small subset of species in an ecosystem. However, further literature supports our assessment. In a systematic literature review, Güsewell and Koerselman (2002) compiled shoot N and P concentrations of *H. lanatus* and *T. repens*. The N and P concentrations observed in our study are at the upper end of the range of values in their data compilation (this study: 34 ± 5 g kg⁻¹ dw N and 3.0 ± 0.4 g kg⁻¹ dw P for *T. repens*, and 20 ± 7 g kg⁻¹ dw N and 2.6 ± 0.6 dw P for *H. lanatus*; Güsewell and Koerselman (2002): 20 to 32 g kg⁻¹ dw N and 1 to 2 g kg⁻¹ dw P for *T. repens*, and 8 to 19 g kg⁻¹ dw N and 0.7 to 2.9 g kg⁻¹ dw P for *H. lanatus*). Lawniczak et al. (2009) studied how the N:K supply ratio affects the performance of *H. lanatus*. Underscoring our assessment, they observed no indication for K limitation at K shoot concentrations lower and N:K ratios higher than the ones observed in our study (this study: 17 ± 3 g kg⁻¹ dw K and N:K of 1.3 ± 0.5; Lawniczak et al. (2009): shift from N to co-limitation at N:K ratios close to 4, and 4 to 8 g kg⁻¹ dw K). It seems that a factor or a combination of factors other than N, P, and K limits plant growth along the investigated transect. Toxicity and deficiency seems unlikely as we did not observe any symptoms during plant

sampling. Given the P-sufficient status of the plants and the presumably high P-retention capacity of the soils as indicated by high contents of iron minerals, phosphate additions (e.g. by fertilization) might increase rather than decrease As uptake by plants due to elevated As availability as a result of competition for binding to soil solid phases (Bolan et al., 2013).

3.5. Assessment of phytoavailable As and P by soil extractions

A correlation analysis of total and extractable As and P fractions in soil versus the corresponding As or P shoot concentrations was conducted to compare the different element pools in soil with regard to their relation to plant uptake (Figure S10 and S11 in SI). Neither the Olsen extraction nor the ascorbate-citrate extraction was superior to the total soil element concentration for explaining the shoot concentrations. In this study, total As in soil was highly correlated with both the Olsen-extractable and the ascorbate-citrate-extractable As (Pearson, $R = 0.95$ and 0.98 , $p < 0.001$). The same was true for the total with Olsen- and ascorbate-citrate-extractable P (Pearson, $R = 0.91$ and 0.91 , $p < 0.001$). In this case, any extraction targeting one of the pools has similar predicting power as has the total element concentration.

3.6. Implications for livestock grazing

Since the investigated area is used as extensive pasture we employed our results on soils and plants to derive implications for livestock grazing along the transect and on comparably contaminated grasslands in the floodplain of the Ogosta and other rivers. The current European Union tolerable maximum content of As in animal feed made from grass is 4 mg kg^{-1} on a 12% moisture content basis which corresponds to $4.5 \text{ mg kg}^{-1} \text{ dw}$ (European Commission, 2002). The contents in 27%, 17%, and 0% of *T. repens*, *H. lanatus*, and *A. spica-venti* samples, respectively, exceeded this level. According to the review by McKone and Ryan (1989), an average dairy cow held on pasture takes in $0.41 \pm 0.24 \text{ kg dw soil}$ with the $16.9 \pm 3.7 \text{ kg dw pasture}$ it consumes per day, equivalent to 2.4 wt% of total herbage being soil. Using this information and the interpolated As topsoil concentrations to consider the soil taken in with the plant matter, we estimate exceedance of the EU quality standard by 82%, 78%, and 63% of *T. repens*, *H. lanatus*, and *A. spica-venti* samples, respectively. Therefore, assuming that a mixture of the three plant species makes a good surrogate for the herbage, the actual herbage as taken in by dairy cows might exceed the EU quality standard. When grazed by sheep, even more pronounced exceedance of the quality standard are expected for a mixture of the three plant species; sheep take in more soil than cows because they graze closer to the ground surface (Abrahams, 2012; de Vries et al., 2007).

Corresponding to the variation of TFs observed along the studied transect, the relative contribution of soil and plants to As intake by dairy cows via soil ingestion and pasture consumption, respectively, was estimated (Figure 9). For dairy cows taking in a mixture of the three plant species the intake via soil would likely dominate, but intake via pasture consumption might not be negligible. As an example, for an equal mixture of the three plant species and using the median TFs, the percentage of As intake via pasture is estimated to be 17%. Dominance of As intake through soil ingestion is consistent with the few available animal studies for cattle grazing on pastures contaminated

by metalliferous mining (Abrahams and Thornton, 1994; Bruce et al., 2003; Thornton and Abrahams, 1983). To which extend As taken in with soil versus pasture finally contributes to the total As absorbed by the epithelial cells in the digestive tract is unclear as this is determined by the respective bioavailability, hardly investigated for ruminants.

Figure 9: Soil-to-shoot transfer factors (TF) of As observed in this study (top panel, median indicated as filled red circle) and estimation of As uptake via soil ingestion and pasture consumption for dairy cow feeding on a hypothetical pasture of given TF (bottom panel). The fraction taken in via pasture as a function of TF was calculated according to Equation 2 with average intake of pasture and soil according to the data set compiled by McKone and Ryan (1989) which includes data from 8 different studies (mean $\pm$ SD): $I_{\text{pasture}} = 16.9 \pm 3.7 \text{ kg dw d}^{-1}$ and $I_{\text{soil}} = 0.41 \pm 0.24 \text{ kg dw d}^{-1}$ (equal to 2.4 wt% of total herbage being soil). The dashed red lines indicate the lowest and highest soil intake as percentage of total herbage intake reported in McKone and Ryan (1989), which are 0.6 wt% ($I_{\text{pasture}} = 16 \text{ kg dw d}^{-1}$ and $I_{\text{soil}} = 0.1 \text{ kg dw d}^{-1}$) and 4.2 wt% ($I_{\text{pasture}} = 16.5 \text{ kg dw d}^{-1}$ and $I_{\text{soil}} = 0.72 \text{ kg dw d}^{-1}$), respectively.

4. Conclusions

In agreement with earlier reports, strong As contamination gradients were observed in the Ogosta river floodplain. Analysis of plant samples revealed that these contamination gradients were reflected in the herbaceous vegetation. Considering that the geochemical conditions of the contaminated floodplain soils along the Ogosta River are similar to the studied transect, elevated As contents in the vegetation are to be expected at other strongly contaminated sites. The translocation of As to the shoot of *T. repens*, *H. lanatus*, and *A. spica-venti* was restricted and calculated soil-to-shoot transfer factors were below 0.07, apart from few exceptions. A comparison with studies from the literature revealed that the observed soil-to-shoot transfer factors for *T. repens* and *H. lanatus* were comparably low. Corresponding to the low As transfer factors, no indication for interference of As and P uptake was identified based on the element concentrations in the plant shoots despite the high As:P ratios in the easily extractable fractions (Olsen extraction).

Large areas of the floodplains along the Ogosta River are used as pastureland. Based on rough estimations, we suggest that for livestock grazing on similar contaminated pastures, As intake via soil ingestion might be even more important than the intake via pasture herbage. Our results indicate that the identification of highly contaminated areas is prerequisite for implementing effective usage restriction to minimize the entrance of As into the food chain, both via uptake by plants and involuntary soil ingestion.

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