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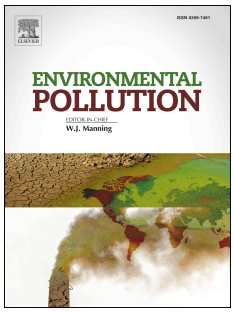

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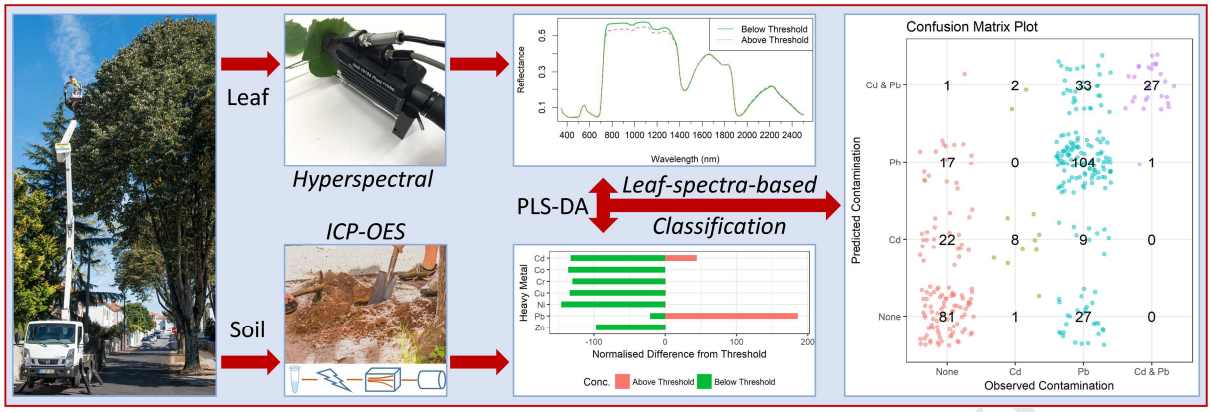

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Vegetation reflectance spectroscopy for biomonitoring of heavy metal pollution in urban soils

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Abstract

Heavy metals in urban soils may impose a threat to public health and may negatively affect urban tree viability. Vegetation spectroscopy techniques applied to bio-indicators bring new opportunities to characterize heavy metal contamination, without being constrained by laborious soil sampling and lab-based sample processing. Here we used *Tilia tomentosa* trees, sampled across three European cities, as bio-indicators i) to investigate the impacts of elevated concentrations of cadmium (Cd) and lead (Pb) on leaf mass per area (LMA), total chlorophyll content (Chl), chlorophyll *a* to *b* ratio (Chla:Chlb) and the maximal PSII photochemical efficiency (Fv/Fm); and ii) to evaluate the feasibility of detecting Cd and Pb contamination using leaf reflectance spectra. For the latter, we used a partial-least-squares discriminant analysis (PLS-DA) to train spectral-based models for the classification of Cd and/or Pb contamination. We show that elevated soil Pb concentrations induced a significant decrease in the LMA and Chl*a*:Chl*b*, with no decrease in Chl. We did not observe pronounced reductions of Fv/Fm due to Cd and Pb contamination. Elevated Cd and Pb concentrations induced contrasting spectral changes in the red-edge (690~740 nm) region, which might be associated with the proportional changes in leaf pigments. PLS-DA models allowed for the classifications of Cd and Pb contamination, with a classification accuracy of 86% (Kappa=0.48) and 83% (Kappa=0.66), respectively. PLS-DA models also allowed for the detection of a collective elevation of soil Cd and Pb, with an accuracy of 66% (Kappa=0.49). This

study demonstrates the potential of using reflectance spectroscopy for biomonitoring of heavy metal contamination in urban soils.

Keywords: soil heavy metal contamination; leaf functional trait; vegetation reflectance spectroscopy; red-edge position; bio-indicator

Capsule

Applying leaf reflectance spectroscopy to urban trees allows for biomonitoring of heavy metal pollution and the classification of pollutants in urban soils.

Introduction

Soil contamination is a widely spread problem across Europe (European Commission, 2006). Among the most frequent soil pollutants are heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), zinc (Zn), antimony (Sb), cobalt (Co) and nickel (Ni), which accumulate on the soil surface and transfer to deeper soil layers where they can infiltrate into the groundwater (Vince et al., 2014). Plants growing on heavy metal polluted soils passively take up heavy metals, jeopardizing their growth and negatively affecting other organisms feeding on the plants (Panagos et al., 2013; Tóth et al., 2016). Furthermore, elevated concentrations of these heavy metals in agricultural or urban soils endanger food safety and public health (Poggio et al., 2009; Tóth et al., 2016).

Urban soils typically contain elevated concentrations of Cd, Cu, Zn and Pb, originating from anthropogenic activities such as traffic and industrial emissions (Gallagher et al., 2008; Li et al., 2001; Poggio et al., 2009; Pourkhabbaz et al., 2010; Vince et al., 2014). Cd and Pb are the most common heavy metals resulting from road traffic, which is attributed to the historical use of Pb as a gasoline additive (Kovarik, 2005) and Cd accumulation which is mainly due to abrasion of tires (Andersson et al., 2010; Vince et al., 2014). Cd and Pb are toxic for plants, animals and humans (Pandit et al., 2010; Poggio et al., 2009). Cd accumulates in human body and can cause nephropathy, pulmonary lesions and lung cancer after long period of exposure (Poggio et al., 2009). Pb increases blood pressure and

damages liver, kidney and fertility, and most severely it reduces brain functioning and induces hyperactivity and hearing loss in children (Poggio et al., 2009). Therefore, it is vital to detect elevated concentrations of Cd and Pb in urban soils.

Measuring heavy metals is typically based on the collection of soil or road dust samples, which is labor intensive and costly, especially when monitoring heavy metal contamination at larger spatial scales (Wei and Yang, 2010). In European countries, the estimated total annual cost related to monitoring and remediating soil contaminants is 17.3 billion euros (European Commission, 2006), and around 81% of the expenditures is spent on remediation measures (Liedekerke et al., 2014). Consequently, only up to 15% is available to be spent on site investigations (Liedekerke et al., 2014), implying that there is a need for more cost-effective investigation methods to evaluate spatial and temporal heterogeneity of soil pollution. Soil near-infrared (NIR) spectroscopy has been applied for the detection of heavy metals at relatively low cost. However, this method requires intensive soil sampling (Pandit et al., 2010; Shi et al., 2014). Therefore, a spatially explicit characterization of heavy metal contamination at large scales is constrained by the capacity of sampling and sample processing, especially in urban areas characterized by sealed soil surfaces and highly heterogeneous land-use types.

Bio-indicators are living organisms that can be used to assess the quality of the environment (Holt and Miller, 2010; Parmar et al., 2016). Urban vegetation can be used as bio-indicators for monitoring air and soil

pollution (Ho, 1990; Khavanin Zadeh et al., 2013; Sawidis et al., 2011). Plants concentrate metal elements in their above ground parts, which are indicative of elevated soil heavy metal concentrations. Furthermore, heavy metals can inhibit plant growth (Giulia et al., 2013; Horler et al., 1980), and decrease chlorophyll content and biomass productivity (Gallagher et al., 2008; Manios et al., 2003). Cd and Pb often limit plant growth by altering leaf internal structures (Giulia et al., 2013; Pourkhabbaz et al., 2010). For instance, Cd can reduce cell wall extensibility and relative water content (Barceló and Poschenrieder, 1990). Pb can reduce not only the leaf expansion but also the total chlorophyll content and efficiency of PSII electron transport (Kastori et al., 1998). Overall, heavy metal toxicity causes multiple direct and indirect effects on various physiological functions and on the morphology of plants (Barceló and Poschenrieder, 1990), reflected in changes of leaf functional traits.

Metal induced morphological and physiological changes can further alter vegetation absorbance and reflectance characteristics (Horler et al., 1980). Typically, heavy metal contamination induces most notable changes in the visible and NIR spectral regions, and thus reflectance spectroscopy holds great promise for evaluating the impact of heavy metal contamination on vegetation (Clevers et al., 2004; Kooistra et al., 2004, 2003; Rosso et al., 2005). By applying reflectance spectroscopy to monitoring candidate bio-indicators located at multiple sites in urban areas, researchers have been able to detect polluted sites (Khavanin Zadeh et al., 2013). Previous studies have investigated the effect of individual metals on vegetation spectral

responses, e.g., canopy reflectance in response to manipulated pot-soil Cd changes (Rosso et al., 2005). However, different metals may induce similar or contrasting spectral responses (Amer et al., 2017; Horler et al., 1980; Manios et al., 2003). Some studies have focused on spectral response in specific spectral bands such as the red-edge region (690~740 nm), which has been used to estimate plant chlorophyll variations under stress due to heavy metals (Clevers et al., 2004; Rosso et al., 2005). The red-edge position (REP) is defined as the position generating the maximum slope (inflection point) of the reflectance spectra (or maximum first derivative reflectance) in the red-edge region (Clevers et al., 2004; Horler et al., 1983), and has been found to be negatively related to soil Pb concentration (Clevers et al., 2004; Kooistra et al., 2004). Overall, associating soil heavy metal pollution with a range of plant functional and reflectance characteristics provides a cost-effective method for assessing heavy metal pollution. However, there is still a lack of vegetation reflectance spectroscopy studies that bio-monitor Cd and Pb contamination across a variety of urban environments, especially for monitoring contamination due to multiple metals.

Here we tested *Tilia tomentosa* as a bio-indicator for elevated soil Cd and Pb concentrations. Selecting 187 study trees cross three European cities (Leuven, Porto and Strasbourg), our objectives were: i) to assess the impacts of elevated concentrations of Cd and Pb on leaf mass per area (LMA), total chlorophyll content (Chl), chlorophyll *a* to *b* ratio (Chla:Chlb) and the maximal PSII photochemical efficiency (Fv/Fm); and ii) to investigate the

feasibility of using leaf reflectance spectroscopy and partial-least-squares discriminant analysis for biomonitoring soil Cd and Pb contamination.

Materials and Methods

Sampling of leaf and soil and heavy metal measurements

We conducted soil and leaf sampling in summer 2017 and randomly selected 19 sites and 187 *T. tomentosa* trees across three medium sized cities (Leuven (Belgium): n = 64; Porto (Portugal), n = 67; Strasbourg (France): n = 56). We randomly selected trees for sampling, and the trunk diameter ranged 5-130 cm. For each tree, we sampled the top soils (0–10 cm) at three random locations surrounding the trunk, and the three locations are mixed for metal measurements. We sampled 15 leaves at three random positions in each tree and stored the leaf samples in a cool box with ice. We performed soil sampling once, while leaf sampling was performed multiple times throughout the growing season for a subset of trees in Leuven and Strasbourg.

Heavy metal concentrations in the soil were measured by digesting 50 mg of dried and sieved soil with 7.5 ml concentrated hydrochloric acid and 2.5 ml concentrated nitric acid. The digested solution was diluted to 10 ml and measured with ICP-OES. For quality control of soil metal analysis, an internal soil standard was run parallel with the soil samples, which deviated less than 5% of the known composition. In this study, we focused on Cd and Pb, as these were the heavy metals that reached the toxicity thresholds (Table 1).

149 *Identification of contamination based on soil heavy metal thresholds*

150 Soil heavy metal contamination levels were identified based on published
151 threshold standards (Tóth et al., 2016) released by the Ministry of the
152 Environment, Finland (MEF, 2007). We grouped the samples into two classes
153 - non-contaminated and contaminated, subjected to individual metals (Table
154 1). Soil samples and corresponding leaf spectral observations (section 2.3)
155 were grouped into four classes according to Pb contamination following the
156 MEF standard (MEF, 2007). The four classes included class 0 being non-
157 contaminated (Pb < 60 mg/kg), class 1 of low contamination (60 ⩽ Pb < 200
158 mg/kg), class 2 of medium contamination (200 ⩽ Pb < 750 mg/kg) and class
159 3 of high contamination (Pb ⩾ 750 mg/kg).

160 We also defined four contamination classes subjected to both Cd and
161 Pb contamination by re-grouping of the Cd and Pb binary classes (Table S1),
162 i.e., four CdxPb classes including the non-contaminated (class 0), Cd
163 contaminated only (class 1), Pb contaminated only (class 2) as well as when
164 both Cd and Pb are over the thresholds (class 3).

165 *Leaf reflectance and functional traits*

166 Leaf reflectance was measured using an ASD FieldSpec 3 spectroradiometer
167 (ASD Inc., Longmont, CO, USA) connected to a Plant Probe and Leaf Clip
168 Assembly (ASD Inc., Longmont, CO, USA). It allows for reflectance
169 measurement in a spectral range of 350 – 2500nm with a band width of 1
170 nm. Next, we measured the leaf maximal PSII photochemical efficiency
171 (Fv/Fm, ratio of the variable fluorescence to the maximal fluorescence) using

a chlorophyll fluorescence meter (Handy PEA, Hansatech Instruments Ltd., Pentney, UK), combined with a leaf clip that allows for dark adaption (25 min). Then, we measured the leaf area using a flatbed scanner, followed by oven dry for 3 days, allowing to determine leaf mass per area (LMA). In total, aggregated per tree and sampling time, collected leave samples allowed for further statistical analysis on a sample size of 333 for reflectance and functional traits. The 333 observations of reflectance spectra and functional traits were grouped into their contamination classes subjected to the soil heavy metal contamination classes as defined in the Section 2.2.

A random subset of the leaf samples (n=53) were used to determine the total chlorophyll (Chl) and carotenoid (Car) content. Leaf round discs with a diameter of 28.6 mm were punched from the leaf samples using a paper punch. Chla, Chlb and Car were extracted with a mortar and pestle in 80% acetone and their concentrations determined by measuring the solution absorbance (A) at wavelengths 470, 646.8 and 663.2 nm using a UV-VIS spectrophotometer (Shimadzu 1650 PC, Kyoto, Japan) according to Eqs. (1-3) (Lichtenthaler, 1987).

$$Chla = 12.25 * A_{663.2} - 2.79 * A_{646.8} \#(1)$$

$$Chlb = 21.50 * A_{646.8} - 5.10 * A_{663.2} \#(2)$$

$$Car = \frac{1000 * A_{470} - 1.82 * Chla - 85.02 * Chlb}{198} \#(3)$$

For quality control of chlorophyll analysis, we performed parallel measurements in 12 samples, and the average standard error was lower than 5%.

Spectral and statistical analysis

To highlight the metal-induced spectral variations, we calculated the reflectance relative differences between group means for the contaminated and non-contaminated classes subjected to Cd and Pb contamination. We also applied first derivatives to the reflectance, focusing mainly on the red-edge region, to derive the red-edge inflection point (REIP) and evaluate the metal induced red-edge shifts (Clevers et al., 2004).

Partial least squares (PLS) regression is a multivariate method for relating two data matrices, X and Y, i.e., explanatory and response matrices, by extracting latent variables (components) to model the variations of both matrices (Wold et al., 2001). The PLS regression can reduce high dimensional data (e.g. hyperspectral) to a small number of latent variables which serve as new predictors on which the response variable is regressed (Rosipal and Krämer, 2006). Partial least squares discriminant analysis (PLS-DA) is a variant used when the response variable is categorical. We used PLS-DA for the classification of metal contamination classes. PLS-DA models were applied to four types of data, (i) the original reflectance spectral, and three pre-processed spectral data including (i) first derivative (ii), standard normal variate SNV and (iii) continuum removal (CR) precede applying the PLS-DA models. PLS-DA model calibration was first initiated on the entire dataset for the full spectrum with 10 components. The initial model was

trained using a 10-fold cross-validation with 99 times of permutations, allowing for determination of the optimal number of components and the spectral bands yielding a variable importance in projection (VIP) $\geqslant$ 0.8.

For an independent validation, the entire dataset was randomly split into the training and test subsets, with a sample size being 2/3 (n=215) and 1/3 (n=118) of the total observations (n=333), respectively. The VIP $\geqslant$ 0.8 spectral bands were then used to train and test models on the two subsets, respectively.

PLS-DA Model classification accuracy was evaluated using the overall accuracy (Eq. 4) and kappa coefficient (Eq. 5), as well as for assessing the classification for individual classes using the producer's (Eq. 7) and user's accuracies (Eq. 8),

$$Accuracy = (TP + TN)/(TP + TN + FP + FN) \#(4)$$

$$Kappa = \frac{p_a - p_e}{1 - p_e} \#(5)$$

$$p_e = \frac{(TN + FP) \times (TN + FN) + (FN + TP) \times (FP + TP)}{(TP + TN + FP + FN)^2} \#(6)$$

$$Producer\ Accuracy = TP/(TP + FP) \#(7)$$

$$User\ Accuracy = TP/(TP + FN) \#(8)$$

where the letters *T* and *F* denote *true* and *false*, respectively, and *P* and *N* denote *positive* and *negative*, respectively, p_a is the actual agreement (identical to accuracy), whereas p_e is the expected agreement by chance (random accuracy) that can be calculated as Eq. (6).

We used linear mixed models to test whether elevated soil heavy metals affect the leaf functional traits. We defined the metal contamination classes, i.e., binary or multi-class, as the fixed effect factor and defined city and sampling site as random effect factors in the mixed models. All analyses were performed in the R programming environment (R Core Team, 2016). The R package 'lme4' (Bates et al., 2015) was used for running the mixed models, and the package 'lsmeans' (Lenth, 2016) was used for post-hoc analysis of pairwise comparisons between the contaminated classes based on Tukey's test. PLS-DA was implemented using the package 'mixOmics' (Rohart et al., 2017).

Results and Discussion

Heavy metal effects on leaf functional traits

Elevated Pb and Cd concentrations had a significant effect on LMA of *T. tomentosa* trees (Table 2). Soil Cd contamination did not induce significant changes in LMA (Fig. 1a), whereas Pb contamination significantly decreased LMA (Fig. 1b). Generally, Cd and Pb stress leads to damages to chloroplasts and thylakoid membranes in plants (Shen et al., 2016; Wu et al., 2014), which often causes reduced leaf growth such as small leaf size and small stomata (Shi and Cai, 2009), as well as thin cuticles of leaf surfaces (Pourkhabbaz et al., 2010). Therefore, elevated Pb concentrations could have reduced leaf thickness and thus decreased LMA. Cd also induces changes in leaf structural properties, while Cd concentrations measured in this study might still be below the threshold that induces significant inhibition of leaf expansion.

253 Elevated soil Pb induced significant changes in leaf total Chl content,
254 Chl*a* to Chl*b* ratio (Chla:Chlb) and Fv/Fm, whereas Cd and other metals did
255 not yield significant changes (Table 2). Decrease in leaf Chl content is often
256 associated with photoinhibition and reduction of the photosynthetic capacity
257 (Shen et al., 2016). Chla:Chlb decreased significantly along with the increase
258 in soil Pb concentration (Fig. 2), suggesting that Chla was more suppressed
259 compared to Chlb (Nie et al., 2016). Similarly, a significant reduction of
260 Chla:Chlb has been found in *Torreya grandis* (Shen et al., 2016) and *Typha*
261 *latifolia* plants (Manios et al., 2003) treated with a high concentration of Cd
262 and Pb, suggesting increases in chlorophyll hydrolysis due to the toxic effect
263 (Manios et al., 2003). Results may differ for different plant species, for
264 instance in a greenhouse environment, Horler et al. (1980) observed a
265 significant decrease of Chla:Chlb in pea leaves due to elevated Cd
266 concentrations, but no changes following elevated Pb (Horler et al., 1980).

267 Cd and Pb contamination induced a decrease in Fv/Fm (Fig. 3a, b),
268 whereas Fv/Fm appeared to be not sensitive to low-level Pb contamination
269 (Fig. 3d), suggesting that Cd and Pb stress may induce photosynthesis
270 inhibition. Similarly, Cd was found to affect Fv/Fm in the wetland plant
271 species *Salicornia virginica* (Rosso et al., 2005) and in the turf grass species
272 *Festuca arundinacea Schreb* (Huang et al., 2017). Generally, the observed
273 decrease in Fv/Fm in plants subjected to Cd/Pb stress is associated with the
274 photoinhibition of PSII, as a result of the overproduction of reactive oxygen
275 species (ROS) (Huang et al., 2017; Shen et al., 2016). However, a significant
276 decrease in Fv/Fm may not always be observable if Cd/Pb concentration does

not exceed a high threshold (Huang et al., 2017; Shen et al., 2016). Giulia et al. (2013) found that a high soil Pb concentration did not decrease Fv/Fm in *Q. ilex* plants, and they argued that these metals may not significantly alter functionality of the photosynthetic apparatus. Similarly, Shi and Cai (2009) reported that Fv/Fm was not affected in peanut plants treated with a high concentration of Cd. Therefore, the effect of heavy metals on Fv/Fm might depend largely on metal type, concentration and plant species.

Mixed models for multi-class CdxPb and Pb contamination showed much more pronounced effects on LMA and Chla:Chlb than on Fv/Fm and leaf total Chl content (Table 3), which suggests that heavy metals induced more structural changes and proportional changes in leaf biochemicals than the quantity changes of individual components. An increase in leaf total Chl content and Fv/Fm was observed at a relative low-level Pb or Cd×Pb contamination (Table 3), suggesting that heavy metals impose complicated effects on photosynthesis and that Cd and Pb may increase the PSII quantum yield within a certain range of low concentrations (Ouyang et al., 2012; Shen et al., 2016).

The effect of soil heavy metals on leaves or the content of heavy metal accumulation in the leaves might be related to the age of trees (Doganlar et al., 2012). To test whether tree age difference affect the observed effects of Cd and Pb on leaf functional traits in this study, we used trunk diameter as a proxy of tree age and added it as an additional random factor in the mixed models (Table S2 and Table S3). Results suggest that the observed effects of

300 Cd and Pb on T. *tomentosa* leaves was not significantly influenced by tree
301 age.

302 *Reflectance and first derivatives in response to heavy metals*
303 Elevated soil Cd concentrations yielded relatively large variations in leaf
304 reflectance centered at the 500, 680 and 720 nm bands (Fig. 4a), whereas
305 elevated Pb yielded large variations at the 550 and 700 nm bands (Fig. 4b).
306 In the red-edge region, Cd had a large effect on reflectance at the red-edge
307 center (~720 nm), whereas Pb had a large effect on reflectance ranging from
308 the red absorption to the beginning of the red-edge bands (680~700 nm).
309 Over the full spectrum, soil Pb contamination induced larger variations
310 (±10%, Fig. 4b) compared to Cd contamination (±5%) (Fig. 4a), which
311 might be attributed to the fact that Pb contamination was severer than Cd in
312 this study. Cd concentration was slightly higher than the threshold (1 mg/kg),
313 but was much lower than the 'low guideline' of contamination level (10
314 mg/kg) at which ecological or health risks present (Tóth et al., 2016).

315 The decrease in the NIR region (750~1400 nm) was associated with
316 elevated Cd and Pb concentrations. This might be attributed partly to the
317 decreased LMA because contaminated trees often have a much thinner outer
318 epidermal layer and thus thinner leaves (Pourkhabbaz et al., 2010), although
319 the effect of Cd on LMA observed in this study was marginal (Fig. 1). Metal-
320 induced decreases in leaf NIR reflectance might be associated mainly with
321 the changes in leaf internal structural properties which decrease the internal

light scattering and increase the transmittance of leaves (Horler et al., 1980; Kumar et al., 2001).

The first derivative reflectance in the visible-to-NIR bands showed two major peaks centered at 530 and 720 nm (Fig. S1). In the red-edge spectral region, Cd contamination induced a shift of absorbance features towards the shorter wavelengths (Fig. S1a). In contrast, Pb contamination induced a red-edge shift to the longer wavelengths (Fig. S1b). In addition to the red-edge bands, Pb contamination also yielded large variations in the first derivative reflectance at the green bands, suggesting a more pronounced change of the overall shape of reflectance (cf. Fig. 4). As shown in the first derivative reflectance, Pb contamination also induced a shift in the green edges (both sides of the green peak) compared to Cd contamination. This might explain the observed decrease in the Chla:Chlb ratio (Fig. 2), since absorption at the green edge bands is related to Chlb variations (Kumar et al., 2001).

The extracted REIP showed contrasting changes in the Cd and Pb contaminated trees, with decreasing and increasing trends, respectively (Fig. S2), which confirms the contrasting effects of Cd and Pb contamination on the red-edge reflectance. Heavy-metal induced REIP changes, or red-edge shifts, have been found to depend to some degree on plant species and sampling sites (Kooistra et al., 2004). Normally, a decreased REIP can be observed when plant stress induces a reduction in leaf total Chl content (Horler et al., 1983). However, here we did not observe obvious Chl reduction associated with Cd or Pb contamination. Therefore, the REIP

variations observed here were more likely associated with the proportional changes in the Chla:Chlb ratio, in combination with changes in leaf structures.

PLS-DA model calibration for binary and multi-class classifications

In the binary classifications, the PLS-DA calibration models for Cd-contamination classification yielded a total accuracy of 84.1~86.5% (kappa = 0.46~0.49, Table S4). PLS-DA models for Pb contamination yielded a total accuracy of 72.7~77.8% (kappa = 0.46~0.57). For the multi-class classification of CdxPb-mixed contamination, PLS-DA models yielded a total accuracy of 43.2~66.1% (kappa = 0.24~0.49, Table S4). PLS-DA models for the multi-class classification of Pb yielded a total accuracy of 52.0~64.0% (kappa = 0.29~0.43). The best classifications for individual metals are illustrated in confusion-matrix plots (Fig. 5).

The best model for Cd correctly classified the Cd class 0 with a producer and use accuracy of 86% and 97%, respectively, and were 77% and 43% for the Cd class 1 (Fig. 5a). The producer and use accuracy for the Pb class 0 were 88% and 67%, respectively, and 77% and 90% for the Pb class 1 (Fig. 5b). The best model for Cd×Pb yielded a relatively low user accuracy in predicting the classes 1 and 3 (Fig. 5c), which however, accounts for a very small proportion of the total observations. The best model for multi-class Pb contamination yielded a relatively high producer accuracy for the classes 0 and 3 (Fig. 5d), with 80% and 100%, respectively. In contrast, the model yielded a higher user accuracy for the classes 0 and 1 than for the classes 2 and 3. The low user accuracy for the Pb classes 2 and 3 was mainly

368 due to the small sample size of high Pb concentrations, which consists of only
369 17 and 4 observations for the classes 2 and 3, respectively.

370 Overall, the high producer accuracy, paired with relatively low user
371 accuracy for a relatively high metal concentration was rather encouraging,
372 since our models slightly tended to overestimate the observed contamination
373 rather than underestimate the elevated contamination. This implies a high
374 probability of detecting the elevated concentrations of soil heavy metals.

375 *PLS-DA model validation using full spectrum and VIP-bands*
376 Compared to model calibration accuracies, model validation based on the full
377 spectrum produced comparable accuracies (Table S5). In binary
378 classifications, models for Pb contamination yielded higher kappa coefficients
379 than the models for Cd contamination. In multi-class classifications, model
380 validation showed improved total accuracies and kappa coefficients (Table
381 S5), suggesting the potential of using calibrated PLS-DA models for detecting
382 elevated soil Cd and Pb concentrations.

383 Validation of models trained with the VIP ($\geqslant$ 0.8) bands showed
384 slightly improved kappa values and total accuracies compared to the full use
385 of bands (Table S5). The importance of individual spectral bands in the
386 classification is indicated by the VIP scores for individual metals (Fig. 6). Cd
387 contamination yielded relatively high VIP scores at the red-edge (730 nm)
388 and SWIR bands (1300 nm, 1650 nm) compared to Pb contamination,
389 suggesting unique spectral responses to elevated soil Cd in these bands (Fig.
390 6). Pb contamination yielded higher VIP scores at the green (530 nm) and

391 the beginning of red-edge (700 nm), suggesting that Pb contamination
392 induced more pronounced responses in the visible bands. For the binary
393 classifications, VIP-based PLS-DA models yielded higher accuracies for Pb-
394 contamination classification (kappa = 0.66) than for Cd (kappa= 0.39, Table
395 S5). For multi-class classifications, the VIP-based PLS-DA models yielded
396 comparable accuracies by using a much less amount of bands compared to
397 the use of full spectral bands.

398 Model validation results showed that selecting a set of influential bands
399 (VIP $\geqslant$ 0.8) allowed for maintaining classification accuracy and improving
400 model-use and computational efficiencies. Within a limited number of
401 observations, by randomly dividing independent training and testing subsets
402 of observations, our results suggest that spectrally calibrated PLS-DA models
403 have great potential of applying to future scenarios for monitoring heavy
404 metals.

405 *Comparison between reflectance pre-processing methods*
406 The kappa coefficient is a balanced measure compared to the use of the
407 producer-, user- and total accuracies, especially when the observations in
408 difference classes are highly imbalanced such as in this study. Hence, we
409 evaluated the three spectra-preprocessing methods according to the kappa
410 values. Model calibration and validation both showed that the first derivatives
411 yielded the highest kappa values compared to the use of the original and
412 SNV reflectance data (Table S4 and Table S5).

Using a different number of components might induce some degree of variation in model accuracies, although we used the cross-validation (CV) procedure. In addition to the CV-optimized number of components, model calibration and validation were repeated by using a fixed number of components (Table S6, Table S7 and Table S8). Results showed that the first derivative reflectance yielded the highest kappa coefficients, followed by the CR reflectance and the original reflectance (Fig. S3). The SNV reflectance did not yield improvement compared to the original reflectance data, suggesting that the SNV process may mask subtle spectral responses subjected to individual metals. Overall, PLS-DA models based on the first derivative reflectance produced the best classifications, which also suggests that heavy metals have induced complicated effects on leaf biochemical and structural properties that lead to light absorption changes/shifts over the full spectrum.

First derivative spectra of leaves have been proven to be effective in eliminating background signals and for resolving overlapping spectral features (Demetriades-Shah et al., 1990), which is useful to detect plant stresses or estimate pigment changes (Rundquist et al., 1996; Smith et al., 2004). Also, first derivative reflectance has better discrimination power compared to the original reflectance by characterizing the rate of change of reflectance with respect to wavelengths (Bao et al., 2013; Lassalle et al., 2018; Smith et al., 2004). Typically, derivative analysis may facilitate the detection of changes that might be masked in the original spectra by the presence of plant intrinsic co-variations (Horler et al., 1983). For instance, derivative spectra in the visible region may enable to detect subtle changes

in leaf pigment balance associated with physiological disorders or vegetation types (Bandaru et al., 2016; Demetriades-Shah et al., 1990; Pu, 2011).

Derivative analysis can be particularly useful for remotely biomonitoring heavy metal using reflectance spectra measured from above the vegetation canopy (Wang et al., 2018). Canopy spectra first derivatives eliminate the additive noises (baseline shifts) induced by illumination instability, canopy structural or soil background influences (Demetriades-Shah et al., 1990; Gnyp et al., 2014; Kochubey and Kazantsev, 2012; Pu, 2011), thereby improving the accuracy for quantification of canopy biochemical or physiological changes (Jin and Wang, 2016; O'Connell et al., 2014). Moreover, PLS modeling further facilitates the use of features of the full derivative spectrum for the characterization of vegetation undergoing changes or stresses.

Apparently, PLS-DA models for Pb-contamination classifications exclusively produced higher kappa values than for Cd contamination classifications, across different cases of spectra-preprocessing methods, model calibration (Fig. S3a) and validation (Fig. S3b), as well as when using a subset of VIP-bands (Fig. S3c). This can be attributed to the data imbalance between the Cd- and Pb-contamination levels, which, however, shows a great promise of the proposed approach for spectroscopic detection of elevated soil heavy metals, given that a diverse set of observations are used for model calibration.

Conclusions

This study used *T. Tomentosa* trees growing in three European cities as bio-indicators of soil heavy metal contamination, and evaluated whether tree spectra responses were able to reflect the elevated metal concentrations. Results showed that elevated soil Cd and Pb concentrations led to decrease in the leaf mass per area (LMA) and the chlorophyll *a* to *b* ratio (Chla:Chlb), while no significant reduction in leaf total chlorophyll (Chl) and the maximal PSII photochemical efficiency (Fv/Fm). Soil Pb contamination was severer and showed more pronounced effect on LMA, Fv/Fm, Chl and Chla:Chlb than did the Cd contamination in the studied sites.

Cd and Pb contamination induced specific changes in leaf reflectance and the reflectance first derivatives, particularly in the red-edge spectral region. Partial least squares discriminant analysis (PLS-DA) models calibrated using leaf reflectance showed promise for detecting soil Cd and Pb contamination in urban areas. PLS-DA models based on reflectance first derivatives allowed for the best classification of Cd and Pb contamination. This study shows that elevated soil heavy metals can be monitored by measuring leaf spectra of trees. This holds great potential for mapping urban heavy metal contamination by measuring urban vegetation using high-resolution spectrometers onboard airborne or drone platforms. Future work should investigate whether our findings can be extrapolated to broader scales by using canopy level reflectance data and a diverse set of plant species as bio-indicators. Multi-temporal investigations of the quantitative relationships between the practical content of heavy metals in leaves and reflectance

spectroscopic measures are also needed to understand metal translocation from soil to vegetation and for dynamic biomonitoring of heavy metal contamination.

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Declarations of interest

None.

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Figure Captions

Fig. 1. Boxplots with the leaf mass per area (LMA) differences between the binary classes (0 = non-contaminated, 1 = contaminated) of (a) Cd and (b) Pb contamination, as well as among multiple classes of (c) Cd×Pb and (d) Pb contamination. Significance levels are indicated according to the post-hoc Tukey's test of the applied mixed models.

Fig. 2. Boxplots with the leaf chlorophyll a to b ratio (Chla:Chlb) differences between the binary classes (0 = non-contaminated, 1 = contaminated) of (a) Cd and (b) Pb contamination, as well as among multiple classes for (c) Cd×Pb and (d) Pb contamination. Significance levels are indicated according to the post-hoc Tukey's test of the applied mixed models.

Fig. 3. Boxplots show the chlorophyll fluorescence Fv/Fm differences between the binary classes (0 = non-contaminated, 1 = contaminated) of (a) Cd and (b) Pb contamination, as well as among multi-class classifications of (c) Cd×Pb and (d) Pb contamination. Significance levels are indicated according to the post-hoc Tukey's test of the applied mixed models.

Fig. 4. Leaf mean reflectance of the contaminated (1) and non-contaminated (0) trees subjected to (a) Cd and (b) Pb, and their reflectance relative difference ((X1-X0)/X0) between the contaminated and non-contaminated leaves.

Fig. 5. Predicted versus observed classes for (a) Cd binary classification, (b) Pb binary classification, (c) Cd×Pb classification and (d) Pb multi-class classification. Here the first derivative reflectance data were used for (a), (b) and (c), the original reflectance were used for (d). Numbers indicate the confusion matrix of classification.

Fig. 6. The variable importance in projection (VIP) scores for the spectral-based PLS-DA models for binary classification for Cd and Pb contamination, and for multi-class classification of Pb and CdxPb contamination. VIP $\geqslant$ 0.8 highlights the spectral bands contributing significantly to the PLS-DA models.

Tables

Table 1. Measured soil heavy metal content and the threshold values for classification of contamination. Cd and Pb were the major contaminates in this study, and Pb was the only metal that reached the highline and thus Pb contamination was classified into three sub-classes.

Metal	Range (mg/kg)	Threshold (mg/kg)	Number of observations (n)			
			Class 0	Class 1/Pb 1	Pb 2	Pb 3
Cd	0-3.9	1	294	**39**		
Pb*	0-2170.8	60	132	**201/**180	17	4
Co	0-15.9	20	333	0		
Cr	0-120.9	100	327	6		
Cu	0-159.1	100	330	3		
Ni	0-76.8	50	331	2		
Zn	10-265.8	200	329	4		

*, Pb contamination sub-levels:
1) Low contamination (60 ≤ Pb < 200 mg/kg);
2) Medium contamination (200 ≤ Pb < 750 mg/kg);
3) High contamination (Pb ≥ 750 mg/kg).

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Table 2. Results of mixed models for testing the effect of soil heavy metals on leaf functional traits, including the leaf mass per area (LMA), Fv/Fm, total chlorophyll content (Chl) and Chla:Chlb ratio. Modeled random effects were city and sites. Chlorophyll data were only available for a subset of the samples, where only Cd and Pb reached the thresholds of contamination.

		Mixed Model		Tukey's Test Class 0 – 1	
Trait	Metal	*F*-value	*P*-value	Estimate	*P*-value
LMA	Cd	1.11	0.292	0.249	0.292
	Cr	6.68	**0.010**	-1.319	**0.010**
	Cu	0.16	0.691	0.284	0.691
	Ni	0.23	0.632	0.425	0.632
	Pb	67.08	**<0.001**	1.284	**<0.001**
	Zn	0.70	0.404	0.521	0.404
Fv/Fm	Cd	0.02	0.901	-0.0013	0.901
	Cr	0.01	0.905	0.0027	0.905
	Cu	0.01	0.911	0.0034	0.911
	Ni	0.08	0.772	-0.0109	0.772
	Pb	5.84	**0.016**	-0.0162	**0.016**
	Zn	0.08	0.784	-0.0074	0.784
Chl	Cd	2.31	0.138	18.091	0.138
	Pb	6.78	**0.013**	-9.238	**0.013**
Chla:Chlb	Cd	0.45	0.509	0.181	0.509
	Pb	23.58	**<0.001**	0.331	**<0.001**

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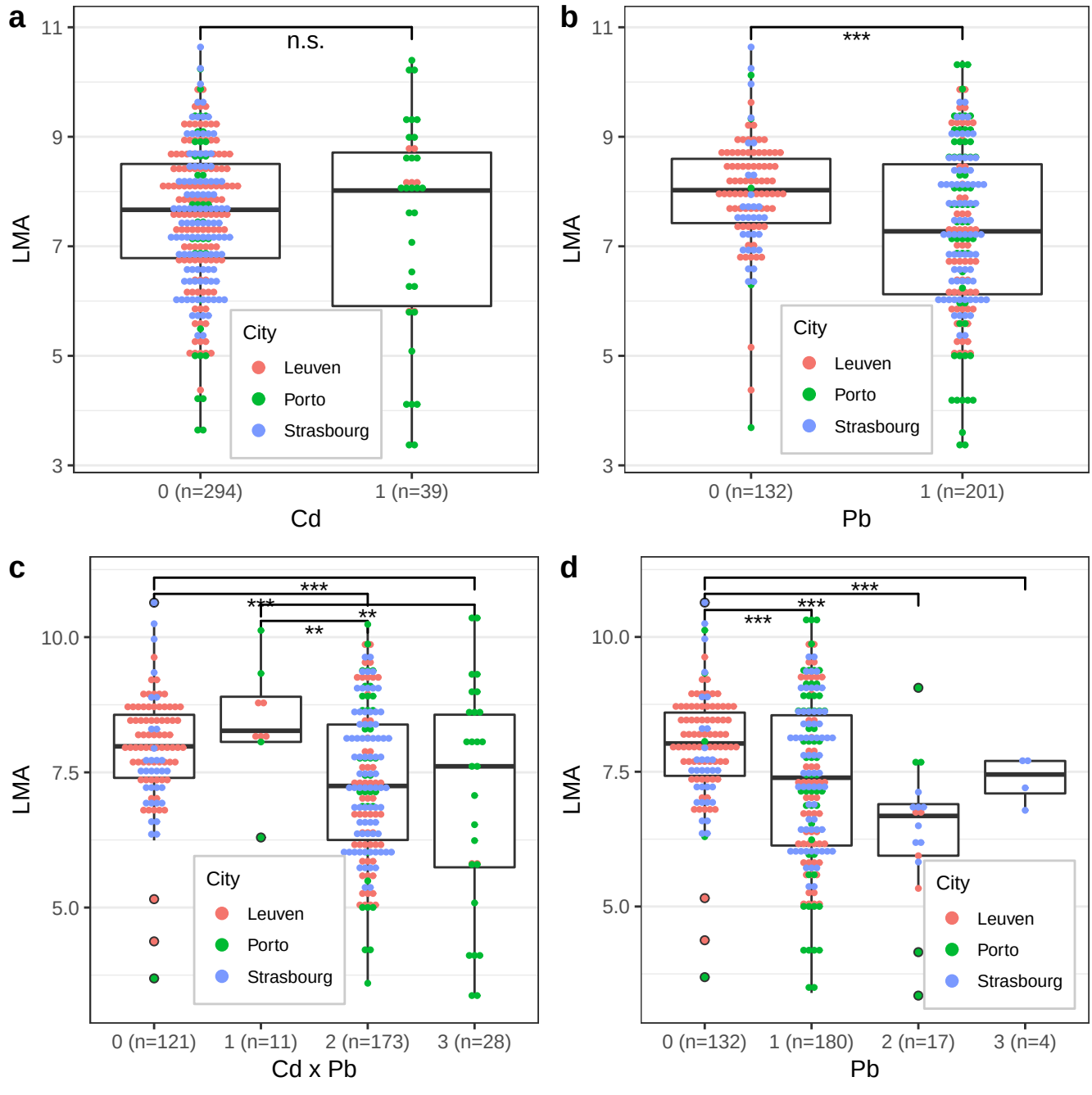
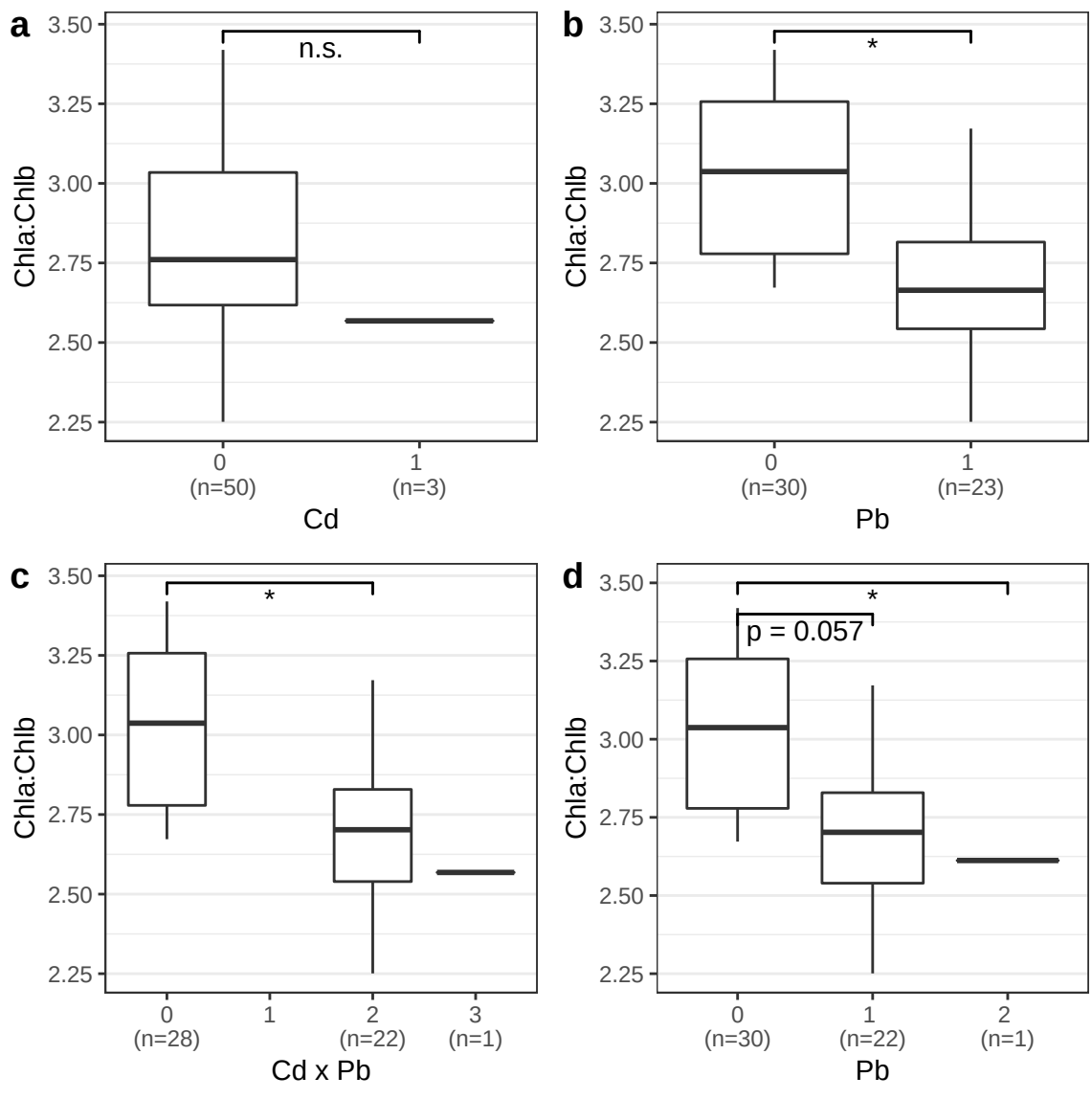
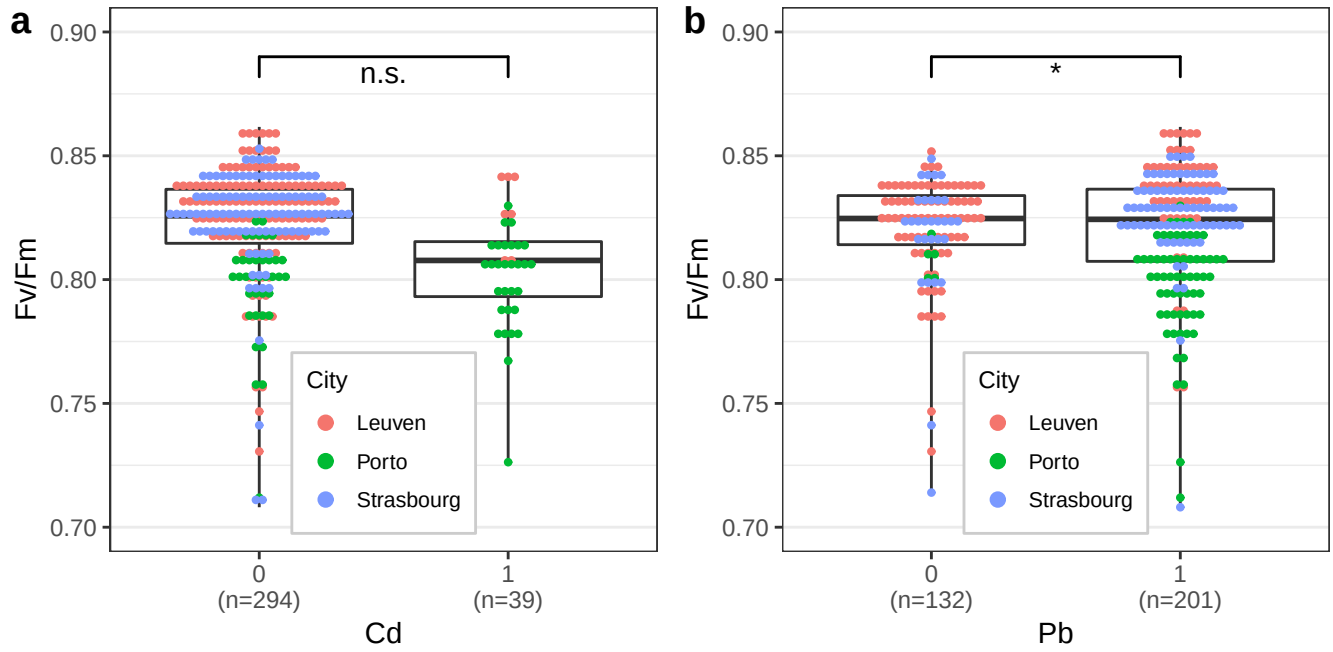
Yu, K., Van Geel, M., Ceulemans, T., Geerts, W., Ramos, M. M., Serafim, C., Sousa, N., Castro, P. M. L., Kastendeuch, P., Najjar, G., Ameglio, T., Ngao, J., Saudreau, M., Honnay, O., Somers, B. (2018). Vegetation reflectance spectroscopy for biomonitoring of heavy metal pollution in urban soils. Environmental Pollution, 243, 1912-1922. . DOI : 10.1016/j.envpol.2018.09.053

Table 3. Mixed models for testing the effect of multi-level Cd×Pb and Pb contamination on leaf functional traits, including the leaf mass per area (LMA), Fv/Fm, total chlorophyll content (Chl) and Chla:Chlb ratio. The modeled random effects are city and site. Chlorophyll data were only available for a subset of the samples, where only Cd and Pb reached the threshold of contamination.

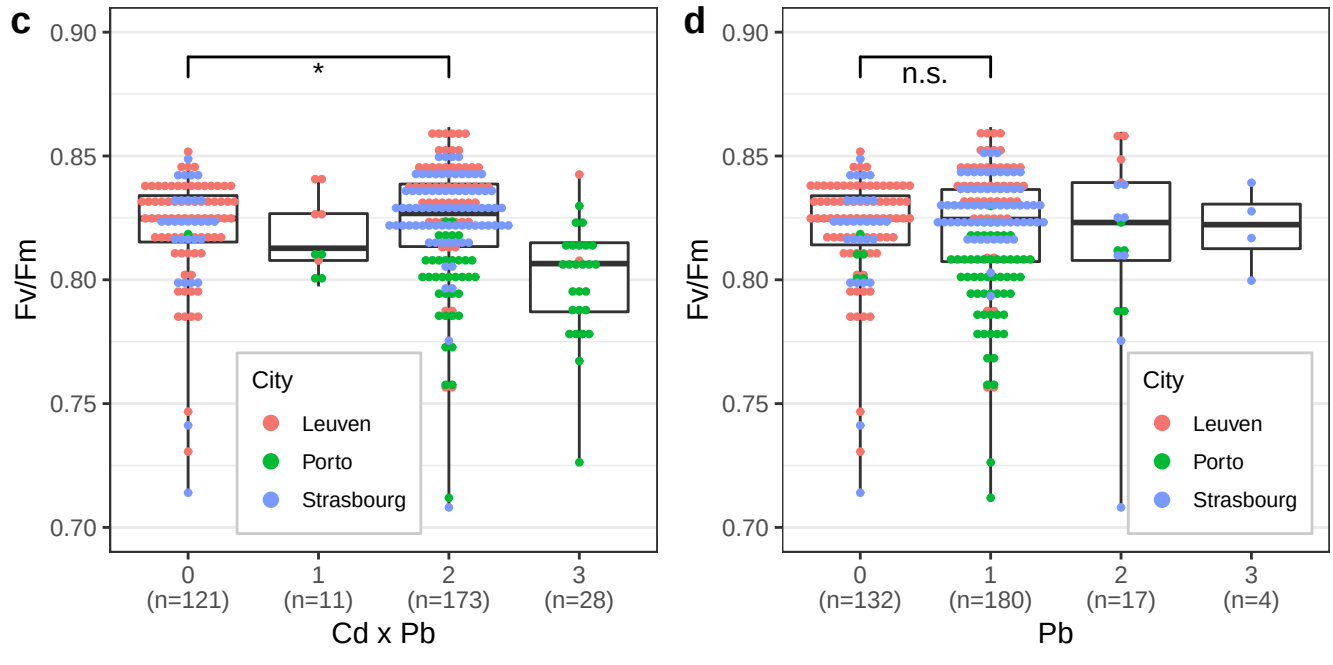
Mixed model				Tukey's Test											
				Class 0 – 1		Class 0 - 2		Class 0 - 3		Class 1 - 2		Class 1 - 3		Class 2 – 3	
Trait	Metal	*F*-value	*P*-value	estimate	*p*	estimate	*p*	estimate	*p*	estimate	*p*	estimate	*p*	estimate	*P*
LMA	Cd×Pb	23.74	**<0.001**	0.025	1.000	1.256	**<0.001**	1.722	**<0.001**	1.231	**0.006**	1.698	**0.001**	0.466	0.246
	Pb	26.29	**<0.001**	1.21	**<0.001**	1.831	**<0.001**	2.444	**<0.001**	0.621	0.104	1.234	0.104	0.613	0.731
Fv/fm	Cd×Pb	2.42	0.066	-0.0211	0.661	-0.0184	**0.044**	-0.0143	0.713	0.0028	0.999	0.0069	0.987	0.0041	0.987
	Pb	2.00	0.113	-0.0168	0.070	-0.0109	0.863	-0.0106	0.980	0.0059	0.971	0.0062	0.996	0.0003	1.000
Chl	Cd×Pb	5.86	**0.006**			-10.275	**0.014**	12.558	0.495					22.834	0.108
	Pb	6.40	**0.004**	-8.108	0.057	-32.96	**0.012**			-24.852	0.070				
Chla:Chlb	Cd×Pb	11.47	**<0.001**			0.332	**<0.001**	0.319	0.319					-0.012	0.998
	Pb	12.01	**<0.001**	0.323	**<0.001**	0.495	0.072			0.172	0.709				


a
n.s.
LMA
11
9
7
5
3
City
Leuven
Porto
Strasbourg
0 (n=294)
1 (n=39)
Cd
b
***
LMA
11
9
7
5
3
City
Leuven
Porto
Strasbourg
0 (n=132)
1 (n=201)
Pb
c
***
***
**
**
LMA
10.0
7.5
5.0
City
Leuven
Porto
Strasbourg
0 (n=121)
1 (n=11)
2 (n=173)
3 (n=28)
Cd x Pb
d
***
***
***
LMA
10.0
7.5
5.0
City
Leuven
Porto
Strasbourg
0 (n=132)
1 (n=180)
2 (n=17)
3 (n=4)
Pb


a
b
c
d
Chla:Chlb
3.50
3.25
3.00
2.75
2.50
2.25
n.s.
*
p = 0.057
0
(n=50)
1
(n=3)
Cd
0
(n=30)
1
(n=23)
Pb
0
(n=28)
1
2
(n=22)
3
(n=1)
Cd x Pb
0
(n=30)
1
(n=22)
2
(n=1)
Pb

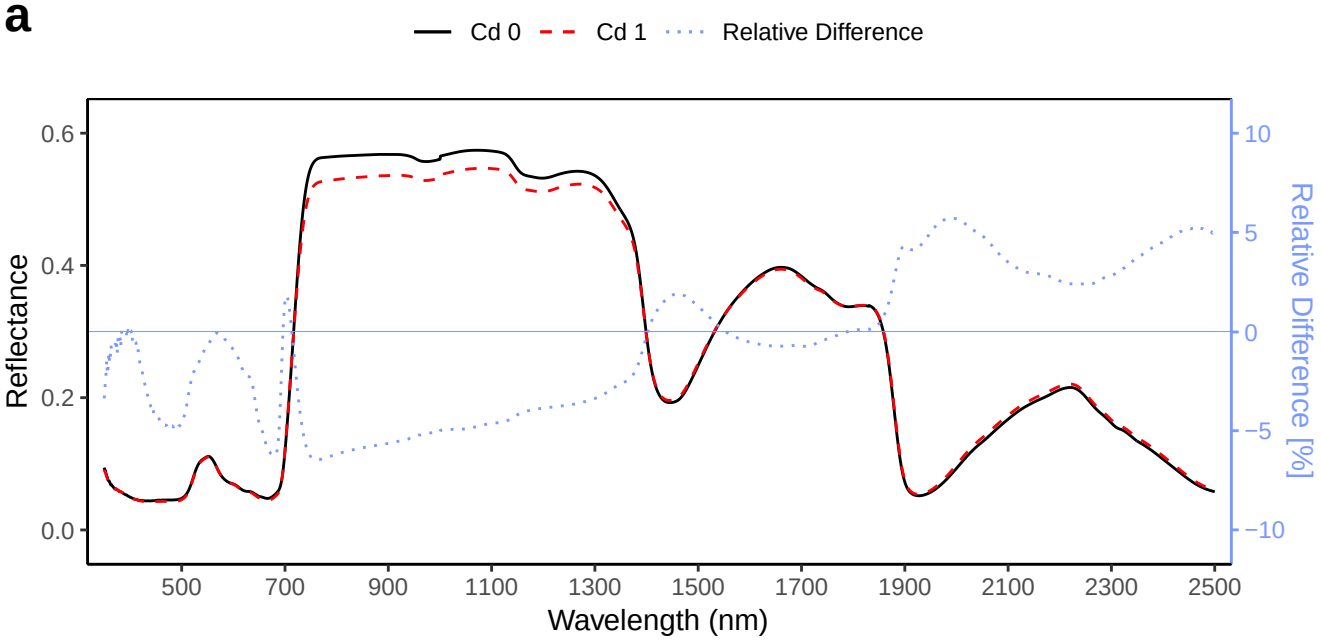

a
0.90
0.85
0.80
0.75
0.70
Fv/Fm
n.s.
City
Leuven
Porto
Strasbourg
0
(n=294)
1
(n=39)
Cd

b
0.90
0.85
0.80
0.75
0.70
Fv/Fm
*
City
Leuven
Porto
Strasbourg
0
(n=132)
1
(n=201)
Pb

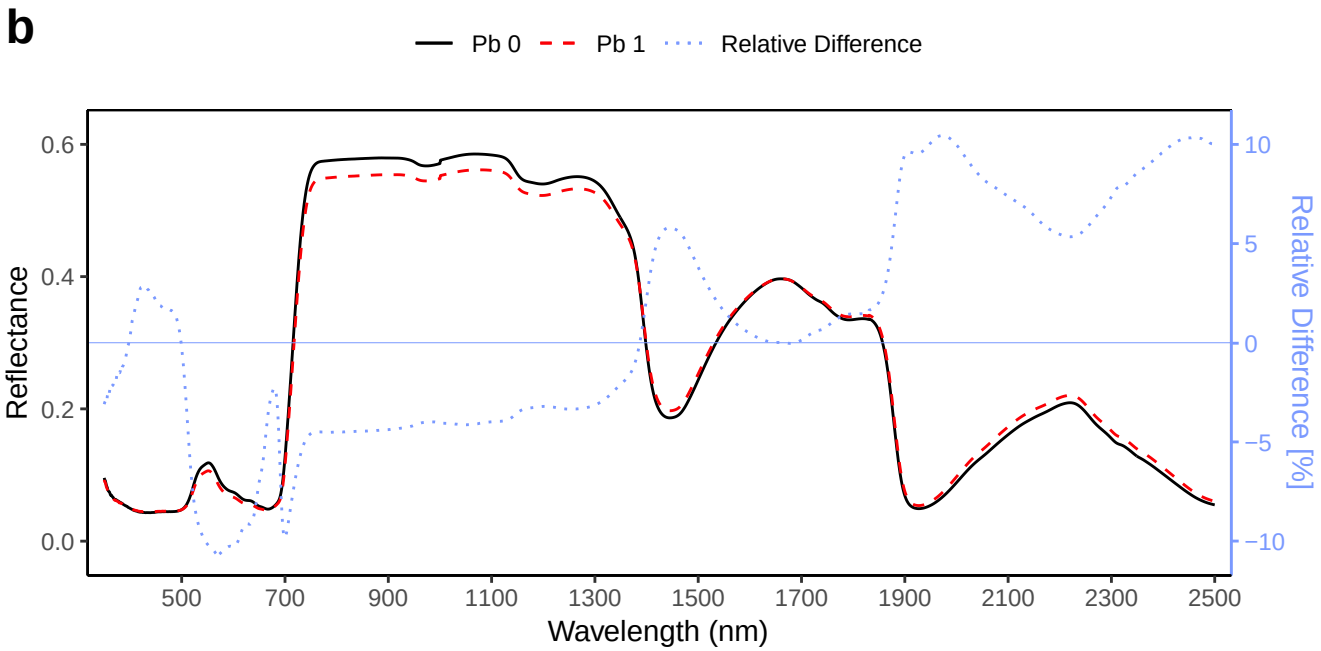

c
0.90
0.85
0.80
0.75
0.70
Fv/Fm
*
City
Leuven
Porto
Strasbourg
0
(n=121)
1
(n=11)
2
(n=173)
3
(n=28)
Cd x Pb

d
0.90
0.85
0.80
0.75
0.70
Fv/Fm
n.s.
City
Leuven
Porto
Strasbourg
0
(n=132)
1
(n=180)
2
(n=17)
3
(n=4)
Pb

a

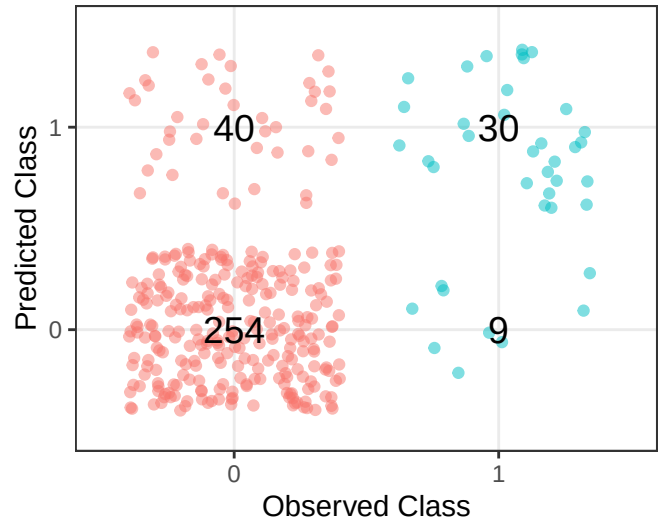


b



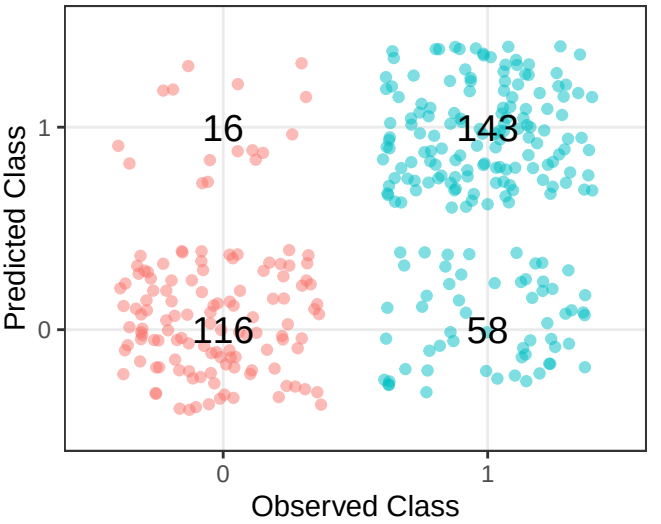
a

Class	ProducerAccuracy	UserAccuracy
0	86.39%	96.58%
1	76.92%	42.86%



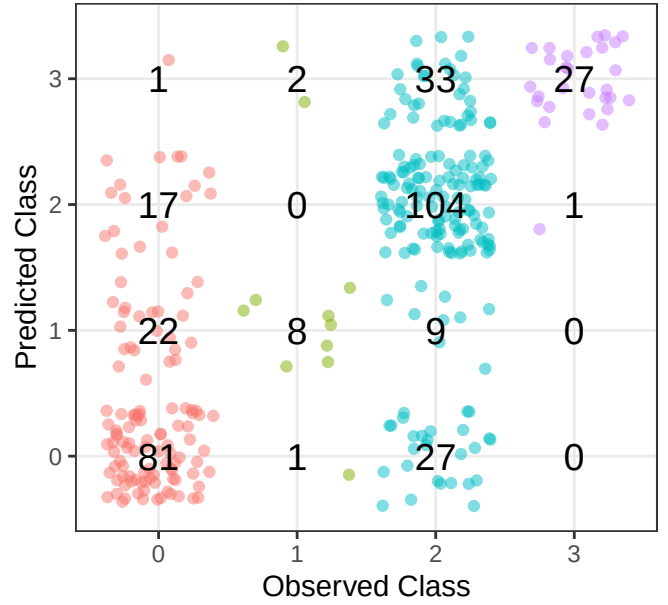
b

Class	ProducerAccuracy	UserAccuracy
0	87.88%	66.67%
1	71.14%	89.94%



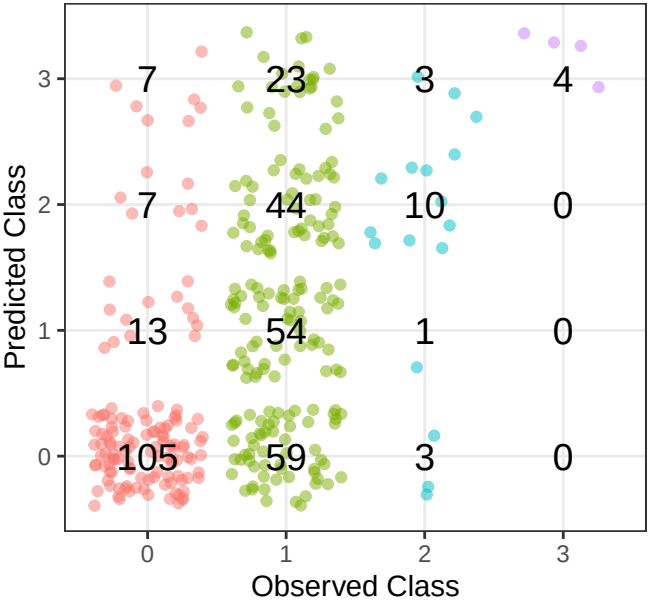
c

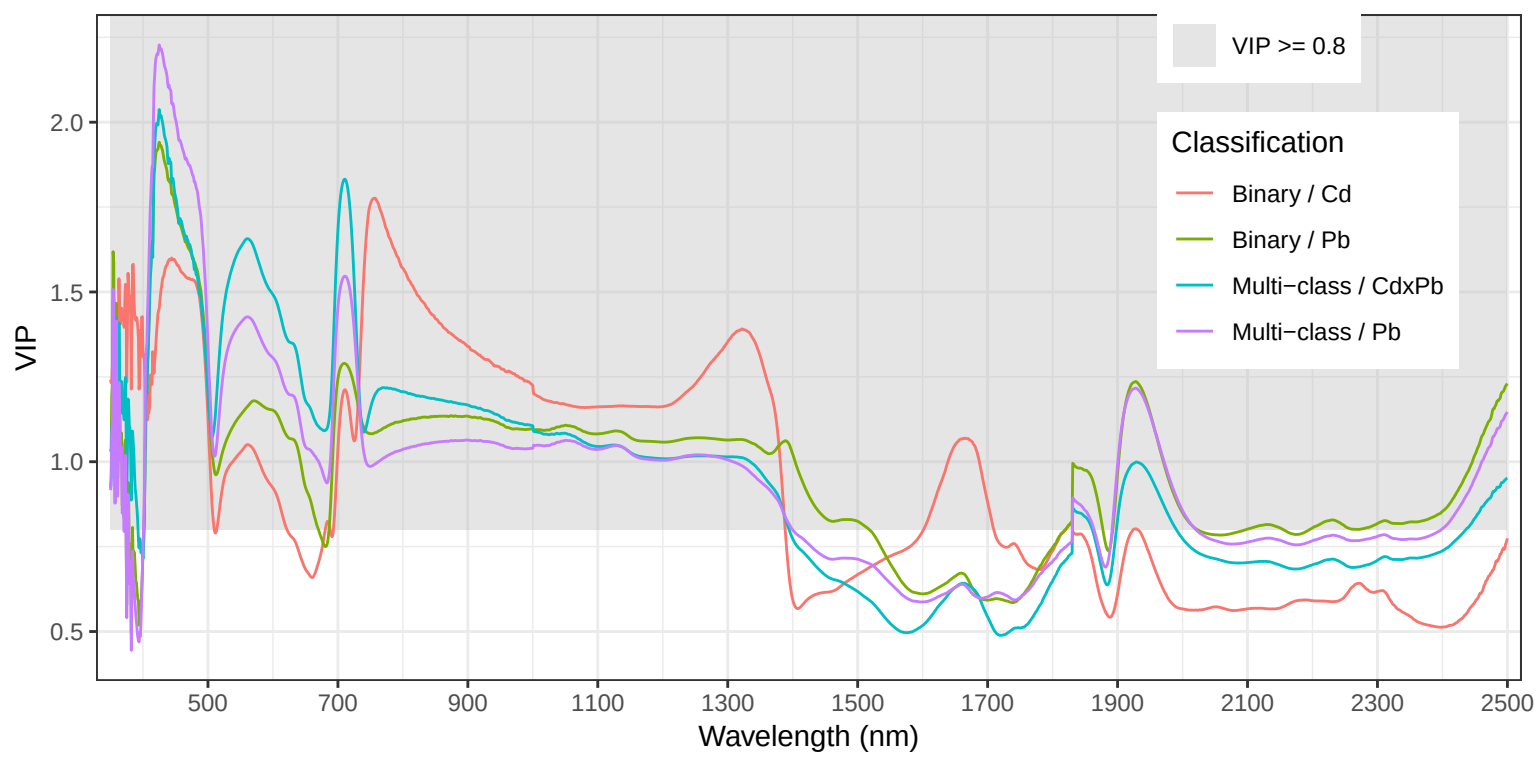
Class	ProducerAccuracy	UserAccuracy
0	66.94%	74.31%
1	72.73%	20.51%
2	60.12%	85.25%
3	96.43%	42.86%



d

Class	ProducerAccuracy	UserAccuracy
0	79.55%	62.87%
1	30.00%	79.41%
2	58.82%	16.39%
3	100.00%	10.81%




VIP >= 0.8
Classification
Binary / Cd
Binary / Pb
Multi−class / CdxPb
Multi−class / Pb
VIP
2.0
1.5
1.0
0.5
500
700
900
1100
1300
1500
1700
1900
2100
2300
2500
Wavelength (nm)

- Cd and Pb pollution in urban soils reduces the leaf mass per area of Tilia trees
- Soil Cd and Pb pollution reduces the leaf chlorophyll a to b ratio of Tilia trees
- Soil Cd and Pb pollution alters leaf spectral properties in the red-edge region
- PLS-DA models based on leaf spectra allow for the detection of Cd and Pb pollution