Ecological Indicators 112 (2020) 106110

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind

Original Articles

Black pine (*Pinus nigra*) barks: A critical evaluation of some sampling and analysis parameters for mercury biomonitoring purposes

Valentina Rimondi[a,b,*], Pilario Costagliola[a,b], Renato Benesperi[c], Marco Benvenuti[a,b], Marc W. Beutel[d], Antonella Buccianti[a,b], Laura Chiarantini[a,e], Pierfranco Lattanzi[b], Daniela Medas[f], Pierluigi Parrini[a]

[a] Dipartimento di Scienze della Terra, Università di Firenze, Via G. La Pira 4, 50121 Firenze, Italy
[b] CNR-IGG, Via G. La Pira 4, 50121 Firenze, Italy
[c] Dipartimento di Biologia, Università di Firenze, Via G. La Pira 4, 50121 Firenze, Italy
[d] University of Merced, 5200 Lake Road Merced, CA 95343, USA
[e] Centro di Servizi di Microscopia Elettronica e Microanalisi (M.E.M.A), Università di Firenze, Via G. Capponi, 50121 Firenze, Italy
[f] Dipartimento di Scienze Chimiche e Geologiche, Università di Cagliari, S.S. 554 bivio per Sestu, 09042 Monserrato (CA), Italy

ARTICLE INFO

Keywords:
Airborne mercury
Biomonitoring
Tree barks
Sampling parameters
Soils

ABSTRACT

Tree barks are increasingly used as biomonitors of airborne pollutants. However, many authors stress the poor comparability of the results achieved in different studies. This drawback is mainly caused by a poor understanding of the critical sampling parameters to be considered. To minimize the biases that could be introduced during sampling, in this study the barks of *Pinus nigra* J.F. Arnold from thirteen sites were investigated in the abandoned Mt. Amiata mercury (Hg) mining district (Southern Tuscany, Italy) and surroundings. The influence of some sampling and analyzing parameters on Hg content was critically assessed. At each site, a total of eight bark samples were taken from a single tree at two heights (70 cm and 150 cm from soil) and at four different sides of the trunk, corresponding to the four cardinal directions; a composite soil sample was also collected. Mercury contents in barks range from 0.1 to 28.8 mg/kg, and are correlated with soil Hg contents (1–480 mg/kg), indicating that barks record both gaseous Hg concentrations in air, and wind-transported Hg-bearing particulate. For each tree, samples at 70 cm and 150 cm show Hg contents of the same order of magnitude, even if values for 150 cm are slightly less dispersed, possibly because barks at 70 cm are more influenced by random soil particles. There is no statistically significant dependence of Hg content on direction and tree age. Simulated rain events cause a negligible loss of Hg from barks. Results suggest that a convenient sampling practice for *Pinus nigra* is to collect a bark slice (typically 1–2 mm) within the outermost 1.5 cm layer.

1. Introduction

Heavy metals (HM) monitoring receives a growing importance in the recent scientific literature due to the adverse effects of these elements on environmental quality. As a consequence, new methods for HM observation and quantification in the environment are constantly sought. Methods based on the use and installation of sophisticated instruments in the field may be expensive and/or complicated. By contrast, biomonitoring can be generally sustained with low expenditures and in regions that lack conventional monitoring networks. Biomonitoring is indeed perceived by the general public as more reliable than monitoring accomplished by technical devices (cf. Burger et al., 2007), which are generally positioned on site for relatively short periods, and may be subjected to failures.

Among different biological indicators, tree tissues still have a relatively small niche when compared to other more widely used substrates, such as mosses and, in particular, lichens (Leavitt and Clair, 2015, and references therein). Costagliola et al. (2017) recently discussed the pros and cons of the use of lichens versus tree barks. The major disadvantages in the use of lichens are the slow regeneration rates, the irregular and patchy distribution, and the relatively weak tolerance to mycophytotoxic pollutants (Berlizov et al., 2007).

An alternative and complementary methodology is represented by tree barks. Tree barks are excellent adsorbents of airborne pollutants, including toxic metals (Panichev and McCrindle, 2004; Baltrénaité et al., 2014; Chrabąszcz and Mróz, 2017; Costagliola et al., 2017; Kang

* Corresponding author at: Dipartimento di Scienze della Terra, Università di Firenze, Via G. La Pira, Firenze 4 50121, Italy.
E-mail address: valentina.rimondi@unifi.it (V. Rimondi).

https://doi.org/10.1016/j.ecolind.2020.106110
Received 29 August 2019; Received in revised form 10 January 2020; Accepted 13 January 2020
Available online 10 February 2020
1470-160X/

et al., 2019), consequently affecting their biogeochemical cycle (see Yang et al., 2018 for Hg investigation). In addition, they are usually available in large amounts and the sampling is generally facilitated by a straightforward species identification, sample treatment, and ubiquity of some genera, which makes it possible to cover large areas.

In spite of these potential advantages, until recently the use of tree barks for environmental monitoring is not widespread, even if growing in number. The lack of an unanimously accepted sampling protocol, and the scarce knowledge of the critical sampling parameters to be considered limit their application. As recently stressed by Cosma et al. (2016) and Rajfur (2019), bark can represent a promising long-term biomonitor for contamination, but only if a proper sampling strategy is established. Indeed, data reproducibility is deemed scarce due to different sampling protocols (e.g., Lodenius, 2013). For other biomonitoring substrates, like mosses, many efforts were made to produce unified sampling protocols, which are periodically revisited (Fernández et al., 2015). By contrast, for barks each researcher follows his/her own procedure, which is designed upon the specific circumstances. The use of barks as a biomonitoring substrate is now mature enough to establish a harmonized protocol. This issue is strategic for any further development in considering bark for biomonitoring studies and as a reservoir for airborne metals (Chiarantini et al., 2016).

The present study does not pretend to fulfill the goal of a complete protocol. The aim is to provide some basic suggestions, derived from the experience developed by this research group (Chiarantini et al., 2016, 2017; Costagliola et al., 2017) on the Hg distribution and speciation in barks of *Pinus nigra* J.F. Arnold from the Mt. Amiata area (Tuscany, Italy). The employment of pine bark species is motivated by the following: i) evergreen species are expected to accumulate higher Hg contents than deciduous species (Yang et al., 2018); ii) bark morphology allows to select and analyze specific slices at known depth from the bark-air interface (Chiarantini et al., 2016); iii) the selected species is quite widespread in the study area. Specifically, the study is focused on the critical assessment of some sampling parameters that may influence Hg concentration in barks, including: sample thickness, sampling height from the ground, tree age, orientation with respect to the prevailing wind directions, and rain effect. The Mt. Amiata district represents a key area to investigate the parameters that may affect Hg content in barks. In the recent past, this region was one of the most important mining districts for Hg in the world (Rimondi et al., 2012), and hosts a diffuse and widespread anomaly of Hg. Implications for a more generalized use of tree barks as biomonitors of airborne pollutants are discussed.

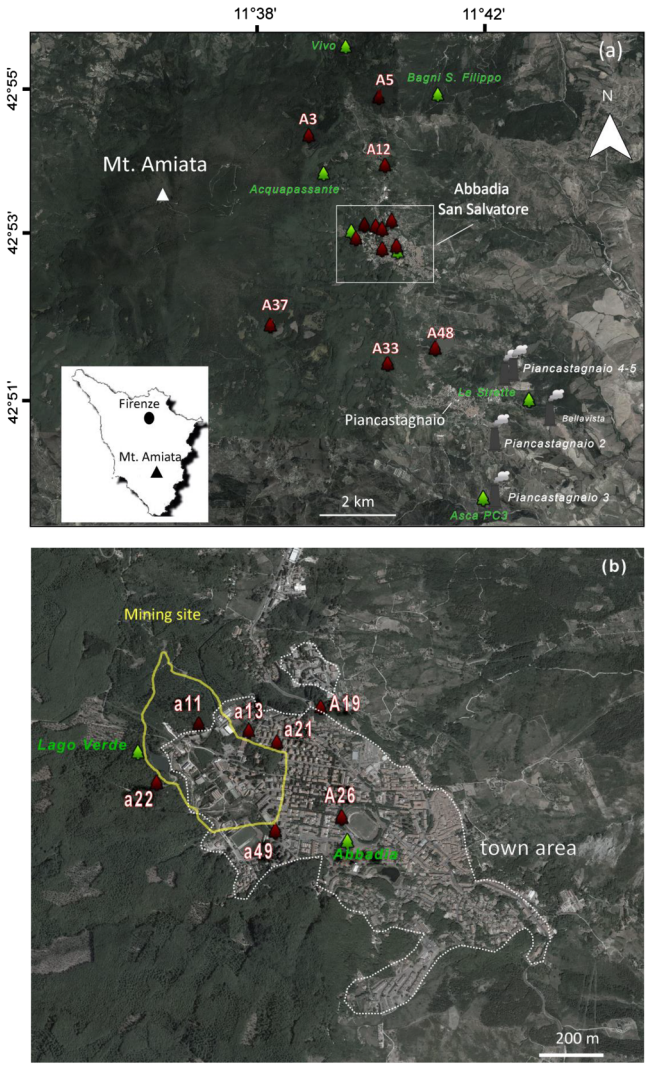

Fig. 1. Satellite image (from Google Earth) of the study area (a) and magnification of the Abbadia San Salvatore area (b) with location of sampling sites of this study (red symbols). The location of samples described by Chiarantini et al. (2016) is shown by green symbols.

2. Methods

2.1. Sampling campaign

In this work, barks of Black Pine (*Pinus nigra* J.F. Arnold) trees in the Mt. Amiata region (Southern Tuscany, Italy) and the underlying soils were sampled in July 2016. In this area two industrial activities, past Hg mining (discontinued in the 1980s) and ongoing geothermal energy production, affect local atmospheric Hg levels (Ferrara et al., 1998; Loppi, 2001; Vaselli et al., 2013; Rimondi et al., 2015; Cabassi et al., 2017); the second source is deemed definitely subordinate (Benvenuti and Costagliola, 2016; Lattanzi et al., 2019). Airborne Hg occurs in this area (as elsewhere) as both particulate and gaseous phase including gaseous elemental Hg - GEM or simply Hg°, and, less commonly, oxidized compounds – GOM (Chiarantini et al., 2016, 2017).

We selected thirteen sampling sites, seven next to the mining area of Abbadia San Salvatore (shortly, Abbadia, the main Hg mine of the Mt. Amiata region), and six in local reference areas not directly affected by mining works (Fig. 1a and b).

For each site, we collected one sample of soil and eight bark specimens from a single tree. Soils were collected as composite samples (three splits from random locations around the tree), out from the tree canopy, after removal of tree litter on the first 10 cm of depth. Tree age could possibly influence Hg accumulated in tree barks. Hence we decided to sample trees whose ages were precisely known by local forest rangers. The number of tree barks employed for site characterization is variable among different studies (e.g., Yang et al., 2018; Navrátil et al., 2017; Suchara and Sucharová, 2008; Sanjo et al., 2004), and may require standardization in future studies. In this specific study, many sample sites are located within the town limits or in other inhabited areas, where we could find only a single tree of known age. We decided therefore to sample just one single plant per location. Barks may trap Hg as a gaseous species from the surrounding atmosphere, or as particulate. Local soil dust is the most likely contribution to particulate (Chiarantini et al., 2016, 2017); in principle, this contribution should be more important for the bark portion closest to soil, and dependent on local wind directions. For this reason, barks were collected at two different heights, 70 and 150 cm, and in the four cardinal directions for each height, for a total of 104 bark samples. We decided to exclude heights higher than 150 cm height to favour easy and fast bark sampling by handpicking, which is an useful requisite for extensive biomonitoring campaigns. Sample thickness is an important parameter to be considered. Several studies demonstrated that the concentration of chemical species across bark thickness is uneven (Chiarantini et al.,

2016; Cosma et al., 2016; Birke et al., 2018; Peckham et al., 2018). Specifically, Chiarantini et al. (2016) found that the maximum Hg content in *Pinus nigra* barks is recorded within the first 1.5 cm from the surface. Aglietti (2017) reports that in thicker (~3 cm) samples Hg concentration is indeed lower than in the first 1.5 cm. The outer bark of *Pinus* genus typically consists of easily split layers, having a thickness of some mm.; for this study, 1 or 2 of these layers within the first 1.5 cm from the bark surface were sampled.

Bark samples were identified according to a pre-defined grid, whereby lower case a (e.g., a11) refers to samples collected within the Abbadia former mining and smelting site (Fig. 1b), and capital A (e.g., A3) is associated to samples collected outside this area (Fig. 1a). The sample label is completed by a letter corresponding to the cardinal point, and by a number (i.e., 70 or 150) indicating the height of sampling (e.g., A3-N70).

As recalled before, in the Mt. Amiata district the local Hg background is typically high for several km^2 (Rimondi et al., 2012 and references therein). The natural matrices affected by this anomaly include soils, water and air. It could be thus conceivably assumed that each tree positioned in this area is exposed to a diffuse Hg source (cf. McLagan et al., 2019). As a consequence: a) the Hg concentration in air and soils surrounding the tree is not strongly dependent upon direction; b) minor punctual Hg sources (i.e., vehicular traffic, incinerators, etc.), which may have some relevance in areas comparatively less polluted than the Mt. Amiata district (e.g., urban areas), are not able to influence the general concentration of Hg in air (as both particulate and gaseous Hg), since they represent a minor contribute. With these premises, any variations of the Hg concentration in barks is expected to be primarily the result of direction-dependent transport of Hg (as both Hg° and Hg particulate) from the source to the receptor (bark), rather than dependent upon the spatial distribution of the Hg anomaly around the receptor. Accordingly, the Mt. Amiata district is the ideal place where any effect of wind direction on bark composition could be measured, minimizing possible biases due to pollutant source area and its geometry and the relative position of the receptor.

2.2. Analytical methods

Soils were air dried at room temperature for approximately one month. About 1 kg of material was disaggregated, homogenized, and sieved to < 2 mm; this fraction was selected in accordance with Italian regulations for HM analysis in soil (D.Lgs. 152/06). The sieved fraction was then pulverized and analyzed for Hg. Low-Hg (< 10 mg/kg) samples were directly analyzed by means of the thermal decomposition technique using USEPA method 7473 on a Milestone DMA-80. Soils with high Hg concentration (> 10 mg/kg) exhibited high analytical variability using the DMA, likely due to the well-known "nugget effect" associated with the analysis of small sample masses of highly contaminated soils (Kocman et al., 2006). To minimize this effect, a larger mass of sample (1 g) was digested in a sand bath with aqua regia (HCl/HNO_3 3:1) and analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES, Perkin Elmer Optima 8000 instrument) equipped with a hydride generator (Perkin Elmer B0507957). Two of these "high Hg" samples were processed in duplicate to evaluate method precision, which was < 15%. The analytical accuracy, checked against DOLT4, TORT3 (DMA) and CCRMP STDDS-1 (ICP-OES) international standards, was < 5% and < 10% for thermal and acid digestion analysis, respectively.

The sampled bark slices were shredded with a ceramic knife, homogenized by mixing, and split into two portions; one was used for determining water loss at 110° C until a constant weight was achieved (Chiarantini et al., 2016), and the other for Hg analysis by thermal decomposition (DMA-80). To estimate the analytical precision, three replicate analyses of the same slice were routinely performed; results were generally reproduced within 15–20% of the average value. Accuracy was evaluated using international standards (DOLT3, TORT3), and was within 10%. All analytical results for barks are presented on a dry weight (dw) basis, recalculated as reported by Chiarantini et al. (2016).

To measure the possible effects of Hg leaching by rainwater, two samples of barks selected at different heights and/or orientations from ten sampling stations showing low (0.2–0.3 mg/kg), moderate (1–1.7 mg/kg), high (6.7–9.6 mg/kg), and extreme (> 10 mg/kg) Hg content were subjected to a leaching test. In the test, about 0.2 g of crushed bark was mixed with 25 ml of deionized water (equilibrated with atmospheric CO_2) for 24 h, with a bark/water ratio of about 1:125 by weight. Considering that barks of the *Pinus* species have a density of about 0.4 g/cm^3 (Mile and Smith, 2009), this ratio in volume corresponds to about 1:50. Leached solutions were measured for Hg via USEPA method 1631 on a MERX-T autoanalyzer (Brooks Rand, Seattle, USA). Background Hg contamination in leaching bottles was negligible, ranging from 0.13 to 0.17 ng/L (n = 3), well below measured samples (> 10 ng/L). Ongoing precisions and recovery samples spiked with known amounts of Hg were run during the analytical run and recovery ranged from 96.8 to 109% (n = 5).

2.3. Meteorological data and Hg air concentrations

The total yearly amount of rain at Abbadia is about 650 mm, and the rainiest month is November, with about 80 mm precipitation. No significant precipitation occurred in the seven days preceding sampling.

Wind direction data were collected from records (period 2014–2016) of the station of Abbadia San Salvatore-Laghetto Verde (TOS 11000114) of the regional meteorological network (www.sir.toscana.it). Based on these data, the wind rose in the study area is reported in Fig. S1. Prevailing winds come from the W direction (280°), while subordinate directions are from NE (40°) and SE (140°).

The study area was surveyed for GEM concentrations by means of a portable analyzer Lumex RA-915 (Vaselli et al., 2013, 2017; Cabassi et al., 2017). A few of those measurement points were close to sampling sites of this study. More recently, McLagan et al. (2019) reported GEM air concentrations in the area estimated from passive sampler analysis. All barks considered in the present study are located in proximity of a passive sampler (maximum distance 20 m), and sampling occurred when the passive samplers were in place. Therefore, we assume that GEM concentrations reported by McLagan et al. (2019) for the closest sampler to each of our sampling sites represent a reasonable approximation of air concentrations at the specific time and site of bark sampling. Table 1 reports the values considered for this study.

2.4. Statistics

By taking into account the structure of the database, characterized by the Hg concentrations (mg/kg) and different controlling factors given by the dissimilar heights and cardinal directions of sampling, a non-parametric (distribution free) analysis of variance was used. The Kruskal–Wallis H-Test for One-way Analysis of Variance (ANOVA) by ranks is a frequently used nonparametric test when it is necessary to determine if three or more independent samples belong to the same or different populations (Siegel and Castellan, 1988; MacFarland and Yates, 2016). The choice was motivated by the limited number of data for each group and by the presence of skewness in the frequency distribution of the chemical variable. It is necessary to stress here that this procedure should be applied under the hypothesis of independence of the samples, a condition that appears to be sustainable in our experiment.

We employed a robust regression as an alternative to least squares regression to evaluate dependence structure between variables, especially when data include outliers or skewness (a frequent condition in our study), and when model assumptions (normal distribution, homoscedasticity, etc.) are not fulfilled (cf. Varmuza and Filzmoser, 2009).

Table 1
Mercury concentrations of soils and barks in the study area, and age of sampled trees. Hg° contents in air are also reported. ^Data from McLagan et al. (2019).

Sample ID	Tree age (years)	Height (cm)	Hg in bark (mg/kg dw)				Hg in soil (mg/kg)	Hg° in air range ^
			N	S	E	W		(ng/m^3)
A3	80	70	1.5	1.4	1.2	1.1	4	1.68–1.84
		150	1.5	2.3	1.7	1.4		
A5	60	70	0.2	0.2	0.3	0.2	2	2.09–2.53
		150	0.1	0.1	0.2	0.1		
A12	70	70	1.2	2.7	2.9	1.7	4	2.06–2.69
		150	1.5	1.6	1.8	1.5		
A19	100	70	11.9	13.6	15.2	14.4	66	3.82–4.24
		150	12.8	20.0	11.4	18.6		
A26	80	70	12.1	11.4	8.8	8.5	72	14.5–29.8
		150	12.9	13.2	9.9	11.7		
A33	67	70	1.6	1.3	1.8	1.7	4	2.67–3.26
		150	1.0	0.7	1.2	0.5		
A37	100	70	0.5	1.8	0.9	2.2	1	1.86–2.07
		150	0.7	0.8	0.7	1.4		
A48	60	70	0.4	0.5	0.3	0.4	1	2.03–3.03
		150	0.4	0.2	0.3	0.1		
a11	100	70	28.8	7.1	14.9	9.8	480	9.86–15.7
		150	7.4	6.6	7.1	8.9		
a13	100	70	14.5	20.7	4.1	14.1	186	16–17.9
		150	21.3	21.8	19.2	15.8		
a21	100	70	9.4	5.8	8.0	1.9	97	11.6–17.8
		150	8.1	7.3	7.9	7.0		
a22	150	70	4.4	11.2	8.4	6.0	66	7.48–14.8
		150	5.8	8.1	8.8	6.7		
a49	100	70	11.8	9.1	12.3	8.8	163	24.7–116
		150	9.9	11.0	11.6	10.4		

3. Results and discussion

3.1. Soils

Total Hg in soils is comprised between 1 and 480 mg/kg (Table 1), i.e. much higher than the average abundance of Hg in the Earth's crust (20 to 60 μg/kg; Kabata-Pendias and Mukherjee, 2007) and in soils from different world regions (50–500 μg/kg; Kabata-Pendias and Pendias, 2001; Wang et al., 2012). In agreement with previous soil data (Rimondi et al., 2012, 2014; Chiarantini et al., 2016; Protano and Nannoni, 2018), these results confirm the presence of a diffuse Hg anomaly centered around the Mt. Amiata district.

As expected, the highest concentrations generally occur close to the town of Abbadia, where the principal mines and the metallurgical plants were located (Rimondi et al., 2015). According to Rimondi et al. (2014) and Protano and Nannoni (2018), at Abbadia Hg in soil generally occurs in volatile form (presumably, Hg°), or as highly insoluble compounds such as sulphides (HgS, cinnabar and metacinnabar). Organic-bound Hg may also be present (Protano and Nannoni, 2018). More soluble Hg compounds, such as $HgCl_2$ and HgO, were also identified (Rimondi et al., 2014). Their abundance is particularly significant in correspondence of the calcines, where such Hg compounds formed during retorting, or later in secondary processes (Rimondi et al., 2014). All samples for this study were collected at a distance > 50 m from remaining calcine piles, therefore we believe that in the sampled soils the main Hg species are Hg° and HgS.

3.2. Barks

Total Hg in barks varies between 0.1 and 28.8 mg/kg dw (Table 1). These concentrations are similar and even higher than those measured by Chiarantini et al. (2016) in the same area, thus confirming that *Pinus nigra* barks can accumulate large quantities of Hg. Arnold et al. (2018) and Peckham et al. (2018) report much lower (~0.01 mg/kg) concentrations in *Pinus nigra* barks exposed to environments where Hg concentrations in air and soil were lower than those typical of the Mt. Amiata area (see also further discussion). Published Hg concentrations in barks of pine (Suchara and Sucharová, 2008; Martín et al., 2013; Navrátil et al., 2017) and other species (e.g., Obrist et al., 2009; Rykowska and Wasiak, 2011; Yang et al., 2018) are usually well below 1 mg/kg, except for values up to 1.18 mg/kg reported by Schulz et al. (1999) for *Pinus sylvestris* L.

The overall distribution of the Hg concentration in barks (average of the eight values for each tree) from different sites follows that of soils (Fig. 2): the highest values are all centered close to the former Abbadia mine and smelting site, whereas lower Hg contents are observed in the reference areas, north and south of the town. The site a11 is a notable exception to this trend, since Hg in bark is apparently low (11.3 mg/kg dw as average value) compared to the extreme Hg content observed in the related soils (480 mg/kg; Table 1). However, we remark that along the N direction, up to 28.8 mg/kg dw Hg are found at the 70 cm height (Table 1), representing the highest value of the entire dataset, hence in

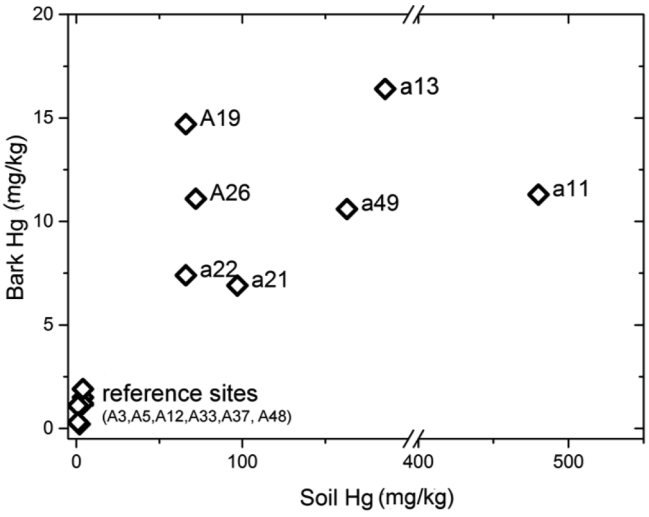

Fig. 2. Correlation of Hg in barks (average of the eight values for each tree) vs. Hg in soil at the same site.

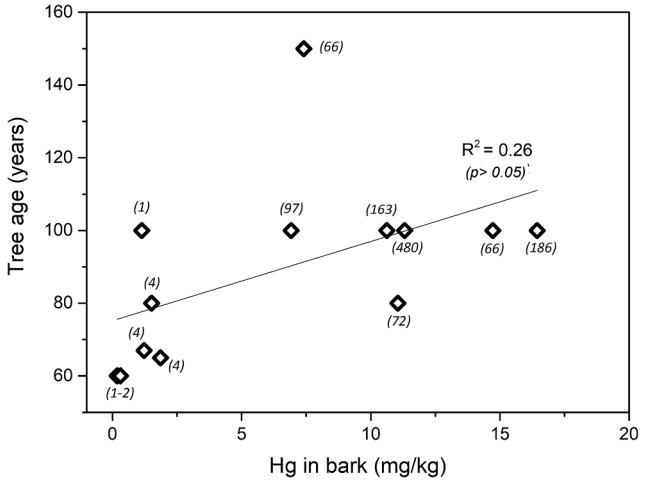

Fig. 3. Plot of bark Hg contents (average of the eight values for each tree) vs. estimated tree age (numbers next to symbols indicate Hg contents in the corresponding soils in mg/kg).

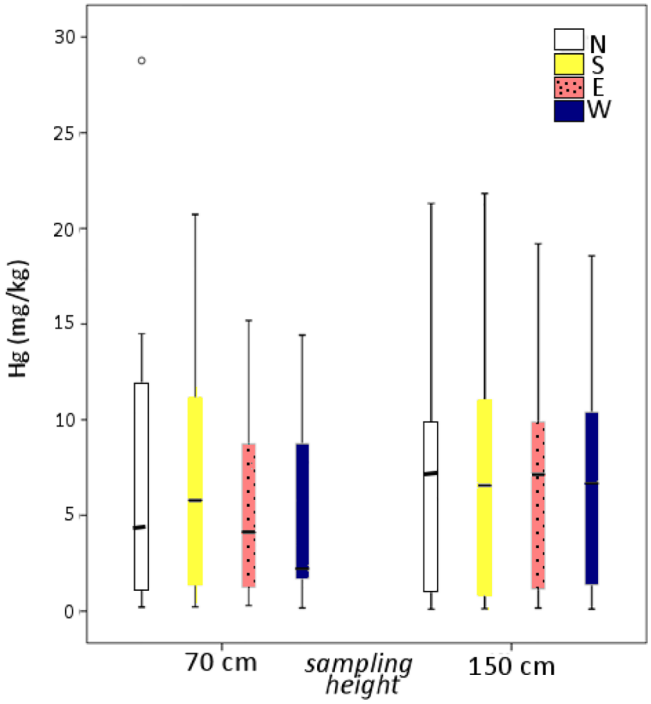

Fig. 4. Boxplots of Hg contents in barks as a function of sampling heights and cardinal direction.

agreement with the underlying soil. It is likely that this subsample is particularly impacted by particulate Hg due to soil resuspension (see below).

This distribution is also roughly consistent with estimates of Hg° concentration in air (Table 1): with the exception of sample A19, all barks with Hg contents ≥7 mg/kg come from areas where Hg concentration in air is ≥7.5 ng/m^3; for lower Hg air concentrations, Hg contents in bark are always < 2 mg/kg. We compared the Hg concentration in barks (average of the eight values for each tree) with the age of the trees. Fig. 3 indicates that correlation of Hg contents in barks with tree age is not statistically significant ($R^2 = 0.26$; $p > 0.05$). Specifically, trees with different ages grown on soils with similar Hg contents have also similar contents in barks; conversely, trees with the same age grown on soils with different Hg contents also show different contents in barks. These results suggest that the time required by barks to collect Hg from the surrounding environment is appreciably shorter than tree age. We tentatively suggest that the time required by *Pinus nigra* barks for (dynamic) equilibration with the surrounding environment is in the order of few years.

The comparison between barks sampled at different heights from the ground (70 cm and at 150 cm) and with different orientation with respect to the cardinal points is presented in the boxplots of Fig. 4. It appears that most of the data are overlapping, and sampling height and different cardinal direction apparently do not affect the Hg concentration. This explorative result is confirmed by the application of the Kruskal-Wallis and median tests (Siegel and Castellan, 1988; MacFarland and Yates, 2016), whose results allow us to accept the null hypothesis of no differences ($p \gg 0.05$) in the action of the controlling factors.

The relationship between the different cardinal directions from which the samples were collected for a given height of sampling was further investigated by checking the results of robust linear regression (cf. Varmuza and Filzmoser, 2009). Results are reported in Table S1 and S2.

Almost systematically, the correlation between sample height and cardinal direction (estimated by R^2) is slightly better for samples picked up at a height of 150 cm with respect to those positioned at 70 cm (Table S1). In addition, the equations of the linear model for the samples at 150 cm have a small intercept (less or equal to the detection limit), and a slope close to unity. The correlations observed for the samples at 70 cm of height are significant, but lower than those observed for the samples positioned at 150 cm. Considering now wind directions, the wind rose at Abbadia (Fig. S1) indicates that the main windward direction is west, and the main downwind directions are south and north. The Hg concentration of the samples picked up on the west side of the trees at 150 cm is however well correlated with the Hg content of barks collected on the north and south side of the tree trunks (Table 1). These features point out that at 150 cm the Hg content in barks is virtually unaffected by sample orientation, even in an area where wind directions are rather focused as at Abbadia. The effect of prevailing winds at Abbadia contributes to generate a broadly elliptical shape of the isoconcentration curves of Hg in air presented by McLagan et al. (2019), with an E-W oriented major axis. Our results indicate that Hg content in barks is unaffected by this overall trend. This feature further simplifies bark sampling procedure, since samples may be picked up without a particular care to cardinal points. By contrast, a drawback is that barks appear poorly effective in recognizing pollutant provenance.

As previously noted, Chiarantini et al. (2016) suggested a correlation between Hg in soils and barks in the Mt. Amiata area. Such a correlation was also observed elsewhere (Suchara and Sucharová, 2008; Martín et al., 2013; Navrátil et al., 2017). In Fig. 5 the robust correlation between the concentration of Hg in soils and barks for the two different sampling heights adopted in the present study is shown. The correlation is higher for the samples collected at 70 cm height ($R^2 = 0.86$) with respect to the same data set at 150 cm ($R^2 = 0.78$). The same scheme is maintained if data are transformed by using the natural logarithm (R^2 equal to 0.84 versus 0.75, respectively; not shown). This result suggests that the Hg concentrations in bark samples at 70 cm could be more influenced by local soil contribution, including particle resuspension and/or re-emission of gaseous Hg, accordingly to the decreasing vertical Hg gradient measured by Feigis et al. (2019) with passive Hg samplers deployed at different heights over polluted soil.

The concentration of pollutants in barks is expected to vary from the phloem toward the bark surface. For pollutants that are generally poorly bioavailable, such as in the case of Hg, the fraction that is deposited on the bark surface normally largely exceeds the systemic fraction (Siwik et al., 2010). As demonstrated by Rimondi et al. (2014) and Protano and Nannoni (2018), the Hg speciation in solid materials (calcine, sediments, soil) at Abbadia includes several phases; insoluble

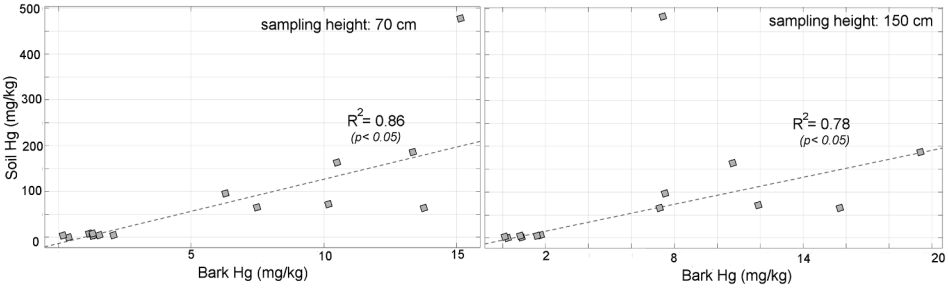

Fig. 5. Correlation between Hg in soils and average values of bark sampled at 70 (a) and 150 (b) cm.

species (cinnabar and metacinnabar) are common in sediment and soil. Chiarantini et al. (2017) found that Hg sulphides are indeed the most abundant phases on the bark surface, where Hg° sporadically has been reported. From the outermost bark layer to deeper layers, there is an increase of the organic-bound fraction (thiol-containing molecules or tannins). As previously noted, this change in speciation from the surface toward the inner bark layers is accompanied by a change in Hg concentration, which is maximum within the first 1.5 cm from the surface, and then decreases (Chiarantini et al., 2016; Aglietti, 2017). Therefore, sampling thickness should be explicitly taken into account when using barks for biomonitoring. In agreement with Aglietti (2017), we suggest taking a bark slice within the first 1.5 cm from the surface. In the case of *Pinus Nigra*, whose bark may be easily split in thin layers having a thickness of some mm., we suggest sampling one or two such layers within the first 1.5 cm.

3.3. Leaching tests

The results from leaching tests (Table 2) showed Hg concentrations in water ranging from 10.4 to 309 ng/L, with more contaminated bark corresponding with higher Hg concentration in leach water. But when evaluated on the basis of Hg mass leached relative to Hg mass in bark (dw), greater leaching was associated with less contaminated bark. The percent of Hg in bark leached to water was ~1.4% for lightly contaminated bark, 0.4–0.6% for moderately contaminated bark, and ~0.2% for highly contaminated bark. Results indicate that surface bark could release low amounts of Hg to the surrounding environment during precipitation events, and that the amounts lost are negligible compared to the mass of Hg stored in bark tissue. On the other hand, our experiments do not consider mechanical removal by rain of previously adsorbed particulate (Catinon et al., 2012), which could enhance Hg release from bark. Therefore, we support the suggestion by those authors that bark sampling for monitoring airborne contaminants is best performed in dry seasons. It should be noted that we cannot exclude that some Hg° originally trapped in barks could have been revolatilized from the organic substrata as reported for barks of some deciduous trees (Hanson et al., 1997), eventually promoted by sunlight irradiation.

Table 2
Leaching data for selected bark samples. *Average of the two heights for each sample.

Sample	Location	Leached Hg (ng/L)	Leached Hg to bark Hg (%)*
A5	E150	10.4	0.9
	N70	15.2	
A48	E70	26.5	1.4
	W70	34.7	
A37	N70	33.2	1.4
	E150	187	
A33	N150	52.4	0.6
	W70	46.6	
A3	W150	26.5	0.4
	E70	46.2	
A12	W150	78.3	0.4
	S150	46.8	
a22	S150	14.1	0.2
	W70	216	
a49	S150	173	0.2
	W70	190	
A26	N150	196	0.2
	W70	150	
a13	W150	309	0.2
	E70	95.4	

4. Conclusions

The results of this study confirm previous data on the ability of *Pinus nigra* bark to accumulate significant amounts of Hg, reflecting local soil and air concentrations. Since barks accumulate different forms of airborne Hg (both Hg° and particulate Hg), they represent a long term proxy for cumulate Hg exposure. A critical assessment of sampling parameters indicates that measured concentrations are essentially independent of tree age and wind direction. The maximum concentrations are found in the outermost bark layers; as a practical guideline for this species (*Pinus nigra* J.F. Arnold) we suggest taking a slice within the first 1.5 cm from the surface.

There is a broad consistency between bark samples taken at different heights (70 and 150 cm), but those taken at 150 cm show less variability in relation to sampling orientation, possibly because samples at lower height are more affected by random soil particles or Hg° re-emission by soils. Leaching tests suggest that rain should not significantly affect Hg concentration in bark, when (as it happens at Mt. Amiata) Hg speciation is dominated by insoluble phases.

CRediT authorship contribution statement

Valentina Rimondi: Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing, Resources. **Pilario Costagliola:** Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing, Resources. **Renato Benesperi:** Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Marco Benvenuti:** Resources, Visualization, Writing - review & editing. **Marc W. Beutel:** Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing, Resources. **Antonella Buccianti:** Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing, Formal analysis. **Laura Chiarantini:** Visualization, Writing - review & editing. **Pierfranco Lattanzi:** Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing, Resources. **Daniela**

Medas: Visualization, Writing - review & editing. **Pierluigi Parrini:** Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing, Visualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

A preliminary data analysis for this study was performed by L. Aglietti for his thesis at Università di Firenze. We would like to thank Dr. Liying Zhao, Christian Tran and Byran Fuhrmann at the University of California, Merced's Environmental Analytical Laboratory for assistance with measuring mercury in bark samples and leaching tests. We gratefully acknowledge the editor Steven Ripp and the constructive criticism by two anonymous referees.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106110.

References

Aglietti, L., 2017. Biomonitoraggio di Hg aerodisperso: standardizzazione del metodo. Tesi di Laurea magistrale in Scienze e tecnologie geologiche, Università di Firenze, 111 pp. (in Italian).

Arnold, J., Gustin, M.S., Weisberg, P.J., 2018. Evidence for nonstomatal uptake of Hg by aspen and translocation of Hg from foliage to tree rings in Austrian Pine. Environ. Sci. Technol. 52, 1174–1182.

Baltrénaité, E., Baltrenas, P., Lietuvninkas, A., Sereviciene, V., Zuokaite, E., 2014. Integrated evaluation of aerogenic pollution by air-transported heavy metals (Pb, Cd, Ni, Zn, Mn and Cu) in the analysis of the main deposit media. Environ. Sci. Pollut. Res. 21, 299–313.

Benvenuti, M., Costagliola, P., 2016. Il distretto mercurifero del comprensorio amiatino: nuovi dati sull'impatto ambientale nel sistema fluviale Paglia-Tevere. Geologia dell'Ambiente 4/2016, 4 pp. (in Italian).

Berlizov, A.N., Blumb, O.B., Filby, R.H., Malyuka, I.A., Tryshyn, V.V., 2007. Testing applicability of black poplar (Populus nigra L.) bark to heavy metal air pollution monitoring in urban and industrial regions. Sci. Total Environ. 372, 693–706.

Birke, M., Rauch, U., Hofmann, F., 2018. Tree bark as a bioindicator of air pollution in the city of Stassfurt, Saxony-Anhalt, Germany. J. Geochem. Explor. 187, 97–111.

Burger, J., Gochfeld, M., Kosson, D.S., Powers, C.W., 2007. A biomonitoring plan for assessing potential radionuclide exposure using Amchitka Island in the Aleutian chain of Alaska as a case study. J. Environ. Radioact. 98, 315–328.

Cabassi, J., Tassi, F., Venturi, S., Calabrese, S., Capecchiacci, F., D'Alessandro, W., Vaselli, O., 2017. A new approach for the measurement of gaseous elemental mercury (GEM) and H_2S in air from anthropogenic and natural sources: Examples from Mt. Amiata (Siena, Central Italy) and Solfatara Crater (Campi Flegrei, Southern Italy). J. Geochem. Explor. 175, 48–58.

Catinon, M., Ayrault, S., Boudouma, O., Asta, J., Tissut, M., Ravanel, P., 2012. Atmospheric element deposit on tree barks: the opposite effects of rain and transpiration. Ecol. Indic. 14, 170–177.

Chiarantini, L., Rimondi, V., Bardelli, F., Benvenuti, M., Cosio, C., Costagliola, P., Di Benedetto, F., Lattanzi, P., Sarret, G., 2017. Mercury speciation in Pinus nigra barks from Monte Amiata (Italy): an X-ray absorption spectroscopy study. Environ. Poll. 227, 83–88.

Chiarantini, L., Rimondi, V., Benvenuti, M., Beutel, M.W., Costagliola, P., Gonnelli, C., Lattanzi, P., Paolieri, M., 2016. Black pine (Pinus nigra) barks as biomonitors of airborne mercury pollution. Sci. Total Environ. 569–570, 105–113.

Chrabąszcz, M., Mróz, L., 2017. Tree Bark, a valuable source of information on air quality. Pol. J. Environ. Stud. 26, 453–466.

Cosma, C., Iurian, A.-R., Incze, R., Kovacs, T., Zunic, Z., 2016. The use of tree bark as long term biomonitor of ^{137}Cs deposition. J. Environ. Radioact. 153, 126–133.

Costagliola, P., Benvenuti, M., Chiarantini, L., Lattanzi, P., Paolieri, M., Rimondi, V., 2017. Tree barks as environmental biomonitors of metals - the example of mercury. Adv. Environ. Pollut. Res. 2017, 11–18.

Feigis, M., Mistry, S., Snow, M., Lei, Y., Mitchell, C., Wania, F., 2019. Assessing the atmosphere-surface exchange of gaseous elemental mercury using passive air samplers. In: Abstract Book SETAC Europe 29th Annual Meeting, p. 368.

Fernández, J.A., Boquete, M.T., Carballeira, A., Aboal, J.R., 2015. A critical review of protocols for moss biomonitoring of atmospheric deposition: sampling and sample preparation. Sci. Total Environ. 517, 132–150.

Ferrara, R., Mazzolai, B., Edner, H., Svanberg, S., Wallinder, E., 1998. Atmospheric mercury sources in the Mt. Amiata area. Italy. Sci. Total Environ. 213, 13–23.

Hanson, P.J., Tabberer, T.A., Lindberg, S.E., 1997. Emissions of mercury vapor from tree bark. Atm. Environ. 31, 777–780.

Kabata-Pendias, A., Mukherjee, A.B., 2007. Trace Elements from Soil to Human, Springer-Verlag Berlin Heidelberg, 567 pp.

Kabata-Pendias, A., Pendias, H., 2001. Trace Elements in Soils and Plants, third ed., CRC Press LLC, pp. 403.

Kang, H., Liu, X., Guo, J., Wang, B., Xu, G., Wu, G., Kang, S., Huang, J., 2019. Characterization of mercury concentration from soils to needle and tree rings of Schrenk spruce (Picea schrenkiana) of the middle Tianshan Mountains, northwestern China. Ecol. Indic. 104, 24–31.

Kocman, D., Bloom, N.S., Akagi, H., Telmer, K., Hylander, L., Fajon, V., Jereb, V., Jaćimović, R., Smodiš, B., Ikingura, J.R., Horvat, M., 2006. Preparation and characterization of a soil reference material from a mercury contaminated site for comparability studies. J. Environ. Manage. 81, 146–154.

Lattanzi, P., Beutel, M.W., Costagliola, P., Fagotti, C., Rimondi, V., 2019. Tracing the impact of geothermal plants in the Monte Amiata area, Tuscany, Italy: evidence from Hg contents in stream sediments and tree barks. In: Proceedings European Geothermal Congress (EGC 2019), Den Haag, The Netherlands, Paper 217.

Leavitt, S.D., St. Clair, L.L., 2015. Bio-monitoring in Western North America: what can lichens tell us about ecological disturbances? In: D. Upreti, P. Divakar, V. Shukla, R. Bajpai (Eds.), Recent Adv. Lichenol., Springer, New Delhi, pp. 119–138.

Lodenius, M., 2013. Use of plants for biomonitoring of airborne mercury in contaminated areas. Environ. Res. 125, 113–123.

Loppi, S., 2001. Environmental distribution of mercury and other trace elements in the geothermal area of Bagnore (Mt. Amiata, Italy). Chemosphere 45, 991–995.

MacFarland, T.W., Yates, J.M., 2016. Introduction to Nonparametric Statistics for the Biological Sciences Using R, Springer, 329 pp.

Martín, J.A.R., Nanos, N., Miranda, J.C., Carbonell, G., Gil, L., 2013. Volcanic mercury in Pinus canariensis. Naturwissenschaften 100, 739–747.

McLagan, D.S., Monaci, F., Huang, H., Lei, Y.D., Mitchell, C.P., Wania, F., 2019. Characterization and quantification of atmospheric mercury sources using passive air samplers. J. Geophys. Res.- Atmos. 124, 2351–2362.

Mile, P.D., Smith W.B., 2009. Specific gravity and other properties of wood and bark for 156 tree species found in North America. Res. Note NRS-38, U.S. Department of Agriculture, Forest Service, Northern Research Station, Newtown Square, PA, 35 pp.

Navrátil, T., Šimeček, M., Shanley, J.B., Rohovec, J., Hojdová, M., Houška, J., 2017. The history of mercury pollution near the Spolana chlor-alkali plant (Neratovice, Czech Republic) as recorded by Scots pine tree rings and other bioindicators. Sci. Total Environ. 586, 1182–1192.

Obrist, D., Johnson, D.W., Lindberg, S.E., 2009. Mercury concentrations and pools in four Sierra Nevada forest sites, and relationships to organic carbon and nitrogen. Biogeosciences 6, 765–777.

Panichev, N., McCrindle, R.I., 2004. The application of bio-indicators for the assessment of air pollution. J. Environ. Monitor. 6, 121–123.

Peckham, M.A., Gustin, M.S., Weisberg, P.J., Weiss-Penzias, P., 2018. Results of a controlled field experiment to assess the use of tree tissue concentrations as bioindicators of air Hg. Biogeochemistry 142, 265–279.

Protano, G., Nannoni, F., 2018. Influence of ore processing activity on Hg, As and Sb contamination and fractionation in soils in a former mining site of Monte Amiata ore district (Italy). Chemosphere 199, 320–330.

Rajfur, M., 2019. Assessment of the possibility of using deciduous tree bark as a biomonitor of heavy metal pollution of atmospheric aerosol. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-019-06581-1.

Rimondi, V., Bardelli, F., Benvenuti, M., Costagliola, P., Gray, J.E., Lattanzi, P., 2014. Mercury speciation in the Mt. Amiata mining district (Italy): interplay between urban activities and mercury contamination. Chem. Geol. 380, 110–118.

Rimondi, V., Chiarantini, L., Lattanzi, P., Benvenuti, M., Beutel, M.W., Colica, A., Costagliola, P., Di Benedetto, F., Gabbani, G., Gray, J.E., Pandeli, E., Pattelli, G., Paolieri, M., Ruggieri, G., 2015. Metallogeny, exploitation and environmental impact of the Mt. Amiata mercury ore district (Southern Tuscany, Italy). Ital. J. Geosci. 134, 75–88.

Rykowska, I., Wasiak, W., 2011. Bioconcentration of mercury and heavy metals by the bark of maple-leaf plane tree. Proceedings of ECOpole 5, 103–108.

Sanjo, H., Kenichi, S., Wang, Y., Masunaga, S., 2004. Evaluation of outer bark and inner bark of Ginkgo biloba Linn. as indicators for mercury pollution. Mater. Geoenviron. 51, 1358–1361.

Schulz, H., Popp, P., Huhn, G., Stärk, H.J., Schüürmann, G., 1999. Biomonitoring of airborne inorganic and organic pollutants by means of pine tree barks: temporal and spatial variations. Sci. Total. Environ. 232, 49–55.

Siegel S., Castellan N.J., 1988. Nonparametric Statistics for the Behavioral Sciences, McGraw-Hill, Inc., New York.

Siwik, E.I.H., Campbell, L.M., Mierle, G., 2010. Distribution and trends of mercury in deciduous tree cores. Environ. Pollut. 158, 2067–2073.

Suchara, I., Sucharová, J., 2008. Mercury distribution around the Spolana chlor-alkali plant (central Bohemia, Czech Republic) after a catastrophic flood, as revealed by

bioindicators. Environ. Pollut. 151, 352–361.
Varmuza, K., Filzmoser, P., 2009. Multivariate Statistical Analysis in Chemometrics, CRC Press, 321 pp.
Vaselli, O., Higueras, P., Nisi, B., Esbrí, J.M., Cabassi, J., Martinez-Coronado, A., et al., 2013. Distribution of gaseous Hg in the mercury mining district of Mt. Amiata (Central Italy): a geochemical survey prior the reclamation project. Environ. Res. 125, 179–187.
Vaselli, O., Nisi, B., Rappuoli, D., Cabassi, J., Tassi, F., 2017. Gaseous elemental mercury and total and leached mercury in building materials from the former Hg-mining area of Abbadia San Salvatore (Central Italy). Int. J. Environ. Res. Pub. He. 14, 425–447.
Wang, J., Feng, X., Anderson, C.W., Xing, Y., Shang, L., 2012. Remediation of mercury contaminated sites–a review. J. Haz. Mat. 221, 1–18.
Yang, Y., Yanai, R.D., Driscoll, C.T., Montesdeoca, M., Smith, K.T., 2018. Concentrations and content of mercury in bark, wood, and leaves in hardwoods and conifers in four forested sites in the northeastern USA. Plos One 13, e0196293.