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Potential of *Castanea sativa* for biomonitoring As, Hg, Pb, and Tl: A focus on their distribution in plant tissues from a former mining district

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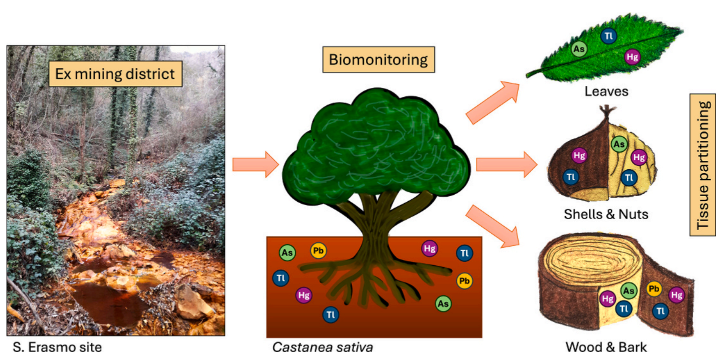
HIGHLIGHTS

- Ore bodies are potential sources of contamination affecting ecosystem functioning.
- Chestnut was tested for Tl, Hg, As, and Pb biomonitoring of rocks and soil.
- Chestnut trees showed high tolerance to very high soil metal(loid) concentrations.
- All metal(loid)s showed a tissue-specific allocation pattern, also in edible fruits.
- Chestnut biomonitoring showed its potentiality in geochemical surveying.

GRAPHICAL ABSTRACT



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ABSTRACT

Bioavailability of potentially toxic elements (PTEs) from the Earth's crust in the soil, e.g., As, Hg, Tl, and Pb, can pose a potential environmental and health risk because of human activities, especially related to mining extraction. The biomonitoring allows to detect PTE contamination through their measurement in living organisms as trees. However, the choice of which plant species and tissue to analyse is a key point to be evaluated in relation to PTE absorption and translocation. The aim of this work was to assess the As, Hg, Tl, and Pb distribution in *Castanea sativa* Mill. plant tissues, given its importance for both biomass and food production. The study identified two sites in the Alpi Apuane (Italy), with similar environmental conditions (e.g., elevation, exposure, forest type, and tree species) but different soil PTE levels. The topsoil was characterized, and the PTE fractions with different bioavailability were measured. The PTE concentrations were also analysed in chestnut plant tissues (leaves, bark, wood, nuts, and shells) in parallel with and evaluation of plant health status through the determination of micro and macronutrient concentrations and the leaf C and N isotope composition ($\delta^{13}C$ or $\delta^{15}N$). Chestnut trees showed a good health status highlighting its suitability for Tl, As, and Pb biomonitoring, displaying a tissue-specific PTE allocation. Thallium and Hg were detected in all plant tissues at similar concentrations, As was found in leaves, wood, and nuts while Pb only in the bark. The $\delta^{15}N$ negatively

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correlated with leaf Mn and Tl concentrations, suggesting possible changes in N source and/or plant metabolism due to the high contamination level and acid soil pH. Thallium in La Culla site trees was associated with its presence in the carbonate rocks but not in the topsoil, highlighting the potentiality of chestnut in providing valuable information for geochemical surveying.

1. Introduction

The ore bodies are mineralogically and/or geochemically anomalous rock volumes in the Earth's crust where one or more elements, e.g., As, Sb, Hg, and Tl, may be enriched in relation to the average crustal composition (Wedepohl, 1995). The ore bodies have provided essential resources for the development of human society but, at the same time, can pose a potential environmental and health risk depending on the presence of PTEs and their chemical forms. The increasing demand for different commodities poses serious challenges on how to manage and moderate this risk. Thus, quantifying and understanding the linkages between PTEs both from natural and anthropogenic sources, and the exposure pathways that mediate their connection with biological receptors, is a prime topic for mitigating the risk.

The detection of PTEs in natural samples such as plants is relevant for the wide and prompt availability of materials to be analysed (Zaghloul et al., 2020). The biomonitoring assesses the potential hazardous exposure to contaminates through the systematic measurement of compounds in living organisms, presenting a valuable tool for both environmental and human health monitoring (Costa and Teixeira, 2014). The application of biomonitoring requires adequate sample collection and cautious data interpretation to improve knowledge and to increase the protection of public and environmental health (Abdullahi et al., 2022). Plants might be useful bioindicator for the monitoring of ecosystem contaminations through qualitative (e.g., visible symptoms) and quantitative (e.g., PTE tissue concentrations, plant physiology measurements) criteria (Zaghloul et al., 2020). However, possible mechanisms of exclusion, accumulation, and/or partitioning of some elements adopted by specific plants/varieties must be considered to select bioindicators with a high sensitivity for the investigated contaminant (Gautam et al., 2022). At present, proxies for investigating the environmental contamination using trees are defined by leaves, bark, and wood with different time resolutions for the exposure to PTEs (Cocozza et al., 2016). Therefore, the most sensitive plant tissue to be used for biomonitoring must be also accurately selected.

The hypothesis of this study is that plant tissues can reveal specific signals of environmental contamination based on specific mechanisms of metal(loid) partitioning. Therefore, different plant matrices were considered to assess the best outcome of tissue-environmental pollution monitoring for PTEs. For this purpose, a widely studied area in the southern Alpi Apuane (northern Tuscany, Italy) was chosen, i.e., the Sant'Anna tectonic window (SAtw) where pyrite ore is characterized by high levels of PTEs such as Tl, As, and Hg (D'Orazio et al., 2017) occurring in the densely inhabited areas of the Versilia coast. The area has been subjected to recent detailed geological and hydrogeological studies, following the discovery of the Tl-rich nature of pyrite ores and the detection of a severe Tl contamination of the drinkable water from the public distribution system of the Valdicastello Carducci-Pietrasanta zone (Biagioni et al., 2017).

The monitoring of plant health status allows to assess the effect of pollutants on plant performance, reflecting their sensitivity to the contamination and environmental changes (Zaghloul et al., 2020). Carbon and nitrogen stable isotope compositions ($\delta\ ^{13}C$ and $\delta\ ^{15}N$) are considered powerful tools to study ecological processes over space and time and record environmental changes (Cotrufo and Pressler, 2023). Among the environmental factors affecting stable isotope signature in plants, Soba et al. (2021) found significant correlations between $\delta^{13}C$ and $\delta^{15}N$ and heavy metals, especially Pb, Cr, and Cd, highlighting the effectiveness of leaf stable isotope profiles as a novel complementary heavy metal biomonitoring tool. Moreover, plant health status can be monitored also considering the level of macro and micro essential nutrients and their fluctuations under stress (Barker and Pilbeam, 2015).

Among the tree species located in the study areas, chestnut was chosen for its dual role as a tree used for biomass and food production. *Castanea sativa* Mill. is the predominant species of its genus found in Europe and Turkey and is one of the main cultivated trees of the genus for its starchy nuts. The presence of metal(loid)s, specifically Pb, As, Cd, Cr, and Hg, has been already reported in nuts from trees located in contaminated soils in China (Wu et al., 2019). Therefore, the efficacy of chestnut trees in pollutant uptake deserves to be investigated. The analysis of chestnut woody rings reflected the atmospheric Hg changes in areas affected by mining activity and geothermal power production (Fornasaro et al., 2023) suggesting the efficacy of this species in metal biomonitoring of air contamination remarking the need to assess its response under an ore contamination. Therefore, the concentration of macro- and micro-nutrients, and PTEs in leaves, bark, wood, nuts, and shells of chestnut trees, and in the topsoil, were analysed in two different sites (S. Erasmo and La Culla) characterized by similar environmental parameters (i.e., elevation, exposure, forest type, and tree species) but different levels of PTE contamination related to the geochemical history of the site. The main non-essential metal(loid)s considered were Tl, As, Hg, and Pb for their high- to very high-concentrations in ores and rocks (e.g., D'Orazio et al., 2017; Vezzoni et al., 2020) and topsoils, over the average concentrations in the upper crust (0.75 mg kg^{-1}, 2.0 mg kg^{-1}, 0.056 mg kg^{-1}, and 17 mg kg^{-1}, respectively; Wedepohl, 1995) and the regulatory limits for Italy (1/10 mg kg^{-1}, 20/50 mg kg^{-1}, 1/5 mg kg^{-1}, 100/1000 mg kg^{-1} in urban/industrial soils, respectively; Legislative Decree 152/06). The PTE partitioning among different plant tissues was studied in relation to their bioavailability in soil rather than only considering their total amount.

Thus, the aims of this work were: i) to assess the PTE compartmentalization in different plant tissues evaluating possible differences due to the presence of a high or a low level of natural background contamination; ii) to evaluate the effect of different PTE levels on chestnut health status using C and N stable isotopes and nutrient analyses.

2. Material and methods

2.1. Study area

The study area is located in the south-western part of the Alpi Apuane, a mountain range of approximately 50 km long, reaching nearly 2000 m a.s.l., and located at < 10 km from the coast of the Ligurian Sea (Fig. 1). The Alpi Apuane are one of the rainiest areas of Italy (≥3000 mm year^{-1}), due to its morphological and geographical position, which favors the rapid cooling of humid air masses of Atlantic and western Mediterranean origin. Rainfall is mainly concentrated in the autumn and spring (Doveri et al., 2019; Amaddii et al., 2023). From a geological point of view, the Alpi Apuane are the largest tectonic window in the inner northern Apennines, where the sedimentary to anchi-metamorphic succession of the Falda Toscana unit overlaps greenschist facies metamorphic rocks belonging to the Massa and Apuane units (Molli et al., 2020). The study area is located near the contact between the Apuane and the overlying Falda Toscana units, in a zone known with the name of SAtw. In this zone, the Apuane unit mainly consists of a Cambrian-lower Ordovician quartzite, phyllitic quartzite, and phyllite (Filladi inferiori Fm; Paoli et al., 2017; Pieruccioni et al., 2023 and references therein), intruded by middle Permian magmatic rocks

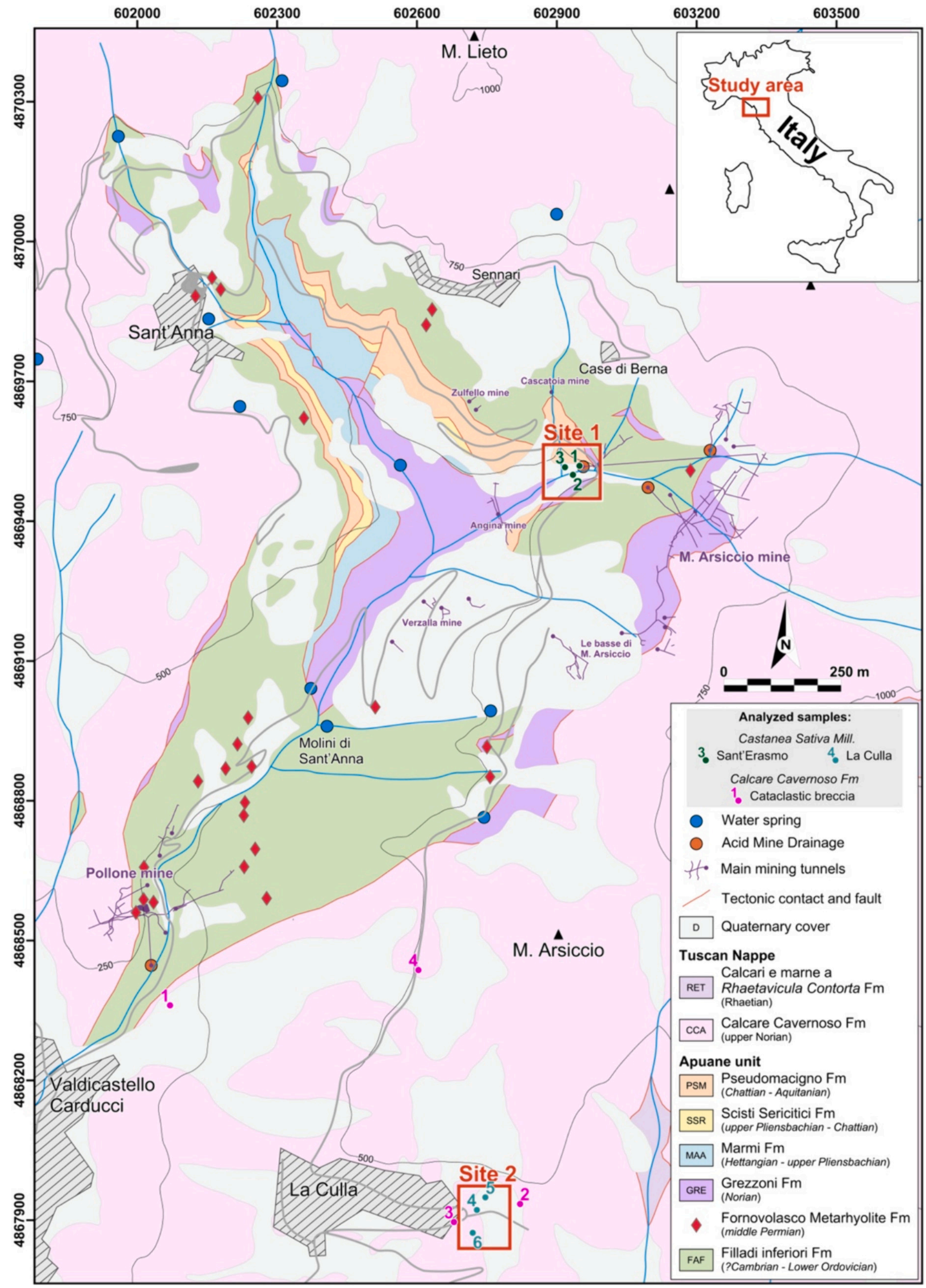

Fig. 1. Geological map of the Sant'Anna tectonic window (modified after Conti et al., 2010) and localization of sampling areas (Site 1: S. Erasmo; Site 2: La Culla, green and blue dots indicate plant and soil sampling points, pink dots indicate rock sampling points). The map also shows the location of the main abandoned mines, acid mine drainages, water springs.

(Fornovolasco metarhyolite Fm). The Paleozoic rocks locally suffered hydrothermal alteration (tourmalinization and sericitization) related to the Permian magmatic cycle (Vezzoni et al., 2020 and references therein). The metasedimentary covers are mainly made up of metadolostone (Grezzoni Fm), marble (Marmi Fm), calcschist with intercalations of green phyllite (Scisti Sericitici Fm), and quartz-feldspathic metasandstone (Pseudomacigno Fm; Conti et al., 2010). The Falda Toscana unit is almost exclusively represented by Upper Norian evaporites (Calcare Cavernoso Fm) (Molli et al., 2018). The contact between Apuane and Falda Toscana units is marked by a cataclasite containing clasts of both the metamorphic and non-metamorphic units (Conti et al., 2012, 2019; Cornamusini et al., 2024). The SAtw, as well as the southern Alpi Apuane, hosts several small polymetallic ore bodies (Lattanzi et al., 1994; D'Orazio et al., 2017). The ore bodies are hosted in the hydrothermalized Filladi inferiori Fm (Vezzoni et al., 2020) except for a minor Cu(-Au) ore hosted in the metadolostone (Grezzoni Fm) (Lattanzi et al., 1994).

The volumetrically most important ore type consists of pyrite ± baryte ± Fe-oxides. The main ore bodies were exploited by the Pollone and Monte Arsiccio mines, whereas minor bodies (e.g., Verzalla and Cascatoia mines) occur widely in the hydrothermalized basement of the SAtw, although the modal abundance of pyrite ± baryte is highly variable (Vezzoni et al., 2020). Sometimes, a Pb-Zn(-Ag) ore is spatially associated with the pyrite ± baryte ± Fe-oxides ore (e.g., Pollone mine), or it occurs as small bodies (e.g., Zulfello mine). These ore-types were discontinuously exploited almost from the Renaissance time (1500s) until the end of the 1980s, when mining activities at Pollone and Monte Arsiccio mines ceased (Biagioni et al., 2016; D'Orazio et al., 2017).

The Alpi Apuane ores have been the subject of renewed scientific interest due to the discovery of Tl-sulfosalts and the Tl-rich nature of pyrite ores (D'Orazio et al., 2017 and reference therein). These discoveries allowed the identification of high concentrations of Tl and other PTEs (e.g., As, Hg, and Sb) in the acid mine drainages (AMDs) of the Pollone and Monte Arsiccio mines (Perotti et al., 2018), as well as the occurrence of Tl between approximately 2 and 10 μg L^{-1} in the public aqueduct of Valdicastello Carducci and part of that of Pietrasanta town. After the report to the public authorities, in October 2014, the Mayor of Pietrasanta disposed a "Not to drink" order for the inhabitants of Valdicastello Carducci. The source of contamination was identified in the Molini di Sant'Anna water spring, with a Tl content of up to 37 μg L^{-1}. The southern Alpi Apuane has, therefore, become a prime case for studying the mechanism of the passage of Tl and other PTEs from the lithosphere to the hydrosphere and biosphere (Biagioni et al., 2017; D'Orazio et al., 2020; Ghezzi et al., 2023).

Two sampling areas were selected for this investigation, characterized by similar elevation and climatic conditions, but different geological and site-specific features, such as the presence of AMDs, i.e., S. Erasmo and La Culla sites (Fig. 1). Sweet chestnut (*Castanea sativa* Mill.) is the most diffused chestnut in Europe and a very common species in the woods of the southern Alpi Apuane, cultivated for the nuts, which were traditionally employed as food or to produce flour for making bread. The nuts are normally 2–3, contained in a thorny shell, the bur; each nut has a shell, the coriaceous epicarp (Bounous and Marinoni, 2010). In each area, three sampling points for the soil and plant tissues were selected based on chestnut tree dimension and sociological position in the forest as well as the distance to the AMD of S. Erasmo tunnel (Monte Arsiccio mine).

2.1.1. S. Erasmo sampling site

The S. Erasmo sampling site is in the Monte Arsiccio mining area in the upper part of the Baccatoio stream at approximately 490 m a.s.l. The site is located below the pyrite-rich orebodies and hydrothermalized Filladi inferiori Fm, where it crops out a metasandstone attributed to the Pseudomacigno Fm. The Baccatoio stream originates from an abandoned tunnel of the Monte Arsiccio mine (Ribasso Pianello tunnel) and, in the upper part, receives AMDs from the S. Olga and S. Erasmo tunnels. The sampling area is in a valley downstream of the lowest AMD (S. Erasmo tunnel). Acid mine drainages and Baccatoio stream waters have recently been investigated, and, at present, their physicochemical parameters are well defined. The S. Erasmo AMD has an average flow rate of ~ 1.8 L s^{-1} although it shows large variation between 0.08 L s^{-1} and 6.0 L s^{-1} in close temporal relation to rainfall. The other physicochemical parameters reflect similar variability with an acidic pH, from strong to moderate (between 1.5 and 5.8), electrical conductivity (EC) from low to high (between 313 and 8970 μS cm^{-1}), and several elements exceeding the limits set by the Italian Regulation for groundwater (e.g., Mn, Fe, As, Ni, Cd, Sb, and Tl). As already described, AMDs flow directly into the stream; therefore, the upper part of the Baccatoio stream waters reflects similar parameters, although with lower concentrations of metal (loid)s (Petrini et al., 2016; Perotti et al., 2018; Ghezzi et al., 2021).

Three chestnut trees were selected at different distances from the S. Erasmo tunnel. Chestnut tree#1 was approximately 10 m in front of the S. Erasmo tunnel, tree#2 at approximately 25 m along the Baccatoio stream, and tree#3 at approximately 40 m in proximity to a left tributary of the Baccatoio stream (Fig. 1).

2.1.2. La Culla sampling site

The second sampling site was located east of La Culla village, approximately 450 m a.s.l. The bedrock is largely covered by the soil and the site is terraced and actively cultivated for chestnuts. No streams or water springs are present. The sparse outcrops are carbonate-rich cataclastic breccia characterized by metamorphic and non-metamorphic clasts. Specifically, Filladi inferiori clasts can be found in the cataclasite. At present, geochemical analysis of cataclastic breccia are not available and we collected four samples at different distance from the tectonic contact with the Apuane unit to evaluate composition (see Fig. 1, pink dots). Also at this site, three chestnut trees were randomly selected, as reported in Fig. 1 using the criteria described above (tree #4, tree#5, and tree#6).

2.2. Plant and soil sampling

For each selected tree, leaves, bark, wood, and nuts were collected in October 2022. Bark and wood were collected at 1 m from the bottom with a clean scalpel by sampling the xylem in the last 3 cm of growth. Leaves, bark, and nuts were surface washed with MQ-H_2O. The shell (pericarp) was removed from the nuts and processed separately from the inner part, hereinafter referred to shells and nuts. Plant tissues were oven-dried at 45 °C for a week. A topsoil bulk sample (0–20 cm deep), composed by three subsamples, was collected near the roots of each tree. Soil samples were air dried and then the 2 mm fraction was collected using a clean sieve.

2.3. Soil characterization

Soil texture was analysed by a Mastersizer 2000 (Malvern Panalytical Ltd., Malver, UK) determining silt, sand, and loam fractions. For total organic carbon (TOC), the soil samples were treated with HCl: H_2O (1:1 v:v) and TOC was determined by dry combustion using a Flash Smart NC Soil elemental analyser (Thermo Fisher Scientific, Waltham, MA, USA). Soil pH and EC were measured in water extract 1:2.5 and 1:5, respectively, using specific electrodes (ASA-SSSA, 1996).

2.4. Mineral element quantification in rocks, plant tissues, and soil, metal extractability in soil

For each rock sample of cataclastic breccia, about 200 mg of powder was dissolved into 30 mL perfluoralkoxy (PFA) vials at about 160 °C with a HF:HNO_3 mixture (4:1 v:v). The total concentration of Ni, Zn, and Tl was determined using inductively coupled plasma spectrometry (ICP-OES 5900 Agilent, Santa Clara, CA, USA) and expressed as mg kg^{-1}.

Oven-dried leaf, bark, wood, nuts, and shell samples were ground to a fine powder by a mill (Tube Mill, IKA®-Werke GmbH & Co. KG, Staufen, Germany). An amount of 0.30 g of powder from each plant and soil sieved sample was digested by a microwave (ETHOS 900, FKV, Torre Boldone, BG, Italy) using a HNO_3:H_2O_2 mixture (5:2 v:v), following the EPA Method 3051a for the soils and the EPA Method 3052 for the plants. The total concentration of K, Ca, Mg, Fe, Mn, Na, Zn, Tl, Pb, Cd, As, Hg, Cu, V, Ni, and Cr was determined as reported for rocks. The available fractions of PTEs in soil were determined as reported by Pedron et al. (2009) for Tl and Pb, and Grifoni et al. (2017) for As and Hg. In short, the available fractions of Tl and Pb in soil samples were determined after a sequential extraction procedure using ultrapure milli-Q water, followed by KNO_3 1 M, and EDTA 1%. Instead, the available fractions of As and Hg were determined using sequential extractions with $NaNO_3$ 0.1 M followed by KH_2PO_4 0.1 M for As and NH_4Cl 0.1 M at pH 7 and $(NH_4)_2S_2O_3$ 0.27 M for Hg.

2.5. Leaf isotopic analyses

Carbon and N isotopic ratios were determined in leaf samples to identify stress related patterns occurring in plant physiology. Samples (1–2 mg DW) were combusted into an elemental analyser (Model NA 1500, Carlo Erba, Milan, Italy) and carried by a He stream to an isotope ratio mass spectrometer (Isoprime Ltd., Cheadle, UK). The isotope ratios of C ($R = {}^{13}C/{}^{12}C$) and N ($R = {}^{15}N/{}^{14}N$) were measured to calculate C and N isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$) referring to the Vienna–Pee Dee Belemnite (VPDB) and atmospheric N_2 standards, respectively, as follows: $\delta^{13}C$ or $\delta^{15}N = R_{sample}/R_{standard} - 1$. Moreover, the percentage of C and N and the C/N ratio were also calculated.

2.6. Statistical analysis

Data were tested for normal distribution using Shapiro-Wilk test and eventually transformed before *t*-test analysis ($P < 0.05$). All the statistical analyses and graphs were done with Prism 10 software (GraphPad, La Jolla, CA, USA). Correlation matrixes were realized using R studio “corrplot” package.

3. Results

3.1. Cataclastic breccia geochemical analyses

The geochemical analyses of the cataclastic breccia showed variable contents of Tl from around 4.5 to 12.1 mg kg^{-1} (Table 1), enriched respect to the average upper continental crust composition of 0.75 mg kg^{-1} (Wedepohl, 1995). The enrichment is most remarkable if compared with the typical values of carbonate rocks, around 0.14 and 0.37 mg kg^{-1} (e.g., Gao et al., 1998). Thallium content decreased moving away from the tectonic contact with the underlying Filladi inferiori, and the two samples in La Culla sampling site had a content between 4.5 and 5 mg kg^{-1}. The Zn content was variable between 35 and 92 mg kg^{-1}. The Ni content was below the limit of quantification, in agreement with the low level of Ni content in the pyrite ± baryte ± Fe-oxides Alpi Apuane ores hosted in the Filladi inferiori Fm (e.g., D’Orazio et al., 2017). The Pb content was between 2.11 and 8.3 mg kg^{-1}.

Table 1
Tl, Zn, Ni, and Pb concentrations in cataclastic breccia samples (mg kg^{-1} dry weight). <LQ = below the limit of quantification.

Parameter	Rock samples			
	1	2	3	4
Tl	12.1	4.5	4.9	8.8
Zn	43	35	63	92
Ni	<LQ	<LQ	<LQ	<LQ
Pb	5.3	3.21	2.11	8.3

3.2. Soil analyses

The physical-chemical characterization of S. Erasmo and La Culla topsoils highlighted a high variability among samples, especially in S. Erasmo site (Table 2). The EC was similar for both sampling areas (on average 1.39 dS m^{-1} in S. Erasmo and 1.74 dS m^{-1} in La Culla). The pH was on average 7.3 in La Culla, while it largely varied in S. Erasmo with values ranging from 4.0 to 6.8. The analysis of soil texture highlighted a higher fraction of sand (on average 69%) and a lower fraction of silt (on average 25%) in La Culla than in S. Erasmo (on average 46% of sand and 45% of silt). Indeed, the soil was classified as loam-sandy loam in S. Erasmo and loam-loamy sand in La Culla.

A high variability among the sampling points was also retrieved in TOC, macro- and micro-element concentrations, particularly in S. Erasmo site. The greatest differences were found in point of sampling 1 in S. Erasmo where some elements, such as K, Fe, and V, were higher compared to the other sampling points in both study areas while other elements, such as Ca, Mg, Mn, Cr, and Ni, were lower.

The analysis of total and potentially bioavailable PTEs highlighted a high level of contamination in S. Erasmo site, particularly in the sampling point 1 (Tables 2 and 4). Thallium was only found in S. Erasmo topsoil samples with total values ranging from 1808 to 17.8 mg kg^{-1} DW (Table 2). However, despite the high variability in total Tl concentration, the amount bioavailable for plants was similar among the three sampling points (Table 3). The different pH of the sampling points affected the availability of Tl in different extractants. Indeed, Tl was easily available in water in sampling point 1 due to the low soil pH, while it was not detected in EDTA. On the contrary, Tl was not available in water in sampling points 2 and 3 while it was mostly available in EDTA. The lower pH of sampling points 1 and 3 also favoured the availability of Tl in KNO_3 while it was undetected in this extractant in sampling point 2, which showed a soil pH almost neutral. Arsenic was only quantifiable in soil samples of S. Erasmo site, with total values ranging from 120 to 850 mg kg^{-1} DW (Table 2). The fraction most easily available ($NaNO_3$) was low in all sampling points, while the fraction less available (KH_2PO_4) was higher (Table 3). Mercury was detected in both study areas with the highest concentration in S. Erasmo with total values ranging from 1.31 to 24.1 mg kg^{-1} DW while the average total value measured in La Culla was 0.82 mg kg^{-1} DW (Table 2). The fraction most easily available (NH_4Cl) was similar in both study areas. On the contrary, the fraction less available ($(NH_4)_2S_2O_3$) was higher in S. Erasmo, particularly in sampling points 1 and 2 (Table 3). Lead was detected in both study areas with the highest values in S. Erasmo with total values ranging from 131 to 467 mg kg^{-1} DW while the average total value measured in La Culla was 194 mg kg^{-1} DW (Table 2). At both site, Pb was unavailable in water in all sampling points and in KNO_3 in three sampling points. It was mostly available in EDTA with the highest values measured in La Culla with the only exception of S. Erasmo sampling point 1 in which the fraction available in EDTA was only 0.40 mg kg^{-1} DW (Table 3).

3.3. Plant analyses

In S. Erasmo, the concentration of As was on average 2.49 ± 0.01 mg kg^{-1} DW in leaves (Fig. 2A) and 3.04 ± 0.48 mg kg^{-1} DW in wood (Fig. 2G), while it was below the limit of quantification in bark samples (Fig. 2D). Arsenic was non quantified in leaf, bark, and wood samples collected in La Culla (Fig. 2A, D, G), in agreement with its not quantification in topsoil samples (Table 2). Mercury was found in all the analysed plant tissues, with no significant differences between S. Erasmo and La Culla (0.82, 0.86, and 0.82 mg kg^{-1} DW in leaves, bark, and wood, respectively, as average values of both study areas, Fig. 2A, D, G). Lead was only retrieved in the bark samples collected in S. Erasmo (3.87 ± 2.67 mg kg^{-1} DW Fig. 2D). Thallium was quantified in all plant tissues without significant statistical differences between S. Erasmo and La Culla samples, except for the leaves, due to the high variability among

Table 2
Topsoil parameters measured in each sampling points of S. Erasmo (1, 2, and 3) and La Culla (4, 5, and 6) sites. Avg = average value, EC = electrical conductivity, TOC = total organic carbon; <LQ = below the limit of quantification, DW = dry weight. *t*-test *P*-values comparing S. Erasmo and La Culla site are reported in the table (*, $P < 0.05$; ns, not significant).

Parameter	Unit	S. Erasmo				La Culla				*P*-value
		1	2	3	Avg	4	5	6	Avg	
EC	dS m^{-1}	2.72	1.24	0.22	1.39	2.15	1.24	1.84	1.74	ns
pH		4.0	6.8	5.6	5.5	7.4	7.4	7.2	7.3	*
TOC	%	13.1	6.7	6.5	8.8	4.2	6.1	4.3	4.9	ns
Ca	g kg^{-1} DW	1.87	28.3	0.91	10.4	1.69	19.7	1.81	7.8	ns
K		12.8	0.89	0.78	4.8	2.24	2.55	0.79	1.86	ns
Mg		0.57	7.3	2.39	3.4	4.3	5.0	3.4	4.2	ns
Fe		53.3	26.7	18.3	33	22.0	18.5	16.6	19.0	ns
Mn	mg kg^{-1} DW	155	5219	1059	2144	553	751	542	616	ns
Zn		84	170	229	161	95	152	65	105	ns
Na		1240	1385	308	977	149	195	71	139	*
Cr		<LQ	0.82	16.5	5.8	33.5	24.9	19.8	26.0	*
Cu		16.5	15.3	30.0	20.6	26.2	29.1	24.8	26.7	ns
V		80	15.2	20.4	39	42.8	36	28.5	36	ns
Ni		3.7	5.0	13.2	7.3	27.5	22.9	14.1	21.5	*
Cd		<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	–
As		850	121	120	364	<LQ	<LQ	<LQ	<LQ	–
Hg		19.7	24.1	1.31	15.0	0.84	0.86	0.78	0.82	ns
Pb		467	131	335	311	183	175	223	194	ns
Tl		1808	69	17.8	632	<LQ	<LQ	<LQ	<LQ	–

Table 3
Concentrations and average values (mg kg^{-1} dry weight) of potentially available Tl, As, Hg, and Pb, extracted by sequential procedures, and residual fractions in the topsoil of each sampling points of S. Erasmo (1, 2, 3) and La Culla (4, 5, 6) sites. *t*-test *P*-values comparing S. Erasmo and La Culla site are reported in the table. Avg = average value, <LQ = below the limit of quantification.

PTE	Extractant	S. Erasmo				La Culla				*P*-value
		1	2	3	Avg	4	5	6	Avg	
Tl	Water	0.60	<LQ	<LQ	<0.60	<LQ	<LQ	<LQ	<LQ	–
	KNO_3	4.7	<LQ	0.45	<2.6	<LQ	<LQ	<LQ	<LQ	–
	EDTA	<LQ	8.1	4.8	<6.5	<LQ	<LQ	<LQ	<LQ	–
	Residual	1803	61	12.6	625	<LQ	<LQ	<LQ	<LQ	–
As	$NaNO_3$	0.08	0.06	0.06	0.07	<LQ	<LQ	<LQ	<LQ	–
	KH_2PO_4	7.9	0.30	0.30	2.83	<LQ	<LQ	<LQ	<LQ	–
	Residual	842	121	119	364	<LQ	<LQ	<LQ	<LQ	–
Hg	NH_4Cl	0.31	0.29	0.60	0.40	0.21	0.48	0.24	0.31	ns
	$(NH_4)_2S_2O_3$	7.0	4.0	0.09	3.7	0.45	0.35	0.16	0.32	ns
	Residual	12.4	19.8	0.62	15.0	0.19	0.03	0.38	0.82	ns
Pb	Water	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ	–
	KNO_3	<LQ	<LQ	1.62	<1.62	0.75	<LQ	0.22	<0.49	–
	EDTA	0.40	24.0	90	38	97	71	105	91	ns
	Residual	467	107	244	272	86	104	118	103	ns

plant replicates, even thought it was higher in S. Erasmo than in La Culla also in other plant compartments (22.9 ± 15.9 vs. 6.1 ± 0.5 mg kg^{-1} DW in bark and 8.8 ± 3.6 vs. 4.7 ± 0.5 mg kg^{-1} DW in wood, Fig. 2D, G). Plant microelements (Fe, Na, Mn, Zn, and Cu) were generally lower in La Culla than in S. Erasmo plants (Fig. 2B, E, H). However, the only significant difference was the lower Mn concentration in leaves and wood and the lower Zn in wood of La Culla trees. The macroelement concentrations were similar in S. Erasmo and La Culla plant samples (Fig. 2C, F, I).

In S. Erasmo, As was on average 3.33 ± 0.83 mg kg^{-1} DW in chestnut nuts (Fig. 3A) while it was below the quantification limit in the shell samples (Fig. 3D). Arsenic was not quantified in the shell and nut samples collected in La Culla (Fig. 3A, D), as well as in the other plant tissues. Mercury was found in both shells and nuts (Fig. 3A, D), and it was higher in S. Erasmo nuts than in La Culla ones (on average 0.55 mg kg^{-1} DW). Lead was undetected in shell and nut samples in both the study areas. Interestingly, thallium was found in both shells and nuts with similar concentrations in S. Erasmo and La Culla samples. The microelement concentrations were generally lower in La Culla than in S. Erasmo chestnuts (Fig. 3B, E), particularly Fe, Mn, Zn, and Cu in the nuts and Mn and Zn in the shells. The macroelement concentrations were similar in S. Erasmo and La Culla in both nut and shell samples (Fig. 3C, F). Cadmium, Cr, Ni, and V were not quantified in all plant samples (data not shown).

The analysis of C and N isotopic compositions in leaves highlighted lower average values of $\delta^{13}C$ and $\delta^{15}N$ in S. Erasmo compared to those in La Culla (Table 4). In addition, the percentage of C and N was similar in both study areas while the C/N ratio was higher in La Culla trees.

3.4. Correlation matrix and regression plots

A correlation matrix among the element concentrations and the isotopic compositions in the leaves is reported in Fig. 4. Regarding the PTEs, As concentration was negatively correlated with Ca concentration, Hg did not show any significant correlation, Tl showed a highly significant positive correlation with Mn concentration while a negative correlation with the $\delta^{15}N$. Lead was not quantified in leaves, therefore it is not reported in the matrix. Regarding the other elements, Mn was negatively correlated with the $\delta^{15}N$, as reported for Tl. Iron had a negative correlation with %N while it positively correlated with the C/N ratio. Finally, Mg was negatively correlated with the $\delta^{13}C$.

The analysis of regression between $\delta^{15}N$ and Tl concentration and

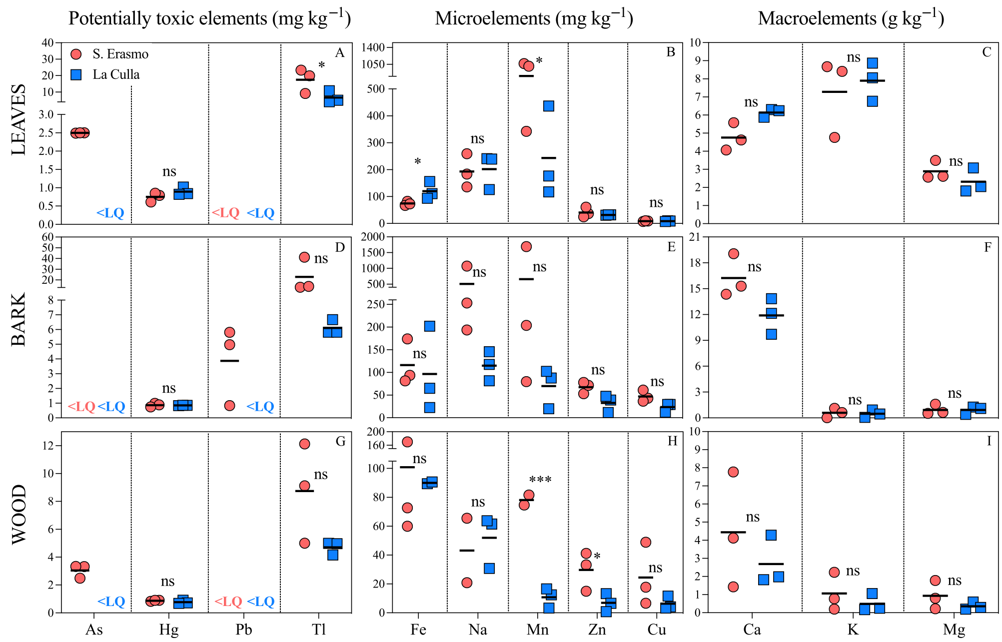

Fig. 2. Concentration of potentially toxic elements (A, D, and G), microelements (B, E, and H), and macroelements (C, F, and I) in the leaves, bark, and wood of chestnut trees in S. Erasmo and La Culla sites. The mean values are reported as a black line for each dataset. *t*-test *P*-values comparing S. Erasmo and La Culla site are reported in the figure (*, $P < 0.05$; ***, $P < 0.001$; ns, not significant). <LQ = below the limit of quantification.

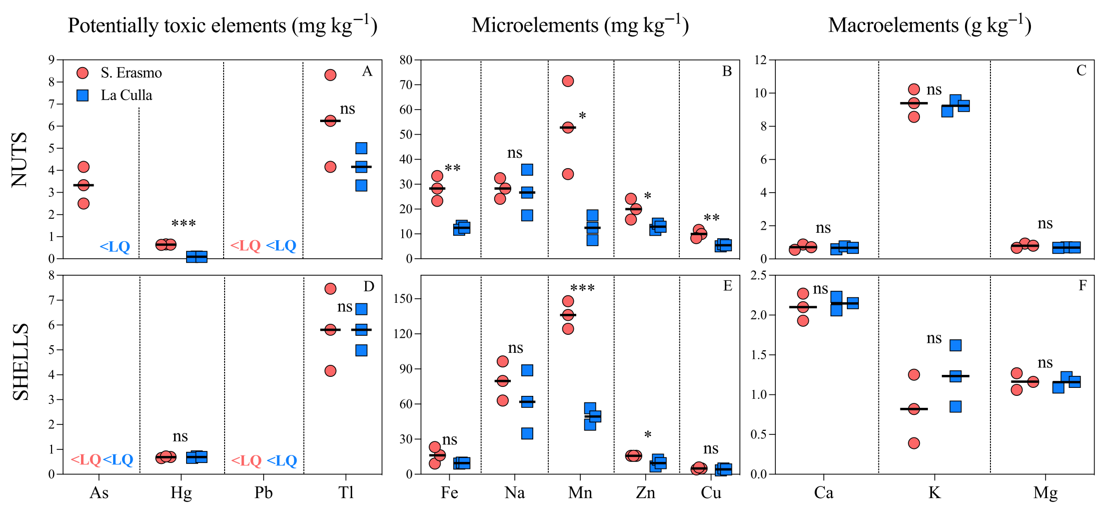

Fig. 3. Concentration of potentially toxic elements (A, D), microelements (B, E), and macroelements (C, F) in the nuts and shells of chestnut plants from S. Erasmo and La Culla sites. The mean values are reported as a black line for each dataset. *t*-test *P*-values comparing S. Erasmo and La Culla site are reported in the figure (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant). <LQ = below the limit of quantification.

Table 4
Carbon and N isotopic composition (δ^{13}C and δ^{15}N), percentages of organic C and total N, and C/N ratio in chestnut leaves from S. Erasmo (1, 2, 3) and La Culla (4, 5, 6) sites. *t*-test *P*-values comparing S. Erasmo and La Culla site are reported in the table (*, $P < 0.05$; ns, not significant).

Parameter	S. Erasmo				La Culla				*P*-value
	1	2	3	Avg	4	5	6	Avg	
δ^{13}C	−30.48	−30.74	−30.43	−30.55	−30.34	−29.45	−28.20	−29.33	ns
C (%)	48.40	48.92	48.19	48.50	46.14	48.83	50.49	48.49	ns
δ^{15}N	−3.89	−1.54	−3.36	−2.93	−2.39	−1.69	−1.35	−1.81	ns
N (%)	2.52	2.78	2.38	2.56	1.92	2.35	2.31	2.20	ns
C/N ratio	19.19	17.62	20.25	19.02	23.97	20.74	21.86	22.19	*

between δ^{15}N and Mn concentration (Fig. 5A, B) highlighted linear relations between both parameters in the leaves. Moreover, a linear relation was found between δ^{13}C and Mg concentration (Fig. 5C).

4. Discussion

Thallium, As, Hg, and Pb were tissue-specific detected in chestnut

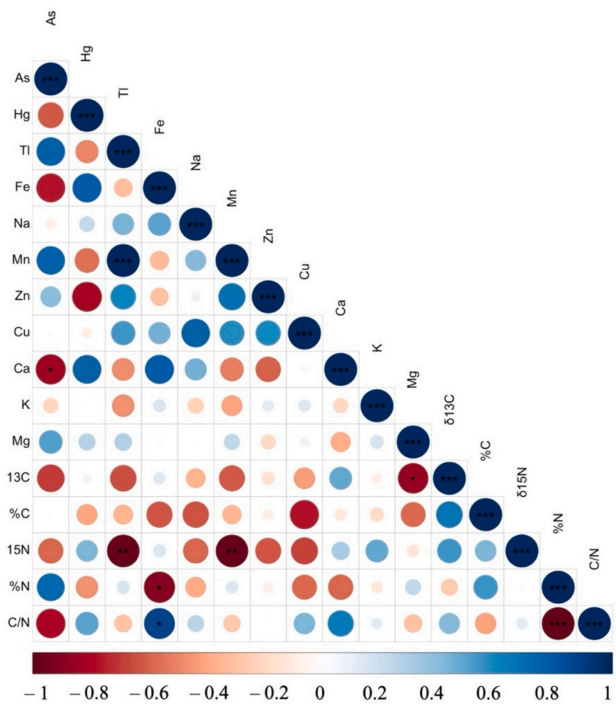

Fig. 4. Pearson correlation matrix among leaf elements and isotopic measures (*, *P*-value < 0.05; **, *P*-value < 0.01; ***, *P*-value < 0.001).

highlighting the potentiality of this tree species for the biomonitoring of contaminants of environmental and human concerns in a well-known geochemically contaminated area. The metal(loid) distribution found among the different plant tissues highlighted the importance of carefully select the most effective plant tissue for the biomonitoring of these non-essential elements. Moreover, the absence of any negative effect of toxicity symptom also in trees located in topsoil with a very high level of contamination highlighted the great tolerance of chestnut for Tl, As, Hg, and Pb.

The PTE quantification in topsoil highlighted a concentration of Tl, As, and Hg above the Italian Regulation limits for soil at S. Erasmo site, exceeding not only the limits set for urban area but also for industrial sites (Legislative Decree 152/06). This geochemical soil anomaly reflects the high content of these metal(loid)s in the pyrite ± baryte ± Fe-oxides Alpi Apuane ores (D'Orazio et al., 2017) and, in general, in the hydrothermalized basement rocks of the SAtw (Vezzoni et al., 2020). The S. Erasmo site is located below the outcrop of the main Monte Arsiccio orebodies along a valley in which the Baccatoio stream is fed by AMDs and crosses through mining dumps. The current environmental conditions reflect a natural geological input to which anthropogenic source(s) related e.g. to mining activities were added. Thus, it is not surprising that chestnut trees had quite high levels of PTEs, particularly Tl, especially in S. Erasmo site. Although literature revealed that, in general, the high bioavailable concentration of PTEs in soils found correspondence with an easier accumulation in plants and other organisms (Karbowska, 2016), Tl uptake by plants was found to be species dependent. Several brassicaceous plants have been reported to behave as (hyper)accumulators of Tl (Pavlíčková et al., 2006; Vaněk et al., 2010; Al-Najar et al., 2003) and also some conifers are able to accumulate Tl in needles, leaves, and wood (Vaněk et al., 2011; Wang et al., 2022). Our study showed peculiar uptake capabilities and high values of Tl also in chestnut trees. The highest values of Tl found in S. Erasmo plants were probably also determined by the lower pH and the higher TOC found in this area, which can improve the Tl bioavailability (Jia et al., 2013). Chestnut showed a high capacity of Tl accumulation since the concentration of this PTE was up to 23 and 12 mg kg^{-1} in the leaves and the wood of plants located in S. Erasmo site, respectively. Instead, in pine trees Wang et al. (2022) found values up to 4 mg kg^{-1} in the leaves and 2 mg kg^{-1} in the wood and Vaněk et al. (2011) up to 0.8 mg kg^{-1} in the wood from Tl-polluted pyrite mine and smelting and primary Zn smelting sites, respectively. This element was highly concentrated also in the chestnut nuts showing a higher concentration than in other edible parts of vegetables collected in the same area, such as cabbage, fennel, onion, and tomato (D'Orazio et al., 2020; Ghezzi et al., 2023).

Geologically, the La Culla sampling site is quite different from the S. Erasmo site. Its bedrock is a carbonate-rich cataclasitic breccia, and there are no ore bodies and AMDs in the neighbouring area (Fig. 1). The topsoil samples have low levels of PTEs, with Tl and As below the quantification limits. However, Tl was found in chestnut plants also in this site. Previous studies on this area have shown a general increase in the concentration of PTEs downward in the soil profile, such as in the sub-soil (Petrini et al., 2016; Ghezzi et al., 2023) similar to what has been reported in other localities (e.g., Voegelin et al., 2015). However, in some cases, a contrast behaviour has been described (e.g., Vaněk et al., 2011). In the absence of external contributions (e.g., AMDs, mining dumps) in La Culla site, the occurrence of Tl in chestnut trees should be linked to a geological source. Indeed, the geochemical analyses of the cataclastic breccia revealed the occurrence of Tl in the Calcare Cavernoso Fm, never previously reported. This lithology is characterized by a medium-high permeability, and it is easily exposed to karst processes (e.g., Doveri et al., 2021). Therefore, the origin of Tl found in trees located in La Culla site might be identified in the cataclastic Calcare Cavernoso Fm probably containing clasts of Tl-bearing mineralized lithologies (i.e., pyrite ore and/or hydrothermalized Filladi inferiori; Vezzoni et al., 2020). The pedogenesis as well as the interaction rock-water may have made Tl (and other PTEs) bioavailable for the chestnut trees. Thus, it is interesting to note that chestnut trees provided chemical data consistent with the ones of the bedrock, suggesting to possibly identify PTE anomalies in rocks, which have not yet been identified. Furthermore, the first identification of Tl in the Calcare Cavernoso Fm, that hosts one of the most important aquifers in this area (e.g., Doveri et al., 2021), opens new assessments of the occurrence of Tl in groundwaters, and the relevance to increase geological knowledge for

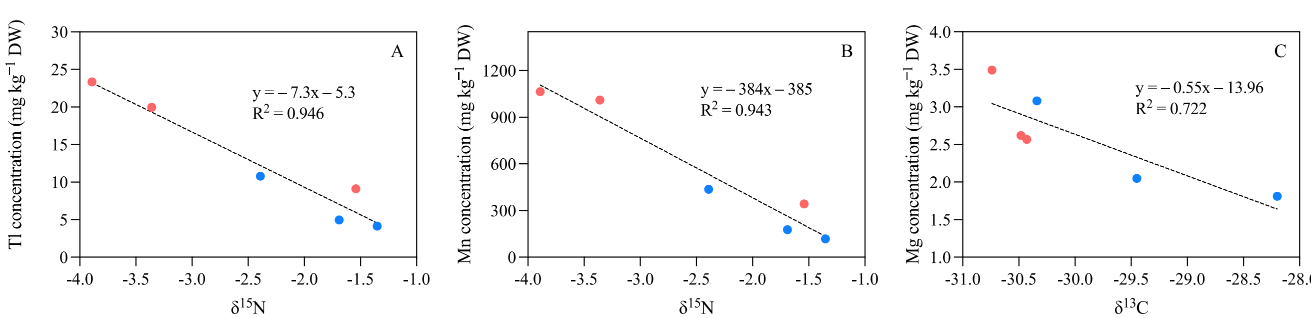

Fig. 5. Regression plots in chestnut leaves between nitrogen isotopic composition ($\delta^{15}N$) and concentration of Tl (A) and Mn (B) and between carbon isotopic composition ($\delta^{13}C$) and Mg concentration (C). Red dots represent plants from S. Erasmo while blue dots correspond to those of La Culla. DW = dry weight.

the managing of natural resources and environmental concerns.

Arsenic was only quantified in S. Erasmo, in agreement with topsoil analyses, showing a specific pattern of compartmentalization in plant tissues (i.e., present in leaves, bark, and nuts, not quantified in wood and shells). Despite the high soil As concentration, this metalloid concentration in the leaves was lower compared to the values found in the other woody species such as maple, oak, or birch (Mleczek et al., 2017). In our study, the As was highly concentrated in the edible part, showing concentrations higher than those found in the leaves. It has been observed that As accumulation and partitioning can be affected by sulfur-mediated thiol metabolism (Dixit et al., 2016). Indeed, chestnut nuts are well known to contain high level of sulfur (Antoniewska-Krzeska et al., 2023). The localization of both As and Hg in chestnut nuts was already found in China by Wu et al. (2019) in soils with a lower As contamination. Interestingly, As was only quantified in nuts while it was unquantifiable in the shells. This result agreed with the compositional analysis obtained using the proton induced X-ray emission (PIXE) technique in which As was only found in nuts while it was undetected in outer and inner shells (Corregidor et al., 2020).

Mercury was found in both study sites in all the analysed plant compartments at similar concentrations. This metal was highly concentrated in the edible part, particularly in S. Erasmo site. Its concentration in the wood was higher compared to the values found in chestnut by Fornasaro et al. (2023) (i.e., 0.70–0.90 mg kg^{-1} in this study compared to 0.01–0.20 mg kg^{-1} from the study of Fornasaro et al. (2023)) probably due to the higher Hg concentration in soil (i.e., 0.40–28.1 mg kg^{-1} in this study compared to 1.7–8.0 mg kg^{-1}).

Regarding the other elements, the main differences were found in Mn concentration that was higher in every plant compartment in S. Erasmo site compared to La Culla site. Tl and Mn trends indicated similar relations with N biogeochemical cycling as highlighted by the regression analysis. However, controversial results are reported in literature regarding this aspect. Chen et al. (2021) suggested that the negative relationship between $\delta^{15}N$ and Mn concentration at the leaf level might be influenced by vegetation and soil type. It has been reported that Mn availability increases with decreasing soil pH and Mn deficiency is most common in soils with a high organic matter content (> 6.0%) and pH above 6.5 (Schulte and Kelling, 1999). Hence, the extremely low soil pH values found in two of the three sampling points of the S. Erasmo site could increase Mn availability to plants, leading to the highest leaf Mn values. Moreover, it has been demonstrated that Tl toxicity is associated with an increase in absorption and accumulation of Mn (Espinosa et al., 2023), partly explaining the highly significant positive correlation between Mn and Tl concentrations. However, it is also possible that this correlation is due to an indirect effect of low pH in increasing the availability of both these elements for plant uptake. According to this hypothesis, an accumulation of Mn has been observed in the wood and nut tissues from S. Erasmo site. On the other hand, it is known that Mn is involved as a cofactor and activator of nitrite reductase (Mukhopadhyay and Sharma, 1991) and Mn deficiency restricts the uptake and transport of NO_3^- and inhibits the activities of N-metabolism-related enzymes (Gong et al., 2011; Tao et al., 2023). It is well known that the soil N sources are isotopically differentiated, being NO_3^- generally ^{15}N more diluted than NH_4^+ due to large fractionation occurring during the nitrification process (Evans, 2001; Robinson, 2001); hence, a change in NO_3^- uptake by plants is expected to affect the values of $\delta^{15}N$ in the leaf. After NO_3^- root uptake, the first step of N assimilation consists in NO_3^- reduction to NO_2^- by the enzyme nitrate reductase, whose activity is dependent on Mn concentration. All these processes can be associated with isotopic fractionations, which modify the $\delta^{15}N$ of plant tissues. Moreover, also Tl toxicity has been associated with changes in N metabolism, with a decrease in the protein content and an accumulation of amino acids in the leaves (Espinosa et al., 2023). Overall, our results suggest that the negative relationships between leaf $\delta^{15}N$ with both Mn and Tl leaf concentrations could be partly due to changes in N source and/or the effects of Mn on the N uptake, translocation, and metabolism.

Carbon isotope composition ($\delta^{13}C$) in leaves is used as a time-integrated indicator of CO_2 partial pressure at the carboxylation site (C_c) and of intrinsic water-use efficiency at different temporal scales (Seibt et al., 2008; Scartazza et al., 2014). Our results showed a negative relationship between $\delta^{13}C$ and Mg concentration in the leaves of the collected plants. It is well known that Mg plays a major role in regulating growth and photosynthetic performance in plants (Hauer-Jákli and Tränkner, 2019), therefore changes in leaf Mg concentration are expected to affect carbon isotope discrimination during the photosynthetic processes and hence leaf $\delta^{13}C$. These results agreed with Tränkner et al. (2016), who reported a decrease of $\delta^{13}C$ values in the shoot biomass of barley plants with increasing Mg supply. The authors attributed this relationship to possible changes in the ratio between C_c and atmospheric CO_2 concentration due to restriction in CO_2 diffusion by stomatal (g_s) or mesophyll (g_m) conductance. In this regard, a reduction in g_m could be related to changes in leaf density (Flexas et al., 2012) due to the accumulation of leaf soluble carbohydrates in plants affected by Mg deficiency (Cakmak et al., 1994). Moreover, the lowest leaf Mg concentrations, associated with the highest $\delta^{13}C$ values, were observed in La Culla site. However, we cannot exclude that the presence of available non-essential metals negatively affecting the plant photosynthetic capacity (Clijsters and Van Assche, 1985) causing a decrease of C_c and hence of leaf $\delta^{13}C$, especially in the S. Erasmo site.

5. Conclusions

Our results highlighted the importance to individuate the optimal plant species and tissue for the biomonitoring of Tl, As, Hg, and Pb. The presence of Tl in the plants from La Culla site, in which this PTE was not found in topsoil, highlighted the possibility that chestnut trees could provide information about the bedrock composition of the study area and thus its potential application for geochemical investigations. The Tl content of samples collected from the cataclastic Calcare Cavernoso Fm support this assertion, although additional field, petrographic, and geochemical data will need to be acquired. The negative relationship found between leaf $\delta^{15}N$ and both Mn and Tl concentrations in the leaves highlighted a possible change in N source uptake in presence of high level of contamination and low soil pH.

In conclusion, after the discovery of Tl-rich nature of the pyrite ores of the Alpi Apuane, the increasing data stress the role of geology, from field survey to geochemical analyses, and the relationship with ecosystems in managing natural resources and environmental concerns.

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CRediT authorship contribution statement

Silvia Traversari: Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Claudia Cocozza:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **Francesca Vannucchi:** Writing – review & editing, Methodology, Investigation. **Irene Rosellini:** Writing – review & editing, Methodology, Investigation. **Manuele Scatena:** Writing – review & editing, Methodology, Investigation. **Francesca Bretzel:** Writing – review & editing, Methodology, Investigation. **Eliana Tassi:** Writing – review & editing, Methodology, Investigation. **Andrea Scartazza:**

Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Simone Vezzoni:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization.

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.

Data availability

Data will be made available on request.

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