Thallium-Transfer from Artificially Contaminated Soil to Young Downy Oak Plants (QUERCUS PUBESCENS WILLD.)

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Abstract— *The aim of this study concerns the observation over time of some young downy oak plants (Quercus pubescens Willd.), grown in a soil artificially contaminated with thallium, to determine i) thallium uptake and concentrations in individual parts (roots, trunks and leaves); ii) thallium transfer capacity from soil to plants; iii) the behavior of growth of affected plants by thallium contamination.*

The value of Bio-concentration Factor (BF) shows the ability of plants to accumulate and concentrate thallium from artificially contaminated soil. Values of BF greater than 1 explain the tendency of Quercus Pubescens Willd. to accumulate thallium in higher concentration than soil. The translocation factor (TF), calculated as the percentage ratio of thallium concentration in aerial parts to thallium concentration in roots, yet asserts a total transfer of thallium through the roots to aerial parts of the plants These data once again demonstrate the roots collapse in the fifth phase (200 days) and the lost of the ability to keep thallium in soil.

The microbial biomass carbon was lower in contaminated soils compared to the controls, and the entity of reduction was proportional to depth. The upper layer showed a decline of microbial population of almost 70%, while in the latter end of soil microbial population was reduced of 30% compared to control.Simultaneously, variations of the enzyme activity in the soil samples showed an increase of arylsulphatase, cellulase and β-glucosidase activity but only in the latter part of top soil (10-15 cm) while other enzymes exhibited a remarkable reduction of their activity in both soil layers, compared to the control.

I. INTRODUCTION

Thallium (Tl) is a little studied heavy metal although it has been reported to be extremely toxic for all organisms in both oxidation states: the more stable mono form Tl(I) (+1 thallous), which tends to create stable toxic complexes with sulphur-containing compounds, and trivalent form Tl(III) (+3 thallic) (Pavlickova et al., 2006; Queirolo et al., 2009; Mercurio and Hoffman, 2011).

Despite its minor consideration, thallium has been classified as one of the most pollutant metals for mammals (Queirolo et al., 2009) and his toxicity seems to be similar to Hg and higher compared to Cd, Pb, Cu and Zn. (Lehn and Bopp,1987;Ralph et al., 2002; Lan et al., 2005; Vanek et al., 2011; Alvarez-Ayuso et al., 2013). Thallium is widely distributed in the environment in very low concentrations. The average Tl concentration is 0.1 -1.0 μg g^{-1} in the lithosphere, 0.01–0.02 μg l^{-1} in seawater, and 0.01-14 μg l^{-1} in fresh water (Queirolo et al., 2009). Furthermore, there is an increasing demand for thallium in the high-technology and future-technology fields (Nriagu, 2003), and by limited data available in literature on its pollution, it seems that Tl level in soils may increase near thallium-emitting industrial sources and hazardous waste sites. Thallium is released into the environment from natural processes, such as the oxidation of pyrite containing thallium impurity, and from industrial operations, which use high temperature processes, as in steel industry, coal combustion, smelting processes and cement production (Bojakowska et al., 2013;Stafilov et al., 2013; Karbowska et al., 2014).

Generally, the average of geogenic Tl content in soil is less than 1 μg g^{-1}all over the world (Tremel et al., 1996; Madejon, 2013), even if, high concentration of thallium (>50 μg g^{-1}) has been reported in Silesian and Krakowian Provinces soil in Poland (Lis et al., 2003; Yang et al., 2015), in Guizhou Province in China (Xiao et al., 2004a, 2004b; He et al., 2007; Jia et al., 2013), and in Republic of Macedonia (Stafilov et al., 2013) especially in the vicinity of heavy metals mining or volcanic areas. Furthermore in 1997, very high thallium concentrations have been found in some arable soils in France (Tremel et al., 1997) and Turkey (Sasmaz et al., 2007).

In Italy, high thallium concentration has been reported in the Apuan Alps (Biagioni et al., 2013, 2014a, 2014b, 2014c), in the Julian Alps (Fellet et al., 2012), and in volcanic soil of Ischia Island (Frattini et al., 2006). Thallium phytoavailability depends on plant species, its form of binding or chemical speciation and total concentration in soil (Pavlìčková et al., 2006; Vanek et al., 2011; Markert at al., 2013). In addition to above-mentioned factors, it is important to consider other intrinsic and external factors, such as soil pH, temperature, soil water content, soil concentration of other elements (e.g. K^+) (Kwan et al., 1990; Escarré et al., 2011), soil organic material and cation exchange capacity of rhizosphere (Wenzel et al., 2009; Jia et al., 2013).Thallium toxicity is mainly due to its similarity to potassium (K+), thus thallium can replace it in some metabolic processes (Wedepohl, 1995; Galvan-Arzate and Santamaria, 1998). Therefore, Tl has a strong affinity to sulfhydryl, amino- and imino-groups, which are normally included in peptides and amino acids, thereby inhibiting and inactivating enzymes activity (Pavlìčková et al., 2005 and 2006; Queirolo et al. 2009). In addition, inhibiting K-controlled activities of enzymes and membrane processes, thallium can deregulate the mitochondrial respiratory chain (Augustynowicz and Tokarz., 2014).As a result of this complex framework, thallium bioaccumulation can significantly vary among plant species because certain of them are more susceptible to accumulate it (Tremel et al., 1997;Pavlìčková et al., 2005; Jia et al., 2013), according to physiological adaptation, and only a few numbers of plants are able to grow in metal-polluted areas.Thallium in soil may be uptaken by plants, then translocated and bioconcetrated into their organs, considering that competitive interactions between pollutants and nutrients may reduce roots ability to absorb essential elements (Dominguez et al., 2009). Brassicaceae crops are Tl accumulator, as a result of their substantial potential to accumulate elevated amounts of Tl (Pavlìčková et al., 2006 and 2007; Madejon et al., 2007; Vanek et al., 2011; Wang et al., 2013). This aptitude highlights their potential for phytoremediation (Escarré et al., 2011; Vanek et al., 2011; Jia et al., 2013) that should be a bright strategy to improve not only the revegetation process in soil highly polluted but also to immobilized thallium preventing its release and migration into groundwater and surface water (Mueller, 2001; Paoletti and Günthardt-Goerg, 2006; Escarré et al., 2011). On the other hand, this peculiar aptitude confirms that food is probably the major source of thallium exposure of the general population (Sabbioni et al., 1994; White and Sabbioni, 1998). For this reason thallium contaminated soils and thallium transfer into the food chain, represent a significant threat to human health (Apostoli et al., 1988; Borges and Daugherty, 1994).

The Valdicastello-Pietrasanta site in Tuscany (Italy) is characterized by dismissed mine sites that release high concentration of thallium in soil and surface water, due to the oxidation of pyrite containing thallium impuritywhich can affect soil and water and increase Tl hazard. The Tl fate in this "extreme" mine environment, due to high thallium concentrations in both soil and water, has not been exhaustively studied neither in both field or mesocosm experiments.Therefore, because only a few numbers of previous studies on thallium transfer from soil to plant have been performed, we decided to assess 1) the Tl effect on the *Quercus pubescens* Willd. an autochthon plant possible candidate for future re-vegetation process (Bran et al., 1990; Wisniewsi and Dickinson, 2003; Paoletti and Günthardt-Goerg, 2006) in vicinity of mine sites; 2) the Tl uptake and translocation in soil-oak system(Hermle et al., 2006);3) distribution of major elements (Ca, Mg, K, Na, P and S) in different oak organs (e.g. root, trunk and leaves) in the control plants and Tl-exposed ones; 4) the consequences on both microbial biomass C and N content and enzymes activities in forest natural soil affected by Tl exposition.

II. MATERIALS AND METHODS

2.1 Experimental design

54 seedlings of *Quercus pubescens* Willd. were placed in pots after 20 days from germination, in the greenhouse at 25-20°C day-night temperature, with a 14 hour photoperiod. The plants were shared in groups of 9 in 6 plastic pots (37 x 27 x 16 cm) of 15.900 cm^3, that were filled with 16 kg of natural soil MO1-AMS, (Vittori Antisari et al., 2014). The solution of Tl for the experiment was prepared by 0.5 g of Tl_2O_3 in 10 L of deionised water. This suspension, because the Tl_2O_3 is insoluble in water, was shaken every day and allowed to stand for 10 days. After this period, the surnatant was collected and analysed for Tl concentration (140μg Tl L^{-1}) and then used for the experiment. The assumption is that the Tl in solution derived by the hydrolysis of Tl_2O_3 forming between pH 6.5-7.5 $Tl(OH)_2^+$ and $Tl(OH)_3$ according to Lin and Nriagu (1998a; 1998b) (Figure 1) and that the Tl used for the experiment is under trivalent form.

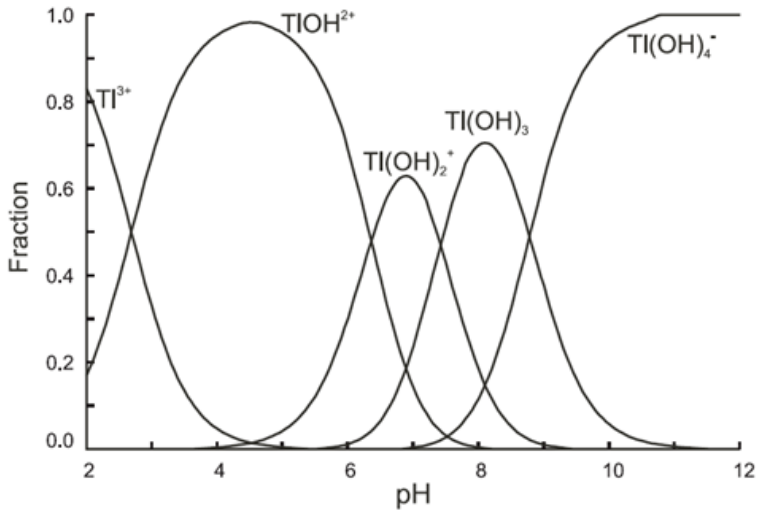

FIGURE 1: THE DISTRIBUTION OF Tl(III) HYDROXIDE COMPLEX AS A FUNCTION OF pH

To assure a homogeneous spread of the solution and to avoid contaminations of the epigeal parts of plants, irrigation has taken place with a syringe of 20 ml inside the topsoil, every time in different positions.

Every 40 days, after 8 cycles of irrigations with the solution and 9.6 mg of Tl were added in each pot, a plant, from each pot, spiked and control treatment, was explanted and the rizospheric soil was sampled for a total of three oak plants. Moreover, different portions of soil were collected in the upper part (0-10 cm), and at the bottom of the pots (10-15 cm),in addition rhizosphere soil were collected by gently shaking of the soil aggregates onto root.

Later trunks, roots and leaves were gently removed and cleaned with distilled water, having special attention to remove soil from roots. Every single sample has been weighed. The height of epigeal parts and the length of roots of every plant were measured. Next, the samples were collected in paper bags. Soils and vegetal samples were put in ventilated oven at 50°C for 48 hours and then finely grinded in a ball mill.

2.2 Thallium and major elements determination

Samples of soil and vegetation have been previously dried in a ventilated oven and then finely ground in a ball mill. The thallium and major nutrients concentration in approximately 250 mg of soil and vegetable sample was determined by Inductively Coupled Plasma Emission Spectrometry (ICP-OES, Ametek, Spectros), after mineralization in a microwave oven (Start D 1200, Milestone, USA) with aqua regia (1:3, v/v; HNO_3:HCl Suprapur grade, E. Merck, Germany) for the soils and with 1:3, v/v; H_2O_2:HNO_3Suprapur grade, E. Merck, Germany) for the vegetable; brought to 20 ml of final volume with with deionised water (18MΩ, Milli-Q, Millipore, USA), then filtered on Wathmann 42. ICP-OES calibrations were performed by the standard solution of Bureau of Collection Recovery (BCR-909) and some internal standard (AMS-MO1 and AMS-ML1)(Vittori Antisari et al., 2014).

2.3 Bio-concentration and Translocation Factors, Tolerance Index

The bio-concentration factor (BF), according to Zayed et al. (1998), was calculated as the ratio between thallium concentration (mg kg^{-1}) in the aerial parts (trunks and leaves) of oaks and the thallium concentration (mg kg^{-1}) of solution used for contamination experiment.

$$BF = \frac{\text{Thallium concentration in the aerial parts of plants (mg kg}^{-1})}{\text{Thallium concentration in the solution (mg kg}^{-1})}$$

The translocation factor (TF) was calculated as the percentage ratio of thallium concentration in the aerial parts and thallium concentration in the roots, and it is useful to evaluate the capability of plant to accumulate the metal absorbed by roots.

$$TF = \frac{\text{Thallium concentration in the aerial parts of plants (mg kg}^{-1})}{\text{Thallium concentration in the roots (mg kg}^{-1})} \times 100$$

The tolerance index (TI), which measure the ability of the plant to grow in the presence of metal, according to Wilkins (1978) was calculated as the percentage ratio of dry weight of the plants grown with contaminated solution and the dry weight of the plants grown without metal (control).

$$Tl = \frac{\text{Dry weight of the plants grown in thallium solution (g)}}{\text{Dry weight of the plants grown in control solution (g)}} \times 100$$

2.4 Soil microbial biomass and enzyme activities

Microbial biomass and its activity are important agents in controlling the overall biological activity of the soil. This is due to the fact that microbial biomass carbon (C_{mic}) responds rapidly to environmental changes and it can be used as indicator of soil disturbance. The classical technique for determining the microbial biomass size is that of chloroform fumigation, that causes the rupture of the 99% of microbial cells and release of cell walls and cellular contents into the soil. Under certain conditions, the size of biomass can be determined by the size of carbon dioxide flush of fumigated samples, compared with the unfumigated control.

Microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) were therefore estimated using the Fumigation Extraction (FE) method after 240 days of thallium contamination. Two portion of each sample of moist soil were weighed (10g oven dry weight) after which the first portion (not fumigated) was immediately extracted with 40 ml of 0.5 M K_2SO_4 for 30 min by oscillating and then filtered (Whatman n.42); while the second one (fumigated) was exposed to chloroform ($CHCl_3$) for 24h at 25°C. After incubation, residual $CHCL_3$ was removed from fumigated soils by repeated evacuations. Then, as previously described, they were extracted by shaking 30 minutes with 0.5 M K_2SO_4.

An extraction efficiency (K_{ec} factor) of 0.45 has been used to calculate the microbial biomass carbon value and 0.54 for microbial biomass nitrogen.

Soil enzyme activities were measured according to the methods of Marx et al. (2001) and Vepsäläinen (2001), based on the use of fluorogenic methylumbelliferyl (MUF)-substrates. Soils were analysed for β-cellobiohydrolase (EC 3.2.1.91), N-acetyl-β-glucosaminidase (EC 3.2.1.30), α-glucosidase (EC 3.2.1.21), β-glucosidase (EC 3.2.1.20), acid phosphatase (EC 3.1.3.2), and xylosidase (EC 3.2.2.27) using 4-MUF-β-D-cellobioside, 4-MUF-N-acetyl-β-glucosaminide, 4-MUF-α-D-glucoside, 4-MUF-β-D-glucoside, 4-MUF-phosphate, and 4-MUF-7-β-D-xyloside as substrates, respectively. The enzyme specific activity (per unit of Corg) was calculated in order to keep the amount of organic matter as an internal control (Trasar-Cepeda et al., 2007). The ratio of acid phosphatase (which mineralizes P) to chitinase (N-acetyl-β-d-glucosaminide) was calculated as a potential index to link enzyme activity to soil development (Caldwell, 2005).The four enzyme activities involved in carbon cycle were expressed per unit of soil organic carbon by a synthetic index (SEIc= 1,4β-cellobiohydrolase + α-glucosidase + β-glucosidase + xylosidase). A moist sample (equivalent weight to 2 g oven-dry material) was weighed into a sterile jar and 50 ml of Na-acetate buffer pH 5.5 were added. A homogenous suspension was obtained by homogenising with UltraTurrax at 9600 rev min^{-1} for 3 min. Aliquots of 100 µl were withdrawn and dispensed into a 96 well microplate (three analytical replicates $sample^{-1}$ $substrate^{-1}$). Finally, 100 µl of 1 mM substrate solution were added giving a final substrate concentration of 500 µM. Fluorescence was measured after 0, 30, 60, 120, 180 min of incubation at 30 °C. Fluorescence (excitation 360 nm; emission 450 nm) was measured with an automated fluorimetric plate-reader (Fluoroskan Ascent).

III. RESULTS

3.1 Thallium concentration in soil

The uppermost layer of soil showed significantly increase of thallium content until 200 days, in which a plateau of accumulation was detected, showing at this time a saturation point of the system, while a slow continuous increase was evident in soil bottom of the pots and in the rhizosphere samples (Table 1).

TABLE 1
THALLIUM AVAILABILITY CONCENTRATION IN SOIL MEASURED AFTER AQUA REGIA EXTRACTION. FOR EACH PHASE DETERMI-NATIONS WERE REPLICATED ON THREE PLANTS. RESULTS ARE COMBINED WITH STANDARD DEVIATION (n=3)

Phase	Tl content (mg kg^{-1})		
	Top soil	Aggregates at root contact	Sub soil
Initial	0.71±0,22	0.68±0,12	0.66±0,07
1	9.58±1,8	14.1±0,9	3.45±0.59
2	17.9±2.7	16.1±5.9	12.8±0.3
3	43.7±8,8	40.8±8,6	20.7±1,1
4	56.4±1.1	37.6±0.2	17.3±1,7
5	72.1±0.1	38.6±0.6	40.3±3,4
6	72.8±0.3	55.2±0.2	45.7±4,7

This major adsorption of thallium into top soil was determined with a peak of 72.8 mg kg-1 after 240 days of Tl-exposure. Instead, the Tl concentration increases in the rhizosphere, where its concentration significantly changed from 16.1 to 40.8 (Table 1). The sub soil was enriched at the end of experiment (40.3 and 45.7 mg kg-1 after 200 and 240 days of Tl exposure).

Tl average concentration detected in soil after AR determination was similar to the theoretical calculated dose used for each treatment and a positive correlation (R^2=0.97) was shown by mass balance of the thallium concentration added in soil by the contaminated solution and the thallium concentration analysed in soil after mineralization (Table2).

TABLE 2
AVERAGE OF THALLIUM CONTENT IN TOP & SUB SOIL COMPARED WITH CALCULATED THALLIUM ADDED EVERY CYCLE (40 DAYS).

Phase	Tl content (mg kg^{-1})	
	Added to soil in 120 cc	Top & Sub soil
Initial	0	0
1	0,96	0,62
2	1.92	1.45
3	2.88	3.07
4	3.84	3.50
5	4.80	5.34
6	5.76	5.63

3.2 Oak morphological parameters, Tl and major elements concentration

Generally, the progressive addition of thallium to the soil affected the oak growing, showing a decreasing trend of dry matter. After 120 days from start of Tl exposure treatment, a significant ($p<0.05$) reduction of dry matter of roots was observed, and after 200 days of trunk, while no significant weight losses of leaves were detected.

Accordingly, as derived from the measurement of dry matter of both root and trunk, a reduction of length was related to the Tl-exposure (Table 3). After 160 days of treatment, a significant ($p<0.05$) decrease of length of both root and trunk was measured. At the end of the experiment, the plants grown in contaminated soil had a length of root 34.8% and almost a 29% of trunk less than the control (Table 3).

TABLE 3

Phase	Treatment	Lenght (cm)		% reduction	
		Roots	Stem	Roots	Stem
Initial	Control	4.3±0.2	7,5±0.4	100,0	100,0
	Thallium	4.3±0.3	7,5±0.4		
1	Control	6.8±1.2	8,3±0.9	100,0	96,4
	Thallium	6.8±0.6	8,0±1.1		
2	Control	9.1±0.7	9,9±0.7	96,7	91,9
	Thallium	8.8±1.1	9,1±0.9		
3	Control	12.9±0.7	12,5±1.2	90,7	86,4
	Thallium	11.7±0.8	10,8±1.1		
4	Control	16.7±1.4	17,9±1.3	74,9	70,9
	Thallium	12.5±2.1	12,7±0.9		
5	Control	22.7±2.9	20,3±0.6	60,4	75,4
	Thallium	13.7±3.5	15,3±4.2		
6	Control	25.0±1.0	25,3±1.5	65,2	71,1
	Thallium	16.3±1.2	18,0±2.0		

The highest Tl content was found for trunk that progressively accumulated it reaching 192.5 μg g^{-1} after 240 days. Low Tl concentration in leaves, corresponding to 92.5 μg g^{-1} was detected. On the other hand, the root showed a different trend of accumulation up to 105.7 μg g^{-1} after 200 days and then at the end of experiment a plummet to 54.9 μg g^{-1} was detected (Table 4).

TABLE 4

THALLIUM AVAILABILITY CONCENTRATION IN THE PARTS OF THE OAK PLANTS AT THE SIX PHASES OF THE GROWTH CYCLE MEASURED AFTER AQUA REGIA EXTRACTION. FOR EACH PHASE DETERMINATIONS WERE REPLICATED ON THREE PLANTS. RESULTS ARE MEAN ± STANDARD DEVIATION (n=3). ND, NOT DETERMINED, VALUES LOWER THAN DETECTION LIMIT (DL) <0,03 μg g^{-1}.

		Phase						
		Initial	1	2	3	4	5	6
Number of days for treatment	partial	0	40	40	40	40	40	40
	cumulative	0	40	80	120	160	200	240
Tl content (μg g^{-1} ss) in	Leaves	ND	ND	ND	3.86 ±1.51	14.7 ±8.8	32.0 ±8.4	92.5 ±44.1
	Stem	ND	0.79 ±0.51	6.69 ±2.47	37.0 ±3.8	61.0 ±17.9	141.4 ±46.7	192.5 ±36.6
	Roots	ND	2.00 ±0.57	15.4 ±6.1	33.9 ±1.1	73.3 ±9.7	105.7 ±39.9	54.9 ±1.69

The major elements concentration stocked in organs of oak in both control and Tl-exposure treatment was shown in Table 5. In the control plants a marked decrease was observed after 120 days from beginning of incubation for all major elements that has been interpreted as an adaptation of the plants to the new condition of growth. The Tl-exposed plants showed a significant higher content of Ca in the root than that determine in the control and in leaves from 120 days from beginning of experiment. In the root of Tl exposed plants higher concentration of major elements were detected.

TABLE 5

MAJOR ELEMENTS (Ca, Mg, K, Na, P, S) AVAILABILITY CONCENTRATION IN THE PARTS OF THE OAK PLANTS AT THE SIX PHASES OF THE GROWTH CYCLE MEASURED AFTER AQUA REGIA EXTRACTION. FOR EACH PHASE DETERMINATIONS WERE REPLICATED ON THREE PLANTS. RESULTS ARE MEAN ± STANDARD DEVIATION (n=3). VALUES EXPRESSEDIN μg g^{-1} ss.

Element	Plant organs	Phase							
		Initial control	1	2	3	4	5	6	Final control
Ca	Leaves	10321	8615 ±1455	16322 ±2456	16831 ±2540	12249 ±6451	26584 ±5312	19848 ±402	9814 ±1577
	Stem	6661	7493 ±2879	8897 ±2425	9239 ±2388	9292 ±2225	15427 ±2031	14329 ±1509	13567 ±978
	Roots	4483	13601 ±4103	10381 ±2769	9201 ±1659	11378 ±1944	20098 ±9524	9567 ±370	7443 ±3726
K	Leaves	8796	9085 ±1189	8623 ±1144	9262 ±446	7493 ±1883	7748 ±527	10001 ±2639	5800 ±619
	Stem	7640	6368 ±685	7986 ±1724	5775 ±93	5412 ±277	6187 ±94	6118 ±463	2930 ±176
	Roots	6535	7551 ±1028	7125 ±922	6312 ±238	5978 ±135	6060 ±1430	4029 ±103	2803 ±508
Mg	Leaves	2297	2375 ±359	2557 ±518	2642 ±781	2183 ±851	2875 ±880	2209 ±187	1140 ±111
	Stem	1472	917 ±74	1066 ±174	1220 ±175	940 ±187	1803 ±94	1880 ±58	815 ±116
	Roots	987	1391 ±245	1661 ±368	1349 ±106	1253 ±220	2830 ±1655	1595 ±54	595 ±67
Na	Leaves	573	259 ±83	388 ±100	419 ±162	183 ±35	192 ±22	277 ±98	200 ±23
	Stem	785	341 ±118	491 ±290	293 ±58	367 ±97	463 ±293	330 ±58	112 ±17
	Roots	303	692 ±609	948 ±771	324 ±28	450 ±105	673 ±272	444 ±13	181 ±142
P	Leaves	2566	1861 ±381	1403 ±20	1363 ±288	1241 ±204	1161 ±172	1408 ±95	776 ±88
	Stem	1703	1062 ±488	880 ±380	1051 ±100	1184 ±156	750 ±243	707 ±103	484 ±93
	Roots	1468	1681 ±533	1306 ±334	1705 ±189	1622 ±24	739 ±365	872 ±34	326 ±131
S	Leaves	1525	1264 ±52	2154 ±414	1753 ±654	1122 ±452	62.2 ±24.7	39.3 ±25.3	40 ±7
	Stem	812	548 ±87	555 ±277	456 ±57	440 ±83	26.2 ±19.0	35.7 ±4.5	8.6 ±1.9
	Roots	632	691 ±165	715 ±282	757 ±34	673 ±73	26.3 ±12.8	58.0 ±2.8	9.1 ±1.0

3.3 Bio-concentration (BF) and Translocation Factors (TF), Tolerance Index (TI)

The Bio-concentration Factor (BF) values greater than 1 explained the tendency of *Quercus Pubescens* Willd. to accumulate thallium in its organs than that of soil (Table 6). The Translocation Factor (TF) yet asserted a thallium translocation through the roots to aerial parts of the plants (Table 6). These data once again demonstrate the roots collapse in the fifth phase (200 days) and the lost of the ability to keep thallium in soil. While the Tolerance Index (TI) showed that plants grown were

highly affected by thallium addition to soil, showing a decrease of growth of almost 60% compared to control (not contaminated soil) (Table 6).

TABLE 6

Phase	BF tot (roots/topsoil)	BF (aero/topsoil)	TF (aero/root)
1	0.208	0.086	0.41
2	0.863	0.376	0.44
3	0.775	0.934	1.21
4	1.317	1.340	1.02
5	1.466	2.404	1.64
6	0.755	3.913	5.18

3.4 Soil microbial biomass C and N and enzyme activities

We notice that the microbial biomass carbon was lower in contaminated soils compared to the controls, and the entity of reduction was proportional to depth. The upper layer showed a decline of microbial population of almost 70%, while in the latter end of soil microbial population was reduced of 30% compared to control (Table 7). Despite this trend, microbial biomass C:N ratio remains constant on 12.

TABLE 7
MICROBIAL BIOMASS CARBON AND NITROGEN (C_{mic} and N_{mic}) IN TOP SOIL (0-5 cm) AND SUB SOIL (10-15 cm)

Depth (cm)	Samples	C mic (mg kg^{-1})	N mic (mg kg^{-1})
0 - 5	Control	499.2 ± 89.2	41.3 ± 38.8
	Contaminated	129.4 ± 105.7	9.8 ± 11.6
10 -15	Control	448.3 ± 131.7	39.6 ± 16.5
	Contaminated	345.9 ± 102.5	28.4 ± 9.7

Simultaneously, variations of the enzyme activity in the soil samples showed an increase of arylsulphatase, cellulase and β-glucosidase activity but only in the latter part of the pots (10-15 cm) (Figure 2) while other enzymes exhibited a remarkable reduction of their activity in both soil layers, compared to the control.

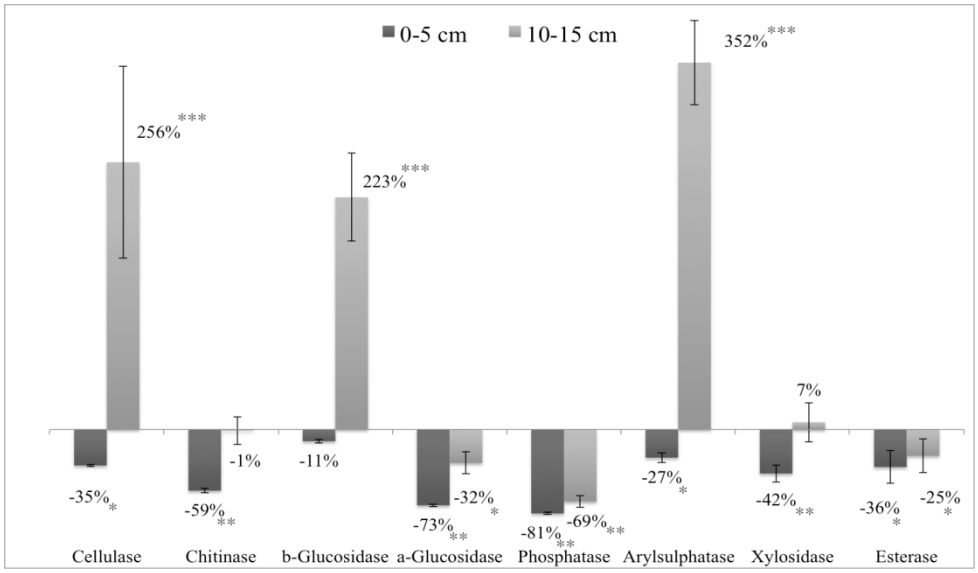

FIGURE 2. VARIATION OF ENZYME ACTIVITY IN TOP SOIL (0-5 cm) AND SUB SOIL (10-15 cm). ONE-WAY ANOVA WAS USED TO DETERMINE STATISTICAL SIGNIFICANCE OF THE DIFFERENCES BETWEEN TREATMENT AND THE CONTROL MEANS. $p<0.001$*, $p<0.01$**, $p<0.05$*.**

IV. DISCUSSION AND CONCLUSION

Evidence shows that Tl(III) is slowly converted to a monovalent state because of its strong oxidizing properties. It is not yet clarify how the plants up taken the Tl from soil, if they taken it in trivalent form or the Tl(III) is reduced to Tl(I) onto the root surface. The hypothesis is that Tl(I) may be taken up by plants in the same mechanism of K uptake due to similar ionic radii and valence. The toxicity of Tl(III) is difficult to estimate because it easy reduced and this form seem to be more toxic than Tl(I) (Ralph and Twiss, 2002).

In this framework, the our experiment was address to evaluate the Tl(III) toxicity in soil-plant system. The Tl added into soil is accumulated and the average shows a good correlation between the calculated dose and measured one. A slight mobility of thallium has been observed in our experiment where the Tl added into soil was mainly immobilized in the upper part of soil (0-10 cm). Laboratory leaching test showed that the Tl mobility depends on composition of the aqueous phase of soil and the pH value between 5-6 strongly influences its mobility in soil (Lin and Nriagu (1998a; 1998b). The oak-soil maintains constant the pH value (7.2) for whole time of experiment, decreasing the leaching of Tl. Furthermore, there are some indications that thallium binds strongly to organic matter of soil showing low mobility into soil.

This hypothesis is further supported by the bio-concentration factor (BF) that comparing the thallium calculated dose added to the soil to the thallium concentration in aerial parts of the plants, revealed a complete transfer of thallium to the epigeal parts of plants. Values of BCF higher than resulted in a rising accumulation of thallium in aerial parts of *Quercus Pubescens* Willd. This tendency of preferential accumulation in the aerial parts of the plants is even more evident by calculating the value of the Translocation Factor (TF), which related the transfer of thallium from roots to aerial parts of plants, confirming the capability to accumulate thallium in the above ground tissues. This phenomenon could be an effect of a collapse of roots maybe caused by the necrosis of cellular structure, highly compromised by thallium concentration. This is probably due to thallium competition with potassium in membrane transporter that can cause deregulation of osmosis and of the transport of small molecules. The inefficiency in roots absorption resulted evident in Table 2, where a fall of thallium concentration in roots is associated with an increase of thallium absorption in leaves and trunks. Roots collapse is well demonstrated by the comparison between dry matters of contaminated plants to the controls. A significant reduction of weight and length of roots confirmed again the suppose death of roots tissues in contaminated soil. The dry weight of roots helped us to calculate the Tolerance Index of *Quercus Pubescens* Willd. Grown in contaminated soil. The ratio between the weights of plants raised in contaminated soil to that of plants grown into uncontaminated soil showed a tolerance of almost 50% up to 200 days of treatment, moment after which, as mentioned above, the collapse of roots is supposed. The results of the analysis showed that addition of thallium to the soils significantly influenced physiology of plants and their growth.

At the end of the experiment, we decided to assess the effect of thallium contamination on the microbial metabolism, assessing thallium effect on enzyme activities of soil. Soil enzymology can indeed serve as an indirect although very sensitive assessment of soil health. First of all, we noticed a decrease in soil microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) with increasing levels of thallium in soil. However, the ratio C:N remained stable on a value of 12, meaning that thallium contamination did not change the microbial population composition. However, the decrease of total microbial population confirms thallium toxicity in affecting soil health. The Figure 2 reports data on soil enzyme activities. A massive increase in the activity of arylsulphatase, cellulase and β-glucosidase is evident, especially in the latter part of the pots (10-15 cm). Cellulase and β-glucosidase are involved in catalysing the hydrolysis and biodegradation of plant debris incorporated to the soil in glucose as final product. This increased activity supports our hypothesis of increased roots decomposition into the soil that should induce hydrolyses action (Marinari and Vittori Antisari, 2010). Bacteria secrete Arylsulphatases into the external environment leading to S mobilization that has a main role in plant nutrition. A recent research suggested that plants control the activity of Arylsulphatases in the rhizosphere through root exudates in promoting both transcriptional and post transcriptional level. It could be possible that the increase of quantity of roots debris had stimulated a rise of secretion of arylsulphatase, to contrast the S fall due to contamination of thallium.

The experimentation performed during the growth early stages of young downy oak plants have shown that the contamination of thallium triggered to a deregulation of several processes in soil, such as microbial metabolism, soil retention ability and roots absorption. Thallium contamination interferes also with microbial ecology that can be considered an important indicator of soil quality. The lasts results are preliminary data collection on the influence of thallium contamination on enzyme activities that will be surely further explore in next experiments.

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