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Abstract:
Contamination of heavy metals in soils may strongly affect the environmental quality. Lipid peroxidation caused by heavy metals in plants was investigated as a relevant bioassay of toxicity. Soils and wild plants were collected from an abandoned mine area in northeast Italy, and the concentration of different heavy metals (Ni, Cr, Cu, Pb, Zn, Fe and Mn) were measured and analyzed. Soils affected by mining activities presented total Zn, Cu, Pb and Fe concentrations above toxic thresholds. Metal-induced oxidative stress was evidenced by the generation of reactive radicals, followed by an increase in malondialdehyde (MDA) production. The formation of MDA is considered as a good indicator of lipid peroxidation. We found that thiobarbituric acid reactive substances (TBARS) concentration in plant tissues differed significantly among species and plant organs. The higher concentration of metal in soil corresponded with the higher concentration of MDA in the plant. Moreover, the results revealed that the MDA content is correlated with metal accumulation. The combined results of metal concentration and translocation coefficients in plants show that the investigated plants are rather highly tolerant towards environmental pollution. This suggests that they could be useful in phytoremediation of metal contaminated sites.

Dear Editors-in-Chief (Journal of Geochemical Exploration):

I would like to submit the attached manuscript, "Toxicity Assessment of Contaminated Soils from a Mining Area in Northeast Italy by Using Lipid Peroxidation Assay," for consideration for possible publication in the special issue - Pollution and reclamation of mining site soils (Gexplo). This paper has not been published or accepted for publication. It is not under consideration at another journal. An earlier version of this work was presented in the general assembly of the European Geosciences Union (EGU) which was held in Vienna (Austria) from 02 – 07 May 2010. That paper was modified to reflect the comments received at EGU.

Kind regards,

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Research highlights

1. Heavy metal contamination of soils in a mine site,

2. Heavy metal transfer from soil to plant,

3. Effect of toxic heavy metals on Plant,

4. Heavy metals induce oxidative stress damage,

5. Lipid peroxidation is an indicator of oxidative stress.

Toxicity Assessment of Contaminated Soils from a Mining Area in Northeast Italy by Using Lipid Peroxidation Assay

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Abstract

Contamination of heavy metals in soils may strongly affect the environmental quality. Lipid peroxidation caused by heavy metals in plants was investigated as a relevant bioassay of toxicity. Soils and wild plants were collected from an abandoned mine area in northeast Italy, and the concentration of different heavy metals (Ni, Cr, Cu, Pb, Zn, Fe and Mn) were measured and analyzed. Soils affected by mining activities presented total Zn, Cu, Pb and Fe concentrations above toxic thresholds. Metal-induced oxidative stress was evidenced by the generation of reactive radicals, followed by an increase in malondialdehyde (MDA) production. The formation of MDA is considered as a good indicator of lipid peroxidation. We found that thiobarbituric acid reactive substances (TBARS) concentration in plant tissues differed significantly among species and plant organs. The higher concentration of metal in soil corresponded with the higher concentration of MDA in the plant. Moreover, the results revealed that the MDA content is correlated with metal accumulation. The combined results of metal concentration and translocation

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coefficients in plants show that the investigated plants are rather highly tolerant towards environmental pollution. This suggests that they could be useful in phytoremediation of metal contaminated sites.

Abbreviations: MDA (Malondialdehyde); TBA (Thiobarbituric acid); TBARS (Thiobarbituric acid reactive substances); ROS (Reactive oxygen species); PUFA (Polyunsaturated fatty acids).

Keywords: Heavy metals, *Taraxacum officinale*, *Salix*, Mining pollution, lipid peroxidation.

1. Introduction:

Contaminants such as heavy metals are threatening human health by their impact on water and food quality and ecosystems (Lim *et al*., 2008). Bioavailable heavy metals can enter the food chain through primary producers reducing growth cycle and altering some biochemical pathways in plants (Loureiro, *et al*., 2006). Moreover, heavy metals induce oxidative stress by generation of hydrogen peroxide, superoxide radical, hydroxyl radical and singlet oxygen, collectively termed reactive oxygen species (ROS) (Verma and Dubey, 2003). The chemical nature of these species can create severe damage to cells (Timbrell, 2009). Formation of ROS and oxidative stress in cells is associated with the development of many pathological states (Wahsha and Al-Jassabi, 2009). This has contributed to the creation of the oxidative stress concept; in this view, ROS are unavoidable toxic products of O_2 metabolism, and aerobic organisms have evolved antioxidant defenses to protect against this toxicity (Alfonso and Puppo, 2009). Oxidative stress can increase sharply in cells either due to

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the decrease in the activity of the antioxidant defense systems or to the overproduction of ROS (Mukherjee *et al*., 2007; Soffler, 2007). One of the most deleterious effects induced by ROS in plants is lipid peroxidation, which can directly cause biomembrane deterioration. Malondialdehyde (MDA), one of the decomposition products of polyunsaturated fatty acids (PUFA) of membrane is regarded as a reliable indicator of oxidative stress (Yadav, 2010). Furthermore, increased lipid peroxidation production and the formation of free radical species, was observed in plants exposed to different heavy metals as chromium, lead, copper and zinc under laboratory conditions (Sinha *et al*., 2005; Aravind and Prasad, 2003; Verma and Dubey, 2003; Baryla *et al*., 2000).

The objectives of this work were firstly, to study wild plants growing on mine soils, and implement the biotoxicity assay on specimens collected in the field, instead of grown in the lab, for better understanding of the stress level in plants living in natural conditions. Bioassessment is a tool that has been used to detect different contaminants and their mixtures in the environment. (Loureiro, *et al*., 2006). A second objective was to assess the concentration and bioavailability of the following metals: Ni, Cr, Cu, Pb, Zn, Fe and Mn in soils and plants of a mined area in North-East Italy (Imperina Valley), with indication of the potential phytoremediation suitability of the selected plants.

2. Materials and methods

2.1 Site description

The Imperina Valley is located in the mountain district of Belluno (North-East Italy), with an altitude ranging between 543m and 990m above sea level. The geological

substrate consists of rocks of the metamorphic basement (Pre-Permian), in tectonic contact with dolomite rocks (Dolomia Principale, Upper Triassic). The mineralized area is located along the tectonic contact; it consists of a deposit of mixed sulphides, composed primarily of cupriferous pyrite, pyrite and chalcopyrite, with minor amounts of other metallic minerals (Frizzo and Ferrara, 1994). Full information on the geological and environmental setting is available in Giordano (2008) and Campana *et al*. (2007). The vegetation cover is mainly constituted of mixed forests (*Abies alba*, *Picea abies*, *Fagus sylvatica* and *Ostrya carpinifolia*), with clearances where herbaceous and shrubby vegetation prevails over the arboreal one (Dissegna *et al*., 1997). The mining activities took place in Imperina Valley from the XV century until 1962, when the mine was closed.

2.2 Field sampling

A previous soil survey was carried out in 2008 in the mined area and the conterminous zone. Eight representative soil profiles were opened, described following National guidelines and sampled for laboratory analyses. Full information on soil properties and classification is available in Fontana *et al.* (2010). Successively, all locations were sampled for topsoil and plants in the period between spring-summer 2009 and spring 2010. A plant inventory was recorded, and the relative abundances were estimated visually according to Wahsha (2008).

2.2.1 Soil sampling

According to the procedures described by Hood and Benton Jones (1997) and Margesin and Schinner (2005), soil samples were collected randomly from the upper

horizon at a depth of approximately 30 cm. Each soil sample was a composite of 5-7 subsamples collected in a given sector. In total, 50 subsamples were taken at the site, mixed, packed in containers, and then, all samples were transported to the laboratory. Upon arrival at the laboratory the samples were air dried at room temperature for 7-10 days, homogenized and sifted through a stainless-steel sieve of 2mm mesh diameter before the determination of physico-chemical soil properties and quantification of soil heavy metal contamination.

2.2.2 Plant sampling

At least five specimens of selected plant species were sampled at each site with their corresponding soils. Samples were packed in plastic bags not completely closed, to allow gas exchange and transported to the laboratory according to Benton Jones (2001). The identification of species was realised according to Pignatti (1982) as the following: *Taraxacum officinale* Weber, *Salix purpurea* L., *Salix caprea* L., and *Salix elaeagnos* Scop.. All plants were divided into leaves, steams and roots, and plant material was gently washed with tap and then rinsed with distilled water to remove all soil particles. Samples were dried in ventilated oven at 50 °C for 2 days. Dried plant tissues were ground into fine powder and then stored for further analysis according to Benton Jones (2001).

2.3 Research Methodology

Physical and chemical determinations were conducted according to the guidelines of the Italian Ministry of Agriculture and Forestry. All chemicals used in this study were of analytical grade and purchased from Sigma-Aldrich Co., USA.

Soil pH in water was measured potentiometrically following the protocol of Violante and Adamo (2000), carbonates by gas-volumetric determination according to Boero (2000), organic carbon and organic matter based on the method described by Walkley and Black (1934), cation exchange capacity, total acidity and base saturation were analyzed following the methods reported by Gessa and Ciavatta (2000) and soil texture was determined following the pipette method (Genevini *et al*.,1994). For both samples of soil and plants, the concentrations of Ni, Cr, Cu, Pb, Zn, Fe and Mn were determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) according to the method reported by Margesin and Schiner (2005), after acid digestion in microwave 1600-ETHOS Milestone in closed Teflon containers. Two standard certified reference materials (SOIL5 from the International Atomic Energy Agency and MESS3 from National Research Council Canada) were analyzed as a part of the quality control.

2.4 Estimation of lipid peroxides

For quality control and assurance for lipid peroxidation evaluation, 10 plant specimens were collected from areas with no industrial or agricultural activities, following the method by Wahsha (2008), from the university garden in case of *Taraxacum officinale*, and from a natural area in a municipality in the province of Venice, in case of *Salix* species. The MDA content was determined by the TBA reaction with minor modification of the method of Heath and Packer (1968) by Taulavuori *et al*. (2001). Briefly, a 0.30g crushed sample was homogenized in 20 mL of 0.25% thiobarbituric acid (TBA) in 10% TCA, using mortar and pestle. The mixture was heated at 95° C for 30 min and then quickly cooled in an ice bath and centrifuged at 10000g for 10 min. The absorbance of the supernatant was read

spectrophotometrically at 532 nm using a spectrophotometer Hach DR 2000, and correction for unspecific turbidity was done by subtracting the absorbance of the sample at 600 nm. A total of 0.25% TBA in 10% TCA served as blank. The concentration of lipid peroxides together with the oxidatively modified proteins of plants were quantified and expressed using Beer's law with an extinction coefficient of 155 mM^{-1} cm^{-1}.

2.5 Statistical analysis procedure

Results were subjected to one-way analysis of variance (ANOVA), and represent means ± S.D. Differences in mean values between groups were assessed by Tukey's test. If data were not normally distributed and data transformation did not correct for normality, a Kruskal-Wallis one way analysis of variance on ranks was performed for the source of statistically significant difference. Statistical significance was considered at $p < 0.05$. The data were analyzed statistically using Sigma Stat statistical software version 3.5.

3. Results and discussion

3.1 Chemophysical properties of soil samples

Selected soil variables (pH, cation exchange capacity (CEC), organic carbon content and soil texture) were analysed in all soil samples and a summary of these analyses is given in Table 1. Many characteristics of the soils studied vary; for example, the pH values oscillate from about 4 to nearly 8, due to the nature of the soil parent material; the highly acidic pH values found in some soils are probably due to the alteration process of iron sulfides (pyrite and chalcopyrite) in the soil (Delgado *et al*., 2009).

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Another parameter in the table is the CEC which has shown generally low values for all the soil samples (from 5.8 to 23.8 cmol kg^{-1}), except for the profile on dolomite (34.3, profile 8). Moreover, the organic carbon content is generally lower in the mine soils than in controls (7, 8). Finally, the soil texture is typically loamy, sandy-loamy or silty-loamy.

Table 1: Selected chemophysical properties of the examined soils.

Profile	pH (in water)	CEC (cmol kg^{-1})	Organic Carbon (g kg^{-1})	Texture sand %	silt %	clay %
1	4.5	10.4	5	40	46	14
2	7.8	15.5	12	46	43	11
3	7.5	9.0	8	71	25	4
4	5.3	5.8	4	41	53	6
5	7.3	23.8	41	68	27	5
6	7.6	11.0	8	44	47	10
7	5.3	14.4	22	61	29	10
8	7.7	34.3	33	15	69	15

Table 2 summarizes the results of the average concentrations of Ni, Cr, Cu, Pb, Zn, Fe and Mn in the soils tested. The total concentrations of most of the investigated metals (Cu, Pb, Zn and Fe) in the soil samples were significantly higher (ANOVA P ≤ 0.05) than those of control soils according to the Italian legislation (D.L. 152/2006), showing that the mine dump is not contaminated by Cr and Ni, on the other hand, the table shows a high contamination by Zn, Cu, Pb and Fe in different sites.

Table 2: Concentration of metals in soils of Imperina Valley ± standard deviation; [1] reference average values (adapted from Angelone and Bini, 1992); [2] threshold limits in the Italian legislation (D.L. 152/2006 Annex 5); [3] certified reference material. <DL = less than the detection limit.

	Ni	Cr	Cu	Pb	Zn	Fe	Mn
Sampling site:	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	%	mg kg^{-1}
1	< DL	< DL	3726.3±21.0	20815.0±93.3	1554.0±18.6	53.1±0.3	177.6±2.0
2	< DL	20.5±1.3	3367.1±41.6	14634.9±71.0	1188.3±8.5	31.9±0.3	280.0±1.2
3	60.4± 1.2	101.8±0.9	526.4±2.8	227.8±5.2	472.0±3.3	6.2±0.1	1166.2±5.4
4	< DL	< DL	3974.6±17.8	14619.3±87.3	2423.0±21.3	58.2±0.4	174.6±2.5
5	46.6±1.5	92.8±0.7	499.7±2.9	293.7±3.4	430.8±2.1	5.3±0.0	1139.5±6.7
6	< DL	< DL	2333.9±19.2	11668.3±67.3	2566.3±36.7	48.3±0.4	161.4±1.8
7	57.7±1.0	162.2±1.5	97.9±1.1	52.1±4.4	103.1±1.4	4.0±0.6	1024.7±16.6
8	13.9±0.5	33.3±0.4	282.8±8.1	342.7±5.2	576.8±9.4	1.3±0.0	193.0±0.0
Italian average[1]	46	100	51	21	89	4	900
International average[1]	40	200	20	10	50	-	850
Excessive values[1]	100	100	100	100	250	-	1500
Residential Limits[2]	120	150	120	100	150	-	-
Industrial Limits[2]	500	800	600	1000	1500	-	-
Mess 3[3]	48.54±1.2	115.67±0.8	38.98±0.9	< DL	156.88±1.3	4.39±0.0	310.06±3.0
Certified composition of Mess 3[3]	46.9 ±2.2	105±4	33.9±1.6	21.1±0.7	159±8	4.34±0.11	324±12
Soil 5[3]	11.56±0.8	27.19±0.8	79.57±0.7	174.01±4.0	365.94±5.4	4.53±0.0	890.09±17.8
Certified composition of Soil 5[3]	-	28.9±2.8	77.1±4.7	129±26	368±8.2	4.45±0.19	852±37

The calcophilous behavior of lead, zinc and copper, which tend to form compounds

with sulfur, as chalcopyrite ($CuFeS_2$), blende (ZnS) and galena (PbS), commonly

found in the Imperina valley ore deposits (Frizzo and Ferrara, 1994), is confirmed by

the correlation between couples of these elements (Cu/Pb 0.768; Pb/Zn 0.709) , whose

correlation coefficients are significant at $P<0.05$. Ni and Cr are negatively correlated

with Cu (-0.796; -0.68), Pb (-0.893; -0.526) Zn (-0.750; -0.758), although Cr is

significantly correlated only with Zn. Iron and Mn are not significantly correlated

each other neither with any other element, although they share the same geochemical

behavior (Bradl, 2005).

3.2 Heavy metals accumulation in plants

The concentrations of heavy metals in plant species of Imperina Valley are presented in Table 3. Results of the present study indicated that willow accumulated significant quantities of heavy metals in both leaves and roots, irrespective of the species. Dandelion too accumulated heavy metals in leaves, consistently with data from literature (Simon *et al*., 1996; Savinov *et al*., 2007). Ni and Cr present concentrations below the phytotoxicity threshold reported by Kabata-Pendias (2001); this is consistent with concentration levels recorded in the soil (Table. 2). Mn concentrations are within the "normal" values (Kabata-Pendias, 2001) for all samples. Fe concentrations are highly oscillating with a range between 67 and 926 mg kg^{-1} in willow, and up to 1636 kg^{-1} in dandelion. However, this metal is not considered toxic unless at very high concentration (He *et al.*, 2005). Cu concentrations in both leaves *and* roots of willow are above the toxicity threshold (Kabata-Pendias, 2001), while in the stem, the Cu concentrations are relatively low, except in *S. caprea*. Willows proved to have the ability to accumulate Pb in roots more than in the aerial parts, and in the leaves more than in the stems, with the exception of *Salix purpurea*, where Pb is easily translocated to leaves. Regarding Zn, our results show that the highest concentrations are recorded in *Salix* leaves, and decrease gradually from stems to roots, acting against the Pb concentration trend. Zn concentrations in *Salix* exceed the toxicity level recommended by Kabata-Pendias (2001). Moreover, *S. purpurea* presents lower concentrations for the elements Cu, Pb, Zn, than *S. caprea* and especially *S. eleagnos*.

Table 3: Concentration of heavy metals in *Salix* and *Taraxacum* tissues (mg kg^{-1} dry weight). <DL = below detection limit; L= leaves, S= stem, R= root. Sampling period summer 2008/2009.

Profile	Plant		Ni	Cr	Cu	Pb	Zn	Fe	Mn
3	*Salix eleagnos*	L	4.75±0.29	3.33±0.29	27.83±0.14	26.41±1.96	516.62±3.14	569.52±5.42	69.92±0.71
		S	< DL	2.84±0.24	16.31±0.12	7.22±1.55	387.99±2.18	66.66±0.38	16.56±0.10
		R	3.68±0.25	3.15±0.15	80.51±0.70	32.71±2.13	227.77±1.54	427.15±2.39	26.38±0.14
	Salix purpurea	L	< DL	3.69±0.23	27.82±0.28	25.57±1.19	231.77±1.09	660.72±2.66	81.80±0.56
		S	< DL	2.66±0.20	14.12±0.14	< DL	189.95±0.73	79.14±0.35	19.68±0.08
		R	< DL	3.03±0.28	15.82±0.17	< DL	92.67±0.34	217.29±1.13	34.76±0.14
4	*Salix caprea*	L	< DL	3.71±0.17	39.88±0.18	156.14±1.96	338.96±0.86	926.58±5.94	104.05±0.35
		S	10.65±0.48	2.39±0.32	50.22±0.23	99.35±2.74	239.73±1.25	122.11±0.66	10.84±0.03
		R	< DL	2.89±0.08	55.51±0.77	573.08±18.72	155.67±0.85	650.14±2.55	13.37±0.05
2	*Taraxacum officinale*	L	<DL	3.51±0.26	42.73±0.46	78.81±3.63	79.06±0.30	1068±1.27	16.57±0.97
6	*Taraxacum officinale*	L	<DL	<DL	53.88±0.45	128.99±2.87	159.94±0.61	1636±6.28	88.12±0.97

It is noteworthy to point out, however, that willows' ability to accumulate heavy metals in different parts is independent of the species; rather, it depends on local factors as soil and microclimate (particularly water availability) and on plant physiology and ageing (Baker and Brooks, 1989). Moreover, a counteracting behavior of essential and toxic heavy metals is likely to occur as an effect barrier of the roots. Concerning *Taraxacum*, data on heavy metals in roots are not available for the same sampling period, and therefore only heavy metal concentrations in leaves are reported in Table 3. Data show that this plant is able to accumulate metals (Cu, Pb, Zn) in leaves at concentrations above the toxicity threshold indicated by Kabata-Pendias (2001).

We have calculated also the translocation factor of willows species, i.e. the ratio between heavy metal concentration in leaves and in roots. The results indicate that *Salix* translocate and retain heavy metals in the aerial parts, in particular zinc (*S. eleagnos*: $TF_{Zn} = 2.27$; *S. purpurea* $TF_{Zn} = 2.5$; *S. caprea* $TF_{Zn} = 2.18$). Instead, Pb and Cu are scarcely translocated ($TF_{Cu} = 0.34\text{-}1.76$; $TF_{Pb} = 0.81\text{-}0.27$) owing to the root barrier effect, as it has been found for Cr (Bini *et al*., 2008).

Translocation factors calculated for *Salix* samples suggest that heavy metals present different mobility within the plant. The less mobile among them is Pb (average TF =

0.54), which tends to be blocked in the root part, indicating it is unessential for plant nutrition, and suggesting some exclusion mechanism by plants. Mn, Zn and Fe appear to be the most translocated among the elements considered (average TF = 2.53, 2.32 and 1.93, respectively), while the other metals (Ni, Cr, Cu) do have similar concentrations in leaves and roots (average TF ≈ 1).

The metal translocation capacity combined with rapid growth and a higher biomass than herbaceous plants, nominate willows as good candidates for phytoremediation of polluted soils, as already mentioned by Greger and Landberg (2003)

3.3 Lipid peroxidation product quantification

One important consequence of excessive free radical production after high accumulation of heavy metals in plant tissues is that many organic molecules, including polyunsaturated fatty acids (PUFA), are attacked, therefore resulting in membrane lipids peroxidation production (Alfonso and Puppo, 2009 ; Joshi *et al*., 2005), as shown in Figure 1. Since it is known that lipids are macromolecules most susceptible to oxidative stress, several studies reported that ROS can initiate lipid peroxidation through the action of hydroxyl radicals (Armstrong, 2008; Katoch and Begum, 2003).

Fig.1

Lipid peroxidation reactions are usually free radical-driven chain reactions in which one radical can induce the oxidation of PUFA that act as substrate molecules (Jung *et al*., 2008). Such a chain reaction is initiated by the abstraction of a hydrogen atom from a reactive methylene group of PUFA residue (Bergman *et al*., 2003). Molecular

oxygen rapidly is added to the carbon centered radical (Dufour *et al*., 2007). This lipid peroxides can abstract a hydrogen atom from another PUFA as in the first step (Yin & Dong, 2003). This reaction is termed propagation, implying that one initiating hit can result in the conversion of numerous PUFA to lipid peroxides (Sathishsekar & Subramanian, 2005). Further, membrane destabilization is directly correlated with MDA production, which is one of the decomposition products of the PUFA (Barreraa *et al*., 1996). MDA concentration is a widely used method to analyse lipid peroxidation in biological material (Taulavuori *et al*., 2001; Zielinska et *al*., 2001). The results of our biochemical assay provide evidence that the degree of LPO levels in leaves and roots of *T. officinale* (Table 4) varies depending on the level of heavy metals pollution in the corresponding profile (Table 2) and a significant increase in MDA content and heavy metals concentration in investigated plants were observed (Fig.2).

Fig.2

Control *T. officinale* exhibited normal levels (Savinov *et al*., 2007) of LPO and it was 0.2063 µM in leaves and 0.1450 µM in roots. There was a dramatic increase in MDA level in leaves and root homogenate from *T. officinale* collected from Imperina Valley. The contents of MDA were maximum in plants root from profile 6 and leaves from profile number 1, intermediate in plant samples from profile 2, 3, 4, and 5. This agrees with data on soil pollution (Table 2). Using Kruskal-Wallis one way analysis of variance on ranks we could find statistically significant differences (P = 0.001) in the average MDA contents among plants from different profiles.

Table 4: Contents of MDA in *T. officinale* (leaves and roots) from different profiles in Imperina Valley. Data represent mean values ± S.D. based on three independent determinations. Sampling period spring 2010

Group	MDA Concentration (mg/L)	
	Leaves	**Roots**
Control	0.206 ± 0.013	0.145 ± 0.005
Profile 1	1.060 ± 0.221	0.636 ± 0.048
Profile 2	0.515 ± 0.037	0.479 ± 0.082
Profile 3	0.454 ± 0.079	0.366 ± 0.030
Profile 4	0.549 ± 0.075	0.702 ± 0.158
Profile 5	0.243 ± 0.128	0.747 ± 0.164
Profile 6	0.542 ± 0.161	0.816 ± 0.131

In agreement with previous results by Savinov *et al*. (2007), the increase of MDA production in *T. officinale* was expected because when heavy metal levels increase in soil their absorption by roots will increase, and the lipid peroxidation through the possible excessive generation of free radicals will be incremented. *T. officinale* responds to the increased heavy metal contents by intensification of LPO processes, with their parameters related with the concentrations of Cu, Zn, Pb and Fe in the soil, as a result of an imbalance in the homeostasis of the antioxidant defense system (Alfonso and Puppo, 2009). Under such conditions, seed production and the phenotypic heterogeneity of *Taraxacum* populations increase, as observed by Savinov *et al*. (2007).

Lipid peroxidation in leaves, stems and roots of *Salix* plants, measured as MDA content, are given in Table 5. Compared to control, heavy metals induced oxidative stress in willows were evident from the increased lipid peroxidation in roots, stems and leaves, indicating an enhanced MDA production, with MDA increasing in leaves in comparison to roots and stems. This is in agreement with data reported by Kuzovkina *et al*. (2004) and Ali *et al*. (2003). A maximum concentration of 41.64 μM

MDA in *S. purpurea* leaves collected from profile 1 and 30.78 µM in the roots of the same species from profile 3 was observed, indicating severe cell injury. Generally, in both parts of the plant, the MDA content were found positively related with metal accumulation (Kruskal-Wallis ANOVA, $P < 0.05$). Therefore, the high level of MDA observed in investigated plants under metal stress might be attributed to the peroxidation of membrane lipids caused by ROS due to metal stress indicating a concentration-dependent free radical generation (Ali *et al* ., 2003).

Table 5: Contents of MDA in *Salix* species from different profiles in Imperina Valley. All the values are mean of three replicates $\pm$ S.D. Statistical analysis by one-way ANOVA followed by Tukey's multiple comparison. Sampling period summer 2010.

Group	MDA concentration (µM)		
Profile 1	**Root**	**Stem**	**Leave**
S. purpurea	30.42 ± 0.30	18.70 ± 0.51	41.64 ± 0.12
S. elaeagnos	29.50 ± 0.54	30.10 ± 0.47	37.00 ± 0.09
Profile 2			
S. elaeagnos	27.00 ± 0.85	35.44 ± 0.10	40.00 ± 0.90
Profile 3			
S. purpurea	30.78 ± 0.75	31.04 ± 0.26	33.04 ± 0.31
S. elaeagnos	24.92 ± 0.51	24.36 ± 0.77	26.94 ± 0.02
Profile 4			
S. purpurea	25.30 ± 0.66	27.80 ± 0.81	28.40 ± 0.92
S. caprea	29.45 ± 0.32	28.90 ± 0.22	31.74 ± 0.14
Profile 5			
S. caprea	20.37 ± 0.74	24.80 ± 0.38	27.50 ± 0.33
S. elaeagnos	19.90 ± 0.55	24.51 ± 0.03	28.70 ± 0.94
Control			
S. purpurea	20.47 ± 0.64	18.70 ± 0.34	23.08 ± 1.10
S. caprea	18.40 ± 0.41	18.10 ± 0.70	20.32 ± 0.52
S. elaeagnos	19.20 ± 0.90	18.21 ± 1.20	24.10 ± 0.82

4. Conclusions

The soils in the mining site examined are highly contaminated by trace elements, mainly Cu, Zn, Pb and Fe. The observed ability of *Salix* species and *T. officinale* to

continue growth in the presence of heavy metals and to accumulate metals in their tissues, and particularly in leaves, demonstrated their tolerance to moderate to high levels of metals. Therefore, they have good potential to be used in phytoextraction projects. Our results show that *T. officinale*, *S. purpurea, S. caprea* and *S. elaeagnos* exposed to contaminated soils result in an increment in lipid peroxidation in their tissues, suggesting an important role of oxidative stress in the pathogenesis of heavy metals-induced cellular toxicity, and they can be a promising bioindicator for such research. The LPO process proved a useful tool to asses the state of populations of wild-growing species, as well as it reflects the anthropogenic heavy metal pollution in ecosystems.

5. Acknowledgments

6. References

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Caption to figures

Fig.1: Lipid peroxidation steps. Adapted from Wahsha (2008)

Fig. 2: Concentration of two metals of interest (A) Lead; B) Zinc) in topsoils and MDA concentration in leaves of *Taraxacum officinale*; six sampling sites are considered.
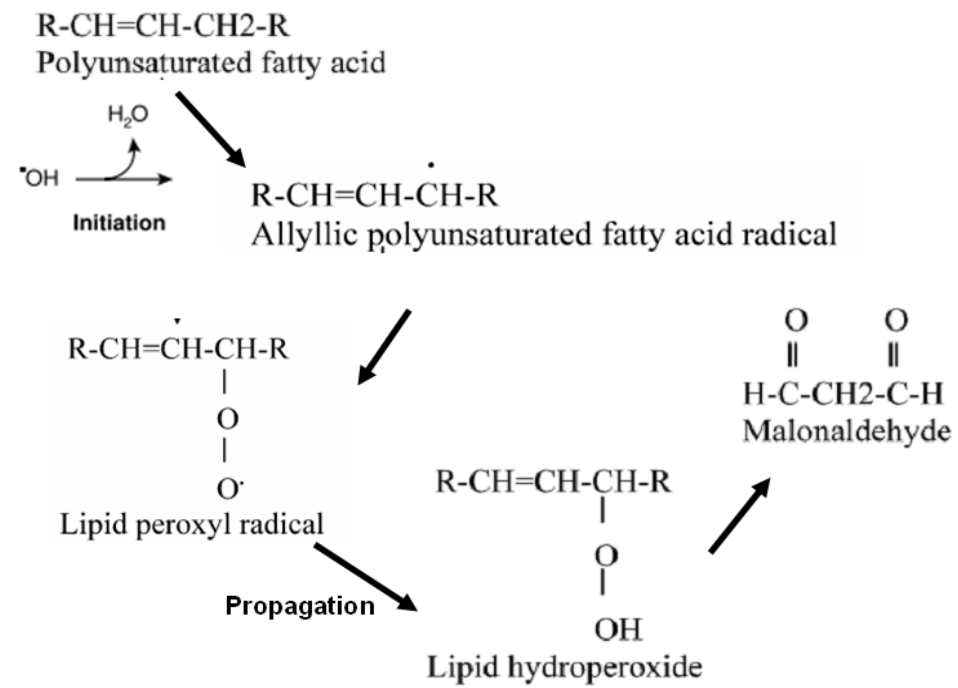
Figure 1

Figure 2