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Effects of heavy metals on morphological characteristics of *Taraxacum officinale* Web growing on mine soils in NE Italy

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ABSTRACT

Plants growing on metal contaminated soils can uptake heavy metals and accumulate them in their tissues; the accumulation of potentially toxic elements can produce adverse effects on plant morphology and health. In this study, plants of *Taraxacum officinale* Web growing on mixed sulphides (Cu, Fe, Pb, Zn) mine waste in NE Italy were studied in order to assess the levels of potentially toxic heavy metals (Cd, Cr, Cu, Fe, Pb, Zn) in plants in relation to soil, and to investigate the accumulation ability and morphological response to environmental stress. *T. officinale* accumulates relatively high amounts of different metals in both shoots and roots, with positive translocation factor ($TF \geq 1$). Micromorphological observations on the leaf anatomy of contaminated plants revealed significant reduction in the leaf thickness, changes in intercellular spaces and in cell structural organization in comparison to plants grown on unpolluted soil. The recorded morphological changes appear to be related to contamination levels in soils.

1. Introduction

The restoration of metal-contaminated sites is one of the most important environmental issues. Soil pollution by chemicals poses serious hazards to surface and ground waters, plants and humans, and presents relevant social, sanitary and economic costs (only in the U.S. up to 250$ m^{-3} soil; Adriano et al., 1995; Bini, 2010). Metal accumulation in soil diminishes soil fertility, microbial activity and plant growth (Lehoczky et al., 1996). Moreover, trace elements are very persistent, can interact with plant roots by adsorption or release from the soil particles, and therefore increase the risk of long-term soil pollution and of toxic effects on organisms (Rosselli et al., 2006).

The assessment of soil contamination by metals has been extensively carried out through plant analysis (Blaylock et al., 2003; Brooks, 1998; Ernst, 1996; Wenzel et al., 1993); both wild and cultivated plant species have been frequently used as (passive accumulative) bioindicators for large scale and local soil contamination (Baker, 1981; Baker and Brooks, 1989; Bargagli, 1993; Zupan et al., 1995, 2003).

In the last decades, attention has been deserved to plants as tools to clean up metal-contaminated soils by the low cost and environmental friendly technique of phytoremediation (Adriano et al., 1995; Baker et al., 2000). This technology is focused on the ability of plants to accumulate high heavy metal concentrations (up to 100 times the normal concentration) in their aerial parts (i.e. they are hyperaccumulator plants as defined by Baker, 1981). The plant ability to uptake metals was firstly applied in phytomining projects (Brooks and Robinson, 1998; Ernst, 1993; Helios-Rybicka, 1996; Mc Grath, 1998; Vergnano Gambi, 1992), and only successively, when environmental contamination became a global concern, it was recognized as an useful tool for remediation projects (Adriano et al., 1995; Bini, 2010, 2005; Bini et al., 2000b; Mc Grath, 1998; Salt et al., 1995). Indeed, tolerant or accumulator populations of higher plants may colonize naturally or even anthropogenic metal-enriched areas, accompanying the disappearance of sensitive plants. Therefore, they may be utilized in restoration of such areas. The choice of plants is a crucial aspect for the remediation techniques. Up to now, more than 400 plants that accumulate metals are reported, *Brassicaceae* being the family with the largest number of accumulator species (Bini, 2010; Marchiol et al., 2004; Mc Grath, 1998).

Heavy metal accumulation is known to produce significant physiological and biochemical responses in vascular plants (Mangabeira et al., 2001). As stated by Preeti and Tripathi (2011), there is a direct relationship between chemical characteristics of soil, heavy metals' concentration and morphological and biochemical responses of plants. Yet, metabolic and physiological responses of plants to heavy metal concentration can be viewed as potentially adaptive changes of the plants during stress.

Plants growing on abandoned mine sites and naturally metal-enriched soils (e.g. serpentine soils) are of particular interest in this perspective, since they are genetically tolerant to high metal concentrations, as reported by several authors (Bini, 2005; Brooks, 1998; Brooks et al., 1977; Giuliani et al., 2008; Maleci et al., 1999; Pandolfini et al., 1997; Vergnano Gambi, 1992), who studied

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endemic serpentine flora (*Alyssum bertoloni*, *A. murale*, *Silene paradoxa*, *Stachys serpentini*, *Thymus ophioliticus*) at various sites in the world. All these authors agree that morphological, physiological and phytochemical characters of serpentine plants are strongly dependent on the substrate composition (what Jenny, in 1989, called "the serpentine syndrome"), and that they are likely metal accumulator or tolerant ecotypes.

Understanding the mechanisms of metal bioaccumulation by plants species and of metal bioreduction by microorganisms is a clue to the efficiency of phytoremediation techniques. The localization and the chemical form of metals in cells are key information for this purpose (Kidd et al., 2009; Sarret et al., 2001). After their assimilation by plants, heavy metals could interfere with metabolic processes and are potentially toxic (Lopareva-Pohu et al., 2011); phytotoxicity results in chlorosis, weak plant growth, yield depression, and may be accompanied by disorders in plant metabolism such as reduction of the meristematic zone (Maleci et al., 2001), plasmolysis and reduced chlorophyll and carotenoids production (Corradi et al., 1993). Mangabeira et al. (2001) studied the ultrastructure of different organs of tomato plants (root, stem, leaf) which showed visible symptoms of Cr toxicity, and argued that Cr^{VI} induces changes in the ultrastructure of these organs. Similar findings were reported by Vasquez et al. (1991) for Cd in vacuoles and nuclei of bean roots. Since both these metals are known to be inessential to plant nutrition, it is suggested that they are likely confined in roots by a barrier-effect as defence strategy during stress. Conversely, essential metals such as Zn and Cu are easily translocated to the aerial parts, as reported by Fontana et al. (2010).

Among wild plants, the common dandelion (*Taraxacum officinale* Web) has received attention (Bini et al., 2000a; Królak, 2003; Simon et al., 1996; Zupan et al., 2003) as bioindicator plant, and has been also suggested in remediation projects (Turuga et al., 2008), given its ability to uptake and store heavy metals in the aerial tissues. *T. officinale* is a very common species, widely diffused in Central and Southern Europe, easy to identify and greatly adaptable to every substrate (Keane et al., 2001; Malawska and Wilkomirski, 2001). Moreover, this species is commonly collected to be used in cooking as fresh salad or boiled vegetable, and is used also in ethnobotany and traditional pharmacopoeia (Rosselli et al., 2006). Therefore, when grown on heavily contaminated soils, it may be potentially harmful if introduced in dietary food, as it occurs in many countries.

Previous studies of our research group (Bini et al., 2000a; Fontana et al., 2010) investigated the heavy metal concentration of soils developed from mine waste material, and the wild plants (*Plantago major*, *Silene dioica*, *Stachys alopecuros*, *Stellaria nemorum*, *T. officinale*, *Vaccinium myrtillus*, *Gymnocarpium dryopteris*, *Gymnocarpium robertianum*, *Salix caprea*, *Salix eleagnos*, *Salix purpurea*) growing on those contaminated soils, in order to determine the extent of heavy metal dispersion, and the uptake by both known and unreported metal-tolerant plant species. The results showed that *T. officinale* is a species tolerant to high metal concentrations, and suggested to use it as a bioindicator plant. Metals accumulated preferentially in roots, but also leaves proved accumulator organs, being able to store up to 200 mg kg^{-1} Pb and 160 mg kg^{-1} Zn, showing only little damages (e.g. reduced foliar surface, reduced plant development).

In this work we report the results of a study carried out on wild *T. officinale* growing on soils of abandoned mine sites in NE Italy, with the following objectives:

- to determine heavy metal concentration in *T. officinale* leaves and roots;
- to calculate the Bioaccumulation Factor (BCF) and the Transfer Coefficient (TF) from soil to plant (roots and shoots); and
- to highlight possible damages at anatomical and cytological level on the aerial part of the plant.

2. Materials and methods

2.1. Site description

The Imperina Valley mining area is located in the mountain district of Belluno (NE Italy), with an altitude ranging between 543 m and 990 m 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 exploitation area is located along the tectonic contact; it consists of a deposit of mixed sulphides (Fe, Cu–Pb–Zn), composed primarily of cupriferous pyrite, pyrite and chalcopyrite, with minor amounts of other metallic minerals. Waste dump materials are dispersed over a large area in the territory, and contain relatively high amounts of toxic metals, with these average values: Cu = 1.3%, Pb = 0.2%, Zn = 1%, Cd = 8 mg kg^{-1}, Cr = 75 mg kg^{-1}, and Ni = 62 mg kg^{-1} (Campana et al., 2007). The recorded metal amounts confirm the waste composition to be determined by weathering products of primary minerals (cupriferous pyrite, sphalerite, galena), where Cr and Ni are present in traces. Full information on the geological and environmental setting is available in Fontana et al. (2010) and references therein.

Mining activity in the investigated areas dates back at least to the Middle Age and flourished in the 19th and 20th centuries, until final closure in 1962.

Copper and sulphur were the main products extracted. Since the beginning of the 15th century, and until the final closure, copper was extracted and processed directly in situ through roasting, a method with a severe impact on the area due to acid rain formation and intensive wood cutting, that left bare soils. Afterwards, in the last century, vegetation cover was naturally re-established, pedogenetic processes started again, and a new soil type, a Spolic Technosol (WRB, 2006), began to form.

The vegetation cover varies strongly at different sites, depending on landscape morphology and elevation, climate and microclimate, age of mine waste and soil evolution. It is mainly constituted of mixed woods (*Abies alba*, *Picea abies*, *Fagus sylvatica*, *Quercus* spp., *Fraxinus ornus* and *Ostrya carpinifolia*), with clearances where herbaceous and shrubby vegetation (the most abundant is willow) prevail over the arboreal one. Some of herbaceous species, as plantain (*P. major*), dandelion (*T. officinale*), moon plant (*S. dioica*) and fescue (*Festuca inops*) are pioneer and very resistant plants which colonize highly degraded areas, especially at sites where mine activity ceased a few decades ago, and soil is highly infertile and phytotoxic.

2.2. Field sampling and laboratory analyses

Preliminary investigations carried out in 2008/2009 in the mined area and the conterminous zone allowed identification of sites with different geo-morpho-pedological conditions, vegetation coverage and anthropogenic impact. Three contaminated areas, each with two sites (1–2, riverbed upstream; 3–4, roasting area; 5–6, permanent meadow downstream) and a not-contaminated site over dolomite (background control), were selected (Fig. 1) and sampled according to the procedures described by Hood and Jones (1997) and Margesin and Schinner (2005).

In the period between spring–summer 2010, soil pits were opened and described following Italian national guidelines (Fontana et al., 2010). All locations were sampled for topsoil (0–30 cm) and wild dandelion plants. Afterwards, samples of both soils and plants were recovered to the laboratory for routine and geochemical analyses. Full information on field sampling and laboratory methods is available in Wahsha et al. (2012).

2.2.1. Plant sampling

At four of the previously selected sites (sites 2, 4, 6, and control), during spring 2011, *T. officinale* specimens have been collected