Journal of Trace Elements in Medicine and Biology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

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

Impact of airborne zinc pollution on the antimicrobial activity of olive oil and the microbial metabolic profiles of Zn-contaminated soils in an Italian olive orchard☆

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ARTICLE INFO

Keywords:
Air zinc pollution
Antimicrobial activity
Biolog®
Olive oil
Phenolic compounds
Zinc

ABSTRACT

The growing of microbial resistance leads to a great interest about some natural alternatives to synthetic compounds. This study was carried out in two olive orchards (*Olea europaea* L., cv. Coratina) South Italy (Basilicata region), one located in a polluted area near a fertilizers factory releasing Zn and the other in a control unpolluted site, both managed with similar cultivation techniques. Olive oil samples were studied from both areas during 2014 and 2015. The soil microbiological status of the polluted and unpolluted orchards has been characterized and the antimicrobial effects of olive oils extracted from polluted plants (PP) and control plants (CP) against some phytopathogens have been explored. Results showed that the antibacterial activity of PP oil was significantly higher than CP and this could be due to the high content of some phenolic compounds elicited by air and soil Zn pollution (especially in the layer 0–20 cm). There is no detectable antifungal activity of the studied oils. The metabolic activity (both total and for each carbon substrate group), diversity and evenness of PP soil bacterial communities were significantly different from CP soil, while the effects of soil depth was negligible. The same parameters measured on soil fungal communities are lower in PP soil at 0–20 cm soil depth. The current research clarified the impact of atmospheric Zn pollution on the antimicrobial activity of olive oil and the soil microbial metabolic profiles. The bioactive substances extracted from olive oils growing in Zn-polluted area might be used as antibiotics.

1. Introduction

Olive oil (*Olea europaea* L.) is well known and accepted as the main component of the Mediterranean diet [1,2]. It is well known that the fruits, oil and leaves of olive trees have a nutritional and medicinal uses [3]. Olive oil dietary consumption is often associated with the lower incidences of atherosclerosis, cardiovascular and neurodegenerative diseases, and certain types of cancer [4–6]. These positive effects are mainly due to various phenolic compounds and polyunsaturated fatty acids, which play an essential role in the taste and flavour of olive oil [7].

The phenolic compounds of olive oil have a promising antimicrobial activity against a wide range of pathogens [7–10], and oleuropein [11,12] and hydroxytyrosol [13] are the more effective among them. Romero et al. [14] reported that the dialdehydic form of decarboxymethyl oleuropein aglycon and the dialdehydic form of decarboxymethyl ligstroside aid in inhibiting *Helicobacter pylori*. Bubonja-Sonje et al. [15] indicated that the polyphenols in olive oil can be used as a natural alternative for the prevention of food spoilage by *Listeria monocytogenes*.

In the case olive trees grown in polluted environments, it should be important to define the physico-chemical and microbiological soil status, to ensure the absence of any contaminants in soils and oil. For this purpose, the metabolic diversity of soil microbial communities can be estimated by different methods [16], such as the determination of community-level physiological profiles (CLPPs), having a high discriminating power between microbial communities from different soil environments or subjected to various sources of pollution [17–19].

The aims of the current study was i) to explore the antimicrobial effects of olive against 3 G+ and 3 G- bacteria and 7 phytopathogenic

Abbreviations: 1–D, Simpon's diversity index; AWCD, average well colour development; CFU, Colony-forming unit; CP, control plot; E, Shannon's evenness index; G+, gram-positive; G−, gram-negative; H', Shannon's diversity index; PGI, percentage of growth inhibition; PP, polluted plot; S, richness; U, McIntosh's diversity index; Z, McIntosh's evenness index; OMW, olive mill wastewaters

☆ This work was supported by a STSM Grant from Zinc-Net COST Action TD1304.

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https://doi.org/10.1016/j.jtemb.2018.02.017
Received 12 October 2017; Received in revised form 14 February 2018; Accepted 16 February 2018

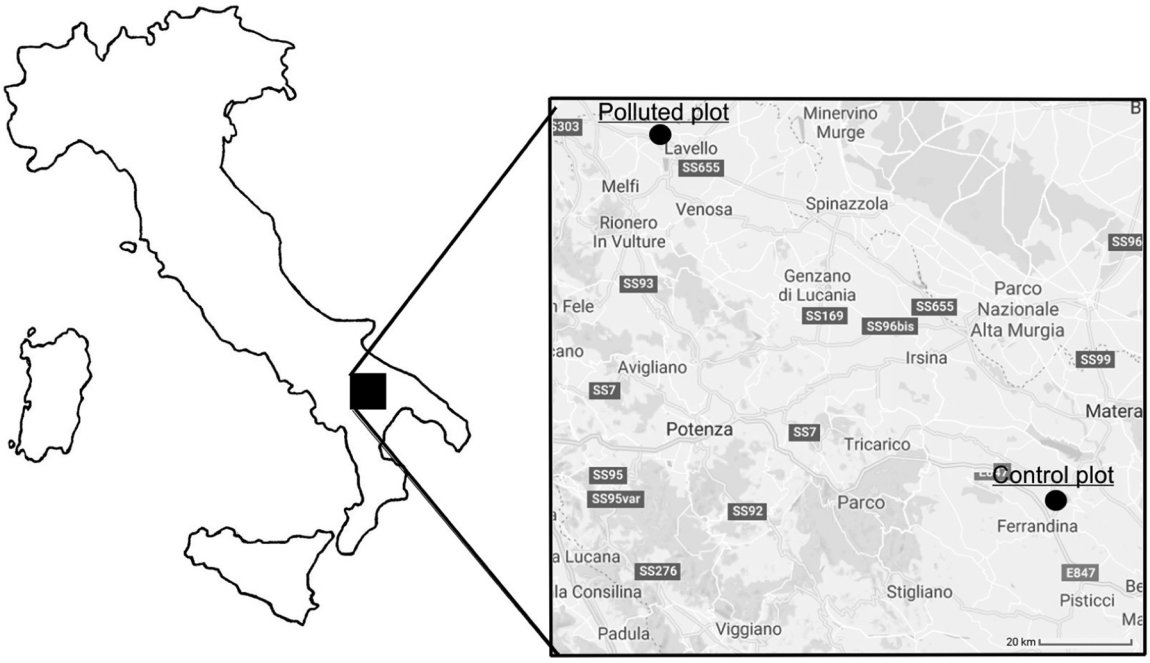

Fig. 1. The studied polluted and control areas.

fungi; and ii) to characterize the soil microbiological status, in terms of microbial metabolic activity and diversity, comparing a zinc-polluted and an unpolluted olive orchard.

2. Materials and Methods

2.1. Plant material and experimental design

The studied 35-year-old olive trees (*Olea europaea* L., cv. Coratina), planted in 1992 at distances of 6 × 3 m, were located in a polluted plot in Lavello (Southern Italy, Basilicata region, N 40°38′, E 15°48′) (Fig. 1). The region is characterized by an arid Mediterranean climate largely influenced by its mild and gentle topography. Polluted plants (PP) were located at 0.5 km from a phosphate fertilizer producing factory located in the industrial area of San Nicola, converting crude phosphate into a granule phosphate fertilizer. During the phosphate attack by sulphuric and phosphoric acids, Zn is released from the industry chimney in the form of inorganic particulate, with an emission at source of 450 kg h^{-1}. The factory emission of Zn in the particulate was found at concentrations of 36.8 Zn g t^{-1} of raw material (mean 2000-2012). Generally, wet deposition dominates during winter, in coincidence with high precipitation. It can occur as washout from smoke below clouds or rainout of particulate taken up by clouds. By contrary, dry deposition dominates in summer period, in association with low precipitation. Therefore, vegetation developed around the factory is continuously exposed to air Zn pollution.

The plot with control plants (CP), was located in Ferrandina (Southern Italy, Basilicata region − N 40°30′, E 16°27′), 90 km south-east of Lavello, in an inland rural area without any industry (Fig. 1). Olive CP were of cv. Coratina and similar in age, plant density and training system to PP. In the control plot, atmospheric Zn was found at concentrations below the instrument detection limits.

2.2. Soil sampling

In October 2015, CP and PP soils were sampled. For each treatment, five composite bulk samples soil were randomly collected from the top soil layers (0-20, 20-40 and 40–60 cm) in the inter-row areas (8-m apart) located at the center of each plot in order to avoid border interferences. Each composite sample was formed by five 7 cm-diameter cores sampled within a 0.50 cm-radius to minimize spatial variability and pooled on site [20,21]. After removal of crop residues, the soil samples were stored immediately at 4 °C in sterilized plastic pots.

2.3. Soil physicochemical parameters

On soil composite samples (soil layers: 0-20, 20-40 and 40–60 cm), soil texture, pH, electric conductivity, organic matter, and contents of Ca, Mg, Na, K and P (Olsen) were determined according to the official methods of the Italian Society of Soil Science [22].

The concentration of metals in the microwave-digestible residual was determined in soil composite samples (soil layers: 0-20, 20-40 and 40–60 cm) after digestion of 1 g in Teflon vessels with 3 ml of HNO_3 70%, 4 ml of HF 40% and 1 ml of $HClO_4$ 70%. Samples were first heated from room temperature to 200 °C for 10 min and kept at this temperature for 15 min in a closed high-pressure microwave system Ethos SEL (Milestone Inc., Schelton, CT, USA). After digestion, vessels were allowed to cool to ambient temperature. Another microwave digestion system ETHOS TC (Milestone, Italy) equipped with VAC-4000 was utilized, and samples were boiled to near dryness in order to evaporate the exceeding acids of HF and $HClO_4$. Operating parameters and power of microwave digestion systems were set according to the procedures reported in the instrumentation manual. The residual solution was subsequently transferred into a 25 mL volumetric flask, and 5 ml of 50% HNO_3 (trace metal grade) were added to preserve the sample for trace metal analyses. Samples were kept at −18 °C until analyses were carried out. Determination of Zn was achieved with inductively coupled plasma mass spectrometer ICP-MS ELAN 6000 DRCe with a cross flow nebulizer (Perkin Elmer, Waltham, MA, USA). ^{103}Rh and ^{187}Re at the concentration 10 µg L^{-1} were used as internal standards. Blank solutions were prepared to correct for contaminants contained in reagents used during sample dissolution. Calibrating standard solutions were used for the calibration of the ICP/MS instrument at different concentrations of metals. The calibration curves were linear in the whole calibrating range ($r \geq 0.9996$). Measures have been replicated thrice and recoveries have been determined using certified soils.