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Spent metal working fluids produced alterations on photosynthetic parameters and cell-ultrastructure of leaves and roots of maize plants

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HIGHLIGHTS

- Metal working fluids (MWFs) cause serious damage in the photosynthetic parameters.
- Plant growth promoting rhizobacteria (PGPR) inoculation protects plants from damage caused by MWFs.
- MWFs cause severe ultrastructural damage in chloroplasts, and reduce the number.
- MWFs cause serious damage to the ultrastructural radical parenchyma, but not in vascular tissues.
- Bacterial inoculation minimises the ultrastructural effects caused by MWFs, as well as the number of chloroplasts.

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ABSTRACT

In this work we assess the capacity of maize (*Zea mays*) plants to phytoremediate spent metal working fluids (MWFs) and its effects on photosynthesis and ultrastructure of mesophyll and root cells. A corn-esparto fibre system patented by us has been used to phytoremediate MWFs in hydroponic culture. Furthermore, a plant growth promoting rhizobacteria (PGPR) has been used to improve the process. The results show that this system is capable of significantly reducing the chemical oxygen demand, under local legislation limits. However, plant systems are really damaged, mainly its photosynthetic system, as shown by the photosynthetical parameters. Nevertheless, strain inoculated improves these parameters, especially Hill reaction. The ultrastructure of photosynthetic apparatus was also affected. Chloroplast number decreased and becomes degraded in the mesophyll of MWFs treated plants. In some cases even plasmolysis of chloroplast membrane was detected. Early senescence symptoms were detected in root ultrastructural study. Severe cellular damage was observed in the parenchymal root cells of plants grown with MWFs, while vascular bundles cell remained unchanged. It seems that the inoculation minimises the damage originated by the MWFs pollutants, appearing as less degenerative organelles and higher chloroplast number than in non-inoculated ones.

1. Introduction

Terrestrial and aquatic ecosystems are affected by a number of noxious organic compounds, mostly of an anthropogenic origin, such as polychlorinated biphenyls (PCBs), dibenzo-*para*-dioxines (PCDDs) or the polycycled aromatic hydrocarbons [1]. The generation, distribution and accidental spills of different organic pollutants (weedkillers, insecticides, acaricides, hydrocarbons) have originated the environment deterioration, with a direct or indirect accumulation in soils, water and air. Its accumulation rate is higher than the planet capacity to remove these xenobiotics compounds [2].

Metal working fluids (MWFs) are widely used for cooling and lubricating during the machining process. The worldwide annual usage is estimated to exceed 2×10^9 L and the waste could be more than ten times the usage, as the MWFs have to be diluted prior to use [3]. MWFs can be divided into two main types, oil based and water based. Oil based MWF are the straight and soluble oils, and the water based are the synthetic and semi-synthetic oils [4]. The latter type of MWF is currently the main group used in engineering applications and has resulted in increased amounts of organic chemicals being present in the MWF wastewater. The complexity

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of the composition of spent MWF streams has created immense difficulties for the waste disposal companies which deal with this type of waste [3].

European Union demands that MWF manufacturers and suppliers provide products that are both safe to use and ecologically acceptable during their production and use. Legislation regarding the regulation of MWFs relates not only to health and safety but also to environmental concerns. The European Union Water Directive (2000/60/EC) has prioritised substances and identified actions to be taken in order to minimise the impact on the environment.

Spent MWF handling in the area where this work was made (city of Madrid, Spain) is regulated by the regional law10/1993 on industrial waste discharges into urban sanitary sewer system. This law details the features that spent MWFs should have to be released into the environment. Chemical oxygen demand (COD) and pH values are normally, of all parameters regulated by the law, the ones which are above the allowed values, and therefore must be reduced prior to release to the environment. However, the decrease in COD and the pH does not necessarily imply that the spent MWF does not have biotoxic effects.

Plants are the main organisms exposed to the spent MWFs released to the environment; they are exposed during all the development stages, from germination to reproduction [5]. Plants can take up these compounds by roots, by particle-phase deposition on the waxy leaf cuticle or by their uptake in the gaseous phase through stomata [6,7]. The ability of plants to absorb, translocate, transform and accumulate these compounds, is a limiting factor for its phytotoxicity and may affect quantitatively and qualitatively to several biochemical and physiological processes related to biomass production [8].

Free-living soil bacteria beneficial to plant growth are usually referred to as plant growth promoting rhizobacteria (PGPR), capable of promoting plant growth by colonising the plant root [9,10]. PGPR are also termed as plant health promoting rhizobacteria (PHPR) [11]. Some PGPR strains inoculated on plant roots trigger the plant's defensive metabolism. This physiological status of the plant has been termed priming [12]. Primed plants show faster and/or stronger activation of defence responses when subsequently challenged by microbes, insects, or abiotic stress (as presence of contaminants), and this is frequently linked to development of local and systemic immunity and stress tolerance [13].

The main objectives of our present study were to explore the effect of spent MWFs on photosynthetic process by measuring the fluorescence emission of photosystem II, and the flow of electrons between photosystems through the Hill reaction. The effects on cell ultrastructure of leaves and roots were also studied. The possible protective effect of plant growth promoting rhizobacteria (PGPRs) inoculated at root level was explored in both cases.

2. Materials and methods

2.1. Metal-working fluid (MWFS)

The MWFS, used as the model effluent in this study, was an operationally exhausted synthetic fluid (Houghton Iberica S. A., Spain), used as a coolant and lubricant in large-scale continuous metal working processes, to machine tungsten carbide and steel. In brief, the main chemical constituents include a formaldehyde-based biocide, alkyl benzotriazole (metal passivator), C16/C18-fatty alcohol polyglycol ether (corrosion inhibitors); isopropanolamine (lubrication agents), and 3-iodo-2-propynylbutylcarbamate. Fresh MWFS is typically supplied as a concentrate, which is diluted with water to form a 2% (v/v) working fluid prior to use in machining operations. John Deere Ibérica S. A. provided the MWFS used in this study from its plant of Madrid. The company supplied us a residue known as MWFs water or spent MWFs: this residue was previously remediated in the company using physicochemical procedures. However, chemical oxygen demand (COD) and pH values of these fluids were over the regulatory values, according to the regional Law 10/1993 on industrial waste discharges into urban sanitary sewer system, being allowed to be released to the environment.

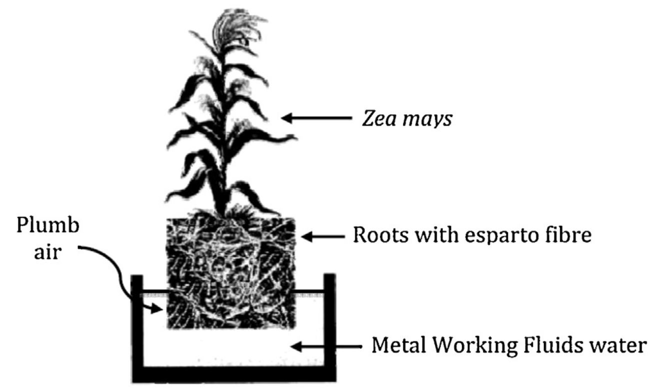

Fig. 1. Phytoremediation system [16].

2.2. Strain used in the inoculated experiments

The microorganism inoculated, called Aur6, was isolated from the Lupin rhizosphere and come from the Universidad San Pablo CEU collection. Taxonic affiliation of this bacteria was performed through the partial sequencing of the gene 16S rRNA, and its later comparison by the BLASTN 2.2.26 [14] algorithm in the GenBank database. The sequence of the bacteria was deposited in the GenBank with the number: HM486749, affiliated with the genus *Pseudomonas fluorescens*. This strain is a PGPR (plant growth promoting rhizobacteria) able to produce auxins and siderophores [15]. This microorganism was chosen for this experiment for its capacity to grow in MWFS.

2.3. Design of the phytoremediation experiment

Seeds of maize (*Zea mays* cv. Eleonora) were surface-sterilised with ethanol (70%) during 30 s, followed by sodium hypochlorite (5%) during 6 min and rinsed five times with sterilised deionized water. Sterilised seeds were deposited in petri dishes with agar-agar. After two days, when seeds had germinated, seedlings were transferred to 1 L-containers with 11.5 g of esparto fibre inside a metal grille (Fig. 1 [16]), where seeds were deposited and supplemented with 1 L of tap water with 1 g of Hoagland (Sigma–Aldrich) medium. These materials were not sterilised, because this experiments' aim is to have an industrial application, so must be the cheapest and easiest possible. Each container had 20 seedlings. The experiment consisted of 12 containers: three without MWFs water and non-inoculated (control containers); three without MWFs water and inoculated; three with MWFs water (final COD 1726 ppm) and non-inoculated and three with MWFs water (final COD 1726 ppm) and inoculated. Inoculation was performed in the corresponding container after 12 days by adding the microorganisms up to 10^7 cfu mL^{-1}. Five days later, COD and pH of the resulting MWFs phytoremediated were measured in order to assess the success of the phytoremediation process and some physiological determinations were made in the maize plants.

The assays were carried out in a controlled environment chamber with a 25/18 °C day/night temperature, a PPFD of 600 mmol/(s m^2) and a 60–70% relative humidity.