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IMPACTS OF ALUMINIUM, MOLYBDENUM, VANADIUM, ZIRCONIUM, TUNGSTEN AND GALLIUM ON THE GROWTH AND ENZYME PRODUCTION OF ASCOMYCETOUS AND BASIDIOMYCETOUS FUNGI

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ABSTRACT

Metals may influence fungi, which have a key role in global carbon recycling and are promising organisms for bioremediation. The oxidative non-specific enzyme laccase is essential in the fungal degradation of polluting compounds and therefore the impact of these metals on laccase should be known. To assess impacts of Al, Mo, V, Zr, W or Ga, the growth and production of oxidative enzymes of three basidiomycetous and two ascomycetous fungi were tested on indicator color plates. The plates contained ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) and alternatively Al (20-100 mg kg^{-1}), Ga (10-50 mg kg^{-1}), Mo (10-50 mg kg^{-1}), Zr (10-50 mg kg^{-1}), W (10-50 mg kg^{-1}), or V (5-20 mg kg^{-1}). All tested basidiomycetes *Phlebia radiata*, *Pleurotus pulmonarius* and *Physisporinus rivulosus*, produced oxidative enzymes with Al, W, Ga, Zr, Mo and V and without added metals. Ascomycetes, an *Alternaria* sp. and a *Fusarium* sp., did not produce oxidative enzymes. The growth of *P. radiata*, *P. pulmonarius* and *P. rivulosus* was sensitive to Al, Mo and W and tolerant to Zr. The growth of *P. radiata* was sensitive to Ga. The growth of *P. rivulosus* was sensitive to V. The growth of *P. pulmonarius* was tolerant to V, Ga and Zr. The growth of both the ascomycetes *Alternaria* sp. and *Fusarium* sp., was sensitive to all six metals (Al, Mo, V, Zr, W, Ga). The basidiomycetous fungi were more tolerant than ascomycetous fungi to all tested metals, indicating that they are more suitable for bioremediation of harmful organic xenobiotics than the ascomycetous fungi in metal-contaminated soil.

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1. INTRODUCTION

Impacts of metals on the functioning of fungal communities in contaminated soils are a concern. Large amounts of metals are released globally into the environment from mining, industry and from wastes [1-7]. Many wood-rotting and litter-decomposing basidiomycetous fungi and some soil and litter-dwelling ascomycetous fungi produce extracellular oxidative non-specific enzymes that have the ability to depolymerize and modify the aromatic biopolymer lignin [8-11]. These enzymes also can degrade xenobiotics whose structures resemble the aromatic backbone of lignin molecules [12,13]. Extracellular enzymes are needed in the cycling of nutrients in well-balanced ecosystems [13-17]. Fungi need some metals, e.g. Cu for laccase, but if they are present in higher amounts they are toxic to fungi [18]. Some metals are non-essential for fungi. Some metals can have deleterious impacts on fungi [19-22]. They can inhibit the production of non-specific enzymes and thus inhibit he degradation of persistent xenobiotic organic compounds when fungi are used in the bioremediation of multi-xenobiotic contaminated soil.

The selected metals Al, Mo, V, Zr, W and Ga for this study have various uses. Gallium (Ga) has been used in semiconductors [23]. Aluminium (Al) is a light metal and the most abundant metal on Earth. Al is used in various food packages, equipment and airplanes [24,25]. Molybdenum (Mo) is an important nutrient for plants and it is used in chemical fertilizers [26-28]. Vanadium (V) is present in fossil fuels as in oil and coal. Vanadium is released into the environment when fossil fuels are burned [29-31]. Tungsten (W) is used in electronic devices, X-ray tubes and as an additive in steel [32,33]. Zirconium is used in nuclear fuel rods in nuclear power plants [34,35]. Only a little is known about the impacts of Al, Mo, V, Zr, W and Ga on the growth of fungi and their ability to produce extracellular oxidative non-specific enzymes.

The aim of this study was to test impacts of aluminium, molybdenum, vanadium, zirconium, tungesten and gallium on the growth and the production of oxidative enzymes of three basidiomycetous and two ascomycetous fungi. The selected basidiomycetous fungi were lignin-degrading wood-rotting fungi *Phlebia radiata*, *Pleurotus pulmonarius* and *Physisporinus rivulosus*. *P. radiata* is an efficient lignin-degrading fungus that produces laccase and lignin-degrading peroxidases [36], but its ability to grow in soil is poor [37], *Pleurotus* spp. can grow in unsterilized soil [37]; *P. rivulosus* is known as a selective lignin-degrading fungus [38], and it can grow in unsterilized soil [37]. The ascomycetous fungi selected for this study were an *Alternaria* sp. and a *Fusarium* sp., isolated from field soil in Finland and representing common fungi in the soil environment.

2. MATERIALS AND METHODS

2.1. Tested Fungi

The three basidiomycetous and two ascomycetous fungi were selected from the Fungal Biotechnology Culture Collection (FBCC) at the Department of Food and Environmental Sciences/ Microbiology and Biotechnology Division at the University of Helsinki in Finland. The basidiomycetous fungi were *Phlebia radiata* FBCC43 (ATCC64659), *Physisporinus rivulosus* (syn. *Obba rivulosa*) FBCC939 (T241i) and *Pleurotus pulmonarius* FBCC1465. The ascomycetous fungi belong to the genera *Alternaria* (HAMBI 3289) and *Fusarium* (HAMBI 3292) and were obtained from the HAMBI culture collection at the Department of Food and Environmental Sciences/ Microbiology and Biotechnology Division at the University of Helsinki in Finland. The selected ascomycetous fungi were isolated from arable soil in southern Finland.

2.2. Indicator Color Plate Tests

Impacts of metals on the growth and production of extracellular oxidative enzymes with three basidiomycetous and two ascomycetous fungi were tested in the presence of Al (20, 50, 100 mg kg^{-1}), Mo (10, 20, 50 mg kg^{-1}), V (5, 10, 20 mg kg^{-1}), Zr (10, 20, 50 mg kg^{-1}), W (10, 20, 50 mg kg^{-1}) or Ga (10, 20, 50 mg kg^{-1}) on the indicator color plates. The chemicals were $AlCl_3$ (Fluka), $H_3[P(MoO_{10})]_4$ (Merck), V_2O_5 (J.T. Baker), $ZrOCl_2 \bullet 8 H_2O$, $Na_2WO_4 \bullet 2 H_2O$ (Merck) and $(Ga)_2(SO_4)_3$ (Riedel de Haen). The basal medium for indicator color plates contained 250 mg kg^{-1} ABTS (2, 2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid, Sigma-Aldrich, USA) as the color indicator. The basal medium contained 10 g of glucose, 2 g KH_2PO_4, 0.5 g $MgSO_4 \bullet 7 H_2O$, 0.1 g $CaCl_2$, 0.5 g of NH_4–tartrate, 2.2 g dimethylsuccinate, 0.1 g of yeast extract and 25 g of agar per liter. The pH was 5. The test plates were incubated at 25°C. The diameter of the growth and the formation of the color change were measured at 90° to the test plates. The average of the four measurements was calculated in the each indicator color plate. The diameters of the growth and the color zones were compared to those of control ABTS plates without added metal. The color zone indicates production of oxidative extracellular laccase enzyme.

2.3. Statistical Tests

ANOVA was used to test statistical differences between the growth or enzyme production of certain fungi with Al, Mo, V, Zr, W or Ga on ABTS color indicator plates compared to the ABTS plates without added metal. A t-test was conducted as a post-hoc test [39]. Statistical tests were calculated with Excel (Microsoft).

3. RESULTS

Figure 1 displays the growth of three basidiomycetous, *P. radiata*, *P. pulmonarius* and *P. rivulosus* fungi with Al, Mo, V, Zr, W or Ga compared to without added metal. All three basidiomycetous fungi grew with and without all six tested metals (Al, Mo, V, Zr, W, Ga).

The growth of *P. radiata* decreased 71% with Al (50 mg kg^{-1}), 29% with Mo (10 mg kg^{-1}), 28% with W (10 mg kg^{-1}) and 59% with Ga (10 mg kg^{-1}), indicating that *P. radiata* is sensitive to Al, Mo, W and Ga. The growth of *P. radiata* increased in the presence of V (10-50 mg kg^{-1}) and Zr (10-50 mg kg^{-1}), indicating that *P. radiata* even benefitted from the presence of V and Zr. The growth of *P. pulmonarius* decreased 23% with Al (20 mg kg^{-1}), 73% with Mo (20 mg kg^{-1}) and 72% with W (50 mg kg^{-1}). The growth of *P. pulmonarius* remained stable or even increased with V (5-20 mg kg^{-1}), Zr (10-50 mg kg^{-1}) and Ga (10-50 mg kg^{-1}). The growth of *P. rivulosus* decreased 62% with Al (50 mg kg^{-1}), 46% with Mo (10 mg kg^{-1}), 26% with W (10 mg kg^{-1}) and 33% with V (10 mg kg^{-1}). The growth of *P. rivulosus* remained stable or even benefitted from Zr (10-50 mg kg^{-1}) and Ga (10-50 mg kg^{-1}).

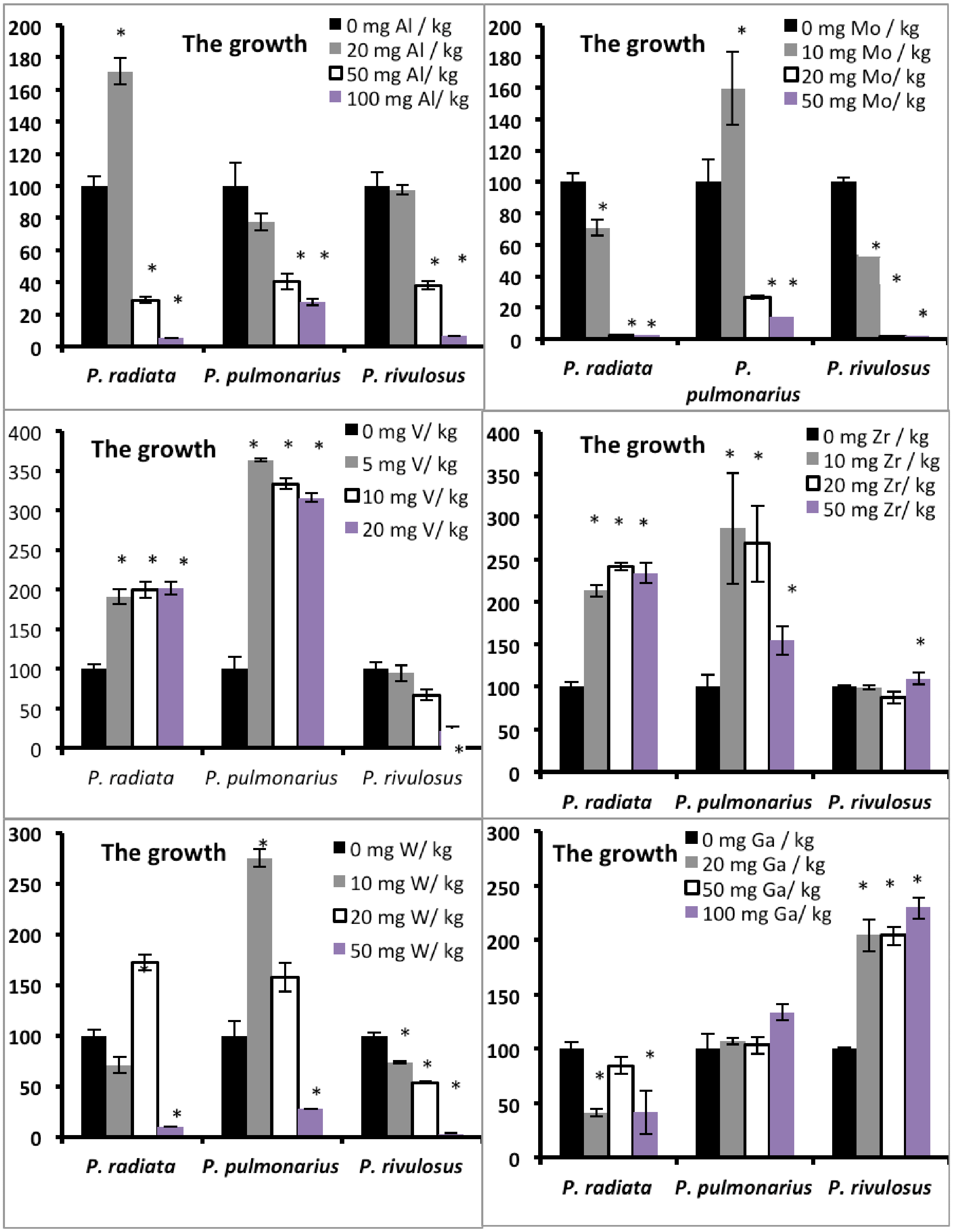

Figure 1 Growth of three basidiomycetous fungi with Al, Mo, V, Zr, W or Ga compared to without added metal on ABTS agar plates (n = 3). The error bar is standard error of mean. Asterisks (*) indicates statistically significant differences between with and without added metal.

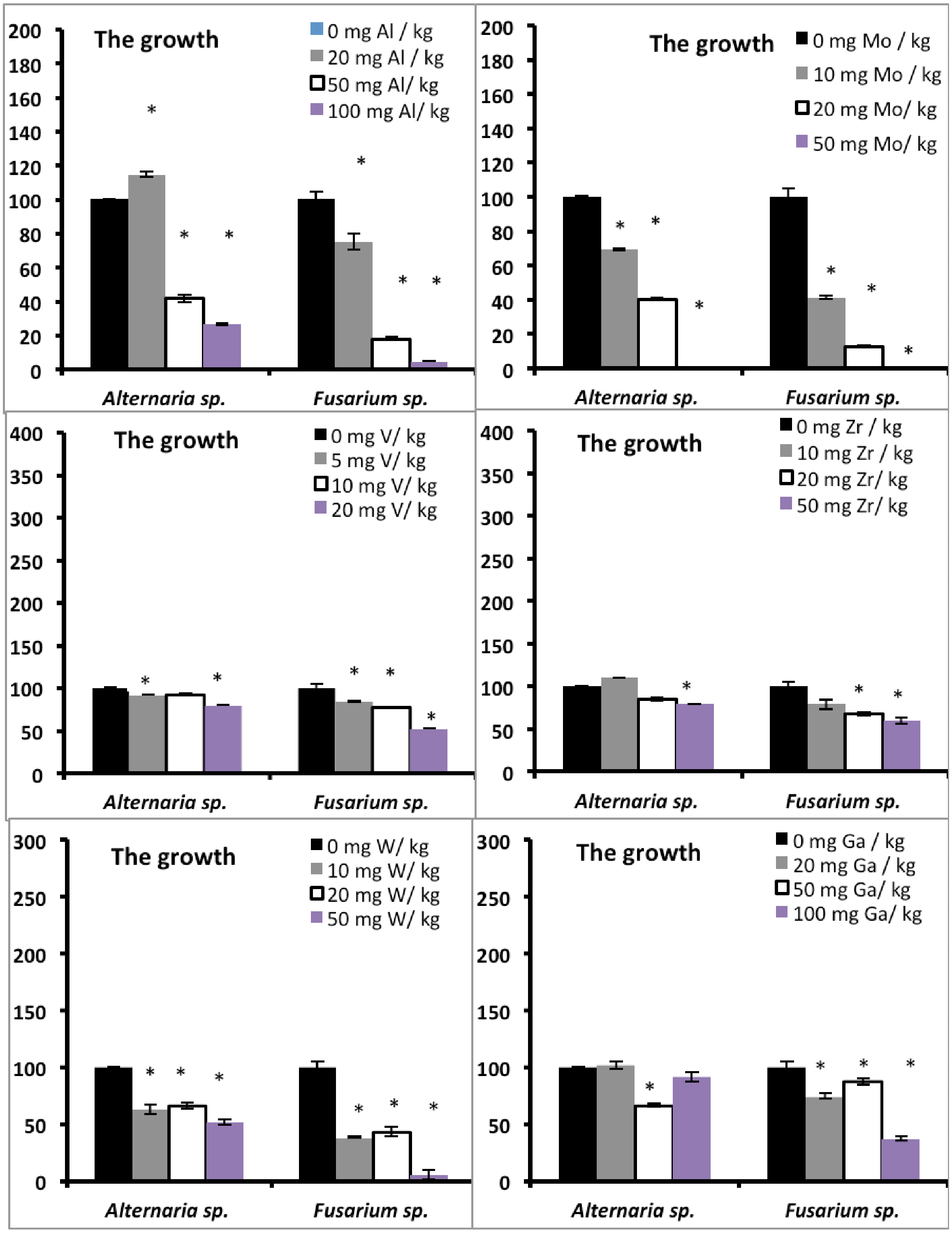

Figure 2 Growth of three ascomycetous fungi with Al, Mo, V, Zr, W or Ga compared to without added metal on ABTS agar plates (n = 3). The error bar is standard error of mean. Asterisks (*) indicates statistically significant differences between with and without added metal.

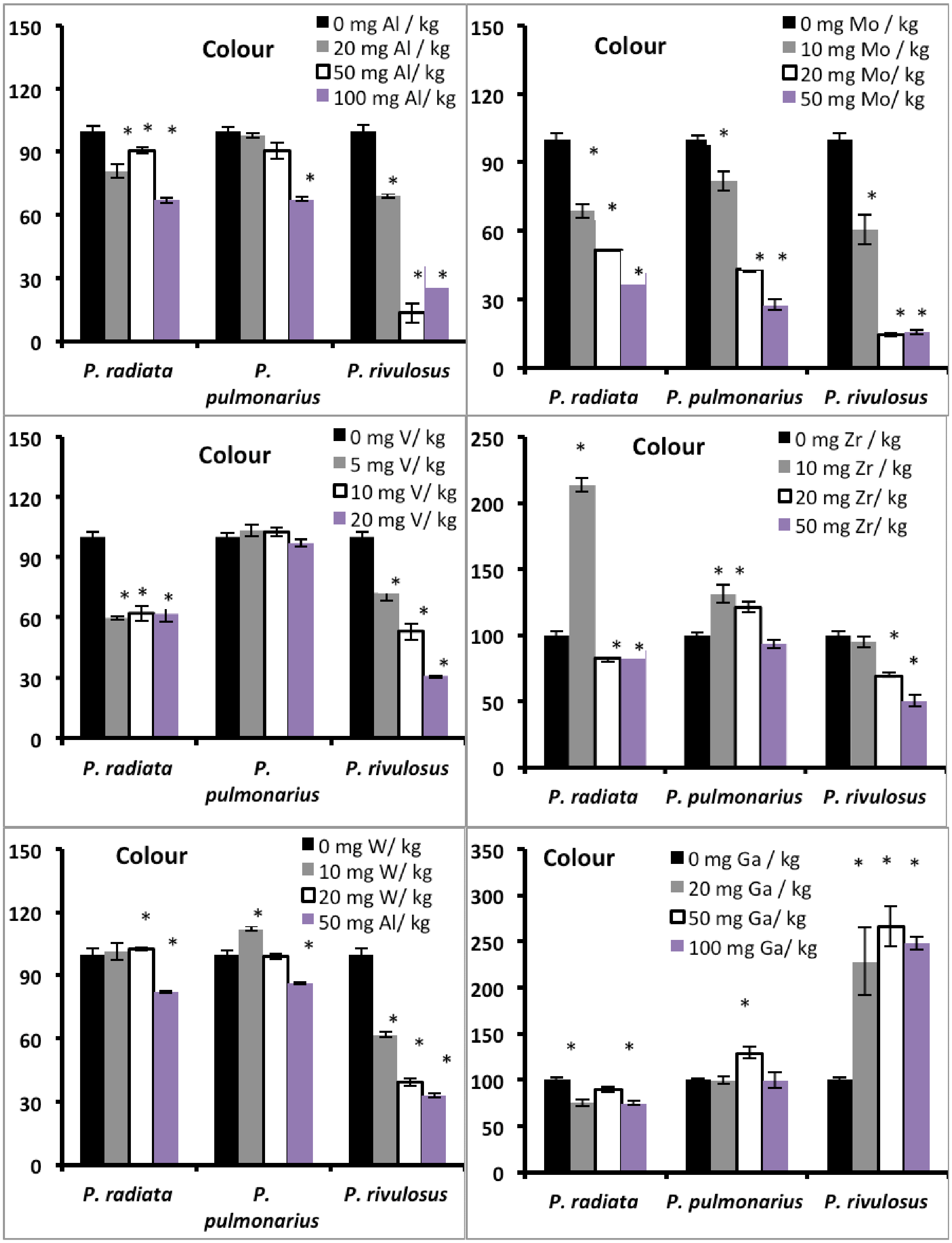

Figure 3 Color zone formation of three ascomycetous fungi with Al, Mo, V, Zr, W or Ga compared to without added metal on ABTS agar plates (n = 3). The error bar is standard error of mean. Asterisks (*) indicates statistically significant differences between with and without added metal.

Figure 2 displays the growth of two ascomycetes, *Alternaria* sp. and *Fusarium* sp. with Al, Mo, V, Zr, W or Ga compared to without added metal. The growth of *Alternaria* sp. decreased 58% with Al (50 mg kg^{-1}), 31% with Mo (10 mg kg^{-1}), 21% with V (20 mg kg^{-1}), 15% with Zr (20 mg kg^{-1}), 37% with W (10 mg kg^{-1}) and 33% with Ga (50 mg kg^{-1}) indicating that *Alternaria* sp. is sensitive to all six metals studied (Al, Mo, V, Zr, W, Ga). The growth of *Fusarium* sp. decreased by 25% with Al (20 mg kg^{-1}), 59% with Mo (10 mg kg^{-1}), 16% with V (5 mg kg^{-1}), 21% with Zr (10 mg kg^{-1}), 61% with W (10 mg kg^{-1}) and 26% with Ga (20 mg kg^{-1}), indicating that *Fusarium* sp. is sensitive to all studied metals (Al, Mo, V, Zr, W, Ga).

Figure 3 displays the formation of color zone with Al, Mo, V, Zr, W or Ga compared to without added metal. All three tested basidiomycetous, *P. radiata*, *P. pulmonarius* and *P. rivulosus*, fungi produced a color zone in the presence of the tested metals (Al, Mo, V, Zr, W, Ga) and without added metals, indicating that all three basidiomycetous fungi produced extracellular oxidative enzymes. The formation of a color zone with *P. radiata*, *P. pulmonarius* and *P. rivulosus* decreased 33-64% with Al (100 mg kg^{-1}), 18-86% with Mo (10-50 mg kg^{-1}), 13-67% with W (50 mg kg^{-1}) and 9-47% with Zr (50 mg kg^{-1}) compared to without added metal, indicating that production of oxidative enzymes by all three tested basidiomycetous fungi are sensitive to Al, Mo, W and Zr. Formation of color zone with *P. radiata*, and *P. rivulosus* decreased 27-69% with V (5-20 mg kg^{-1}) and with *P. radiata* decreased 11-25% with Ga (10-50 mg kg^{-1}) compared to the control. Formation of a color zone with *P. pulmonarius* and *P. rivulosus* remained stable or even increased in the presence of Ga (20-100 mg kg^{-1}) and with *P. pulmonarius* in the presence of V (5-20 mg kg^{-1}) compared to without added metal. Tested ascomycetous *Alternaria* sp. and *Fusarium* sp. fungi did not form a color zone in the presence of the tested metals (Al, Mo, V, Zr, W, Ga) or without metals, indicating that the tested ascomycetous fungi do not produce extracellular oxidative enzymes.

4. DISCUSSION

All three tested basidiomycetous, *P. radiata*, *P. pulmonarius* and *P. rivulosus*, fungi produced a color zone with Al, Mo, V, Zr, W and Ga and without added metals, indicating that all three basidiomycetous fungi produce extracellular oxidative enzymes. Tested ascomycetous *Alternaria* sp. and *Fusarium* sp. fungi did not form a color zone with Al, Mo, V, Zr, W and Ga or without tested metals, indicating that the tested ascomycetous fungi do not produce extracellular oxidative laccase enzymes. Basidiomycetous and some ascomycetous fungi produce laccase [13,40]. *Phlebia radiata* [41], *Pleurotus pulmonarius* [42] and *Physisporinus rivulosus* [43] produce laccase. A novel finding was that the production of laccase with *P. radiata*, *P. pulmonarius* and *P. rivulosus* is sensitive to Al, Mo, W and Zr, with *P. radiata* and *P. rivulosus* sensitive to V and with *P. radiata* to Ga. The production of laccase with *P. pulmonarius* and *P. rivulosus* tolerated or even benefitted with Ga (20-100 mg kg^{-1}) and with *P. pulmonarius* with V (5-20 mg kg^{-1}). Fungus *Curvularia inaequalis* produced the vanadium-containing chloroperoxidase enzyme [44]. Our results indicate that production of laccase with *P. pulmonarius* has a mechanism to tolerate Ga and V, and with *P. rivulosus* to tolerate Ga. Our results indicate that the production of laccase was vulnerable with basidiomycetous *P. radiata*, *P. pulmonarius* and *P. rivulosus* in the presence of Al, Mo, Zr and W and can change cycling of carbon and biodegradation of xenobiotics in soil.

The growth of *P. radiata*, *P. pulmonarius* and *P. rivulosus* was sensitive to Al, Mo and W and tolerant to Zr. The growth of *P. radiata* was sensitive to Ga. The growth of *P. rivulosus* was sensitive to V. The growth of *P. pulmonarius* and *P. rivulosus* was tolerant to Ga. The growth of ascomycetes *Alternaria* sp. and *Fusarium* sp., was sensitive to all six studied metals Al, Mo, V, Zr, W and Ga. The growth of *Trichoderma aureoviride*, *Trichoderma harzianum* and *Trichoderma viride* was inhibited 50 % with Al in the concentration range (100-150 mg dm^{-3}) [45], which is close to inhibitory concentrations for all our three tested basidiomycetous and two ascomycetous fungi. The growth of ectomycorrhizal fungi *Pisolithus* sp. and *Cantharellus cibarius* was almost completely inhibited with 200 µg Al dm^{-3} in liquid culture [46], which is about two orders of magnitude lower concentration than in the present study. The growth of *Aspergillus terreus*, *Cladosporium cladosporioides*, *Clonostachys rosea*, *Paecilomyces lilacinus*, *Penicillum citrinum* and *Rhizopus arrhizus* in 180 mg V dm^{-3} containing malt extract agar decreased 45%, 55%, 60%, 30%, 0% and 20%, respectively, compared to the control [47]. This V concentration is up to an order of higher concentrations than those for V (5-20 mg kg^{-1}), where the growth of tested ascomycetous fungi in our study was inhibited. *Penicillium simplicissimum* tolerated 8000 mg W dm^{-3} in liquid culture [48], which is up to two orders of magnitude higher than the concentration of W (10-50 mg kg^{-1}) that inhibited our tested basidiomycetous and ascomycetous fungi. The growth of two tested ascomycetous fungi was inhibited up to an order of magnitude lower concentration (10-50 mg Mo kg^{-1}) in the present study compared to the growth of *Alternaria alternata*, *Aspergillus flavus* and *Cladosporium herbarum*, which were tolerant to 500 mg Mo dm^{-3} in liquid culture [49].

The contamination limits set by the Finnish Government are 150-250 mg V kg^{-1} in soil [50], which is higher than the concentration of V, where the growth of basidiomycetous *P. rivulosus* and two tested ascomycetous fungi is inhibited. The Finnish Government has not set the contamination limit values for Al, Mo, Zr, W, or Ga in the soil. The growth of tested ascomycetes was more sensitive to V, Zr and Ga than the growth of *P. pulmonarius.* Our results indicate that *P. pulmonarius* is suitable for the biodegradation of organic xenobiotics in V, Zr and Ga contaminated soil. Our results also indicate the change of fungal communities in the metal Al, Mo, V, Zr, W and Ga contaminated soil. Basidiomycetous fungi are more tolerant than ascomycetous fungi to grow and produce oxidative enzymes, indicating that basidiomycetous fungi are more suitable for the bioremediation of organic xenobiotics than ascomycetous fungi in metal-contaminated soils.

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