

VALORISATION OF FOREST BIOMASS SIDE-STREAMS IN ADD VALUE GREEN-PRODUCTS FOR HORTICULTURAL INDUSTRY

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Ph.D. Henrique Manuel Filipe Ribeiro

**THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE IN
ENVIRONMENTAL ENGINEERING**

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2020

In memory of my grandfather,

António Nunes de Carvalho (1937-2019)

“This is the most simple and basic component of life: our struggles determine our successes”

Manson, M. (2016). *The subtle art of not giving a f*ck* (First edition.). New York, NY: HarperOne, an imprint of HarperCollinsPublishers

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Abstract

Horticulture industry uses peat as the main constituent in growing media formulations due to its ability to support efficient plant production. However, peat is a non-renewable resource at its actual extraction rate, and environmental issues associated with greenhouse gases emission from peat harvest raised peatland ecosystem conservation awareness through environmental initiatives, organizations and politics worldwide, limiting its use. There has been an increasing demand for environmentally friendly peat alternatives focused on locally available, organic and renewable materials from industrial side-streams, mainly wood-based and forest biomass. Therefore, woody raw-materials physical, chemical and biological properties are important to determinate further pre-treatment identification and choice. This work evaluates bark-based growing media suitability from non-native forest species in Mediterranean region, *Acacia melanoxylon* – residual biomass from invasive species control – and *Eucalyptus globulus* – a pulpwood industrial waste-stream. Ageing, a zero-waste treatment, allowed *A. melanoxylon* mature bark to effectively replace half of container medium volume as peat alternative. Low-temperature hydrothermal treatment, a faster process, enabled *E. globulus* bark to substitute quarter container medium volume, ensuring equal plant performance as commercial material. Both raw-materials sieve size manipulation promoted its incorporation as aeration growing media component. Given the wood-based raw-materials nature, Nitrogen amendment should be provided according to plant and cultivation system's needs. Furthermore, by replacing the 'end-of-life' biomass material into new potential horticultural products, circular economy approach was applied throughout this study. Thus, *A. melanoxylon* juvenile bark extracts phytotoxic effect showed a promising non-synthetic and natural bio-herbicide for weed control. In response to the potential circularity of invasive species biomass resources into add-value horticultural products, the present study outcome underlines *Acacia* species biomass commercial valorisation as alternative management tool to support the costs of control, avoiding the potential risk of conflict between economic exploitation and negative environmental impact.

Keywords

Peat alternatives; forest residual biomass; non-native species; waste-flow valorisation; circular economy.

Resumo

A indústria hortícola utiliza a turfa como constituinte principal na formulação de substratos devido às suas características ideais para a produção eficiente de plantas. A turfa é um recurso não renovável à escala de extração atual, e problemas ambientais relacionados com a emissão de gases com efeito de estufa devido a esta extração aumentaram o apelo à conservação do ecossistema das turfeiras através de iniciativas, organizações e políticas ambientais, limitando o seu uso. Existe uma crescente procura por materiais alternativos à turfa e “amigos do ambiente”, centrados em recursos localmente disponíveis, orgânicos e renováveis, provenientes de resíduos industriais, concretamente da biomassa florestal. Consequentemente, a caracterização física, química e biológica das matérias primas florestais torna-se essencial para uma escolha eficiente do pré-tratamento exigido. Este trabalho avalia a aptidão de substratos provenientes da biomassa de espécies florestais não-nativas na região Mediterrânica, como *Acacia melanoxylon* (biomassa residual proveniente do controlo de espécies invasoras) e *Eucalyptus globulus* (subproduto da indústria da celulose e papel). O envelhecimento, um tratamento livre de resíduos, permitiu a substituição de metade do volume, em vaso, de turfa por casca madura de *A. melanoxylon* sem comprometer a qualidade da planta. O tratamento hidrotérmico a baixa temperatura possibilitou a mistura de um quarto de volume de casca de *E. globulus*, com crescimento da planta igual ao substrato comercial. O controlo da granulometria promoveu a incorporação das cascas como agentes de arejamento do substrato. A fertilização azotada deverá ser aplicada tendo em conta as necessidades da planta e do sistema de cultivo. A casca juvenil de *A. melanoxylon* revelou-se um promissor bio herbicida natural para o controlo de infestantes. Em resposta à potencial circularidade da biomassa de espécies invasoras em produtos hortícolas de valor acrescentado, o presente estudo menciona a valorização comercial de biomassa residual de espécies de Acácia como ferramenta alternativa de gestão para suportar os custos do controlo de invasoras, reduzindo o risco de conflito entre aproveitamento e exploração económicas e o impacto ambiental negativo.

Palavras chave

Alternativas à turfa; biomassa residual florestal; espécies não-nativas; valorização fluxo de resíduos; economia circular.

Resumo alargado

A turfa é um solo orgânico formado pela decomposição de matéria vegetal em condições anaeróbias e de alagamento, criando o ecossistema das turfeiras. Este ecossistema teve a sua origem há 10 000 anos, e atualmente cobre uma área de 5 milhões Km² (aproximadamente 3% da superfície terrestre), equivalente à floresta Amazónica. As turfeiras exercem um papel fundamental na regulação do ciclo de carbono; são responsáveis pelo armazenamento de um quarto de carbono total do solo e de 10% da reserva mundial de água doce. Porém, a colheita de turfa envolve a drenagem das turfeiras durante o processo de extração, provocando a libertação de gases com efeito de estufa para a atmosfera e, conseqüentemente, acelerando o efeito das alterações climáticas. Além do impacto climático negativo, a biodiversidade encontra-se ameaçada pela degradação deste ecossistema único, e a turfa é um recurso não renovável à escala de extração corrente. Atualmente, a indústria hortícola depende da turfa para a formulação de substratos devido às suas características ideais para a produção eficiente de plantas. No entanto, o uso da turfa como meio de cultivo encontra-se ameaçado por fatores que afetam a procura deste recurso finito a nível global, como: i) distribuição geográfica das turfeiras, na Europa limitada aos países do Norte (Noruega, Irlanda e países Bálticos), fazendo com que países mais afastados (Alemanha, Holanda e Bélgica) dependam da distância e do aumento do custo de transporte; ii) apelo à conservação do ecossistema das turfeiras através de iniciativas, organizações e políticas ambientais para a descarbonização da indústria turfeira, levando os líderes da convenção no Acordo de Paris a instituir o fim da extração de turfa até 2050; iii) crescimento da população mundial, exigindo a contínua produção de alimentos, com a indústria hortícola a ter um papel fundamental no sistema global de produção alimentar, o que cria espaço para a formulação de substratos a partir de materiais alternativos à turfa. Um substrato hortícola ideal deve conter propriedades físicas (equilíbrio da relação ar-água), químicas (baixos pH e teores de sais e nutrientes) e biológicas (ausência de pragas, agentes patogénicos, infestantes e toxicidade, e estabilidade biológica) favoráveis a um crescimento saudável da planta. Atualmente, existe uma crescente procura por novos materiais alternativos à turfa e sustentáveis, centrados em recursos localmente disponíveis, orgânicos e renováveis, provenientes de resíduos industriais (circularidade do fluxo de resíduos aplicada à indústria hortícola), concretamente da biomassa florestal. Após

a recolha da biomassa, esta necessita de ser pré-tratada para remoção de compostos fitotóxicos, e posteriormente fertilizada para um balanço estável da disponibilidade de azoto para os microrganismos e plantas. Consequentemente, a caracterização física, química e biológica das matérias primas florestais torna-se essencial para uma escolha eficiente do pré-tratamento exigido na utilização como substrato orgânico. Este trabalho tem como objetivos principais: i) caracterizar a biomassa de *Acacia melanoxylon* (madeira e casca) proveniente de ações de controlo de plantas invasoras, e discutir os possíveis usos; ii) avaliar o potencial do fluxo de resíduos florestais de casca de espécies não-nativas na região Mediterrânica, de *Acacia melanoxylon* (resíduo da gestão de controlo de plantas invasoras) e de *Eucalyptus globulus* (subproduto da indústria da celulose e papel), como componentes na formulação de substratos hortícolas através da avaliação, análise e otimização dos pré-tratamentos; iii) investigar as propriedades alelopáticas dos extratos da casca na produção de alternativas não químicas para o controlo de infestantes.

Esta investigação permitiu concluir que a idade do povoamento de *A. melanoxylon* afetou as propriedades da casca. Quanto à formulação de substrato hortícola, a casca madura envelhecida possibilitou a substituição de metade do volume do vaso como substrato alternativo à turfa, sem comprometer a qualidade da planta. O envelhecimento é um tratamento sem produção de resíduos secundários, porém, poderá demorar até 8 semanas para garantir um material não tóxico. Através do ajuste da granulometria das partículas, a casca grosseira poderá ser adicionada como agente de arejamento, enquanto a casca fina aumenta a retenção de água no substrato. Existe um pré-tratamento alternativo que utiliza temperatura, tempo e água como solvente – tratamento hidrotérmico – no entanto o teor de saponinas da casca madura não permitiu a execução desta alternativa, devido ao difícil manuseamento do material. Os extratos residuais do tratamento hidrotérmico da casca jovem, ricos em compostos fenólicos, exibiram um promissor efeito alelopático para o controlo natural de infestantes. Em resposta à potencial circularidade da biomassa de espécies invasoras em produtos hortícolas de valor acrescentado, o presente estudo menciona a valorização comercial de biomassa residual de espécies de *Acácia* como ferramenta alternativa de gestão para suportar os custos do controlo de invasoras, reduzindo o risco de conflito entre aproveitamento e exploração económicas e o impacto ambiental negativo.

O estudo das condições ideais do tratamento hidrotérmico da fibra de *E. globulus* destacou a temperatura como fator principal na remoção dos compostos fitotóxicos, demonstrando ser um processo rápido e eficaz. Devido à baixa temperatura utilizada, o efeito alelopático dos extratos residuais não foi considerado. Como agente de arejamento do substrato, a mistura de um quarto de volume de casca de *E. globulus*, após tratada, assegurou o crescimento da planta igual ao obtido com um substrato comercial. Uma quantidade equivalente de casca de *E. globulus* envelhecida, misturada num vaso, também confirmou o mesmo resultado positivo da resposta da planta. No entanto, poderá demorar até 4 semanas a concluir o processo de envelhecimento da casca. Portanto, ambos os tratamentos devem ser considerados para a valorização da fibra de *E. globulus*, pois este é um recurso com grande potencialidade na recirculação de materiais entre as indústrias da celulose e hortícola.

Adicionalmente à reconversão do ‘fim-de-vida’ da biomassa em novos produtos para utilização hortícola, o conceito de economia circular foi aplicado ao longo deste estudo, visando também a procura de alternativas sustentáveis para a recirculação de resíduos dos pré-tratamentos. Assim, os futuros trabalhos a desenvolver passarão por: i) estudar o promissor efeito bio herbicida natural da casca jovem de *Acacia* para o controlo de infestantes; ii) incorporar a casca jovem residual depois da extração na formulação de discos com efeito inibidor do crescimento de infestantes, para cobertura em vaso; iii) ajustar a espessura do disco e testar a impregnação de sementes para facilitar a sua propagação e sementeira, ou através da diversificação do molde numa estrutura de iv) vasos biodegradáveis para redução do uso de plástico. Estes trabalhos futuros vão ao encontro da emergente tendência de aplicações comerciais a partir de materiais “verdes”, reconhecendo a importância e necessidade da transferência de conhecimento entre a investigação aplicada à indústria, interligando as partes interessadas na obtenção de benefícios ambientais globais.

Palavras-chave

Alternativas à turfa; biomassa residual florestal; espécies não-nativas; valorização fluxo de resíduos; economia circular.

Contributions

Journal articles produced during PhD studies

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Chemetova, C., Gominho, J., Fabião, A., Ribeiro, H., 2019. Hydrothermally treated *Eucalyptus globulus* bark: an innovative organic material for plant substrates. *Acta Horticulturae* 1266, 207-214. <https://doi.org/10.17660/ActaHortic.2019.1266.29>

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Chemetova C., Barroso G., Gominho J., Fabião A., Ribeiro H. Green application for an industrial by-product: aged *Eucalyptus globulus* bark-based substrates. Submitted to *Acta Horticulturae* on 25th of November (2019).

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Conferences, symposiums and workshops

The professional exchange and presentations on ongoing research were regularly conducted in various occasions.

Flanders' Food Inspiration day: New resources, New opportunities, Gent, Belgium, November 2019.

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15th International Conference on Environmental Science and Technology, Rhodes, Greece, August 2017.

International Symposium on Growing Media, Soilless Cultivation and Compost Utilization in Horticulture, Portland, Oregon USA, August 2017.

Workshop “Gestão de espécies invasoras em Portugal: onde estamos e para onde queremos ir?” at Coimbra College of Agriculture, Portugal, July 2017

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CHAPTER 1

General Introduction



Acacia melanoxylon bark



Eucalyptus globulus bark

General Introduction

Global peat demand

Peat moss is an organic soil which is made of decomposed plant matter in water-logged conditions creating the peatland ecosystem (Drake et al., 2016). Peatlands have been growing for the last 10 thousand years, covering an estimated area of 5 million km² (approximately 3% of land surface). They may occur from high altitudes to coastal areas and from tropical rainforests to permafrost towards northern circumpolar regions (Méndez et al., 2015; UN Environment, 2019). Peatlands have a major role in carbon cycle regulation due to storage of a quarter of world soil Carbon (Bonaguro et al., 2017) - sink for carbon dioxide (CO₂) and source of atmospheric methane (CH₄) - as well as capacity to store around 10% of world's freshwater resource (Evans et al., 2019). They are mainly used for agriculture, forestry and grazing, and peat soil is extracted for horticulture and energy production (CIFOR, 2020; Gruda, 2012).

However, peat harvesting involves peat bogs drainage during its extraction process. The carbon stored under the water is released as greenhouse gas (GHG) to atmosphere, thus accelerating climate change through CO₂ emission (Vandecasteele et al., 2018). Figure 1 displays the 25 parties responsible for 95% of global CO₂ equivalent emission from peatland drainage. Indonesia is responsible for more than 40% of global peatland emissions, followed by EU 16 member states (accounting for 17%), and Russian Federation (with ca. 10%). Finland, Germany, Poland and Sweden hold 50% of EU peatland emissions. In addition to GHG emission, biodiversity is threatened by peatland ecosystem degradation, and peat is a non-renewable resource at the timescale of its extraction (Gruda, 2012; UN Environment, 2019).

Environmental concern around peat industry has been growing worldwide (Blok, 2019; Vandecasteele et al., 2018; Wetlands International, 2015). Efforts to reduce CO₂ emissions from peatlands increased political regulatory changes. In Europe, the convention leaders in Paris Agreement set the threshold of zero peat harvest by 2050 (Blok, 2019; IPS, 2019). Global initiatives and organizations (international, regional and country level) aiming to protect and restore natural peatland ecosystems have been growing worldwide, examples are listed in Table 1. Indonesia strictly influenced peatland industrial activity, due to its strong impact at global GHG emission (Figure 1), and recently set the sustainability commitment to restore 2.4 million ha of peatland by

2030 (Hergoualc'h et al., 2018). Climate change forecast have been acting as a driving force for decisions on peat extraction policies, especially in northern peatlands (Carlile et al., 2015); arctic temperatures are rising twice as fast as global average and, by 2050, climate models may suggest the loss of 35% of permafrost zones (Wetlands International, 2015). These are essential steps towards Sustainable Development Goals achievement (FAO, 2015), currently aligned with European Green Deal (United Nations, 2019) to reach European continent carbon neutrality by 2050. At European level, it means practical actions (connecting economic, institutional, technological, social and legal factors) for a 50% cut in GHG compared to 1990 levels (FAO, 2015).

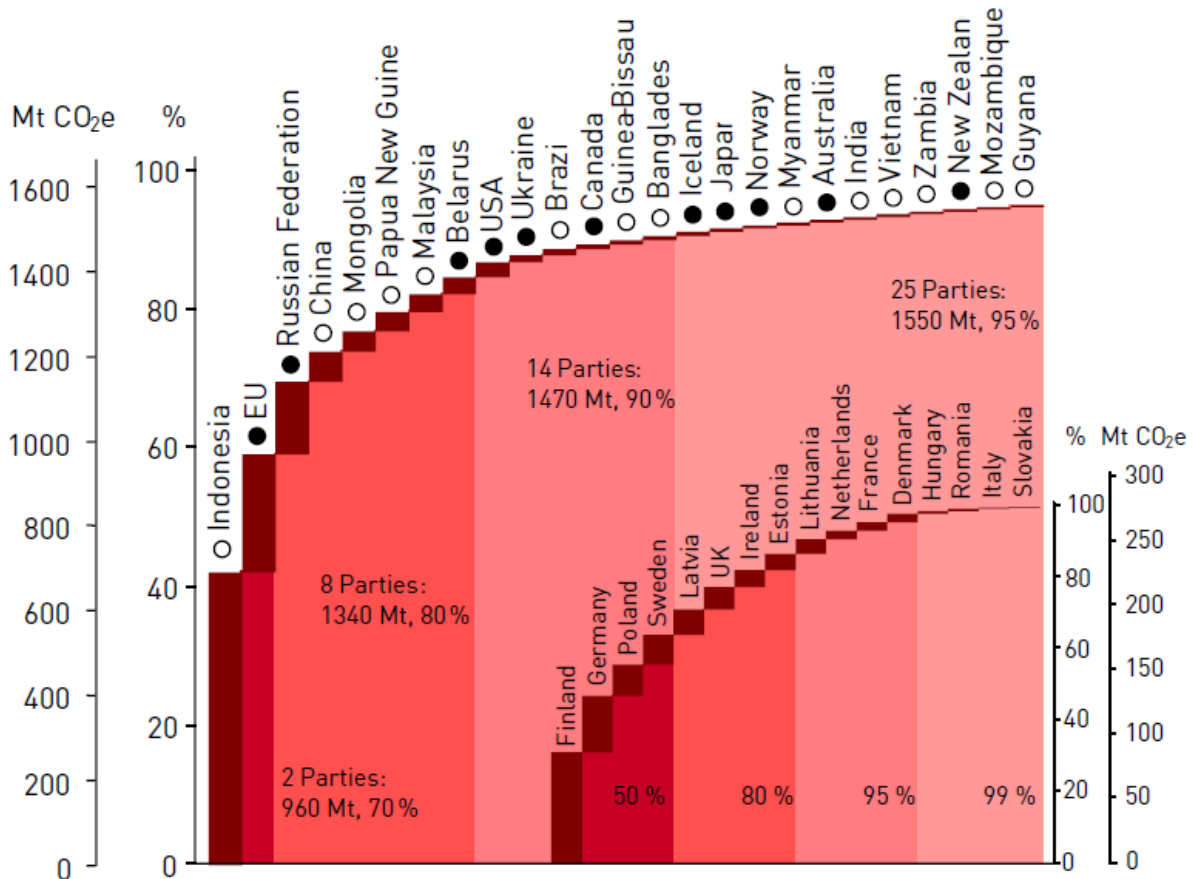


Figure 1. The 25 key parties with emission from drained peatlands in descending order. Greenhouse gas emissions in a cumulative way in Mt CO₂e per year (Mt = 1 000 000 tonnes) and as percentage of the total global emissions from degrading organic soils. 70, 80, 90 and 95 percent marks are crossed. The inset depicts the relative contributions of the 16 EU countries that are together responsible for 99 % of EU and 17 % of global peatlands emissions. (source: Wetlands International (2015))

Table 1. Worldwide organizations and initiatives aiming to improve the conservation, restoration and sustainable management of peatlands focus on economic, environment and social aspects.

Organizations and initiatives				
Type	Name	Parties	Main focus	Source
Natural Sciences and Engineering Research Council of Canada	Arctic Development and Adaptation to Permafrost in Transition	Canada	Integrated Permafrost Systems Science approach - how changing permafrost and snowfall affect peatlands, water and wildlife, and the implications for peat industry	(ADAPT, 2020)
NGO for Canadian peat moss producers and marketers interest representation	Canadian Sphagnum Peat moss association	Canada	Provides support and advocacy for CSPMA members and leadership in environmental and social stewardship, and economic well-being related to the use of Canadian peatland	(CSPMA, 2020)
Non-profit scientific research organization	Centre for International Forestry Research	Indonesian part of Borneo and New Guinea	Geo-platform tool on how the land cover of the Indonesian part of Borneo and New Guinea island has changed over time. Aiming to improve the governance of peatlands by increasing transparency and accountability for peat mining companies	(CIFOR, 2020)
Non-structural institution directly under and responsible to the President of the Republic of Indonesia	Peatland Restoration Agency	Indonesia	Empowering communities to join in restoration of 2 million ha of degraded peatlands by 2020	(BRG, 2020)
For-profit Organization	Katingan Peatland Restoration and Conservation Project	Indonesia	Peatland conservation and restoration project covering around 120 thousand ha of peatland	(PT Rimba Makmur Utama, 2016)
Non-profit Organization	Global Environmental Centre	Malaysia	Promotes sustainable peatland management strategy - Peatlands conservation and rehabilitation projects in Southeast Asia	(GEC, 2020)

International partnership with 33 organizations	Global Peatlands Initiative	Global	Global assessment of peatland extent and carbon content followed by a more detailed analysis of sustainable peatland management options	(GPI, 2020)
International NGO of scientific, industrial and regulatory stakeholders	International Peatland society	Global	Organization of events, publish peat and peatland knowledge, share data and provide facts and opinions for decision-makers	(IPS, 2019)
NGO for European peat industry interest representation	Growing Media Europe AISBL	EU	Promotes optimum legislation for the manufacturing as well as the free and fair trade of growing media within Europe	(GME, 2020)
Trade Association	Growing Media Association	UK	The Growing Media Initiative - mechanism to encourage growing media manufacturers to develop and market effective peat replacements	(GMA, 2020)
Gardening charity	Royal Horticultural Society	UK	Transparency and traceability - Informative guides to gardening community on how to grow more with less peat	(RHS, 2020)
Charity	National trust	UK	Protecting peatlands through research, conservation and sensitive management - Northern Ireland	(NT, 2020)
Charity	Waste and Resources Action Programme	UK	Develop a good practice guide provides practical advice to compost producers about the production of quality composts that are consistently fit for purpose as growing media constituents	(WRAP, 2020)
Charity	Friends of the earth	UK	Local campaigns of peat reserves protection – Ballymaloe, Thorne and Hatfield Moors bog nature reserves	(FE, 2020)

Increasing the world's demographic trend is a well-known forecast; by 2050, United Nations predicts that global population will reach around 9.7 billion (United Nations, 2019). The exponential urbanization rate in contrast with rural abandonment will persist, and projections point that around 70% of world's population will live in urban areas (Gruda, 2019; United Nations, 2018), mainly in Asia and Africa, demanding an additional 40% of food resources to sustain the world's challenge (Martin et al., 2016). Combined with globalization, the urbanization process detached citizens from food production sites and made them dependent on imported food. However, in parallel, urban population has been growing their interest in urban farming concept (Edmondson et al., 2020). Furthermore, climate change (rising temperatures, extreme weather events and soil erosion) is projected to result in farm yield losses (FAO, 2015). New plant-based diet patterns and consumption habits have been adopted by developed countries increased food safety awareness (Blok, 2019). These above-mentioned factors combination impact food security, climate change and social justice, urging the need of agri-food production system immediate actions, with proactive and innovative solutions to guarantee the quality and safety of world's food supply (European Commission, 2019; Williams et al., 2019).

Horticulture industry can ensure high quality plant production and contribute to global food security, playing the fundamental role in global food production systems, consequently demographic projection has direct impact in horticulture sector demand (Blok, 2019; European Commission, 2019; IPS, 2019). Horticultural or soilless plant production includes both water culture systems known as "hydroponics" and containerized plant systems using a porous rooting medium known as "substrate" or "growing medium", thus a desired growing medium must hold physical (balanced air-water relationship), chemical (low pH, salinity and nutrient content) and biological (free from pests, pathogens and weeds, biologically stable and non-toxic) properties for a healthy root and plant growth (Gruda, 2019). Since early 60's peat moss is the main raw-material used for horticulture growing media due to its inherent characteristics suitable for plant growth (Abad et al., 2001; Bonaguro et al., 2017; Gruda, 2012; Gruda and Schnitzler, 2004; Neumaier and Meinken, 2015; Zhong et al., 2018), such as: low pH and nutrient level, absence of pathogens, non-phytotoxic, biological stability and reliable high quality. *Sphagnum* moss is the most suitable peat for horticulture, existing in northern peatlands within the permafrost zones of Canada, Denmark, Greenland,

Finland, Norway, Sweden and Russia, as well as Baltic States and Germany (UN Environment, 2019). The limited peat moss geographical availability might rise transportation cost to peat dependent countries (e.g. US horticulture sector is mostly dependent from Canadian peat, while EU main peat exporters are Baltic states, Germany and Sweden) (Abad et al., 2001). From the total 100 million m³ of peat extracted per year, 40 million m³ are used in horticultural production (Gruda, 2012), with peat moss amounting to 70% of this value, and the latest projections estimated that the volume demand will double by 2050 (Blok, 2019). In addition, peat depending countries as China will influence EU and US peat markets in a faster rate, forced by food demand by fast-growing Asian population (European Commission, 2019; UN Environment, 2019).

Summarizing, the peat moss resource availability for 2050 are limited by:

- Geographic peatlands distribution, and transport distance and cost
- Environmental values, ecology and climate change protection
- Political decisions, regulations
- Growing population, food demand

As response to the above limitations, since the 90's of last century, the rise of "green movement" has been attracting considerable interest within the horticulture industry stakeholders. This "green movement" is driven by negative peat scarcity scenarios (Gruda, 2019), as well as the sustainable trend of agri-food sector (Blok, 2019; Neumaier and Meinken, 2015), which created the new circular horticulture concept. It comprises the framework of producing more quantity and quality food with less inputs focussed on efficient resource use, and re-introduction of organic side-streams as new raw-materials (European Commission, 2019). Sustainability in circular horticulture should be based on following aspects: improving farming practices, reducing GHG emission, re-introducing waste-stream flows while maintaining crop yield, understanding consumer habits change, gradual shift to plant-based diets, and introduction of novel products to reduce chemicals and peat dependence (Bar-Tal et al., 2019). Consequently, numerous growing media components, derived from organic side-streams generated by agriculture, livestock farming, forestry, other industries (e.g. coconut or bamboo production) and municipalities have been introduced

worldwide as organic alternatives to peat, mostly to be used as mixture or to be added to peat-based growing media (Caron et al., 2014; Neumaier and Meinken, 2015).

Alternative organic growing media

As for general growing media choice, the alternative organic components must have the ideal performance according to (i) physical, chemical and biological properties essential for plant growth, and (ii) production systems practical requirements driven by plant biology and applied plant technology. The physical properties have the biggest influence on air and water availability to the roots and rely on growing media structure (particle size, shape and texture) to describe aeration and water-holding capacity of different materials (Wallach, 2019). Chemical properties such as pH and electrical conductivity have the largest impact on nutrient availability, but this parameter can be manipulated by growers for an efficient plant nutrient provision (Barrett et al., 2016). Especially organic materials phytotoxicity, biological stability and nutrient immobilization are the most limiting factors for direct use as “fresh” raw material. Usually organic material biological behaviour pre-requires secondary processing or pre-treatment before its use (Gruda, 2019). Pre-treatments can represent additional investment cost for growing media manufactures. However, they might be compensated by environmental carbon footprint savings.

The challenge of peat replacement deal to its ideal performance for plant growth and “ready-to-use” characteristic, with no pre-treatment need. Nevertheless, peat-based growing media can be partly or even fully substituted and the focus on organic components have gain research attention due to general low-cost and availability, as well as the sustainability concept within a circular horticulture approach (Bar-Tal et al., 2019). The most commercialized organic materials are coir, compost, bark and wood-based materials such as fiber (Gruda, 2012).

Coir

Coir is a by-product from coconut (*Cocos nucifera* L.) industry; it is the fibrous material derived from the mesocarp of the fruit, and is one of the most abundant plant-derived organic waste-stream from tropical and subtropical countries as Sri Lanka, India,

Philippines, Vietnam, Mexico, Ivory Coast and Guyana (Carlile et al., 2015). Depending on the origin, during the production process coir is soaked with salt water to facilitate gridding, which might increase salt levels (K, Na and Cl content), thus often a washing pre-treatment is needed with fresh water or buffering with calcium nitrate $\text{Ca}(\text{NO}_3)_2$ previously to horticultural use (Neumaier and Meinken, 2015). In addition, coir must be aged to guarantee material stability.

Coir chips, fiber and pith (Figure 2) are some of the materials that can be obtained for use in growing media. Coir is mostly used as aeration agent due to low bulk density and high air capacity ($40\text{-}70\% \text{ vv}^{-1}$) lowering the water logging risk in growing media mixtures. It has a good water holding capacity and rewetting properties and tends to retain its initial structure after wet and dry cycles. Since coir contains more lignin and less cellulose than peat it is more resistant to microbial breakdown, reducing the risk of nitrogen immobilization and consequently offering biological stability (Barrett et al., 2016).



Figure 2. Coconut fruit, coir chips and fibers aspect (Source: Garden (2020))

Worldwide, coir has been used as mixture or standalone growing media since late 80's. Typically, coir fibers can replace about $20\% \text{ vv}^{-1}$ of peat in growing media and

coir ships might completely substitute peat for course growing media. In 2017, the annual coir production was about 5 million m³ and predictions estimate an increase of 7 times total coir demand for horticultural use by 2050 (Blok, 2019). However, the main disadvantage of coir production is the long distance from coir suppliers and EU and US growers, reflected in high transportation environmental impact and cost (Gruda, 2019).

Compost

Compost is a general term describing the resulted material from bio-oxidative aerobic and thermophilic process (Figure 3) in which microbial populations decompose the original raw-material into a more physically, chemically and biologically stable final product (Zhong et al., 2018). Compost properties may vary with initial raw-material characteristics, and duration and nature of composting methodology, dictating the end-product quality regarding biological oxygen demand, organic matter and nutrient contents, and pathogen free guarantee. Numerous composted materials have been globally used as organic growing media due to its strong environmental incentive to be re-used; organic wastes with no suitable disposal options, despite landfill or incineration plants (Raviv et al., 2019), have been composted, such as: municipal solid waste (Abad et al., 2001), sewage sludge (Ostos et al., 2008), animal manure (Ribeiro et al., 2009), food industry by-products (sugar cane, olive mill, grape marc, corn cobs, rice and peanut hulls) (Santos et al., 2016; Zhong et al., 2018), and marine costal residue (*Posidonia oceanica* L.) (Parente et al., 2014). This section does not include forest biomass composted waste and bark.

Composts are generally good sources of P and other micronutrients, and have higher water holding capacity than peat, although a limiting factor of their use might be very high soluble salts content and pH, therefore recommending them as a component of growing media mixtures (Raviv et al., 2019). In addition, composts must be stable and mature; a mature compost which undergone a long and well-controlled composting process is more stable than young ones that still contain readily biodegradable compounds leading to plant root oxygen and N deficiencies. The environmental value-added benefits of using compost should be mentioned as biofertilization and bio stimulation plant growth (Abad et al., 2001; Santos et al., 2016).



Figure 3. Composting process (thermophilic phase) from unknown material (Source: RODALE, 2020)

Depending on the final product characteristics, composts can be mixed 10-50% vv^{-1} with other materials (Barrett et al., 2016). Over the last 40 years, composts have been widely used as a growing media component in the Netherlands, United Kingdom, Italy and Germany for horticulture hobby market, and in Canada and United States for the professional horticultural sector (Carlile et al., 2015). In 2017, the annual compost demand was about 1 million m^3 (excluding bark) and is estimated to increase up to five times by 2050 (Blok, 2019). However, the high compost bulk density increases the weight of media, rising its transportation cost, and to guarantee pathogen free environment as well as stability it might take long periods of composting time, which can be a disadvantage for compost use (Gruda, 2019).

Bark and wood-based materials

After coir, bark, mainly from trees included in the *Pinaceae* family, dominates the alternative growing media market in areas where peat is scarce, and high transportation

distance and cost are determinant, such in southern Europe maritime pine (*Pinus pinaster*), south eastern USA loblolly pine (*Pinus taeda* L.), Pacific Northwest douglas-fir (*Pseudotsuga menziesii*), Australia slash pine (*Pinus elliottii*) and New Zealand Monterey pine (*Pinus radiata*) areas (Barrett et al., 2016; Carlile et al., 2015). Bark is a by-product from wood manufacturing, considered a low-cost and readily available material (Depardieu et al., 2016). As with coir, bark can be produced in various particle sizes to suit different growing media characteristics regarding air and water holding capacities (Gruda, 2019). Depending on final material characteristics and crop culture, up to 50% vv^{-1} of bark might be added for container plant production (Neumaier and Meinken, 2015).

Current bark demand is equal to compost and might be 10 times higher by 2050 (Blok, 2019). However, there are current global aspects influencing bark use in horticulture, such as (i) since early 2000's North America pine bark supply have been gradually decreasing due to decay of lumber industry, in combination with bark production for bioenergy purposes, increasing the competition and cost of pine bark resources (Barrett et al., 2016); and (ii) the emerging pests (pine wood nematode) and diseases (pitch canker) affecting southern Europe maritime pine trees have been monitored and studied concerning extend of infection transmission trough pine-based resources (European Commission, 2019). This may reflect in lack of trust regarding quality and safety issues of bark source origin.

Since the 80's of last century, the use of woody forest materials as alternative component has been growing in European horticulture industry, and after 2000 in USA (Carlile et al., 2015). Wood-based materials include wood chips, wood fiber (Figure 4) and sawdust, which are wood industry by-products. As with bark, wood-based materials are mainly from spruce (*Picea* spp.) and pine (*Pinus* spp.) trees. Usually, they are characterized by higher pH than peat, very high total porosity and air holding capacity, retaining insufficient water with tendency to shrink over time. Therefore, the irrigation regimes should be adjusted to achieve ideal watering conditions and this component is mostly used to optimize physical properties of other materials as aeration agent (increasing air-filled porosity) (Gruda and Schnitzler, 2004).



Figure 4. Wood fiber raw material from pulpwood industry waste-stream

Wood fiber companies produce commercial fiber materials using secondary processing methods (e.g. Fibrilla di Legno – Fibrilla®, Kekkila BvB – PrimeFLOW®, Florentaise – Hortifiber®, Lambert Peat Moss – Ecopeat®, Klasman – Green Fiber®, Berger – Natural Fiber Wood®) (Gruda and Schnitzler, 2004). The types of machinery to process wood chips to fiber are hammer mills, disk refiners and extruders. Economically, the extruder is the one requiring higher initial investment. However, the high pressure-temperature environment generated, modifies the fiber to a desired structure and creates a stable, sterile and consistent final product (Barrett et al., 2016). Wood fiber can be added up to 25% vv^{-1} as aeration growing media component (Caron et al., 2014). By 2050, wood fiber demand is expected to increase from 2 to 25 million m^3 (Blok, 2019).

Phytotoxicity and biological limitations

Both bark and wood-based materials, when fresh harvested or produced (without secondary processing methods) are usually pre-treated mainly via (i) composting, (ii) ageing or (iii) leaching/washing, to eliminate phytotoxic compounds that affect plant growth (Gruda, 2012). Such phytotoxins are secondary metabolites (terpenes, tannins and phenolic compounds) inherent to tree composition; in natural environment they have a protection effect against tree diseases, pests or infections. (i) Composting involves the entire process where materials have been piled, turned, aerated and amended with nitrogen. (ii) Ageing is a stockpiling and weathering process for several months after harvest. (iii) Leaching or washing raw material is a procedure where water (with established volume ratio) is used to extract phytotoxic compounds (the liquid extract may be collected for other uses). Other methods using temperature as main agent for phytotoxic compounds elimination may be used and combined (e.g. steam explosion, solarization and hydrothermal treatment) depending on initial raw-material chemical constitution (Gruda, 2019).

The fresh untreated bark and wood materials possess high Carbon (C) decomposition rate by microorganisms, or mineralization, which leads to high microbial Nitrogen (N) immobilization, competing for available N with plants (Carlile et al., 2015; Domeño et al., 2009; Neumaier and Meinken, 2015). The mineralization rate is influenced by production systems management (temperature and moisture) and properties of raw-materials (C/N ratio, lignin content and pH). The bark and wood material Nitrogen draw-down capacity or stability can be evaluated by applying a known mineral N (nitrate and/or ammonium) concentration and then evaluating the relative immobilization rate after a certain incubation period (Barrett et al., 2016). The most important factors to understand growing media stability involve knowing both the amount of N applied and the rate of N release from the raw material. The growing media stability might be balanced by blending with more stable components, by adding N sources (before and during plant growth) or by a composting process where N amended is adjusted (Vandecasteele et al., 2018).

Other organic materials

In areas where forestry activity is reduced and arable farmland is abundant, crop-derived growing media alternatives have been studied, mainly straw-like plant fibers, such as: flax (*Linum usitatissimum*) shives, miscanthus (*Miscanthus sinensis* L.), reed (*Phragmites australis*), giant reed (*Arundo donax* L.), switchgrass (*Panicum virgatum* L.) and willow (*Salix* spp.) (Carlile et al., 2015; Gruda, 2019; Vandecasteele et al., 2018). Each plant species holds a specific chemical, physical and biological profile regarding potential use in growing media formulations. As they are not initially produced for horticultural use, the new organic materials research should consider site specific environmental factors (e.g. edaphoclimatic characteristics).

Sustainable growing media trends

Based on the latest prediction (Blok, 2019), Figure 5 presents percentage of total organic (peat, coir, wood fiber, bark and compost) and inorganic (perlite, stone wool and soils/tuffs) growing media used in 2017 (left chart) compared to expected demand for 2050 (right chart), including possible new constituents. Organic materials are the most used, and within inorganic ones the soils/tuffs are the majority (representing 80% of the total inorganic), which are low quality materials with limitations to use. Currently, peat moss is the most used organic material and is expected to keep the highest demand by 2050, however being less representative, dropping almost half of demanded proportion, from more than 65% in 2017 (40 million m³ year) to around 35% in 2050 (80 million m³ year). Such reduction is mainly attributed to peat industry environmental issues aligned with increased trend to use alternative and sustainable organic growing media made from side-stream raw-materials (Hergoualc'h et al., 2018; Vicente et al., 2013). Wood fiber is expected to have the highest increase, reaching almost four times the demand by 2050, followed by coir and bark.

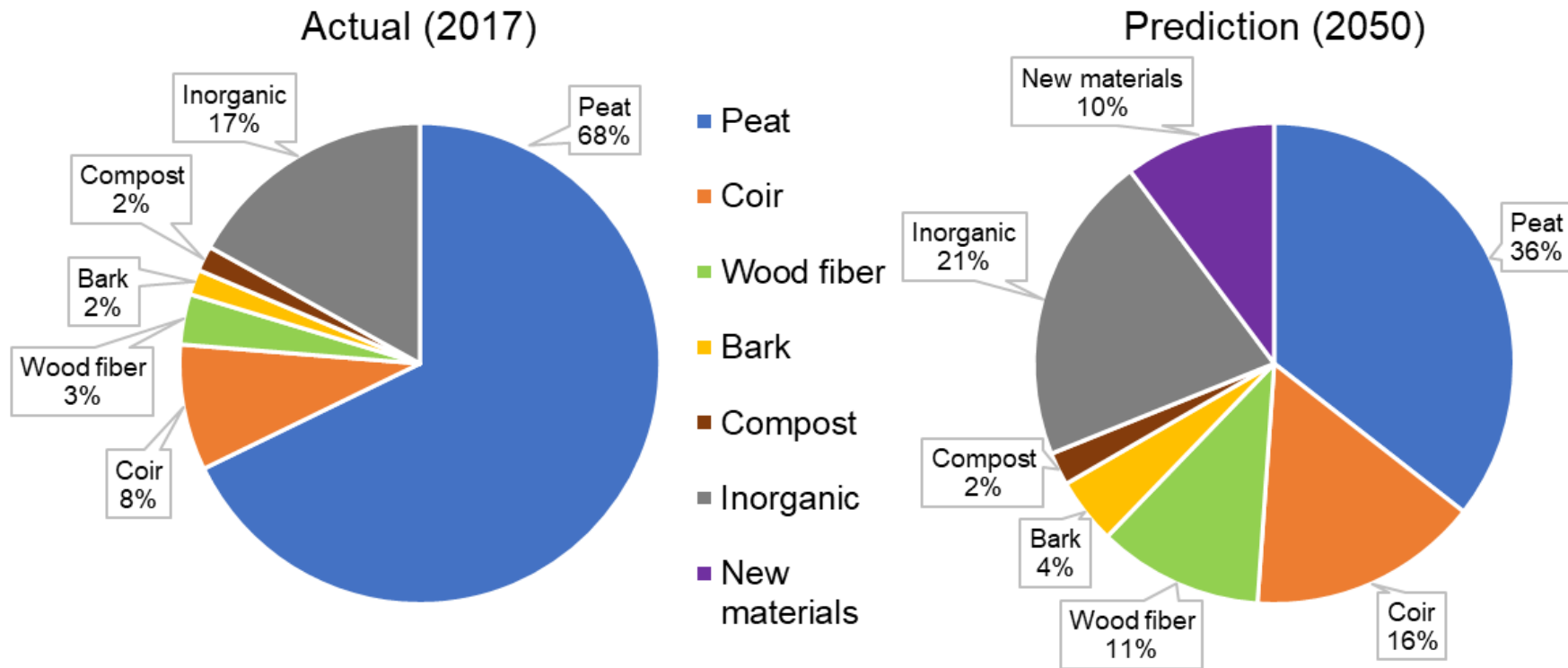


Figure 5. Global growing media material demand in 2017 (left chart) and predicted for 2050 (right chart), expressed in percentage of total amount (Adapted from Blok (2019))

One tenth of total growing media is the margin predicted for new materials introduction in horticulture industry by 2050 (Figure 2, right chart). Worldwide, there is an impressive amount of data concerning the use of alternative growing media, the suitability of these materials being difficult to compare (Barrett et al., 2016; Vandecasteele et al., 2018). Containerised plant production has various requirements to develop “standard mixtures” regarding consistent raw-materials supply streams, growing systems and growth seasons, while plant response is strictly dependent on plant types tested and proportions of mixtures (Zhong et al., 2018). The scientific community underline the need of correct characterization and use of standard methodology to overlap the challenging transition from testing to commercial production of alternative materials (Vicente et al., 2013).

The rising trend of novel, locally available and “environmentally friendly” raw materials, either from forestry or farm industries, have a beneficial “sustainable label” for nutrient recovery (biomass nutrient content is returned to plants) within circularity approach applied to horticulture industry. In the next 20 years nutrient use will increase by 30-40% (FAO, 2015) and to reach the most profitable and efficient situation, the optimization of cultivation systems regarding N management must be adapted in parallel with novel materials use to avoid deficiency or surplus of available N for efficient plant nutrition (Vandecasteele et al., 2018).

Novel wood growing media components

In southern European countries, especially in Mediterranean region, peat is not produced, and non-native tree species are abundant such *Eucalyptus globulus* and *Acacia melanoxylon*. The biomass by-products from forest activities and management are potential candidates as alternative and local sourced growing media components.

***Eucalyptus globulus* bark: forest waste-stream**

Eucalyptus globulus, commonly named Tasmanian blue gum, is native from South-eastern Australia and Tasmania. It is mainly cultivated in temperate and

Mediterranean region for pulp and paper production and explored in intensive short-rotation plantations (10-14 year long) (CELPA, 2017; Parada and Fernández, 2017). In Europe, it is distributed over 1.3 million hectares of forested area, especially in Iberian Peninsula (covering more than 1 million hectares), reaching around 845 thousand hectares only in Portugal (ICNF, 2015).

In Portugal, *Eucalyptus globulus* is the predominant species for pulp and paper production and around half million tons of *E. globulus* bark are generated as a by-product every year (CELPA, 2017). Currently, bark is directly used as solid biofuel in power plants inside of pulp and paper industries (Domingues et al., 2013). Other potential uses to valorise bark waste-stream have been investigated, such as:

- i. incorporation in the pulping process (Miranda et al., 2012; Neiva et al., 2016),
- ii. source of chemical compounds with biological and pharmacological activities (Domingues et al., 2013; Parada and Fernández, 2017),
- iii. incorporation into the soil to improve its structure and fertility (Yadav et al., 2002).

E. globulus bark is a smooth and fibrous material (Figure 6) representing in average 11% of stem dry weight (Quilhó and Pereira, 2001). Research on its use as a growing media component focused on bark cell wall characteristics. As a forest waste-stream, it is chemically rich in phenolic, triterpenic and other secondary metabolites which are phytotoxic for plant growth (Domingues et al., 2013; Yadav et al., 2002). In addition, as a wood fiber material, Nitrogen availability to plants might be limited due to high microbial activity. It is therefore essential to study and understand the bark biological stability before its use (Cunha-Queda et al., 2007).



Figure 6. Understory *Eucalyptus globulus* bark naturally released from tree trunks, laying over the soil at a plantation site in Cercal do Alentejo, South of Portugal (ca. 184 km to the south of Lisbon)

***Acacia melanoxylon* bark: non-native tree control residue**

Australian acacias were widely introduced around the world outside their native range. At least one-third of their ca. 1012 species were used for several purposes, including ornamental, economic, or environmental, the latter mainly related to sand dune stabilization or improvement of disturbed lands (Richardson and Kluge, 2008). Eight species of introduced Australian acacias were considered invasive in Europe, being very well represented within the Mediterranean area, especially in France, Spain and Portugal (Brito et al., 2015; Lorenzo et al., 2016; Souza-Alonso et al., 2018). *Acacia melanoxylon* R. Br., generally named Tasmanian blackwood, native from south-eastern Australia, is one of those species, being a fast-growing tree with high silvicultural performance and good adaptation to degraded soils (Vicente et al., 2013). These characteristics probably led to its introduction in Portugal in the late XIXth century and further use

in forestation, e.g. in Algarve mountains (Carneiro et al., 2013). *A. melanoxyton* is a nitrogen-fixing tree, able to promote land rehabilitation of bare soils (Shackleton et al., 2018), but highly invasive and adaptable.

Disturbances caused by habitat degradation and wildfires promoted *A. melanoxyton* invasion in several areas of Portugal (Carneiro et al., 2013). Some invaded areas occur in the Sintra Mountain (North of Lisbon) following wildfires involving existing stands. Eradication demands high economical investments and continuous field management over long periods of time, due to the necessity of several follow up control actions (Shackleton et al., 2018). Traditional control methods may reveal positive results when, simultaneously, synthetic herbicide and tree cutting are performed. Knowledge of the best phenological stage to manage is crucial, e.g. ring-barking method is only effective when the cambium is active and is appropriate for juvenile trees (≤ 7 years) with smooth or unbroken bark (Souza-Alonso et al., 2017).

While *Acacia* wood has been studied for biofuels for energy applications (Carneiro et al., 2016), and for agriculture and horticulture sectors as organic substrates formulation from composted whole tree residues (Brito et al., 2015), or as green mulch/bio-herbicides for weed control and crop protection (Souza-Alonso et al., 2018), the use of acacia bark may, however, have better prospects than those for timber. Bark obtained from fallen trees, or from debarking or girdling of trees aiming their elimination (Figure 7), is usually a waste material that may reach large volumes requiring adequate disposal. Most recent literature addresses new applications for *Acacia* bark residues, such as:

- i. fertilizer and nutrient source for crops (Souza-Alonso et al., 2018),
- ii. bioactive compounds with antioxidant and antifungal activity (Luís et al., 2012),
- iii. nanotechnology applications (Taflick et al., 2017),
- iv. textile dyeing (Linhares and Amorim, 2017).

The potential of such bark studies focused on chemical characteristic of this material, which is ultra-rich in naturally produced phytochemicals such phenols, flavonoids and tannins (Souza-Alonso et al., 2018). As for *E. globulus* bark chemical profile, these compounds may have a phytotoxic effect for plant growth

when used as growing media. Therefore, the study and characterization of *A. melanoxylon* bark chemical and biological properties are mandatory before testing for plant cultivation.



Figure 7. Debarked *Acacia* trees in the Buçaco mountain, Central Portugal (ca. 228 km to the North of Lisbon)

Nevertheless, most of the above-mentioned studies did not necessarily prove the economic feasibility of using *Acacia* residues at a large, commercial scale for the proposed aims, which must still be an objective to perform in the near future to allow the development of control strategies based in the valorisation of the residual biomass. The potential risk of conflicts between economic uses and environmental objectives must be, however, carefully regulated taking into consideration the priority of invasion control.

Zero waste approach: bark pre-treatment potential

There is insufficient information or data in literature on the potential valorisation of bark-based growing media from *Eucalyptus globulus* and *Acacia*. Both novel bark-based growing media materials, due to natural physical, chemical and biological characteristics, must be pre-treated before their final use to (i) remove the phytotoxic effect - allowing healthy plant growth, and (ii) create a biological stable environment - enabling enough plant Nitrogen availability.

Regarding the bark pre-treatment in a context of zero waste and circularity within horticultural sector, there might be a possibility to reuse the extracted (toxic) phytochemicals. This feature opens the hypothesis to test the phytotoxic activity of plant materials obtained from *E. globulus* and *A. melanoxylon* to control weed emergence and establishment. Weed management is one of the most critical and costly aspects for container nursery production (Mathers, 2003). The continued use of synthetic herbicides intensifies undesired consequences, such as damage of planted crops, emergence of herbicide resistant weeds, and concern on synthetic herbicide leaching and run-off. Overall, it increases environmental pollution with impacts on human health and ecosystems (Knox et al., 2012). For these reasons, many agrochemicals used for decades have been banned (EC, 834/2017), leading to synthetic herbicides reduction and gradual adoption of natural bio-herbicides meeting the European Union agenda (FAO, 2015) for agriculture and horticulture sectors (Souza-Alonso et al., 2018). Consequently, social, economic and regulatory issues might influence nursery producers to assume sustainable production methods. Thus, the allelopathy potential of

natural plant extracts can be directly used as eco-friendly weed management alternatives.

Objectives and structure

The present thesis is the interface between forest waste-streams valorisation and alternative peat replacement in growing media for horticulture. Waste biomass from non-native species in Mediterranean region is a potential source for alternative wood-based growing media components used in containerised plant production meeting circularity of horticultural sector. The physical, chemical and biological properties before and after pre-treatments were investigated regarding growing media performance and suitability for plant growth.

The research topic was divided in three main objectives:

- I) Characterize *Acacia melanoxylon* biomass (wood and bark) from exotic plant control actions and discuss the possible uses.
- II) Explore the potential of bark forest waste-streams, from paper industry by-product (*Eucalyptus globulus*) and exotic plant control management (*Acacia melanoxylon*), as growing media components used in horticulture trough the evaluation of bark-based growing media phytotoxicity: pre-treatment analysis and optimization.
- III) Investigate bark extracts allelopathic properties to produce non-chemical alternatives for container weed control.

Each objective of this list was dealt with in research papers published (or submitted) to ISI or Scopus indexed international journals and are covered in chapters 2-7. Chapters 2-4 are related to *Acacia* studies, while chapters 5-7 focus on *Eucalyptus globulus* investigation, as illustrated in Figure 8.

A. melanoxylon residue

E. globulus waste-stream



Figure 8. Graphical structure of thesis scheme

Description of chapters

Chapter 2 “**Towards sustainable valorisation of *Acacia melanoxylon* biomass: characterization of mature and juvenile plant tissues**” describes wood and bark tissues from control actions on *Acacia melanoxylon* R. Br. from two different stands, identifying the influence of stand age and location. Chemical profile and growing media characteristics were analysed to propose potential residue valorisation in the current search for novel solutions for a sustainable *Acacia* control that could economically cover part of management costs, taking into consideration the priority of invasion control.

Chapter 3 “**Aged *Acacia melanoxylon* bark as an organic peat replacement in container media**” investigates *A. melanoxylon* bark use as a local forest-derived material for organic growing media formulation. Different bark granulometries in growing media physical and chemical performance were analysed, as well as the effect of ageing treatment in phytotoxicity removal from fresh bark.

Chapter 4 “**The *Acacia* bark phytotoxic potential: a non-synthetic bio-herbicide**” evaluates *Acacia* bark extracts from juvenile (*A. melanoxylon* and *A. dealbata*) debarked trees as organic bio-herbicides, understanding the influence of solvent and solvent:bark ratio in the extraction yield and final bio-herbicidal activity.

Chapter 5 “**Range analysis of *Eucalyptus globulus* bark low-temperature hydrothermal treatment to produce a new component for growing media industry**” studies *E. globulus* bark as raw-material for alternative growing media production applying the hydrothermal treatment in the phytotoxic compounds removal. Response surface analysis methodology was used to study the optimum theoretical treatment residence time and temperature in order to improve bark physical, chemical and biological properties.

Chapter 6 “**Evaluation of low-temperature hydrothermal treated *Eucalyptus globulus* bark as fiber component for horticultural growing media**” is a follow up development from chapter 5, where two low-temperature hydrothermal treatments were used to formulate blended mixtures with peat. The study

assessed bark-based growing media physical, chemical and biological properties, and studied the plant responses comparing to a commercial substrate.

Chapter 7 “**Green application for an industrial by-product: aged *Eucalyptus globulus* bark-based substrates**” evaluates *E. globulus* bark ageing treatment among successive periods of time aiming phytochemical compounds reduction, and the effect of an initial amendment to balance Nitrogen availability to plants. Aged bark was blended with peat in different volumetric proportions and growing media biological, chemical and physical properties were investigated.

The last chapter 8 gives the general conclusions of the results and illustrates perspectives of follow up projects. Further methods and results, which have not been described or published yet, are included as supplemental material.

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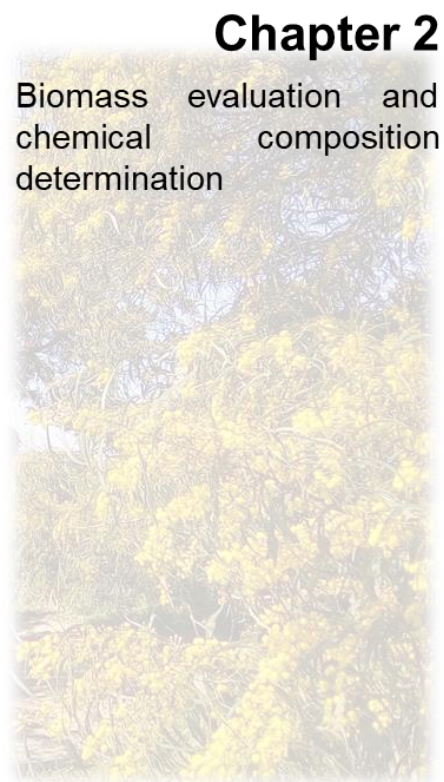
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CHAPTER 2

Towards sustainable valorization of *Acacia melanoxylon* biomass: characterization of mature and juvenile plant tissues

A. melanoxylon residue



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Chemetova C., Ribeiro H., Fabião A., Gominho J. Towards sustainable exotic plant control measures: characterization of mature and juvenile *Acacia melanoxylon* plant tissues.

Towards sustainable valorization of *Acacia melanoxylon* biomass: characterization of mature and juvenile plant tissues

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Abstract

In Mediterranean area, *Acacia melanoxylon* biomass is an abundant waste material from non-native and invasive tree species control actions, requiring suitable disposal. Valorization of such biomass residues requires its complete characterization to best approach the full potential of each plant material that could suit specific applications. This study compares mature and juvenile *A. melanoxylon* plant tissues (wood and bark) from two stands in different locations, regarding their chemical characteristics and organic growing media properties, such as mineral content and phytotoxicity effect for *Lepidium sativum* seeds. Juvenile bark (JB) showed greater total extractives (29%) extracted using solvents of increasing polarity (dichloromethane, ethanol, and water), followed by mature bark (MB) (21%). MB revealed the highest lignin content (> 50%) suggesting material resistance to microbial biodegradation in horticultural applications. High barks phenolic content proved to be phytotoxic for cress seeds (null JB root index), although the toxic substances may be removed. After 1 week, ageing effect reduced MB phytotoxicity (root index > 60%) improving seed performance. Bark presented more mineral elements availability than wood. Wood high cellulose (> 50%), low extractive (< 9%) and moderate total lignin (< 30%) contents can be attractive for pulp production, while bark growth medium profile may potentiate its application for horticultural uses. The future research on novel uses of *A. melanoxylon* plant residues can result in economic benefits that may alleviate management costs.

Keywords: Woody residues; Acacia blackwood; non-native species; biomass management

List of abbreviations (Chapter 2)

ANOVA	Analysis of variances
B	Boron
Ba	Barium
Ca	Calcium
CE	Catechin equivalent
Cr	Chromium
Cu	Copper
EC	Electrical conductivity
Fe	Iron
GAE	Gallic acid equivalent
JB	Juvenile bark
JW	Juvenile wood
K	Potassium
Li	Lithium
LSD	Least significant difference
MB	Mature bark
Mg	Magnesium
Mn	Manganese
MW	Mature wood
Na	Sodium
N_{min}	Mineral Nitrogen
P	Phosphorous
Pb	Lead
RI	Root index
RL	Root length
S	Sulphur
Zn	Zinc

1. Introduction

Worldwide, native plant species and ecosystem services are threatened by invasions of exotic plants, which increase both with time and level of disturbance (Carneiro et al., 2016). In the last two centuries, the Mediterranean region, due to its favourable climate, rich plant species diversity and narrow endemism, has become sensitive to the pressure of invasive flora, frequently following land abandonment and/or wildfire occurrence (Richardson and Kluge, 2008).

Exotic plants from the *Fabaceae* family are examples of the negative impact of invaders from outside their origin (Australian continent), especially in the Iberian Peninsula. In the middle of the 19th century, several Australian *Acacia* species were introduced into Portuguese territory for tannin extraction from bark, and for fuelwood, coastal dune stabilisation (erosion prevention and slope stabilisation), shadowing and for other ornamental purposes (Marchante et al., 2015) that do not depend any more on the maintenance of harvested biomass from species stands. Consequently, some Mediterranean ecosystems and habitats are currently under risk of *Acacia* invasion, such as shrublands, mixed forests, grasslands, prairies, watercourses, coastal and riparian habitats, agricultural lands and crop plantations (Shackleton et al., 2018).

The invasive character of *Acacia* is related to the sprouting ability of most species, to their abundant seed production capacity and to the high persistence of viable seeds within the soil seed bank (Arán et al., 2017). In addition, the capacity to maintain persistent soil seed banks (>50 years), fast germination by taking advantage of natural disturbances (e.g., coastal dune movements exposing the seeds to sun heating, or soil heating by wildfires breaking seed-coat dormancy), vigorous growth rate and the absence of natural enemies, all contribute to colonisation success (Richardson and Kluge, 2008).

In Portugal, *Acacia* stands (mainly *A. melanoxylon* and *A. dealbata*) cover about 30 thousand hectares (Luís et al., 2012). Currently, land afforestation and rehabilitation plans using the most common *Acacia* species are forbidden by law (Decree-law 92/2019, 10th July 2019) and the adoption of effective control or eradication measures is envisaged in that document. Nevertheless, *Acacia* control demands high economic investments and continuous field management

over long periods of time, due to the necessity for several follow-up control actions (Shackleton et al., 2018). Traditional control methods may reveal positive results regarding stump re-sprouting when herbicide application and tree cutting are performed simultaneously (Souza-Alonso et al., 2017); however, root coppicing and seed germination are not prevented. Knowledge of the best phenological stage to manage is crucial, e.g., ring-barking is only effective when the cambium is active and is appropriate for juvenile trees (≤ 7 years) with smooth or unbroken bark (Souza-Alonso et al., 2017). However, similar control actions may result in different levels of control success, considering sprouting and seed germination reduction, and they are site-dependent, where local edaphoclimatic conditions should be considered (Vicente et al., 2013).

Research into the current use of woody residues from *Acacia* is emerging. Cost-effective valorisation may be a strategy to mitigate control-plan costs (Shackleton et al., 2018). The transformation of invasive plant residues into a usable product must provide a new opportunity for economic development (Carneiro et al., 2016), but clear strategies and continuous monitoring programmes must be applied to counteract any pressures towards the promotion of new *Acacia* plantations within the context of such business development. Most of the recent literature addresses the applications of some residues, such as: organic substrate formulation from composted whole-tree residues (Brito et al., 2015), bioenergy applications (Carneiro et al., 2016), residue transformation into green mulch/bio-herbicides for weed control and crop protection (Souza-Alonso et al., 2018), the use of nutrient-enriched leaves and barks as fertilisers and nutrient sources for crops (Souza-Alonso et al., 2017) and flowers (Casas et al., 2019), and bark extractives can contain bioactive molecules with antioxidant and antifungal activity (Freire et al., 2005; Luís et al., 2012). Bark has also gained interest in nanotechnology applications (Taflick et al., 2017), and, due to the high tannin content of most of the species (Seigler, 2003), *Acacia* can play an important role in wastewater and effluent treatments (Souza-Alonso et al., 2017), and in textile dyeing (Linhares and Amorim, 2017).

The first step to understand the new uses of *Acacia* residues requires investigation of their chemical composition. Additionally, an integrated characterisation of *Acacia* residues, considering edaphoclimatic conditions of

harvest location and stand age to identify the main differences between tree constituents, is crucial (Feng et al., 2013). Chemically, the major structural constituents of plant biomass are cellulose, followed by lignin and hemicelluloses (Taflick et al., 2017). However, typically, bark differs from the corresponding wood and generally contains more extractives and polyphenols, but fewer polysaccharides (celluloses and hemicelluloses) (Feng et al., 2013). The extractives are phytochemical compounds (e.g., flavonoids, phenolics, and tannins), essential for normal plant development and self-protection against damage and/or environmental stress, and their content is usually much higher in bark than in wood (Jablonsky et al., 2017). Phytochemical content is an important feature in growing-medium applications due to possible phytotoxic effects on plant growth. Phytotoxicity occurs due to the greater presence of phenolics, terpenes, flavonoids and tannins in freshly harvested bark tissues (Chemetova et al., 2018; Jelassi et al., 2016). Therefore, the evaluation of bark phytotoxicity and mineral and texture analyses determine its possible horticultural use (Brito et al., 2015; Ribeiro et al., 2009).

This study aimed to compare wood and bark tissues from control actions on *Acacia melanoxylon* R. Br. (subgenus *Phyllodineae*) from two different stands, understanding the potential influence of stand age and location. Chemical profile and growing-medium characteristics were analysed to propose potential residue valorisation in the current search for novel solutions for a sustainable *Acacia* control that could alleviate the cost of management.

2. Materials and Methods

2.1. Sampling

Sampling of *A. melanoxylon* was conducted during the winter season of 2017/18. Four groups of samples were prepared: juvenile bark (JB), juvenile wood (JW), mature bark (MB) and mature wood (MW).

Juvenile plants were collected from Tapada da Ajuda, the Instituto Superior de Agronomia (ISA) Campus (38°42'27.5"N, 9°10'56.3"W, Lisbon, Portugal), where the experimental *Acacia* stands were approximately 6 years old. The climatic

conditions (Lisbon metropolitan area) were characterised by a regular winter period according to the official local weather institute (average 11 °C and 80% humidity). Tapada da Ajuda has a gentle west–east slope (less than 5%) and had previously been used as agricultural land, mainly for cereal culture. The soil at the site is a Vertisol from basaltic formations belonging to the Lisbon Volcanic Complex, with the occurrence of coarse elements of basalt and limestone (Medina, 1973), typically corresponding to a soil with neutral pH.

Mature plant residues were collected from Parque da Pena–Monte da Lua (38°47'38.4"N, 9°25'15.2"W, Sintra, Portugal). The *Acacia* used for this study was around 25 years old. The Parques de Sintra–Monte da Lua has a matrix of igneous rocks and is within the area covered by the cultural landscape of the Sintra World Heritage site classified by UNESCO. The soil can be considered a Humic Cambisol with the occurrence of intrusive and extrusive magmatic outcrops belonging to the Sintra Eruptive Massif, moulded by granitic elements (Mesquita et al., 2005) and characterised by siliceous soil with acidic pH.

For all determinations, wood and bark were collected from 50 trees selected randomly in each stand. Juvenile trees (7-10 cm diameter breast height) were ring-barked in the field: bark strips (0.75–1.0 m length and up to 1 cm width) were collected, followed by tree cutting to collect the wood. Mature trees (20-25 cm diameter breast height) were debarked in the field: five strips of bark (0.75–1.0 m length and 3–5 cm width of board shape) per tree were collected, followed by a cross-sectional disc taken from each tree at diameter breast height. The four groups of samples were collected separately (Figure 1), air dried and ground in a knife mill (Fritsch Pulverisette 15 – Fritsch GmbH, Idar-Oberstein, Germany) with an output sieve of 10 × 10 mm, followed by 2 × 2 mm. The 0.4–0.25 mm fraction was used for all chemical analyses. The 0.25–0.18 mm fraction was selected for mineral determination.



Figure 1. Juvenile and mature wood and bark of *A. melanoxyton* residues aspect, (top images) air dried and (bottom image) after gridded with output sieve of 10 x 10 mm²

2.2. Chemical characterisation

2.2.1. Summative analysis

The dry mass content was assessed by oven-drying samples at 105 °C for 24 h, and the ash content was determined by combustion of the oven-dried sample at 550 °C for 5 h in a muffle furnace, according to TAPPI Standard Methods (T211 om-02). Samples (around 3.5 g dry mass) were fully extracted using a solvent sequence of increasing polarity (dichloromethane, ethanol, and water) in a Soxhlet apparatus (T204 cm-07) for total extractive determination. Total lignin was determined as the sum of Klason lignin (T222 om-11) and acid-soluble lignin (UM 205 om-83). Neutral monosaccharide content was determined in the hydrolysate from the lignin analysis. The monosaccharides were separated by high-performance liquid chromatography using a Thermo/Dionex 031824 system equipped with a pulse-amperometric detector; the mobile phase was NaOH plus CH₃COONa with a flux of 1.0 mL min⁻¹ at 25 °C, and the column used was an Aminotrap plus Carbopac PA10 (4 × 250 mm). The monosaccharides were reported as percentages of their total content. The holocellulose content was determined in extractive-free samples by the chlorite method (Browning, 1967) and α-cellulose and hemicelluloses content according to Rowell (2012). Hemicelluloses were calculated as the difference between holocellulose and α-cellulose contents. Chemical analysis determinations were replicated, and results reported as percentages of dry mass material.

The soluble material determination after extraction by 1% sodium hydroxide (0.25 N) was performed (212 om-93). The alkaline solution removes mainly low-molecular-weight carbohydrates present in hemicelluloses, degraded cellulose, and a large fraction of pectins (Feng et al., 2013). The solubility in 1% NaOH indicates the degree of degradation of plant residues by fungus, heat, light and oxidation. High solubility may suggest easy material degradation.

2.2.2. Phytochemical analysis

Extracts were prepared using approximately 1 g of the oven-dried samples and ethanol/water (50:50 v v⁻¹), with a 1:10 m v⁻¹ solid/liquid ratio for 60 min at 50 °C,

using an ultrasonic bath. After filtration, the supernatant extract was used to determine the contents of total phenolics, flavonoids and condensed tannins.

The total phenolic content was determined spectrophotometrically by the Folin–Ciocalteu method using gallic acid as standard (Singleton and Rossi, 1964). An aliquot (100 μl) of the extract was mixed with 4 ml of the Folin–Ciocalteu reagent and after 6 min, 4 ml of 7% Na_2CO_3 solution was added. After 15 min of incubation in a bath at 45 $^\circ\text{C}$, absorbance was read at 760 nm against a prepared blank. A calibration curve was built using gallic acid as a standard (0–150 $\mu\text{g ml}^{-1}$). The total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract (mg GAE g^{-1}).

The flavonoid content was determined using a modified colorimetric aluminium chloride methodology using catechin as standard (Zhishen et al., 1999). An aliquot (1 ml) of the extract was mixed with 4 ml water and 0.3 ml NaNO_2 solution (5% m v^{-1}) and kept in the dark for 5 min. After addition of 0.3 ml AlCl_3 solution (10% m v^{-1}) and mixing for 6 min, the reaction was initiated by adding 2 ml NaOH solution (4% m v^{-1}) and 2.4 ml water was added sequentially and shaken vigorously. Sample absorbance was read at 510 nm after 30 min incubation. The total flavonoid content was expressed as milligrams of catechin equivalent (CE) per gram of extract (mg CE g^{-1}).

Condensed tannins were determined by the vanillin- H_2SO_4 method (Abdalla et al., 2014). An aliquot (1.0 ml) of the extract was mixed with 2.5 ml of vanillin (1.0% m v^{-1}) in absolute methanol and then with 2.5 ml of H_2SO_4 (25% v v^{-1}) in absolute methanol for reaction of the vanillin with the polyphenols in the extract. The blank solution was prepared using the same procedure but without vanillin. Absorbance was recorded at 500 nm after 15 min. The condensed tannin content was calculated from a calibration curve using catechin as standard and expressed in the same units as the flavonoid content (mg CE g^{-1}).

2.3. Preliminary growing-medium tests

2.3.1. Phytotoxicity, electrical conductivity and pH

Phytotoxicity was tested with two sets of samples: one of fresh material, and one kept moistened for one week according to the 'fist test', as defined by European standards (CEN, 2011), corresponding to 65–70% ($w w^{-1}$) moisture content. According to European standards (CEN, 2011), a total of 10 cress (*Lepidium sativum*) seeds were incubated in Petri dishes filled with samples at room temperature (25 °C) in the dark for 3 days. The experiment was carried out in triplicate (1 to 3) using commercial substrate as control (c). Phytotoxicity was evaluated of the fresh and one-week-moistened material, based on root length (RL) to calculate the root index (RI; Eq. 1), as described in previous work (Chemetova et al., 2018):

$$Root\ Index\ (\%) = \frac{\left(\frac{RL1}{RLc} + \frac{RL2}{RLc} + \frac{RL3}{RLc}\right)}{3} \times 100 \quad (1)$$

Electrical conductivity (EC) (CEN, 1999a) and pH (CEN, 1999b) were measured of the water extract (1:5 $v v^{-1}$) of fresh material samples.

2.3.2 Mineral analysis

The nitrogen content was determined (CEN, 2001a) using a modified Kjeldahl method. This procedure can determine ammonium-N, nitrate-N and nitrite-N and organic-N content. Remaining major mineral elements (P, S, K, Ca, Mg, and S) and minor elements (Fe, Cu, Zn, Mn, B, Cr, Pb, Ba, and Li) were removed by aqua regia soluble elements extraction (CEN, 2001b) and quantified via inductively coupled plasma optical emission spectrometry.

2.3.3 Texture analysis

Particle size distribution was determined for samples from the 10 × 10 mm sieve. Vibrational sieving (DIN, 2016) was adopted, using screen sizes of 10, 5, 3, 2, 1, 0.5 and 0.25 mm. All experiments were carried out in triplicate.

2.4 Statistics

Data for growing-medium tests were subject to analysis of variances (ANOVA), followed by least significant difference test (LSD) based on the p-value of 95% of confidence level ($p \leq 0.05$). Means followed by the same letter in a table column indicate no significant differences. Data were analysed using Statistica ® 10.0 (StatSoft, USA).

3. Results and Discussion

3.1. Chemical analysis

3.1.1. Summative analysis

Table 1 describes the summative analysis of *A. melanoxyton* wood and bark (juvenile and mature). The bark fraction presented around 3- to 4-fold higher ash content than the wood. Similar trend differences were reported by Feng et al. (2013) for hardwood and softwood species: cedar bark revealed 9-fold higher ash content compared to the respective wood (5.22% vs. 0.59%), and oak bark almost 23-fold higher (5.22% vs. 0.58%).

In *A. melanoxyton* samples the extractives contents were also greater in bark than in wood; JB presented 29% total extractives followed by 21% in MB, almost 9% in MW and around 6% in JW. Ethanol extracts showed higher yields for all plant materials, followed by water, and dichloromethane extracts registered the lowest yields, since most *Acacia* extractives were composed of soluble hydrophilic compounds, while lipophilic compounds were residual. According to Neiva et al. (2016), *Eucalyptus globulus* showed higher extractive content in bark tissue compared to wood, and polar solvents were also responsible for the highest yields. Other authors (Taflick et al., 2017) found 26% of extractives in the exhausted *Acacia* bark even after the industrial hot water-based process for tannin extraction.

Table 1. Chemical analysis from juvenile and mature wood and bark of *A. melanoxylon*

	Wood		Bark	
	Juvenile	Mature	Juvenile	Mature
Ash (wt % dry basis)	0.9	0.2	2.8	3.9
Total extractives (wt % dry basis)	6.4	8.5	29.2	20.7
Dichloromethane	0.4	0.3	0.9	2.1
Ethanol	3.7	6.0	22.1	12.1
Water	2.3	2.2	7.0	7.3
Total lignin (wt % dry basis)	25.9	30.4	23.6	50.9
Klason lignin	23.2	28.4	22.0	48.8
Soluble lignin	2.8	2.0	1.6	2.1
Monosaccharides (% total monosaccharides)				
Rhamnose	0.4	0.6	0.3	2.8
Arabinose	0.4	2.6	0.3	10.8
Galactose	0.9	2.4	1.2	7.6
Glucose	62.0	70.9	67.5	49.4
Xylose	27.2	15.6	20.8	8.1
Mannose	2.9	1.6	4.0	2.3
Galacturonic acid	1.1	3.1	1.0	16.2
Glucuronic acid	0.1	0.1	0.0	0.6
Acetic acid	4.9	3.1	4.8	2.2
Holocellulose (wt % dry basis)	57.9	52.4	35.4	17.8
α -cellulose	23.2	21.1	13.4	4.0
Hemicelluloses	34.7	31.3	21.9	13.8
1% NaOH_{solubles} (wt % dry basis)	23.6	23.2	53.7	70.4
Total residual lignin (wt % dry basis)	5.3	4.7	10.0	30.5
Klason lignin	4.6	4.2	8.9	29.5
Soluble lignin	0.7	0.6	1.1	1.1

Mature tissues (wood and bark) revealed higher total lignin content: around 30% and 51% in MW and MB, respectively, compared to 26% and 24% in juvenile tissues (JW and JB). *Acacia* bark usually contains more lignin than the corresponding wood (Feng et al., 2013). However, wood extractives and lignin content were higher than previous comparable *A. melanoxylon* studies: Santos et al. (2006) reported around 3.2% total extractives and 17.5% total lignin, and Lourenço et al. (2008) found 4.0–9.5% total extractives and 20.5–22.0% lignin in sapwood and heartwood, respectively, from *A. melanoxylon* samples collected at four different sites to study the influence of heartwood in pulp production.

In all samples, glucose represented the highest percentage of total monosaccharide content, followed by xylose. JW and MW glucose and xylose contents were in accordance with literature findings for *A. melanoxylon* wood (Lourenço et al., 2008), where values ranged from 67–70% and 23–26% for glucose and xylose contents, respectively. Nevertheless, MB fractions showed the highest proportion of galacturonic acid (16.2%) while the percentages of other samples were mostly below 3%. As expected, wood (JW and MW) presented more holocellulose content compared to bark (JB and MB): 57.9%, 52.4%, 35.4% and 17.8%, respectively. For all samples, hemicelluloses presented the largest fraction of total holocellulose content.

The assay of samples after treatment with 1% NaOH solution gave an idea of the facility of the plant materials to be degraded by chemical attack. Seventy percent of the MB mass was removed by alkaline extraction, followed by 54% of JB and around 23% of wood samples. Thus, bark was revealed to be easily attacked by chemicals. However, after 1% NaOH extraction, MB maintained high lignin content, around 30%, followed by 10% in JB and around 4% in wood samples. MB presents an attractive lignin potential, either for biorefinery valorisation (transformation into biofuels and chemicals) or, on the other hand, the presence of lignin can enhance the resistance to microbial biodegradation in horticultural applications (Majeed et al., 2017).

3.1.2. Phytochemical characterisation

Juvenile tissues were revealed to be more phytochemically active than mature tissues (Table 2). In agreement with the total extractive content results in Table 1, JB presented the highest levels of phenolics (485 mg GAE g⁻¹), flavonoids (223 mg CE g⁻¹) and condensed tannins (80 mg CE g⁻¹), while MB presented around half the JB level of phenolics, three-fold lower levels of flavonoids and identical condensed tannin content. In literature, the phenolic content of *Acacia cyclops* pods was reported (Jelassi et al., 2016) to be 318–426 mg GAE g⁻¹. Values of the same magnitude of MW phenolic content (97 mg GAE g⁻¹) were also reported for *A. melanoxyton* aerial plant tissues (wood, bark and leaves) (100–138 mg GAE g⁻¹) (Luís et al., 2012). Nevertheless, the phenolic contents found in our study for bark, either juvenile or mature, were higher than those mentioned in this latter reference. Generally, the percentage of condensed tannins in bark and leaves can range between 1% and 20%, while wood contains 1% or less (Seigler, 2003). Bark tannins can be influenced by species, age, extraction method and particle size, with smaller particles tending to show increased tannin yield due to improved mass transfer (Feng et al., 2013). These facts may explain the great condensed tannin content in bark (more than two-fold higher) compared to wood (Table 2).

Phenolic compounds have gained global interest due to their bioactivity, such as antimicrobial, antioxidant, antiviral and anti-inflammatory effects (Freire et al., 2005; Jablonsky et al., 2017; Luís et al., 2012). According to Luís et al. (2012), *A. melanoxyton* extracts (wood, bark and leaves) are rich in biologically active compounds, including hydroxybenzoic acids, hydroxycinnamic acids and flavonoids.

Condensed tannins or proanthocyanidins are polyphenolic compounds that have been used in the formulation of leather tanning agents, wood adhesives, and resins that might replace synthetic phenolics (Jablonsky et al., 2017). Tannins are also a determinant component in animal diets, and *Acacia mearnsii* (also belonging to subgenus *Phyllodineae*) is the basis of industrial tannin production in South Africa and Brazil (Seigler, 2003). Recent studies (Ogawa and Yazaki,

2018) have pointed out the feasibility of *A. mearnsii* bark tannin extract as a new human dietary supplement.

Table 2. Phytochemicals determination including phenolics, flavonoids and condensed tannins content from juvenile and mature wood and bark of *A. melanoxylon*

	Wood		Bark	
	Juvenile	Mature	Juvenile	Mature
Phenolics (mg GAE g ⁻¹)	299.6	96.7	484.9	249.8
Flavonoids (mg CE g ⁻¹)	201.5	60.4	222.6	79.8
Condensed tannins (mg CE g ⁻¹)	25.9	37.3	79.8	77.3

Some species of the *Acacia* genus show the presence of saponins (Jelassi et al., 2016). These phytochemical substances have been associated with biological activities, including allelopathic effects on other species (Jelassi et al., 2016). According to Souza-Alonso et al. (2018), a natural technique to control weed emergence and decrease the use of synthetic herbicides in organic farming systems could be the application of phytochemical substances with phytotoxic effects from *A. dealbata* residues, although their findings were dependent on target species, and leaves are the plant tissue responsible for the greatest inhibitory effect. Another study (González et al., 1995) pointed out the negative effects of the decomposition of *A. melanoxylon* litter on the germination and growth of native plant species. Laboratory bioassay (Allan and Adkins, 2007) testing of *A. melanoxylon* bark aqueous extracts reduced the growth of an aquatic plant (*Lemna aequinoctialis*) to around 90%.

3.2. Growing-medium suitability

3.2.1. Phytotoxicity, pH and EC determination

Among fresh samples, JB was the most phytotoxic (Table 3) for cress seeds, causing total root inhibition. The phytotoxicity of fresh forested residues is documented (Barrett et al., 2016; Brito et al., 2015; Chemetova et al., 2018; Gruda et al., 2009), and is strongly correlated with phytochemicals: essentially, the presence of phenolic compounds. This statement agrees with the

phytochemicals determination (Table 2) and was confirmed by the RI of all fresh samples (Table 3). An option to remove the phytotoxic effect is the extraction of (toxic) phytochemicals for a variety of applications (section 3.1.2), and at the same time reducing the phytotoxic effect on seeding plants. On the other hand, treatments can be applied to reduce the toxic effect of bark, such as: composting (Brito et al., 2015), ageing (Jackson et al., 2010), low-temperature hydrothermal treatment (Chemetova et al., 2018), and washing/leaching (Gruda et al., 2009) the growing media.

After one week, JW and MW show similar root growth performance, presenting a maximum RI of 18% (Table 3). JB maintained its inhibitory effect, although MB increased the RI from 15% in fresh to 63%. Phytotoxicity assays used peat moss as control (RI = 100%). A short ageing period (one week) was sufficient to remove some toxic compounds from MB, reducing its toxicity. Longer periods (2, 4 and 8 weeks) may be tested to remove these toxic compounds totally.

Table 3. Cress seed root length index (fresh and after aging 1 week), fresh samples pH and electrical conductivity (EC). Means followed by the same letter, in a column, do not differ at $P \leq 0.05$ by the LSD-test

Plant material		Root length index		pH	EC (mS m ⁻¹)
		Fresh	1 week		
		(%)			
Wood	Juvenile	10.2 a	18.1 b	5.3 b	27.7 b
	Mature	10.5 a	11.3 b	4.8 c	9.3 c
Bark	Juvenile	0.0 b	< 2.0 c	5.3 b	86.6 a
	Mature	15.4 a	62.7 a	5.6 a	10.0 c

JW, JB and MB samples presented pHs within the recommended range (5.3–6.5) (Barrett et al., 2016) for growing-medium performance. The pH of MW was slightly acidic; however, pH adjustment is a common nursery and greenhouse management procedure (Brito et al., 2015; Ribeiro et al., 2009). EC values were within the acceptable range (<60 mS m⁻¹) (Chemetova et al., 2018), with exception of JB.

Regarding preliminary phytotoxicity, pH and EC results, MB can be considered the most suitable material for growing-medium application.

3.2.2. Mineral analysis

The mineral composition of *Acacia* biomass is shown in Tables 4 and 5 for major and minor elements, respectively. Bark samples showed greater abundances of major elements than did woods (Table 4). Regarding mineral nitrogen content (N_{\min}), wood fractions presented low values (less than 2.0 g kg^{-1}), while bark ranged between 7 and 12 g kg^{-1} , in JB and MB, respectively. JB and MB also revealed significantly higher content of Ca, Na, Mg and S than did wood samples, which had values of the same magnitude as those previously reported in the literature (e.g., Brito et al., (2015)).

The use of forest residues in the horticultural industry has been gradually increasing, although microbial N immobilisation may still be the main constraint (Barrett et al., 2016). Wood's poor N_{\min} content can increase microbial N immobilisation and subsequent instability (weak growing-medium performance), making wood residues less suitable for horticultural applications. On the other hand, amount and availability of major elements in bark can be an advantage, reducing external fertiliser requirements (Ribeiro et al., 2009), suggesting the use of bark as a growing-medium component.

Table 4. Major elements present in bark and wood of *A. melanoxylon*. Legend: mineral nitrogen (N_{\min}), phosphorous (P), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S). Means followed by the same letter, in a column, do not differ at $P \leq 0.05$ by the LSD-test.

Plant material		Major elements						
		N_{\min}	P	Na	K	Ca	Mg	S
		(g kg ⁻¹)						
Wood	Juvenile	2.0 c	0.2 b	0.9 c	4.7 b	1.2 b	0.2 c	0.5 c
	Mature	1.3 c	0.1 c	0.5 d	2.6 c	0.5 c	0.2 c	0.3 c
Bark	Juvenile	6.9 b	0.6 a	2.1 b	5.6 a	9.4 a	1.0 b	1.2 b
	Mature	11.9 a	0.2 b	2.5 a	4.7 b	9.1 a	1.8 a	2.0 a

The presence of Fe, Cu, Zn, B, Pb, Ba and especially Mn in MB (Table 5) was significantly higher than in the other plant materials. The main differences in minor elements can be considered to be site-dependent; juvenile tissues were collected from a neutral pH soil (section 2.1), while MB came from acidic soils. The availability of mineral elements is affected by pH; thus, at low pH (high H⁺ concentration) at least the elements Fe, Cu, Zn and Mn may become easily available for plants (Barrett et al., 2016).

In general, bark plant tissues presented greater mineral element concentrations than did wood, although special attention should be given to biomass provenance. Regarding its potential application in organic growing media for horticultural use, the mineral content of bark can be adjusted easily for efficient plant nutrient provision (Brito et al., 2015; Ribeiro et al., 2009).

Table 5. Minor elements present in barks and wood of *A. melanoxylon*. Legend: iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), boron (B), chromium (Cr), lead (Pb), barium (Ba), lithium (Li). Means followed by the same letter, in a column, do not differ at $P \leq 0.05$ by the LSD-test.

Plant material		Minor elements								
		Fe	Cu	Zn	Mn	B	Cr	Pb	Ba	Li
		(mg kg ⁻¹)								
Wood	Juvenile	35.1 bc	4.0 b	3.1 b	3.4 b	0.4 c	1.9 ab	0.2 c	3.3 c	1.1 b
	Mature	20.8 c	4.8 b	4.3 b	15.8 b	0.2 c	2.0 a	0.4 c	3.4 c	0.2 b
Bark	Juvenile	62.0 b	4.9 b	6.1 b	10.1 b	6.8 b	1.5 b	2.3 b	13.4 b	2.8 a
	Mature	122.1 a	12.7 a	26.2 a	657.5 a	17.8 a	1.5 b	3.9 a	29.6 a	0.7 b

3.2.3. Sieving pattern

Texture analysis is illustrated in Figure 2. Wood presented a higher proportion of coarser particles (>2 and ≤10 mm): JW 51% and MW 68%, (Figures 2 (a) and (b)), compared to 8% and 24% in JB and MB (Figures 2 (c) and (d)), respectively. On the other hand, bark usually generates more fines than does wood (Jackson et al., 2010). The distribution curve shows 70% of particles <1 mm in JB (Figure 2 (c)) and 50% in MB (Figure 2 (d)).

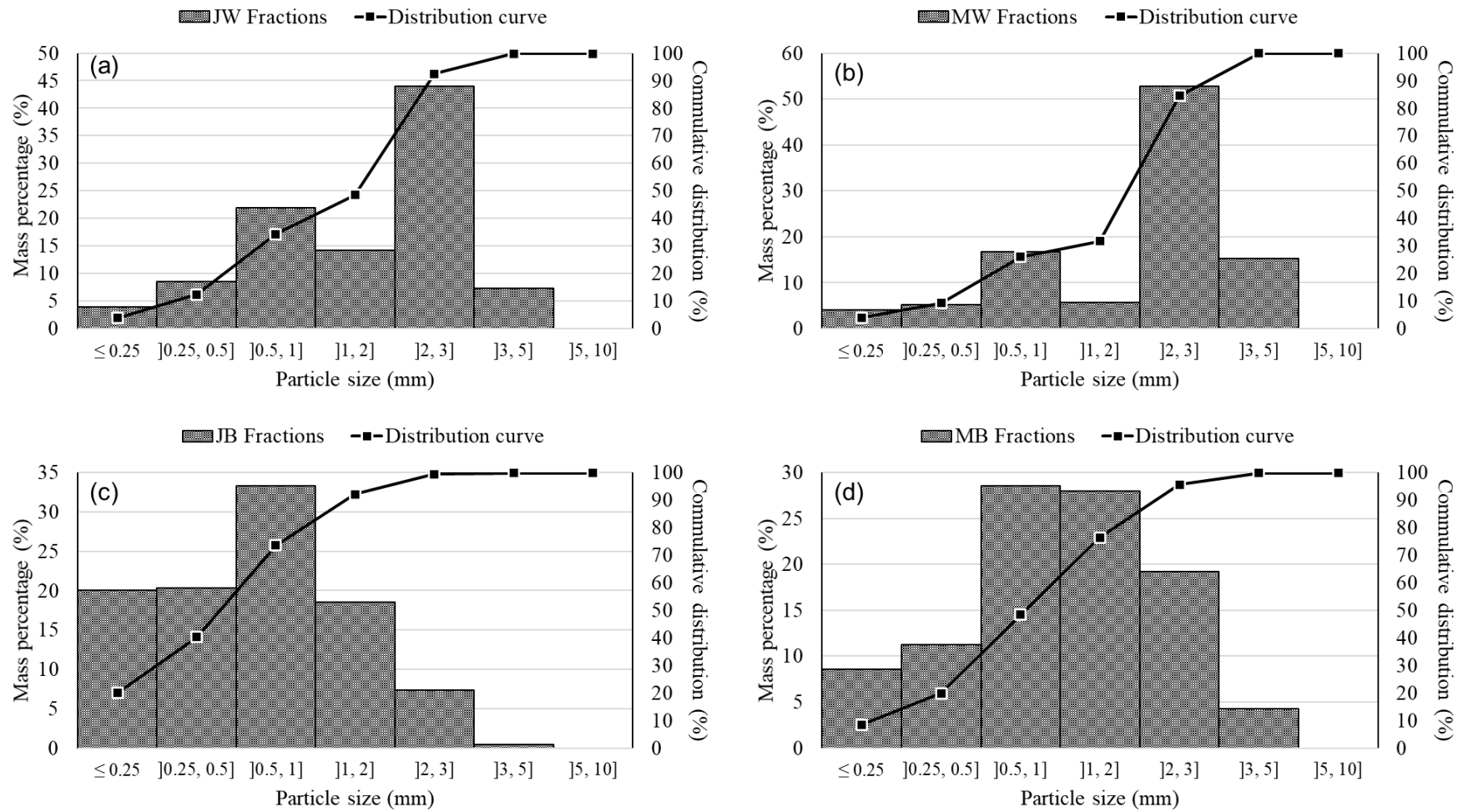


Figure 2. Histogram with percentage of fractions and distribution curve of **(a)** juvenile wood (JW), **(b)** mature wood (MW), **(c)** juvenile bark (JB) and **(d)** mature bark (MB)

In the application of woody residues as horticultural substrates, the greater the proportion of fines (particles <1 mm), the higher is the water available for plant per unit volume, due to the greater surface contact of particles when compared to the coarse fraction; however, fines may also promote substrate aeration deficiency (Gruda and Schnitzler, 2004). In this context, particle size distribution is determinant in the prediction of the physical performance of the substrate and its viability for plant growth (Brito et al., 2015).

According to Jackson et al. (2010), substrate particles <0.5 mm have a stronger influence on air–water relationships than the proportion of coarse particles (>0.5 mm). JB (Figure 2 (c)) presented 40% of particles <0.5 mm, thus may be suitable for growing-medium formulation for plants with higher water demands, paying attention to the potential root O₂-deficiency. On the other hand, MB (Figure 2 (d)) presented only 20% of particles <0.5 mm, and consequently presents a potential for use in substrate aeration enhancement.

Producing a growing medium with a particle size fine enough to maintain an adequate air–water quality for plant growth may be too expensive, primarily due to the energy costs associated with grinding (Barrett et al., 2016). Unlike mineral content, physical properties are difficult to manipulate, the texture of bark therefore indicating it to be preferential as a growing-medium material.

4. Conclusions

This comparative study between mature and juvenile *A. melanoxyton* plant residues explored their major chemical profile differences and their suitability for growing-medium formulation. Juvenile bark had the highest extractive yield, and mature plant tissues revealed greater ash and total lignin content. More than half the chemical expression of mature bark was lignin, and after 1% NaOH extraction it maintained its high lignin content, suggesting its resistance to microbial biodegradation. The phytochemical profile of juvenile bark underlined its potential as a source of valuable phenolic substances. Mature bark showed lower phytotoxic effects on cress seed growth after ageing, and regarding its pH, electrical conductivity, mineral content and particle size distribution was proved

to possess the suitable characteristics for organic substrate formulation. The feasibility of using *A. melanoxylon* residues to alleviate eradication/control costs must be supported by cost analyses to determine whether this process is an economically viable and sustainable valorisation of this valuable biomass.

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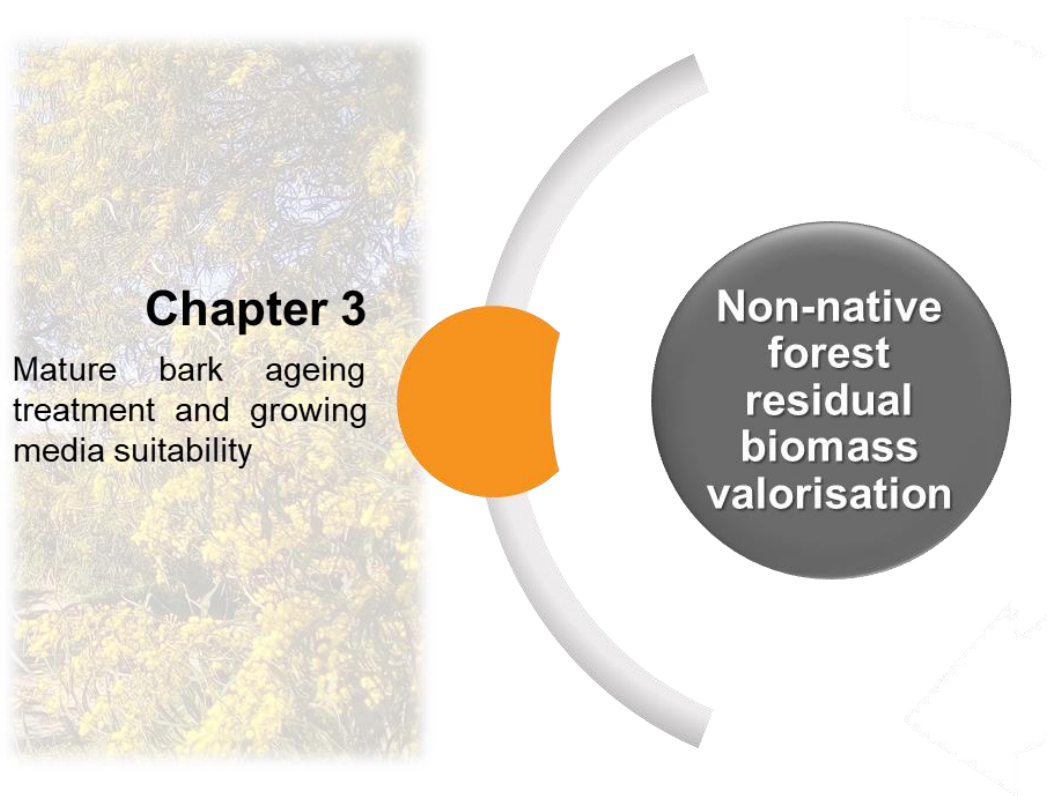
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CHAPTER 3

Aged *Acacia melanoxylon* bark as an organic peat replacement in container media

***A. melanoxylon* residue**



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Aged *Acacia melanoxylon* bark as an organic peat replacement in container media

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Abstract

The continuous search for peat substitution by local available organic materials, to reduce horticulture industry environmental foot-print, is growing worldwide. In Mediterranean region, *Acacia melanoxylon* bark is an abundant waste material from non-native tree species control actions, lacking suitable disposal. This study explores the potential use of *A. melanoxylon* bark as an alternative material for container media. Bark anatomy was characterized, the effect of different bark sieve sizes (4, 6, 8, and 10 mm) and the ageing treatment among successive periods of time (at 0, 4 and 8 weeks) were evaluated regarding substrate physical and chemical performance, as well as phytotoxic effect on tested seeds. Fresh bark was phytotoxic (cress root index < 22 %) due to phenolic and extractives presence in bark material. Ageing bark during 8 weeks might eliminate those toxic elements which promoted cress roots growth equal to peat (root index > 99 %). Ageing process may motivate Nitrogen immobilization which raises pH (up to 6.2) and dropped electrical conductivity (minimum of 14 mS m⁻¹), however initial substrate amendment is required prior to potting, thus providing enough nutrients according to microbial-plant needs. Physical substrate performance was strongly correlated with particle size distribution: coarse bark (10 mm) increase air filled porosity, enhancing substrate aeration, while fine bark (4 mm) retain equal water content as commercial peat. Aged *A. melanoxylon* bark can be blended up to 50% with peat and produce plants as great as in commercial peat-based substrates.

Keywords: Ageing process; phytotoxicity; particle size; aeration; water availability; bark-based substrate

List of abbreviations (chapter 3)

AB	Aged bark
AB 4 mm	8 weeks-old aged fine bark
AB 10 mm	8 weeks-old aged coarse bark
AFP	Air filled porosity
ANOVA	Analysis of variance
B	Boron
BD	Bulk density
C	Carbon
Ca	Calcium
Cu	Copper
DBH	Diameter at breast height
DM	Dry mass
DW	Dry weight
EAW	Easy available water
EC	Electrical Conductivity
f	Fiber
FAA	Ethanol-formalin acetic acid solution
FB	Fresh bark
FB 4mm	Fresh fine bark
FB 10 mm	Fresh coarse bark
Fe	Iron
FW	Fresh weight
GI	Growth inhibition
GR	Germination rate
H₂PO₄⁻	Dihydrogen phosphate ion
K	Potassium
LSD	Least significant difference
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen

Na	Sodium
NH₃⁻-N	Nitrate-Nitrogen
NH₄⁺-N	Ammonium-Nitrogen
P	Phosphorous
p	Parenchyma cells
r	Rays
rd	Rhytidome
RI	Root index
RL	Root length
SO₄²⁻	Sulfate
TP	Total porosity
WBC	Water buffer capacity
Zn	Zink

1. Introduction

Peat represents the most common organic constituent used in Europe by growing-media producers due to its distinctive physical, chemical and biological characteristics (Bonaguro et al., 2017). Peat is considered a limited resource, although abundant in boreal regions of Northern Hemisphere, and its extraction has become an environmental issue. In Mediterranean countries peat is imported from northern Europe (Finland, Lithuania, Estonia, Sweden, and other neighbor countries) rising the transportation cost. Therefore, the increasing demand for more sustainable and local resources solution is attracting researchers and growing-media producers, which progressively have been adopting local forest-derived materials as an emerging alternative container media component (Vandecasteele et al., 2018).

In Europe, the introduction of Australian native species from *Acacia* genus for ornamental and economic purposes in late 19th century (Hussain et al., 2011). Most of them rapidly became invasive plants, especially in Mediterranean areas, with an urgent need for control management (Brito et al., 2013). *Acacia melanoxylon* (subfamily *Mimosoideae*) invasive character is not only related to

the abundant seed production (with long persistence within the soil seed bank) but also largely associated with the sprouting capability (from the stump and/or from the roots) (Carneiro et al., 2016), which increases the resistance to control measures and enhances the costs of eradication. Bark obtained from fallen trees, or from debarking of trees aiming their elimination (Gutierrez et al., 2011), is usually an abundant waste material requiring adequate disposal.

Tree barks are anatomically complex biomass formed by the activity of two meristems, the vascular cambium and phellogen. Bark consists of the secondary phloem (innermost part of bark), and the rhytidome (the outermost parts of bark); the secondary phloem comprises the sieve-tube elements associated with companion cells, sclerenchyma cells (fibers and sclereids) and parenchyma cells (Evert, 2006); the rhytidome includes the last formed periderm (with phellem, phelloderm and phellogen) and all of the dead tissue isolated by it (Angyalossy et al., 2016). According to Roth (1981), bark from subfamily *Mimosaceae* forms a group with many anatomical features in common, and the most characteristic is the secretory system, which is well developed in form of secretory cells. Few references recorded bark anatomy of *A. melanoxyton*, *i.e.* Tavares et al. (2011) analyzed the fiber biometry and their variation within and between trees growing in different site condition.

The chemical constitution of *A. melanoxyton* bark tissues is based in lignin and extractives, and a preliminary test using fresh bark as growing-media resulted in partial inhibition of cress (*Lepidum sativum*) seed growth (Unpublished results). The inhibitory effect was related to the presence of compounds such, phenolics, flavonoids and tannins, secondary metabolites naturally produced by the tree.

Forest-based materials, alternative to peat, are associated to inherent biological and chemical limitations, such as the presence of phytotoxic substances in fresh harvested biomass (Bonaguro et al., 2017), high microbial Nitrogen (N) immobilization (Naasz et al., 2009; Vandecasteele et al., 2018) and Carbon (C) respiration (Buamscha et al., 2008; Chemetova et al., 2018). The biological features can be prevented by fertilization adjustment, *i.e.* adding N-source to avoid N immobilization is a common practice and recommended from the beginning of container seedling process (Caron and Michel, 2017).

Composting fresh raw-material is a common procedure before the substrate final use (Barrett et al., 2016). Brito et al. (2013) composted *A. dealbata* and *A. melanoxyton* biomass to produce organic soil amendments and horticultural substrates, however pointing the need of a long period (> 231 days) to achieve compost maturation. Additionally, these authors observed pH and electrical conductivity (EC) oscillation during the storage process. A decrease of aeration levels and overall physical performance of *Acacia* composts over time requires attention to adequate the irrigation regime to new growing mixtures (Bakry et al., 2013).

An alternative technique to reduce phytotoxicity is ageing (Altland et al., 2018). It is an attractive procedure due to its simplicity, chemical-free nature, and positive results after short time intervals, depending however on raw-material characteristics, the volume used and storage conditions (Barrett et al., 2016). Buamscha et al. (2008) reported that ageing *Pseudotsuga menziesii* bark promoted substrate stability and greater geranium growth, compared to fresh bark. *A. melanoxyton* bark reduced its toxicity on cress seed after 1 week (Unpublished results).

The physical properties of organic growing substrates are key factors for successful plant growth due to their influence in container media ability to store and supply adequate air and water (Bakry et al., 2013). These can be optimized for ideal substrate characteristics, although gradual degradation over time may occur, depending on growing mix stability and chemical profile (Carlile et al., 2015). Thus, particle size distribution plays an important role in adequate aeration of plant root zone (Kaderabek et al., 2017), as well as in media water-holding capacity (Caron and Michel, 2017).

This study addresses to the *A melanoxyton* bark valorization as a local forest-derived material for organic substrate formulation, and consequently meeting environmental and economic sustainability in a win-win context, decreasing the need of peat, and alleviating costs associated to control efforts of the invasive plant. The objectives are to (i) investigate the effect of different bark granulometries in growing-media physical and chemical performance and, (ii)

evaluate the phytotoxicity removal from fresh *A. melanoxyton* bark over consecutive ageing periods.

2. Materials and Methods

2.1 Bark collection

During winter season of 2015/16, bark samples of *A. melanoxyton* were collected from a stand located at Sintra, Portugal (38°47'38.4"N, 9°25'15.2"W) managed by the Parques de Sintra - Monte da Lua Company. The site has a matrix of igneous rocks and is within the area covered by the cultural landscape of Sintra-World Heritage site classified by UNESCO. The soil at the site is a Humic Cambisol (FAO, 2015) with the occurrence of intrusive and extrusive magmatic outcrops belonging to the Sintra Eruptive Massif, molded by granite (Gutierrez et al., 2011) and characterized by siliceous soils with acidic pH.

The *Acacia* stand used for this study was around 25 years-old, according to the records of the stand manager (Parques de Sintra - Monte da Lua Company). For all determinations, with anatomical analysis exception, bark was collected from 50 trees selected randomly in the stand. The wood from these trees is mostly used as fuelwood, and the debarking process was performed in the site. Five strips of bark (0.75 to 1.0 m length and 3 to 5 cm width in board shape) per tree were collected using simple hand tools and, after mixture, a representative sample with 300 L was taken and transported to the laboratory. Sampled bark was dried at 35°C in an electric oven for 7 days and, after drying, crushed up to 10 cm size pieces for further use.

2.1.1. Anatomical analysis

For anatomical analysis, 20 randomly trees were selected. A cross-sectional disc was taken from each tree at breast height (DBH) and bark samples were taken in two opposite radii and fixed in ethanol-formalin acetic acid solution (FAA). A total of 40 bark samples of *A. melanoxyton* were embedded in polyethylene glycol 1500 (Barbosa et al., 2010) to be sectioned using a sliding microtome Leica SM

2400; thin transverse sections (15–20 mm thick) were obtained and stained with a triple staining of chrysodine/acridine red and astra blue for the differentiation of the tissue (blue for nonlignified tissues; yellow and red for lignified tissues). Light microscopic observations were made using Leica DM LA and photomicrographs were taken with a Nikon Microphot-FXA. Terminology followed Angyalossy et al. (2016).

2.1.2. Texture analysis

Bark pieces were grinded in a knife mill (Fritsch pulverisette 15 – Fritsch GmbH, Idar-Oberstein, Germany) with four different output sieves: 10 mm; 8 mm; 6 mm and 4 mm. Particle size distribution of the four different grinded barks was assessed for different granulometry fractions, adapting vibrational sieving method (DIN, 2016) using screen sizes of: 10, 5, 2, 1 and 0.5 mm. All experiment was carried out in triplicate.

2.2. Bark ageing

Samples from the different grinded barks (10, 8, 6 and 4 mm) were moistened according to the “fist test” as defined by European standards (CEN, 2011), corresponding to 65 to 70% ($w w^{-1}$) of moisture content, and stored in black plastic bags at 25 °C in a dark chamber for a period of 8 weeks. Each bag contained a total volume of 10 L of moistened bark and was opened and homogenized weekly. At weeks 0, 4 and 8 a sample was collected for ageing effect evaluation. Moisture content was confirmed at the sampling time and additional water was added as needed.

2.3. Chemical characteristics

The pH, EC, and water-soluble Potassium (K), Phosphorous (P), Calcium (Ca), Magnesium (Mg), Sodium (Na) and mineral N *i.e.* Ammonium (NH_4^+ -N) and Nitrate (NO_3^- -N), were measured in water extract 1:5 by volume, according to the

European Standards (CEN, 2001a, 1999a, 1999b). The dry mass (DM) was assessed by oven-drying bark at 105 °C for 24 hours and the ash content was determined by combustion of the oven-dried sample at 550 °C for 5 hours in a muffle furnace. The difference between DM and ash was considered the organic matter content.

2.4. Substrates formulation

From the bark aging essay results, four different barks were selected: fresh fine bark (FB 4 mm); 8 weeks-old aged fine bark (AB 4 mm), fresh coarse bark (FB 10 mm) and 8 weeks-old aged coarse bark (AB 10 mm). Each bark was mixed with peat in the following proportion (bark:peat) by volume: 10:90, 25:75 and 50:50. A total of 12 bark-based substrates were formulated. Peat moss slightly decomposed, H2-H5 von Post scale, Floragard Co. (Germany), amended with 4 g L⁻¹ of calcitic lime and 4 g L⁻¹ of dolomitic lime to adjust pH (between 5.6 – 5.8) was used. Peat-only substrate was also used as control.

2.4.1. Physical characteristics

Water-air substrate properties as defined by Wallach (2008), were determined according to the European Standards (CEN, 2001b). The following physical properties were obtained: total porosity (TP); bulk density (BD), air-filled porosity (AFP) as the amount of air at a suction of 1 kPa, easy available water (EAW) as the difference between the water content at suctions of 1 and 5 kPa, and water buffer capacity (WBC) as the difference between the water content at 5 and 10 kPa.

2.4.2. Phytotoxicity essay

According to the European Standards (CEN, 2011b), a total of 10 cress (*Lepidium sativum*) seeds were incubated in Petri dishes filled with samples, at room temperature (25 °C) in the dark, for 3 days. The experiment was carried out in

triplicate (1 to 3) and using peat as control (c). Phytotoxicity was evaluated based on the average germination rate (GR, Eq.1), and root length (RL) to calculate root index (RI; Eq. 2) using RL of triplicates and control.

$$\text{Germination rate (\%)} = \frac{(GR_1 + GR_2 + GR_3)}{3} \times 100 \quad (1)$$

$$\text{Root Index (\%)} = \frac{\left(\frac{RL_1}{RL_c} + \frac{RL_2}{RL_c} + \frac{RL_3}{RL_c}\right)}{3} \times 100 \quad (2)$$

2.4.3. Pot experiment

Substrates mixtures were pre-plant fertilized with 15 mmol NO₃⁻-N L⁻¹, 8 mmol K L⁻¹, 4 mmol Ca L⁻¹, 1.5 mmol Mg L⁻¹, 1.25 mmol Sulfate (SO₄²⁻) L⁻¹, 1.5 mmol Dihydrogen phosphate (H₂PO₄⁻) L⁻¹, 15 μmol Iron (Fe) L⁻¹, 8 μmol Manganese (Mn) L⁻¹, 4 μmol Zink (Zn) L⁻¹, 25 μmol Boron (B) L⁻¹, 0.75 μmol Copper (Cu) L⁻¹, 0.5 μmol Molybdenum (Mo) L⁻¹. Pots with a volume of 290 mL (Ø = 8.5 cm; height = 8.2 cm) were filled with substrates mixtures and a total of 10 Chinese cabbage (*Brassica napa* subsp. *pekinensis*) seeds per pot were sown and grown for 5 weeks. During the entire experiment time range, all pots were placed in an unheated glass greenhouse, distributed in a complete randomized design. Pots were daily irrigated with deionized, in order to avoid the effect of different compounds usually present in tap water. The experiment was carried out in triplicate (1 to 3) and using peat as control (c). At the end of the growing period, sample fresh weight (FW), dry weight (DW, at 65 °C for 48 h), growth inhibition percentage (GI; Eq. 3) using average FW of triplicates and average FW control, and root visual rating (1= the worst; 5=the best) were calculated and recorded.

$$\text{Growth inhibition (\%)} = \frac{(FW_c) - (FW_{1-3})}{(FW_c)} \times 100 \quad (3)$$

2.5. Statistics

Data were subject to analysis of variances (ANOVA), followed by least square difference test (LSD) based on the p-value with 95% of confidence level (p ≤ 0.05). Data were analyzed using Statistica ® 10.0 (StatSoft, USA).

3. Results and discussion

3.1. Bark anatomy

*A. melanoxylo*n bark is brownish grey to dark grey, hard and longitudinally furrowed. Figures 1 and 2 represent some features of the anatomical structure of the bark. The rhytidome was composed by several periderms interspersed with phloem cells; in each periderm, the phellem consisted of tabular cells with thick tangential walls and the phelloderm included layers of rectangular to round cells, some with thin walls and others uniformly thickened, both filled with a great amount of extractives (Fig. 1A). The main tissues of the phloem are the sieve-tube elements and companion cells (conducting tissues), axial parenchyma (storing tissues) and radial parenchyma cells (storing-radial conducting tissues) and fibers (tissue of mechanical support) (Fig. 1B).

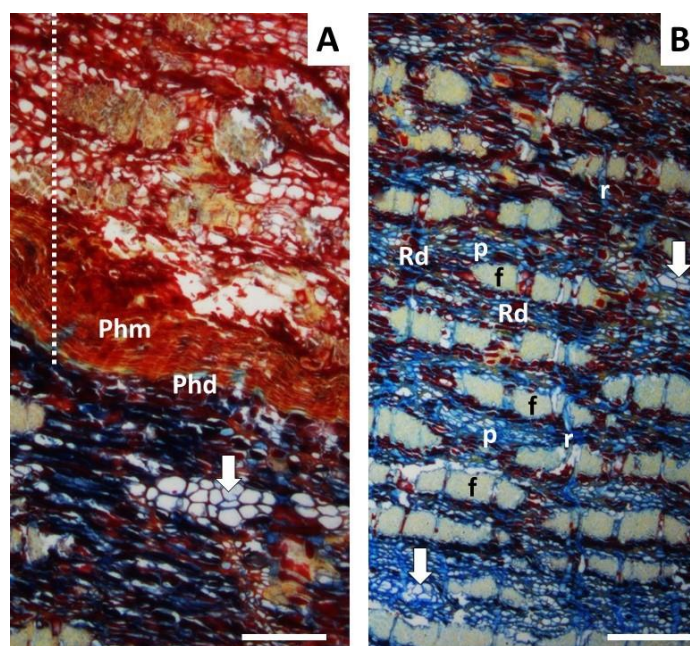


Figure 1. *A. melanoxylo* bark. **A)** Rhytidome (--) and last formed periderm showing phellem (Phm), phelloderm (Phd) and phloem with groups of secretory cells (arrow); **B)** Phloem with fibres (f), parenchyma cells (p), rays (r), dilated rays (Rd) and secretory cells (arrows). Scale bar A= 120 μ m & B=160 μ m

The general anatomy of *A. melanoxylo* bark had similarities with other genus of the *Mimosaceae* described by Roth (1981), with some particular findings: secondary fibers (f) in alternate plates sometimes forming more or less tangential bands interrupted by the rays (r) and alternated with bands of sieve tubes and parenchyma cells (p) (Fig. 1B); secretory cells well developed and arranged in

groups or in tangential bands through the phloem (Figs. 1A and B, 2A); sclerified cells (sclereids) scattered or irregularly dispersed in the outer phloem (Figs. 2B); reduced dilatation growth in the non-conducting phloem, mainly restricted to the rays, which are slightly too dilated (Rd), due to the cell enlargement and anticlinal divisions; in minor extent axial parenchyma cells also undergo some tangential dilatation; some extent of sclerification of both axial and radial parenchyma cells (Fig. 1B); cell contents (phenolic deposits of red and brown color) are particularly abundant through the rhytidome *i.e.* phellem, phelloderm and also in the parenchyma tissues of the non-conducting phloem (Figs. 2A and B).

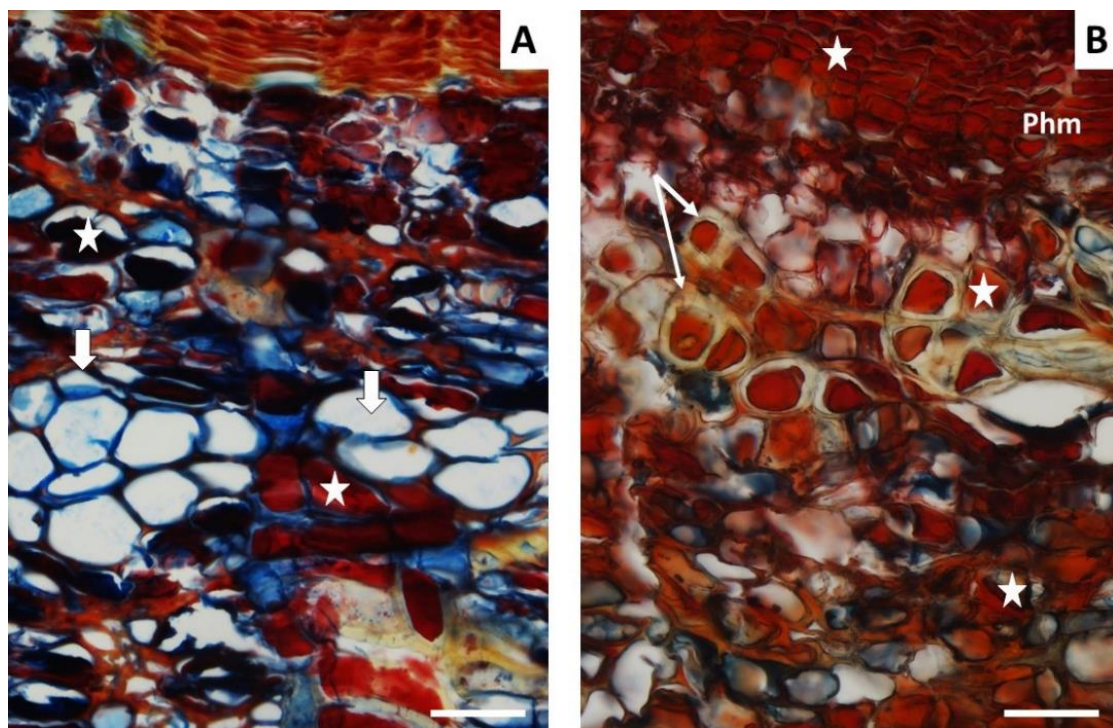


Figure 2. *A. melanoxylo* bark. **A)** Conspicuous secretory cells (arrows) in the outer phloem filled of extractives (*); **B)** Outer phloem with abundant sclerified cells (arrows) and tubular cells of phellem (Phm), both filled with red contents (*). Scale bar **A & B**= 50 μm

Within the anatomical features described above, special attention is addressed to the conspicuous and abundant secretory cells in clusters/or tangential bands imposing a characteristic pattern on bark (Figs. 1A and B; Fig. 2A); secretory cells with variable arrangement are often of high diagnostic value for certain families such as *Sapotaceae*, *Papilionoideae*, *Caesalpinoideae*, *Moraceae* or *Meliaceae* (Roth, 1981). These specialized parenchyma cells synthesize and store diverse secondary metabolites *i.e.* oils and tannins (Evert, 2006). The ample presence of

these cells over the entire *A. melanoxylon* bark justified the great amounts of extractives determined by authors in a previous study (Unpublished results).

3.2. Sieving pattern

The cumulative distribution curves of Figure 3 reveal a decrease of particles smaller than 2 mm with increasing sieve size, from 98% in 4 mm bark (Fig. 3A) to 85%, 65% and 53% in 6 mm (Fig. 3B), 8 mm (Fig. 3C) and 10 mm bark (Fig. 3D), respectively. The contrary trend is observed for particles greater than 2 mm, gradually decreasing from almost 50% in 10 mm bark (Fig. 3D) to 35, 15 and 2% in 8 mm (Fig. 3C), 6 mm (Fig. 3B) and 4 mm bark (Fig. 3A), respectively.

The particle distribution depends on raw-material fractionation ability, and particles smaller than 4 mm should be carefully balanced: higher particle abundance within 2-4 mm, and intermediate proportion of fines (< 2 mm) (Caron and Michel, 2017). The arrangement of particles determines the dimension and organization of the pore space and influence the water and air retentions (Naasz et al., 2009). Fines presence in growing-media is strongly correlated with the primary process of bark deconstruction. A right choice of sieve size is an important step to meet the final substrate quality purposes (Carlile et al., 2019).

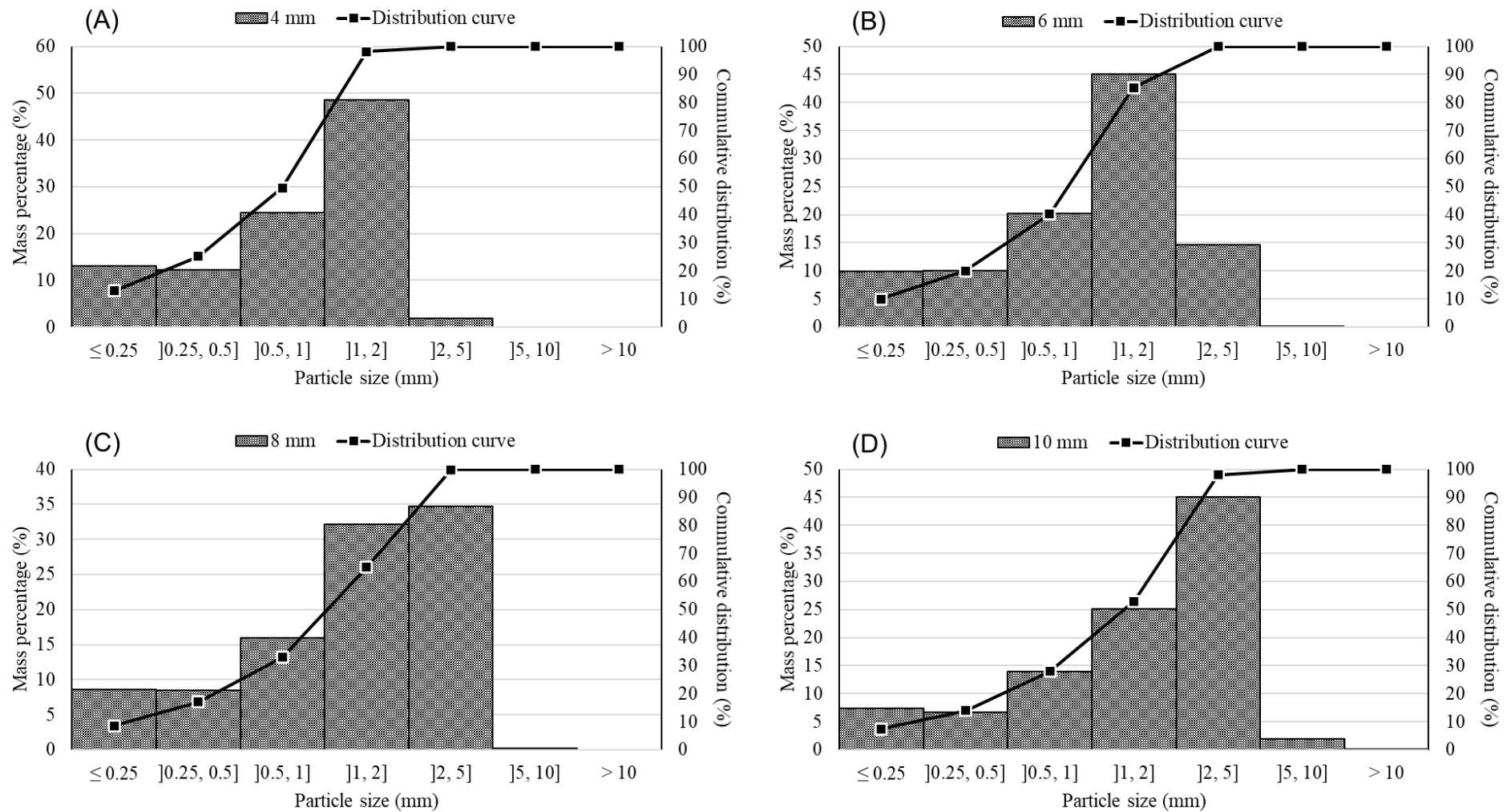


Figure 3. Particle size distribution of *A. melanoxyton* bark grinded through output sieves of **A)** 4 x 4 mm², **B)** 6 x 6 mm², **C)** 8 x 8 mm² and **D)** 10 x 10 mm²

3.3. Fresh vs. aged bark

All fresh bark was phytotoxic for cress seeds (Table 1). Root length index (RI) values were significantly lower at week 0 than in the following ageing weeks. However, seeds germination was not affected, since no statistical differences between barks germination rate (GR) were found. Phytotoxicity of *A. melanoxyton* bark may be attributed to phenolic compounds presence, confirmed by abundant deposits in cell tissues (Figs. 1 and 2), which is an inevitable consequence of fresh bark material with active secondary metabolites production.

Ageing had a positive effect on phytotoxicity reduction. At week 4, RI in different barks (RI > 92 %) was statistical equal to RI in peat-based control (RI =100%). At week 8, RI was higher than 99% in all barks, showing that the inhibitory effect of bark was totally extinct, except in the 8 mm bark.

Ageing is recognized (Buamscha et al., 2008; Carlile et al., 2015; Kaderabek et al., 2017) as an efficient, low-cost, and fast process, recommended for phytotoxins removal from freshly harvested barks. Table 1 results suggest an ageing period of 8 weeks for *A. melanoxyton* bark (AB) use as substrate component with residual phytotoxicity risk.

Table 1. Phytotoxicity determination: cress seeds germination rate (GR) and root length index (RI) at 0, 4 and 8 weeks of *A. melanoxyton* bark ageing period. Means followed by the same letter, in column, do not differ at $P \leq 0.05$ by the LSD-test.

Sieve size (mm)	Ageing (weeks)	GR	RI
		(%)	
4	0	100.0 a	15.5 c
	4	93.3 a	97.6 ab
	8	96.7 a	98.8 ab
6	0	100.0 a	18.6 c
	4	96.7 a	91.9 ab
	8	100.0 a	113.7 a
8	0	100.0 a	21.7 c
	4	93.3 a	78.0 b
	8	93.3 a	90.5 ab
10	0	96.7 a	19.7 c
	4	96.7 a	93.3 ab
	8	100.0 a	103.1 ab

The FB 4 mm presented statistical higher EC, N_{\min} and P contents (Figs. 4B, C and D) compared to other grinded bark. High fines proportion (Fig. 3A) in 4 mm bark may promote nutrient availability, due to greater particle surface contact area when compared to the coarser ones. On the other hand, pH is positively correlated with particle size enlargement (Fig. 4A).

All grinded AB presented statistically equal N_{\min} content. Ageing dropped N_{\min} up to 6 mg L^{-1} (Fig. 4C) and reduced P values below 2 mg L^{-1} (Fig. 4D), which declined EC to a minimum of 14 mS m^{-1} (Fig. 4B), and consequently increased pH up to 6.2 (Fig. 4A). Bark N_{\min} and P decay during ageing has been commonly reported and associated to microbial growth activity that involves N immobilization by microorganisms and P depletion (Carlile et al., 2019). An increase in pH followed by EC reduction agrees with the literature findings for aged pine bark substrate (Kaderabek et al., 2017), which over the course of 3 months pH increased from 4.2 to 4.5; authors attribute this correlation to aerobic activity stimulated by pine bark natural process.

Generally, nutrient elements availability depends on pH, and when substrate pH tends to basic (low H^+ concentration) decreases EC, and vice-versa (Vandecasteele et al., 2018). The N immobilization risk in bark occurs due to microbial activity in fresh material (Buamscha et al., 2008; Chemetova et al., 2018). However, nutritional balance can be strategically regulated by adding and incorporating N sources into the bark with N fertilizers, depending on plant need (Ribeiro et al., 2009; Vandecasteele et al., 2018).

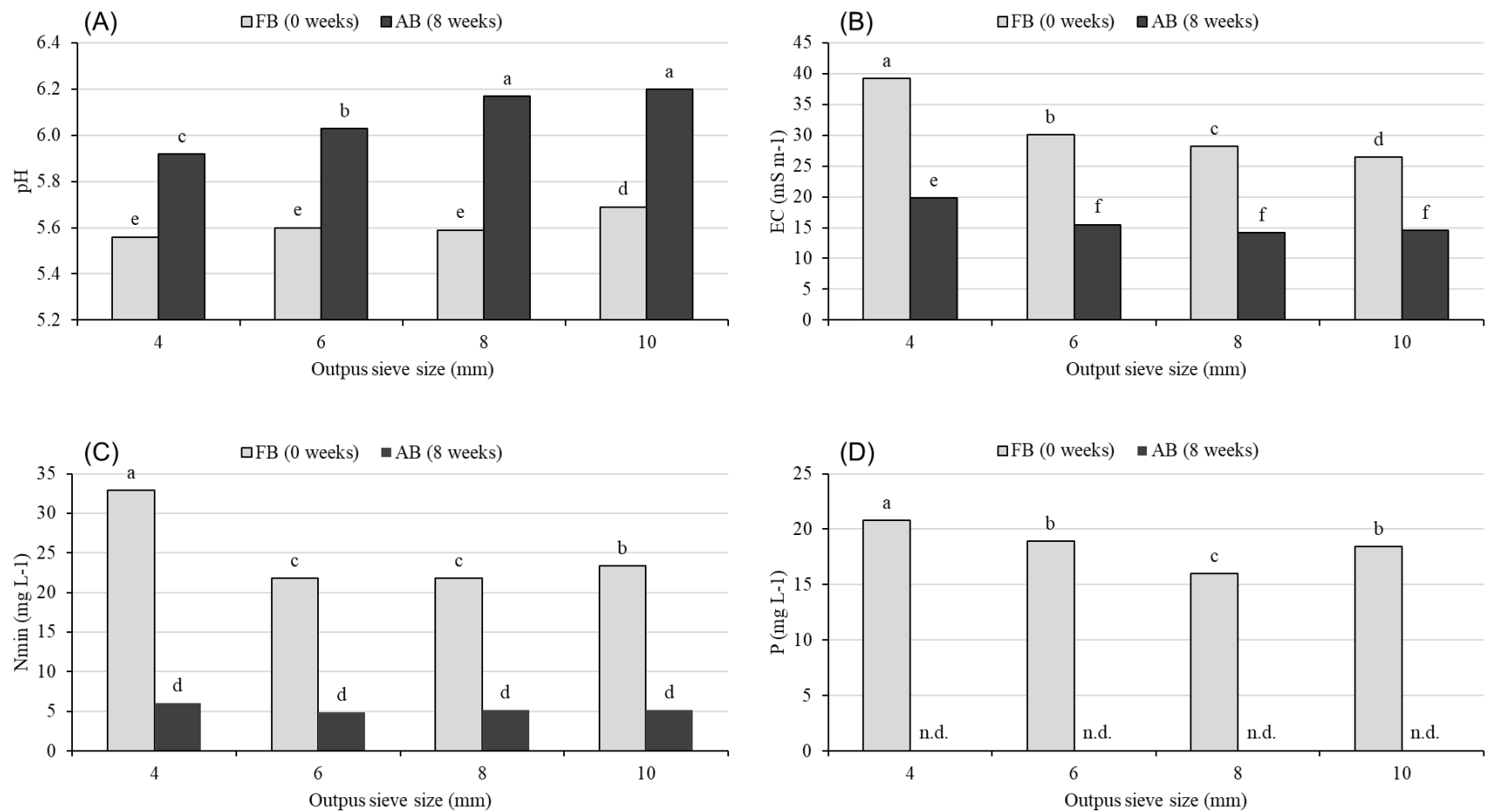


Figure 4. Fresh bark (FB; 0 weeks) and aged bark (AB; 8 weeks) from the four granulometric sizes (4, 6, 8 and 10 mm); **A)** pH, **B)** electrical conductivity (EC), **C)** mineral nitrogen (Nmin) and **D)** phosphorus (P) determination. Bars followed by the same letter do not differ at $P \leq 0.05$ by the LSD-test. n.d. = not detected, below quantification limit (< 2 mg L⁻¹)

3.2. Bark based growing media

3.2.1. Physical properties

Bark progressive addition (10, 25 and 50%) in FB and AB-based substrates increased BD, while TP decreased (Table 2), in agreement with previously mentioned by Carlile et al. (2019). Despite the significant differences, BD values fitted the recommended upper limit ($< 400 \text{ g L}^{-1}$) and TP values were greater than 85% of total substrate volume, which are similar values to aged pine bark substrates: $\text{BD} < 200 \text{ g L}^{-1}$ and TP ranging from 86 to 88% (Altland et al., 2018). These parameters can be adapted by managing the compaction at potting moment (Barrett et al., 2016).

Coarser bark (10 mm) significantly increased aeration (AFP) in FB and AB-based substrates, while finer bark (4 mm) did not affected AFP, pointing a strong correlation between particle size distribution (Figs. 3A and D) and aeration properties. In container media, as fine particles increase, the proportion of smaller pore sizes in the total pore volume increases, reducing substrate AFP (Kaderabek et al., 2017). Ageing had little effect on bark-based substrates AFP (Table 2), which is in agreement with findings for aged pine bark substrates (Altland et al., 2018). All substrates had an AFP within the recommended range ($20\text{-}30\% \text{ v v}^{-1}$), except substrates with 25 and 50% of coarser bark (10 mm), that presented slightly higher aeration. However, to prevent plant-microbial respiration competition, Caron and Michel (2017) suggested AFP above 30% to promote biological stability in organic mixtures.

Peat-based substrate presented the highest available water content. Bark addition tend to reduced easy available water (EAW) and water buffering capacity (WBC), mainly in the coarser bark (10 mm). EAW in substrates with 50% of coarser bark (10 mm) was as low as 11.8 and 14.9%, in fresh and aged bark respectively, fairly below the recommended values ($20\% \text{ v v}^{-1}$) and leading to possible hydraulic conductivity changes (i.e., the ability to transfer water inside the container) (Altland et al., 2018). Considering all AB 4 mm based substrates, the EAW values were within, or close to, the acceptable range.

Bark is rarely used as a stand-alone growing-media constituent, is normally added to optimize physical properties of media mixtures (Barrett et al., 2016). According to air-water relationships (Table 2), coarser (10 mm) bark-based substrate may optimize air supply to the roots, while finer (4 mm) bark mixes retain similar water as commercial peat. For nursery and greenhouse growers, the water logging risk is residual, however, is recommended frequently checking bark physical properties to ensure material consistency (Kaderabek et al., 2017). The adoption of adequate irrigation intervals regime, considering container geometry, may help to achieve the desired media performance (Bakry et al., 2013; Barrett et al., 2016).

Table 2. Substrates physical properties: bulk density (BD), total porosity (TP), easily available water (EAW), water buffering capacity (WBC) and air-filled porosity at 10 cm of water column (AFP). Means followed by the same letter, in column, do not differ at $P \leq 0.05$ by the LSD-test. Acceptable range, adapted from Caron and Michel (2017)

Substrate	Sieve size (mm)	Bark (%)	BD (g L ⁻¹)	TP	EAW	WBC	AFP
FB based	4	10	139 ef	91.3 cd	21.4 cde	5.4 bc	30.7 bcd
		25	165 c	89.6 f	21.3 cde	4.2 fg	29.3 bcde
		50	212 a	86.6 h	19.6 f	3.9 g	26.1 f
	10	10	144 de	91.0 de	20.2 def	5.1 cd	30.8 bcd
		25	164 c	89.7 f	18.7 f	4.4 efg	31.8 b
		50	196 b	87.6 g	11.8 h	0.7 h	38.1 a
AB based	4	10	134 f	91.6 bc	23.2 ab	6.0 a	26.4 ef
		25	150 d	90.6 e	22.0 bcd	5.1 cd	27.7 def
		50	170 c	89.3 f	19.4 f	4.8 de	28.2 cdef
	10	10	132 fg	91.7 b	22.2 bc	5.7 ab	28.1 cdef
		25	146 de	90.9 de	19.6 ef	4.7 d	31.2 bc
		50	168 c	89.5 f	14.9 g	0.5 h	35.5 a
Peat based	-	0	125 g	92.2 a	24.3 a	5.9 a	27.2 ef
Acceptable Range			< 400	> 85	20 - 30	4 - 10	20 - 30

3.2.2. Chemical properties

pH values slightly increased with bark addition, from 6.1 to 6.7 (Table 3), standing within the recommended range (5.5-6.5), except for substrate with 50% of AB 10 mm. The ideal pH for plants can vary quite extensively (Barrett et al., 2016), however most plant nutrients tend to be available within a narrowed pH range.

Similar to pH, the optimum EC (salt solubility level) may be plant specific. The EC values (7-12 mS m⁻¹) were below tolerance limit (<50 mS m⁻¹), thus suggesting low concentration of total salts in bark blends.

Analogous to Figure 4 trend, ageing may induce N immobilization reflected by N_{min} decrease from 13 to 5 mg L⁻¹ (Table 3). N_{min} decrease is linked with successive bark volume addition (10, 25 and 50%) in AB substrates. In the other hand, substrate with 50% of FB 4 mm presented statistical equal N_{min} (22 mg L⁻¹) to commercial peat, due to the initial rich N content (Fig. 4). N availability is the most limiting factor that affects substrate biological stability (Chemetova et al., 2018), thus a previous knowledge on N requirements is fundamental to overcome N limitations (Buamscha et al., 2008).

Table 3. Substrates chemical properties: pH, electrical conductivity (EC), mineral nitrogen (N_{min}), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na). Means followed by the same letter, in column, do not differ at P ≤ 0.05 by the LSD-test. Acceptable range, adapted from Barret et al (2016). n.d. = not detected, below quantification limit (< 2 mg L⁻¹).

Substrate	Sieve size (mm)	Bark (%)	pH	EC (mS m ⁻¹)	N _{min}	mg L ⁻¹				
						P	K	Ca	Mg	Na
FB based	4	10	6.2 ef	6.5 i	4.8 fg	2.9 f	19 bc	24 bcd	2.8 cde	21 de
		25	6.4 bdd	8.4 bcd	6.4 ef	2.4 g	26 ab	18 d	2.2 efg	34 bc
		50	6.2 def	11.8 a	21.6 a	7.0 b	35 a	23 cd	2.7 cdef	45 a
	10	10	6.3 cde	7.4 gh	10.2 d	4.8 d	25 ab	23 cd	3.1 bcd	15 ef
		25	6.3 cde	7.8 efg	6.9 e	2.3 g	23 bc	22 cd	2.2 efg	32 bc
		50	6.4 bc	8.9 b	17.7 b	5.1 d	27 ab	21 cd	2.1 efg	36 b
AB based	4	10	6.1 fg	7.5 fgh	12.9 c	5.7 c	14 cd	31 ab	3.5 abc	11 fg
		25	6.4 bc	7.9 def	6.0 ef	3.4 e	22 bc	27 abc	2.8 cde	23 de
		50	6.5 ab	8.5 bc	5.1 fg	3.4 e	22 bc	19 d	n.d.	27 cd
	10	10	6.2 efg	7.2 h	12.8 c	4.8 d	15 cd	33 a	3.8 ab	11 fg
		25	6.2 cde	7.0 hi	5.3 efg	2.6 fg	19 bc	23 cd	2.4 defg	17 ef
		50	6.7 a	8.0 cde	4.2 g	n.d.	23 bc	22 cd	n.d.	17 ef
Peat based	-	0	6.0 g	7.8 efg	22.4 a	7.7 a	8 d	32 a	4.3 a	3.4 g
Acceptable range			5.5-6.5	< 50	50-250	19-75	51-400	16-80	16-80	<100

Despite significant differences, the water-soluble P content of all substrates, including peat, was extremely low (< 8 mg L⁻¹), close to residual level, and distant from recommended range (19-75 mg L⁻¹). Identical result was found for Mg content. Water-soluble K and Ca were the elements with the greatest

representation, although K stands below the lower limit (51 mg L^{-1}), Ca content fulfilled the requirements. Nutritional composition of bark blends suggests that all substrates must be fertilized before seedling to balance nutrient needs and is likely start with low nutrient initial level and gradually increase fertilizer rate according to plant demand (Ribeiro et al., 2009).

3.2.3. Phytotoxicity

Substrates with 50% of FB (4 mm and 10 mm) cause early growth inhibition on cress seed (Fig. 5), showing RI values statistically different than other bark-based substrates, either made from fresh or aged bark, and standing below the peat-based control. This result agrees with previous bark phytotoxicity findings (Table 1), pointing out the phytotoxins reduction over ageing time. The addition of 50% (vv^{-1}) of aged bark to peat guarantees a non-toxic substrate and matches the maximum volume of composted materials generally incorporated into potted mixtures (between 10-50% vv^{-1}) (Barrett et al., 2016).

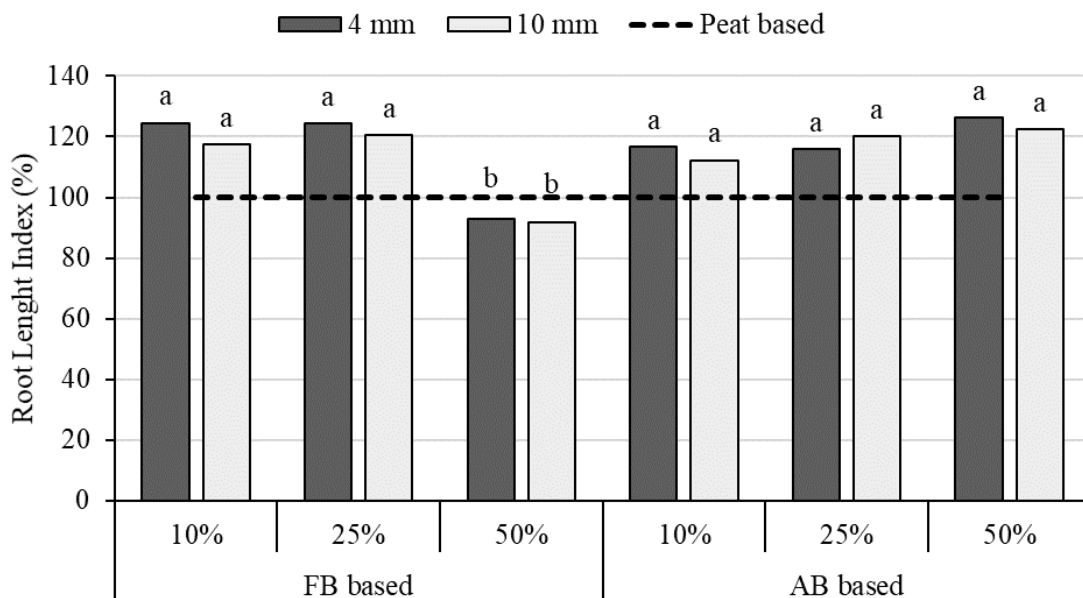


Figure 5. Phytotoxicity: cress seed root length index of fresh bark (FB) and aged bark (AB) based substrates, fine (4 mm) and coarse (10 mm) grinded sizes, mixed at 10, 25 and 50% volumetric proportion with peat. Bars followed by the same letter, do not differ at $P \leq 0.05$ by the LSD-test

Ageing and composting are widely performed treatments which reduce or may completely remove fresh bark phytotoxic effect (Carlile et al., 2019). The main advantage of ageing treatment, compared to composting, is the simplicity (economical attractiveness) of whole process operation: from pile storage and

maintenance to prepare a stable toxic-free substrate without turning or aeration need (Buamscha et al., 2008).

3.2.4. Potted plant response

Pot experiment results (Table 4) showed the lowest chinese cabbage performance in both substrates with 50% of FB (4 mm and 10 mm), which are in accordance with cress seed growth findings (Fig 5). Phytotoxicity increased with FB addition (10, 25 and 50 %); growth inhibition (GI) in FB 4 mm raised from 5% to 65%, in substrates with 10% and 50% of FB, respectively (Table 4), which corresponded to a loss of cabbage fresh weight (FW) higher than 20 g pot⁻¹. Similar trend was found in FB 10 mm. Cabbage dry weight (DW) and root rating presented same tendency with a weak plug colonization by roots in substrates with 50% of FB. As supported by previous studies (Bonaguro et al., 2017; Brito et al., 2013; Carlile et al., 2015; Chemetova et al., 2018) plant performance was affected by phytotoxic elements presence in fresh bark-based substrates.

Table 4. Pant responses: chinese cabbage fresh weight per pot (FW), dry weight per pot (DW), roots rating and growth inhibition (GI). Means followed by the same letter, in column, do not differ at $P \leq 0.05$ by the LSD-test.

Substrate	Sieve size (mm)	Bark (%)	FW (g pot ⁻¹)	DW (g pot ⁻¹)	Roots rating [1-5]	GI (%)
FB based	4	10	36.6 ab	3.0 abc	4.6 ab	5.0 cd
		25	34.3 bc	2.8 c	4.4 ab	11.1 cd
		50	13.4 e	1.2 e	2.1 d	65.2 a
	10	10	36.7 ab	3.0 abc	4.8 a	4.9 cd
		25	33.1 c	2.7 c	4.8 a	14.1 c
		50	23.1 d	1.9 d	3.5 cd	40.1 b
AB based	4	10	37.4 a	3.2 abc	4.8 a	3.1 cd
		25	38.0 a	3.2 abc	4.6 ab	1.6 cd
		50	38.7 a	2.9 abc	4.2 ab	0 d
	10	10	38.6 a	3.3 abc	5 a	0 d
		25	38.8 a	3.1 abc	4.5 ab	0 d
		50	36.8 ab	2.8 bc	3.8 cd	4.7 cd
Peat based	-	0	38.6 a	3.2 abc	4.8 a	0 d

Ageing completely removed bark phytotoxicity (Table 4). AB-based substrates revealed plant responses statistically equal to peat (except roots rating in 50% AB 10 mm), which allowed the production of strong seedlings and chinese cabbage plants with a well-developed root system (total root plug colonization). Alternative container media made from forest biomass such *Eucalyptus globulus* bark (Chemetova et al., 2018), *Pseudotsuga menziesii* bark (Buamscha et al., 2008), pine tree (Barrett et al., 2016), and pine bark (Naasz et al., 2009) used around 25-30% (v v⁻¹) of the organic material in the mixtures without compromise plant development. AB-based substrates presented equal plant performance to those grown in peat-based substrate, encouraging up to 50% v v⁻¹peat replacement in commercial media formulations by aged *A. melanoxyton* bark.

4. Conclusion

The *A. melanoxyton* fresh bark phenolic and extractives presence promoted cress seed inhibition. Ageing might remove these phytotoxic elements and a period of 8 weeks allowed cress roots development as great as peat control. However, ageing process induced nutrients immobilization, and consequently, external nutritional provision is suggested to adequate N balance within microbial-plant needs. Physical performance can be regulated by choosing the initial bark sieving size. Coarser bark (10 mm) addition may increase air porosity and potentiate coarse aged bark use as aeration agent, replacing peat in growing media with deficient root zone air supply. Finer bark (4 mm) incorporation may be suitable for a growing media with water requirements similar to peat. Up to 50% of aged *A. melanoxyton* bark might replace commercial peat in container media without compromising plant and root growth. This option is an environmental convenient solution with two main driving forces: exotic *A. melanoxyton* biomass valorization that may stimulate their profitability and control, and the use of local available woody biomass to counteract dependence on peat in horticulture industry.

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CHAPTER 4

The *Acacia* bark phytotoxic potential: a non-synthetic bio-herbicide

A. melanoxylon residue



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The *Acacia* bark phytotoxic potential: a non-synthetic bio-herbicide

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Abstract

A natural technique to control weed emergence and decrease the use of synthetic herbicides could be the application of phytochemical substances with phytotoxic effects from *Acacia* bark. In Mediterranean area, *A. melanoxylon* and *A. dealbata* bark are an abundant waste material from non-native tree species control actions, requiring suitable disposal. The presence of phytotoxic substances in young harvested biomass is greater than in mature plant tissues, thus for organic substrates formulation ageing treatment is only effective (phytotoxicity reduction after 8 weeks) for *A. melanoxylon* mature bark, which potentiate young bark for different valorization. This study explores *A. melanoxylon* and *A. dealbata* bark extracts from young debarked trees, regarding their bio-herbicide effect on germination and development of cress seeds. Extraction conditions were performed under fixed time (t: 45min) and a maximum temperature of 120 °C, using the following solvents: ethanol, water and an equal volume of ethanol:water. The extract yield was greater in water extracts (40 g dry extract L⁻¹), followed by 50% ethanol:water (36 g dry extract L⁻¹) and ethanol (28 g dry extract L⁻¹). In water extract essay, the extract yield remained constant even after bark percentage gradual reduction over the extraction ratio conditions (liquid:bark ratio from 1:5, 1:10 and 1:15), suggesting a possible final liquor saturation. However, all bark extracts inhibited the tested seed growth (root index < 8%) compared to deionized water control (root index = 100%). The bio-herbicide activity of water soluble *Acacia* bark phytochemical substances increases its phytotoxic effect as extract concentration increase.

Key-words: Bark extracts; *A. melanoxylon*; *A. dealbata*; phytochemicals concentration; seed inhibition

List of abbreviations (Chapter 4)

CH₂Cl₂	Dichloromethane
EtOH	Ethanol
GR	Germination rate
R	Ratio
RI	Root index
RL	Root length
T	Temperature
t	Time
H₂O	Water

1. Introduction

Weed infestation is considered a limiting factor for horticultural food production systems which decrease crop yield and therefore fruit yield. To feed the increasing global population and meeting the sustainable development goals, the use of synthetic herbicide for weed control must be reduced, while the search for alternative herbicide is growing worldwide (Mohammadi, 2013).

A natural and environmentally friendly method to control weed growth is the use of biochemicals produced from specific species which have a phytotoxic effect on target weeds, so called allelopathic phenomena (Jelassi et al., 2016). Most allelochemicals are secondary metabolites inherent to native species and activated for plant self-defense, which are commonly presented in external plant tissues (Feng et al., 2013). Tree barks contain great amount of those phytochemicals, thus making them potential alternative weed control options (Ogawa and Yazaki, 2018).

In Southern Mediterranean countries, some ecosystems are currently under Australian *Acacia* invasion risk (Vicente et al., 2013). *A. melanoxylon* and *A. dealbata* bark are an abundant waste material from non-native tree species control actions (e.g. ring-barking control method), requiring suitable disposal

(Carneiro et al., 2013). A possible valorization of this biomass has been analyzed, and previous research (Chemetova et al., 2019; Souza-Alonso et al., 2018) revealed *Acacia* bark suitability as natural weed control. The allelopathic effect was attributed to the presence of naturally produced compounds such, phenolics, flavonoids and tannins (Chemetova et al., 2019; Ogawa and Yazaki, 2018).

The natural allelochemicals are mostly water-soluble, although different chemical composition resulted when extraction is fractioned among solvents of increasing polarity (Feng et al., 2013). A common biological assay for plant allelochemicals phytotoxicity activity evaluation are the germination tests using model-sensitive species (e.g. *Lepidum sativum* and/or *Lactuca sativa*) (Lorenzo et al., 2016). The application of *Acacia* bark extracts is a promising approach due to its simplicity, easy extraction method and ready availability of raw-material, however the minimum concentration of the extract that has phytotoxicity activity must be determine (Mohammadi, 2013).

The objective of this research was to evaluate the potential of *A. melanoxylon* and *A. dealbata* bark extracts from juvenile debarked trees, regarding their bio-herbicide effect on germination and growth of cress seeds.

2. Materials and Methods

A. dealbata and *A. melanoxylon* bark collection was conducted during the winter season 2017-2018. Both barks were sampled from juvenile tree stands (up to 6 years-old). *A. melanoxylon* bark was collected from Tapada da Ajuda, the Instituto Superior de Agronomia (ISA) Campus (38°42'27.5"N, 9°10'56.3"W) (Lisbon, Portugal), while *A. dealbata* was collected from Mata Nacional do Bussaco (40°22'36.7"N 8°22'05.4"W) (Luso, Portugal).

The bark samples were collected separately after on-site ring-barking method application, air dried and ground in a knife mill (Fritsch pulverisette 15 – Fritsch GmbH, Idar-Oberstein, Germany) with an output sieve of 10 x 10 mm², followed by 2 x 2 mm². The 0.4-0.25 mm fraction was used for extractive content chemical analysis, while the coarser bark from output sieve of 10 x 10 mm² was used for testing bio-herbicide effect.

The dry mass content was assessed by oven-drying samples at 105 °C for 24 hours, and the ash content was determined by combustion of the oven-dried sample at 550 °C for 5 hours in a muffle furnace, according to TAPPI Standard Methods (T211 om-02). Samples (around 3.5 g dry mass) were full extracted using a solvent sequence with crescent polarity (dichloromethane, ethanol, and water) in a Soxhlet apparatus (T204 cm-07) for total extractives determination. The extraction analysis was done in duplicate and results were reported as percentage of the dry mass material.

For bio-herbicide effect evaluation, the extraction was performed using a stainless-steel reactor (max ca. 4 L) with fluid recirculation. The conditions were set according to previous pre-tests (data not shown) under fixed time 45 min and a maximum temperature of 120 °C using ethanol, water and an equal volume of ethanol water as solvents. For water extraction, different liquid-to-solid ratio (R) were tested: R1:5, R1:10 and R1:15. From the obtained liquors, solvents were left to evaporate, and distilled water was added as dissolution agent.

Phytotoxicity of extracts was evaluated using *Lepidium sativum* as model plant. According to European Standards (CEN, 2011), a total of 10 seeds were placed on top of petri dish filled with perlite and a filter paper impregnated with 50 ml of the water dissolved extracts (0.25 g, 0.5 g and 1.5 g per petri dish), the incubation was performed at room temperature (25 °C) in the dark, for 3 days, and water as a control. The allelopathic effect was evaluated based on the average germination rate (GR), and root length (RL) to calculate root index (RI).

3. Results and Discussion

Both *Acacia* barks presented equal ash content, around 3% dry basis, however extractives content was greater in *A. dealbata* compared to *A. melanoxylon* bark, 36% and 29% dry basis, respectively. According to Taflick et al. (2017), exhausted *Acacia* bark showed 26% of extractives even after the industrial hot water-based process for tannin extraction.

Figure 1 describes the extractives distribution, within the total extracted content (% total extractives), by solvent. In both *Acacia* species, ethanol extracts showed

higher yields, three times greater than water extracts, and dichloromethane extracts registered the lowest yields, meaning that the most of *Acacia* extractives were composed by hydrophilic compounds, while lipophilic compounds were residual.

The stainless-steel reactor extraction data focusses on *A. melanoxylon* bark as a representative species for further determination of the minimum concentration of *Acacia* extract that has bio-herbicide activity. Table 1 presents total water (100% H₂O) extract (g L⁻¹) variation with decreasing bark material in the reactor, and different solvents for the higher liquid-to-solid ratio R 1:5. The extract saturation may occur among the water extraction even after bark percentage successive reduction over the extraction ratio conditions successive reduction, from R1:5 to R1:15, the final extract difference was less than 12%, respectively, using same reaction condition. *Acacia* is widely known as tannin rich species (Souza-Alonso et al., 2018), and water is more suitable solvent for extracting tannin from the bark than ethanol (Taflick et al., 2017).

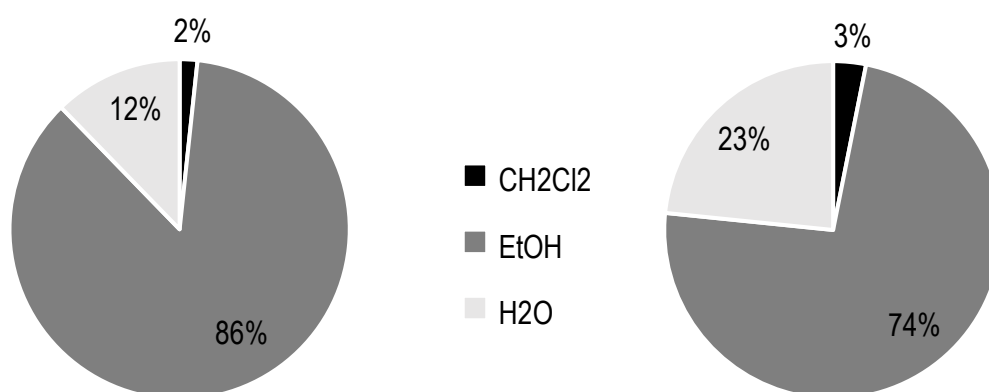


Figure 1. Soxhlet extraction: *A. dealbata* (left) and *A. melanoxylon* (right) bark extractives content (% total extractives) by solvent: dichloromethane (CH₂Cl₂), ethanol (EtOH), and water (H₂O)

Between solvents at R1:5, water presented the highest yield (40 g L⁻¹), while ethanol reduces the total final extract, reaching minimum of 28 g L⁻¹ in 100% EtOH. Figure 1 reports ethanol as a solvent with greater extractive content, however, this finding may only be attributed to sequence of soxhlet extraction; while, when bark is extracted only one time (Table 1), there is evidence that water

can extract most of semi-polar and polar substances. The influence of particle size may be also considered between extraction methods. Literature findings (Sowndhararajan et al., 2015) highlighted that polar solvents are responsible for the higher yields.

Table 1. Stainless-steel reactor extraction: *A. melanoxylon* bark total extract (g L⁻¹) using liquid-to-solid ratio (R1:5, R1:10 and R1:15); and water (100% H₂O), water:ethanol (50% EtOH:H₂O), and ethanol (100% EtOH) solvents.

Solvent	Liquid:solid ratio		
	R1:5	R1:10	R1:15
	400g : 2000 ml	200g : 2000 ml	133g : 2000 ml
Bark extracts (g L ⁻¹)			
100% H ₂ O	40.2	37.0	35.5
50% EtOH:H ₂ O	35.7	-	-
100% EtOH	27.5	-	-

Phytotoxicity of extracts from R1:5 ratio extraction is presented in Table 2. All extracts presented an allelopathic effect on tested seeds (RI ≤ 12%). Up to 0.5 g extract petri⁻¹ all seeds germinated (GR = 100%), however the bio-herbicide activity of soluble substances rises its phytotoxic effect as extract concentration increases. The solvents 50% H₂O:EtOH and 100% H₂O had a quicker bio-herbicidal effect among the concentration increment, reaching cress seed inhibition (GR and RI= 0%) at extract concentration 1.5 g petri⁻¹, while ethanol allows cress seed germination (GR = 86%) and showed the highest cress growth.

Acacia bark water soluble extract had greater yield (Table 1) and combining its inhibitory effect at minimum extract concentration of 30 g L⁻¹, could be a promising bio-herbicide which, simultaneously, promotes sustainable management of juvenile *Acacia* tree stands, and may alleviate the costs of invasive plant control.

Table 2. *A. melanoxyton* bark extract phytotoxicity on cress seeds: germination rate (GR), root length (RL) and root index (RI); using 0.25 g, 0.5 g and 1.5 g extract per petri dish.

Solvent	[Extract] (g petri ⁻¹)	GR (%)	RL (cm)	RI (%)
100% H ₂ O	0.25	100	0.59	8
	0.5	100	0.23	3
	1.5	0	0	0
50% H ₂ O:EtOH	0.25	100	0.64	8
	0.5	100	0.31	4
	1.5	0	0	0
100% EtOH	0.25	100	0.88	12
	0.5	100	0.83	11
	1.5	86	0.45	6
Control H ₂ O	-	100	7.55	100

4. Conclusions

A. melanoxyton and *A. dealbata* bark can be extracted using polar solvents. Water can extract most of semi-polar and polar substances. For the studied conditions, the extract saturation may occur among the water extraction even after bark ratio successive reduction. All bark extracts were phytotoxic for tested cress seeds. The bio-herbicide activity of water-soluble substances rises its phytotoxic effect as extract concentration increase, and 30 g L⁻¹ extract may establish the minimum concentration of *Acacia* extract required for full cress seeds germination and growth inhibition. The tested innovative bio-herbicide approach contributes for synthetic herbicide replacement using natural allelochemical, which also addresses agroecological value of sustainable control and management of non-native plants.

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CHAPTER 5

Range analysis of *Eucalyptus globulus* bark low-temperature hydrothermal treatment to produce a new component for growing media industry

E. globulus waste-stream

Chapter 5

Bark fiber hydrothermal
treatment optimization



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Range analysis of *Eucalyptus globulus* bark low-temperature hydrothermal treatment to produce a new component for growing media industry

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Abstract

The use of industrial *Eucalyptus globulus* bark residues for organic growing media formulation was studied. Hydrothermal treatments were tested using Response Surface Methodology approach. Model design consisted of twelve combinations of temperature (T: 60-140°C) and residential time (t:20-60') to evaluate the effect on bark properties. Temperature had a significant effect in C mineralization and N immobilization rates, where the lowest responses (111.8 mmol CO₂ kg⁻¹d⁻¹ and NIR=4.1 mmol N kg⁻¹d⁻¹, respectively) compared to IEB (214.6 mmol CO₂ kg⁻¹d⁻¹ and 8.9 N kg⁻¹d⁻¹, respectively) were suggested after modeling at 40°C during 70'. Industrial bark was phytotoxic and treatments were effective for phytotoxicity removal. Industrial bark presented high air content but low water availability; treatments had no effect on bark physical properties and the use of demineralized water may have leached nutrient content. Results from pot experiment recommend the use of 25% (v v⁻¹) of treated barks in future growing media formulations.

Keywords

Forest residues; hydrothermal treatment; phytotoxicity; microbial activity; growing media

List of abbreviations (Chapter 5)

AFP	Air-filled porosity
ANOVA	Analysis of variances
BD	Bulk density
CCD	Central Composite Design
CMR	Carbon Mineralization rate
DM	Dry mass
DW	Dry weight
EAW	Easy available water
EC	Electrical conductivity
FW	Fresh weight
HTEB	Hydrothermal treated Eucalyptus globulus bark
IEB	Industrial Eucalyptus globulus bark
k	Number of factors
NIR	Nitrogen Immobilization rate
OM	Organic matter
PM	Peat moss
R2	Regression coefficient
R2Adj	Adjusted regression coefficient
RL	Root length
RLI	Root length index
RSM	Response Surface Methodology
T	Temperature
t	time
TP	Total porosity
WBC	Water buffer capacity

1. Introduction

In European horticultural industry, the peat presents almost 80% of total plant and seedling growing media (Gruda, 2012a). It is available in Northern Europe

as a cheap resource widely used either pure or as the main constituent of growing media (Barrett et al., 2016; Gruda, 2012a, 2012b). No other natural raw-material offers that many advantages as growing media: regular consistency, lightweight, good air and water-holding capacities, low pH and nutrient content (easy to control) and a biological stable structure. However, peat is a limited resource with a great demand, and the extraction of peat bogs causes negative impacts on environment, decreasing the carbon sink in peat bogs and releasing greenhouse gases into the atmosphere through degradation and oxidation of the unsaturated peat layer, which produces an estimated annual emission equivalent of 15 million tons of carbon (Barrett et al., 2016; Gruda, 2012a, 2012b).

The increased environmental awareness of peatland conservation has stimulated intensive research aiming to reduce the use of peat in growing media (peat-reduced growing media), replacing it, totally or in part, by new materials. Recently, organic materials derived from agricultural and municipal waste streams, as well as industrial by-products have become common (Barrett et al., 2016; Gruda, 2012a; Ribeiro et al., 2009). Their characterization and performance interpretation have been supported by multiple approaches, making them difficult to compare and dependent on crop culture used (Barrett et al., 2016; Depardieu et al., 2016).

Organic materials alternative to peat that are already commercialized include composted biowastes, coir, bark and wood fiber (Gruda, 2012a). For instance, from coconut husk, different products are available based on particle size, including chips of various sizes, fiber of various lengths or coir dust material. However, the raw material used in these products is produced out of Europe, for example in India, Sri Lanka, Vietnam, Philippines and Ivory Coast (Maher et al., 2008). The long transportation distance makes this alternative less attractive and less “environmentally friendly” when compared to locally sourced materials. For this reason, European countries are focused in pine, spruce and other softwoods as the main source of raw materials for plant growth substrates, regarding the availability of residues from forest harvest or from wood processing industries (Barrett et al., 2016; Gruda, 2012b; Jackson et al., 2010; Ribeiro et al., 2009).

In Portugal, *Eucalyptus globulus* is the predominant species for pulp and paper production (Domingues et al., 2010; Neiva et al., 2016) and around 500,000 tons of *E. globulus* bark (CELPA, 2015) are generated as industrial residue used mainly as a solid biofuel in power plants inside of pulp and paper industries (Domingues et al., 2013; Gruda, 2012a; Neiva et al., 2016). Other actual or potential uses to valorize bark residues were investigated by several researchers such incorporation in the pulping process (Neiva et al., 2016), source of chemical compounds with biological and pharmacological activities (Domingues et al., 2010, 2013), or incorporation into the soil to improve its structure and fertility (Murphy et al., 2010).

E. globulus bark as raw-material for substrate production alternative to peat was investigated in this paper. In literature, there is no information or data on this potential valorization of *E. globulus* bark.

Phytotoxicity is a common problem to solve when the forest residual biomass is used as a component of growing media, since the presence of phenolic compounds, terpenes and tannins are typical in the chemical composition (Caron et al., 2010; Gruda, 2012b). Previous studies demonstrated that *E. globulus* bark is rich in phenolic, triterpenic and other inhibitory compounds (Domingues et al., 2010; Neiva et al., 2016). A different approach has been suggested to eliminate the phytotoxicity from the forested materials and to create a biological stable environment for plant growth (Gruda, 2012b). Buamscha et al. (2008) studied the differences in plant growth between fresh and aged Douglas fir (*Pseudotsuga menziesii*) bark used as growing media and found out that plants were smaller in fresh bark than in the aged one. Gruda et al. (2009) reported improvements in germination rate and radicle growth after washing/leaching pine tree bark based substrates. Cunha-Queda et al. (2007) and Jackson et al. (2010) proposed composting pine bark to remove phytotoxicity and to increase low electrical conductivity. From the techno-economical point of view, the more raw-material transformation required the higher associated cost, potentiating low-temperature hydrothermal treatment as an attractive process due to its simplicity and rapid implementation, while using water as the main reagent (Barrett et al., 2016).

The aim of this study was to investigate the effect of hydrothermal treatment in the phytotoxic compounds removal from the industrial *E. globulus* bark. A range analysis of theoretical treatment residence time and temperature was carried out in order to improve *E. globulus* bark physical, chemical and biological properties. Based on the initial treatments, was evaluated the viability of adding up to 25% of *E. globulus* bark for structural improvement of commercial peat-based growing media.

2. Material and Methods

2.1. Raw-material

Industrial *E. globulus* bark (IEB) was collected from The Navigator Company pulp mill (Setúbal, Portugal). Raw-material was gridded in a knife mill ($\emptyset \square 6\text{mm}$). Granulometry revealed more than half (64% of total weight) bark fibers distributed within particle sizes of 5 to 1 mm, around 11% of fines ($< 1\text{ mm}$), and 25 % of coarse particles ($> 5\text{mm}$). Peat moss (PM) slightly decomposed (H2-H5 on Von post scale) from Floragard Co. (Germany), amended with 4 g L^{-1} of calcitic lime and 4 g L^{-1} of dolomitic lime to adjust pH (to 5.6 – 5.8), was used as a commercial standard.

2.2. Low-temperature hydrothermal treatment

Hydrothermal treatment of industrial *E. globulus* bark (HTEB) was performed using Response Surface Methodology (RSM) and Central Composite Design (CCD), which consisted in modeling the simultaneous effect of two process variables, temperature (T) and retention time (t).

Table 1 summarizes the experimental design matrix with natural (T and t), and the correspondent coded (X_1 and X_2) factor values for three-factor levels (-1, +1 and α). Based on preliminary tests of independent variables, the input parameters were minimum and maximum $T=60$ and $140\text{ }^\circ\text{C}$ ($X_1=-1$ and $+1$), and $t=20$ and 60 minutes ($X_2=-1$ and $+1$). To maintain rotatability in full factorial design, when the number of factors (k) is equal to 2, α value was calculated by Eq. 1:

$$\alpha = 2k^{[1/4]} = 1.414 \quad (1)$$

Determination of experiment number (runs) is essential to achieve desired responses with reliable measurements. Physical, chemical, and biological properties were the responses of the system, and the number of model runs required that guarantees the feasibility of CCD was found to be 12 (four factorial, four star and four central).

Table 1. Experimental design matrix

	Runs	Independent variables			
		Coded		Natural	
		X ₁	X ₂	T (°C)	t (min)
Factorial k ²	I	-1	-1	60	20
	II	-1	1	60	60
	III	1	-1	140	20
	IV	1	1	140	60
Star	V	-α	0	43	40
	VI	α	0	157	40
	VII	0	-α	100	12
	VIII	0	α	100	68
Central	IX	0	0	100	40
	X	0	0	100	40
	XI	0	0	100	40
	XII	0	0	100	40

The HTEBs were performed in autoclave reactor/conditions placing 5 individual hermetic vessels (1 L) containing 90 g (10% moisture content) of bark and 900 ml of water. Under the present experimental condition, the autoclave pressures ranged between 0 (run V; T=43°C) to 0.6178 MPa (run VI; T=157°C). Treatment liquor fractions were collected and frizzed for further analysis. Water excess from the solid treated material was removed by centrifugation. All experiments were performed in randomized order to minimize uncontrolled factors.

2.3. Physical and chemical analysis

Water-air relationships, as defined by Wallach (2008), were determined according to the European Standard (CEN, 2011a). The physical properties were obtained: total porosity (TP); bulk density (BD), air-filled porosity (AFP) as the amount of air at a suction of 1 kPa, easy available water (EAW) as the difference between the water content at suctions of 1 and 5 kPa, and water buffer capacity (WBC) as the difference between the water content at 5 and 10 kPa. Electrical conductivity, pH, and water-soluble K, P, Ca, Mg, Na and mineral N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) were measured in water extract 1:5 by volume, according to the European Standards (CEN, 1999a, 1999b, 2001).

The dry mass (DM) content was assessed by oven-drying bark at 105 °C for 24h and the ash content was determined by combustion of the oven-dried sample at 550 °C for 5 hours in a muffle furnace. Afterward, the difference between DM and ash was considered the organic matter (OM) content.

2.4. Biological properties

2.4.1. Carbon mineralization and nitrogen immobilization rates

Due to the possible sterilization effect of the hydrothermal treatments, amended PM (an inoculum of microorganisms) was added to the HTEBs in the carbon mineralization and nitrogen immobilization studies. HTEBs were mixed with amended PM in the volumetric proportion of 3:1 (v v^{-1}) bark/peat. Simultaneously, an only-PM control was performed and its results were used to calculate the microbial activity of the HTEBs only. All bark-based mixtures and only-PM control were conditioned to moisture content equivalent to the water retained at 1 kPa and N-fertilized (300 mg mineral N L^{-1}).

Carbon mineralization ($\text{mmol CO}_2 \text{ kg}^{-1} \text{ OM day}^{-1}$) was evaluated by the release of CO_2 using a laboratory static incubation-titrimetric determination method (Buamscha et al., 2008; Zibilske, 1994). 40 ml of fertilized mixtures (30 mL bark + 10 ml PM) were placed in 100 ml plastic containers which were subsequently put into 1.5 L glass jar containing a vessel with 20 ml of distilled water to avoid

desiccation and a vessel with 20 ml of 1M NaOH solution to trap evolved CO₂. The jars were sealed with air-tight glass lids and incubated at 25 °C for 14 days. At days 0, 2, 7 and 14, the vessel with 20 ml of a 1M NaOH solution was removed and replaced with fresh NaOH. The amount of CO₂ trapped by NaOH was quantified by a titrimetric determination, including a control vessel without sample used as a blank (Buamscha et al., 2008; Ribeiro et al., 2010).

Nitrogen immobilization rate (mmol N kg⁻¹ volatile solid day⁻¹) was evaluated by aerobic laboratory incubation. 400 ml of fertilized mixtures (300 mL bark + 100 mL PM), were placed in closed 2 L plastic containers (16x16x8 cm) and incubated aerobically for 4 weeks at 25°C. The containers were periodically aerated to guarantee the aerobic environment. Water content was controlled by regularly weighing the containers for all treatments during the incubation period and adding distilled water whenever necessary. At days 0, 2, 7 and 14, 40 mL were sampled from each container and mineral N (NH₄⁺-N and NO₃⁻-N) was quantified as described in chemical analysis in 2.3.

2.4.2. Phytotoxicity test

According to the European Standards (CEN, 2011b), a total of 10 cress (*Lepidium sativum*) seeds were incubated in Petri dishes filled with bark sample, at room temperature (25 °C) in the dark, for 3 days. The experiment was carried out in triplicate, using amended peat as the control. Phytotoxicity was evaluated based on root length (RL; where 1-3 are the triplicates and c is the control) and root length index (RLI, Eq.2) determination.

$$\text{Root length index(\%)} = \frac{\left(\frac{RL1}{RLc} + \frac{RL2}{RLc} + \frac{RL3}{RLc}\right)}{3} \times 100 \quad (2)$$

2.4.3 Pot experiment

Hydrothermally treated barks were mixed with PM in a standard volumetric proportion, 25% bark and 75% PM (v v⁻¹), for wood-based growing media (Barrett et al., 2016; Depardieu et al., 2016), and fertilized: 15 mmol NO₃⁻ L⁻¹, 8 mmol K L⁻¹

¹, 4 mmol Ca L⁻¹, 1,5 mmol Mg L⁻¹, 1,25 mmol SO₄²⁻ L⁻¹, 1,5 mmol H₂PO₄⁻ L⁻¹, 15 μmol Fe L⁻¹, 8 μmol Mn L⁻¹, 4 μmol Zn L⁻¹, 25 μmol B L⁻¹, 0,75 μmol Cu L⁻¹, 0,5 μmol Mo L⁻¹. Pots with a volume of 290 mL (Ø = 8.5 cm; height = 8.2 cm) were filled with HTEB mixtures and a total of 10 Chinese cabbage seeds (*Brassica napa* subsp. *pekinensis*) per pot were sown and grown for 5 weeks. During the entire experiment time range, all pots were placed in an unheated glass greenhouse, distributed in a complete randomized design. Pots were daily irrigated with deionized water. At the end of the growing period, sample fresh weight (FW) and dry weight (DW, at 65 °C for 48 h) were calculated and recorded.

2.5. Statistics

The experimental data (response variables) were fitted to second order polynomial equation (Eq. 3), as follows:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i < j} \beta_{ij} X_i X_j + \varepsilon \quad (3)$$

Where Y represent the response variables; X_i and X_j are the coefficients related with the two (k=2) factors (independent variables); β₀ is the intercept term, a constant; β_i is the coefficient of the linear effects, β_{ii} is the coefficient related to quadratic effects, β_{ij} is the coefficient of the interactions between the factors and ε the residual associated with the experiment.

The regression coefficient (R²), adjusted regression coefficient (R²_{Adj}) and *Lack of fit* test were used in the determination of the adequacy of the quadratic model. The statistical significance of model was evaluated by F-test by analysis of variance (ANOVA), as well as the significance of linear, quadratic and interaction model sources based on the p-value with 95% confidence level (p≤0.05).

Experimental design and statistical analysis of data were performed using the Statistica ® 10.0 (StatSoft, USA).

3. Results and discussion

3.1. *Eucalyptus globulus* industrial bark characterization

The industrial eucalyptus bark (IEB) physical, chemical, and biological properties are presented in Table 2. Physical properties analysis showed BD of 74.8 g L⁻¹ and high total pore space (TP) of 95% (v v⁻¹); both parameters were within recommended values for organic substrates (Noguera et al., 2003). However, IEB was characterized by very high AFP around 80%, low water availability (EAW lower than 2%) and null water reserves (WBC=0 %). According to Gruda (2012b), TP is about 85-95% (v v⁻¹) for a common organic substrate depending on shape, arrangement and particle size distribution. Particle size distribution is important to describe the physical quality of substrate: IEB presented 64% of fibers distributed within particle sizes of 5 to 1 mm and around 11% of fines (< 1 mm). Jackson et al. (2010) recommended that wood fiber substrate should contain mainly particles in the range from 1 mm to ≈ 6 mm and 10 to 15% of fines.

Chemical analysis presented EC value (22.3 mS m⁻¹) within acceptable range (< 60 mS m⁻¹) whereas pH revealed to be slightly acidic (4.9) and below the recommended substrate range (between 5.3 - 6.5) (Miner, 1994), however liming substrates to increase pH is a common nursery and greenhouse management practice (Jackson et al., 2010). No mineral N was detected and low availability of P and Ca was registered, while other macronutrients such as K (167.3 mg L⁻¹), Mg (21.8 mg L⁻¹) and Na (38.4 mg L⁻¹) were within acceptable range values for a substrate. Similar results were found in previous studies with wood fiber materials as spruce and pine barks (Neumaier and Meinken, 2015), and authors highlighted that nutrient content can be adjusted easily to provide an efficient plant nutrient provision.

Table 2. Physical, chemical and biological properties of industrial eucalyptus bark (IEB)

Property	Parameter	Unit	Average \pm Stand. dev	Acceptable range ²
Physical	Bulk density (BD)	g L ⁻¹	74.8 \pm 3.7	< 400
	Total porosity (TP)		95.3 \pm 0.3	> 85
	Air-filled porosity (AFP)	% (v v ⁻¹)	79.3 \pm 1.1	10 - 30
	Easy available water (EAW)		1.9 \pm 0.4	20 - 30
	Water buffering capacity (WBC)		0 \pm 0	4 - 10
Chemical	pH		4.9 \pm 0	5.3-6.5
	Electrical Conductivity (EC)	mS m ⁻¹	22.3 \pm 1.7	< 60
	Mineral Nitrogen N		<i>nd</i> ¹	50 - 250
	Phosphorus P		7.7 \pm 0.6	19 - 75
	Potassium K	mg L ⁻¹	167.3 \pm 14.1	51 - 400
	Calcium Ca		8.5 \pm 1.0	16 - 80
	Magnesium Mg		21.8 \pm 1.1	16 - 80
	Sodium Na		38.4 \pm 3.7	< 100
Biological	Root length index (RLI)	%	0 \pm 0	-
	C mineralization rate (CMR)	mmol CO ₂ kg ⁻¹ d ⁻¹	214.6 \pm 44.3	-
	N immobilization rate (NIR)	mmol N kg ⁻¹ d ⁻¹	8.9 \pm 1.2	-

¹*nd* = not detected, below quantification limit (< 2 mg L⁻¹)

²Acceptable range, adapted from Miner (1994) and Noguera et al. (2003)

Very high biological activity (C mineralization couple with N immobilization) was verified in IEB. Measurements took into account the 14th-day incubation and results showed total C mineralization of 3004.4 mmol CO₂ kg⁻¹ and N immobilization of 124.4 mmol N kg⁻¹, corresponding to MR= 214.6 mmol CO₂ kg⁻¹d⁻¹ and IR= 8.9 mmol N kg⁻¹d⁻¹, respectively. Mineral N availability is the most limiting factor in biological evaluation and the appropriate incubation period is needed (Buamscha et al., 2008). Organic materials are generally biologically unstable. Pine bark residues from sawmills were characterized by a high level of microbial activity which led to a lack of N in the substrate (Neumaier and Meinken, 2015), therefore external N inputs are strongly recommended.

In the germination essays, the IEB revealed phytotoxic for cress seeds with total early root growth inhibition (RLI= 0%) when compared to peat (PM) used as the reference (RLI=100%). Previous research underlined that phytotoxicity is a common issue associated with fresh forest biomass mainly caused by phenolic compounds present in fresh wood fiber, already reported in pine based substrates (Caron et al., 2010; Gruda et al., 2009; Murphy et al., 2010), spruce bark (Barrett et al., 2016; Neumaier and Meinken, 2015), and *Pseudotsuga menziesii* bark (Buamscha et al., 2008).

To eliminated the phytotoxicity present in forest residues Gruda et al. (2009) recommended leaching pine tree substrates six times to improve growing medium performance and Barrett et al. (2016) suggested the aging, composting or washing wood fiber materials.

3.2. Experimental design and model fitting

3.2.1. Biological experimental results

Polynomial model fitting was tested for cress seeds root growth, C mineralization and N immobilization rates and Chinese cabbage growth in growing-media containing 25% of HTEBs. The experimental design matrix and the corresponding results for RLI, CMR, NIR, FW, and DW are presented in Table 3.

ANOVA results of fitting experimental data of CMR and NIR models showed that both, linear and quadratic temperatures, were significant ($p \leq 0.05$). While for FW and DW models only quadratic temperatures were significant. Moreover, ANOVA results underline the strong influence of temperature in hydrothermally treated *E. globulus* bark biological properties. Previous works focused in *E. globulus* bark treatments for polyphenolic compounds extraction (Domingues et al., 2013) and delignification processes (Neiva et al., 2016) also pointed out the influence of temperature as the main effect. Heat treatments are reported (Gruda et al., 2009) as the most effective in toxins removal, as well biological stability improvements. CMR and NIR models also presented significant interaction term. All linear and quadratic terms were kept for models accuracy enhancement.

Table 3. Experimental results for cress seeds root length index (RLI), carbon mineralization rate (CMR) and nitrogen immobilization rate (NIR); Chinese cabbage fresh weight (FW) and dry weight (DW) using a pot experiment with volumetric proportion of 25% HTEBs and 75% PM ($v v^{-1}$)

Runs	Dependent variables				
	RLI	CMR	NIR	Pot experiment	
				FW	DW
%	$\text{mmol CO}_2 \text{ kg}^{-1} \text{d}^{-1}$	$\text{mmol N kg}^{-1} \text{d}^{-1}$	mg pot^{-1}		
I	95.9	196.4	6.22	1127	212
II	92.2	143.8	6.02	1269	246
III	85.4	260.6	12.89	1326	237
IV	70.2	427.8	20.85	1194	214
V	67.0	198.0	8.35	1120	196
VI	93.9	608.8	21.07	374	58
VII	78.4	280.2	12.11	1946	371
VIII	90.7	212.3	11.48	806	144
IX	105.1	225.0	9.93	2091	360
X	103.8	170.9	10.34	1543	288
XI	100.9	181.3	11.85	2018	363
XII	96.1	218.4	9.73	2171	364

The experimental data were used to estimate the coefficients of the polynomial second order equations (Table 4), where X_1 and X_2 are the independent variables, temperature and time, respectively.

Table 4. Approximated polynomial equations for root length index (RLI), C mineralization rate (CMR), N immobilization rate (NIR), Chinese cabbage fresh weight (FW) and dry weight (DW) in terms of coded factors (X_1 and X_2 , temperature and time, respectively) and respective coefficients of determination (R^2 and adj R^2)

Polynomial model equations	R^2	R_{adj}^2
$RLI = -4.898 + 1.366X_1 - 0.006X_1^2 + 1.869X_2 + 0.019X_2^2 + 0.004X_1X_2$	0.496	0.076
$CMR = 739.07 - 10.511X_1 + 0.053X_1^2 - 8.107X_2 + 0.017X_2^2 + 0.069X_1X_2$	0.876	0.773
$NIR = 18.033 - 0.189X_1 + 0.001X_1^2 - 0.257X_2 + 0.0006X_2^2 + 0.002X_1X_2$	0.922	0.858
$FW = -2203 + 70.83X_1 - 0.35X_1^2 + 48.15X_2 - 0.62X_2^2 - 0.09X_1X_2$	0.765	0.569
$DW = -346.6 + 12.534X_1 - 0.062X_1^2 + 6.678X_2 - 0.085X_2^2 - 0.018X_1X_2$	0.770	0.578

The coefficient (R^2) and adjusted coefficient of determination (adj R^2) provide an indication of the total variability of response explained by the regression model. RLI model did not fit the data (Table 4) due to poor correlation ($R^2=0.50$), where less than 1% (adj $R^2=0.08$) of cress seeds growth can be associated to independent variables and significant lack of fit (p -value=0.02). Despite no relation with independent variables, for the chosen temperature window, the hydrothermal treatments were effective in phytotoxic compounds removal (Table 3) with RLI ranging from 67-105% in comparison with RLI=0% in IEB (Table 2). The experimental region may be approximated (e.g. lowering temperature window) to fit new RLI results.

Model fitting for CMR and NIR variables were valid for the present work (Table 4). Both models had correlation values close to the unit, CMR presented an $R^2=0.88$ and NIR $R^2=0.92$. Interpretation of adj R^2 indicates that 77% of C mineralization and 86% of N immobilization rates were attributed to hydrothermal (independent) variables studied. The good quality of the CMR and NIR models was also confirmed by the no significant (p -value ≥ 0.05) lack of fit value, 0.05 and 0.08, respectively.

According to experimental conditions, plant FW and DW models were valid (table 4), demonstrating satisfactory correlation, since both R^2 values were greater than 0.75. The lack of fit was no significant (p -value ≥ 0.05) for FW and DW, with p -values=0.25 and 0.11, respectively.

The correlation between predicted and observed values (Fig. 1.) was plotted for CMR (Fig. 1 (a)), NIR (Fig. 1 (b)), FW (Fig. 1 (c)) and DW (Fig. 1 (d)). Graphics from Fig. 1 (a) and (b) express straighter point alignment near 45° line, since they represent good CMR and NIR model accuracy. Fig. 1 (c) and (d) show reasonable point dispersion within graphics area, supporting that prediction for FW and DW models are reliable.

Plots visual interpretation suggests a similar behavior for CMR (Fig. 1 (a)) and NIR (Fig. 1 (b)) results with higher concentration of points in the lower graphical quadrant. According to Buamscha et al. (2008), biological activity promotes simultaneous release of CO₂ and proportional capacity to immobilize mineral N. Plants FW (Fig. 1 (c)) and DW (Fig. 1 (d)) values are practically coincident, due to subsequent nature of the measurements.

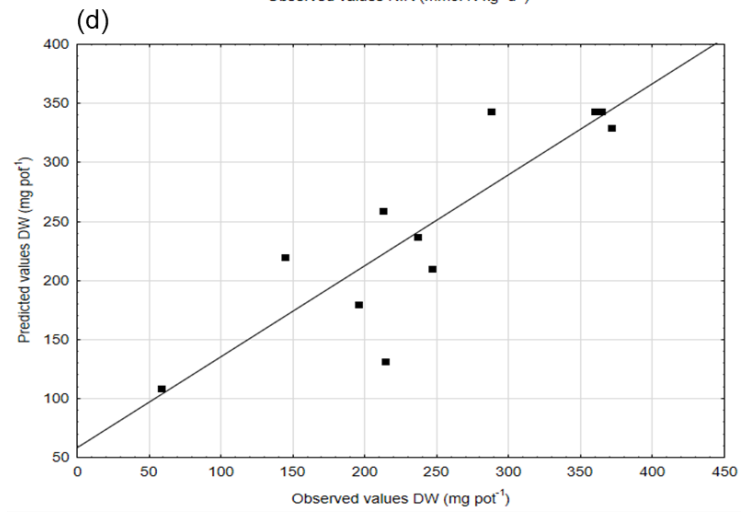
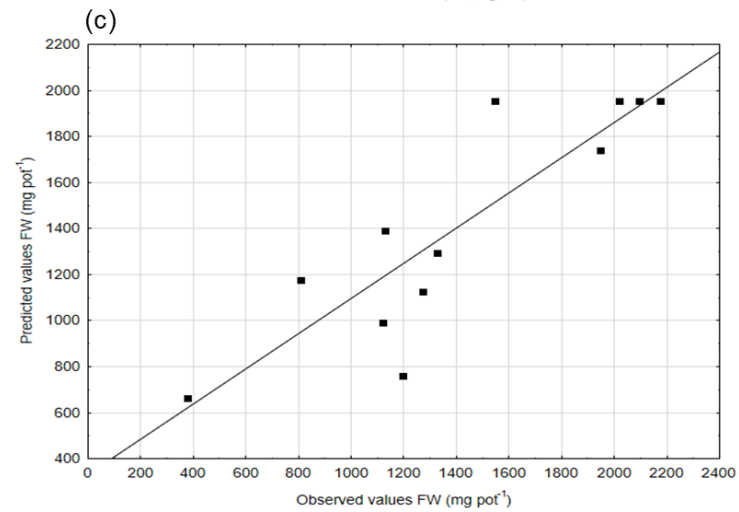
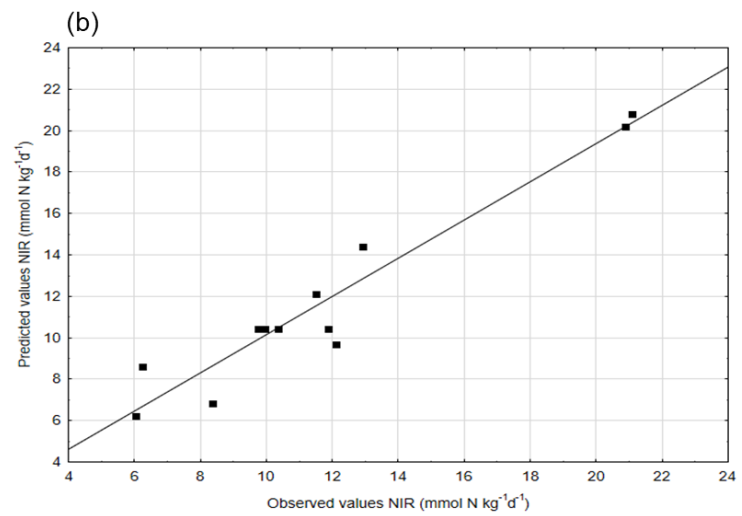
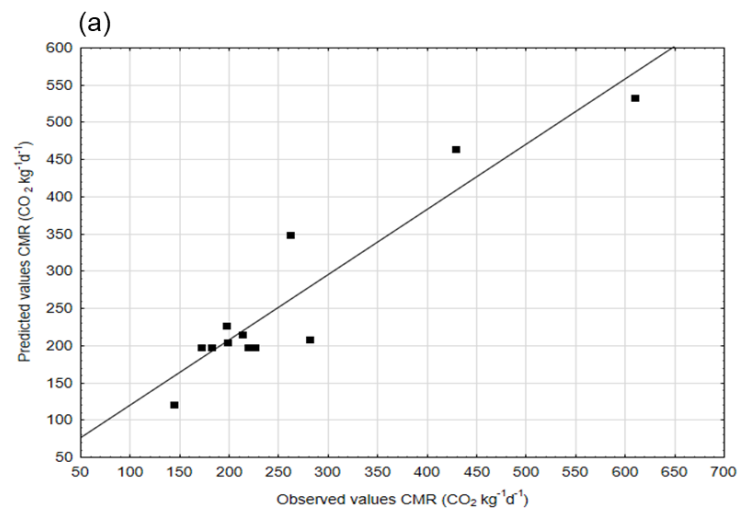


Figure 1. Observed and predicted model values of **(a)** C mineralization rate (CMR), **(b)** N immobilization rate (NIR), **(c)** Chinese cabbage fresh weight (FW), and **(d)** Chinese cabbage dry weight (DW).

3.2.2. Physical and chemical experimental results

Both studied factors, temperature and time (linear and quadratic), did not affect physical and chemical response variables. Results are confirmed by no significant ($p \leq 0.05$) ANOVA outcome (data not shown). In addition, very low correlation (R^2 less than 0.40 and adj R^2 near zero) was found among all physical and chemical experimental and predicted results.

The HTEBs presented physical properties (Table 5) similar to the untreated industrial eucalyptus bark (IEB, Table 2): high porosity (TP around 96%); high aeration (AFP ranging from 74 to 86%); and low water availability (EAW from 1.4 to 4.5% and WBC less than 0.3%). HTEBs had an average pH of 5.4, within the recommended range for most of the substrates (Miner, 1994), and a very low EC, when compared to IEB (Table 2). EC values were less than 7.2 mS m^{-1} , suggesting that soluble salts and nutrients were most likely leached after treatments.

Table 5. Experimental results for physical and chemical parameters: total porosity (TP) air-filled porosity (AFP), easy available water (EAW), water buffering capacity (WBC), pH, electrical conductivity (EC)

Response variables	Range	Average	S.D.
TP (% v v ⁻¹)	95.8-96.8	96.3	0.3
AFP (% v v ⁻¹)	74.1-85.6	81.5	3.0
EAW (% v v ⁻¹)	1.4-4.5	2.3	0.8
WBC (% v v ⁻¹)	0-0.3	0.15	0.1
pH	4.7-5.9	5.4	0.3
EC (mS m ⁻¹)	5.6-7.2	6.1	4.3

3.3. Response Surface Analysis

Fig. 2 show the response surface (3D) plots that illustrate the relationship between hydrothermal (independent) and biological bark responses (dependent) variables. As noticed in Fig. 2 (a) and (b), same graphical trend is repeated for CMR and NIR surfaces. Greater values were found with increased temperature

and time (darker color), where the factorial IV and star VI runs drawn curvature for maximal response. Extreme hydrothermal conditions, focused on higher temperatures ($T > 140^{\circ} \text{C}$), may lead to aggressive bark structural rupture promoting material decomposition via micro-organisms need for mineral N. At the same time, high extraction temperature may release triterpenic compounds, fatty acids, fatty alcohols and aromatic compounds (Domingues et al., 2013; Neiva et al., 2016). Response surface plots (Fig 2) allowed to visualize the lower biological rates (lighter color) in a window temperature between 40° to 80°C and time ranging from 40 to 80 minutes for CMR (Fig 2 (a)), and similar NIR (Fig 2 (b)) window.

In accordance with Table 3, the central runs (IX to XII) revealed the maximum response for FW and DW that were responsible for the critical maximum of curvatures (darker color around the center) in Fig. 2 (c) and (d), respectively.

In order to produce a substrate with minimum biological activity, hydrothermally treated bark surface responses for CMR and NIR Fig. 2 (a) and (b) were combined and near-optimal operating conditions were found at $T=40^{\circ}\text{C}$ and $t=70$ min. This could provide economical and energy savings while avoiding the longest time and highest treatment temperature.

3.4. Expected outcomes

Hydrothermal treatment can be modeled ($T=40^{\circ} \text{C}$ and $t=70\text{min}$) to reduce microbial activity expressed in the lowest CMR ($111.8 \text{ mmol CO}_2 \text{ kg}^{-1}\text{d}^{-1}$) and NIR ($4.1 \text{ mmol N kg}^{-1}\text{d}^{-1}$) compared to IEB ($214.6 \text{ mmol CO}_2 \text{ kg}^{-1}\text{d}^{-1}$ and $8.9 \text{ N kg}^{-1}\text{d}^{-1}$, respectively). Fertilization should be applied before the final use of present wood fiber material (Barrett et al., 2016; Gruda, 2012a).

Despite the lack of significance of the model fitting, after hydrothermal treatments, RLI may drastically increase from 0% (Table 2) in IEB to greater than 100% (Table 4). Leaching and/or washing organic media to mitigate the effect of phytotoxins are widely recommended methodologies (Barrett et al., 2016; Gruda et al., 2009).

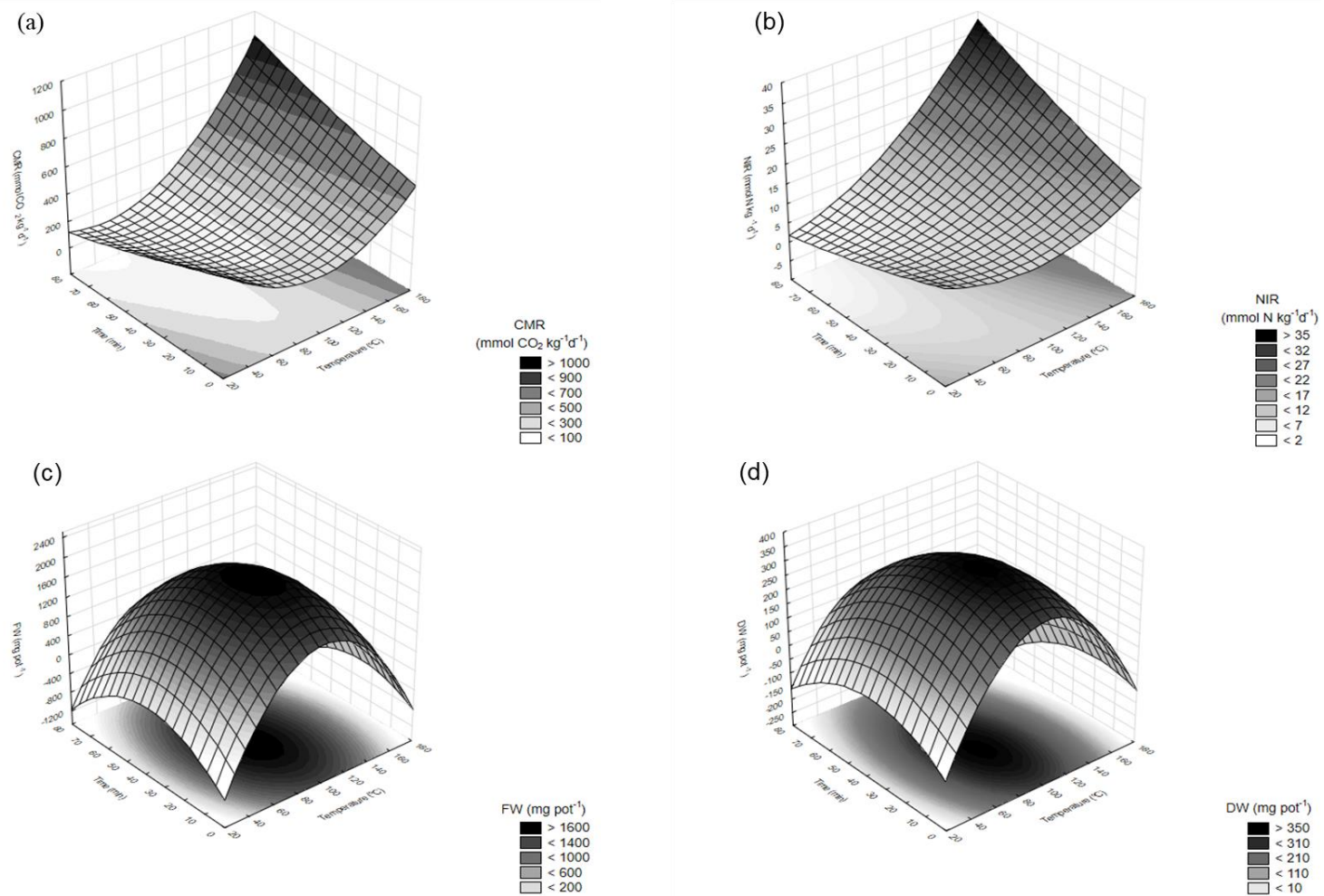


Figure 2. 3D surface plot of **(a)** C mineralization rate (CMR), **(b)** N immobilization rate (NIR), **(c)** Chinese cabbage fresh weight (FW), and **(d)** Chinese cabbage dry weight (DW)

After treatments, pH value might increase from 4.9 (Table 2) in IEB to a maximum of 5.7 (Table 5) achieving an acceptable value for substrate use (Miner, 1994). Treatments did not show relevant changes in nutritional bark content. The use of demineralized water as a main reagent may leach soluble salts and macronutrients, resulting in low EC and residual concentration of macronutrients (P, K, Ca, Mg and Na).

Regarding physical properties, AFP, TP, and water availability (EAW and WBC) did not vary between treatments, maintaining bark high air content ($>74\% \text{ v v}^{-1}$) as well as TP ($> 96\% \text{ v v}^{-1}$) (Table 5). These results may suggest that *E. globulus* bark fiber can be used as a component for aeration improvement of peat-based substrates.

Previous studies with wood-based substrates showed that organic media are normally added to commercial peat at proportions up to 25% (v v^{-1}) (Barrett et al., 2016; Depardieu et al., 2016; Jackson et al., 2010). Within the experimental conditions, the lowest plant growth (FW of 374 mg pot^{-1} and DW of 58 mg pot^{-1}) (Table 3) was observed in growing-media containing HTEB submitted to the highest temperature (run VI; $T = 157^\circ \text{ C}$). This result may be a consequence of greater N deficiency that promotes plant retardation (Depardieu et al., 2016). However, positive plant performances can be achieved by remaining HTEBs growing media.

4. Conclusions

Hydrothermal treatments were effective regarding phytotoxicity removal from IEB. Regarding experimental design used, temperature effect was significant for both bark biological responses. The lowest C mineralization and N immobilization rates were found by modeling theoretical optimal hydrothermal treatment at $T=40^\circ \text{ C}$ and $t=70\text{min}$. After hydrothermal treatments, bark maintains a very high air content that can be a plus in aeration improvement when added to commercial peat-based substrates. HTEB may be suitable as an alternative substrate component, encouraging up to 25% (in volume) peat substitution.

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Supplementary material captions

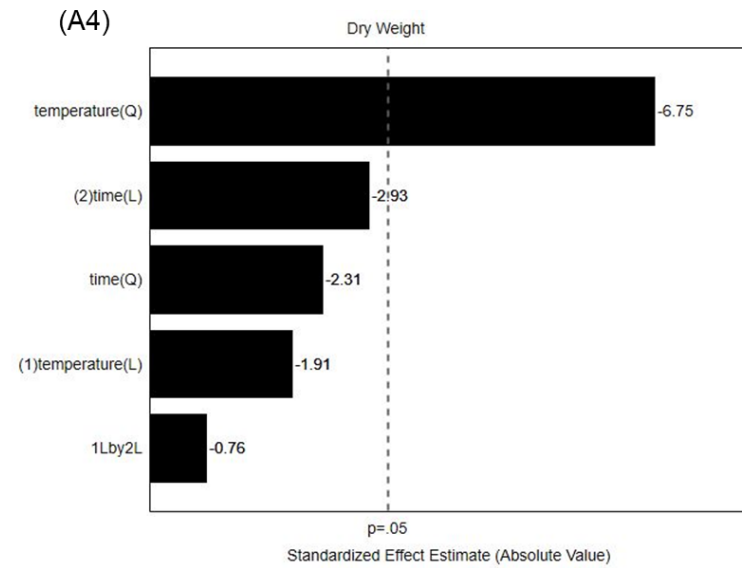
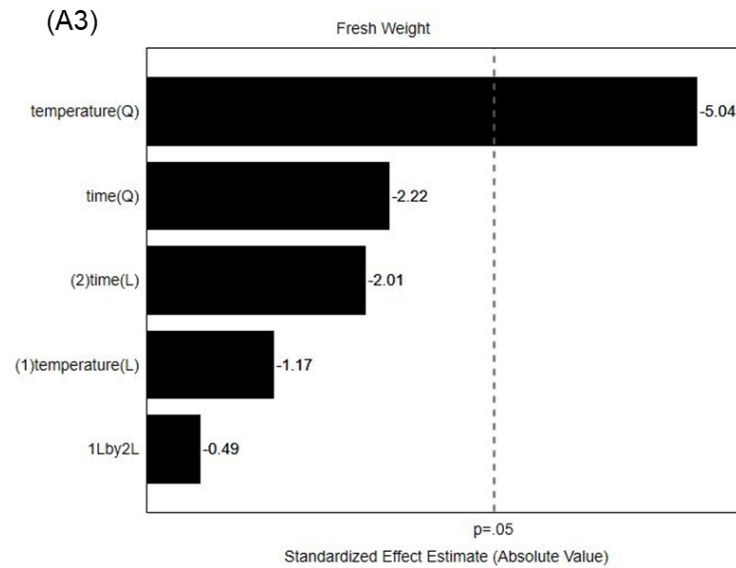
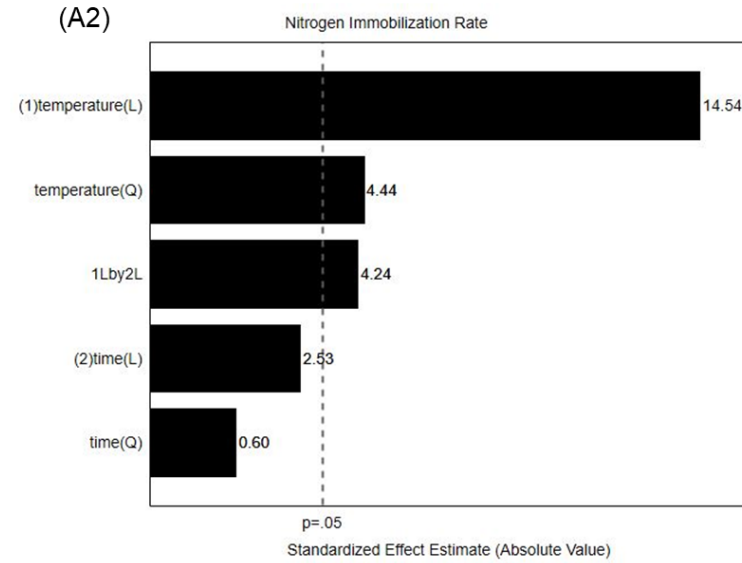
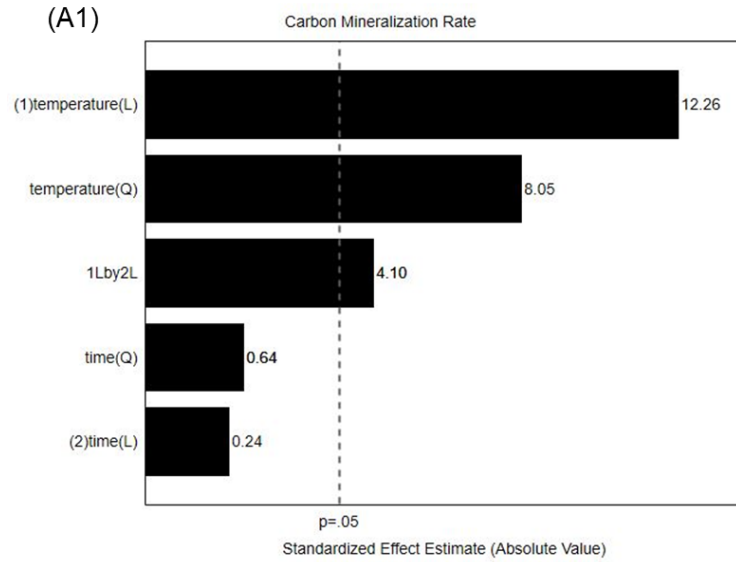
Figure (A.1) Pareto chart of standardized effects for C mineralization rate model

Figure (A.2) Pareto chart of standardized effects for N immobilization rate model

Figure (A.3) Pareto chart of standardized effects for Chinese cabbage fresh weight model

Figure (A.4) Pareto chart of standardized effects for Chinese cabbage dry weight model

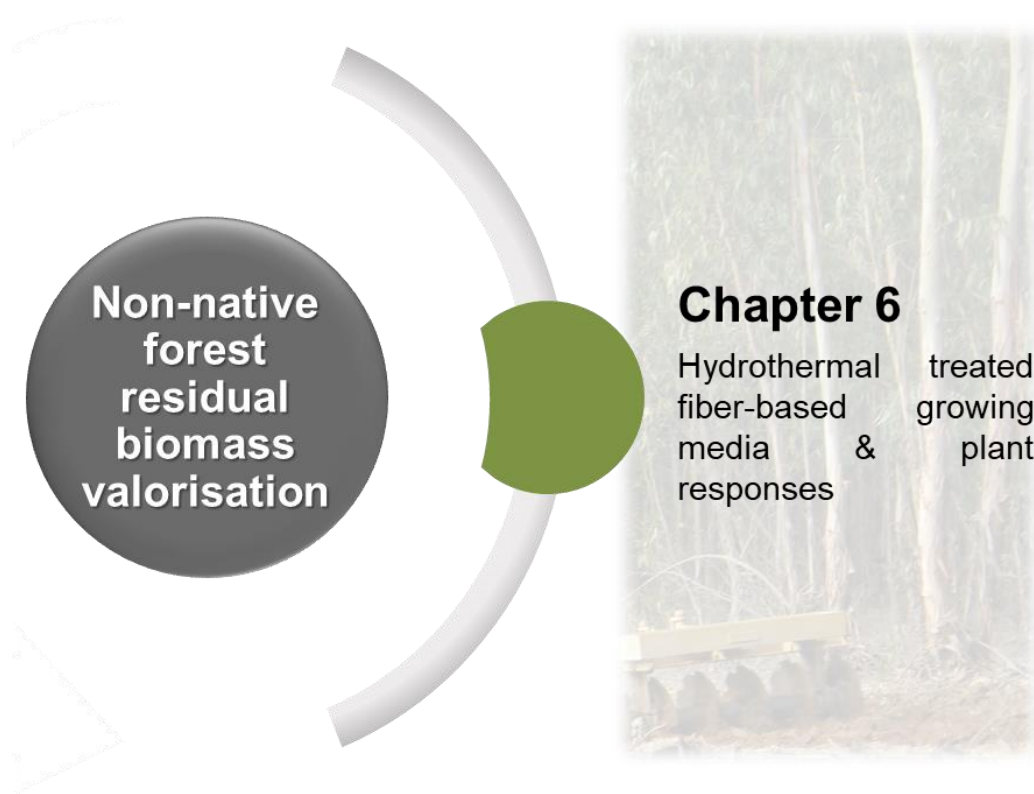
Supplementary material



CHAPTER 6

Evaluation of low-temperature hydrothermal treated *Eucalyptus globulus* bark as fiber component for horticultural growing media

E. globulus waste-stream



This chapter was prepared to be submitted and published.

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Evaluation of low-temperature hydrothermal treated *Eucalyptus globulus* bark as fiber component for horticultural growing media

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Abstract

Worldwide, the circular economy approach increased the need of waste-streams minimization, promoting by-products re-circulation into the value chain which creates sustainable industrial synergies. *Eucalyptus globulus* bark fiber is a waste from pulp and paper industry that can be re-used in horticultural applications. This work aims to use low-temperature hydrothermal treated *E. globulus* bark as a fiber material for growing media formulation. Three types of bark fiber were used: industrial *E. globulus* fresh bark (IEB) milled to 6 x 6 mm² particle size, and two low-temperature hydrothermally treated barks (HTEB1: 20' 60 °C; HTEB2: 40' 100 °C). The three fiber materials were blended at 25 and 50% (v v⁻¹) (B25; B50) with peat. IEB was phytotoxic for *Lepidum sativum* seeds, causing low germination (91%) and root growth inhibition. HTEB1 and HTEB2 reduced significantly phytotoxicity with germination rates of 98 and 100%, and Munoo Liisa index around 90% compared to commercial substrate. Pot experiment, using Chinese cabbage as a model plant, revealed lower germination (95%) in IEB blends than in treated ones, and commercial substrate (CS) (98-100%), reinforcing the IEB phytotoxic. B50 decreased plant growth, maybe due to lower water retention, as well as nitrogen immobilization inherent to woody biomass. B25 showed shoot weight, and root growth statistically equal or higher than CS, encouraging use of this blending proportion of low-temperature hydrothermally treated bark in future growing media formulation.

Keywords: Woody biomass, fiber, processed bark, phytotoxicity, substrate blend

List of abbreviations (Chapter 6)

AFP	Air-filled porosity
ANOVA	Analysis of variances
B	Boron
B25	Fiber blended at 25% (v v-1) with peat
B50	Fiber blended at 50% (v v-1) with peat
BD	Bulk density
Ca	Calcium
CS	Commercial substrate
Cu	Copper
DAW	Difficult available water
DM	Dry mass
DW	Dry weight
EAW	Easily available water
EC	Electrical conductivity
Fe	Iron
FW	Fresh weight
GR	Germination rate
H₂PO₄⁻	Dihydrogen phosphate
HTEB	low-temperature hydrothermally treated bark
HTEB1	low-temperature hydrothermally treated bark (20' 60 °C)
HTEB2	low-temperature hydrothermally treated bark (40' 100 °C)
IEB	Industrial Eucalyptus globulus fresh bark
K	Potassium
LSD	Least significant difference test
Mg	Magnesium
MLV	Munoo Lisa Vitality Index
Mn	Manganese
Mo	Molybdenum
Na	Sodium
NH₄⁺-N	Ammonium-Nitrogen
NIR	N immobilization rate

NO₃-N	Nitrate-Nitrogen
P	Phosphorous
RL	Root length
RR	Respiration rates
SO₄²⁻	Sulfate
TP	Total porosity
WBC	Water-buffering capacity
Zn	Zink

1. Introduction

Currently, the challenges to feed the world in 2050 should be aligned with technological innovation in a system where producing more with less inputs, resources efficiency and zero waste are the driving forces to achieve sustainable development goals (FAO, 2015a). The industrial operations should play a crucial role in building their activities towards a circular economy approach (Gruda, 2019); waste-streams must be converted into opportunities to create value, which potentiates micro-level synergies, e.g. between horticultural and pulp and paper industries.

In Iberian countries, *Eucalyptus globulus* is the predominant species for pulp and paper production (Neiva et al., 2014). In 2017, European pulp and paper industry used 149 M m³ of wood, where 13 M m³ belonged to *E. globulus* wood (CELPA, 2017). In bleached pulp production bark remains excess fiber, and 7 to 20% of *E. globulus* stem's dry weight is bark (Neiva et al., 2014). Consequently, total *E. globulus* bark surplus resulted in 0.91 to 2.6 M m³ y⁻¹. As industrial by-product it is normally burned for energy production in power plants within industrial facilities (Neiva et al., 2016), remaining a low added value product application. Alternative bark valorization has been investigated, such as: raw-material in the pulping process (Neiva et al., 2016), natural textile dyeing (Rossi et al., 2017), source of biological and pharmacological compounds (Domingues et al., 2013), and soil amendment (Murphy et al., 2010). Due to its fibrous nature, *E. globulus* bark physical properties may suggest that *E. globulus* bark can be used as a

component for aeration improvement of horticulture growing media (Chemetova et al., 2018). However, limited research documents the physical, chemical and biological properties of *E. globulus* bark-based growing media for horticultural use.

Horticultural industry grows along with world population, and growing media demand is predicted to increase by four times in next 30 years, although, due to increased environmental awareness of peatlands conservation, by 2050 peat extraction will be prohibited by European regulation, thus peat moss mining will end (FAO, 2015b). Indeed, peat is a limited resource, and the extraction of peat bogs causes negative impacts on environment, decreasing the C sink in peat bogs and releasing greenhouse gases into the atmosphere through degradation and oxidation of the unsaturated peat layer (Barrett et al., 2016). Intensive research aiming new peat alternative materials in horticultural industry has been done (Carlile et al., 2019; Chemetova et al., 2019; Gruda et al., 2009; Ribeiro et al., 2009). Wood-based fibers from forest harvest residues and wood processing industries by-products have been studied as growing media components (Caron and Michel, 2017).

The physical properties of organic growing medium are the main characteristics for successful plant growth due to their influence in container media ability to store and adequately supply air and water (Wallach, 2019). Wood fiber materials are generally regarded as presenting very high air-filled porosity and low bulk density; when blended, fibers improve aeration and reduce shrinkage of peat-based substrates (Buamscha et al., 2008). Despite the good physical performances, phytotoxicity is a common issue of woody biomass caused by natural chemical barriers (Chemetova et al., 2019). In the natural environment, the role of these chemical compounds has a protection effect against diseases or infections of native plants, although they also act as toxins for other cultivations in growing media applications (Gruda, 2012). Secondary process treatments are required to eliminate toxic compounds and promote stability before wood material effective use, and they are broadly depending on raw material, such as: ageing *Pseudotsuga menziesii* bark (Buamscha et al., 2008) and *Acacia melanoxylon* bark (Chemetova et al., 2019), composting pine bark (Jackson et al., 2010) and

whole tree *Acacia* (Brito et al., 2013), and washing/leaching pine tree based substrate (Gruda et al., 2009).

Chemically, *E. globulus* bark is rich in phenolic, triterpenic and other inhibitory compounds (Domingues et al., 2013; Neiva et al., 2016, 2014), being toxic for plants (Chemetova et al., 2018). Chemetova et al. (2018) proposed low-temperature hydrothermal treatment to reduce toxicity and microbial activity in *E. globulus* bark, making this treated bark a potential component for horticulture growing media. In addition, low-temperature hydrothermal treatment is attractive due to its simplicity, low construction material cost requirement, rapid implementation with null material corrosion and chemical free nature.

The objective of this research is to study two low-temperature hydrothermal treated *E. globulus* barks, in comparison with fresh untreated bark, evaluating (i) physical, chemical and biological properties of bark-based growing media and, (ii) plant performance in bark-based growing media contrasting to settled commercial substrate.

2. Materials and Methods

2.1. Raw material and treatment selection

Fresh industrial *E. globulus* bark (IEB) was collected from The Navigator Company pulp mill (Setúbal, Portugal) in November 2015, and grinded in a knife mill with an output sieve size of 6 x 6 mm².

Based on previous tests (Chemetova et al., 2018), two low-temperature hydrothermal treatments were used (HTB1: 60 °C for 20'; HTB2: 100 °C for 40'). The HTB1 and HTB2 were performed in autoclave-reactor conditions placing 5 individual hermetic vessels (1 L) containing 90 g (10% moisture content) of bark and 900 ml water each. Excess water of treated material was removed by centrifugation. All experiments were performed in randomized order to minimize uncontrolled factors.

2.2. Growing media formulation

IEB, and HTB1 and HTB2 were blended with peat moss slightly decomposed (H2-H5 on Von post scale) amended with 4 g L⁻¹ of dolomitic lime to adjust the pH (5.6-5.8), in volumetric proportion of 25 and 50% (bark/peat) (B25; B50). All substrates blends were fertilized with 15 mmol NO₃-N L⁻¹, 8 mmol K L⁻¹, 4 mmol Ca L⁻¹, 1.5 mmol Mg L⁻¹, 1.25 mmol Sulfate (SO₄²⁻) L⁻¹, 1.5 mmol Dihydrogen phosphate (H₂PO₄⁻) L⁻¹, 15 μmol Iron (Fe) L⁻¹, 8 μmol Manganese (Mn) L⁻¹, 4 μmol Zink (Zn) L⁻¹, 25 μmol Boron (B) L⁻¹, 0.75 μmol Copper (Cu) L⁻¹, 0.5 μmol Molybdenum (Mo) L⁻¹.

2.3. Physical, chemical and biological properties

The pH, EC, and water-soluble Potassium (K), Phosphorous (P), Calcium (Ca), Magnesium (Mg), Sodium (Na) and mineral N i.e. Ammonium (NH₄⁺-N) and Nitrate (NO₃⁻-N), were measured in water extract 1:5 by volume, according to the European Standards (CEN, 2001a, 1999a, 1999b). The dry mass (DM) was assessed by oven-drying bark at 105 °C for 24 hours and the ash content was determined by combustion of the oven-dried sample at 550 °C for 5 hours in a muffle furnace. The difference between DM and ash was considered the organic matter content. The incubation experiment (14 days) for N immobilization and respiration rates (NIR and RR) measurements (Buamscha et al., 2008; Fangueiro et al., 2012), were performed according to Chemetova et al. (2018).

The physical properties as defined by Wallash (2019), were determined according to European Standards (CEN, 2001b): total porosity (TP), bulk density (BD), easily available water (EAW) as the difference between the water content at suctions of 1 and 5 kPa, water-buffering capacity (WBC) as the difference between the water content at 5 and 10 kPa, air-filled porosity (AFP) as the amount of air at a suction of 1 kPa, and shrinkage.

2.4. Plant response: petri dish and pot experiment

According to the European Standards (CEN, 2011), a total of 10 cress (*Lepidium sativum*) seeds were incubated in Petri dishes filled with samples, at room temperature (25 °C) in the dark, for 3 days. The experiment was carried out in triplicate (1 to 3) and using commercial substrate as control (C). Phytotoxicity was evaluated based on the average germination rate (GR, Eq.1), and root length (RL) to calculate Munoo Lisa Vitality Index (MLV; Eq. 2) using RL and GR of triplicates and control.

$$\text{Germination rate (\%)} = \frac{(GR_1 + GR_2 + GR_3)}{3} \times 100 \quad (1)$$

$$\text{Munoo Liisa Vitality Index (\%)} = \frac{(GR_1 \times RL_1 + GR_2 \times RL_2 + GR_3 \times RL_3)}{3 \times GR_C \times RL_C} \times 100 \quad (2)$$

Pots with a volume of 290 mL ($\emptyset = 8.5$ cm; height = 8.2 cm) were filled with substrates blends and a total of 10 Chinese cabbage (*Brassica napa* subsp. *pekinensis*) seeds per pot were sown and grown for 5 weeks. During the entire experiment time range, all pots were placed in an unheated glass greenhouse, distributed in a complete randomized design. Pots were daily irrigated with deionized water, in order to avoid the effect of different compounds usually present in tap water. The experiment was carried out in triplicate (1 to 3) and using CS as control. At the end of the growing period, sample fresh weight (FW), dry weight (DW, at 65 °C for 48 h), germination rate and root visual rating (1= the worst; 5=the best) were calculated and recorded.

2.5. Statistics

Data were subject to analysis of variances (ANOVA), followed by least significant difference test (LSD) based on the p-value with 95% of confidence level ($p \leq 0.05$). Data were analyzed using Statistica ® 10.0 (StatSoft, USA).

3. Results and discussion

3.1. *E. globulus* bark fiber properties

Fresh bark was phytotoxic for cress seeds (Figure 1), causing low germination rate (GR) and root growth inhibition with MLV of 2% (equivalent to 0.1 cm of root length). The hydrothermal treatment of barks reduced bark toxicity, with GR of 98.3 and 100%, and MLV of 89 and 93%, in HTB1 and HTB2, respectively. The HTB1 and HTB2 statistically equal results to CS may indicate the efficient removal of toxic elements on both treatments. Research on aqueous washing of pine bark (Gruda et al., 2009) also recorded reduction of toxins levels, associated with decline of resin acids, fatty acids and phenols contents. *E. globulus* bark chemical composition had demonstrated high extractable inhibitory compounds (Neiva et al., 2016) and GR and MLV of fresh bark fiber (Figure 1) are in accordance with previous phytotoxicity findings (Chemetova et al., 2018). Thus, as strongly recommended for other wood-based fibers (Brito et al., 2013; Buamscha et al., 2008; Gruda, 2012; Jackson et al., 2010), *E. globulus* bark must be treated before use as growing media.

All fiber materials were biologically unstable in contrast with peat which showed a very low microbial activity (Table 1). After 14 days incubation, higher N immobilization (NIR) and respiration rates (RR) were measured in IEB and HTB2, followed by HTB1. Although treatments removed part of organic material, maximum NIR in HTB2 ($0.9 \text{ mmol N L}^{-1} \text{ d}^{-1}$) might be explained by fiber structural fragmentation, associated to higher treatment temperatures, that increased cellulose digestibility (Neiva et al., 2016) and sturdily promoted microorganism activity (Depardieu et al., 2016). The high amount of N consumption by microorganism in wood fiber materials have been broadly reported (Carlile et al., 2019; Chemetova et al., 2018; Gruda, 2019). Biological activity promotes simultaneous release of CO_2 and proportional capacity to immobilize N, and if there is a microbial need for N, it may occur soon after potting and therefore N fertilization should be applied before pot planting (Buamscha et al., 2008).

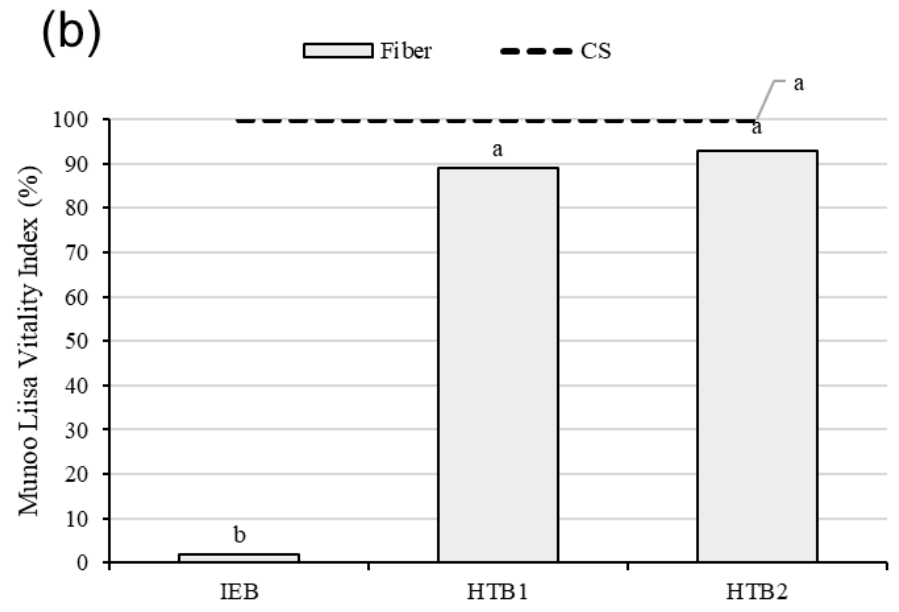
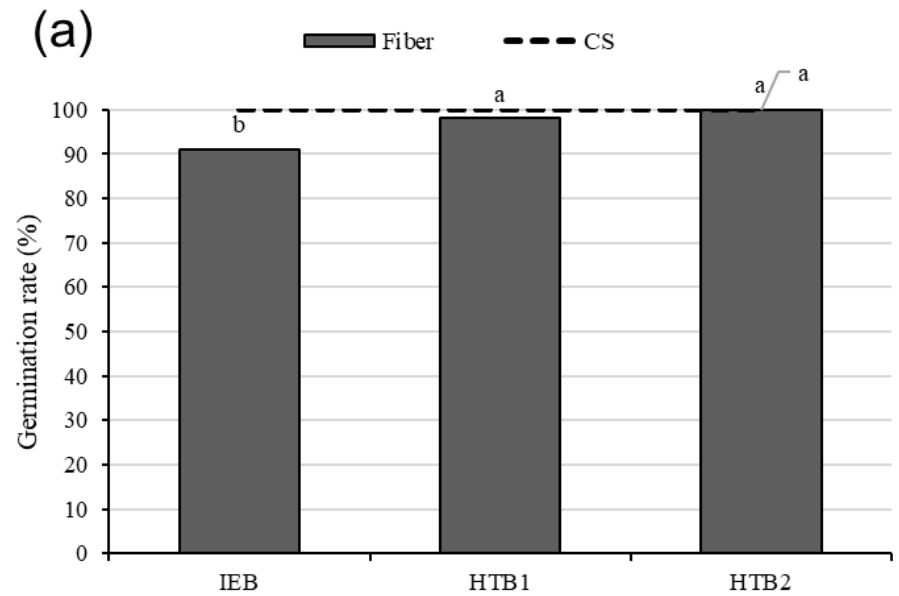


Figure 1. Phytotoxicity evaluation of fiber material industrial fresh bark (IEB), both low-temperature hydrothermal treated barks (HTEB1, HTEB2) and commercial substrate (CS): **(a)** germination rate and **(b)** Munoo-Lisa Vitality Index.

Table 1. Chemical properties of fiber barks and peat moss: nitrogen immobilization rate (NIR), respiration rate (RR), pH, electrical conductivity (EC), mineral nitrogen (N_{\min}), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na). Means followed by the same letter, in column, do not differ at $P \leq 0.05$ by the LSD-test. n.d. = not detected, below quantification limit ($< 2 \text{ mg L}^{-1}$)

Raw-material	NIR $\text{mmol N L}^{-1} \text{ d}^{-1}$	RR $\text{mmol CO}_2 \text{ L}^{-1} \text{ d}^{-1}$	pH	EC mS m^{-1}	N_{\min}	P	K mg L^{-1}	Ca	Mg	Na
IEB	0.80 a	15.4 a	4.9 c	22 a	n.d.	8	167 a	9 a	22 a	38 a
HTB1	0.63 b	14.1 a	5.8 a	6 b	n.d.	n.d.	32 b	3 a	5 b	3 b
HTB2	0.90 a	15.7 a	5.5 b	6 b	n.d.	n.d.	27 b	3 a	4 b	3 b
Peat	0.00 c	1.3 b	4.0 d	5 b	n.d.	n.d.	6 b	3 a	1 c	3 b

Acidic pH of 4.0 was verified in peat moss (before liming), followed by IEB with 4.9, and treatments significantly increased bark pH (Table 1). No mineral nitrogen was detected in bark fibers nor in peat (levels below the quantification limit of 2 mg L^{-1}), and IEB had higher levels of water-soluble P, K, Ca, Mg and Na. The use of demineralized water in the low-temperature hydrothermal treatments may have leached the soluble elements, decreasing their concentration in HTB1 and HTB2, consequently reducing the EC. However, all materials had low EC values and low levels of available nutrients, except for K and Mg. It is noticeable that peat, HTB1 and HTB2 showed statistically equal chemical composition regarding water-soluble nutrients.

Air-water relationships are shown in Figure 2 and Table 2. The treatments did not affect fiber physical properties, with all barks presenting low bulk density (BD) and water availability, very high total porosity (TP) and aeration (AFP) greater than 80% (v v^{-1}). On the contrary, peat had low AFP ($8\% \text{ v v}^{-1}$) and high water availability. Wood fibers relative lightweight and very high air capacity have been recognized for good drainability and aeration improvement in peat-based substrates (Caron and Michel, 2017). Similar shrinkage was observed in all bark fibers.

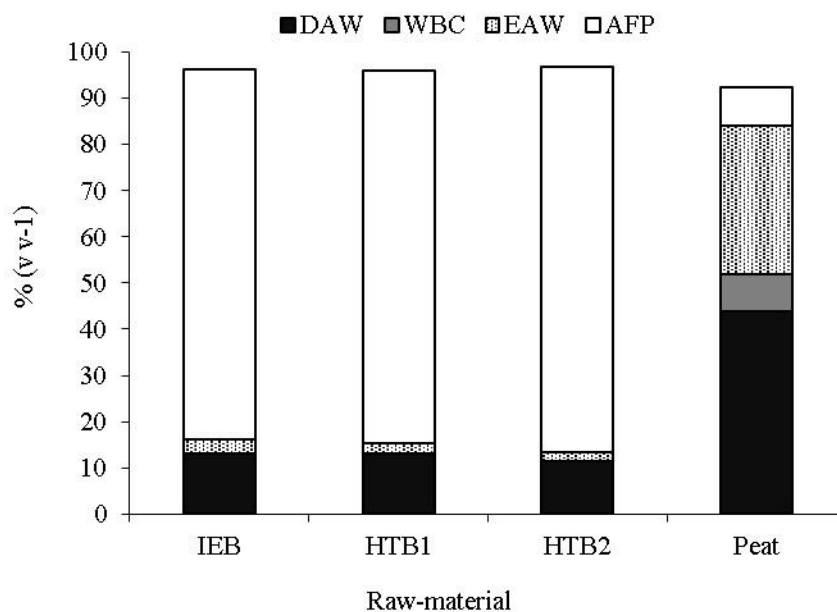


Figure 2. Raw-materials physical properties, air-filled porosity (AFP), easy available water (EAW), water buffering capacity (WBC), and difficult available water (DAW)

Table 2. Eucalyptus barks and peat physical properties: bulk density (BD), total porosity (TP) and shrinkage. Means followed by the same letter, in column, do not differ at $P \leq 0.05$ by the LSD-test

Raw- material	BD	TP	Shrinkage
	g L^{-1}	$\% (\text{v v}^{-1})$	
IEB	58.0 c	96.3 a	8.2 b
HTB1	64.7 b	95.9 a	11.2 b
HTB2	52.3 d	96.7 a	8.1 b
Peat	119.8 a	92.4 b	34.9 a

3.2. *E. globulus* bark fiber-based growing media properties

All bark-based growing media were fertilized with a complete nutrient solution (section 2.2), and with an extra amount of 100 mg N L^{-1} (total 200 mg N L^{-1}) compared to CS (100 mg L^{-1}), to compensate potential bark mineral N competition documented in previous work (Chemetova et al., 2018) and confirmed in Table 1. All substrates pH values were within recommended range

(5.3-6.5) and EC values were lower than the threshold value of 60 mS m⁻¹ (Table 3). The fiber-based blends nutrient content was within or slightly higher than the recommended range for a growing media (Barrett et al., 2016), consequently no limitations to plant growth caused by nutrient deficit were expected.

Table 3. Bark fiber-based blends and commercial substrate pH, electrical conductivity (EC), and nutrients: mineral nitrogen (N_{min}), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na). Means followed by the same letter, in column, do not differ at P ≤ 0.05 by the LSD-test. Acceptable range adapted from Barret et al (2016).

Substrate	Bark type	pH	EC mS m ⁻¹	N _{min}	P	mg L ⁻¹			
						K	Ca	Mg	Na
B25	IEB	5.7 a	59 a	193 a	50 a	313 ab	154 c	61 a	22 c
	HTB1	5.6 a	56 ab	203 a	45 a	257 c	164 bc	58 a	12 d
	HTB2	5.7 a	58 ab	197 a	39 a	268 c	155 c	65 a	12 d
B50	IEB	5.7 a	58 a	196 a	34 a	336 a	167 bc	69 a	32 b
	HTB1	5.7 a	53 b	209 a	35 a	279 bc	172 bc	62 a	12 d
	HTB2	5.7 a	53 b	203 a	36 a	282 bc	192 b	64a	11 d
CS	-	5.9 a	37 c	94 b	26 a	181 d	264 a	22 b	40 a
Acceptable range	-	5.5-6.5	< 50	50-250	19-75	51-400	16-80	16-80	<100

Bark addition to peat had a significant effect on substrates physical properties (Table 4). Bark increased total porosity and improved peat aeration. AFP raised from 8.3 % v v⁻¹ in peat (Table 4) to an average of 26.9% in B25, and 46.1% in B50 blends. Following an inverse trend, gradual bark addition decreased water availability. Shrinkage was also reduced by bark increment and tended to meet previous results from Table 2. Shrinkage is often related to hydrophobic effects caused by drying and it is a problem mainly for outside plant production. In these cases, due to channeling, irrigation water drains very fast through the cracks or the void between container wall and the substrate (Caron and Michel, 2017). Concerning standard range for substrate physical properties, B25 blends fitted in recommended values. Generally, it is assumed that high AFP promote air supply to the roots but can compromise water availability (Jackson et al., 2010), but with addition of 25% of bark, aeration may improve while water availability remains adequate (Table 4). Gruda et al. (2013) pointed out the relevance of higher

irrigation frequency when wood fibers are used as a component of growing media to maintain container water content.

Table 4. Bark fiber-based blends physical properties: bulk density (BD), total porosity (TP), air-filled porosity (AFP), easy available water (EAW), water buffering capacity (WBC), available water (AW) and shrinkage. Means followed by the same letter, in column, do not differ at $P \leq 0.05$ by the LSD-test. Acceptable range adapted from Caron and Michel (2017)

Substrate	Bark type	BD	TP	AFP	EAW	WBC	AW	Shrinkage
		g L ⁻¹			% (v v ⁻¹)			
B25	IEB	109.3 b	93.3 b	28.0 bc	23.1 b	5.1 bc	28.2 b	24.1 bc
	HTB1	110.2 b	93.2 b	29.1 b	23.4 b	5.1 bc	28.5 b	26.5 b
	HTB2	113.6 b	93.0 b	23.7 c	26.0 b	5.2 b	31.2 b	25.6 b
B50	IEB	96.8 c	94.0 a	45.1 a	16.4 c	4.3 bc	20.7 c	19.4 cd
	HTB1	94.8 c	94.1 a	46.1 a	16.3 c	3.6 c	20.0 c	15.5 d
	HTB2	92.9 c	94.3 a	47.1 a	15.8 c	4.2 bc	20.0 c	17.9 d
CS	-	139.1 a	91.5 c	10.5 d	30.9 a	8.3 a	39.2 a	39.7 a
Acceptable Range		< 400	> 85	10 - 30	20 - 30	4 - 10	24 - 40	< 30

3.3. Plant response

3.3.1. Petri dish test using cress

Figure 3 (a) and (b) shows the results of the cress seed germination rate and Munoo-Liisa vitality index, using the commercial substrate as control. Equal GR (100%) was recorded for all substrates, but there were significant differences concerning MLVI. Increasing the percentage of bark in the mixture led to MLVI reduction, with lower values in B50 substrates. In B50 substrates, IEB presented the lowest MLV (73.8%), against 80.3% in HTB1 and 88.5% in HTB2, underlining the positive effect of treatments on phytotoxicity reduction. B25 substrates showed high MLV values (>90%), suggesting that mixing 25% of bark with peat-based growing media may present favorable results for further consideration in substrates formulation, which is in accordance with previous determination (Chemetova et al., 2018). The percentage of bark in a mixture could influence the extent of phytotoxicity and generally recommended peat substitution by wood

fiber materials is up to 30% (v v⁻¹) (Barrett et al., 2016; Brito et al., 2013; Gruda, 2012).

3.3.2. Pot growth test with Chinese cabbage

Potting test, using Chinese cabbage (Table 5), revealed lower germination rate (95%) in IEB blends than in HTB1, HTB2 and CS (98-100%), reinforcing the fresh bark phytotoxicity. B25 blends showed shoot weight and roots rating statistically equal or higher than CS. B25 increased Chinese cabbage growth may be due to favorable conditions for plant and root development as bark incorporation improved substrate aeration while maintained adequate water availability (Table 4). In addition, it seems that the N surplus applied (100 mg N L⁻¹) was enough to counteract bark N immobilization in B25 blends. Plant growth was lower in substrates with 50% bark (B50), probably related with unsuitable water retention properties (Table 4), as well as N immobilization (Buamscha et al., 2008; Depardieu et al., 2016) due to a higher percentage of fibers.

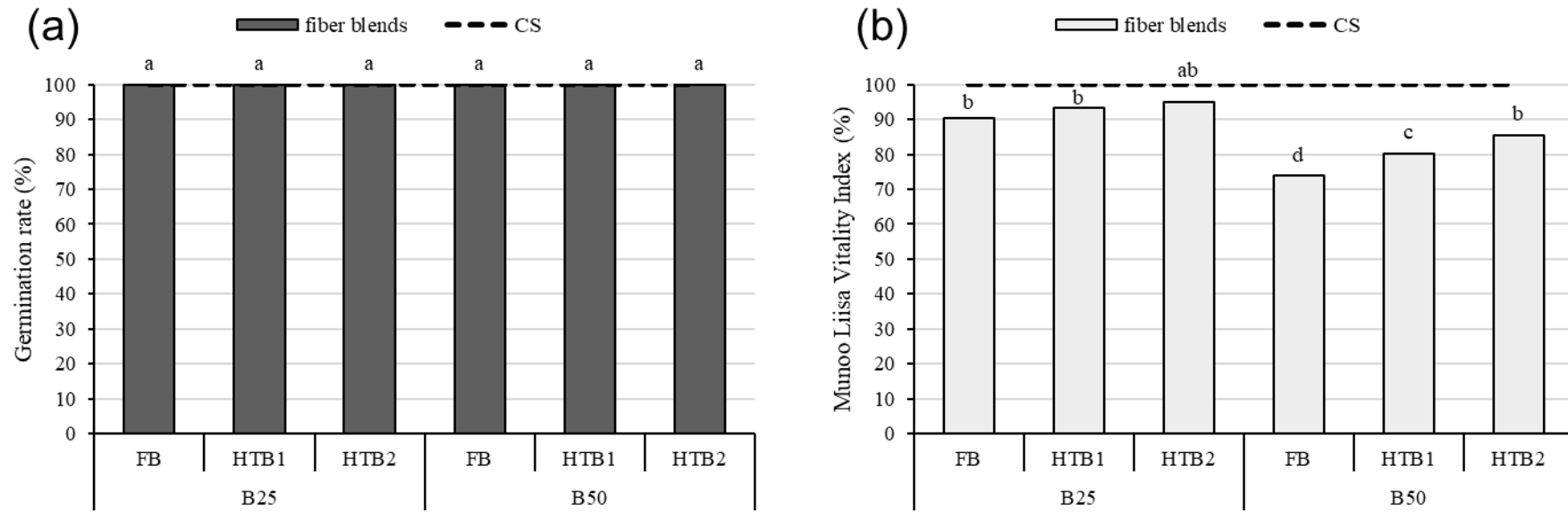


Figure 3. Phytotoxicity evaluation of fiber growing media blends and commercial substrate (CS): **(a)** germination rate and **(b)** Munoo-Lisa Vitality Index

Table 5. Potted plant response: Chinese cabbage germination rate (GR) fresh weight per pot (FW), dry weight per pot (DW), roots rating. Means followed by the same letter, in column, do not differ at $P \leq 0.05$ by the LSD-test.

Substrate	Bark type	GR %	FW g pot ⁻¹	DW	Root rating [1 – 5]
B25	IEB	95 b	17.4 ab	2.9 ab	4.7 a
	HTB1	100 a	15.7 ab	2.8 ab	4.0 a
	HTB2	100 a	18.5 b	3.3 a	3.7 ab
B50	IEB	95 b	9.0 c	1.5 c	2.7 cd
	HTB1	100 a	8.3 c	1.5 c	2.7 cd
	HTB2	98 ab	6.9 c	1.2 c	2.4 d
CS	-	100 a	14.3 b	2.6 b	3.2 bc

4. Conclusions

The study shows that hydrothermal treatments were effective regarding phytotoxicity removal from *E. globulus* fresh bark. Due to less energy and time consumption, and less N immobilization, the HTB1 treatment (20' and 60°C) seems to be the most adequate. Blending 25% (in volume) of treated bark with peat shows simultaneously improvements in growing media aeration properties, while adequate water content is maintained. An additional N-fertilization (100 mg N L⁻¹) in 25% bark fiber-based blends (to counteract N-immobilization), allowed a Chinese cabbage growth (shoot weight and roots rate) statistically equal or higher than in commercial substrate, encouraging use of this proportion of hydrothermally treated bark in substrates formulation.

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CHAPTER 7

Green application for an industrial by-product: aged *Eucalyptus globulus* bark-based substrates

E. globulus waste-stream



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Chemetova C., Barroso G., Gominho J., Fabião A., Ribeiro H. Green application for an industrial by-product: aged *Eucalyptus globulus* bark-based substrates.

Green application for an industrial by-product: aged *Eucalyptus globulus* bark-based substrates

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Abstract

A sustainable practice for *Eucalyptus globulus* bark valorization applying circular economy approach - a valuable waste stream from industrial pulpwood production in temperate regions - might be this fiber conversion into a new raw-material component for horticultural substrate industry. Forest-based materials, as alternatives to peat in growing media, are associated with inherent biological and chemical limitations, such as the presence of phytotoxic substances in fresh biomass and high microbial N immobilization. This study evaluates *E. globulus* bark ageing treatment over successive periods of time (at 0, 4 and 12 weeks), and the effect of an initial amendment (220 mg N L⁻¹), regarding substrate biological, chemical and physical properties. Fresh bark (FB) was phytotoxic: cress seed germination rate (GR) and root index (RI) were close to 0%, compared to peat control (GR and RI of 100%). Ageing gradually removed the toxic effect; after 4 weeks FB showed GR=85% and RI>80%. On the 4th week of ageing period bark chemical properties fitted within the recommended range for substrate use, excepting mineral N content. The ageing process may promote N immobilization, however, and therefore initial substrate amendment is required prior to potting, thus providing enough nutrients according to plant needs. After seven days of incubation, aged bark pre-amended led to a lower N immobilization rate (0.02 mmol N L⁻¹ day⁻¹) than aged bark without N-supplement (0.29 mmol N L⁻¹ day⁻¹). The gradual addition of bark enhanced substrate aeration while water availability decreased. However, compaction risk may be reduced. Aged *E. globulus* bark can be blended up to 25% with peat and produce plants as good as in commercial peat-based substrates.

Key-words: biomass waste; peat replacement; phytotoxicity; N immobilization; bark blending.

List of abbreviations (Chapter 7)

AB	Aged bark at week 4
ABN	4-week aged N-amended bark
AFP₁₀	Air-filled porosity
ANOVA	Analysis of variances
B	Bark
BD	Bulk density
BN	N-amended bark
EC	Electrical conductivity
FB	Fresh bark
GR	Germination rate
LSD	Least significant difference test
NR	Nitrogen immobilization rate
Pm	Peat moss
PmN	N-amended peat moss
RI	Root index
RL	Root length
TP	Total porosity

1. Introduction

Industrial bark from pulpwood production is valuable biomass (Yadav et al., 2002), however its potential use constitutes a challenge due to its heterogeneous structure and diverse chemical composition, and thus is still considered as waste by-product and mainly used as a cheap source of energy (heat and power generation) in pulp mills (Feng et al., 2013). In Mediterranean countries, *Eucalyptus globulus* is the major species for pulp and paper production, where 20% of the total bleached pulp remains in the bark waste stream (Neiva et al., 2016). Portugal produced 2.6 M tons of pulp in 2017, which generates around 0.52 M tons of bark (CELPA, 2017).

In Europe, around 1.9 M m³ of bark is used in the horticulture industry (Carlile et al., 2015). Forest-based materials, including tree barks, are associated to natural chemical and biological limitations when used as horticultural substrates (Kaderabek et al., 2017). These restrictions are mainly attributed to natural chemical composition (presence of phenolic compounds, terpenes and tannins) (Feng et al., 2013; Neiva et al., 2016) and high microbially-mediated nitrogen immobilization (Buamscha et al., 2008) as well as respiration (Chemetova et al., 2018). *E. globulus* bark is a fibrous material which has been tested for horticultural applications, such as soil conditioner (Yadav et al., 2002), alternative component in container substrate formulation (Chemetova et al., 2018; Cunha-Queda et al., 2007) or in block systems for substrate seedlings (Freitas et al., 2010). In two of the above mentioned studies, fresh *E. globulus* bark was composted, with a minimum of four (Yadav et al., 2002) up to six (Cunha-Queda et al., 2007) months, and involved windrow/stock pile preparation, with urea incorporation and mechanical turning to produce a toxin free and biologically stable substrate. In addition to the lengthy treatment to achieve a desired compost maturity, Cunha-Queda et al. (2017) reported a very high electrical conductivity (EC) at the end of the storage process, which is a limitation of composted bark further application. The previous authors study (Chemetova et al., 2018) found an efficient bark phytotoxic effect elimination using low temperature hydrothermal treatment, however aqueous extract residue resulted after treatment, thus waste steams must be minimized.

An alternative treatment to remove bark phytotoxic effect is ageing (Carlile et al., 2015). It is an attractive procedure due to its simplicity, chemical-free nature, zero waste production, and positive results after short time intervals, depending however on bark characteristics, volume used and storage conditions (Altland et al., 2018). Ageing is widely used with barks from hardwood and softwood species, such as *Pinus palustris* (Kaderabek et al., 2017), *P. taeda* L., *Pinus radiata* (Altland et al., 2018), *Pseudotsuga menziesii* (Buamscha et al., 2008), and *Acacia melanoxylon* (Chemetova et al., 2019). However, there is no literature information or data on use of aged *E. globulus* bark.

Substrate physical properties directly influence plant growth performance due to their impact in container media ability to store and supply adequate air and water

(Wallach, 2019). These can be optimized for ideal substrate characteristics, although gradual degradation over time may occur, depending on substrate mix stability and chemical profile (Altland et al., 2018).

The objective of this research was to evaluate potential *E. globulus* bark use as component of substrates for potted plants, investigating the phytotoxicity removal from fresh bark over consecutive ageing periods, and the effect of initial N amendment on substrate biological, chemical and physical properties.

2. Materials and Methods

Fresh *E. globulus* bark (FB) was collected from the Navigator Company pulp mill (Setúbal, Portugal) in Spring 2016, air dried and ground in a hammer mill ($\emptyset = 6\text{mm}$).

Amended (8 g L^{-1} of limestone) peat moss slightly decomposed (H2-H5 on Von post scale) was used as control. Bark and peat samples were amended with initial N supplement (220 mg N L^{-1}). Consequently, four different raw-materials were tested: bark (B), peat moss (Pm), N-amended bark (BN) and N-amended peat moss (PmN).

For ageing procedure, samples were moistened according to the “fist test” as defined by European standard (CEN, 2011), corresponding to 72 to 77% ($w w^{-1}$) of moisture content, and stored in black plastic bags at $25\text{ }^{\circ}\text{C}$ in a dark chamber for a period of 12 weeks. Each bag contained a total volume of 10 L of moistened bark and was opened and homogenized weekly. At weeks 0, 4 and 12 a sample was collected for ageing effect evaluation. Moisture content was confirmed at the sampling time and additional water was added as needed.

The phytotoxicity was evaluated according to the European standards (CEN, 2011), using cress (*Lepidum sativum*) as test plant.

Electrical conductivity (EC), pH, and water-soluble mineral N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) were measured in the water extract (1:5 by volume), according to the European standards (CEN, 1999b, 1999a, 2001).

According to Wallach et al. (2019), the following physical properties were determined: total porosity (TP), bulk density (BD), air-filled porosity at 10 cm of water column (AFP₁₀), easily available water between 10 and 50 cm of water column (EAW), water-buffering capacity between 50 and 100 cm of water column (WBC).

Based on data analysis from ageing effect evaluation, fresh bark at week 0 (FB) and aged bark at week 4 (AB), and 4-week aged N-amended bark (ABN), were selected for bark-based substrate formulation. Bark samples were blended with peat in volumetric proportion 25 and 50% (bark/peat).

The incubation experiment (7 days) for N immobilization rate (NR) measurements was adapted from literature (Buamscha et al., 2008; Fangueiro et al., 2012).

Data were subject to analysis of variances (ANOVA), followed by least significant difference test (LSD) based on the p-value with 95% of confidence level ($p \leq 0.05$). Data were analyzed using Statistica ® 10.0 (StatSoft, USA).

3. Results and Discussion

At week 0, fresh bark raw-materials (B and BN) were phytotoxic for cress seeds (Table 1). All parameters which tested seed performance, germination rate (GR), root length (RL) and root index (RI), were significantly lower at week 0 than in the following weeks. *E. globulus* fresh bark phytotoxic effect is in accordance with previous findings (Chemetova et al., 2018) and may be attributed to the presence of inhibitory substances (e.g. phenolic compounds, terpenes and tannins) present in bark tissues (Neiva et al., 2016).

The bark phytotoxic effect gradually decreased. After 4 weeks, B presented RI>80%. At week 12, RI in B raw-material was statistically equal to the control and the 12 weeks-old BN achieved cress seed performance greater than peat, suggesting the total removal of inhibitory substances. Buamscha et al. (2008) reported that ageing *Pseudotsuga menziesii* bark promoted substrate stability and greater geranium growth, compared to fresh bark. *A. melanoxyton* bark was not toxic to cress seed after ageing for 8 weeks (Chemetova et al. 2019).

Table 1. *E. globulus* bark (B) and N-amended bark (BN), peat moss (Pm) and N-amended peat moss (PmN) effect on cress seeds: germination rate (GR), root length (RL), and root length index (RI). Means followed by the same letter, in column, do not differ at $P \leq 0.05$ by the LSD-test

Ageing (week)	Raw-material	GR	RL	RI
		(%)	(cm)	(%)
0	B	5 c	0.1 e	1 f
	BN	10 c	0.4 e	6 f
	Pm	100 a	5.9 ab	100 bc
	PmN	100 a	5.6 b	94 bc
4	B	85 b	4.8 d	81 de
	BN	100 a	4.6 f	77 e
	Pm	100 a	5.9 ab	100 bc
	PmN	100 a	5.6 b	94 bc
12	B	80 b	4.9 cd	89 cd
	BN	100 a	6.4 a	115 a
	Pm	100 a	5.5 bc	100 bc
	PmN	100 a	5.6 b	102 b

Chemical analysis (Table 2) revealed that after aging, B and BN pH values rose from slightly acidic (week 0) to a more basic pH at the end of aging period, above the suggested limit of Barrett et al. (2016) (> 6.6). EC values followed the inverse trend, decreasing over ageing weeks, although were below the maximum recommended ($EC < 50 \text{ mS m}^{-1}$). Commonly, nutrients availability depends on pH, and when substrate pH tends to basic (low H^+ concentration) decreases EC, and vice-versa (Vandecasteele et al., 2018).

Both barks showed N decay during the aging process, however N immobilization occurred at a faster rate in BN, from 226 to 5.1 mg L^{-1} , at 0 and 12 weeks, respectively. Bark microbial population may promote N immobilization during ageing process compared to peat; however, initial substrate amendment is required prior to potting, thus providing enough nutrients according to plant needs (Ribeiro et al., 2009; Vandecasteele et al., 2018).

Table 2. pH, electrical conductivity (EC) and water-soluble nutrients (water extract 1:5 by volume) mineral N (N_{\min}) from *E. globulus* bark (B) and N-amended bark (BN), peat only (Pm) and N-amended peat (PmN). Acceptable range adapted from Barrett et al. (2016). Means followed by the same letter, in column, do not differ at $P \leq 0.05$ by the LSD-test.

Aging (week)	Raw-material	pH	EC	Nmin
			(mS m^{-1})	(mg L^{-1})
0	B	5.2 h	32.0 f	7.7 d
	BN	4.7 i	76.1 a	226.8 b
	Pm	6.7 d	8.1 h	7.6 d
	PmN	6.2 f	54.4 cd	227.8 ab
4	B	6.4 e	24.3 g	6.6 d
	BN	6.9 c	57.1 bc	132.2 c
	Pm	6.5 d	8.6 h	7.6 d
	PmN	6.0 g	57.9 b	246.6 a
12	B	7.2 b	25.4 g	7.4 d
	BN	7.9 a	36.0 e	5.1 d
	Pm	6.9 c	10.0 h	6.4 d
	PmN	6.3 ef	52.4 d	238.6 ab
Suggested range		5.5-6.6	< 50	50-250

Based on tables 1 and 2 findings, the 4th aging week was considered for further analysis. The pH values of all substrate blends (Figure 1) slightly increased with bark addition, however remained within recommended range (5.5-6.6) (Barrett et al., 2016). The EC values increase with bark increment and stand below tolerance limit ($<50 \text{ mS m}^{-1}$), and the highest value was achieved by ABN blended at 50%. Analogous to Table 1 findings, ABN blended at 50% also presented higher N_{\min} content ($>50 \text{ mg L}^{-1}$), and consequent lower NR ($0.02 \text{ mmol N L}^{-1} \text{ day}^{-1}$) compared to AB blended at 50% without N-supplement ($0.29 \text{ mmol N L}^{-1} \text{ day}^{-1}$). As the biochemical composition of the substrate, the N availability is one of the most limiting factor that affects substrate biological stability (Ribeiro et al., 2009), thus a previous knowledge on N requirements is fundamental to overcome N limitations (Buamscha et al., 2008).

Bark addition in FB, AB and ABN blends slightly decrease BD (Figure 2), although this parameter can be adapted by managing the compaction at potting (Barrett et al., 2016). The peat-based substrate presented the highest water content. Bark blends reduced the EAW and WBC, however AFP increased with bark increment.

Bark is rarely used as a stand-alone substrate constituent, is usually added to optimize physical properties of substrate mixtures (Barrett et al., 2016). According to air-water relationships (Figure 2), the addition of bark may optimize air supply to the roots, which makes the water logging risk residual for a nursery and greenhouse growers. The adoption of adequate irrigation intervals, also considering container geometry, may help to achieve the desired media performance (Altland et al., 2018). Pot growth assay evaluation using Chinese cabbage as model plant (data not shown) suggested that aged *E. globulus* bark N-supplemented can be blended up to 25% with peat and produce plants as good as in commercial peat based substrates.

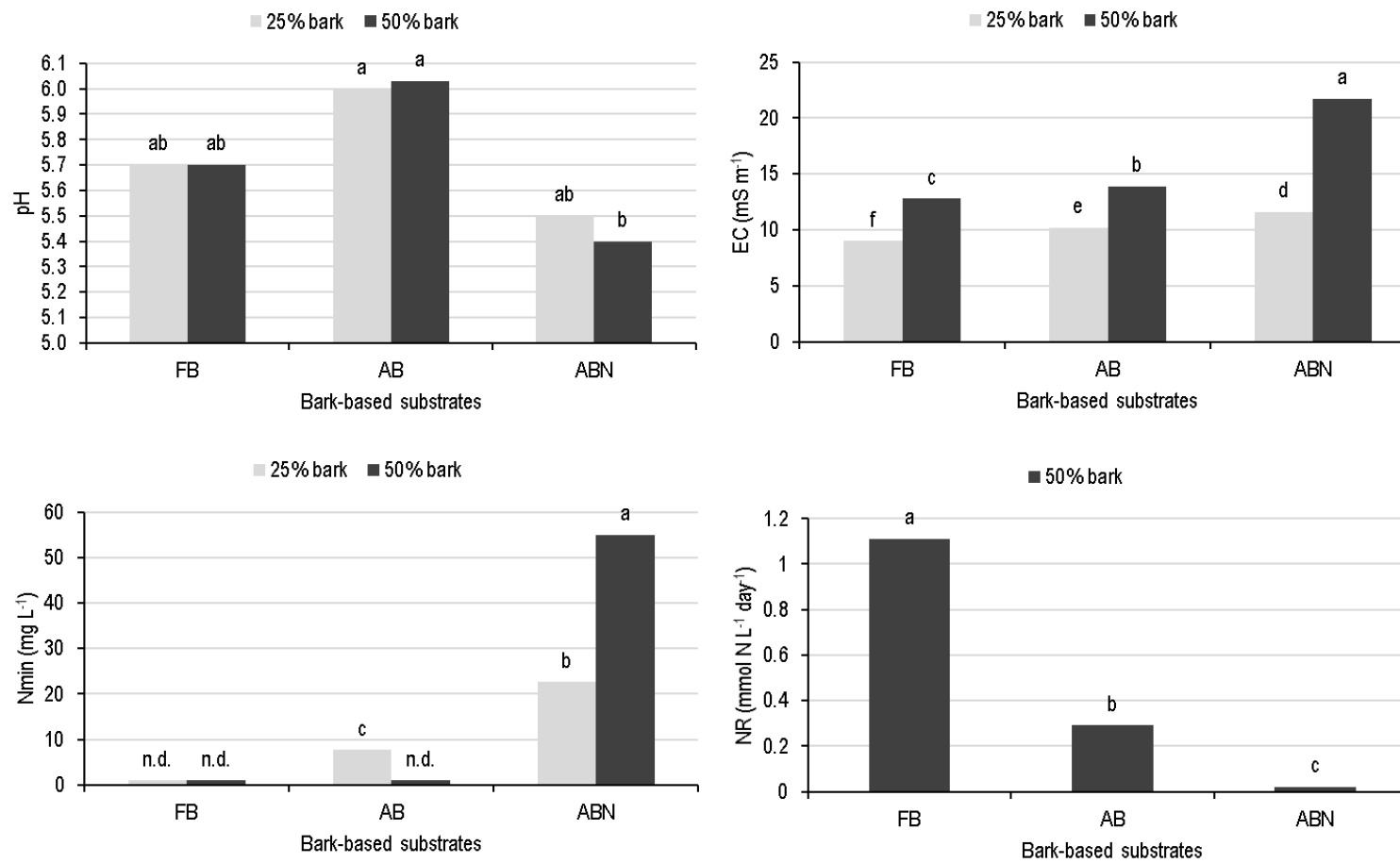


Figure 1. pH, electrical conductivity (EC), water-soluble mineral N (N_{min}) and nitrogen immobilization rate (NR) of *E. globulus* bark-based substrates made from 0-week and 4-week bark (FB; AB) and N-amended 4-week bark (ABN), blended at 25 and 50% vv⁻¹ with peat. Means followed by the same letter, on the bar top, do not differ at P ≤ 0.05 by the LSD-test. n.d. = not detected, below quantification limit (< 2 mg L⁻¹)

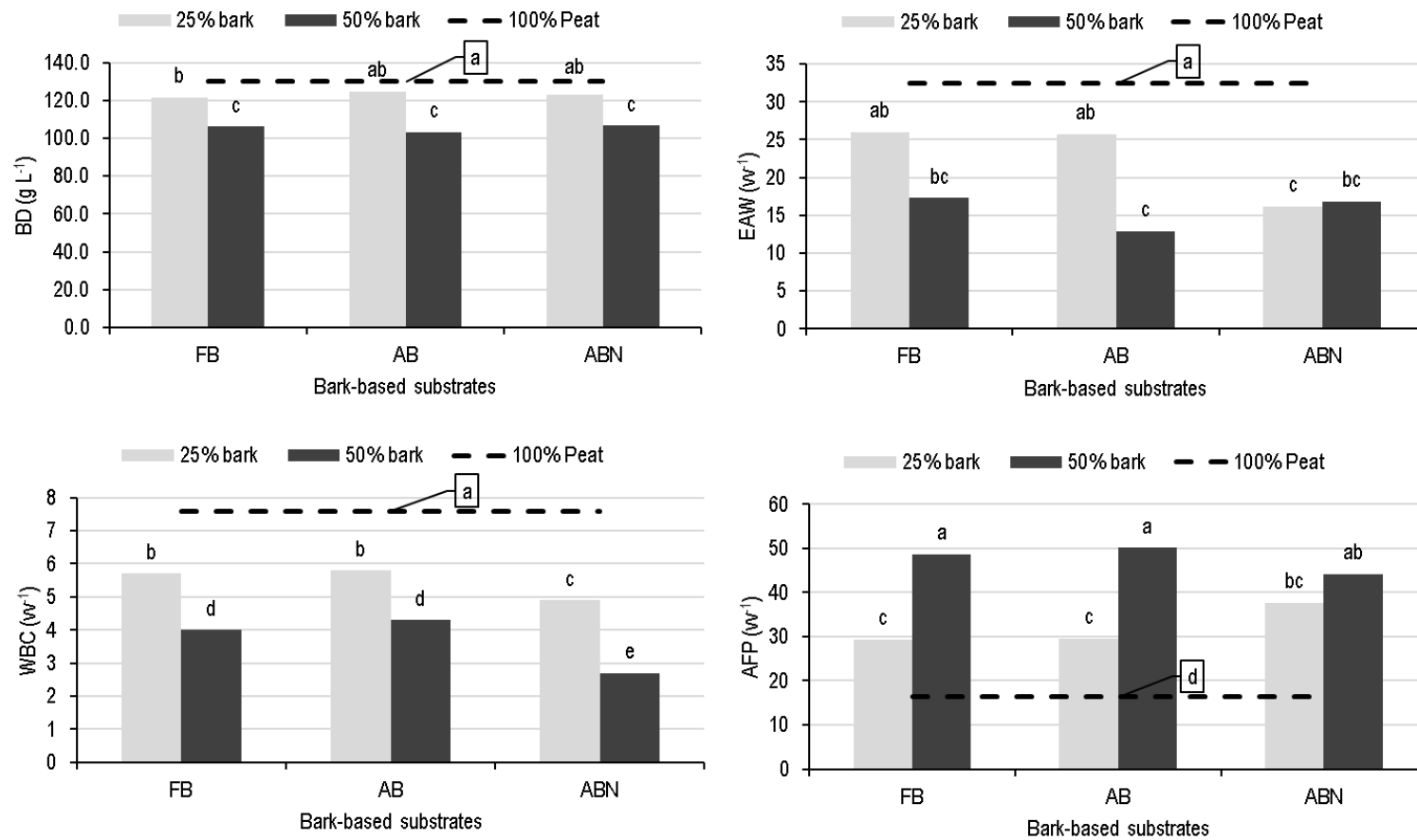


Figure 2. Bulk density (BD), easy available water (EAW), water buffering capacity (WBC) and air-filled porosity (AFP) of 100% peat-based and *E. globulus* bark-based substrates made from 0-week, 4-week bark (FB; AB), and N-amended 4-week bark (ABN) blended in 25 and 50% vv⁻¹ with peat. Means followed by the same letter, on the bar top and dashed line, do not differ at P ≤ 0.05 by the LSD-test

4. Conclusions

E. globulus fresh bark was phytotoxic for cress seeds inhibiting their growth. Ageing can remove this phytotoxic effect and at 4 weeks, aged bark allowed cress roots to develop and grow. N immobilization was associated with aged bark, and consequently external nutritional provision is suggested to provide enough N for plant growth. Mixing up to 25% of amended aged bark to peat can improve substrate aeration and allows plant growth as good as in commercial peat-based substrate.

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CHAPTER 8

Conclusions

A. melanoxylon residue

Chapter 2

Biomass evaluation and chemical composition determination

Chapter 3

Mature bark ageing treatment and growing media suitability

Chapter 4

Allelopathic potential of juvenile bark extracts

E. globulus waste-stream

Chapter 5

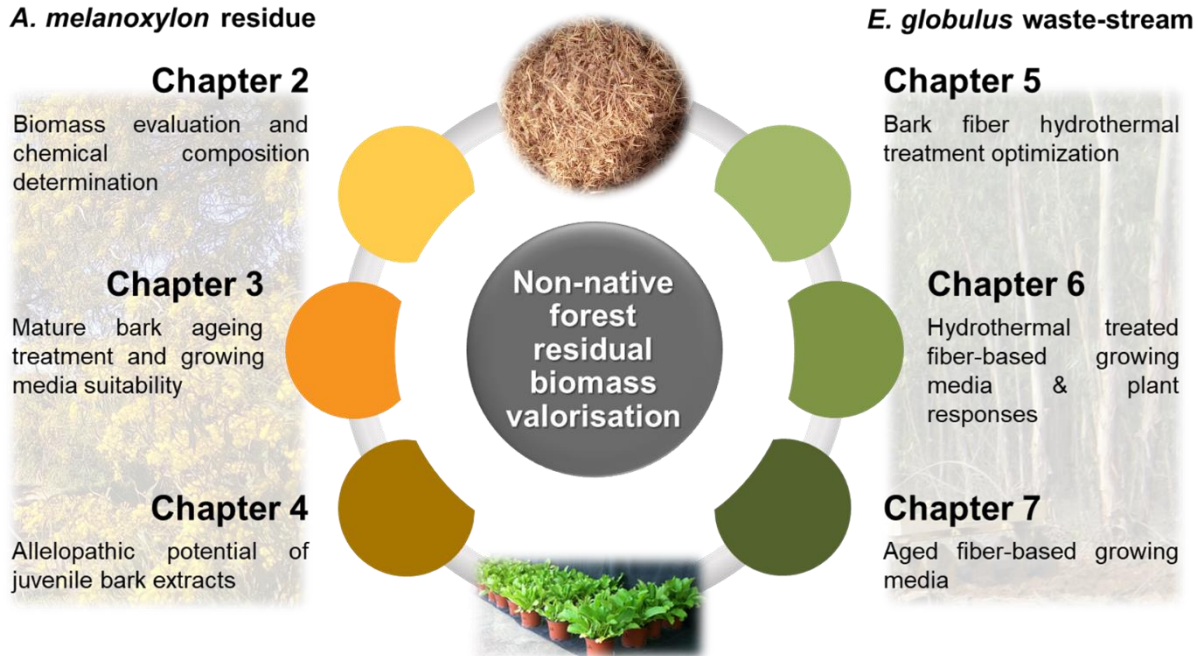
Bark fiber hydrothermal treatment optimization

Chapter 6

Hydrothermal treated fiber-based growing media & plant responses

Chapter 7

Aged fiber-based growing media



Conclusions

The novel uses of forest biomass waste-streams from non-native tree species in Mediterranean region, both from *Acacia melanoxylon* and *Eucalyptus globulus*, are driven by initial raw-material physical, chemical and biological characteristics. The research provides an understanding of the pre-treatment choice that better suits each raw material for horticulture applications. According to literature review included in this study, wood-based growing media components when fresh harvested or produced must be pre-treated to remove phytotoxic compounds and should be N-amended to guarantee enough N available to plants.

A. melanoxylon tree stand age affects its bark properties (Chapter 2) and regarding bark-based growing media suitability, aged mature bark can successfully replace half of container medium volume as a peat alternative component (Chapter 3). Ageing is considered a zero-waste treatment, but it may take as much as 8 weeks to guarantee toxic free bark material and, by adjusting sieving size, coarse bark may be added as an aeration component, while finer can retain water within container medium system. There is an alternative pre-treatment using temperature, time and water as solvent – hydrothermal treatment (HT) – but mature bark saponin content did not allow HT execution due to material handling constrains. Nevertheless, juvenile *A. melanoxylon* bark extract from HT demonstrated an allelopathic effect for organic weed control (Chapter 4). Furthermore, in Mediterranean countries, *A. melanoxylon* is considered an invasive species and its potential biomass valorisation must be underlined as an alternative management tool to support the costs of control, avoiding the potential risk of conflict between economic exploitation and negative environmental impact.

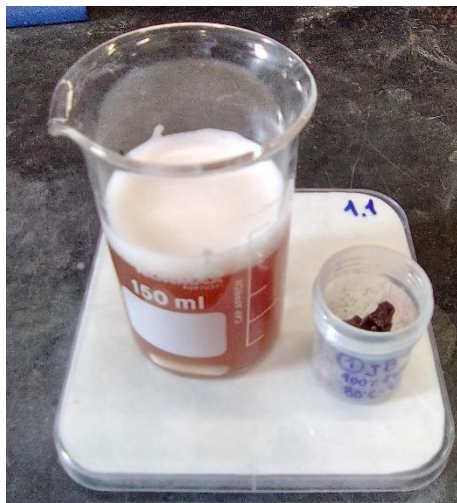
Assessing *E. globulus* fiber optimal HT conditions highlighted temperature as the main factor in phytochemicals removal (Chapter 5) and proved to be a fast and effective technique for fiber-based growing media output. Due to low temperature needed, the HT fiber extract allelopathic potential was not considered. As a container medium aeration agent, blending HT fiber up to quarter medium volume assured same plant performance as commercial materials (Chapter 6. Equivalent replaced fiber amount was achieved by blending aged *E. globulus* fiber to peat

(Chapter 7); this environmentally friendly approach may take 4 weeks before aged fiber-based growing media use. Therefore, both pre-treatments are adequate and should be considered for *E. globulus* fiber material which has large potential in waste-flow re-circulation from pulp and paper to horticulture industry.

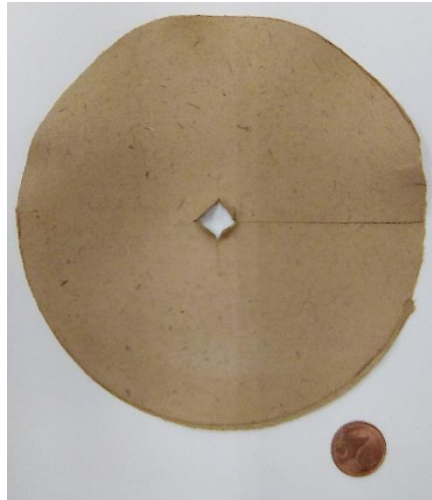
Despite the treatment choice, the studied growing media materials must be externally N amended, and nutritional provision should be adapted according to plant and cultivation system's needs. Furthermore, irrigation regime must be also combined and optimized to prevent water resource losses and transform the new growing media components incorporation into practical and economic realities. There is an enormous opportunity for the horticulture industry to include forest resources into soilless culture, whilst improving the productivity and efficiency of containerized plant production. In an increasing climate uncertainty world, the horticultural sector started to be guided by 4R's (reduce, reuse, recycle and recover) resource approach, where new materials are chosen when they are (i) easily available, (ii) financial and (iii) environmentally sustainable.

Within the framework of this thesis, circularity in horticulture was closely applied throughout the search of sustainable alternatives for peat using local, organic and renewable raw-materials based on forest waste-streams biomass. Additionally, the nutrient recycling flow from the initial biomass raw material is returned to plants via its reconversion into growing media. The strong environmentally sustainable approach is the driving force for further add-value applications development (Figure 1), such as: (i) the promising bio-herbicidal potential from juvenile *Acacia* bark extracts might be improved by identification and isolation of the specific phytotoxic component(s); (ii) the extracted *Acacia* bark might be blended with the respective wood (richer in cellulose) to produce "weed disks" with mulching effect – an organic and non-synthetic pot weed control; (iii) the disk thickness adjustment may allow thinner layers to be coated with specific seed content aiming seed propagation, or diversify disk shape to (iv) bio-pot configuration. The above listed future works are becoming increasingly attractive for commercial applications made from "green" materials and will implement the circular economy as base concept, recognizing the importance of applied research to industrial needs, and through stakeholders engagement to achieve positive environmental benefits.

Future work development:



Bio-herbicide



Weed disk



Impregnated seed medium



Bio-pot

Figure 1. Circularity approach to future horticultural industry applications