Department of Agriculture, Food and the Marine
An Roinn Talmhaíochta, Bia agus Mara

NDP NATIONAL DEVELOPMENT PLAN

Food Institutional Research Measure

Final Report

'Mushrooms and Fungi, Functional and Life Enhancing Reservoirs (MUFFLER)'

DAFM Project Reference No: 13F418

Start date: 01/03/2014

End Date:31/05/2016

Principal Coordinator and Institution: Catherine Collins

Email: Catherine.Collins@lit.ie

Collaborating Research Institutions and Researchers: None

Please place *one* "x" below in the appropriate area on the research continuum where you feel this project fits

Basic/Fundamental → Applied → Pre Commercial						
X						
1	2	3	4	5	6	7

Please specify priority area(s) of research this project relates to from the National Prioritisation Research Exercise* (NRPE) report;

Priority Area (s)	**(H) Food for Health**

Key words:
Mushrooms; fungi; ligninolytic enzymes; bioactives

1. Rationale for Undertaking the Research

This section should outline the rationale for carrying out the research and identify the need / problem to be addressed

A functional food may be defined as a food that improves health or well-being. From time immemorial, mushrooms have been recognized not only as a culinary wonder but also for having valuable health benefits. In addition to their value as flavoursome high protein low fat food, mushrooms can be described as a functional food given that they are a valuable source of nutraceuticals, anti-oxidants, anti-cancer, prebiotic, immunomodulatory, anti-inflammatory, cardiovascular, anti-microbial and anti-diabetic activities. Given that mushrooms have so many medicinal properties it is not surprising that fungi have made an enormous contribution to medicine. Approximately 38% of the 22,000 known bioactive, microbial metabolites are of fungal origin. Medicine was revolutionised by the discovery of the antibiotic penicillin from a fungus, which ultimately saved countless lives and lead to the birth of the antibiotic industry. Today, a cholesterol lowering drug which was originally isolated from a fungus has consistently been the bestselling pharmaceutical drug worldwide over the past few years. Mining for new pharmaceuticals including anti-cancer and anti-viral from fungi is an on-going process. Fungi inhabit a whole range of habitats and in doing so produce a plethora of enzymes which are secreted into the extracellular environment. Many of these enzymes have been exploited for industrial processes such as food, textile, the pulp and paper industries as well as bioremediation of environmental pollutants. Mushrooms and fungi represent a relatively unexplored species with only an estimated 5-10% known to mankind, making them an enormous reservoir for the discovery of novel bioactives and industrial enzymes. Ireland's oceanic and temperate climate together with some of its unique habitats acts as a wonderful host for the growth of mushrooms and fungi. To date there has been no study done on metabolites produced by fungi unique to Irish habitats or an evaluation of edible mushrooms from Ireland as a functional food. This study aimed to address this deficit by collecting fungi from Irish habitats, analysing them chemically and biologically. Such a study could identify new bioactives and enzymes for industry. Also by evaluating the functional food properties of edible mushrooms it will make the Irish consumer more aware of the health benefits of eating such mushrooms.

2. Research Approach

Specify the research methodologies employed, emphasising novel techniques and also outline any modifications from the original approved project proposal

Master Project 1: Wild mushroom collection and analysis

Sample collection

Twenty-seven species of wild edible Irish mushrooms were collected from Irish Habitats including Curragh Chase Park, Co. Limerick and Scariff, Co. Clare. In addition to the proposed wild edible mushrooms collected, two strains of commercially available strains of button mushrooms, (*Agaricus bisporus*) were purchased for comparison to wild commonly available species of mushrooms. All mushroom samples were cleaned, photographed and

catalogued, before freezing drying. Frozen glycerol stocks were prepared of these mushrooms so that they can be grown in laboratory conditions at a later date for future work.

Figure 1 Foraged Wild Mushrooms ready for cataloguing and processing

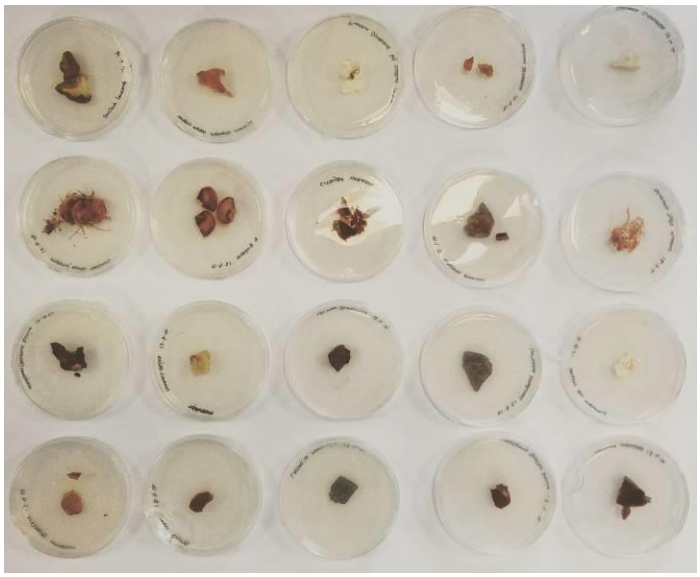


Figure 2 Generation of mushroom stocks from wild edible strains.

Sample Processing and Crude compositional analysis

To determine moisture content, the difference in mass of sample prior to, and immediately after freeze drying process which was calculated. Dried samples were finely milled and passed through a 1mm mesh sieve to remove large particles. Powdered samples were sealed in airtight containers in the absence of light for further extraction and compositional quantification.

Figure 3 Freeze drying process to determine moisture content of mushrooms

Fat Content

Total lipids were extracted using solvents Chloroform:Methanol 2:1, V/V. Solvent extract was evaporated to dryness in pre weighed vials to determine total lipid content. All extractions were performed in triplicate. Supercritical fluid extraction (SCFE) on two samples was also carried out to determine total lipid content. This allowed a comparison between the solvent method and the more environmentally friendly method of SCFE where Supercritical Carbon Dioxide is used as a solvent to extract non-polar compounds without the need for harsh or toxic chemicals.

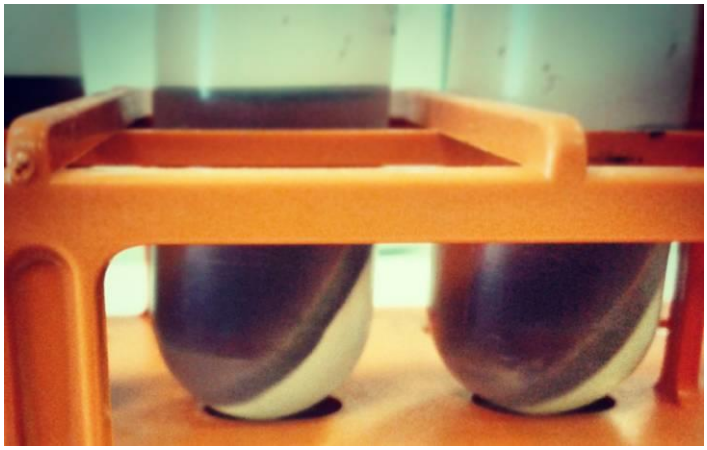


Figure 5 Extraction of total lipid using Chloroform and methanol

Figure 6 Supercritical fluid extraction unit in Shannon ABC laboratory

Crude Protein

Lowry protocol was successful in the determination of total protein in samples whereby phenol reagent is used to induce a colour change in the presence of proteins.

Total Carbohydrate & free sugar analysis by High Performance Liquid Chromatography

Total carbohydrate content was determined using a method described by Dubois method which uses phenol and sulphuric acid to generate a colour product directly related to the amount of carbohydrate present. Free sugars were determined using a method whereby samples were extracted in 80% aqueous ethanol, and was analysed using High Performance Liquid Chromatography (HPLC) coupled to a refractive index detector. The column used was a normal phase, supelcosil NH2 Polyamide column.

Sugar molecules were determined by comparison with retention time refractive index signals of authentic standards. Samples spiked with samples were also run and compared with external calibration curves. Quantification was achieved by measuring total area of sample target peaks, which were compared with generated standard curves of fructose, sucrose, mannitol, trehalose and arabinose.

Figure 7 Dubois, total Carbohydrate extracts developing colour change

Vitamin C

Vitamin C content was determined using HPLC rather than the originally proposed spectrophotometric assay, as levels of ascorbic acid detected in samples were so low, higher sensitivity was needed to detect it within samples. HPLC was used coupled to a DAD detector, Ascorbic acid was extracted in mild formic acid by saponification and centrifugation. An Ace 5 C18 column was used to detect target ascorbic acid in neat and spiked samples, a standard curve was constructed for comparison and quantification.

Vitamin E

Vitamin E Tocopherols were determined using HPLC coupled to a Diode Array Detector and a normal phase polyamine II column from YMC. Samples were extracted by hexane. Adaptions included use of diode array detection instead of Fluorescence detection.

Ergosterol

Ergosterol was extracted from Mushrooms using Chloroform and methanol. Ergosterol profiles were determined in saponified samples i.e. the lipid extract was treated under alkaline conditions to release the fatty acid chains from their glycerol backbone so that they could be analysed. Saponified Lipids were filtered and injected onto a Poroshell C18 column in HPLC machine coupled to a Diode Array Detector to achieve separation of Ergosterol from other lipids. Ergosterol was determined in all samples in triplicate extractions and compared with a standard curve constructed from an authentic ergosterol standard.

Fatty acid profiles

Fatty acids profiles were determined by high performance Liquid Chromatography Mass Spectrometry (LCMS) using both retention times of authentic standards and molecular weights. Quantification was achieved by comparing sample peak area to standard curve peak areas.

Fatty Acids Analyzed

Eicosapentaenoic acid (EPA)
Docosapentaenoic acid (DPA)
Docosahexaenoic acid (DHA)
Palmitic
Araquidonic
Oleic
Lauric
Linoleic
Linolenic
Stearic
Myristic

Figure 8 Lipids during hot saponification step before fatty acid or Ergosterol analysis could be carried out

Total Phenolic and Flavonoid Content

Total phenolic content was determined spectrophotometrically using Folin Ciocalteu phenol reagent to initiate a colour change in the presence of phenolic compounds. This was determined by comparison to a standard curve constructed of gallic acid authentic standard.

Total flavonoid content was determined in all samples by spectrophotometry whereby a reaction containing aluminium chloride initiated a development of colour in the presence of flavonoid content. Content in samples was quantified by comparison with a standard curve of quercetin. Results were expressed in mg/ml quercetin equivalent.

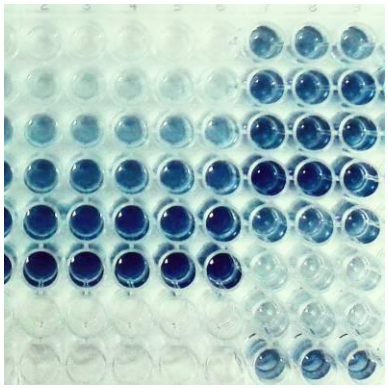


Figure 9 Total Phenolic content plate assay

Figure 10 Total Flavonoid plate assay

Bioactive screening

All bioactivity activity screens were carried out using water (polar) and dichloromethane (non-polar) extracts of Mushroom samples for the following assays.

Antioxidant capacity

Antioxidants mop up free radicals in cells hence preventing cellular damage and oxidation. Antioxidant Activity was screened in all extracts, through a number of assays namely the DPPH, FRAP, ORAC and Ferrous ion chelating activity assays.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay is a spectrophotometric procedure where mushroom sample extracts were compared with a standard (trolox). Trolox is a vitamin E analogue with known anti-oxidant properites. The DPPH assay measures the ability of extracts to scavenge the stable DPPH radical in a reaction carried out and measured on a 96-well micro-titre plate.

Figure 11 DPPH plate assay, Straw yellow to blue colour change is indicative of ability of mushroom extract to scavenge the stable DPPH radical

Ferric Reducing Ability of Plasma (FRAP) Assay

This assay measures the Ferric Reducing Ability of Plasma (FRAP). The FRAP assay originally is designed to analyse the ferric ion reducing power of extracts. Results were calculated based on the constructed linear regression for the standard curve. All results were expressed in mM Fe(II).

Figure 12 FRAP plate assay being plated using multi-channel pipette.

ORAC

The Oxygen Radical Absorption Capacity (**ORAC**) assay is the method used to quantify antioxidant activity of extracts. This assay uses the free radical sensitive fluorescent protein fluorescein as the substrate and 2,2 azobis (2-amidinopropane) dihydrochloride (AAPH) as the free radical oxidant generator. If an antioxidant is present in an extract it will act as a protecting agent, inhibiting or slowing the degradation (oxidation) of fluorescein by the AAPH radical. ORAC activity was calculated based on a Trolox standard curve.

Ferrous ion chelating activity

Ferrous ion chelating activity was measured in extracts based on ability of samples to chelate iron.

Antimicrobial activity screen

Antimicrobial activity of all extracts was screened using a disk diffusion assay whereby a zone of clearance or inhibition of growth of strains of bacteria and yeast was measured against positive controls. The positive controls consisted of a number of commonly used anti-biotics, which like the extracts, were pipetted onto diffusion disks designs to disperse compounds evenly into the surrounding soft top agar. The diameter of the zones of clearance were representative of the microbial growth inhibiting compounds present

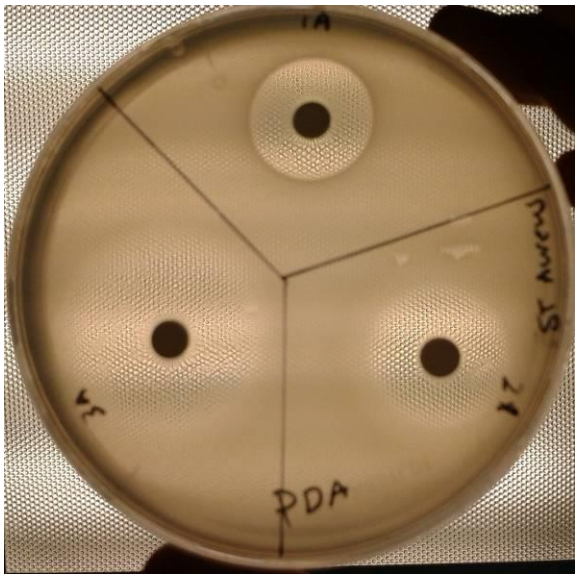


Figure 13 Positive control antibiotics inhibit growth of bacteria in the area surrounding the diffusion disks on agar plates

Enzymatic activity screen

HMG-CoA Reductase Assay cholesterol lowering activity in mushroom extracts whereby a kinetic read programme was used in a synergy four plate reader to measure the reduction of absorbance at 340nm due to the oxidation of NADPH over time.

ACE inhibitory activity

Blood pressure lowering activity was assessed in extracts of all samples by measuring the ability of the mushroom extracts to inhibit Angiotensin Converting Enzyme (ACE).

Masters Project 2: Collection and analysis of fungi from Irish habitats

Fungal bio-bank generation, culturing and extract generation for MuFFLER screening programme

Initially a number of substrates were collected from various habitats around Ireland including forestry, marine and agricultural sites (Figure 14).

Figure 14: A variety of substrates collected from habitats around Ireland.

From these supports, fungi were extracted by allowing their proliferation and purification on Potato Dextrose Agar and Czapek Dox Agar. By molecular means, each individual fungi (Figure 15) was then identified phylogenetically from 18S rDNA sequence.

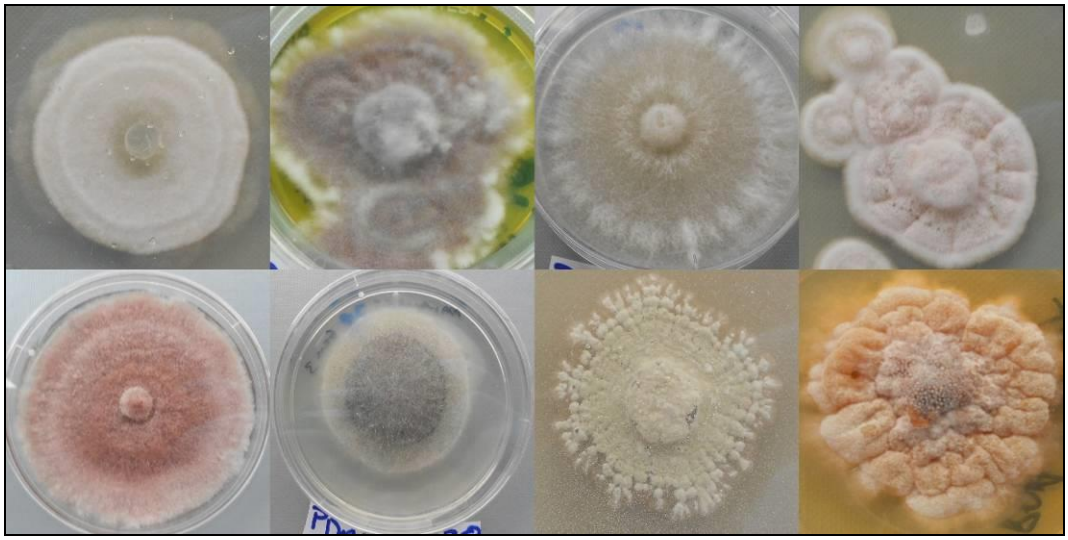


Figure 15: A number of purified fungi, propagated from substrates from Irish habitats.

For subsequent bioactive experiments, polar (water) and non-polar (dichloromethane) extracts were prepared from freeze dried fungal cell biomass.

Antimicrobial activity

The disc diffusion assay (Figure 16) using extracts described above was used to indicate antimicrobial activity of all fungi against medicinally important strains of Gram-positive bacteria (*Staphylococcus epidermidis* ATCC12228, *Staphylococcus aureus* ATCC6538 and *Bacillus cereus* NCTC7464) Gram-negative bacteria (*Escherichia coli* NCTC9001 and *Pseudomonas aeruginosa* NCTC10662) and one yeast species (*Candida albicans*).

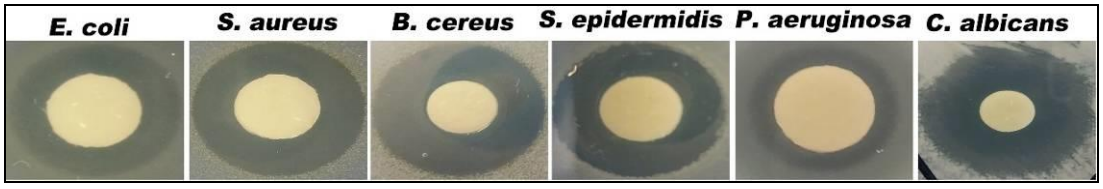


Figure 16: Various antimicrobial properties of fungal extract. Inhibition is assessed on circular zone of clearance around white diffusive disc.

Antioxidant and angiotensin converting enzyme inhibiting compounds

The antioxidant potential of individual fungi was assessed on proxyl radical scavenging, DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging and ferric ion reduction capacities. Neither mode of antioxidant action was considered more relevant than the other, rather an integrated approach was taken using a relative antioxidant index (RACI), to better assess the greatest antioxidant producers based on multiple mechanisms. Additionally, the

best antioxidant producing fungi were also assessed for potential blood pressure lowering capabilities by measuring angiotensin converting enzyme (ACE) inhibition.

Laccase activity and bioremediation potential

An initial screen of laccase producing fungal species was important for selecting suitable laccase producing organisms. The usage of chromogenic substances has been widely used as a rapid and inexpensive testing method, which within either solid or liquid media allows for direct visualisation of potential lignin degrading fungi. Our investigation utilised the chromogenic substrate guaiacol, which in the presence of lignin degrading fungi is oxidised, resulting in a distinct colour change from transparent to burnt orange (Figure 17). The usage of colour changing indicator compounds is generally used due to their ease and non-destructive nature.

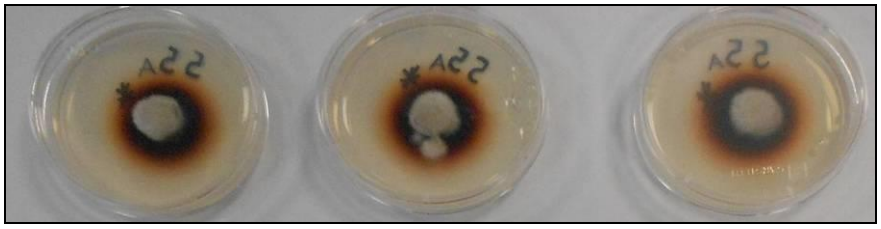


Figure 17: Plate indicator method for oxidation potential in the presence of guaiacol. Oxidation is observed as a burnt orange colouration on media.

However, as various enzymes have oxidative properties upon such colour changing substrates, other lignin degrading enzymes may be responsible for colour changes including lignin and manganese peroxidases. To further assess and confirm the enzymes produced by individual guaiacol oxidising fungi, all were grown within liquid culture, in the presence of various laccase inducing substrates (copper sulphate and Reactive Black 5-RB5). The individual fungi were then assessed for laccase, lignin peroxidase and manganese peroxidase enzyme activities. In addition, the bioremediation potential of the select guaiacol oxidising fungi were assessed by measuring reactive black 5 (textile dye) decolourisation (Figure 18) by both living and dead fungal cells.

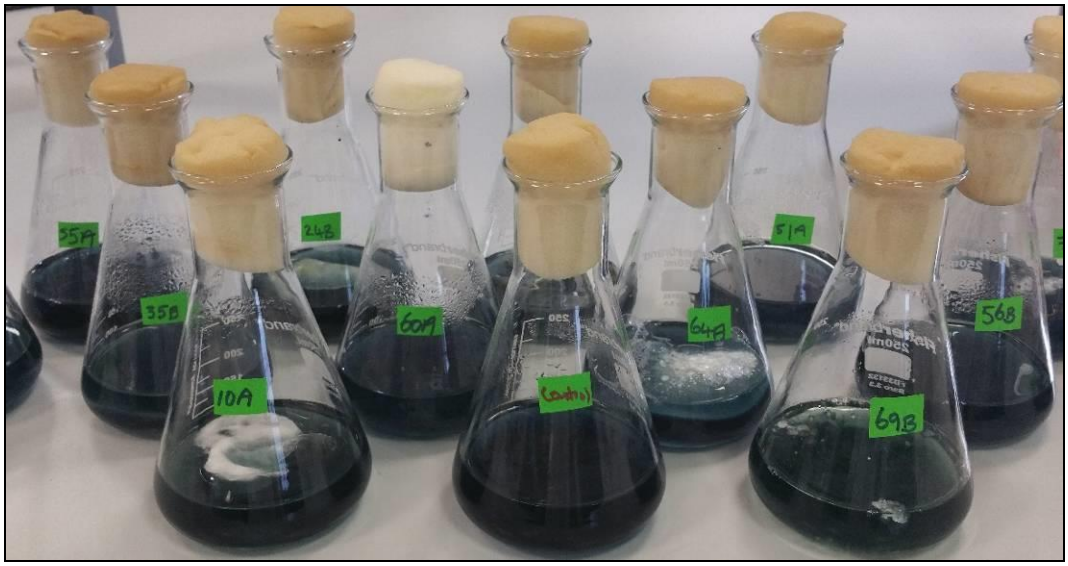


Figure 18: Bioremediation potential of fungi grown in liquid broth and RB5 (Day 1). RB5 appears blue, fungi with the capability to decolourise RB5 will result in disappearance of blue colour. RB5 absorbance and decolourisation can be monitored by UV-vis spectrophotometry.

3. Research Achievements/Results

Outline main results achieved

Masters Project 1: Wild mushroom collection and analysis

Main results achieved. All results have been collated and analyzed; Emily Panter is now incorporating these results and findings into her thesis.

- *Total Moisture content of mushrooms ranged from 65-96% of initial weight, all other results were calculated on a dry weight basis.*
- *Total Lipid ranged from 1-12%*
- *Protein Range 3-18%*
- *Carbohydrate 18-60%*

The results obtained from crude compositional analysis of wild edible mushrooms indicate favourable attributes such as low in total fat and relatively high protein content. Significant amounts of Phenolic compounds and flavonoids which are known for their antioxidative properties. Eight separate Phenolic compounds were quantified in each sample in triplicate. All extracts analysed contained varying levels of phenolic compounds. Gallic acid was the only phenolic compound detected in all samples.

Total Phenolic and Flavonoid content

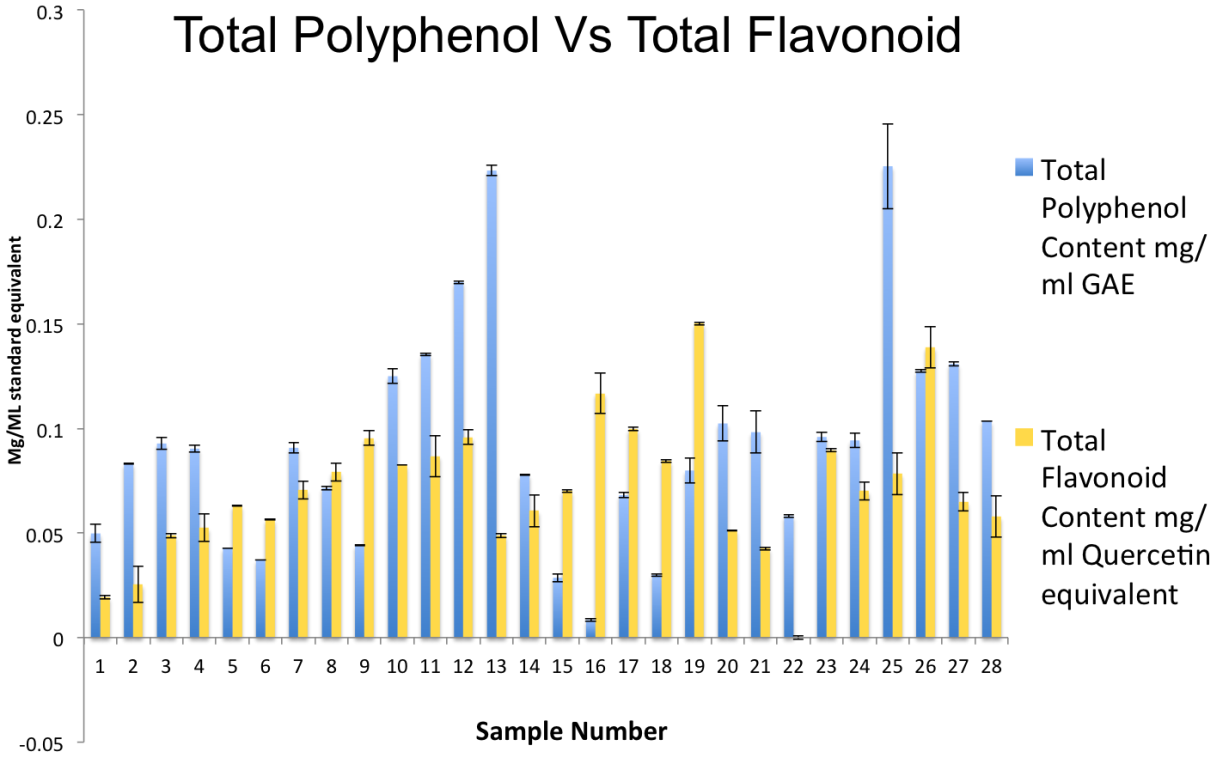


Figure 19 Total polyphenol and total flavonoid content

Sugars

Four separate sugars have been detected and quantified for each sample. Where detected, concentration levels showed broad variations depending on sample species.

Detected sugars are expressed in mg per gram of dry weight (dw)

Mannitol was determined to be the most abundant sugar in most samples. Mannitol is a sugar that generates interest for diet and nutrition, as this particular sugar is only partially absorbed in the lower intestine making it a potential natural source of alternative sweetening agents in the food industry.

Ergosterol was quantified for each sample, they were extracted three times and run in triplicate. Highest Ergosterol content was determined in *Lycoperdon mammiforme*, at a concentration of 687µg/g/dry weight. Ergosterol content found in mushrooms is significant to human bone health, ergosterol which is synthesized naturally in mushroom cells, is converted to vitamin D^2 by UV radiation, this occurs naturally in wild mushrooms exposed naturally to sunlight.

Fatty Acid profiles of 11 fatty acid compounds have been quantified in each mushroom species. Essential fatty acids, **EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid)** were simultaneously detected in *Cantharellus cibarius*, commonly known as the Girolle, this mushroom is considered a delicacy and is particularly good eating.

Fatty acid profiling by LCMS revealed particularly high levels of Stearic, Myristic and Palmitic acid with promising levels of **EPA** fatty acids. Predominance of Unsaturated fatty acids and, in particular, oleic and linoleic acids in all samples suggest that wild edible Irish mushrooms are a dietary source of beneficial fatty acids linked with dietary benefits for cardiovascular health.

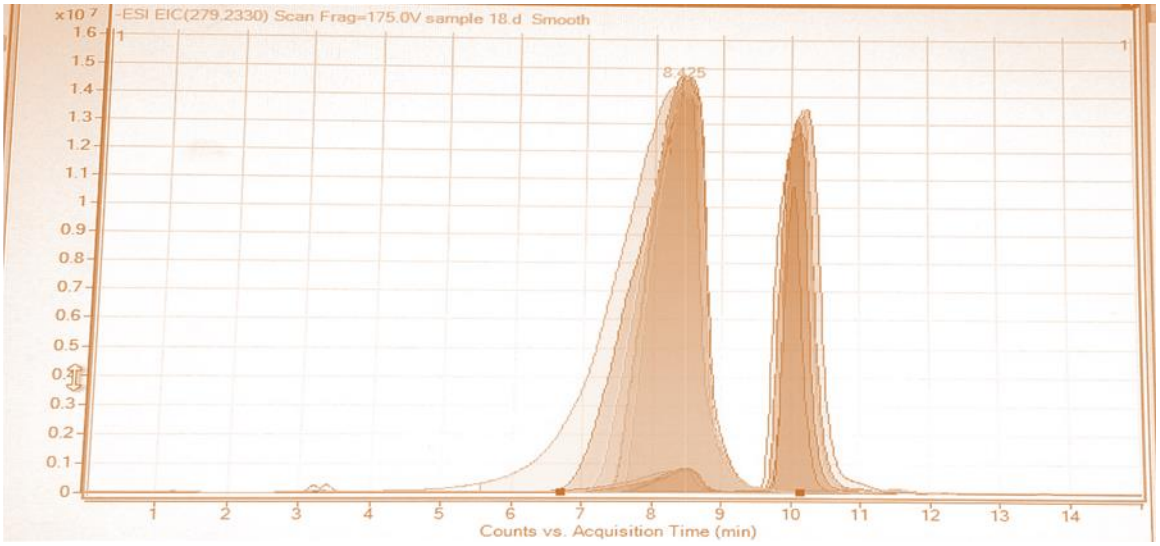


Figure 20 Extracted chromatogram peaks from EPA & DHA fatty acids

Vitamin C

Vitamin C was detected in minute quantities in very few samples, less than 1ppm concentration in most cases. Vitamin C is notoriously difficult to analyse due to its lack of stability in water. It is quite possible therefore that in fresh samples picked and immediately eaten there may be more vitamin C present. It is however short-lived and was only found in tiny amounts in samples. It is however a potent antioxidant and natural preservative, therefore the presence in dried month old samples may suggest that fresh samples are a viable source of Vitamin that can be readily introduced into the human body through diet.

Tocopherols

Tocopherol profiles were created in triplicate for each species, **α and δ** tocopherols were identified and quantified. Highest levels were detected in **α tocopherol** a common form of vitamin E group, which is commonly associated with health giving antioxidant benefits, due to its role as a scavenger of free radicals. Vitamin E is also believed to protect our bodies against degenerative malfunctions, mainly cancer and cardiovascular diseases. Vitamin E, naturally derived sources of vitamin E such as those found in wild Irish mushrooms, consumed in the human diet therefore has potential significant benefits to human health.

Antioxidant activity

Antioxidant activity was determined through a number of antioxidant screens. Water-soluble compounds contained the most potent or highest amount of compounds with

antioxidant potential. Anti-oxidant content was highest in *Coprinus comatus* (Shaggy inkcap). Through natural metabolic processes, harmful free radicals are generated in the human body. In the absence of counteracting antioxidants, free radicals have the potential to damage cell structures which can lead to degenerative diseases such as cancer or cardiovascular disease. FRAP ORAC and DPPH assays are able to screen and quantify protecting agents present in the mushroom extracts, which could be of significant health promoting value in the human diet to eradicate or slow down the degenerative actions of free radicals.

Ferrous Ion Chelating activity assay, confirms the presence of potent metal sequestering agents and chelating compounds in mushroom extracts, which are useful in the treatment of heavy metal poisoning or protection of cellular damage caused by exposure to atmospheric or environmental detrimental, metal contaminants to which humans are exposed on a day-to-day basis. High chelating value was observed in species *Hydnum repandum* (Woody hedgehog).

Antimicrobial assay - no significant antimicrobial activity was observed from the disk diffusion antimicrobial screens.

Most edible mushroom extracts exhibited some level of ACE inhibition which can help lower blood pressure further supporting the health benefits of mushrooms.

Masters Project 2: Collection and analysis of fungi from Irish habitats

Fungal bio-bank generation, culturing and extract generation for MuFFLER screening programme

Ninety fungal isolates were collected and purified (MuFFLER biobank) from Irish habitats. In accordance with other studies, species of the phylum *Ascomycota* contributed mostly to the biodiversity of fungal isolates, followed by *Zygomycota* and lastly *Basidiomycota*, this to our best knowledge being the first account of microfungi biodiversity in Ireland. With regards to the MuFFLER screening programme, both polar and non-polar extracts display a range of various pigment colourations which may suggest they harbour a variety of compounds with commercial applications.

Antimicrobial activity

Antimicrobial assessment of the MuFFLER biobank, revealed 19 fungal isolates to have acceptable (≥10 mm, diameter zone of clearance) antimicrobial inhibitory properties. Most activity resided in the non-polar DCM fungal extracts, most frequently against Gram positive bacterial strains. The greatest zone of inhibition was recorded against *S. aureus* (26 mm) and *B. cereus* (24 mm) from *Mortierella hyaline* #1D a *Zygomycete* fungus which has been previously assessed for antimicrobial compounds. Among the 19 fungi, inhibition

against multiple microorganisms was not uncommon, with 8 showing activity against more than one microbe.

Antioxidant and angiotensin converting enzyme inhibiting compounds

This screen identified numerous antioxidant producing fungi, with various mechanisms of antioxidant potential.

Laccase activity and bioremediation potential

Positive lignin degraders (22) identified via the guaiacol plate indicator method were further evaluated for ligninolytic potential. Using various inducible substrates 11 fungal isolates were found to produce detectible amounts of laccase, with copper sulphate proving to be the most effective inducer. Similarly 12 isolates produced manganese peroxidase enzyme activity, with the highest producer being greatly induced by RB5. Further investigation involving a detailed time course analysis (i.e. 24hr intervals) of culture filtrates would give a more accurate representation of enzyme production at different rates over time, identifying the point at which such activities are highest during growth.
Most (19) of the tested strains were also found to decolourise RB5 dye with the highest strains (11) ranging between 77- 99% decolourisation

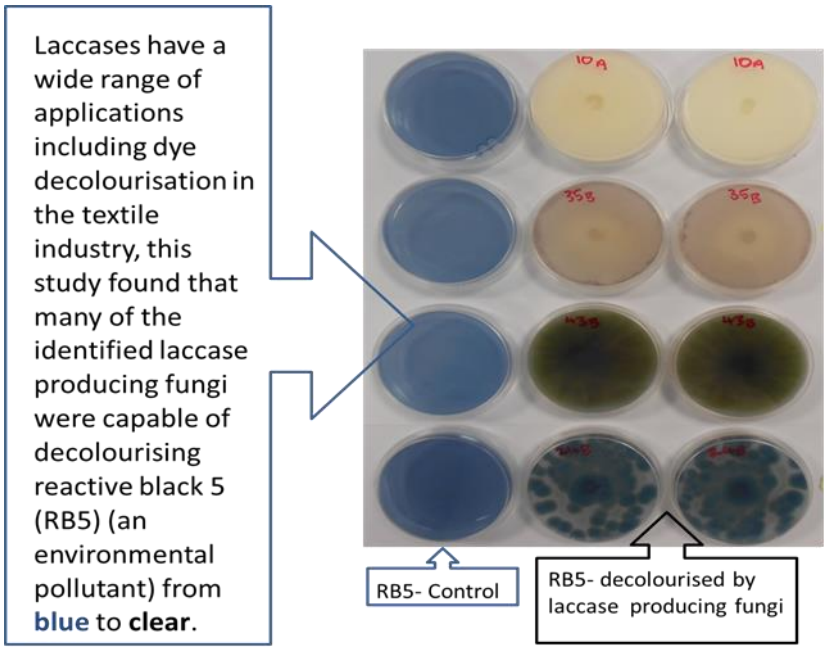


Figure 21: Many identified lignin degrading fungi were found to degrade (blue to clear) RB5 (environmental pollutant).

4. Impact of the Research

A summary of the tangible impact of the research project should be provided under the outcomes' and 'outputs' heading below. In addition, please provide a short narrative synopsis of the benefits / improvements the research has made to the area under investigation particularly as regards end users, e.g. industry, consumers, regulatory authorities, policymakers, the scientific community, etc

This study has investigated the nutritional and bioactive properties of wild Irish mushrooms. This information will make the public more aware of the benefits of eating wild mushrooms. Public interest on maintenance of healthy lifestyle and diet will ensure a niche market within Pharma-Nutritional industries, the potential of which will become more relevant with further research into the beneficial components of wild edible mushrooms.
Also in this study fungi collected from Irish habitats were analysed for a range of bioactives and enzyme activities. Ligninolytic enzymes, like those produced by fungi in our study, can play a vital role in degrading such pollutants, many of which were found to degrade the textile dye Reactive Black 5 (RB5) by more than 80% after 7 days. Investigating these potentially valuable commodities and further research may lead to the identification of new bio-active compounds, while also increasing the awareness of value linked with Irish habitats.

4(a) Summary of Research Outcomes

(i) Collaborative links developed during this research

- Because of our mushroom research we are collaborating with mushroom producer Peter McDonald of Shiele & McDonald Mushrooms, Co. Tipperary and Professor Kevin O Connor of University College Dublin on Industry Co-Funded Agri-Food and Bio-economy Innovation Platform Funding Instrument (2015 call). Phase 1 of this application has been successful.
- Planned further work to involve collaboration with Universidade Federal de São João del-Rei (UFSJ), Praça Frei Orlando, 170, Centro, São João del-Rei, Minas Gerais, CEP: 36307-352. Martin Hayes will travel to UFSJ and test findings of MuFFLER's fungal bioremediation potential against other commercially available pollutants.

(ii) Outcomes where new products, technologies and processes were developed and/or adopted

- A range of fungi, previously undervalued with regards to the natural products they produce have been identified and evaluated for healthcare and industrial applications.
- Twenty seven mushrooms from the wild were accessed for their nutritional content and bioactive properties. This study highlights the benefits of eating mushrooms from the wild. Frozen stocks have been made of these mushrooms allowing them to be grown in the future for further research.

(iii) Outcomes with economic potential

- The ligninolytic enzymes like those produced by fungi in our project could have many industrial applications including the degradation of waste dyes.
- Growing mushrooms on timber logs could be encouraged to generate additional revenue for forestry/land owners.
- The nutritionally favourable nutritional research should be useful in promoting wild mushrooms and reminding land owners of the importance of mushroom habitat and not to overlook this undervalued and underutilized bio-resource
-

(iv) Outcomes with national/ policy/social/environmental potential

- This study has investigated the nutritional and bioactive properties of wild Irish mushrooms. This information will make the public more aware of the health benefits of eating wild mushrooms and will encourage more interest in buying, growing or foraging mushrooms.
- Also in this study fungi collected from Irish habitats were analysed for a range of bioactives and enzyme activities. Ligninolytic enzymes, like those produced by fungi in our study, can play a vital role in degrading pollutants. Many of the fungi collected were found to degrade RB5 dye by more than 80% after 7 days. Applying such bioremediation potential may facilitate the detoxification of industrial waste products resulting in reducing pollution.

4 (b) Summary of Research Outputs

(i) Peer-reviewed publications, International Journal/Book chapters.

Acceptable Format: ***Walsh, D.R., Murphy, O., Cosgrave, J. (2008). Echinococcosis - an international public health issue. Research in Veterinary Science 774, 891-902.***

- Publication in writing entitled: 'Screening of Irish fungi for bioactive compounds with healthcare applications' by Martin Hayes
- It is expected a second publication will follow highlighting the bio-remediation potential of these fungi. However further evaluation is required based on proposed future work.
- Emily Panter expects to publish her findings in a scientific journal on the nutritional and bioactive content of wild mushrooms from Irish habitats and also in a non-scientific article to make the public more aware of the health benefits of wild mushrooms.

(ii) Popular non-scientific publications and abstracts including those presented at conferences

- The MuFFLER project was featured in the Research Enterprise Development newsletter of LIT in December 2015; a profile on the researchers involved and work being carried out was covered in the full colour newsletter which is also available in digital format in Limerick institute of technology website.

- An article on Muffler was also presented in Biotech For Business newsletter issue 10 (June 2016) which was distributed at the Shannon Applied Biotechnology Centre open Innovation conference 2nd of June 2016, Thomond Park Limerick.

Martin Hayes:

Poster presentations

- 'Environ 2015', April 9th 2015.
- 'Shannon ABC Open Innovations Conference', April 2nd 2015 (1st prize winner).
- 'The institute of Chemistry of Ireland Congress', September 19th 2014.
- 'Shannon ABC Symposium', April 15th 2014.

Oral presentations

- Irish Fungal Society, March 22 2016
- SABC Symposium, December 2015
- 'SABC Symposium', July 31st 2014

Emily Panter

- Researcher Emily Panter submitted a poster at the Shannon Applied Biotechnology Centre open innovation conference in Tralee Institute of Technology in April 2015 and in Thomond Park Limerick in June 2016.
- Researcher Emily Panter gave an oral presentation based on her current research at Mary Immaculate College Limerick for the Limerick Postgraduate Research Conference 2015
- Researcher Emily Panter Attended the Irish Fungal Society meeting 2015 in Trinity College Dublin and was awarded first prize for best Oral presentation by a researcher

(iii) National Report

None

(i) Workshops/seminars at which results were presented

Frequently data generated throughout this project was presented to the CHIMERA (CHemIcal and Molecular microbial biotEchnology ReseArch) Research group at Shannon Applied Biotechnology Centre, LIT. This exercise brought awareness to this project to many researchers from numerous backgrounds whilst also enabling a scientific discussion of findings, allowing for feedback and advice for further development.

(v) Intellectual Property applications/licences/patents

None

(vi) Other

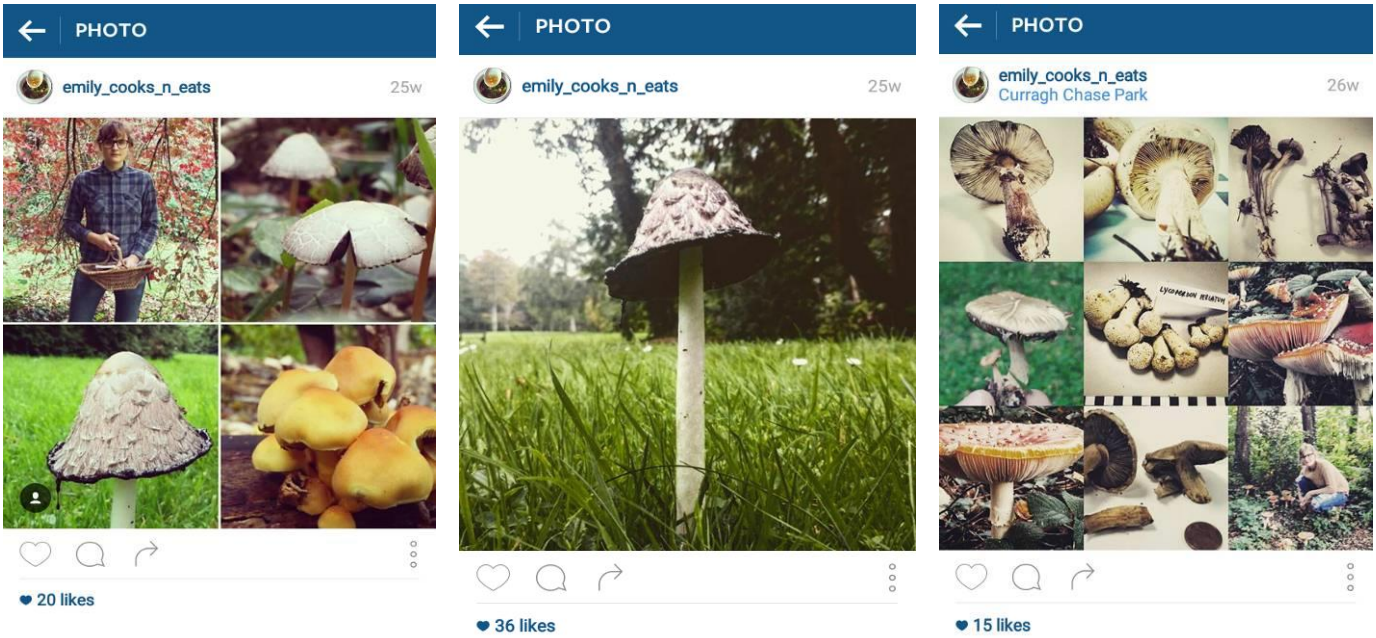
Researcher Emily Panter has conducted informal experiments based on the cultivation growth and harvesting of a number of edible mushrooms including *Hericium erinaceus* otherwise known as lions mane or bearded tooth fungus, *Pleurotus ostreatus*, (oyster mushroom) and *Lentinula edodoes*, (shitake mushroom).

These cultivation experiments based on the inoculation of fresh, beech and sycamore logs with mushroom colonized spawn. The growth process, inoculation to fruition to harvest is being carefully monitored, photographed and publicized using various social media. These experiments and publications aim to reinforce the dissemination of scientific research with more accessible information more easily accessed by the non-scientific and scientific communities alike. The overall message is the promote mushrooms as a healthy food, that can be grown in a garden just like any other vegetable or fruit, and turn garden waste into a viable source of exciting, healthy food.


Left: Emily hammering a mushroom plug into a timber log. Right: Oyster mushrooms growing on straw.


Emily's timber logs becoming impregnated with mushroom mycelia which will develop into mushroom fruiting bodies

Researcher Emily Panter has taken part in a number of days foraging for wild mushrooms and was featured on gathering *Lycoperdon pyreforme & Coprinus comatus,* in Mountshannon Co. Clare, on "Wildfoodlove" blog, which has over 23,000 followers online. This continued interaction with social media is an efficient way to reach many people in the scientific and non-scientific communities with updates and promotion the work involved and on-going in the MuFFLER project



Emily Panter and Martin Hayes were accepted to partake in A Marine Science and Technology Partnership Programme for the island of Ireland, which was conducted by The Strategic Marine Alliance for Research and Training (SMART). The programme took place April 12th-17th 2015 in Cork involved two days of marine science offshore operations on board the RV Celtic Voyager, whereby the researchers gained practical experience in offshore research methods, emphasising a multidisciplinary ecosystems approach to investigating the marine environment. The programme allowed Emily and Martin the opportunity to collect otherwise unobtainable samples for their investigations, while also training them on effective sample collection and processing, deployment and operation of offshore equipment and instrumentation, and data acquisition and interpretation. They also gave an interview to the Limerick Leader newspaper regarding this experience.

Figure 22: To the left- Research vessel Celtic Voyager docked within Cork harbour, Right- Box grab apparatus which was used to collect sea samples on board the Celtic Voyager as part of The Strategic Marine Alliance for Research and Training (SMART) programme.

5. Scientists trained by Project

Total Number of PhD theses: __0__

Please include authors, institutions and titles of theses and submission dates. If not submitted please give the anticipated submission date

Total Number of Masters theses: _2___

Please include authors, institutions and titles of theses and submission dates. If not submitted please give the anticipated submission date

Martin Hayes submitted his Master's thesis in December 2015 at LIT which was entitled "Mushrooms and Fungi, Functional and Life Enhancing Reservoirs". This document has been assessed by viva with an external and internal examiner and Martin has been transferred to the PhD register where he will continue his studies on selected fungi from the Muffler project.

Emily Panter is expected to submit her on her study of wild Irish mushrooms as a Master's Thesis at LIT in September 2016.

6. Permanent Researchers

Institution Name	Number of Permanent staff contributing to project		Total Time contribution (person years)
LIT	Catherine Collins	0.225	
LIT	Patrick Murray	0.110	
Total	**2**	**0.335**	

7. Researchers Funded by DAFM

Type of Researcher	Number	Total Time contribution (person years)
Post Doctorates/Contract Researchers		
PhD students		
Masters students	2	2
Temporary researchers		
Other		
Total	**2**	**4**

8. Involvement in Agri Food Graduate Development Programme

Name of Postgraduate / contract researcher	Names and Dates of modules attended
NONE	

9. Project Expenditure

Total expenditure of the project:	€140,762.13
Total Award by DAFM:	€154,828.13
Other sources of funding including benefit in kind and/or cash contribution(specify):	€0

Breakdown of Total Expenditure

Category	Limerick Institute of Technology Institution 1	Name Institution 2	Name Institution 3	Name Institution 4	Total
Contract staff					
Temporary staff					
Post doctorates					
Post graduates	84,131.53				84,131.53
Consumables	21,806.02				21,806.02
Travel and subsistence	2,167.54				2,167.54
Sub total	108,105.09				108,105.09
Durable equipment	225.51				225.51
Other					
Overheads	32,431.53				32,431.53
Total	**140,762.13**				**140,762.13**

10. Leveraging

Summarise any additional resources'/funding leveraged by this award from other sources e.g. Additional Staff, National/EU funding secured, EI Commercialisation Fund, etc.
None

11. Future Strategies

Outline development plans for the results of the research.

- Martin Hayes is going to further research his findings from this project by doing a more indepth study on ligninolytic enzymes from Muffler fungi. These enzymes have many industrial applications including the removal of harmful dyes from water systems.

- Patrick Murray and Catherine Collins were invited by Professor Kevin O'Connor of UCD to become involved in a proposal for a bio-refinery with other industries including the mushroom producer Peter McDonald of Shiele & McDonald Mushrooms, Co. Tipperary. This proposal was submitted to DAFM in January 2016 and been invited for Phase 2 application.