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Determination of the quantity and homogeneity of UK sources of chitinous by-product streams for improving soil resilience

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CONTENTS

1. Abstract

After cellulose, chitin is the second most abundant polysaccharide on Earth. Agriculturally, chitin-containing soil amendments have the potential to improve crop plant vigour, soil moisture retention, uptake of soil nutrients, induce plant defence mechanisms and increase soil suppressiveness against pests and pathogens. Chitin can be applied to the soil as a pure product or organic composites such as by-products from shellfish waste, spent mushroom substrate (SMS) or black soldier fly (BSF) frass and pupal cases. Such by-products have huge potential in circular economies but the basis of their application requires thorough investigation to ensure optimal and safe usage. The objectives of this project were to (i) conduct a review of existing published and grey literature, (ii) conduct physico-chemical analysis of three chitinous by-products, namely a shellfish by-product compost, BSF by-product and SMS, (iii) investigate the legislative issues surrounding the application of chitinous soil amendments by food processors and environmental agencies and (iv) conduct knowledge exchange activities to improve general awareness of this approach.

Our review identified a significant body of work on chitin, chitosan (the deacetylated form of chitin) and chitinous by-products in relation to different agricultural applications. Chitin and chitosan have negative effects on plant pathogenic fungi, oomycetes and slime moulds, as well as insects and plant parasitic nematodes through direct effects, such as membrane disruption, and indirectly through interactions with the soil biota. Additionally, there are other additional benefits such as enhanced activity of soil microbes, plant growth promotion and removal of heavy metals.

Physico-chemical analysis of BSF by-product, SMS and composted shellfish indicated a significant level of organic matter and key nutrients for crop growth to be present, potentially reducing the reliance on synthetic fertilisers. Additionally, the microbial content of these by-products will provide benefits to crops, as well as improving soil health by being incorporated into the soil microbiome. Technical challenges are present to obtain an accurate assessment of the chitin content in these by-products, with inconsistencies in results obtained and ongoing breakdown of the chitin by microbial activity.

In terms of the future usage and application of by-products of shellfish waste, spent mushroom substrate and black soldier fly frass, it is anticipated that the legislative situation will alter with the implementation of new UK fertiliser product regulations, which are expected in 2023. It is expected that these regulations will include more detailed specifications of the requirements for soil improvers and amendments, and organic fertilisers than the legislation that is currently applicable. Currently, shellfish by-products and black soldier fly frass are classed as animal by-products (ABPs) and government guidelines need to be followed to provide a route to application on land. Spent mushroom substrate (SMS) is classified as a non-meat food waste in the UK, and is subject to different regulations than shellfish or insect by-products. In England, SMS has an exemption (called

a U10 exemption) that allows its spread on agricultural land (maximum of 50 tonnes per ha per year) and in Scotland, the application of SMS to land is regulated by SEPA. Risks regarding allergens present in chitinous by-products are primarily related to the handling of material prior and during processing and should be covered by a COSHH assessment to minimise any risk during handling, transport and processing (e.g. composting) as well as subsequent application to land. The allergen risk after application to soil and in food crops is very low.

In many ways, the application of chitinous by-products resonates with the circular economy, regenerative agriculture and the movement towards net-zero. There are, however, practicalities that need greater development to ensure optimal benefits. Equally, growers need to be fully informed of the legislation surrounding the use of these inputs.

2. Introduction

Livestock manures, sewage sludge and green waste compost have a successful track record of use in agriculture in protecting and enhancing soil; optimising land use; maximising input efficiency; and turning wastes into a valuable resource (Crooks and Litterick, 2020). Biowastes such as shellfish frass, mushroom substrate (spent compost and mushrooms) and black soldier fly waste have potentially similar characteristics - they contain valuable amounts of major crop nutrients, biopolymers such as chitin, organic matter and calcium carbonate (lime), and have the potential to alter the environmental conditions in the rhizosphere to shift the microbial balance in favour of beneficial organisms and to increase natural suppression of plant pathogens and pests (Sharp, 2013; Grimm and Wösten, 2018; De Corato, 2021; Gärttling and Schulz, 2022). Transforming chitin containing by-products into high value products, such as a soil health amendment, will aid in the reduction of carbon emissions and bring economic and environmental benefits to the end user.

This report will explore the potential for upcycling shellfish by-products, black solder fly (BSF) frass and spent mushroom substrate (SMS) by reviewing the scientific and grey literature, report on an analysis of the by-product material in terms of nutrients, chitin content and physico-chemical properties, quantify their carbon sequestration potential, and evaluate the legislative hurdles to be addressed to legally allow these by-products to be utilised as soil health amendments. These materials provide a route to a circular economy (and bioeconomy) that straddles the aquaculture, insect and mushroom farming and agriculture sectors, contributes to a low carbon economy for all sectors, and addresses the key aims of the UK Government to reduce GHG emissions and increase carbon storage.

The key outcomes from this report applicable to growers will be communicated through knowledge exchange activities via the AHDB web site, an AHDB/BBSRC webinar, the AHDB Farm Excellence platform and the What Works Centre for Agriculture and Horticulture.

- **Objective 1:** Review of the scientific and grey literature regarding the benefits of each of the chitinous biowaste streams to soil health, suppression of pests and pathogens of crops, crop nutrition, carbon sequestration, and potential environmental and food safety concerns.
- **Objective 2:** Undertake a physico-chemical analysis of the chitinous by-product streams, quantify the chitin content, and assess the logistics necessary for route from waste material to use in the field.
- **Objective 3:** Review the legislative issues regarding use and acceptability of the chitinous by-product streams by environmental agencies and food processors.
- **Objective 4:** Undertake knowledge exchange activity, summarise the potential benefits of each of the chitinous waste streams, their likely adoption by growers, recommendations for use and any future R&D requirements to increase adoption.

3. Materials and methods

3.1. Literature review

A literature review was conducted on pertinent topic areas using search engines such as Web of Science and Google Scholar. Short interviews were conducted with Insect Farming companies (ECOInsect and Beta Bugs) to discuss the following questions in relation to BSF by-products: -

i. What volume of solid waste (by-product) material do you generate?
ii. What volume of waste does the industry produce as a whole?
iii. Do you see the waste as a significant income stream either for your company or for the Insect farming industry as a whole?
iv. What type of customer purchases your waste and do they specify any particular criteria such as particle size, chemical or biological analysis?
v. What is the logistics in terms of movement and storage of waste, and legislative requirements that need to be satisfied in terms of production/sale/disposal?
vi. Do you have any concerns regarding allergens (in the by-product)?
vii. What are your recommendations for future R&D required in the sale/disposal and use of BSF waste?
viii. Are you aware of any studies using BSF or other insect farming waste as a by-product?

3.2. Physico-chemical analysis of chitinous by-product streams

Samples of the chitinous waste streams and those that have undergone processing (e.g. composted shellfish waste) were sourced and submitted to commercial laboratories for physico-chemical analysis, to determine parameters such as *Salmonella*, *E. coli*, heavy metals, physical contaminants, water extractable and total nutrient content, pH, bulk density, dry matter, organic matter, organic carbon, conductivity and liming potential. In addition, samples were sent to a commercial laboratory to determine their chitin content, and the presence of any allergens present in crustacea and mollusc shellfish waste (pre-composted and post-composted). The route from waste production to the field was assessed in terms of logistics and costs. Samples of composted shellfish by-product, BSF frass and SMS were sent to D&F Associates, Widnes for a physico-chemical analysis equivalent to what would be undertaken if the material was to be put forward for the BSI PAS100 standard (https://standardsdevelopment.bsigroup.com/projects/9017-01020#/section), which is assured in the UK by the Compost Certification Scheme (https://www.qualitycompost.org.uk/). Note that the materials under test were not considered as a compost, hence the full PAS100 testing involving a plant growth test was not undertaken. The name of the tests undertaken at D&F Associates are the PAS100E suite and WATSOLNUT2 option.

Analysis of the % chitin and chitosan content of composted shellfish by-product, BSF frass and SMS was undertaken by Eurofins in Finland via Public Analyst Scientific Services Ltd, Wolverhampton.

3.3. Review of legislative issues regarding use and acceptability of chitinous by-products

A summary of the legislation and health and safety requirements regarding the preparation, handling and use of composted shellfish by-product, BSF frass and SMS is presented, based on experiences of the authors and in discussion with relevant organisations including the Health and Safety Executive (HSE), Red Tractor and others. Discussion of the acceptability of these by-products by food producers and growers is also summarised.

3.4. Knowledge exchange activities

In order to disseminate knowledge associated with this project, the following activities were undertaken: -

i. An article on the project was prepared for press release by Harper Adams University in March 2022
ii. A further article for the AHDB for release in April/May 2022
iii. Results to be disseminated at a BBSRC KE event scheduled for 13th July 2022

4. Results

4.1. Literature review

4.1.1. Chitin – occurrence in nature, description of molecule, function, intro application in crop protection

Chitin is the second most abundant polysaccharide biopolymer after cellulose (Gooday, 1990). Chitin was first isolated from mushrooms in 1811 but wasn't named 'chitin' until around 20 years later when the same material was found in the exoskeleton of insects (Badawy and Rabea, 2011). The term 'chitin' is derived from the Greek word for "tunic" or "envelope", which succinctly summarises its primary role within the exoskeleton of arthropods such as insects, crustaceans, arachnids, cephalopods, the radula of molluscs and the eggs and gut linings of nematodes. Several microorganisms also have chitin in their cell walls, membranes and spores, including fungi and the spines of diatoms (Sharp, 2013).

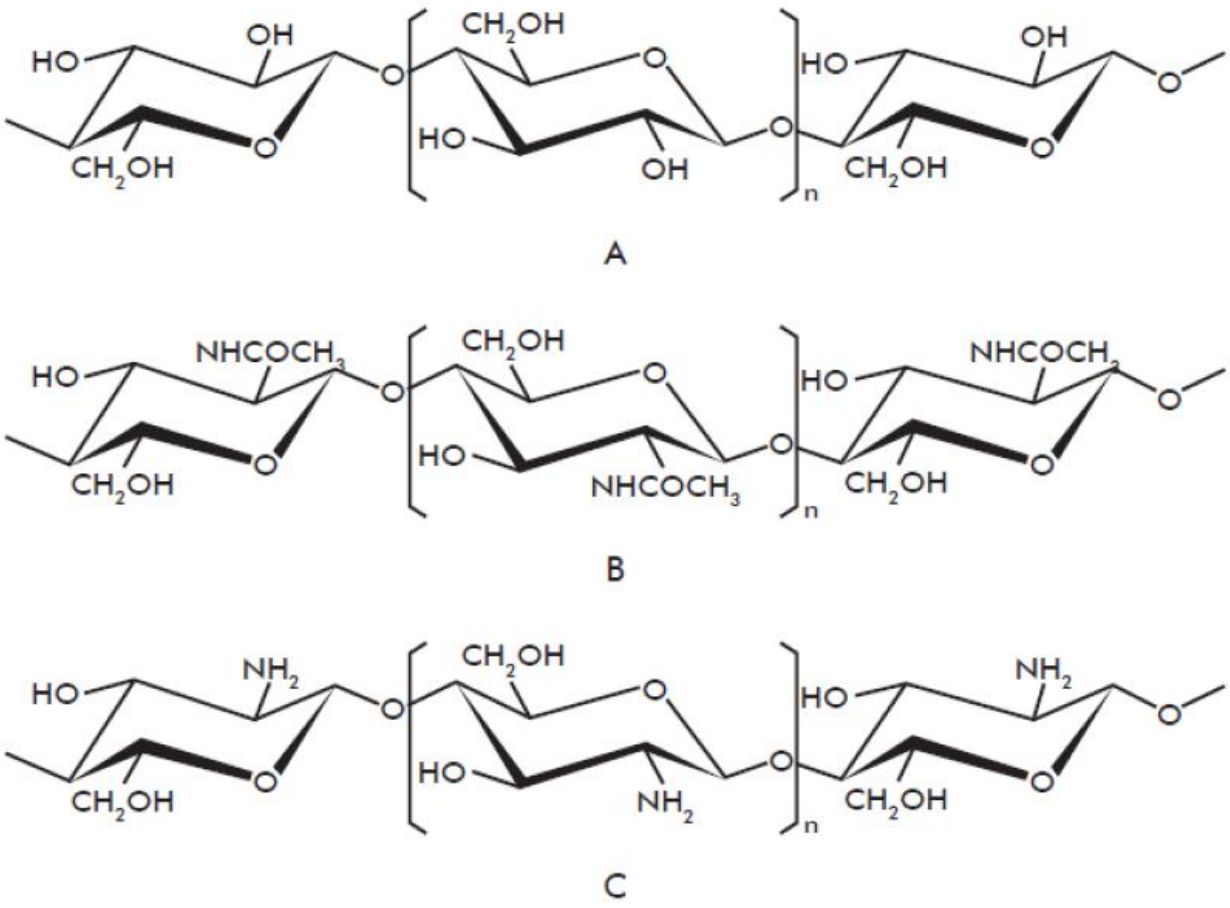

Figure 1 The structural representation of repeating polymer chains of cellulose (A), chitin (B) and chitosan (C). Adapted from Sharp (2013).

Chitin shares structural similarities with cellulose (Figure 1); it is a long-chained, linear, neutrally charged polymeric polysaccharide of (1–4)-linked 2-acetamido-2-deoxy-b-D-glucose (Figure 1) and has three crystalline polymorphic forms; α, β, and γ chitin. Within each form there are different orientations of the microfibril chains: α-chitin has antiparallel chains, β-chitin has parallel chains, and γ-chitin has a mixture of parallel and anti-parallel chains (Rudall and Kenchington, 1973).

The α-chitin form is mostly found in crustacean shells such as prawns, crabs and lobsters, as well as beetle shells and fungi cell walls (Beckham and Crowley, 2011). β-Chitin is primarily found in marine diatoms (Chiriboga *et al*., 2020), molluscs (Hajji *et al*., 2014) and the peritrophic matrix in insects (Liu *et al*., 2019). The occurrence of γ-chitin is not very common, and it has been reported to be present in beetle cocoon fibres (Moussain, 2019) and moth cocoons (Kaya *et al*., 2017).

Chitin has significant mechanical properties as a biopolymer demonstrating structural strength and flexibility for many different species. For example, many organisms utilise chitin as an armour for protection and defence, e.g. insect cuticles and crustacean shells (Hou *et al*., 2020). The structural strength in cuticles and shells is a result of the hierarchical construct of high-strength chitin in a protein matrix reinforced by calcium carbonate and other minerals, with varying amounts of chitin present depending on species and the body part.

The chitin content of a range of organisms is summarised in the table below (Table 1), with variability due to differing chitin content between body parts (e.g. crustacean claws and body) and methods of extraction deployed.

Table 1 Examples of chitin sources with their respective chitinous constituent dry weight compared to total source mass (adapted from Jones *et al*., 2020; Hamed *et al*., 2016; Soetemans *et al*., 2020)

Source	Chitin content
Crustacean shells	
Lobster	16-23%
Crab	25-30%
Krill	34-49%
Molluscs (cephalopods)	
Squid, cuttlefish, octopus	6-40%
Insect cuticles	
Cockroach	18-38%
Butterfly	22-64%
Silkworm	2-44%
Black soldier fly	8-24%
Fungi	
Cultivated mushrooms (basidiocarp)	8-43%
Mycelium	5-35%

Chitin containing by-products from the BSF range from 8% to 24% chitin content depending on the life stage of the insect (Soetemans *et al*., 2020). The amount of chitin in black soldier fly frass has been quoted as 14% (www.Agrigrub.co.uk) although data is scarce on this and variable.

The cellular wall of mushrooms has a relatively high chitin content (Antunes *et al*., 2020), although reports of chitin content vary depending on species of mushroom and the analyses undertaken. Wu *et al*. (2004) extracted chitin from *Agaricus bisporus* stipes (stalks) and found that chitin content reached 27% dry weight after stipes were stored at 25°C for 5 days. Chitin content determined by Vetter (2007) in *A. bisporus* found lower amounts of chitin as a % dry weight, ranging from 4.31% - 9.66% for the stipe and 4.35% - 8.31% for the pileus (cap). Nawawi *et al*. (2019) found 25.4% chitin from the stalk of *A. bisporus* and 15.0% from the cap. Nitschke *et al*. (2011) determined that chitin content in a range of different mushroom species including *A. bisporus* did not exceed 10%.

Waste from the mushroom growing process tends to consist of spent mushroom substrate (SMS) and mis-shapen mushrooms, stipes and the mycelia in the compost. As a result, the chitin content

in SMS is variable depending on the mushroom species and the amount of mushroom material present in the SMS itself.

Chitin and its derivatives are renewable, biocompatible, biodegradable, and non-toxic compounds that have been shown to have a wide range of biological properties including anti-cancer (Salah *et al*., 2013), antioxidant (Yen *et al*., 2008), antimicrobial (Goy *et al*., 2009) and anti-coagulant (Vongchan *et al*., 2003) properties. Additionally, they are used as biomaterials in a wide range of applications such as biomedical purposes for artificial skin, bones, and cartilage regeneration (Dash *et al*., 2011; Parvez *et al*., 2012), for food preservation such as for edible films (Muzzarelli and Muzzarelli, 2005), and for pharmaceutical purposes such as for drug delivery (Riva *et al*., 2011). See Hamed *et al*. (2016), Morris *et al*. (2019) and De Corato (2021) for a broader review of the applications of chitin and its derivatives.

Chitin is obtained from organisms after demineralization and deproteinisation treatments, however these typically rely on the use of an acid treatment to dissolve the calcium carbonate followed by alkaline solution to dissolve proteins (Younes and Rinaudo, 2015). In addition, a decolorization step is often added in order to remove pigments to obtain a colourless pure chitin. Lactic acid has been identified as a more benign approach to chitin extraction, and a recent Innovate UK project – iCRAB (integrated Chitin Ryegrass Acid Biorefinery) aimed to improve the economics of both chitin extraction and lactic acid production by combining the two in a single biorefinery process (https://pennotec.com/the-icrab-project/). One of the limitations in the use of extracted chitin on a large-scale is its water insolubility. Therefore, water-soluble derivatives have been produced, and chitosan (Fig. 1C) is the most important of these, and is obtained after the deacetylation of chitin (Kaczmarek, 2019). Chitosan has widespread uses in medicine, agriculture, food processing, nutritional enhancement, cosmetics, and waste water treatment (Hudson and Jenkins, 2001; Hamed *et al*., 2016; Aranaz *et al*., 2021).

Chitin and chitosan are potent elicitors of plant defence, inducing plants to resist or tolerate a wide selection of diseases and pests (Sharp, 2013; Orzali *et al*., 2017). There is a growing interest for using chitin in agricultural systems to reduce the negative impact of diseases and pests on the yield and quality of crops (El Hadrami *et al*., 2010; Badawyi and Rabea, 2011; Shamshina *et al*., 2019). The production of refined chitin and water-soluble chitosan from the by-products of crustacea and mollusc processing, mushroom farming and insect farming uses methods that use strong acids and alkali that can be ecologically damaging, expensive and therefore limit the overall environmental advantages of any subsequent reduced pesticide use (Sharp, 2013). When chitin and chitin containing materials are added to soil, several processes occur which enable the positive effects of chitin to occur.

The mechanisms behind the enhanced soil suppressiveness of pests and disease have been linked to a change in the structure and activity of the microbiota in soil, which leads to the suppression of plant pathogens (Mendes *et al*., 2011; Cretoiu *et al*., 2013; Andreo-Jiminez *et al*., 2021). Chitinolytic microorganisms use various extracellular enzymes to solubilize chitin (Wieczorek *et al*., 2019), and these chitinase enzymes will also degrade chitin-rich tissues of other organisms such as plant pathogens and pests which possess chitin within their structures (Sharp, 2013). The addition of chitin to soil may also aid beneficial microbial antagonists by providing a stable nitrogen-rich polysaccharide food source that boosts the population to the level where other mechanisms control the plant pathogens (Sharp, 2013). Soil treated with chitin amendments have a microbial community composition very different from untreated soils (Andreo-Jiminez *et al*., 2021), and the suppression of plant pathogens can extend for 2 years after treatment (Cretoiu *et al*., 2013).

It can be considered that it is the diversity and multiplication of chitinolytic bacteria and fungi after adding chitinous material to soil (or when composted) that significantly contribute to the benefits seen in terms of suppressing plant pathogens and pests.

It has also been suggested that chitin decomposition in the soil releases significant amounts of ammonia that may have an effect on plant-parasitic nematodes (Mian *et al*., 1982). However, Rodriquez-Kabana *et al*. (1987) suggested that as the control of nematode populations by chitin addition has also been found over longer periods than would be expected from the short-term release of ammonia, other control mechanisms such as increase of microbial antagonists as discussed above may also play a significant role.

Miya *et al*. (2007) isolated a range of chitin elicitor binding proteins from a number of crops, and these can lead to the expression of a number of defence-related genes in plants leading to the induction of local and systemic defences. Reactions induced by chitin and/or chitosan include ion flux variations, cytoplasmic acidification, membrane depolarisation and protein phosphorylation (Felix *et al*. 1993, 1998), chitinase and glucanase activation (Roby *et al*., 1987; Tayeh *et al*., 2015), lignification (Kawasaki *et al*., 2006; Ali *et al*., 2014), generation of reactive oxygen species (Kuchitsu *et al*., 1995), biosynthesis of jasmonic acid (Nojiri *et al*., 1996), and phytoalexins (Ren and West 1992; Yamada *et al*., 1993), and the expression of early responsive and defence-related genes (Minami *et al*., 1996; Libault *et al*., 2007). Moreover, chitosan induces proteinase inhibitors (Walker-Simmons and Ryan, 1984), phytoalexin biosynthesis (Hadwiger and Beckman 1980) and callose formation (Köhle *et al*., 1985).

Spent mushroom substrate has a variety of uses when applied to soil, and SMS could (partly) replace inorganic fertilisers (Grimm and Wösten, 2018). Mineral fertilisers are superior to SMS with respect to nitrogen, phosphorous, and potassium content. However, nutrient release is slower in the case of

SMS and therefore plants can potentially use them more effectively (Uzun, 2004). In addition, SMS improves soil structure by increasing organic matter, water capacity, microbial activity, soil temperature, and subsequently decreasing soil compaction (Grimm and Wösten, 2018). Soil amendments with composted mushroom substrate provided consistent root knot nematode nematode suppression and a significant increase of tomato plant growth (D'Addabbo *et al*., 2011).

As BSF production is expanding, the valorisation of BSF frass as a potential organic fertiliser is gaining importance (Gärttling and Schulz, 2022). However, little is known on the properties and variability of this by-product from BSF production. Frass is not a uniform product: its quality and composition are strongly affected by the feed substrates utilised in the BSF rearing process (Klammsteiner *et al*., 2020), and also any post-processing that may be necessary for sanitation purposes (Lopes *et al*., 2020) can change its properties considerably (Anyega *et al*. 2021). Quilliam *et al*. (2020) have suggested that fragments of chitin remaining in BSF frass biofertilisers can induce disease resistance in crop plants grown in biofertiliser-amended soil. Several studies show the relationship between the use of insect frass as promoters of resistance to biotic and abiotic stressors (Chavez and Uchanksi, 2021). In addition, BSF frass led to a reduction in wireworm populations when added to soil (Vickerson *et al*., 2016), and inhibited the growth of *Fusarium oxysporum* and *Rhizoctonia solani* (Choi and Hassanzadeh, 2019).

There are several studies of direct action or activation of plant defence responses due to the use of frass as a fertiliser (Poveda, 2021), and the recognition by the roots of microorganisms and biomolecules present in insect frass may be involved in the activation of plant systemic resistance.

4.1.2. Application of chitin for soil health, suppression of pests and pathogens of crops and crop nutrition

Wider reading of the published literature shows that chitin-based soil amendments may offer many benefits to crop production. Firstly, chitin provides a valuable source of carbon and nitrogen for microorganisms and is an important component of the soils' organic matter (Wieczorek *et al.,* 2019). Chitinous composts such as those produced by Angus Horticulture Ltd. contain a mixture of shellfish by-product and wood chip. While wood chip is not the topic of this review, this blend has the potential to increase both saprotrophic and chitinolytic organisms and thus improve nutrient availability. For example, Clocchiatti *et al*. (2020) used alkaline extraction of ergosterol (a fundamental lipid associated with fungal cell membranes) as a proxy for measuring fungal biomass after various organic amendments. Cellulose rich materials such as paper pulp and deciduous woody materials resulted in 'Moderate or strong initial stimulation of fungal biomass followed by gradual decrease or further increase' over the two month period of observation, highlighting potential benefits to soil fertility.

The lignin-cellulose complex present within wood chips undergoes biotransformation and transformation during the composting process leading to the production of humic and fulvic acids (Bekier et al., 2022). Humic substances present in mature composted woody material tend to be characterized by a high degree of stability, so that after being introduced into the soil they can effectively act as an additive, improving its fertility and increasing the resources of organic carbon (Kaluza-Haladyn et al., 2019). Composting woody biomass rich in cellulose and lignin's requires some nitrogen imput (e.g. from the addition of chitinous material), which enhances the biotransformation process, and in the initial period of composting the nitrogen content is too high and will be phytotoxic. With subsequent stages to obtain fully composted material, the nitrogen content decreases and the mature compost demonstrates no phytotoxicity (Sowiński et al., 2022).

Despite this, a recent review by De Corato (2021) highlighted the distinct lack of published work on the effect of chitin on soil quality such as structure and organic matter turn over. Chitin and chitosan may have a role in reducing organic and inorganic contaminants in soil and water (Singh *et al.,* 2020). Industries such as those concerned with electroplating, leather tanning, textiles and paint production have been linked to pollution of water with heavy metals such as chromium, which is toxic and can act as a carcinogen (Saravanan, Gomathi and Sudha, 2013). In their sorption studies, Saravanan, Gomathi and Sudha (2013), chromium ions were effectively removed from waste water with the addition of a chitin/bentonite biocomposite and this was optimal at pH 4. Similar effect has been reported for a chitin/chitosan nano-hydroxyapatite composite in the removal of copper (II) ions from an aqueous solution (Kousalya, Gandhi and Meenakshi, 2010). In addition, to heavy metals, chitin and chitosan have been used to extract pesticides contaminating water. Rahmanifar and Moradi Dehaghi (2013) successfully utilised chitosan beads embedded with silver oxide nanoparticles to remove the neurotoxin pyrethroid insecticide, permethrin, from water with an adsorption capacity of 99%.

Numerous studies have recorded beneficial reductions in soil-borne pests and pathogens following the application of chitin or chitosan in a range of cropping systems. The suppressive activity of chitin may be direct or indirect or a combination of both. Some mechanisms may be specific to certain types of organism e.g. plant pathogenic fungi, while others, such as induced resistance, are likely to be non-specific.

The bacterial and fungal microbial communities in chitin-amended soils are distinct from the microbial communities in non-amended soils (Andreo-Jiminez et al., 2021), however, to better understand the duration of the effects of treatments on disease/pest suppression, and whether there is a long-term impact on beneficial microbes, a time line study is needed, in which microbial populations and their functions are tracked together with levels of disease/pest suppression across multiple time points.

Direct effects of chitosan on fungal pathogens have been observed under *in-vitro* conditions, where mycelial growth is inhibited or retarded with increased chitosan dose. For example, Cheah, Page and Shepherd (1997) recorded a dose dependent reduction in the mycelial growth of *Sclerotinia sclerotiorum* grown on potato dextrose agar (PDA) plates amended with 1, 2 and 4% chitosan; the highest concentration (4%) resulted in a 53% reduction in growth as compared to the control (adjusted to pH 6.3). *Colletotrichum gloeosporioides* is a fungus that causes anthracnose on papaya leading to post harvest losses. Amendment of PDA with 2.5 and 3% chitosan resulted in complete inhibition of the fungus during the 7-day incubation period (Bautista-Baños *et al.,* 2003). Fungi and oomycetes have different sensitivities to chitosan as observed by (Palma-Guerrero *et al.,* 2008) who considered organisms used in biocontrol as well as pathogens. Here, chitosan reduced the mycelial growth of the fungi *Fusarium oxysporum* f.sp *radicis-lycopersici*, *Verticillium dahliae* and the oomycete *Pythium ultimum*, particularly at the highest concentration used (2 mg ml^{-1}). In contrast, *Gaeumannomyces graminis* var. *tritici*, the causal pathogen of the disease take-all in wheat, was relatively insensitive to chitosan even at the highest concentration (7% reduction when compared to the growth of the control).

As well as inhibition of mycelial growth, some authors report reduced spore germination following exposure to chitosan. For example, Palma-Guerrero *et al.* (2008) observed complete inhibition of spore germination for the fungi *Fusarium oxysporum* f.sp *radicis-lycopersici* and *Verticillium dahliae* when exposed to chitosan as a dose of 0.01 mg ml^{-1}.

Chitosan is thought to affect fungi directly through disruption of membranes leading to increased permeability. A recent review paper by Torres-Rodrigurez *et al*. (2021) describes how the amino (NH_3^+) glucosamine groups of chitosan interacts electrostatically with phospholipids in fungi, lipopolysaccharides in Gram-negative bacteria, teichoic acid in Gram-positive bacteria. Such interactions lead to a breakdown in membrane integrity, the loss of cellular metabolites, and ultimately, cell death. There is also antimicrobial activity through the chelation of soil micronutrients such as zinc, copper, and manganese, which increases positive charge and chitosan affinity to membranes.

Chitin and chitosan also have been reported to have activity against plant pathogenic oomycetes and bacteria (Sharp, 2013). In oomycetes, such as *Phytophthora infestans* (causal pathogen of late blight of potatoes and tomatoes) endomembrane integrity is affected, particularly the vacuoles. Huang *et al*., (2021) observed that *P. infestans* had reduced mycelial growth and sporangia production after chitosan application. Additionally, treated isolates showed low tolerance to adverse conditions, while fungicide performance was increased indicating a synergy between chitosan and chemical treatments. Furthermore, transcriptome analysis of treated *P. infestans* confirmed that

chitosan affected cell growth through changes in the activity of genes associated with cell membrane structure and function, metabolism and ribosome biogenesis.

Plasmodiophora brassicae is a protist (protozoan) that causes the disease club root in brassicas. The organism is problematic in arable rotations as it can persist in the soil for up to 15 year as resting spores. A study by Wang *et al*. (2012) showed that two types of chitosan inhibited resting spore germination and lowed disease severity regardless of the dose applied. Evans (1993) found that a chitin-containing product added to soil significantly reduced the clubroot disease score in Chinese cabbage (16.3%) compared to a score of 80% in untreated soil.

Another pathogen, capable of long-term persistence in soil (40+ years) is the fungus *Synchytrium endobioticum* (Chytridiomycota) which causes wart disease in potatoes; hypertrophy associated with roots, shoots and tubers. Wart disease is rarely found in the UK due to being a notifiable disease (quarantine organism - A2 list; EPPO (2022)), the requirement for contaminated fields to be scheduled (under control by phytosanitary bodies) and the use of potato cultivars with resistance to the disease. Despite its quarantine status, wart disease has recently been found in Denmark in 2014 (Van de Vossenberg *et al*., 2021) and Prince Edward Island, Canada in 2021 (Government of Canada, 2022). Early work by Hampson (1989) indicated that chitin or crab shell could completely eliminate infection by *S. endobioticum*. A follow-up study by Hampson and Coombes (1995) confirmed these results, showing that crushed crab shell applied to infested soil at 40 g or 80 g kg^{-1} soil resulted in no infections in the susceptible potato cultivar Arran Victory following harvest at 8 weeks after planting. The authors concluded that this treatment could reliably be used for short term management off the diseases, although the longer-term effects were not known.

A popular theory for pest and pathogen suppression, following chitin-based applications, is through increased populations of chitinolytic microorganisms (bacteria, fungi and actinomycetes); i.e. microorganisms that secrete the enzyme chitinase to break down chitin. Fungal cell walls primarily consist of glucans, chitin, glycoproteins and melanin. Whilst, the polysaccharide, glucans, is the main component, the content of chitin can be 10-20% in some filamentous fungi (Huang *et al.,* 2021; Betchem, Johnson and Wang, 2019; Garcia-Rubio *et al.,* 2019). Therefore, enhanced densities of chitinolytic microorganisms may lead to higher levels of suppression of plant pathogenic fungi, due to cell wall degradation. Evidence of shifts in microbial community composition after the application of an organic chitin rich amendment (a compost containing spores of the basidiomycete *Agaricus bisporus*) are presented in the work of Andre-Jimenez *et al.* (2021). These workers recorded reductions in the infection of sugar beet seedlings by *Rhizoctonia solani* following an application of the chitinous soil amendment. Sequencing of DNA extracted from soil using the Illumina MiSeq platform (16S rDNA for bacteria and ITS2 for fungi) showed that there was a greater abundance of Oxalobacteraceae (bacteria) and Mortierelleceae (fungi) species. In particular, *Mortierella*,

Aspergillus and *Mucor* species are known to have chitinolytic activity which may explain the suppression observed. Furthermore, bacteria found in samples associated with chitinous amendments, such as *Flavobacterium*, *Pseudomonas* and *Microbacterium*, *Devosia* spp. (Hyphomicrobiaceae) and *Rhizobium* spp. (Rhizobiaceae) are associated with the suppression of *R. solani*.

In addition to fungal cell walls, the eggshells of plant parasitic nematodes include a chitinous layer. For instance, the eggshells of the root knot nematode *Meloidogyne javanica* contain 30% chitin (Bird and McClure, 1976), whilst the golden or yellow potato cyst nematode, *Globodera rostochiensis* has 9% chitin (Clarke, Cox and Shepherd, 1967). Chen and Peng, (2019) highlight that chitinolytic microorganisms break down the chitinous layer into chitobiose, which in turn disrupts eggs and young juveniles, leading to increased mortality. However, Spiegel, Chet and Cohn (1987) suggested that chitin degradation results in the production of ammonia and nitrous acid, which are toxic to nematodes. Their hypothesis was based on the observations of nematode suppression soon after amendment.

The production of ammonia due to microbial degradation of chitin can be phytotoxic (Culbreath *et al*., 1985), and these authors have demonstrated that the addition of a carbon source (in their case waste paper pulp) to the soil along with chitin reduced the phytotoxic impact of the chitin. This reduction in chitin phytotoxicity is thought to be due to the additional carbon source preventing a reduction in soil pH by acting as a buffer, providing a stable pH and environment for plant growth. Chitin alone leads to a sharp drop in pH which leads to variability in nutrient availability to young plants, and exacerbates the effects of toxic elements that may be present in the soil. Evans (pers comm) has seen the effect of chitin toxicity on potatoes, through a transient yellowing of leaves, however, the plants do recover and ultimately yield higher than potatoes grown in non-chitin amended soil. Advice has been with the chitinous compost produced by Angus Horticulture Ltd, which comprises shellfish material and wood chips, that the material is applied to land several weeks before planting of any crop, in order to mitigate any potential short-term phytotoxicity due to a flush of ammonia from the initial degradation of chitin.

Observations of nematode suppression through chitinous amendments have predominantly been observed for cyst or root knot nematodes. An unpublished study by Adekoya (2020) developed a systematic map to summarise studies conducted on the application of chitinous amendments for the suppression of plant parasitic nematodes. His study showed that 64% of 107 experimental observations were conducted on root knot nematodes, with a further 15% on cyst nematodes (Figure 2).

Ebrahimi *et al.* (2016) conducted pot experiments to evaluate the effect of various organic amendments including crab shell compost (chitinous waste) on the encysted eggs of the potato cyst nematodes *Globodera pallida* and *G. rostochiensis*. In order to stimulate the activity of chitinolytic microorganisms, crab shell compost was added to the potting soil 8 months prior to the experiment. Batches of PCN cysts were buried in the amended pots for a period of 8, 12 and 16 weeks before being recovered and assessed for egg viability. At each of these intervals there was a significant reduction in the percentage of viable eggs, of both PCN species, from the pots with the crab shell compost when compared with the untreated control; the viability of *G. pallida* eggs was reduced by 18% and *G. rostochiensis* by 20.7%. Similarly hatching assays performed on cysts recovered from the soil amended with the crab shell compost showed that overall hatching was significantly reduced. Given that the PCN assessments for this work was conducted on soil where the crab shell compost was applied 8 months before the experiment, the release of ammonia is unlikely to be the cause of the suppression as the release is short lived.

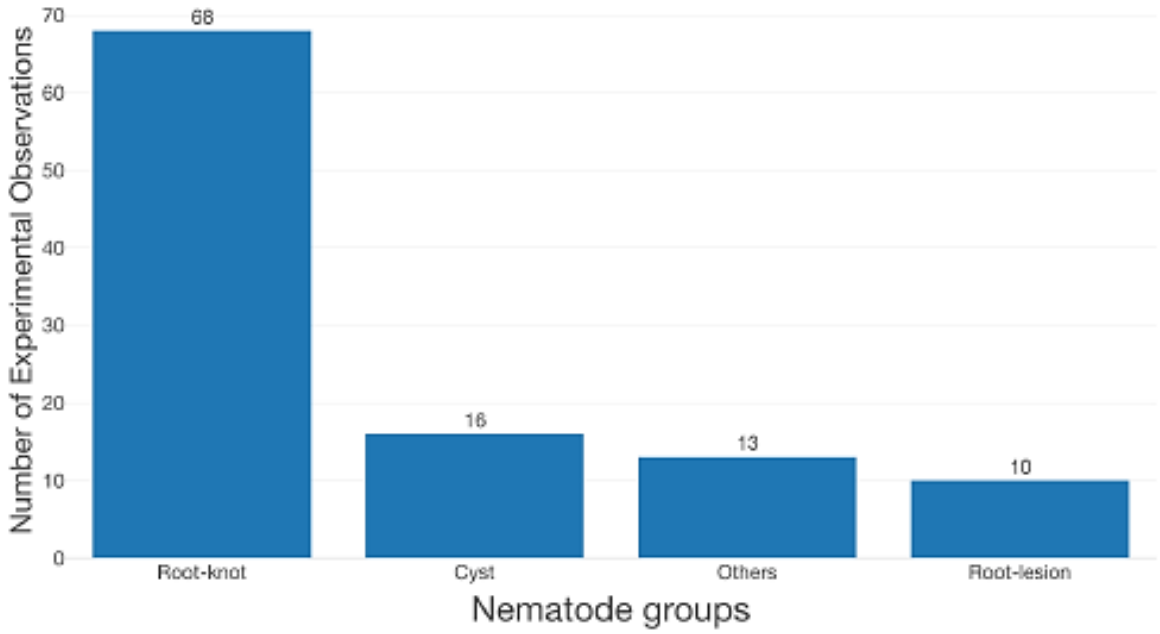

Figure 2 The proportion of experimental observations (EO) (N=107) for root knot (*Meloidogyne* spp.), cyst (*Globodera* and *Heterodera* spp.), root lesion (*Pratylenchus* spp) and other plant parasitic nematodes investigated in relation to suppression with chitinous soil amendments. This data is based on 57 eligible articles

Due to the phase out of many synthetic crop protection products, there is greater interest in alternative pest and disease management approaches. Korthals *et al.* (2014) conducted a long-term study to evaluate the effect of 10 'soil health' treatments including chitin on the suppression of the fungal pathogen *Verticillium dahliae* and root lesion nematodes (*Pratylenchus penetrans*). Chitin was applied in the form of shrimp debris (Gembri) at a rate of 20t/ha and incorporated to 20cm depth. Out of all the treatments investigated, the chitinous material caused the greatest reduction (>50%) of *V. dahliae* microsclerotia and also significantly reduced *P. penetrans* over a 5-year period. Furthermore, a >60% increase in potato yield was observed three years after the application of the chitin treatment.

Chitin and chitosan can have beneficial effects through enhancing the activity of microbial biopesticides such as *Bacillus subtilis* (distributed in the UK as Serenade ASO), *B. thuringiensis*, *Beauveria bassiana* (e.g. Botaniguard) and *Trichoderma* spp (e.g. Trianum P) (Sharp, 2013). Enhanced activity of these antagonists, following chitin or chitosan applications is linked to their secretions of chitinase. Essentially, an additionally supply of chitin provides additional substrate for them to proliferate on. Table 2 shows examples of some of the studies undertaken on the effect of chitin or chitosan on microbial antagonists.

Table 2 Examples of improved activity of biopesticides against pathogens and pests due to chitin applications

Antagonist scientific name	**Type of organism**	**Target pathogen or pest**	**Crop type and main result**	**Citation**
Bacillus subtilis	Bacterium	*Fusarium udum* (Fusarium wilt)	Pidgeon pea – increased emergence and disease reduction	Manjula and Podile (2001)
B. licheniformis **LS674** ***B subtilis*** **HS93** ***T. harzianum***	Bacterium Bacterium Fungus	*Phytophthora capsici* and *Rhizoctonia solani* (root rot)	Pepper (*Capsicum annum*) – Improved suppression of *Phytophthora* or *Rhizoctonia* root rot when HS93 was applied with 0.5% chitin. LS674 and *T. harzianum* had improved suppression against *R. solani* if applied with chitin. Some addition benefits in yield of pepper recorded with these combined applications.	Sid Ahmed *et al.,* (2003)
Beauveria bassiana **(B2 and B4)** ***Pseudomonas fluorescens*** **(TDK1 and Pf1)**	Fungus Bacterium	Leaf miner (*Aproaerema modicella*) Collar rot (*Sclerotium rolfsii*)	Groundnut – Chitin improved the activity of the biocontrol agents against leaf miner and collar rot	Senthilraja *et al.* (2010)

Elicitors are naturally occurring or synthetic molecules involved in the triggering of defense responses in plants to biotic and abiotic diseases. Avirulent pathogens are recognised by their plant hosts through 'Microbe or Pathogen Associated Molecular Patterns (MAMPs/PAMPs) by Pathogen Recognition Receptors (PRRs). PAMPs (synonymous with elicitors) activate signalling pathways leading to structural and chemical defences such as reactive oxygen species (ROS), phytoalexins, callose papillae, cell wall reinforcements and the accumulation of pathogenesis related proteins (PR proteins) (Thakur and Sohal, 2013). Chitin is generally accepted as a plant defense elicitor; plants break down chitin through the secretion of chitinase into oligosaccharide fragments that activate some of the defences described above (phytoalexins, defence gene activation and ROS) (Li *et al*., 2020). For instance, Hayafune *et al.* (2014) undertook NMR spectroscopy and computational work to clarify the molecular basis of the Chitin-elicitor Binding Protein (CEBiP), a type of PRR, in rice (*Oryza sativa*). In nature, CEBiP would bind to chitin found in the cell walls of pathogenic fungi, to subsequently trigger cellular defense mechanisms. The work of Hayafune *et al.* (2014) (See Figure 3) identified that (GlcNAc)8, an oligosaccharide of chitin, was detected by a complex involving CEBiP and chitin-elicitor receptor kinase 1 (OsCERK1).

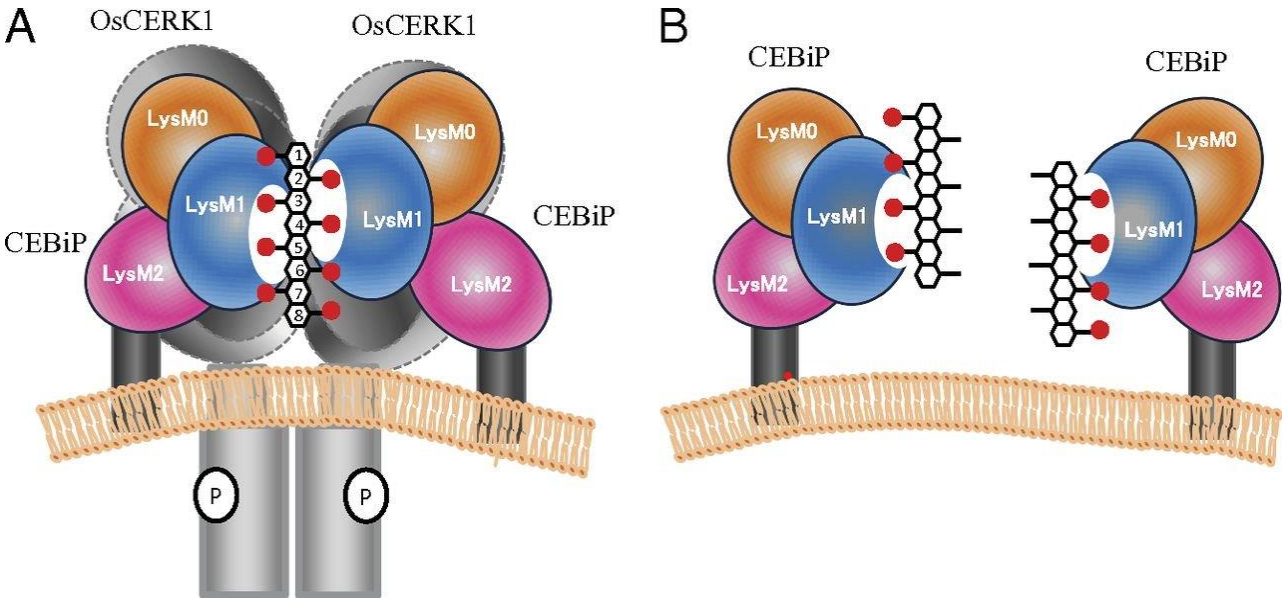

Figure 3 A hypothetical model to show chitin defense activation in rice (*Oryza sativa*) by (GlcNAc)8 (an oligosaccharide of chitin represented in the centre of the diagram); (A) a sandwich-like model of activation based on a complex of chitin elicitor binding proteins (CEBiP) and chitin-elicitor receptor kinase 1 (OsCERK1), (B) a model to show dimerisation activation inhibition by (GlcNβ1,4GlcNAc)4 (a unique oligosaccharide). Figure adapted from Hayafune *et al*. (2013).

Oligochitosan is a water-soluble compound, produced through the hydrolysis of chitosan (Kim and Rajapakse, 2005), that can be applied directly to crop plants through foliar sprays, irrigation systems or seed soaking. In contrast, chitin and chitosan are not soluble and therefore cannot be applied in the same way. The review by Yin, Zhao and Du (2010), highlights how oligochitosan can be used to prime immune systems of various crop plants to lower infections from a variety of fungal, oomycete and viral pathogens. Moreover, Escudero *et al.* (2017) investigated the role of chitosan in tomato plants treated with the nematode egg parasite, *Pochonia chlamydosporia* against the root knot nematode *Meloidogyne javanica*. Interestingly, chitosan is known to increase the sporulation of *P. chlamydosporia* and upregulate the expression of VCP1, a serine protease associated with the parasitism of nematode eggs. This study showed that *P. chlamydosporia* could use chitosan as a carbon source, with greater mycelial production observed, and improved colonisation of tomato roots. Additionally, there was some evidence that chitosan irrigation applied to soils, naturally infested with *P. chlamydosporia*, increased plant growth and reduced *M. javanica* multiplication.

As well as having a role in the suppression of pests and diseases, chitin and chitosan can have direct and indirect effects on plant growth stimulation and yield performance. There are several plausible hypotheses to support this including (i) the provision of a carbon source for soil microbes, leading to a greater breakdown of organic matter and increased soil nutrients; chitin and chitosan contain around 6.1-8.3% nitrogen, (ii) chitin/chitosan improving the uptake of nutrients, (iii) stimulation of arbuscular mycorrhizal fungi or plant growth promoting rhizobacteria (iv) greater water retention in sandy soil through porous structure (hydrogels). Increases in plant growth or yield, following chitin/chitosan applications, have been observed in a range of crops including rice (Boonlertnirun, Boonraung and Suvanasara, 2008), soybean sprouts (Lee, Kim and Kim, 2005), tomato (Egusa *et al.,* 2020) and wheat (Wang *et al.,* 2015)

4.1.3. Summaries of interviews with insect protein suppliers

ECOInsect

An interview was held with Paul Cartwright, CEO of ECOInsect on the 18th February 2022. ECOInsect is a new company, looking to produce black soldier fly (BSF) protein for animal feed. The company has been working with several universities such as Harper Adams University on Innovate UK grants. Since October 2019, ECOInsect began investing in new sites and scaling up their production with the aim of entering the market in 2024. The type of processing used produces a unique leachate, which is being investigated as a biostimulant for enhancing plant growth.

In terms of solid by-product ECOInsect is not anticipating producing large volumes. Tomato waste and brewery spent grain is the main feed stock being used for BSF culture with brewer's grain providing more by-product. By 2024-2026, Mr Cartwright estimated that the company will produce 750 -1000t per annum of by-product from BSF production. Their solid by-product contains frass,

pupal cases and dead adult flies. In terms of the industry as a whole, Mr Cartwright estimates that 10-15% of the income of BSF production will come from sale of the by-product.

Mr Cartwright strongly agreed that the solid by-product of BSF production would be a small income stream for his company, but could provide a significant income stream for the insect farming industry as a whole. The solid product produces a compost or soil improver of a much higher value compost; it is a much finer material that traditional compost. For ECOInsect, there is unlikely to be enough material to make this a major income generator (maybe 15% of total income). On the other hand, ECOInsect is looking for other niche markets (biostimulants) to supply the liquid by-product.

As ECOInsect do not currently supply solid waste, Mr Cartwright could not comment on the type of customer that purchases solid by-product and thee specifications or criteria that they have, such as particle size, chemical or biological analysis. Mr Cartwright suggested that Hexafly (Republic of Ireland) produced higher volumes of by-product. In terms of legislative requirements, Mr Cartwright highlighted the difficulties of classifying the by-product as a fertiliser, compost (PAS 100 accreditation) or a biostimulant. He was not aware of any concerns about allergens associated with the by-products.

In terms of the future R&D required, Mr Cartwright highlighted that he would be interested in studies investigating chitin/by-product in plant protection and/or pharmaceuticals.

Beta Bugs Ltd

An interview was held with Dr Thomas Farrugia, CEO of Beta Bugs Ltd and Des Cave, head of business development on 11th February 2022. Beta Bugs was founded in 2017 by Dr Farrugia and are involved in the breeding and distribution of BSF to insect farming companies.

As Beta Bugs are involved in BSF breeding rather than production, they do not produce substantial amounts of by-product in the form of frass or other BSF body parts such as pupal cases or dead flies. The small amount of by-product that they do produce tends to be sold on an ad hoc basis to a range of clients, with a worm farmer being one that was mentioned. Dr Farrugia and Mr Cave did indicate that the potential for commercial use of BSF by-products such as frass was likely to increase significantly once large scale BSF production was underway in the companies that they supply BSF to. They cited the WWF-Tesco commissioned report (Ffoulkes *et al*, 2021) as a useful indicator of the levels of by-product likely to be available in the future (up to 58,000 tonnes a year by 2050).

Both Dr Farrugia and Mr Cave indicated that processing of the frass by-product would likely be necessary before it is applied to soil, and there would be variability on the nutritional content dependent on the food stock that the BSF larvae are reared on. They thought that addition of the

material to composting of other materials might be one way to utilise the material, as it is unlikely that the frass could be applied 'neat' to agricultural soils as it is currently classed as an animal by-product. However, there is frustration and confusion within the insect farming sector as there is no clear regulation around how to treat insect frass which prevents this being sold or used by farmers as a fertiliser or soil amendment.

Neither Dr Farrugia or Mr Cave were aware of any issues likely to be related to allergens, although it was mentioned that handling of the by-product may pose a risk to someone allergic to allergens and that COSHH measures should be considered.

In terms of future R&D requirements regarding the potential uses of by-products from BSF production, it was suggested that effort should be made to identify the best approaches for incorporating this material into products that could be utilised on agricultural land, whether it be as a biofertiliser or as a component of a composting process to produce compost or soil amendments for use on land. Both considered the known benefits of BSF frass in terms of inducing plant defences and impact on soil-borne diseases to be of significant value and worthy of future research.

Informal discussions with UK researchers

Interviews were also conducted with Dr Elaine Fitches from Durham University and Prof Rob Lillywhite from the University of Warwick. Both are involved in an R&D project to characterise BSF frass, and investigate its use as a plant fertiliser and growth stimulant. Both Dr Fitches and Prof Lillywhite commented on the current uncertainty from a regulatory perspective on the status of BSF frass as an animal by-product and the subsequent legislation being a barrier to its development as a fertiliser and growth stimulant. They have had promising results on growing plants when BSF frass has been incorporated into the growing medium and commented on the need for an industry wide standard feed for BSF larvae to help with consistency of frass by-product. There are analytical issues in terms of determining the chitin content in BSF frass, and doubt was expressed at the claims of 14% chitin content in BSF frass stated by one producer.

The research being undertaken by Prof Lillywhite, Dr Fitches and others in characterising the benefits from BSF frass points to a positive future for this by-product from BSF production, provided the regulatory and legislative issues can be overcome.

4.2. Physico-chemical analysis of chitinous by-product streams

A comprehensive nutrient and physico-chemical analysis were undertaken for composted shellfish material (supplied by Angus Horticulture Ltd, Forfar), spent mushroom substrate (supplied by Monaghan Mushrooms, North Berwick) and black soldier fly frass (supplied by Beta Bugs Ltd, Roslin). The full results are provided in the Appendix, with selected results presented in Tables 3-5 below.

Table 3 Water extractable nutrients (mg/kg) in dry matter of composted shellfish by-product, spent mushroom substrate and black soldier fly frass

Parameter	Composted shellfish	Spent mushroom substrate	Black soldier fly frass
		In dry matter (mg/kg)	
NH_4-N (ammonium-N)	3389.54	591.61	5488.33
NO_3-N (nitrate-N)	2732.21	10.33	7.19
NH_4-N plus NO_3-N	6121.75	601.94	5495.52
Phosphorus as P	188.63	601.00	5616.15
Potassium as K	4141.19	20274.44	5887.77
Calcium as Ca	1986.28	13672.80	239.67
Magnesium as Mg	480.14	2028.38	343.52
Sulphur as S	3458.13	19898.81	3690.84
Boron as Bo	0.66	N/A	0.88
Copper as Cu	N/A	N/A	0.88
Iron as Fe	N/A	12.21	28.76
Manganese as Mn	0.03	4.23	0.40
Molybdenum as Mo	N/A	N/A	N/A
Zinc as Zn	0.46	1812.40	6.07
Chloride as Cl	4804.23	4479.35	3810.67
Sodium as Na	2697.92	1868.74	1493.91

N/A – not analysed as very low level detected when analysing fresh material

The water extractable nutrients and total nutrients present in each of the chitinous by-products tested are summarised in Tables 3 and 4. Each of the by-products evaluated possess beneficial levels of the key nutrients for crop growth and these nutrients will also be beneficial for maintaining microbial populations in the by-products themselves and after adding to soil. In particular, there are significant

levels of nitrogen, phosphorus and potassium as well as other nutrients such as sodium, calcium and magnesium.

The values shown should be considered as illustrative rather than a definitive guide to the likely content of these materials, as each batch of by-product will have its own particular profile. However, what these values show is that the application of these and similar materials to soil will add a beneficial amount of nutrients for the benefit of crops and the soil microbiome, as well as adding beneficial microbes to the soil.

Table 4 Total nutrients (mg/kg) in dry matter of composted shellfish by-product, spent mushroom substrate and black soldier fly frass

Parameter	Composted shellfish	Spent mushroom substrate	Black soldier fly frass
		In dry matter (mg/kg)	
Nitrogen as N	28810	24870	41390
Phosphorus as P	6200	4671	11870
Potassium as K	7067	25430	9192
Calcium as Ca	45160	55910	4505
Magnesium as Mg	8342	5734	3251
Sulphur as S	5801	34630	6531
Boron as Bo	44	17	14
Iron as Fe	16460	3143	610
Manganese as Mn	466	575	80
Molybdenum as Mo	4	3	3
Sodium as Na	4843	2238	2104

Composted shellfish, spent mushroom substrate and BSF frass contain a significant amount of organic matter and carbon (Table 5). The C:N ratios present in the by-products (between 9.5:1 and 16.7:1) suggest that the nitrogen present will be mineralised and be available for plant uptake if these materials were to be added to soil. These C:N ratios are also indicative of a relatively fast microbial decomposition of organic matter in the material, which may explain inconsistent results in chitin analysis (see Section 4.3). The addition of these materials to soil would subsequently help in the breakdown of plant residues and increase nitrogen availability to crops.

Table 5 Physico-chemical properties of composted shellfish by-product, spent mushroom substrate and black soldier fly frass (fresh)

Parameter	Composted shellfish	Spent mushroom substrate	Black soldier fly frass
Dry matter	68.6% m/m	34.6% m/m	77.7% m/m
Moisture	31.5% m/m	65.4% m/m	22.3% m/m
Organic matter (loss on ignition)	32.2% m/m	24.8% m/m	72.8% m/m
Organic carbon (LOI ÷1.72)	18.7% m/m	14.4% m/m	42.3% m/m
pH	7.0	6.0	7.7
Electrical conductivity	473 mS/m @25°C	283 mS/m @25°C	219 mS/m @25°C
NH_4-N : NO_3-N (ratio)	1.24 : 1	57.27 : 1	763.33 : 1
Carbon : Nitrogen (ratio)	9.5 : 1	16.7 : 1	13.2 : 1

4.3. Review of legislative issues regarding use and acceptability of chitinous by-products

Standardisation of chitin content

As mentioned previously, obtaining by-products from the shellfish industry, spent mushroom substrate (SMS) and black soldier fly (BSF) frass will provide a variable chitin content depending on the shellfish material, the composition of the SMS, and the substrate used for rearing the BSF. Consequently, standardisation and consistency of chitin content is difficult and unreliable. The methodology used relies on specialist chemical extraction and analysis, which is time consuming, and expensive if using commercial laboratories. Results obtained from these laboratories have been inconsistent, with problems reported by the laboratories in terms of sample preparation and especially when trying to obtain a chitin content from a complex processed material such as composted shellfish, spent mushroom substrate, and insect frass that is mixed with insect feed material. There is also the likelihood of chitin degradation occurring within the material, and it should be borne in mind that for processed material such as composted shellfish and spent mushroom substrate, microbial degradation of chitin will be an ongoing process within the material.

It has been reported that by-products from the black soldier fly range from 8% to 24% chitin content depending on the life stage of the insect (Soetemans *et al*., 2020). The amount of chitin in black soldier fly frass has been quoted as 14% (www.Agrigrub.co.uk).

The cellular wall of mushrooms has a relatively high chitin content of chitin (Antunes *et al*., 2020), although reports of chitin content vary depending on species of mushroom and the analyses undertaken. Waste from the mushroom growing process tends to consist of spent mushroom substrate (SMS) and mis-shapen mushrooms, stipes and the mycelia in the compost. As a result, the chitin content in SMS is variable depending on the mushroom species and the amount of mushroom material present in the SMS itself.

There is still research to be undertaken to determine whether time since production of the chitinous 'product' – composted material, BSF by-product or mushroom waste substrate – is an important factor in determining the efficacy of the material when added to soil in terms of suppression of pathogens/pests. It is recommended that standard methods of analysis for chitin and chitosan in these types of products should be developed in order to address the inconsistencies in analytical results.

Regulations

The intended purpose of the chitinous by-products discussed in this report would be as soil amendments or fertilisers for application to the soil. Further to the legislation relevant to the usage or application of these by-products that are detailed below, the development of viable saleable products requires consideration of the legislative position relating to their sale or supply. In the UK, the sale of fertilisers is covered by The Fertiliser Regulations 1991 (as amended) (https://www.legislation.gov.uk/uksi/1991/2197/contents/made). Under these regulations, fertilisers do not require to be registered, but the regulations do specify labelling and packaging requirements and there is a responsibility on the manufacturer to declare the nutrient content. Soil health amendments and soil improvers are neither explicitly defined by, nor within the scope of, the UK 1991 regulations, although the definition of a form of "compound fertiliser" is said to exclude "any materials sold or offered for sale for improving soil structure or as growing media, which contain less than 1 % of each of these nutrients", where the nutrients in question relate to nitrogen, phosphorus pentoxide and potassium oxide.

Outside the UK, the sale of fertilisers and soil amendments is regulated by the legislation that is applicable in the country in question. Within the EU, for example, the sale of fertilisers and related products in each Member State is covered by national legislation and by EU-wide legislation. From mid-July 2022, Regulation (EU) 2019/1009 will apply in full and this legislation specifies detailed requirements for soil improvers and for 'fertilising products' that have compost as a component.

A key aspect of legislation relevant to the sale of fertilisers and soil improvers is the specification of the nutrient content on product labels. In general, the applicable regulations specify permitted tolerances between stated nutrient content and the actual nutrient content of the product. The

statement of the nutrient content is also important from the perspective of informing users and purchasers of the product details. Therefore, the development of a saleable fertiliser or soil amendment based on chitinous by-products has an inherent need for consistency and confidence in the characteristics of the product.

Shellfish

By-products from the shellfish industry are classed in the UK as animal by-products (ABPs) and there is comprehensive guidance provided here (https://www.gov.uk/government/collections/guidance-for-the-animal-by-product-industry) and here (https://www.gov.scot/policies/animal-health-welfare/animal-by-products/) on all regulations relating to the use of ABPs.

Relevant to the use of shellfish by-products for use on agricultural land, there are regulations regarding unprocessed by-products and those that have been composted or made into fertiliser. If planning to spread unprocessed chitinous shellfish by-product onto agricultural land, an Environment Agency (EA) or Scottish Environmental Protection Agency (SEPA) waste disposal permit or exemption will be required. Shellfish shells must be processed in some way before direct application to land, unless the soft tissue and flesh has been removed from the shell. Fully removing flesh from shellfish can be difficult to achieve, particularly from crustaceans such as crabs. However, it is still possible to spread shells from crustaceans on land without processing, if the following conditions are met: -

- they have been cooked in a government approved fishery products processing plant
- soft tissue and flesh have been removed to leave no more than 40% volatile solids (this will need to be determined in a laboratory)
- the shells have been crushed (but not reduced to powder)
- if the shells are stored before application to land, farmed animals don't have access to them
- no farmed animal can access the land where the shells are applied for 21 days after application (pigs can't access the land for 60 days)
- the land where the shells are applied is ploughed immediately after application or some other method is used to mix the shells into the soil immediately after application

Shells from molluscs (e.g. oysters, mussels and scallops) can also be spread on land without processing if the conditions above for crustacean shells are met.

If shellfish by-products are to be composted, the compost site needs to be approved and validated by the Animal and Plant Health Agency (APHA) - https://www.gov.uk/guidance/using-animal-by-products-at-compost-and-biogas-sites. There are strict UK standards to be followed and achieved during the composting process, the key ones being the time and temperature requirements when

composting, and the particle size of the material being composted. If composting material in a closed reactor system, a minimum temperature of 60°C must be achieved for 2 days for material with a maximum particle size of 400mm. For smaller maximum particle size of 60mm in a closed reactor, a minimum temperature of 70°C need only be achieved for 1 hour.

If composting material using a housed windrow system, a minimum temperature of 60°C must be achieved for 8 days (during which the windrow must be turned at least 3 times, at no less than 2 day intervals), for material with a maximum particle size of 400mm.

Production of compost in the UK is assured by the Compost Certification Scheme (https://www.qualitycompost.org.uk/) to the BSI PAS100 standard (https://standardsdevelopment.bsigroup.com/projects/9017-01020#/section). Additionally, in England, Wales and Northern Ireland, certification in accordance with the Quality Protocol is applicable. Compost produced in this way is considered a product and is not subject to the need for an environmental permit before application.

Shellfish by-products are classified as Category 3 ABPs and if they are to be made into fertiliser (https://www.gov.uk/guidance/making-fertiliser-from-processed-animal-by-products-abps) must be processed through crushing to obtain a maximum particle size, require heat treatment (at temperatures higher than that for composting), and add an authorised mixing component to processed material before you can use them as fertiliser. Authorised mixing components include wood shavings and limestone chips.

Black soldier fly (BSF)

Black soldier fly frass is a mixture of insect manure, exoskeletons from moulting processes, and residuals from the processed substrate. Frass provides a source of nitrogen, phosphorous and other nutrients, as well as chitin, which can stimulate plant defences. Because insects are considered as farmed animals, the frass is considered as a manure and would therefore be classed as an animal by-product. However, there is no clear regulation around how to treat insect frass which prevents this being sold or used by farmers as a fertiliser. This can be sold to the home market at present, for gardeners to use, but it is considered a 'grey' market (Ffoulkes *et al*., 2021). Frass can be used as a soil conditioner and is currently sold for this purpose. But if frass is to be sold as an agricultural fertiliser, they must first be treated at an APHA-approved plant (https://www.gov.uk/guidance/making-fertiliser-from-processed-animal-by-products-abps). If frass is to be composted the PAS100 specifications for composted materials will need to be adhered to (https://www.qualitycompost.org.uk/). It has been estimated that by 2050 there could be between 3,000 to 58,000 tonnes of frass/year from UK BSF production depending on the size and number of BSF facilities in place (Ffoulkes *et al*., 2021).

Evidence from other countries suggests that a strong domestic market for frass in agriculture and horticulture would provide an important secondary revenue stream within insect farming. Using frass to return nutrients to the soil and displace fossil-based fertiliser has considerable environmental benefits. Consequently, it is recommended that regulators review the use of frass as a fertiliser and soil enhancer in other countries and develop a clear framework for its use in the UK (Ffoulkes *et al*., 2021).

Spent mushroom substrate (SMS)

Spent mushroom substrate is classified as a non-meat food waste in the UK, and as a result is subject to different regulations than shellfish or insect by-products. In England SMS has an exemption (called a U10 exemption) that allows its spread on agricultural land (maximum of 50 tonnes per ha per year) to replace manufactured fertilisers or lime to improve or maintain soil (https://www.gov.uk/guidance/waste-exemption-u10-spreading-waste-to-benefit-agricultural-land).
In Scotland, the application of SMS to land is regulated by SEPA. To allow SMS application to agricultural land a Paragraph 7 exemption licence (for a fee) needs to be obtained (https://www.sepa.org.uk/regulations/waste/activities-exempt-from-waste-management-licensing/).
All Paragraph 7 exemption applications to SEPA must include a "Certificate of Agricultural Benefit" (prepared by a suitably qualified individual), which demonstrates that the application of SMS will result in agricultural benefit or ecological improvement, and no more than 50 ha block of land can have SMS applied to it.

Presence of allergens

Shellfish are one of the leading causes of food allergies and shellfish allergens are common cause of food-induced anaphylaxis in adults (Woo and Bahna, 2011). The two invertebrate phyla of Crustaceans and Molluscs are generally referred to as "shellfish" (Lopata *et al*., 2016). Crustaceans are classified as arthropods together with spiders and insects. Molluscs are a large and diverse group, subdivided into the class's bivalve, gastropod, and cephalopod and include several important seafood groups including mussels, oysters, abalone, snails, and squid. There are also land molluscs within this group which include snails and slugs.

At least 34 shellfish allergens have been identified and characterized from various crustacean and mollusc species and registered in the International Union of Immunological Societies (IUIS) Allergen Database (www. allergen.org). Almost all of the known characterized allergens are found in the edible portions of various shellfish species, with tropomyosin being the major allergenic protein across all edible crustacean and mollusc species (Lopata *et al*., 2016). Tropomyosins are present in muscle and non-muscle cells (Fernandes *et al*., 2015). Most shellfish-allergic individuals cross-react with several crustacean or mollusc species due to the high structural similarity of tropomyosin proteins (Fernandes *et al*., 2015).

The use of shellfish by-products for use as soil amendments raises the potential threat of spreading allergens such as tropomyosin onto the land and consequently another route into the human food chain. However, the measures outlined in the section above on ensuring that flesh on shellfish by-products has been removed through various processing or composting procedures significantly mitigates this threat.

A series of tests for tropomyosin has been conducted on composted shellfish and crops grown in land treated with the composted shellfish by Angus Horticulture Ltd in response to a request by Red Tractor Ltd and a commercial food provider on allergen risks. Samples of shellfish, composted shellfish, harvested potatoes and winter wheat seed from soil that was treated with composted shellfish produced by Angus Horticulture Ltd were sent to a commercial laboratory for testing for the presence of tropomyosin. A sample of the composted shellfish was also sent to the Health and Safety Executive (HSE) laboratory for tropomyosin analysis.

As expected, tropomyosin was present in the raw shellfish but was below detectable levels in the tests on composted shellfish carried out at a commercial laboratory. Tropomyosin from molluscs (but not crustacea) was detected at very low levels (~ 20 ppb) on unwashed potatoes but not on washed potatoes or winter wheat seed grown in land that had been treated with composted shellfish. On submission of samples from land where no composted shellfish had been applied there was a positive result for tropomyosin (~ 20 ppb) on unwashed potatoes. The presence of the allergen in fields where no composted shellfish had been applied suggests that there is a natural low level of tropomyosin present in soil. Prof Andreas Lopata, who leads the Molecular Allergy Research Laboratory in the College of Public Health, Medical and Veterinary Sciences, James Cook University in Australia was approached to give his view on the results obtained, and his opinion is that there is a residual low level of tropomyosin present in soil from molluscs such as snails and slugs, and invertebrates such as earthworms and insects, which is likely to explain the low levels being detected in the positive results from untreated soil and unwashed potatoes.

After obtaining these results the HSE were approached and they offered a different method of analysis (not ELISA) which the HSE state is for tropomyosin detection. Samples of the composted shellfish were sent to the HSE and the results indicated that the two samples of composted shellfish analysed contained tropomyosin at 252.83 ng/g compost (or 0.000025 %) and 417.84 ng/g compost (or 0.000042 %). These figures equate to 252.83 ppb and 417.84 ppb respectively. This is a significant reduction from the 3-5% tropomyosin content typically found in raw shellfish. The HSE Service Manager advised that the levels of tropomyosin detected in the compost were very low and of no cause for concern in terms of allergenicity. A set of comments/suggestions regarding the issue of allergenicity relating to the composting of shellfish and its' use were helpfully provided by the HSE, and are included in the Appendix. A key statement from the HSE was that a maximum of 5,000 ng

of shellfish tropomyosin per gram of compost should be considered, which is 10 times the maximum found in the composted shellfish. When the dilution effect of the addition of composted shellfish to the soil is considered (for example 1 tonne of composted shellfish per ha), the amount of tropomyosin being added to the soil is very low, and as the HSE stated, are of no cause for concern in terms of allergenicity.

Insect based foods pose potential risk to shellfish allergic patients due to homologous proteins including tropomyosin being present (Palmer, 2016). Cross-reactive allergens such as tropomyosin, arginine kinase and myosin have been identified in BSF and their cross-reactivity increase the likelihood of allergic reactions occurring in consumers who would consume BSFL that are allergic to crustaceans (Reese *et al*., 1999; Leni *et al*., 2020). However, as tropomysosin is associated with the soft tissue of insects and other invertebrates, the risk of allergens being present in BSF frass is likely to be very low.

Allergy to mushrooms and other fungus-derived products (e.g. textured mycoproteins) have been reported (Kayode *et al*., 2020). Most allergen reports are to the spores or on eating mushrooms rather than exposure to spent mushroom substrate, and a recent review of occupational diseases associated with mushroom growers (Ficociello *et al*., 2019) suggested that most health issues in this area are include allergic pulmonary diseases and, more rarely, from forms of contact dermatitis. The cause of these clinical manifestations may be found in the exposure to several factors, such as the mushroom growing media which consists of various decaying organic materials (e.g. wheat straw and hay, oat, rice bran, virgin olive pomace, bird or horse manure) leading to the presence of many allergens (bacteria, moulds, mycotoxins, endotoxins) and the direct contact of workers with some fungal species, which are themselves allergens.

Handling of chitinous by-products should be covered by a COSHH assessment to minimise any risk during handling, transport and processing (e.g. composting) as well as subsequent application to land. Likely key risk activities would be the mixing/preparation of compost material with chitinous by-products. Application of chitinous by-products to land should involve safety measures such as an enclosed tractor cab, possibly with filtration, in place. Potential exposure to bystanders and local housing from wind drift of dust may occur so to mitigate this application should be on days with relatively light winds and direction away from local housing.

HSE monitoring within the shellfish processing sector has shown a median atmospheric level of shellfish tropomyosin of 60 ng m^{-3} (50% of all the air monitoring results in the sector are less than 50 ng m^{-3}). The HSE advise that this value should be used as a benchmark with the view of not breaching it and maintaining exposure as low as possible below this value.

Any atmospheric dust level greater than 10mg m^{-3} (gravimetric analysis) is considered hazardous to health and subject to COSHH. Based on the measured levels of shellfish tropomyosin in the shellfish compost supplied by Angus Horticulture Ltd, the relatively high atmospheric level of 10 mg m^{-3} of compost dust would equate to only 2.5-4.2 ng m^{-3} of shellfish tropomyosin. This is a low level of atmospheric exposure to the allergen where shellfish are being processed. More moderate total atmospheric dust levels would reflect commensurately less allergen. Based on these values the HSE recommend a fixed maximum of 5,000ng shellfish tropomyosin per gram of composted shellfish. A little over 10 times the maximum that was found in the composted shellfish material.

5. Discussion

Chitinous by-products have the potential to be used in agriculture for a variety of purposes including improved soil quality, enhanced microbial activity, greater plant vigour/growth and the suppression of an array of pathogens and pests. Considering the various benefits, the application of chitinous by-products should be viewed as a holistic approach for improved soil health rather than a single purpose strategy e.g. crop protection. On the other hand, the sustainability of using synthetic pesticides and fertilisers is questionable, due to increasingly restrictive pesticide legislation, rising fuel costs, diminishing resources and pest or pathogen resistance to pesticides. Chitinous by-products are available from existing and developing industrial processes such as shellfish processing, mushroom cultivation and insect farming (Black Soldier Fly).

The first objective of this project was to conduct a review of the published and grey literature. The review highlights that chitin is present in a variety of different by-products such as crustaceans from shellfish processing e.g. langoustines, spent mushroom substrate (SMS) and black soldier fly (BSF) frass. It was clear, however, that the chitin content in these products can vary depending on the material tested e.g. difference between chitin in mushroom stipes and caps or the life stage of BSF. Furthermore, there are limitations with regard to laboratories that can provide reliable assessments of chitin content.

In terms of benefits of agriculture, published work highlighted benefits for improved breakdown of organic matter leading to higher fertility (Grimm and Wösten, 2018), reduced pollution (Singh *et al*., 2020), greater water retention and increased crop yield (Lee, Kim and Kim, 2005; Egusa *et al*., 2020; Wang *et al*., 2015). Chitin and chitosan also have suppressive activity against fungi, oomycetes, slime moulds, insects and to a lesser extent bacteria and viruses. Realistically, chitinous by-products are most likely to be applied as a soil amendment within the rotation rather than prior to planting specific crops. This means that any activity against soilborne pests and pathogens is likely to be through the elevation of chitinolytic microbes (Chen and Peng, 2019) or increases in beneficial antagonists (Sharp, 2013). Of the publications reviewed, it was clear that chitinous by-products could

reduce significant agricultural threats such as club root (*Plasmodiophora brassicae*), potato cyst nematode (*Globodera* spp.), take all of wheat and barley (*Gaeumannomyces graminis* var *tritici*) and Rhizoctonia diseases (*Rhizoctonia solani*) to name but a few. Further work, is however, needed to compare the performance of chitinous by-products as most research appears to be on pure chitin or chitosan under controlled conditions. Such work would ideally monitor changes in the soil microbiome in order to qualify the effects observed. Once fully understood, the process of by-product application should be optimised.

The chitinous by-products were submitted for physico-chemical analysis to BSI PAS100 standard, and were shown to contain beneficial levels of key crop nutrients such as N, P, K as well as Ca and Mg. They were also high in organic matter and the C:N ratios indicate that the by-products would allow the mineralisation of N, making it available for plant uptake. Many studies have demonstrated the positive effects on plants with the addition of these by-products to soil, and in some cases the beneficial effects can persist over several seasons.

Determining the chitin content in these by-products has proved to be inconsistent, with technical issues reported by a laboratory tasked with this. Standard methods of analysis for chitin and chitosan in these types of products need to be developed in order to obtain an estimate of chitin/chitosan content, and any evidence of chitin/chitosan breakdown by microbial activity. The by-products are also variable in terms of particle size, microbial load and moisture content. It can be hypothesised that the benefits of these by-products as soil amendments are not necessarily due to the chitin content per se, but with the microbial population and diversity within the material which, when added to soil, alters the soil microbiome and leads to the beneficial effects reported in terms of pest and pathogen suppression and improved plant growth.

Our review identified the various types of legislation surrounding the use of different chitinous by-products. For instance, there are clear regulations set out by the Environment Agency (EA) or Scottish Environmental Protection Agency (SEPA) on the use of shellfish by-products in agricultural land in composted or un-composted forms. Shellfish by-products and insect by-products are also associated with allergens, the most notable being the protein tropomyosin. Tropomyosin is associated with anaphylaxis and would need to be negated in crops destined for food. Fortunately, the risk of these allergens can be mitigated by ensuring that the shellfish/insect material is free of flesh and/or composted before use as demonstrated in the study conducted by Angus Horticulture. The handling of these materials poses a potential risk of allergen exposure, and suitable COSHH plans for working with these materials will need to be in place. Guidance from the HSE (see Appendix) suggest that the risk of allergen exposure is very low, and the amount of allergen material being added to the soil is negligible.

The availability of chitinous by-products from the shellfish industry, insect farming and mushroom growing provide an obvious route into a circular economy where what would perhaps be previously considered as 'waste material' can be utilised for agricultural benefit. The burgeoning insect farming industry in the UK could well provide a significant source of chitinous by-product over the next 30 years or so (Ffoulkes *et al*., 2021), which, when added to the amount of shellfish 'waste' and spent mushroom substrate available, could provide a steady source of material for use on agricultural land. Decisions need to be taken regarding the processing of these materials, particularly in light of the legislative situation changing with the implementation of new UK fertilising product regulations, which are expected in 2023. It is expected that these regulations will include more detailed specifications of the requirements for soil improvers and amendments, and organic fertilisers, than the legislation that is currently applicable. Accordingly, further research on the most appropriate processing of these by-products will be necessary, in line with legislative developments.

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8. Appendix

8.1. Full physico-chemical analyses of samples submitted to D&F Associates

Composted shellfish, spent mushroom substrate and black soldier fly frass samples were submitted to D&F Associate for BSI PAS100 standard testing (https://standardsdevelopment.bsigroup.com/projects/9017-01020#/section), which is assured in the UK by the Compost Certification Scheme (https://www.qualitycompost.org.uk/). Note as the materials under test were not to be considered as a compost, the full PAS100 testing involving a plant growth test was not undertaken. The name of the tests undertaken at D&F Associates were the PAS100E suite and WATSOLNUT2 option.

The Table below summarises the acceptable levels of key parameters to be considered for a material to be classified as a compost.

D&F Associates Ltd

PAS100 TEST PARAMETERS - ACCEPTABLE LEVELS

Parameter	Element	PAS 100 upper limit	Unit
Pathogens	*E. coli*	1000	CFU/g
	Salmonella spp	Absent	Absent or Present in 25g
PTE's	Cadmium as Cd	1.50	mg/kg
	Chromium as Cr	100.00	mg/kg
	Copper as Cu	200.00	mg/kg
	Lead as Pb	200.00	mg/kg
	Mercury as Hg	1.00	mg/kg
	Nickel as Ni	50.00	mg/kg
	Zinc as Zn	400.00	mg/kg
Maturity	CO_2 (stability)	16.0	mg CO_2/g OM/d
Physical Contaminants	Glass, metal, plastic & other	0.25	% of 'air-dry' sample > 2 mm
	Plastic	0.12	
	Sharps	R	
	Stones in "mulch"	10.0	% of 'air-dry' sample > 4 mm
	Stones in other than "mulch"	8.0	
Plant Growth Test	Tomato plants germinated	Min. 80	≥ 80 test plants as % of control plants
	Tomato plant top growth	Min. 80	≥ 80 average g / plant, tests as % of controls
	Tomato plant abnormalities	Absent	abnormal tomato plants in test trays
	Germination of tomato seeds sown in control trays	Min. 27	≥ 27 tomato seeds germinated in control trays by 14 days after sowing
	Tomato plant top growth in control trays	Min. 2.00g	≥ 2.00 g per tomato plant in control trays
	Abnormal tomato plants in control trays	Zero	No abnormal tomato plants in control trays
	Weed plants	Zero	per litre compost as received

8.1.1. Composted shellfish

WATER EXTRACTABLE NUTRIENTS

Parameter	As received (fresh)		In dry matter		Method Reference
	Result **	Unit	Result	Unit	
NH_4-N (ammonium-N)	1186.00	mg/l	3389.54	mg/kg	BS EN 13652
NO_3-N (nitrate-N)	956.00	mg/l	2732.21	mg/kg	BS EN 13652
NH_4-N plus NO_3-N	2142.00	mg/l	6121.75	mg/kg	Calculated
Phosphorus as P	66.00	mg/l	188.63	mg/kg	BS EN 13652
Potassium as K	1449.00	mg/l	4141.19	mg/kg	BS EN 13652
Calcium as Ca	695.00	mg/l	1986.28	mg/kg	BS EN 13652
Magnesium as Mg	168.00	mg/l	480.14	mg/kg	BS EN 13652
Sulphur as S	1210.00	mg/l	3458.13	mg/kg	BS EN 13652
Boron as B	0.23	mg/l	0.66	mg/kg	BS EN 13652
Copper as Cu	<0.1	mg/l	N/A	mg/kg	BS EN 13652
Iron as Fe	<0.1	mg/l	N/A	mg/kg	BS EN 13652
Manganese as Mn	0.01	mg/l	0.03	mg/kg	BS EN 13652
Molybdenum as Mo	<0.1	mg/l	N/A	mg/kg	BS EN 13652
Zinc as Zn	0.16	mg/l	0.46	mg/kg	BS EN 13652
Chloride as Cl	1681.00	mg/l	4804.23	mg/kg	BS EN 13652
Sodium as Na	944.00	mg/l	2697.92	mg/kg	BS EN 13652

N/A = Not Analysed

** Elements of this test may be subcontracted

TOTAL NUTRIENTS

Parameter	As received (fresh)		In dry matter		Method Reference
	Result	Unit	Result **	Unit	
Nitrogen as N	10081	mg/l	28810	mg/kg	Modified Kjeldahl, BS EN 13654-1
	1.97	% m/m	2.88	% m/m	
	N/A	mg/l	N/A	mg/kg	Dumas, BS EN 13654-2 [2]
	N/A	% m/m	N/A	% m/m	
Phosphorus as P	2169	mg/l	6200	mg/kg	BS EN 13650
	0.43	% m/m	0.62	% m/m	BS EN 13650
Potassium as K	2473	mg/l	7067	mg/kg	BS EN 13650
	0.48	% m/m	0.71	% m/m	BS EN 13650
Calcium as Ca	15801	mg/l	45160	mg/kg	BS EN 13650
	3.10	% m/m	4.52	% m/m	BS EN 13650
Magnesium as Mg	2919	mg/l	8342	mg/kg	BS EN 13650
	0.57	% m/m	0.83	% m/m	BS EN 13650
Sulphur as S	2030	mg/l	5801	mg/kg	BS EN 13650
	0.40	% m/m	0.58	% m/m	BS EN 13650
Boron as B	15	mg/l	44	mg/kg	BS EN 13650
Iron as Fe	5759	mg/l	16460	mg/kg	BS EN 13650
Manganese as Mn	163	mg/l	466	mg/kg	BS EN 13650
Molybdenum as Mo	2	mg/l	4	mg/kg	BS EN 13650
Sodium as Na	1695	mg/l	4843	mg/kg	BS EN 13650

N/A = Not Analysed

** Elements of this test may be subcontracted

POTENTIALLY TOXIC ELEMENTS

Parameter	As received (fresh)		In dry matter		Method Reference
	Result	Unit	Result **	Unit	
Cadmium as Cd	0.14	mg/l	0.40	mg/kg	BS EN 13650
Chromium as Cr	7.00	mg/l	20.00	mg/kg	BS EN 13650
Copper as Cu [1]	37.79	mg/l	108.00	mg/kg	BS EN 13650
Lead as Pb	6.65	mg/l	19.00	mg/kg	BS EN 13650
Mercury as Hg	0.07	mg/l	0.20	mg/kg	BS ISO 16772
Nickel as Ni	3.85	mg/l	11.00	mg/kg	BS EN 13650
Zinc as Zn [1]	120.72	mg/l	345.00	mg/kg	BS EN 13650

N/A = Not Analysed

** Elements of this test may be subcontracted

PHYSICO-CHEMICAL PROPERTIES

Parameter	As received (fresh)		In dry matter		Method Reference
	Result	Unit	Result	Unit	
Bulk Density [1]	510.43	g/l	349.9	g/l	LO-WI-002
Dry Matter	68.6	% m/m	N/A		Calculated
Moisture	160.5	g/l	N/A		LO-WI-034
	31.5	% m/m	N/A		
Organic Matter (Loss On Ignition)	32.2	% m/m	47.0	% m/m	LO-WI-038
Organic Carbon (LOI ÷ 1.72)	18.7	% m/m	27.3	% m/m	Calculated
pH	7.0		N/A		LO-WI-005
Electrical Conductivity	4730	µS/cm @ 25 °C	N/A		LO-WI-005
	473	mS/m @ 25 °C	N/A		

1 Bulk density in dry matter is termed 'Dry Weight Density' and expressed in (g/l). DWD = fresh bulk density (g/l) - volumetric moisture content (g/l)

N/A = Not Analysed

PATHOGENS

Parameter	As received (fresh)		Method reference
	Result	Unit	
E. coli at 44°C	<5	CFU/g	LO-WI-008
Salmonella spp at 37°C	Absent	Absent or Present in 25g	LO-WI-011

STABILITY / MATURITY

Parameter	As received (fresh)		Method reference
	Result **	Unit	
Carbon dioxide (evolution rate)	3.1	mg CO_2 / g organic matter / day	ORG0020
Proportion of particles <20 mm	100.0	% g/g	

Parameter	As received (fresh)		In dry matter		Method reference
	Result		Result		
NH_4-N : NO_3-N (ratio)	1.24	:1	1.24	:1	Calculated
Carbon : Nitrogen (ratio)	9.5	:1	9.5	:1	Calculated

N/A = Not Analysed

** Test subcontracted

PHYSICAL CONTAMINANTS (air-dry sample)

Sieve apertures [1]	Glass	Metal	Plastic	Other	Description	Total [1]	of which sharps [2]	Stones [3]	Method Reference
mm	g	g	g	g		g	g	g	
31.5	0.00	0.00	0.00	0.00		0.00	0.00	0.00	LO-WI-036
16.0	0.00	0.00	0.00	0.00		0.00	0.00	0.00	
10.0	0.00	0.00	0.00	0.00		0.00	0.00	5.09	
8.0	0.00	0.00	0.00	0.00		0.00	0.00	0.00	
4.0	0.00	0.00	0.00	0.00		0.00	0.00	0.00	
2.0	0.00	0.00	0.00	0.00		0.00	0.00	0.00	
1.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Pan	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
% of total sample > 2 mm	0.00	0.00	0.00	0.00		0.00	0.00	N/A	
% of total sample > 4 mm	N/A	N/A	N/A	N/A		N/A	N/A	1.06	

Contaminants Key - Other

A = Cardboard/Fibreboard B = Rubber C = String/Twine/Rope D = Textiles/Fabric E = Polystyrene F =

[1] 'Total' is for glass, metal, plastic and 'other'. N.B.: excludes stones

[2] Sharps > 2 mm, of any inorganic physical contaminant type (excludes woody fragments)

[3] Stones and other consolidated mineral contaminants

N/A = Not Analysed

PARTICLE SIZE DISTRIBUTION (air-dry sample)

Sieve apertures	Sample	of which Compost	Cumulative		Method Reference
	Retained	Retained	Retained	Passing	
mm	g	g	%	%	
31.5	0.00	0.00	0.0	100.0	LO-WI-036
16.0	0.00	0.00	0.0	100.0	
10.0	15.30	15.30	3.2	96.8	
8.0	15.16	15.16	6.4	93.6	
4.0	66.21	66.21	20.2	79.8	
2.0	94.51	94.51	40.0	60.0	
1.0	71.03	71.03	54.9	45.1	
Pan	215.75	215.75	100.0	0.0	
Total	**477.96**	**477.96**			

N/A = Not Analysed

Note: Moisture at 40°C = 25.85 % m/m

8.1.2. Spent mushroom substrate

WATER EXTRACTABLE NUTRIENTS

Parameter	As received (fresh)		In dry matter		Method Reference
	Result **	Unit	Result	Unit	
NH_4-N (ammonium-N)	63.00	mg/l	591.61	mg/kg	BS EN 13652
NO_3-N (nitrate-N)	1.10	mg/l	10.33	mg/kg	BS EN 13652
NH_4-N plus NO_3-N	64.10	mg/l	601.94	mg/kg	Calculated
Phosphorus as P	64.00	mg/l	601.00	mg/kg	BS EN 13652
Potassium as K	2159.00	mg/l	20274.44	mg/kg	BS EN 13652
Calcium as Ca	1456.00	mg/l	13672.80	mg/kg	BS EN 13652
Magnesium as Mg	216.00	mg/l	2028.38	mg/kg	BS EN 13652
Sulphur as S	2119.00	mg/l	19898.81	mg/kg	BS EN 13652
Boron as B	<0.1	mg/l	N/A	mg/kg	BS EN 13652
Copper as Cu	<0.1	mg/l	N/A	mg/kg	BS EN 13652
Iron as Fe	1.30	mg/l	12.21	mg/kg	BS EN 13652
Manganese as Mn	0.45	mg/l	4.23	mg/kg	BS EN 13652
Molybdenum as Mo	<0.1	mg/l	N/A	mg/kg	BS EN 13652
Zinc as Zn	193.00	mg/l	1812.40	mg/kg	BS EN 13652
Chloride as Cl	477.00	mg/l	4479.35	mg/kg	BS EN 13652
Sodium as Na	199.00	mg/l	1868.74	mg/kg	BS EN 13652

N/A = Not Analysed

** Elements of this test may be subcontracted

TOTAL NUTRIENTS

Parameter	As received (fresh)		In dry matter		Method Reference
	Result	Unit	Result **	Unit	
Nitrogen as N	N/A	mg/l	N/A	mg/kg	Modified Kjeldahl, BS EN 13654-1
	N/A	% m/m	N/A	% m/m	
	2648	mg/l	24870	mg/kg	Dumas, BS EN 13654-2 [2]
	0.86	% m/m	2.49	% m/m	
Phosphorus as P	497	mg/l	4671	mg/kg	BS EN 13650
	0.16	% m/m	0.47	% m/m	BS EN 13650
Potassium as K	2708	mg/l	25430	mg/kg	BS EN 13650
	0.88	% m/m	2.54	% m/m	BS EN 13650
Calcium as Ca	5954	mg/l	55910	mg/kg	BS EN 13650
	1.93	% m/m	5.59	% m/m	BS EN 13650
Magnesium as Mg	611	mg/l	5734	mg/kg	BS EN 13650
	0.20	% m/m	0.57	% m/m	BS EN 13650
Sulphur as S	3688	mg/l	34630	mg/kg	BS EN 13650
	1.20	% m/m	3.46	% m/m	BS EN 13650
Boron as B	2	mg/l	17	mg/kg	BS EN 13650
Iron as Fe	335	mg/l	3143	mg/kg	BS EN 13650
Manganese as Mn	61	mg/l	575	mg/kg	BS EN 13650
Molybdenum as Mo	0.3	mg/l	3	mg/kg	BS EN 13650
Sodium as Na	238	mg/l	2238	mg/kg	BS EN 13650

N/A = Not Analysed

** Elements of this test may be subcontracted

POTENTIALLY TOXIC ELEMENTS

Parameter	As received (fresh)		In dry matter		Method Reference
	Result	Unit	Result **	Unit	
Cadmium as Cd	0.04	mg/l	0.40	mg/kg	BS EN 13650
Chromium as Cr	7.67	mg/l	72.00	mg/kg	BS EN 13650
Copper as Cu [1]	4.37	mg/l	41.00	mg/kg	BS EN 13650
Lead as Pb	0.31	mg/l	2.90	mg/kg	BS EN 13650
Mercury as Hg	<0.01	mg/l	<0.10	mg/kg	BS ISO 16772
Nickel as Ni	0.33	mg/l	3.10	mg/kg	BS EN 13650
Zinc as Zn [1]	20.55	mg/l	193.00	mg/kg	BS EN 13650

N/A = Not Analysed

** Elements of this test may be subcontracted

PHYSICO-CHEMICAL PROPERTIES

Parameter	As received (fresh)		In dry matter		Method Reference
	Result	Unit	Result	Unit	
Bulk Density [1]	307.86	g/l	106.5	g/l	LO-WI-002
Dry Matter	34.6	% m/m	N/A		Calculated
Moisture	201.4	g/l	N/A		LO-WI-034
	65.4	% m/m	N/A		
Organic Matter (Loss On Ignition)	24.8	% m/m	71.6	% m/m	LO-WI-038
Organic Carbon (LOI ÷ 1.72)	14.4	% m/m	41.6	% m/m	Calculated
pH	6.0		N/A		LO-WI-005
Electrical Conductivity	2830	µS/cm @ 25 °C	N/A		LO-WI-005
	283	mS/m @ 25 °C	N/A		

1 Bulk density in dry matter is termed 'Dry Weight Density' and expressed in (g/l). DWD = fresh bulk density (g/l) - volumetric moisture content (g/l)

N/A = Not Analysed

PATHOGENS

Parameter	As received (fresh)		Method reference
	Result	Unit	
E. coli at 44°C	<5	CFU/g	LO-WI-008
Salmonella spp at 37°C	Absent	Absent or Present in 25g	LO-WI-011

STABILITY / MATURITY

Parameter	As received (fresh)		Method reference
	Result **	Unit	
Carbon dioxide (evolution rate)	16.4	mg CO_2 / g organic matter / day	ORG0020
Proportion of particles <20 mm	100.0	% g/g	

Parameter	As received (fresh)		In dry matter		Method reference
	Result		Result		
NH_4-N : NO_3-N (ratio)	57.27	:1	57.27	:1	Calculated
Carbon : Nitrogen (ratio)	16.7	:1	16.7	:1	Calculated

N/A = Not Analysed

** Test subcontracted

PHYSICAL CONTAMINANTS (air-dry sample)

Sieve apertures [1]	Glass	Metal	Plastic	Other	Description	Total [1]	of which sharps [2]	Stones [3]	Method Reference
mm	g	g	g	g		g	g	g	
31.5	0.00	0.00	0.00	0.00		0.00	0.00	0.00	LO-WI-036
16.0	0.00	0.00	0.00	0.00		0.00	0.00	0.00	
10.0	0.00	0.00	0.00	0.00		0.00	0.00	0.00	
8.0	0.00	0.00	0.00	0.00		0.00	0.00	0.23	
4.0	0.00	0.00	0.00	0.00		0.00	0.00	0.14	
2.0	0.00	0.00	0.00	0.00		0.00	0.00	0.00	
1.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Pan	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
% of total sample > 2 mm	0.00	0.00	0.00	0.00		0.00	0.00	N/A	
% of total sample > 4 mm	N/A	N/A	N/A	N/A		N/A	N/A	0.26	

Contaminants Key - Other

A = Cardboard/Fibreboard B = Rubber C = String/Twine/Rope D = Textiles/Fabric E = Polystyrene F =

[1] 'Total' is for glass, metal, plastic and 'other'. N.B.: excludes stones

[2] Sharps > 2 mm, of any inorganic physical contaminant type (excludes woody fragments)

[3] Stones and other consolidated mineral contaminants

N/A = Not Analysed

PARTICLE SIZE DISTRIBUTION (air-dry sample)

Sieve apertures	Sample	of which Compost	Cumulative		Method Reference
	Retained	Retained	Retained	Passing	
mm	g	g	%	%	
31.5	0.00	0.00	0.0	100.0	LO-WI-036
16.0	7.16	7.16	5.1	94.9	
10.0	29.61	29.61	25.9	74.1	
8.0	16.26	16.26	37.4	62.6	
4.0	20.19	20.19	51.6	48.4	
2.0	27.39	27.39	71.0	29.0	
1.0	21.30	21.30	86.0	14.0	
Pan	19.87	19.87	100.0	0.0	
Total	**141.78**	**141.78**			

N/A = Not Analysed

Note: Moisture at 40°C = 65.72 % m/m

8.1.3. Black soldier fly frass

WATER EXTRACTABLE NUTRIENTS

Parameter	As received (fresh)		In dry matter		Method Reference
	Result **	Unit	Result	Unit	
NH_4-N (ammonium-N)	687.00	mg/l	5488.33	mg/kg	BS EN 13652
NO_3-N (nitrate-N)	0.90	mg/l	7.19	mg/kg	BS EN 13652
NH_4-N plus NO_3-N	687.90	mg/l	5495.52	mg/kg	Calculated
Phosphorus as P	703.00	mg/l	5616.15	mg/kg	BS EN 13652
Potassium as K	737.00	mg/l	5887.77	mg/kg	BS EN 13652
Calcium as Ca	30.00	mg/l	239.67	mg/kg	BS EN 13652
Magnesium as Mg	43.00	mg/l	343.52	mg/kg	BS EN 13652
Sulphur as S	462.00	mg/l	3690.84	mg/kg	BS EN 13652
Boron as B	0.11	mg/l	0.88	mg/kg	BS EN 13652
Copper as Cu	0.11	mg/l	0.88	mg/kg	BS EN 13652
Iron as Fe	3.60	mg/l	28.76	mg/kg	BS EN 13652
Manganese as Mn	0.05	mg/l	0.40	mg/kg	BS EN 13652
Molybdenum as Mo	<0.1	mg/l	N/A	mg/kg	BS EN 13652
Zinc as Zn	0.76	mg/l	6.07	mg/kg	BS EN 13652
Chloride as Cl	477.00	mg/l	3810.67	mg/kg	BS EN 13652
Sodium as Na	187.00	mg/l	1493.91	mg/kg	BS EN 13652

N/A = Not Analysed

** Elements of this test may be subcontracted

TOTAL NUTRIENTS

Parameter	As received (fresh)		In dry matter		Method Reference
	Result	Unit	Result **	Unit	
Nitrogen as N	5181	mg/l	41390	mg/kg	Modified Kjeldahl, BS EN 13654-1
	3.22	% m/m	4.14	% m/m	
	N/A	mg/l	N/A	mg/kg	Dumas, BS EN 13654-2 [2]
	N/A	% m/m	N/A	% m/m	
Phosphorus as P	1486	mg/l	11870	mg/kg	BS EN 13650
	0.92	% m/m	1.19	% m/m	BS EN 13650
Potassium as K	1151	mg/l	9192	mg/kg	BS EN 13650
	0.71	% m/m	0.92	% m/m	BS EN 13650
Calcium as Ca	564	mg/l	4505	mg/kg	BS EN 13650
	0.35	% m/m	0.45	% m/m	BS EN 13650
Magnesium as Mg	407	mg/l	3251	mg/kg	BS EN 13650
	0.25	% m/m	0.33	% m/m	BS EN 13650
Sulphur as S	818	mg/l	6531	mg/kg	BS EN 13650
	0.51	% m/m	0.65	% m/m	BS EN 13650
Boron as B	2	mg/l	14	mg/kg	BS EN 13650
Iron as Fe	76	mg/l	610	mg/kg	BS EN 13650
Manganese as Mn	10	mg/l	80	mg/kg	BS EN 13650
Molybdenum as Mo	0	mg/l	3	mg/kg	BS EN 13650
Sodium as Na	263	mg/l	2104	mg/kg	BS EN 13650

N/A = Not Analysed

** Elements of this test may be subcontracted

POTENTIALLY TOXIC ELEMENTS

Parameter	As received (fresh)		In dry matter		Method Reference
	Result	Unit	Result **	Unit	
Cadmium as Cd	0.08	mg/l	0.60	mg/kg	BS EN 13650
Chromium as Cr	0.94	mg/l	7.50	mg/kg	BS EN 13650
Copper as Cu [1]	4.88	mg/l	39.00	mg/kg	BS EN 13650
Lead as Pb	0.30	mg/l	2.40	mg/kg	BS EN 13650
Mercury as Hg	0.01	mg/l	0.10	mg/kg	BS ISO 16772
Nickel as Ni	0.43	mg/l	3.40	mg/kg	BS EN 13650
Zinc as Zn [1]	42.81	mg/l	342.00	mg/kg	BS EN 13650

N/A = Not Analysed

** Elements of this test may be subcontracted

PHYSICO-CHEMICAL PROPERTIES

Parameter	As received (fresh)		In dry matter		Method Reference
	Result	Unit	Result	Unit	
Bulk Density [1]	161.1	g/l	125.2	g/l	LO-WI-002
Dry Matter	77.7	% m/m	N/A		Calculated
Moisture	35.9	g/l	N/A		LO-WI-034
	22.3	% m/m	N/A		
Organic Matter (Loss On Ignition)	72.8	% m/m	93.7	% m/m	LO-WI-038
Organic Carbon (LOI ÷ 1.72)	42.3	% m/m	54.5	% m/m	Calculated
pH	7.7		N/A		LO-WI-005
Electrical Conductivity	2190	µS/cm @ 25 °C	N/A		LO-WI-005
	219	mS/m @ 25 °C	N/A		

1 Bulk density in dry matter is termed 'Dry Weight Density' and expressed in (g/l). DWD = fresh bulk density (g/l) - volumetric moisture content (g/l)

N/A = Not Analysed

PATHOGENS

Parameter	As received (fresh)		Method reference
	Result	Unit	
E. coli at 44°C	>3000	CFU/g	LO-WI-008
Salmonella spp at 37°C	Absent	Absent or Present in 25g	LO-WI-011

STABILITY / MATURITY

Parameter	As received (fresh)		Method reference
	Result **	Unit	
Carbon dioxide (evolution rate)	18.3	mg CO_2 / g organic matter / day	ORG0020
Proportion of particles <20 mm	100.0	% g/g	

Parameter	As received (fresh)		In dry matter		Method reference
	Result		Result		
NH_4-N : NO_3-N (ratio)	763.33	:1	763.33	:1	Calculated
Carbon : Nitrogen (ratio)	13.2	:1	13.2	:1	Calculated

N/A = Not Analysed

** Test subcontracted

PHYSICAL CONTAMINANTS (air-dry sample)

Sieve apertures [1]	Glass	Metal	Plastic	Other	Description	Total [1]	of which sharps [2]	Stones [3]	Method Reference
mm	g	g	g	g		g	g	g	
31.5	0.00	0.00	0.00	0.00		0.00	0.00	0.00	LO-WI-036
16.0	0.00	0.00	0.00	0.00		0.00	0.00	0.00	
10.0	0.00	0.00	0.00	0.00		0.00	0.00	0.00	
8.0	0.00	0.00	0.00	0.00		0.00	0.00	0.00	
4.0	0.00	0.00	0.00	0.00		0.00	0.00	0.00	
2.0	0.00	0.00	0.00	0.00		0.00	0.00	0.00	
1.0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
Pan	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
% of total sample > 2 mm	0.00	0.00	0.00	0.00		0.00	0.00	N/A	
% of total sample > 4 mm	N/A	N/A	N/A	N/A		N/A	N/A	0.00	

Contaminants Key - Other

A = Cardboard/Fibreboard B = Rubber C = String/Twine/Rope D = Textiles/Fabric E = Polystyrene F =

[1] 'Total' is for glass, metal, plastic and 'other'. N.B.: excludes stones

[2] Sharps > 2 mm, of any inorganic physical contaminant type (excludes woody fragments)

[3] Stones and other consolidated mineral contaminants

N/A = Not Analysed

PARTICLE SIZE DISTRIBUTION (air-dry sample)

Sieve apertures	Sample	of which Compost	Cumulative		Method Reference
	Retained	Retained	Retained	Passing	
mm	g	g	%	%	
31.5	0.00	0.00	0.0	100.0	LO-WI-036
16.0	0.00	0.00	0.0	100.0	
10.0	2.59	2.59	1.3	98.7	
8.0	1.10	1.10	1.9	98.1	
4.0	3.69	3.69	3.8	96.2	
2.0	94.16	94.16	52.1	47.9	
1.0	64.02	64.02	84.9	15.1	
Pan	29.47	29.47	100.0	0.0	
Total	**195.03**	**195.03**			

N/A = Not Analysed

Note: Moisture at 40°C = 15.71 % m/m

8.2. Comments and recommendations from the HSE regarding production and use of composted shellfish (but also applicable to BSF frass by-product)

- Major route of exposure to allergens is inhalation. Sensitisation and/or precipitation of symptoms in those already sensitised.
- As an allergen is being added to the compost via the addition of shellfish waste (albeit at a low level), COSHH should apply. Keeping exposure to as low as reasonably practicable
- There are no thresholds for allergens in terms of risk of sensitisation or precipitation of symptoms i.e. no "safe" levels
- Likely key risk activities:
    1. Mixing/preparation of compost material with waste shellfish. Exposure to staff undertaking this process and other staff in vicinity
    2. Application of composted shellfish to fields at a rate of 1 tonne per hectare. Enclosed tractor cab, possibly with filtration, in place. Some potential exposure to bystanders and local housing from wind drift of dust. Apply compost on days with relatively light winds and direction away from local housing (mitigation)
- Our air monitoring within the shellfish processing sector show a median atmospheric level of shellfish tropomyosin of 60 $ng.m^{-3}$ (50% of all the air monitoring results in the sector are less than 50 $ng.m^{-3}$). Therefore, we could take this value as a benchmark with the view of;
    1. never to breaching it either through the nature of work activities or the amount of allergen added to the compost.
    2. Maintaining exposure as low as possible below this value.
- Any atmospheric dust level greater than 10$mg.m^{-3}$ (gravimetric analysis) is considered hazardous to health and subject to COSHH. Based on the measured levels of shellfish tropomyosin in the compost supplied by Angus Horticulture, the relatively high atmospheric level of 10 mg. m^{-3} of compost dust would equate to only 2.5-4.2 $ng.m^{-3}$ of shellfish tropomyosin. This is a low level of atmospheric exposure to the allergen where shellfish are being processed. More moderate total atmospheric dust levels would reflect commensurately less allergen.
- So, on the basis of the above calculations, the HSE would be happy that there is a fixed <u>maximum</u> of around 5,000ng shellfish tropomyosin per gram of compost. A little over 10 times the maximum found in samples submitted.
- Some other comments:
    1. If manufacturing the compost, it might be worth doing some atmospheric monitoring to ensure during manufacturing activities workers are not exposed to greater than 10 $mg.m^{-3}$ of dust.
    2. Add instructions on not applying to fields on windy days and when the wind is directed towards any local housing.