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SCIENTIFIC REPORT

Chitin estimation in agricultural soils

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

The present scientific report has been elaborated in the context of the European Commission mandate requesting for an opinion according to Article 23(6) of Regulation (EC) No 1107/2009 regarding the approved plant protection uses of chitosan and chitosan hydrochloride as basic substances. This scientific report focused on estimating the amount of chitin present in an average agricultural soil, aiming to establish a baseline for its natural availability. Understanding the source and concentration of biotic chitin in soil assisted the estimation of chitosan potentially available in the environment, as requested in one of terms of reference of the concerned EC mandate. Chitin in soil was estimated to range from 27 to 280 kg/ha in the first 0–5 cm layer and 99 to 901 kg/ha in the 0–20 cm layer. Fungi are the main chitin producer followed by insects and nematodes. Soil crustaceans could not be considered in the assessment due to the lack of necessary information and the variability of their presence. The development of a polynomial function to estimate the amount of chitin in such biome can also identify the main predictors of chitin content in similar biomes. This estimate was based on the available scientific literature, and it would require additional validation using field measurements and error analysis on different soil types and conditions, to become a generalised model. Lack of information alongside related uncertainties have also been identified.

KEYWORDS

agricultural soil, basic substance, chitin, chitosan, natural occurrence, plant protection

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CONTENTS

SUMMARY

The present scientific report has been elaborated in the context of the European Commission (EC) mandate[1] requesting for an opinion according to Article 23(6) of Regulation (EC) No 1107/2009, in conjunction with Article 29 of Regulation (EC) No 178/2002, regarding the approved plant protection uses of chitosan[2] and chitosan hydrochloride[3] as basic substances.

As part of one of the terms of reference of the concerned EC mandate, it was requested to estimate the exposure levels of chitosan potentially available in the environment, either naturally or derived from other uses, by also taking into account amongst others the structural similarity between chitin and chitosan and the natural abundance of chitin in the environment.

Accordingly, the present work reported an estimation of the chitin content due to biological sources in (agricultural) **soil**. The biological producers of chitin that were considered in this scientific report were fungi, insects and nematodes.

The calculations and figures presented and discussed in this scientific report accounted for general uncertainties such as:

- the population variability, thus having a scenario of 'high content' and one of 'low content' of chitin in soil;
- fungal population size estimated considering a range of uncertainty of one order of magnitude (10^6–10^7 fungal cells/g of soil);
- insect population size estimated considering a range of uncertainty of one order of magnitude (10^7–10^8 individuals per hectare);
- nematodes population size estimated based on two different studies, one derived from a global biomass estimation and one from an in-field quantification in a related biome.

Fungi were considered the main chitin producer since their cell walls constitutively contain chitin. Based on the current approach their chitin contribution was estimated approximatively between 20–204 kg/ha in the first 0–5 cm layer and 80–815 kg/ha in the 0–20 cm layer.

Insects were also considered as chitin producers due to the presence of this polymer in their cuticles, exoskeleton and exuviae. From the estimation included in this opinion the chitin contribution from arthropods was estimated approximatively between 7–71 kg/ha in the first 0–5 cm layer and 17–173 kg/ha in the 0–20 cm layer.

Finally, nematodes were also considered in this report because chitin appears in their eggshell and pharynx, however, they are expected to contribute only marginally to the total amount of biological chitin in soil with the maximum value being 5 kg/ha.

Soil crustaceans were not considered in the assessment due to the lack of information that was collected and the variability of their presence. Although the size of the crustacean population is difficult to estimate, it will likely constitute a small addition to the overall amount of chitin predicted in soils.

Overall, chitin in soil was estimated to range from **27 to 280 kg/ha in the first 0–5 cm layer** and from **99 to 901 kg/ha in the 0–20 cm** layer based on an average soil bulk density of 1.3 g/cm^3. Fungi are the main producer followed by insects and their respective population sizes are the strongest predictor of chitin content in agricultural soil.

Lastly, it is noted that this estimate was based on the available scientific literature; it would require additional validation using field measurements and error analysis on different soil types and conditions, in order to become a generalised model.

[1]EFSA-Q-2024-00037: https://open.efsa.europa.eu/questions/EFSA-Q-2024-00037.

[2]Commission Implementing Regulation (EU) 2022/456 of 21 March 2022 approving the basic substance chitosan; OJ L 93, 22.3.2022, pp. 138–141.

[3]Commission Implementing Regulation (EU) No 563/2014 of 23 May 2014 approving the basic substance chitosan hydrochloride; OJ L 156, 24.5.2014, pp. 5–7.

1 | INTRODUCTION

The present scientific report has been elaborated in the context of the European Commission mandate requesting for an opinion according to Article 23(6) of Regulation (EC) No 1107/2009, in conjunction with Article 29 of Regulation (EC) No 178/2002, regarding the approved plant protection uses of chitosan and chitosan hydrochloride as basic substances. The EFSA Panel on Plant Protection Products and their Residues (PPR) was tasked to address this mandate and provided the results of its work in the form a statement (EFSA PPR Panel, 2025).

Amongst the mandate terms of reference (ToR) addressed by the PPR Panel, one (ToR 2) related to the comparison between the exposure levels of chitosan and chitosan hydrochloride resulting from the approved uses as basic substances and the exposure levels expected to occur in the environment, either naturally or derived from other uses, also taking into account amongst others the structural similarity between chitin and chitosan (and its hydrochloride), and **the natural abundance of chitin** in the environment.

It is acknowledged that there is a general lack of experimental data on the determination of the natural occurrence of chitin/chitosan in the different environmental compartments. The available scientific literature widely reports that chitin is the second most abundant natural polysaccharide found in the environment, derived mainly from fungi and arthropods, and being biocompatible and biodegradable. Though this is recurring background information, it is not supported by any quantitative estimation, especially when considering specific environments like soil and freshwater compartments. Accordingly, EFSA with the present work carried out a quantitative estimation of ***chitin*** in agricultural soils due to natural biological sources. The biological producers of chitin that were considered in this scientific report were fungi, insects and nematodes.

Such chitin estimation assisted the PPR Panel to have, at least for the soil compartment, a quantitative estimation of the chitosan natural background exposure levels to address the requests under ToR 2 (see details in section 5.4.1 of EFSA PPR Panel statement, EFSA PPR Panel, 2025).

2 | DATA AND METHODOLOGIES

2.1 | Data

All the data collected in this report are retrieved from publicly available scientific literature. In particular, collected information referred to amount of chitin per organism, fungal population absolute and relative abundance, fungal cell composition, insect body composition and population size, nematodes population size and biology. When possible, this information was retrieved specifically for agricultural soil rather than for general soil including all different biomes.

2.2 | Methodologies

To develop an estimation of the amount of chitin naturally present in agricultural soils a polynomial function was developed including in its terms different coefficients encompassing the main sources of chitin; these are identified through the literature as fungi, arthropods and nematodes. To encompass the natural variation of the biological communities that can be found on agricultural soils based on latitude, crop, seasonality and other edaphic factors the estimation is provided as a range using the minimum and maximum values for the population's size. Finally, the estimation is made to a surface of a hectare encompassing two different soil layers of 0–5 cm and 0–20 cm depth, respectively.

2.3 | Definition of polynomial terms for the chitin-estimation model

This is the formula designed to estimate the total amount of natural chitin in agricultural soil considering three main biological sources: fungi, insects and nematodes. The following polynomial model was used:

$$C_S = C_{tf} + C_{tar} + C_{tnm}$$

where

$$C_{tf} = \left(C_f \cdot N_f\right) \Big| C_{tar} = \left(C_i \cdot N_i\right) \Big| C_{tnm} = \left(C_n \cdot N_n\right)$$

and therefore

$$C_S = \left[\left(C_f \cdot N_f\right) + \left(C_i \cdot N_i\right) + \left(C_n \cdot N_n\right)\right]$$

where each term refers to: C_S: chitin in soil, C_{tf}: total chitin from fungi, C_{tar}: total chitin from arthropods, C_{tnm}: total chitin from nematodes, C_f: chitin in fungal cell, N_f: fungal population size, C_i: chitin in individual arthropod, N_i: arthropods population size, C_n: chitin in individual nematode and N_n: Nematodes population size.

Each term between brackets is dimensionally estimated as an amount of chitin per kg of soil or surface area. The final calculation in paragraph 3.5 converted all these units in the most convenient measurement unit to represent the different layers of soil, over a surface of a hectare.

2.3.1 | Definition of polynomial terms for the fungal population

For the fungal parameters (C_f and N_f) further calculations and estimations are performed and are described in Section 3.1. C_f is calculated as follow:

$$C_f = (a \cdot C_a + b \cdot C_b + c \cdot C_z)$$

and furthermore:

$$C_a = \left(d \cdot C_y + e \cdot C_{ff}\right)$$

where each term refers to: C_f: chitin in fungi, *a*: coefficient % Ascomycota, *b*: coefficient % Basidiomycota, *c*: coefficient % Zygomycota, *d*: coefficient % yeast, *e*: coefficient % filamentous fungi, C_a: chitin in Ascomycota, C_b: chitin in Basidiomycota, C_y: chitin in yeast, C_z: chitin in Zygomycota, N_f: fungal population size, C_{ff}: chitin in filamentous fungi.

Finally, each fungal 'C_x', intended as chitin content, is calculated using the following:

$$C_x = [(\text{cBM} \cdot \text{Dm}\%) \cdot \text{CW}\%] \cdot \text{CH}\%$$

where C_x: chitin content for fungal cell type, cBM: cell biomass, Dm%: percentage w/w of cell dry-matter, CW%: percentage w/w of cell wall dry-matter, CH%: chitin percentage of the cell wall.

2.3.2 | Definition of polynomial terms for the insect population

For the total arthropods estimation of chitin in soil (C_{tar}), we applied the following:

$$C_{tar} = \left(C_i \cdot N_i\right) = \left[\left(C_{ma} \cdot N_{ma}\right) + \left(C_{ar} \cdot N_{ar}\right)\right]$$

where: C_{tar}: chitin in all arthropods, C_i: chitin in individual insect, N_i: number of insects, C_{ma}: chitin in microarthropods, C_{ar}: chitin in larger insect, N_{ma}: number of microarthropods, N_{ar}: number of larger insects.
followed by

$$C_x = \text{OBM} \cdot \text{CH}\%$$

where $C_{\cdot}$ represent the chitin content per arthropod group (micro or macroarthropods, respectively *ma* or *ar*), OBM indicate the Organism's Biomass (normally expressed as dry-weight) and CH% refer to the chitin content as dry-weight percentage of biomass.

2.3.3 | Definition of polynomial terms for the nematodes population

And finally, for the estimation of chitin from nematodes C*tnm* is resulting from the following equation:

$$C_{tnm} = \left(C_n \cdot N_n\right) = \left[\left(N_{bm} \cdot \%_{eg} \cdot \%_{cheg}\right) \cdot N_n\right]$$

where C_{tnm}: total chitin from nematodes, C_n: chitin per individual nematode, N_n: number of nematodes/m^2, N_{bm}: nematodes biomass, $\%_{eg}$: percentage of biomass due to eggs, $\%_{cheg}$: percentage of chitin in eggshell.

2.3.4 | Additional parameters defined for chitin calculation

Since these estimations are based on kg of soil or referred to a surface expressed in m^2 area we considered also the following parameters to calculate the final amount of chitin in soil from natural sources:

- Soil Bulk Density = 1.3 g/cm^3 (Walters, 2021)
- Soil Volume (calculated for a surface of 1 ha (10,000 m^2) for the different layers depth considered in this assessment)
  - Sv_1 = 1ha · 0–5 cm: = 500 m^3
  - Sv_2 = 1ha · 0–20 cm = 2000 m^3

- Soil Biomass calculated as the product of soil density and soil volume per each layer
  - S_{BM1} = 650,000 kg of soil per ha
  - S_{BM2} = 2.600000 kg of soil per ha

3 | ASSESSMENT

3.1 | Chitin in fungi

Fungi are a fundamental part of the microbial community in soil, both in terms of abundance and due to their roles in the ecosystem (He et al., 2020). Although they are constitutively made out of chitin (Brown et al., 2020) the single-cell chitin abundance is sensibly different based on the taxonomical group and so is the cell size and shape. Furthermore, the soil fungal population is highly variable both in terms of organism size and composition (Djemiel et al., 2023). In the framework of this estimation, these issues were addressed in different ways. This report accounts for two different population sizes ranging from 10^6 to 10^7 fungal cells/g of soil which represents two representative scenarios in agricultural soil (Tecon & Or, 2017) and (Mendes et al., 2013). Additionally, the soil fungal population was divided into their most representative large taxonomical group (Ascomycota, Basidiomycota and Zygomycota) relevant for the soil community. Furthermore, Ascomycota was divided into its most relevant subgroups such as filamentous fungi and yeasts. Except for the yeasts which are unicellular organisms, all the remaining groups are multicellular organisms that form similar filamentous structures, known as hyphae, to compose the mycelia. *Hyphae* have a tubular structure surrounded by a cell wall and can be divided by *septae* or while sharing a similar diameter across all groups. The main difference between Ascomycota, Basidiomycota and the so called Zygomycota is in fact to be found into the spore dispersion mechanism and structure. Finally, Zygomycota is an old name for a phyla which is currently divided into sub-phyla of uncertain placement. Of these subgroups, Mucoromycota contains several fungal genera that are often retrieved in soil, such as *Mucor* spp. that due to their chitin content and for being direct chitosan producers are particularly relevant for this report. However, the old name of Zygomycota, while often referring to genera belonging to Mucoromycota, was maintained for consistency with other EFSA opinions on similar topics.

For the purposes of this scientific report, it was maintained the division of filamentous fungi amongst these three phyla since the chitin content in their cell wall changes sensibly and so does their relative abundance in a soil fungal community. Finally, many methods can be used to measure and characterise the complex fungal population size and composition in soil such as isolation-based microbiological methods (i.e. count of colony forming units), chemical methods (linked to the amount and composition of fatty-acids and chitin amount) or molecular based, i.e. based on next generation DNA sequecing (NGS) or quantitative polymerase chain reaction (qPCR). In this report fungal chitin content is estimated based on data derived from molecular-based methods in order to avoid isolation-biases and to use older methods which have been proven to have a large degree of errors (Sharma et al., 1977). From the NGS-based literature therefore the population size's range in soil and an average taxonomical composition expressed in terms of relative abundance between groups were extracted. Instead, physiological information such as the cell biomass, cell wall composition in terms of chitin and dry-matter percentage and the diameter of hyphae, as well as the average cell size, was extracted from biological literature. Figure 1, adapted from (Gow & Lenardon, 2023) displays the different structures of the fungal hyphae included in this report.

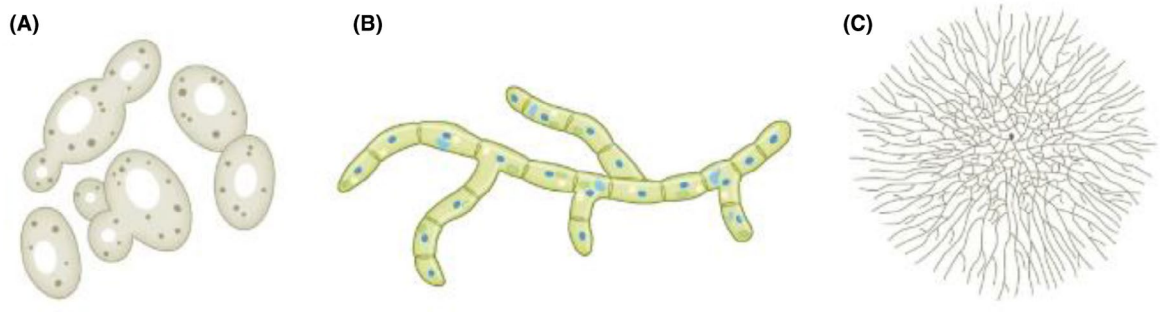

FIGURE 1 Overview on cell structure for different type of fungi: Yeast (A), Hyphae or Pseudohyphae (B) and Mycelia (C). The image is adapted from figure 1 (Gow & Lenardon, 2023) Nature Reviews Microbiology (*Nat Rev Microbiol*) ISSN 1740–1534 (online).

Chitin estimation in yeast

Yeast represents a unicellular fungal cell surrounded by a cell wall partially constituting chitin. Their cell size is generally smaller than filamentous fungi and thus is the amount of chitin constituting their cell wall. To estimate the amount of chitin in yeasts information from a model species of *Saccharomyces cerevisiae* and other yeast was retrieved when available as occurrence in agricultural soil, averaging the results. For this specific cell type a cell biomass of around 100 pg. wet-weight, when it is fully developed, and an indication of dry-weight being generally slightly higher than 50% was retrieved (Cuny

et al., 2022). From Nguyen et al. (1998) it was possible to determine that, for yeasts, around 30% of their dry-matter constitutes the cell wall and of this, a small percentage, around 2%–3% is made of chitin (Nguyen et al., 1998). Using the formula presented before can be therefore calculated an amount of chitin per yeast cell being:

$$C_y = [(\text{cBM} \cdot \text{Dm}\%) \cdot \text{CW}\%] \cdot \text{CH}\%$$

$$C_y = \left[(100\ \text{pg} \cdot 50\text{Dm}\%) \cdot 30\%\right] \cdot 2\%$$

$$C_y = 0.3\ \text{pg of chitin / yeast cell}$$

3.1.1 | Chitin estimation in filamentous fungi

Filamentous fungi are multicellular fungal organisms constituted from many eucaryotic cells forming a tubular structure known as hyphae, which is assembled in a larger tissue called the mycelia. Despite large differences between fungal groups in spores dispersal mechanisms and life-cycle, the amount of chitin can be estimated in a similar way for the fungi belonging to this macro-group, by using a common approximation for the hyphae structure as shown in Figure 2.

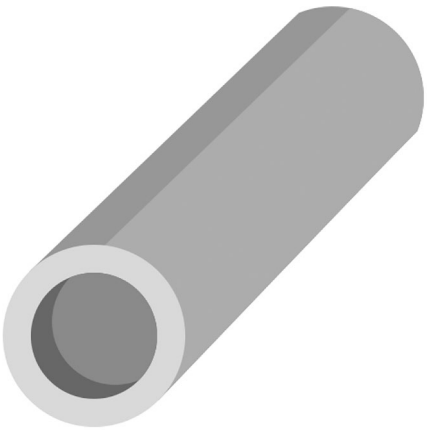

FIGURE 2 Hyphae structure approximation used in the model for different type of filamentous fungi. The difference between external and internal diameter correspond to the width of the cell wall where most of the chitin is stored. Image taken from www.flaticon.com

From the existing literature, different diameters for the hyphae belonging to Ascomycota, Basidiomycota and Zygomycota were retrieved (Lehmann et al., 2019):

- Diameter Hyphae Ascomycota = 4.86 μm
- Diameter Hyphae Basidiomycota = 5.2 μm
- Diameter Hyphae Zygomycota = 4.57 μm

Considering 1 m length for the hyphae (l) we can calculate the volume (V) for 1 m of hyphae for each fungus using the current formulas:

$$V_{\text{hyphae}} = \pi \cdot r_{\text{hyphae}} \cdot l_{\text{hyphae}}$$

- Volume Hyphae Ascomycota = 4.86 μm
- Volume Hyphae Basidiomycota = 5.2 μm
- Volume Hyphae Zygomycota = 4.57 μm

and assuming a density (d) similar to water (1 g/cm^3) and a fungal cell length (100 μm, which translate into around 10,000 cells/meter of hyphae) the cellular biomass can be finally estimated for the different fungal groups:

- Biomass Ascomycota cell: 1.8 ng
- Biomass Basidiomycota cell: 2.1 ng
- Biomass Zygomycota cell: 1.66 ng

With this biomass, retrieving from the literature additional information such as the dry-matter index of 0.2 from Struwe (1982), the percentage of dry-matter constituting the cell wall, being 30% from (Nguyen et al., 1998) or 15%–30% from

(Yarden, 2010) and finally the percentage of chitin in the cell walls of the three major groups Ascomycota (averaged at 30%), Basidiomycota (averaged at 30%) and Zygomycota (averaged at 45%) estimated from Tharanathan and Kittur (2003), Bastiaens et al. (2019), Garcia-Rubio et al. (2019) and Di Mario et al. (2008) it was possible to calculate the amount of chitin for filamentous fungi, by applying the same approach used before for the yeast cell.

$$C_x = [(\text{cBM} \cdot \text{Dm}\%) \cdot \text{CW}\%] \cdot \text{CH}\%$$

and obtain

- C_{ff} = Chitin content in Ascomycota cell: 32.4 pg.
- C_b = Chitin content in Basidiomycota cell: 37.8 pg.
- C_z = Chitin content in Zygomycota cell: 45 pg.

3.1.2 | Estimation of soil fungal population size and relative abundance

In this section both the absolute abundance of fungal cells in agricultural soil and a plausible representation of its community given as relative abundance percentage between the dominant taxonomical groups included in this report were estimated.

Mendes et al. (2013) and Vincze et al. (2024) report a 10^5–10^6 cells/g of soil while Djemiel et al. (2023) report 10^7 genes/g of soil in average for crops. Again, 10^6 cells/g of surface soil from (Tecon & Or, 2017) and a larger range from 10^3 to 10^4 colony forming units/g of soil till 10^8–10^9 genes/g of soil (without copy-number variation normalisation) is also seen in unpublished data. These numbers are highly dependent to the methodology used and subject to many biases and additional variables from the environment which can heavily impact on the retrieved amount of fungi. For this reason, the assessment using two values retained plausible from the literature was conducted: 10^6 and 10^7 fungal cells/g of soil which represent the term N_f in the polynomial formula.

Once the range of the population size was decided, a refinement of this analysis by estimating a potential average fungal composition characteristic of agricultural soil was needed. Different studies were concurring on assigning the dominant fraction to Ascomycota, followed by Basidiomycota and, in lower abundance, Zygomycota and other groups. Amongst the Ascomycota, yeasts are generally present in lower relative abundance compared to filamentous fungi. These comparisons allow to estimate the different coefficients (*a, b, c, d* and *e*) that appeared in the formulas:

$$C_f = (a \cdot C_a + b \cdot C_b + c \cdot C_z)$$

and

$$C_a = \left(d \cdot C_y + e \cdot C_{ff}\right)$$

Based on the metabarcoding approach applied by (Egidi et al., 2019), (Ciccolini et al., 2015), (Gao et al., 2021), (Xiong et al., 2024) and (Gobbi et al., 2022) the following coefficients can be estimated: $a = 0.7$, $b = 0.2$, $c = 0.1$, $d = 0.15$, $e = 0.85$. That translate into an average fungal composition for agricultural soil being around 70% of Ascomycota, of which 15% yeast and 85% filamentous fungi, 20% Basidiomycota and finally 10% of Zygomycota.

3.1.3 | Estimation of chitin in soil due to fungal population

Now that both the absolute and relative abundance of fungi, together with the chitin content of each cell type, have been estimated, it was finally possible to calculate the amount of chitin in soil due to the fungal community applying the formula below and subsequently calculating the amount for the different soil layers over the surface of an hypothetical hectare. The results are reported in Table 1.

TABLE 1 Chitin in soil due to fungal contribution.

Soil layer	N_f cells/g of soil	Soil biomass (kg/ha)	Chitin in soil (kg/ha)	Scenario
0–5 cm	10^6	650,000	**20.4**	Low fungi content
0–20 cm	10^6	2,600,000	**81.5**	
0–5 cm	10^7	650,000	**204**	High fungi content
0–20 cm	10^7	2,600,000	**815**	

3.2 | Chitin in arthropods

Arthropods are ubiquitous in fields and due to their exoskeleton and cuticles, largely composed of chitin, they represent a major source of this polymer in the environment (Chapman, 2013). Compared to fungi there is a much higher variation in terms of size and biomass between insects and this could affect the estimation of chitin. When compared to fungi, the literature regarding the total population size in fields or soil is limited. The group of arthropods can be distinguished conveniently between microarthropods and larger insects, i.e. macroarthropods. These two groups differ greatly in size, with one being in the order of few hundreds' micrometres to 2 mm, while the larger insects can easily reach the centimetres size. Assuming a similar chitin content in terms of body-weight percentage it is also noticeable that these two groups have different population's size which suggests they should be estimated separately. From the formula presented above, and reported here for convenience, the terms that need to be estimated are:

$$C_{tar} = (C_i \cdot N_i) = \left[(C_{ma} \cdot N_{ma}) + (C_{ar} \cdot N_{ar})\right]$$

3.2.1 | Estimation of biomass for arthropods

Regarding the chitin content expressed as percentage of the insect biomass there are several papers reporting overall a range from 6 to 36% of dry-weight, depending on the species (Kaya et al., 2014; Marei et al., 2016) and further confirmed by (Bastiaens et al., 2019) who restricted the range to 10%–15% of dry-weight. In addition, (Tharanathan & Kittur, 2003) reported an average of 40% chitin content in the cuticles only (ranging from 18.4% to 64% in cuticles dry-weight). Considering the wide diversity of the arthropod's community, for the following estimation a chitin percentage of 15% of body dry-weight was used.

Regarding the estimate of body-weight for the two groups in exam (Genoud et al., 2023) found an average of 17.1 mg/individual insect using an approach based on size-independent trap. The value of 17.1 mg was used as an average dry-weight mass per individual in the estimation for larger arthropods biomass. Assuming a similar body composition between microarthropods and large insects, the average biomass for microarthropods was approximated at around 50 micrograms per individual, in dry-weight. With these parameters it was then possible to estimate the amount of chitin per individual with the formula:

$$C_x = \text{OBM} \cdot \text{CH}\%$$

and the result is the following:

$$C_{ma} = 7.5\ \mu\text{g of chitin / individual}$$

$$C_{ar} = 2.56\ \text{mg of chitin / individual}$$

3.2.2 | Estimation of population size for arthropods

The differences within the group of Arthropods between microarthropods and larger insects are also reflected in their population size and not only in their biomass. A few indications of the populations are reported such as the 40 millions insect per acre (which translate into 98 millions per hectare, or approximatively 10^8) from Pedigo & Rice (2014), or (Yoshida & Hijii, 2005) that reported 24,000 to 220,000 microarthropods per m^2 in soil, which is around 10^8–10^9 individuals/ha. Finally, Salavuddin et al. (2015) reported an average of 341.5 microarthropods per kg of soil, which again correspond to 10^8 individuals/ha. Assuming a 1:10 population rate between large insects and microarthropods, their number can be estimated in the order of 10^7. Finally, since these values were obtained in different measurements units (referring to surface and soil quantity), a distinction can be made based on the scaling of population size with the increasing soil depth. Considering the topsoil layer 0 to 5 cm, insects and microarthropods will be present in the expected amount. However, at the deepest layer 0–20 cm, it can be assumed that microarthropods will scale proportionally while larger insect, due to the presence of flying insects only appearing in the topsoil and are not expected to increase in the same way. For this reason, while the microarthropods estimate follows the proportion with depth, large insect will be accounted for a 50% increase compared to the other group (accounting only for soil-bound insects such as ants for example). To summarise, the estimation of the arthropods population, which correspond to our polynomial terms N_i, N_{ma} and N_{ar} is reported in Table 2.

TABLE 2 Arthropods population estimated in soil.

Soil layer	Microarthropods (N_{ma}/Ha)	Large insects (N_{ar}/Ha)	All arthropods (N_i/Ha)
0–5 cm	$2.2 \cdot 10^8$	$2.2 \cdot 10^7$	$2.4 \cdot 10^8$
0–20 cm	$8.8 \cdot 10^8$	$4.4 \cdot 10^7$	$9.32 \cdot 10^8$

As previously done with fungi, due to the uncertainty of the estimation and the plausible dynamics following seasonal variability, two different scenarios were considered in the estimation of chitin; a range of all arthropods that span between 10^7 and 10^8 individuals/ha.

3.2.3 | Estimation of chitin in soil due to arthropods

With both the absolute and relative abundance of arthropods, together with the average chitin content per individual, it is finally possible to calculate the amount of chitin in soil due to the arthropods community (C_{tar}). The formula below is applied to calculate the amount of chitin in the different soil layer over the surface of an hectare. The results are reported in Table 3.

$$C_{tar} = \left(C_i \cdot N_i\right) = \left[\left(C_{ma} \cdot N_{ma}\right) + \left(C_{ar} \cdot N_{ar}\right)\right]$$

TABLE 3 Chitin estimation in soil due to Arthropods.

Soil layer	Number of Arthropods – N_i (individuals/Ha)	Soil biomass (kg/Ha)	Chitin – C_{tar} (kg/Ha)
0–5 cm	$2.4 \cdot 10^8$	650,000	**71.43**
0–20 cm	$9.32 \cdot 10^8$	2,600,000	**172.86**
0–5 cm	$2.4 \cdot 10^7$	650,000	**7.14**
0–20 cm	$9.32 \cdot 10^7$	2,600,000	**17.3**

3.3 | Chitin in nematodes

Nematodes, also known as roundworms, are a diverse group of microscopic, unsegmented worms found in nearly every ecosystem on Earth. They have a large range variation in size, typically going from micrometres to centimetres in length, with most soil-dwelling species being less than 1 mm long. In soil, their population size can be astounding highlighting their ecological significance. Nematodes play crucial roles in nutrient cycling, decomposition and pest control, with some species acting as plant parasites while others prey on harmful microbes or insects. Chitin is a key structural component of their eggshells (Spiegel, 2011) and body pharyngeal cuticle (Neuhaus et al., 2010), providing protection and mechanical support. One important aspect, which affects the estimation of chitin due to nematodes, is the fact that despite their body size, the eggs have a relatively similar size across species and also a fairly similar chitin content.

3.3.1 | Estimation of nematodes chitin content

In the chapter 'Nematode Chitin and Application', written by Qi Chen and Deliang Peng from the book 'Targeting Chitin-containing Organisms' (Fukamizo, 2019), it is possible to see that not only the egg-size is comparable across species, but also that eggs ($\%_{eg}$) constitutes around 10% of the nematode mass and that 20% of the eggshell ($\%_{cheg}$), in average, is constitutively made of chitin. Although this may not scale linearly or be free of exceptions, this value was used as reference value for this estimation.

3.3.2 | Estimation of nematodes biomass or population size

In order to be able to estimate the amount of chitin deriving from nematodes the total population size should first be estimated. In this regard, van den Hoogen et al. (2019) but also Wang et al. (2021) suggested 5000–10,000 nematodes per kg of soil as an average. Since the global number of individuals in van den Hoogen et al. (2019) were estimated accounting for a total biomass of 302.3 Mt., the estimation resulted in 6.87 mg of nematodes per kg of soil (N_{bm}). This value seems lower than other references and for this reason information from Petersen and Luxton (1982), who indicate the population size being in the range of 10^6–10^7 individuals per m^2 (N_n) of land with individual biomass spanning between 1 and 10 μg/nematode (N_{bm}), was condisered. To cope with those uncertainties, it was decided to select, once again, two different scenarios. The first scenario is a low-nematode case study designed using the population size and biomass retrieved in (van den Hoogen et al., 2019), while the second one is a high-nematode case study using the information from (Petersen & Luxton, 1982). All the information extracted in 3.3.1 and 3.3.2 are summarised in Table 4.

TABLE 4 Nematodes biomass and chitin content estimation.

Nematodes population (N_i)	Nematode biomass (N_{bm})	Eggs biomass % ($\%_{eg}$)	Eggshell chitin % ($\%_{cheg}$)	Chitin in nematode (C_n)	Scenario
5000–10,000 individuals per kg of soil	6.87 mg nematodes per kg of soil	10	20	137.4 μg of chitin per kg of soil	Low-Nematodes content (van den Hoogen et al., 2019)
1–10 millions individuals per m^2 of soil	5 μg/individual nematode	10	20	0.1 μg of chitin/ nematode	High-Nematodes content (Petersen & Luxton, 1982)

3.3.3 | Estimation of chitin content from nematodes

With both the chitin content and two different scenarios of population for the nematodes community, their contribution to natural chitin in soil can be estimated. Applying the formula below, the results are reported in Table 5. Due to the way these data are reported in the literature, the high-nematodes scenario is calculated directly per hectare without soil layers distinction:

$$C_{tnm} = \left(C_n \cdot N_n\right) = \left[\left(N_{bm} \cdot \%_{eg} \cdot \%_{cheg}\right) \cdot N_n\right]$$

TABLE 5 Chitin estimation in soil due to Nematodes.

Soil layer	Number of Nematodes - N_n (individuals/Ha)	Soil biomass (kg/Ha)	Total nematodes chitin – C_{tar}	Scenario
0–5 cm	6.87 mg nematodes per kg of soil	650.000	**89** (g/ha)	**Low-Nematodes content (van den Hoogen et al., 2019)**
0–20 cm	6.87 mg nematodes per kg of soil	2.600.000	**356** (g/ha)	
1 m^2	$5 \cdot 10^6$ individuals per m^2	NA	**5** (kg/ha)	**High-Nematodes content (Petersen & Luxton, 1982)**

3.4 | Estimation of chitin per hectare of agricultural soil

To finalise the chitin estimation the different amounts calculated in agricultural soil from the different biological sources considered in this report should be merged: fungi, arthropods and nematodes. To proceed, the values in the original formula were replaced as follow and results were summarised in Table 6 considering the different layers and the range of populations included in the report to compensate for the unavoidable uncertainties and for the natural variation in population size that could occur due to environmental factors such as latitude, climate, soil type, seasonality and crop-type.

$$C_S = C_{tf} + C_{tar} + C_{tnm}$$

TABLE 6 Chitin estimation in soil due to fungi, arthropods and nematodes.

Soil layer	Chitin from fungi (C_{tf})	Chitin from arthropods (C_{tar})	Chitin from nematodes (C_{tnm})	Soil biomass (kg/ha)	Total chitin in soil (C_S)	Scenario
0–5 cm	204 kg/ha	71.43 kg/ha	5 kg/ha	650,000	**280 kg/ha**	High-Chitin content
0–20 cm	815 kg/ha	172.86 kg/ha	5 kg/ha	2,600,000	**901.5 kg/ha**	
0–5 cm	20 kg/ha	7.14 kg/ha	0.1 kg/ha	650,000	**27.24 kg/ha**	Low-Chitin content
0–20 cm	80 kg/ha	17.29 kg/ha	0.5 kg/ha	2.600000	**98.78 kg/ha**	

4 | CONCLUSIONS

Based on the results presented in the current scientific report, it was concluded that in the first 0–20 cm of soil, the expected amount of chitin due to biological sources ranges from about 100 to 900 kg over the surface of a hectare, while for the first 0–5 cm values are ranging from about 30 to 280 kg per hectare. As outlined above, these calculations referred to an average agricultural soil bulk density of 1.3 g/cm^3. Considering these calculations normalised to a soil bulk density 1.5 g/cm^3 as reported in the FOCUS[4] Guidance ((1997) ref), values range from **31 to 320 kg/ha** in the **0–5 cm layer**, and from **113**

[4]FOCUS: Forum for the Co-ordination of pesticide fate models and their Use.

to 1038 kg/ha in the **0–20 cm layer (Boesten et al.,** 1997**)**. In addition, as initially expected, fungi represent the biggest contributor to soil biological chitin followed by arthropods, while nematodes contributed only marginally to the total amount. In this regard, the strongest chitin predictor is considered as being the population size of fungi (N_f) and arthropods (N_i) which can scale exponentially between seasons, soil types and climatic conditions while most of the remaining parameters included the polynomial equation proposed are assumed to follow a linear variation.

ABBREVIATIONS

NGS	next generation DNA sequencing
PPR	EFSA Panel on Plant Protection Products and their Residues
qPCR	quantitative polymerase chain reaction
ToR	Terms of Reference

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