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The chemical and microbiological safety of emerging alternative protein sources and derived analogues: A review

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Abstract

Climate change and changing consumer demand are the main factors driving the protein transition. This shift toward more sustainable protein sources as alternatives to animal proteins is also reflected in the rapid upscaling of meat and dairy food analogues. Such changes could challenge food safety, as new food sources could result in new and unexpected food safety risks for consumers. This review analyzed the current knowledge on chemical and microbiological contamination of emerging alternative protein sources of plant origin, including soil-based (faba bean, mung bean, lentils, black gram, cowpea, quinoa, hemp, and leaf proteins) and aquatic-based (microalgae and duckweeds) proteins. Moreover, findings on commercial analogues from known alternative protein sources were included. Overall, the main focus of the investigations is on the European context. The review aimed to enable foresight approaches to food safety concerning the protein transition. The results indicated the occurrence of multiple chemical and microbiological hazards either in the raw materials that are the protein sources and eventually in the analogues. Moreover, current European legislation on maximum limits does not address most of the "contaminant-food" pairs identified, and no legislative framework has been developed for analogues. Results of this study provide stakeholders with a more comprehensive understanding of the chemical and microbiological safety of alternative protein sources and derived analogues to enable a holistic and safe approach to the protein transition.

KEYWORDS
alternative protein sources, chemical hazards, food safety, microalgae, microbiological hazards

Abbreviations: AGEs, advanced glycation end products; ATCV-1, *Acanthocystis turfacea chlorella virus 1*; ATX, antillatoxin; BMAA, *β*-*N*-methylamino-L-alanine; CECs, contaminants of emerging concerns; CPF, chlorpyrifos; DAB, 2,4-diaminobutyric acid; DMA, dimethylarsinate; EAA, essential amino acids; HAs, heterocyclic amines; MCPD, monochloropropanediol; MCs, microcystins; ML, maximum limit; NF, novel food; NPAAs, non-protein amino acids; PAH4, benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene; PAHs, polycyclic aromatic hydrocarbons; PSTs, paralytic shellfish toxins; REE, rare earth elements; SCaa, sulfur-containing amino acids; STXs, saxitoxins; THC, delta-9-tetrahydrocannabinol; TWI, tolerable weekly intake.

1 INTRODUCTION

Over the past decade, alternative protein sources have gained attention as replacements to animal protein sources as they combine good nutritional characteristics with environmental sustainability (Eshel et al., 2019; Kim et al., 2019; Willett et al., 2019). This trend is the recognizable result of various drivers; the FAO Corporate Strategic Foresight Exercise identified climate change and changing consumer demand (toward healthier and more sustainable food) among the key current and emerging drivers relevant to the agri-food systems (FAO, 2022). The quest for sustainability stems from ongoing pressures of anthropogenic activities on planetary boundaries with a major contribution of those involving the modern agri-food system (Campbell et al., 2017; Rockström, 2009; Steffen et al., 2015). Indeed, in 2015, the agri-food systems accounted for about one-third (34%) of anthropogenic global greenhouse gases (GHGs) emissions and the European Union alone for 6.7% of food system global GHGs emissions (Crippa et al., 2021). Further, in 2018, about 83% of European GHGs from food supply were associated with the consumption of animal-derived food products and proteins (Poore & Nemecek, 2018; Sandström et al., 2018) The environmental pressure of the agri-food system is expected to increase since the global population is forecast to reach 9.7 billion in 2050, about one third more mouths to feed than today (United Nations Department of Economic and Social Affairs, Population Division, 2022). In light of the above, a transition toward alternative dietary patterns is considered a key step toward achieving more sustainable food systems. A shift toward alternative proteins will lead to a marked reduction in GHG emissions from animal sources (49.89 kg GHGs/100 g beef protein) to plant sources (0.84 kg GHGs/100 g pulses protein) (Poore & Nemecek, 2018). Accordingly, this is a central ambition of the EU's Green Deal and "Farm-to-Fork" (F2F) strategy (European Commission, 2020; Food and Agriculture Organization of the United Nations & World Health Organization [FAO & WHO], 2019). Alternative protein sources and derived foods are a very diverse category that encompasses either sources not traditionally consumed in Western countries (i.e., originating from soil, aquatic environment, unicellular organisms, or insects) but also foods from conventional sources produced using innovative techniques (i.e., meat grown in a laboratory from animal cells). To ease the inclusion of alternative proteins in the diet, the global food industry is increasingly committed to the development of animal-like products, hereinafter referred to as analogues. Analogues are aimed to mimic taste, texture, and appearance of the animal counterparts but are made from alternative protein sources (Boukid, 2021). Sales of the plant-based food sector have been growing rapidly since 2020, reaching up to 5.7 EUR billion in Europe and 60.45 USD billion globally (Good Food Institute [GFI], 2022; Global Market Insights, 2020). So far, in Europe, dominant categories are milk- and meat-analogues (38% and 35% of sector sales), but also seafood- and dairy-analogues are on the rise (+102% and 326% sales from 2020) (GFI, 2022). The first analogues to hit the market were soy-based; indeed, soy has been the most common alternative protein source among vegetarians for years (Rizzo & Baroni, 2018). However, the F2F strategy targets the reduction of soybean use and dependence in favor of EU-produced sources (European Commission, 2020). In this respect, other sources are entering the analogues market or are expected to do so; likewise, further increases in production are expected for this sector to keep pace with above-mentioned trends (Banach et al., 2023; Boukid, 2021; He et al., 2020; Lima et al., 2022; Smart Protein Project, 2021). Despite this, several factors hinder the widespread adoption of analogues by the public. These include (1) the high content of calories, saturated fat, and salt, (2) the harsh processing conditions involved in their manufacture, and (3) the lack of harmonized regulatory standards (Aggarwal et al., 2022; Bocker & Silva, 2022; Boukid, 2021; Romulo, 2022). Furthermore, food safety could be challenged by the introduction of new food sources, either through microbiologically and/or chemically contaminated foods or through new and/or increased associations between foods, quantities consumed, demographic characteristics and geographic origin of consumers, and applied processing techniques (FAO, 2022). Yet, current knowledge and data related to chemical and microbial hazards that may contaminate alternative protein sources are relatively limited and scattered.

This review aims to investigate the existing knowledge on the chemical and microbiological safety of emerging alternative protein sources and derived analogues, as well as data gaps. Results provide a more comprehensive understanding of the safety of alternative protein sources, allow a safe by design approach, and guide directions for further research.

2 METHODOLOGY

2.1 Selection of alternative proteins

Protein sources were selected that are recently on the market or expected to come on the market in the near future. Protein sources with a history of use, such as soybean and Spirulina, were, therefore, not included in the analysis. The review focused on emerging alternative protein sources of plant origin. These can be soil-based, such as soybean or lentils, but also aquatic-sourced; in fact, microalgae and duckweed are also considered plant-based proteins (Baek et al., 2021; Zdziebłowska et al., 2024). Therefore, these have been categorized as aquatic-based sources. The selected sources are described in more detail in the sections below. Furthermore, insects were also not included since, recently, several reviews on food safety related to insects have been published (FAO, 2021; Meyer et al., 2021; van der Fels-Klerx et al., 2018).

2.2 | Alternative proteins of plant origin

2.2.1 | Soil-based

Legumes were included as an alternative protein source because leguminous crops have several beneficial effects on the agricultural landscape, as they can help maintain overall biodiversity and increase crop yields (e.g., through intercropping and crop rotation). Furthermore, their ability to biologically fix nitrogen and resist drought favors resource conservation and reduces GHGs emission due to lower fertilizer and water requirements (FAO, 2016). Mainly originating from Asia, Middle East, and Africa, legumes are very ancient crops that are part of the traditional food culture among local populations (FAO, 2016). Overall, nutritional characteristics of legumes indicate good protein quality and balanced amino acids content (essential amino acids [EAA]; in line with FAO/WHO requirements), with a general deficiency in sulfur-containing amino acids (SCaa−); typical of plant proteins (Affrifah et al., 2021; Boukid, 2024; Boukid & Castellari, 2022; Hertzler et al., 2020; Jood et al., 1989; Khazaei et al., 2019; Nasir & Sidhu, 2013; Torres-Tiji et al., 2020). The protein content of raw legumes average 25 g/100 g edible product and reaches up to 90% in protein isolate and concentrates (used for analogues) (Table 1) (Boukid & Castellari, 2022; FAO, 2016). In Europe, legume consumption has a long history; among those included in this review, mung bean protein received "novel food (NF)" authorization from the European Food Safety Authority (EFSA) in 2021 (Turck et al., 2021a). Legumes already employed in analogues are faba bean in meat- and fish-analogues (beyondmeat.com; goodcatchfoods.com), mung bean in egg-analogues (zeroegg.com), and lentils in dairy- and fish-analogues (violifefoods.com; goodcatchfoods.com). Research is also addressing the use of black gram and cowpea as animal protein replacements (Ali et al., 2018; Loushigam & Shanmugam, 2023; Rosida et al., 2022). The multiple characteristics of legumes have led FAO to establish 2016 as the International Year of Pulses (FAO, 2016).

Apart from legumes, quinoa (*Chenopodium quinoa*, Amaranthaceae) is an interesting alternative protein as it is a gluten-free grain-like food crop (usually referred to as pseudocereal), which has wide genetic variability and high stress resistance. Quinoa originates from South America, where it is part of the traditional food culture (Vilcacundo & Hernández-Ledesma, 2017). Nutritional characteristics indicate good protein content and high quality; the EAA content is balanced and in line with FAO/WHO requirements (Venlet et al., 2021; Vilcacundo & Hernández-Ledesma, 2017) (Table 1). As for analogues development, quinoa is already being used for milk analogues (thebridgebio.com), yet its application in meat analogues is being investigated (Shaghaghian et al., 2022).

Apart from quinoa, hemp (*Cannabis sativa*, Cannabaceae) is currently used in milk analogues (goodhemp.com), with its application in meat analogues being investigated as well (Nasrollahzadeh et al., 2022; Zahari et al., 2020). Hemp is an herbaceous annual plant whose environmental benefits include carbon storage, ability to break disease cycles when used in crop rotation, and prevention of soil erosion (Yano & Fu, 2023). It is a long-standing crop, first domesticated in East Asia, and traditionally grown for multiple uses (food, feed, textiles, paper, construction, cosmetics, and biofuels). However, cultivation has been banned in many countries since the late 1930s due to flowers containing delta-9-tetrahydrocannabinol (THC), a psychoactive substance, leading to its abuse as a drug (Yano & Fu, 2023). Nevertheless, varieties with a low THC content (>0.3%) can be cultivated for industrial uses (Yano & Fu, 2023). Nutritional characteristics indicate protein content of 24 g% in hemp seeds and around 50 g% in hemp meal (/100 g). Besides, protein quality and EAA content are comparable to that of legumes (House et al., 2010) (Table 1).

Leaf proteins included in this review are from *Medicago sativa* (or lucerne, alfalfa) and *Moringa oleifera* and *peregrina* (or drumstick) plants. Leaf proteins have been acknowledged by the FAO as a potential source of high-quality proteins for human consumption due to their abundance of sources and varieties and absence of animal cholesterol (Mielmann, 2013). The soluble fraction contains mainly the protein Rubisco, that is, the most common plant protein, which contains all EAA (EAA/total amino acids in different leaf protein concentrate are up to 53%). Besides, *M. sativa* and *Moringa* spp. have good protein content and quality (Anoop et al., 2023; Hadidi et al., 2023; Mielmann, 2013; Saa et al., 2019) (Table 1). These plants are multipurpose crops already consumed in different countries. Currently, in Europe, Rubisco is extracted from *M. sativa* as a protein powder for use in various food applications, including meat-analogues (Anwar et al., 2007; Robiansyah et al., 2014; Mielmann, 2013) (rubiscofoods.com). Research is also addressing the use of *M.*

TABLE 1 Nutritional characteristics of protein sources included in the review.

	Protein content	Essential amino acids (–)	Protein quality
Legumes			
Cowpea	21 g%	SCaa and Trp	72% (IVPD)
Faba bean	26 g%	SCaa, Met, Lys, Thr, Trp, Val	0.68 (PDCAAS)
Lentils	25 g%	SCaa, Leu, Thr, Val, and Trp	0.80 (PDCAAS)
Mung beans	24 g%	SCaa	0.65 (PDCAAS)
Black gram	25 g%	SCaa	58% (IVPD)
Pseudocereal			
Quinoa	16.7 g%	n/a	0.81 (PDCAAS)
Seed			
Hempseeds	24 g%	Lys	0.51 (PDCAAS)
Leaf proteins			
Medicago sativa	20 g%	SCaa and Trp	0.95 (PDCAAS)
Moringa spp.	35%	Met, Lys, Val, and Trp	0.91 (PDCAAS)
Microalgae			
Chlorella spp.	60% (DW)	SCaa	90% (PER, BV, DC, NPU)
Other microalgae	40% (DW)	SCaa	/
Duckweeds			
Lemna spp.	45% (DW)	Lys	0.70 (DIAAS)
Wolffia spp.	50% (DW)	Scaa, Val, Lys, Leu, and His	0.75 (DIAAS)

Abbreviations: (–), reduced EAAs; BV, biological value; DC, digestibility coefficient; DIAAS, digestible indispensable amino acid score; DW, dry weight; His, histidine; IVPD, in vitro protein digestibility; Leu, leucine; Lys, lysine; Met, methionine; NPU, net protein utilization; PDCAAS, protein digestibility corrected amino acid score; PER, protein efficiency ratio; SCaa, sulfur-containing amino acids; Thr, threonine; Trp, tryptophan; Val, valine.

Source: References: Affrifah et al., 2021; Anoop et al., 2023; Boukid, 2024; Boukid & Castellari, 2022; FAO, 2016; Fu et al., 2021; Hadidi et al., 2023; Hertzler et al., 2020; House et al., 2010; Hu et al., 2022; Jood et al., 1989; Mes et al., 2022; Mielmann, 2013; Moura et al., 2022; Nasir & Sidhu, 2013; Saa R. W. et al., 2019; Torres-Tiji et al., 2020; Venlet et al., 2021; Vilcacundo & Hernández-Ledesma, 2017; Xu et al., 2021.

oleifera for use in meat- and dairy-analogues (Trigo et al., 2023).

2.2.2 | Aquatic-based

Microalgae are a diverse class of photosynthetic eukaryotic and cyanobacterial organisms, consisting of up to millions of different species. They can accumulate nutrients and organic substances using solar energy and CO_2 and convert inorganic substances (e.g., minerals and trace elements) into valuable compounds (e.g., organic biomass) (Matos, 2019). Microalgae have been known to man for millennia with both food and nonfood applications; in Asia, they play an important role in traditional culinary practice (Matos, 2019). In Western countries, they have gained popularity since 1950s for their use as functional foods ingredients and dietary supplements (Christien Enzing et al., 2014; Fu et al., 2021; Matos, 2019). Today, *Chlorella* spp. are commonly marketed as dietary supplements, representing (together with *Spirulina*) the largest share of the global market for algae products. Moreover, these species can be sold whole, without any major processing other than drying (Christien Enzing et al., 2014; Moura et al., 2022). Other microalgae species have gained popularity for their content of specific beneficial compounds, which are now marketed as single fractions. The microalgae *Dunaliella salina* and *Haematococcus pluvialis* are valued for carotenoids content (i.e., beta-carotene and astaxanthin, respectively), whereas *Nannochloropsis* and *Schizochytrium* species for omega-3 fatty acids (Christien Enzing et al., 2014; Matos, 2019). The use of microalgae as food or food ingredients is subjected to country-specific authorization and legislations. In Europe, microalgae need NF approval from the European Commission (EC) after being assessed by EFSA. Overall, *Chlorella* spp. is widely approved as food ingredient across continents, whereas species as *Dunaliella* spp., *H. pluvialis*, *Schizochytrium* spp., *Tetraselmis chuii*, *Chlamydomonas reinhardtii*, *Nannochloropsis* spp., and *Euglena gracilis* differ in authorization between countries (Christien Enzing et al., 2014; Fu et al., 2021; Matos, 2019; Torres-Tiji et al., 2020). In more recent years, microalgae have also been listed as a novel dietary protein source for use in multiple food preparations (including analogues) with *Spirulina* being recognized by WHO as a health-promoting food owing to its nutritional characteristics combined with health benefits (i.e., prevention or cure of some chronic diseases) (Fu et al., 2021; Koyande et al., 2019). Accordingly, the general EAA composition of microalgae is in line with FAO requirements with only SCaa being slightly reduced (SCaa−) (Torres-Tiji et al., 2020). As for *Chlorella* spp., the EAA composition and index (EAA index: 107.5) make this protein source comparable to traditional animal proteins (Fu et al., 2021). Almost all the microalgae species mentioned average up to 40% protein content (dry weight), with *Chlorella* reaching up to 60% (Moura et al., 2022; Torres-Tiji et al., 2020). As for protein quality, *Chlorella* is comparable to traditional plant-based protein sources such as soy (Fu et al., 2021) (Table 1). In terms of analogues development, microalgae proteins are currently being used to manufacture meat- and dairy-analogues, as well as in combination with other alternative protein sources (Singh et al., 2021) (sophiesbionutrients.com).

Apart from microalgae, duckweeds (or water lentils) are a family of aquatic plants including more than 37 species; those of interest as alternative protein sources (aquatic-based leaf proteins) are part of *Lemna* (*minor* and *gibba* spp.) and *Wolffia* (*arrhyza* and *globose* spp.) genera. These plants are among the fastest growing on earth and can easily adapt to different geographic areas and climatic zones (Stacy et al., 2023). Nutritional characteristics indicate 45%–50% protein content (dry weight), good protein quality, and content of all EAA comparable to FAO requirements (Hu et al., 2022; Mes et al., 2022; Stacy et al., 2023; Xu et al., 2021) (Table 1). Duckweeds have a safe history of use in Asia, where they are considered a common food source among the poorest. In Europe, Wolffia plants have been recognized by EFSA as "traditional food from a third country," whereas other species need NF approval (EFSA, 2021; Stacy et al., 2023). So far, a protein concentrate from a mixture of Lemna species has received NF approval from EFSA. Rubisco is currently extracted as protein powder for use in various food applications, including meat analogues (Stacy et al., 2023) (rubiscofoods.com).

2.3 | Systematic literature search

In this review, food safety was assessed through four hazard categories divided in two main groups, namely: (1) the contamination by chemical hazards, biotoxins, and nanoparticles and (2) the contamination by microbiological hazards. The literature search was conducted based on the EFSA's guidelines for systematic reviews on food safety assessments (EFSA, 2010). The two databases, Scopus and Web of Science, were used, using the year of publication from 2000 onward. Both generic and specific keywords were selected and used to define the search queries; these were combined into four separate searches (Table 2). Upon completion of the systematic searches on both databases, the resulting references were downloaded in EndNote, and duplicates were removed with the database tool, followed by a manual check. The remaining unique references were screened for each search separately (Figure 1). The screening process was performed by title, key words, and abstract. References were classified into relevant, maybe relevant, and not relevant. A second reviewer independently screened 10% of the references in each search. Any differences in categorization were discussed, and consensus was reached on inclusion and exclusion criteria. Papers included address the food safety aspects related to hazard occurrences, foodborne illnesses, and human health outbreaks associated with selected protein sources and derived analogues. In all cases, papers on beneficial effects (e.g., anti-inflammatory potential and nutraceutical compound content) and on other diseases (i.e., non-foodborne) were excluded. Furthermore, only papers with full text in English were included. Following this, a full-text screening was carried out for papers categorized as relevant and maybe relevant. Even though the review focused on upcoming protein sources, papers on meat and dairy analogues produced from "known" alternative protein sources were obtained and, if relevant, included. In the end, results and data were manually extracted from the included papers and compiled into Excel files for synthesis of results. A total of 65 papers were included in this review.

3 | RESULTS

3.1 | Soil-based alternative protein sources

A total of 17 papers on soil-based alternative protein sources were deemed relevant, of which 8 were on chemical contamination and 9 on microbial contamination. Details of the distribution of papers per protein source and topic are given in Figure 2.

3.1.1 | Chemical contamination

Most of the results retrieved for this category describe chemical hazards in raw materials rather than in the protein products extracted from these. Most of the findings reported the occurrence of trace elements and heavy metals in legumes. Their concentrations (upper levels) in cowpea, faba bean, lentil, mung bean, and black gram are given in Table 3. All legumes exceeded the maximum limits (MLs) of cadmium in legumes, that is, 0.020 mg/kg, as set in Commission Regulation (EU) 2023/915 on maximum levels of contaminants in food. In addition, cowpeas, lentils, mung beans, and black wheat also exceeded the

TABLE 2 Search categories, search strings, and resulting searches used to conduct the review.

Categories
1. Generic soil-based protein* OR “plant based*” OR “plant protein based*” OR “plant protein*” OR “alternative protein*” OR “novel protein*” OR “meat substitute*” OR “meat analogue*” OR “meat alternative*” OR “novel food*”
2. Generic aquatic-based protein* OR “microalgae*” OR “microalgae based food*” OR “aquatic protein*” OR “alternative protein*” OR “novel protein*” OR “novel food*”
3. Food safety “food safety” OR food* OR safety* OR risk* OR “risk assessment*” OR health* OR exposure* OR outbreak* OR “human consumption*” OR “food safety risk” OR “food safety hazard*” OR “health concern*” OR “health risk”
4. Specific soil-based protein sources pulse OR pulses OR legume* OR fa?a OR “vicia fa?a” OR “fa?a bean*” OR “broad bean*” OR “horse bean*” OR “field bean*” OR “mung bean*” OR “vigna radiata” OR “v. radiata” OR “green gram” OR cowpea* OR “black eye* pea*” OR “vigna unguiculata*” OR “v. unguiculata*” OR “crowder pea*” OR “southern pea*” OR lentil* OR “lens esculenta” OR “l. esculenta” OR “lens culinaris” OR “l. culinaris” OR “cicer lens” OR quinoa* OR “chenopodium quinoa” OR “c. quinoa” OR hemp* OR “lea? protein*” OR “green lea?* protein*” OR “lpc*” OR “edible lea?*” OR “medicago sativa” OR “m. sativa” OR alfalfa* OR lucerne* OR “cowpea lea?*” OR moringa* OR “moringa oleifera lea?”*
5. Specific aquatic-based protein sources microalgae* OR cyanobacteria OR “green algae*” OR “edible algae” OR chlorella* OR “c. vulgaris” OR “c. pyrenoidosa” OR dunaliella* OR “d. salina” OR “d. tertiolecta” OR “aphanizomenon flos-aqua” OR “alga* fraction*” OR “alga* component*” OR duckweed* OR lemna* OR yclospo*
6. Chemical hazards **OR** Biotoxins **OR** Nanoparticles (“chemical hazard” OR pollutant* OR contaminant* OR contamination* OR occurrence* OR hazard* OR residue* OR “chemical contamination*” OR chemical* OR debris* OR processing* OR pesticide* OR “env* pollutant*” OR “industrial residue*” OR “persistent organic pollutant*” OR “ food contact material*” OR “heavy metal*” OR acrylamide* OR furan* OR acrolein* OR “biogenic amine*” OR “monochloropropandiol*” OR “*mcpd*” OR benzene* OR “d-aminoacid*” OR lysinoalanine* OR “plant protection product*” OR herbicide* OR fungicide* OR insecticide* OR molluscicide* OR “growth regulator*” OR biocide* OR “agr* chemical*” OR agrochemical* OR organophosphate* OR organochlorine* OR carbamate* OR pyrethroid* OR chlordane* OR heptachlor* OR “ddt*” OR “mineral oil hydrocarbon*” OR “moh” OR “flame retardant*” OR “endocrine disruptor*” OR dioxin* OR “polychlorinated biphenyl*” OR “pcb” OR “poly and perfluoroalkyl substance*” OR “ pfas*” OR “polycyclic aromatic hydrocarbon*” OR “pah” OR “petroleum product*” OR benzene* OR bisphenol* OR “methyl tert-butyl ether*” OR “mtbe” OR paraben* OR perchlorate* OR arsenic* OR cadmium* OR mercur* OR methylmercury* OR thallium* OR lead* OR copper* OR aluminium* OR nickel* OR zinc* OR disinfectant* OR detergent* OR antiparastitic* OR pharmaceutical* OR drug* OR “nsaid*” OR hormone* OR sedative* OR steroid* OR “beta agonist*”) **OR** (“biotoxin*” OR “biological toxin*” OR “natural toxin*” OR toxin* OR “fungal toxin*” OR mycotoxin* OR aflatoxin* OR alternaria* OR citrinin* OR “ergot alkaloid*” OR fumonisin* OR “kojic acid*” OR ochratoxin* OR patulin* OR sterigmatocystin* OR trichotecenes* OR deoxynivalenol OR “don” OR “t-2 toxin*” OR zearalenone* OR “masked mycotoxin*” OR “plant toxin*” OR phytotoxin* OR alkaloid* OR “pyrrol* alkaloid*” OR “tropane alkaloid*” OR “quinolizidine alkaloid*” OR “poppy seed* alkaloid*” OR “pyridine alkaloid*” OR glycoalkaloid* OR “hydroxypyrimidine glucoside alkaloid*” OR phytoestrogen* OR phytochemical* OR polyphenol* OR “antinutritional factor*” OR antinutrient* OR lectins* OR “cu-galactoside*” OR “phytic acid*” OR tannin* OR catechin* OR lignanamide* OR “trypsin inhibitor*” OR “protease inhibitor*” OR haemagglutin* OR “nucleic acid*” OR “algal toxin*” OR “shellfish toxin*” OR “ shellfish poison*” OR microcystin* OR cyanotoxin* OR dinotoxin* OR “marine toxin*” OR tetrodotoxin* OR “ttx*” OR “*anatoxin-a”) **OR** (nanopart* OR nanomaterial* OR nanotechn* OR “nano* engineered particle*” OR “nano* material*” OR “nano* structure*” OR nanoplastic* OR microplastic*)
7. Microbiological hazards bacteria* OR pathogen* OR virus* OR parasite* OR prion* OR toxin* OR *infection* OR intoxication* OR occurrence* OR hazard* OR “microbiological contamination*” OR “microbiological hazard*” OR “microbial hazard*” OR “biological contamination*” OR spore* OR spoilage*OR “microbial spoilage*”OR bacillus* OR campylobacter* OR clostridium* OR cronobacter* OR enterobacter* OR klebsiella* OR “e coli*” OR escherichia* OR “stec” OR “vtec” OR “ehec” OR listeria* OR “mycobacterium tuberculosis*” OR salmonella* OR shigella* OR staphylococcus* OR vibrio* OR yersinia* OR brucella* OR coxiella* OR plesiomonas* OR shigelloides* OR aeromonas* OR marine bacteria* OR norovirus* OR rotavirus* OR enterovirus* OR hepatitis* OR cryptosporidium* OR cyclospora * OR giardia* OR toxoplasma* OR taenia* OR anisakis* OR trichinella* OR enterotoxin* OR exotoxin* OR neurotoxin* OR botulinum* OR tetan* OR superantigen* OR “toxic-shock syndrome toxin*” OR “tsst” OR “shiga toxin*” OR “stx” OR “microalgal toxin*”

Searches	
Soil-based	**Aquatic-based**
#1 Chemical hazards 1. AND 3. AND 4. AND 6.	#2 Chemical hazards 2. AND 3. AND 5. AND 6.
#3 Microbiological contamination 1. AND 3. AND 4. AND 7.	#4 Microbiological contamination 2. AND 3. AND 5. AND 7.

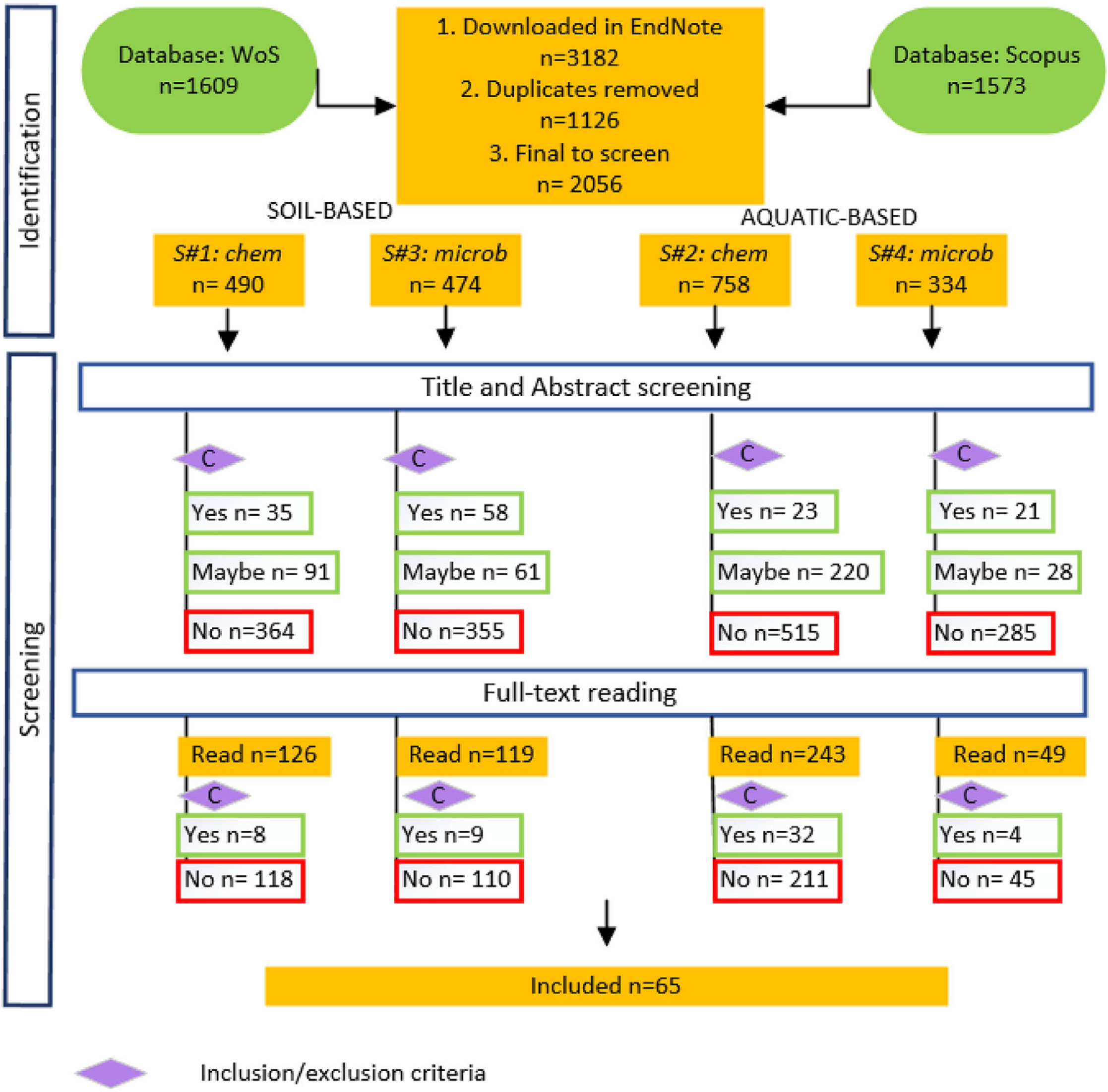

FIGURE 1 Flow diagram of the screening procedure. *Source*: Microsoft Visio Drawing.

limits set for lead in legumes (0.10 mg/kg); cowpeas, lentils, and black wheat also had Pb levels equal to or higher than those set for pulses (0.20 mg/kg) (European Commission, 2023). With regards to other chemical hazards, concentrations of nitrate and nitrite have also been reported for different lentil samples. Nitrate concentrations were 44.98 ± 20.66 (organic lentil samples) and 49.09 ± 26.55 mg/kg (conventional lentil samples); nitrite concentrations were 0.93 ± 0.30 (organic lentil samples) and 0.87 ± 0.44 mg/kg (conventional lentil samples). Moreover, ready-to-eat lentil foods contained nitrites (12–225 mg/kg) and mycotoxins (deoxynivalenol: 1.69 µg/kg, β-zearalanol: 2510 µg/kg) (Baydan et al., 2016; Carballo et al., 2018). Next, faba beans contained mycotoxin HT-2 toxin at a concentration of 1.96–4.71 µg/kg (Reinholds et al., 2021). However, legumes, pulses, and derived foods are not subjected to MLs of nitrates, DON βZAL, T-2, and HT-2 according to the Commission Regulations (EU) 2023/915 and (EC) 1881/2006 (Commission of the European Communities, 2006; European Commission, 2023). To date, the risk associated with dietary exposure to contaminants from consumption of such legumes and derived foods has not been studied yet. In 2021, EFSA recognized mung bean protein as a NF; therefore, its safety at the proposed use (i.e., ingredient in standard food categories up to 200 g NF/kg food) has been approved. Nevertheless, EFSA noted that the cumulative exposure to magnesium (background dietary intake + intake of NF at proposed use) would exceed the adequate intake (i.e., 300–350 mg/day) in adults and the sodium content (i.e., 7320–12,200 mg/kg) would exceed the safe levels of intake (i.e., 2.0 g/ day in adults). However, it was concluded that these levels can already be exceeded with the background diet (Turck et al., 2021a).

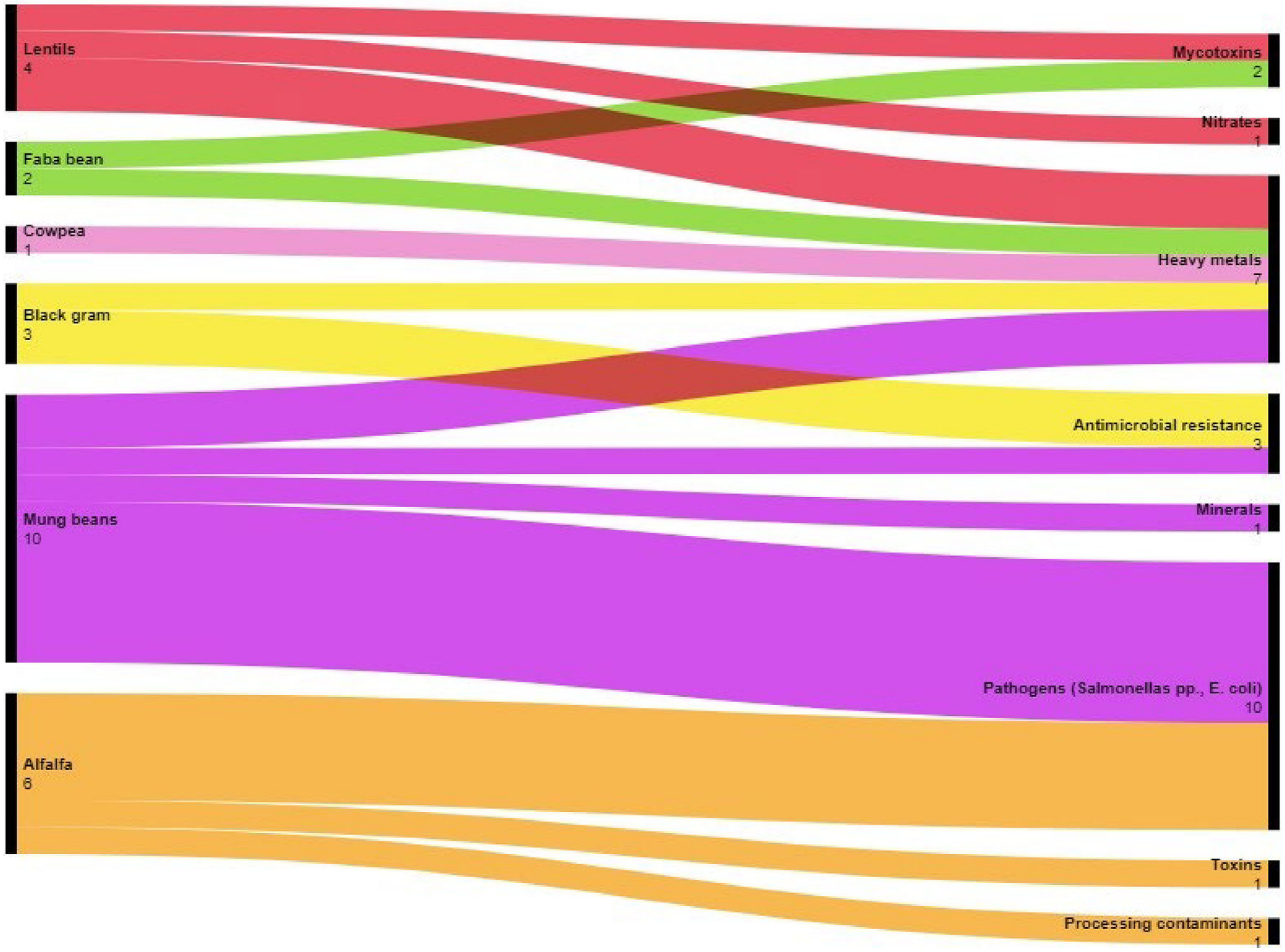

FIGURE 2 Sankey diagram displaying the distribution of papers between the soil-based protein sources and the different topics. The height of the nodes (black rectangles) and the width of the arrows (curved lines) represent flow rate of each "protein source—hazard" pair, that is, higher nodes and wider arrows indicate a larger number of papers. *Source*: RAWGraphs 2.0.

TABLE 3 Concentrations of trace elements and heavy metals in legumes (mg/kg, upper levels).

	Cowpea	Faba bean	Lentils	Mung beans	Black gram
As	0.06		1.80	0.07	1.60
Cd	0.11		0.30	0.08	0.16
Pb	0.20	0.05	1.80	0.14	1.60
Ni	3.80	3.12	4.00	0.08	4.00
Hg			0.01	<0.01 (pp)	
Al			1.17		
B			1.82		
Co			0.01[3]		
Cr			0.01	0.3 (pp)[2]	
Mn			1.04	10.7 (pp)	
Mo			0.30	7.07 (pp)	
Mg				977 (pp)	
Fe			5.88	91.3 (pp)	
Zn			4.15	20.2 (pp)	

Abbreviations: Al, aluminum; As, arsenic; B, boron; Cd, cadmium; Co, cobalt; Cr, chromium; Fe, iron; Hg, mercury; Mg, magnesium; Mn, manganese; Mo, molybdenum; Ni, nickel; Pb, lead; pp, protein product from pulses; Zn, zinc.
Source: References: Hassan, 2023; Hussain et al., 2019; Turck et al., 2021a.

Apart from legumes, papers have been found on alfalfa (*M. Sativa*). In 2009, EFSA acknowledged the safety of an alfalfa protein concentrate as a food supplement at the proposed use of 10 g/day. The opinion reported concentrations of Cd up to 0.243 mg/kg, Pb: 1.937 mg/kg, As: 0.308 mg/kg, Hg: 0.05 mg/kg, and total polycyclic aromatic hydrocarbons (PAHs) up to 327 µg/kg (EFSA, 2009). Moreover, alfalfa can take up cyanobacterial neurotoxins (i.e., non-protein amino acids: *β-N*-methylamino-L-alanine [BMAA] and 2,4-diaminobutyric acid [DAB]) from contaminated waters. Authors pointed out that these can subsequently be transferred to the human food chain (Samardzic et al., 2021). However, alfalfa and derived foods are not subjected to MLs for contamination according to Commission Regulations (EU) 2023/915 and (EC) 1881/2006 (Commission of the European Communities, 2006; European Commission, 2023).

3.1.2 | Microbial contamination

Most of the results retrieved for this category concern sprouts. Although sprouts are not implicated in protein extraction (and analogues production), it was decided to include the findings because of gaps in evidence that the plant parts implicated in such processes have different features. Nevertheless, it is important to further clarify that the findings presented in this section are not directly attributable to the protein sources nor to the derived analogues. Further research is thus needed to establish the effect of processing sprouts into protein or analogues on the hazards identified in the raw material.

Sprouts of mung beans and alfalfa have been responsible for multiple foodborne outbreaks worldwide as a result of microbial contamination, mostly from *Salmonella* spp. and *Escherichia coli* species (Barak et al., 2002; Brankatschk et al., 2014; Gorski et al., 2004; Haijing et al., 2004). Bacterial strains were reported in seeds at concentrations of 3–6 × 10^4, 9 × 10^5, 5–400 × 10^3 CFU/g (alfa alfa), and 1–20 × 10^4 CFU/g (mung bean). Optimal conditions for bacterial growth and replication during germination results in highly contaminated sprouts, even when levels on seeds were minimal (Brankatschk et al., 2014; Haijing et al., 2004). In addition, mung bean sprouts can internalize *Salmonella typhimurium* from contaminated irrigation water at levels between 2.0 and 5.1 log CFU/g, whereas all major plant tissues can internalize *E. coli* O157:H7, allowing its growth and replication (Deering et al., 2011; Ge et al., 2014). Finally, antibiotic-resistant gene transfer between bacteria can occur during mung bean germination (Luo & Matthews, 2023).

Apart from sprouts, other results for this category pointed out the occurrence of antibiotic-resistant strains in black gram-based fermented foods. In details, 48 strains of multidrug-resistant Bacillus cereus and 33 of multidrug-resistant Salmonella spp. have been detected from 105 to 12 samples of commercial food products, respectively (Roy et al., 2007, 2009). The hazards associated with the soil-based alternative protein sources, according to the findings of this review, are summarized in Table 4.

3.2 | Aquatic-based alternative protein sources

A total of 36 papers were included in the results on aquatic-based alternative protein sources, of which 31 are on chemical contamination, 4 are on microbial contamination, and 1 covers both hazard group. Details of distribution of papers per protein source and topic are given in Figure 3.

3.2.1 | Chemical contamination

The results retrieved for this category cover several aspects, which are described below, categorized as microalgae and duckweeds. Figure 4 provides an overview of the hazards associated with microalgae consumption and the potential routes of exposure to humans.

Microalgae

Cyanobacterial toxins production. Several studies have reported the ability of different microalgae species to produce cyanobacterial toxins. Species associated with *Aphanizomenon have* been linked with the production of multiple neurotoxins, such as saxitoxins (STXs), paralytic shellfish toxins, and anatoxins. *Aphanizomenon* species from Finland are reported to produce anatoxin-a at a concentration of 1562–6700 µg/g DW (Osswald et al., 2007), whereas the *A. flos-aquae* can produce STXs (Ampofo & Abbey, 2022; Briand et al., 2003; Carmichael, 2001; Cheung et al., 2013; Mankiewicz et al., 2003; Markou et al., 2021; Mutoti et al., 2022). To date, MLs for the concentration of cyanobacterial toxins in drinking waters have been proposed or are already implemented by different countries worldwide (Ibelings et al., 2015). Additionally, *Aphanizomenon* species have been associated with the production of cylindrospermopsin toxins (Markou et al., 2021; van Apeldoorn et al., 2007). *Aphanizomenon* species have also been recognized as the main toxin-producing microalgae species in a bloom occurring in Spain in 2013 (Casero et al., 2019). Nevertheless, some controversies have been raised about the actual toxin-producing activity of *Aphanizomenon* species. Indeed, Cires and Ballot (2016) pointed out that the attribution of toxin production to some *Aphanizomenon* species has been questionable due to doubtful

TABLE 4 Summary of chemical and microbiological hazards found on soil-based alternative protein sources.

	Elements and metals	Nitrates	Mycotoxins	Cyanobacterial toxins	PAHs	Pathogenic bacteria	Support microbial growth	Outbreaks
Cowpea	√(c)							
Faba bean	√(c)		√(c)					
Lentils	√(c)	√(c)						
Mung beans	√(c)							
Black gram	√(c)							
Alfalfa	√(c)			√(u)	√(c)			
RTE lentil foods		√(c)	√(c)					
Black gram fermented foods						√(amr)		
Sprouts (mung bean, alfalfa)						√(i; amr)	√(c)	√(c)

Abbreviations: amr, antimicrobial resistance; c, contamination; i, internalization; PAHs, polycyclic aromatic hydrocarbons; u, uptake.

phylogeny and frequent misclassification, as well as rarely confirmed occurrence in human outbreaks and blooms. The author reported that in some cases where toxin production was initially attributed to strains of *A. flos-aquae*, these were later reclassified as *A. gracilis*. Indeed, the production of several cyanotoxins is confirmed for the *A. gracilis* species. In addition, the coexistence of *Microcystis aeruginosa* (which produces microcystins [MCs]) and *A. flos-aquae* from Upper Klamath Lake in Oregon has been documented. Therefore, if collected together, MCs from *M. aeruginosa* can contaminate *A. flos-aquae* (Gilroy et al., 2000). Apart from *Aphanizomenon* spp., *Nostoc* species have been associated with production of MCs and non-protein amino acids (BMAA) (Cheung et al., 2013; Markou et al., 2021; Mutoti et al., 2022; van Apeldoorn et al., 2007).

Contamination from culture media. Multiple studies pointed out the need to further assess the impact of cultivation media on microalgae contamination in relation to their absorption and accumulation capacity. Indeed, organic and inorganic compounds such as heavy metals, pesticides, PAHs, polychlorobiphenyls, bird droppings, debris, insects, and rodent hair contaminations can result from cultivation conditions. In addition, as microalgae-based food production involves minimal processing, the removal of contaminants can be difficult and not always complete (Hadi & Brightwell, 2021; Markou et al., 2021; van der Spiegel et al., 2013).

Microalgae grown in agro-industrial wastes and wastewater have the following sorption uptake rates of metals; Cd: 0.02–13.5 mg/g DW, Cr: 226–333 mg/g DW, Co: 0.89–1.3 mg/g DW, Cu: 0.5–7.54 mg/g DW, Pb: 4.49–5.11 mg/g DW, Zn: 72.1 mg/g DW, As: 0.3–1.4 mg/g DW, Hg: 9.2–15.1 mg/g DW, and Ni: 0.4–15.4 mg/g DW. However, the probability of heavy metal contamination may be low because most metals remain attached to the solid fraction after solid/liquid separation during biomass production, thus reducing the amount available to microalgae. In addition, the necessary dilution of digestates to prepare the culture medium further reduces heavy metals concentration (Markou et al., 2018). The biomass of *Scenedemus* spp., used as fertilizers, was analyzed for chemical contaminations from wastewater. Heavy metal occurrences resulted as 1.31 mg/kg DM (Cr), 0.52 mg/kg DM (Hg), 46.5 mg/kg DM (Ni and Pb), 18.6 mg/kg DM (As), 279 mg/kg DM (Cu), and 437 mg/kg DM (Zn), with only Cd exceeding the EU thresholds for mineral-organic fertilizers (Reg EC 2019/1009). Alvarez-Gonalez et al. (2023) analyzed contaminants of emerging concern (CECs), a diverse group of substances that have recently gained attention for their presence in water sources and potential implications for human health and the environment. CECs include pharmaceuticals, personal care products, flame

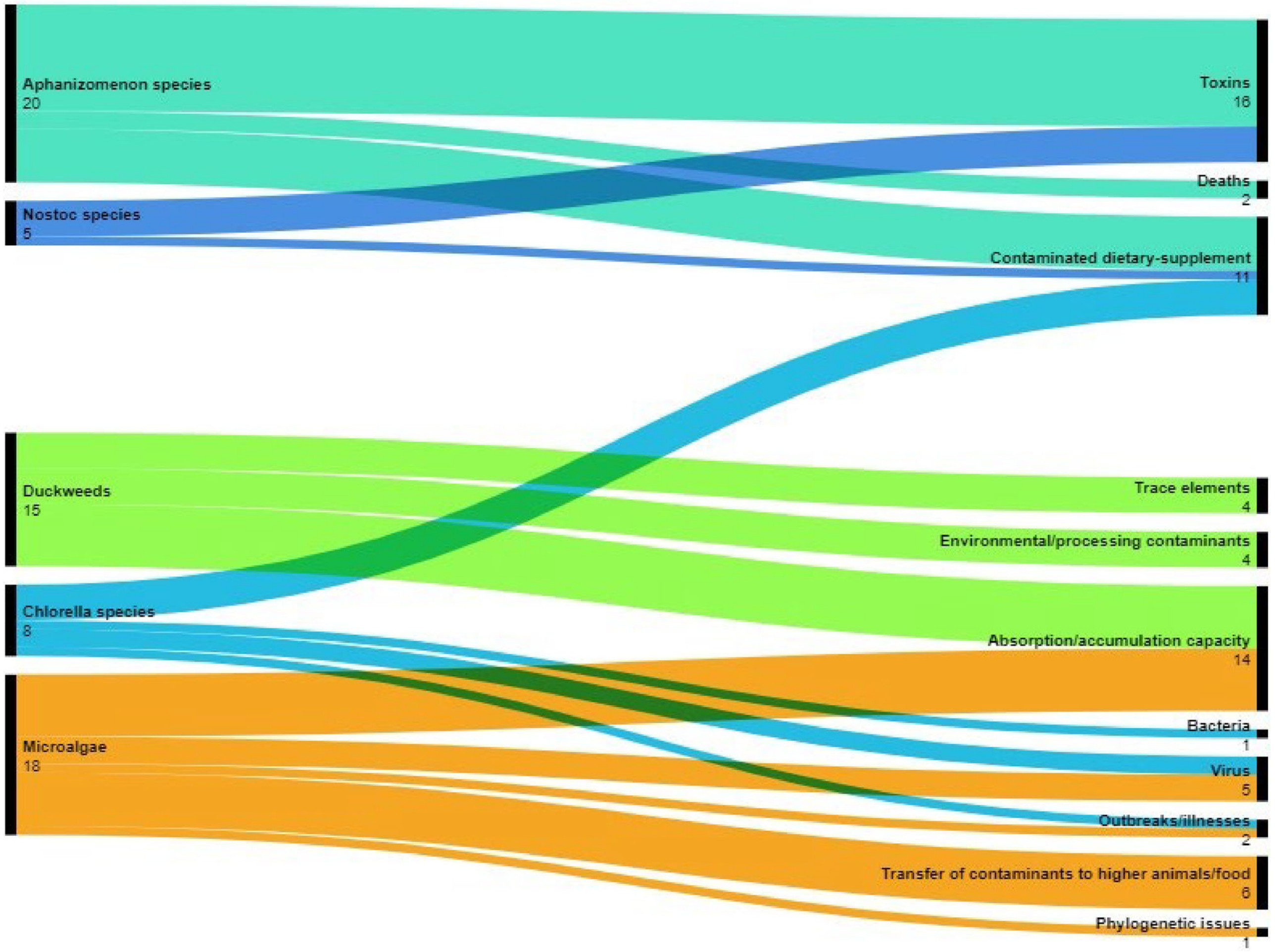

FIGURE 3 Sankey diagram displaying the distribution of papers between the aquatic-based protein sources and the different topics. The height of the nodes (black rectangles) and the width of the arrows (curved lines) represent flow rate of each "protein source—hazard" pair, that is, higher nodes and wider arrows indicate a larger number of papers. *Source*: RAWGraphs 2.0.

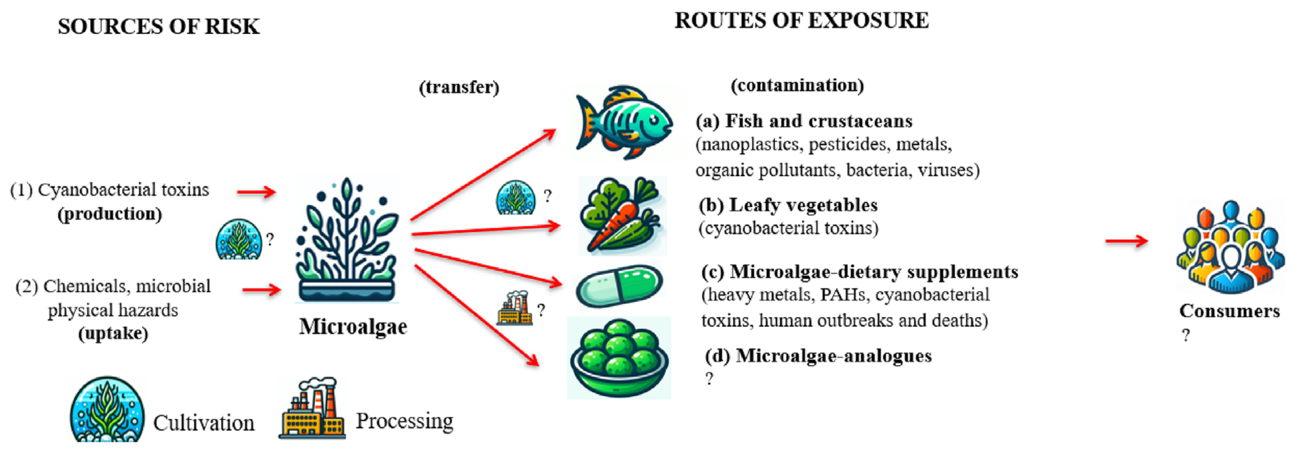

FIGURE 4 Potential food safety hazards, routes of exposure, and knowledge gaps (identified with:?) associated with microalgae consumption. *Source*: Microsoft Bing Image Creator tool.

retardants, pesticides, artificial sweeteners, nanoparticles, and microplastics (Pastorino & Ginebreda, 2021). Their study revealed that hydrocinnamic acid, caffeine, and bisphenol A accumulated in microalgal biomass at concentrations ranging from 0.1 to 25 μg/g DM. Microalgae can also metabolize bisphenol A present in the environment into its glycosides. These can be accumulated in plants and digested in animal intestine (Nakajima et al., 2007). With regards to accumulation of other contaminants such as hormones, antibiotics, parasiticides, mycotoxins, and dioxins, Markou et al. (2018) reported the risk to be low to moderate, but variable and not always negligible. Indeed, in some cases, these contaminants may resist microalgae cultivation processes, and/or their fate is not completely known so far (Markou et al., 2018). Overall, the ability of microalgae to accumulate different kinds of compounds could pose a threat to the entire food chain; the potential transfer of these compounds to higher animals has been assessed for different microalgae. *D. salina* can absorb the nanoplastic amine-modified nanopolystyrene (nPS-NH2) and gradually transfer it to higher-trophic-level organisms, inducing inhibition of digestive α-amylase (Kim et al., 2022). *Isochrysis galbana* can absorb metals, organic pollutants, and pesticides with estimated sorption capacity of 93% toward chlorpyrifos (CPF). Additionally, mussels exposed to the complex microalgae pesticide showed high CPF accumulation (Fernández et al., 2022). Oysters fed with zinc-labeled *Tetraselmis* species can assimilate it with estimated efficiencies of 67% (Lee et al., 2015). Another study reported that oysters fed upon microalgae, *Tetraselmis suecica*, previously exposed to copper, can assimilate it (Amiard-Triquet et al., 2006). Furthermore, terrestrial plants, leafy vegetables, and crops have been found contaminated with MCs from contaminated water, thus confirming cyanobacterial toxins bioaccumulation in aquatic and terrestrial food webs (Mankiewicz et al., 2003; Mutoti et al., 2022; van Apeldoorn et al., 2007).

Contamination of dietary supplements. Several studies have reported contamination of microalgae-based dietary supplements and associated illnesses and deaths in humans. Although these are different from protein foods and analogues, the findings are included because supplements represent virtually the only microalgae-based products widely marketed and consumed to date. Moreover, they involve the same raw material employed in the production of protein foods and (perhaps) undergo similar processing.

With regards to metals, *Chlorella* spp. supplements were found to be contaminated with Al, Pb, As, Ni, Hg, Cd, and inorganic As (descendent order). Al content (1732.8 ± 1991.5 mg/kg) exceeded the EFSA tolerable weekly intake, that is, 1.0 mg/kg bw/day; Hg content (0.41 ± 0.017 mg/kg) exceeded the EU ML, that is, 0.10 mg/kg, whereas Pb content (2.6 ± 1.3 mg/kg) was close to the EU ML, that is, 3.0 mg/kg. Concentrations of rare earth elements averaged 2.03 ± 11.28 mg/kg (European Commission, 2023; Rzymski et al., 2019). The content of arsenic species was reported up to 2.64 μg/g, with inorganic As ranging <0.020–0.127 μg/g and the carcinogenic compound dimethylarsinate ranging <0.020 to 0.035 (Cheyns et al., 2021). PAHs content exceeded the ML from the latest Commission Regulation (EC) 2023/915 for supplements containing spirulina; benzo[a]pyrene ranged 538–873 μg/kg (ML: 10 μg/kg) and PAH4 (i.e., benzo[a]pyrene, benzo[a]anthracene, benzo [b]fluoranthene and chrysene) ranged 2323–3423 μg/kg (ML sum of PAH4: 50 μg/kg) (European Commission, 2023; Muys et al., 2019). With regards to cyanobacterial toxins, multiple papers reported their occurrence in *Chlorella* spp., *A. flos-aquae*, and *Nostoc* supplements and biomasses. Anatoxin-a was found both in *Chlorella* spp. and *A. flos-aquae* supplements at concentrations 0.034 ± 0.002 μg/g (*Chlorella*) and 0.002 ± 0.001 μg/g (*A. flos-aquae)* (Sanchez-Parra et al., 2020). *A. flos-aquae* supplements were also contaminated with multiple MCs at concentrations up to 2210 μg/g (Gilroy et al., 2000; Mutoti et al., 2022; Sanchez-Parra et al., 2020; Vichi et al., 2012; Vinogradova et al., 2011). Additionally, *A. flos-aquae* supplements were also contaminated with neurotoxins (STX and antillatoxin) at ranges 0.46–2.43 μg/g (Mutoti et al., 2022). Overall, *Chlorella* spp. and *A. flos-aquae* supplements tested positive on the MCs/nodularin test (Sanchez-Parra et al., 2020). Manolidi et al. (2019) evaluated the occurrence of BMAA and its isomers DAB and 2,4-diaminobutyric acid and *N*-(2-aminoethyl) glycine *in A. flos-aquae* and *Nostoc* supplements and biomasses. *A. flos-aquae* supplements resulted in BMAA and DAB concentrations of 0.04–0.55 μg/g and 0.08 μg/g, respectively. For *Nostoc* species, which are sold as whole food concentrations were reported in the range of 2.04–21.51 μg/g (21/21 tested samples).

Apart from contaminations, outbreaks associated with microalgae-based supplements consumption have been reported. A 39-year-old women experienced illnesses associated with the consumption of lyophilized blue–green algae-based supplements. Analyses revealed the presence of several MCs (i.e., MC-LR, -YR, -LA, and -RR) in pills (range: 0.8–4.6 ng/mg) and capsules (range: 0.10–1.42); with demethylated forms of MC-RR also present in capsules. Based on the lowest prescribed dose, the total intake of MC would exceed the tolerable daily intake for MC-LR (2.4 μg/day; WHO) for pills only (Bruno et al., 2006). Next, a 34-year-old woman's death was associated with prolonged consumption of *A. flos-aquae* supplements contaminated with MCs (range: 2.62–4.06 μg/g DW). Results from the patient's liver section pointed to the presence of MCs as the most likely cause of the liver failure that resulted in the patient's death (Bruno et al., 2006; Vichi et al., 2012). Lastly, Rzymski et al. (2015) reported two cases of human poisoning in adult females from Poland associated with daily intake of *Chlorella* spp. (and *Spirulina*) supplements from China. Symptoms were atopic dermatitis, rashes, nausea, dizziness, headache and fatigue, abdominal pain, weakness, and so on. Elemental composition analyses of *Chlorella* supplements revealed a high content of metals Al, Cd, Pb, Hg, and Zn. Authors concluded that microalgae are non-toxic per se, and the safety of the final product mostly depends on culturing and manufacturing conditions as well as exposure doses.

Duckweeds

Apart from microalgae, aquatic-based papers were also found for duckweed. The food safety aspects of duckweeds species have been extensively evaluated by EFSA following multiple NF application requests, that is, for "Water lentil powder from *Lemnaceae*," "*Wolffia globosa* powder," "Water lentil protein concentrate from a mixture of *Lemna gibba* and *Lemna minor*," and "*Lemna minor* and *Lemna gibba* as whole plants" (Turck et al., 2021b, 2021c, 2022, 2023). However, only the application for the water lentil protein concentrate from a mixture of *Lemna* species (as ingredient for various foods at maximum concentration of 20 g/100 g) was approved. For the others, the cumulative manganese intake (background dietary intake + intake of NF at proposed use) was of concern, and EFSA could not guarantee the safety. Manganese (Mn) content in the NF was up to 97 mg/kg. Instead, in *Lemna* species powder, their Mn content ranged 128–333 mg/kg, and in *Wolffia globosa* powder, it ranged 56.9–292 mg/kg, with proposed use as ingredients in various foods at maximum levels of 90,000 and 20,000 mg NF/kg, respectively. In whole plants of *Lemna* species, the content ranged 4.8–20 mg/kg, with no specifications of the proposed use other than as leafy vegetables to be eaten either raw or cooked (Turck et al., 2021b, 2021c, 2022, 2023). Apart from manganese content, the presence of other chemical hazards in duckweeds was evaluated. Nitrate content ranged <20 to 63.42 mg/kg in *Lemna* species powder, 1600–5280 mg/kg in *Lemna* species protein concentrate, and 85–2300 mg/kg in *Wolffia globosa* powder (Turck et al., 2021b, 2021c, 2023). Next, *Lemna* species powder contained 1.5 mg/kg of sodium nitrite and 18.4 mg/L of sulfate (Turck et al., 2021b). Whole plants contained 293.1–512.5 mg/kg (nitrate) and 0.5–1.5 mg/kg (nitrite) (Turck et al., 2022). *Wolffia globosa* powder contained ethylenediaminetetraacetic acid up to 380 µg/g (Turck et al., 2021c). As for the NF, dioxins concentrations averaged 0.156 pg TEQ/g (Turck et al., 2023).

With regards to contamination from culture media, duckweeds can accumulate different kinds of contaminants, such as trace elements, metals, cyanobacterial toxins, phenols, and pesticides (Markou et al., 2021; van der Spiegel et al., 2013). Duckweeds grown in agro-industrial wastes and wastewater have the following sorption uptake rates of metals: Cd: 0.2–7.7 mg/g DW, Cr: 0.6–1.2 mg/g DW, Co: up to 21 mg/g DW, Cu: 1–5.5 mg/g DW, Pb: 0.28–10 mg/g DW, Zn: 0.8–20 mg/g DW, As: 0.5–2.2 mg/g DW, and Ni 5.5–12.9 mg/g DW (Markou et al., 2018). In addition, duckweeds can harbor indole alkaloids-producing endophytic bacteria; the content of indole acetic acid (IAA) in *Lemna* species powder and protein concentrate was below MLs set for similar foods categories, whereas in whole plants ranged 58–463 µg/kg DW (Turck et al., 2021b, 2022, 2023).

3.2.2 | Microbiological contamination

The results retrieved for this category mostly cover the identification of microbial hazards and viruses newly associated with microalgae.

Microalgae generally grow in symbiotic association with different types of bacteria. In detail, *Chlorella*-associated bacteria have been reported as *a-Proteobacteria* (40%), *b-Proteobacteria* (12.5%), *g-Proteobacteria* (9%), *Bacteroidetes* and *Actinobacteria* (16%), and *Planctomycetia* (6%). This means that both harmful and beneficial bacteria are associated with *Chlorella* (Chegukrishnamurthi et al., 2020). DNA sequences similar to that of *Acanthocystis turfacea chlorella virus 1* (ATCV-1) have been identified in human oropharyngeal specimens. ATCV-1 has only been known to infect *Chlorella* species. However, it can unexpectedly persist in mammalian macrophages and stimulate inflammatory responses, contributing to pathologies in animals and humans. The subjects with ATCV-1-like sequences in their oropharynx were experiencing decreased visual functions (Hadi & Brightwell, 2021; Petro et al., 2015). In addition, new microalgae-RNA viruses associations have been reported for *Nannochloropsis* and *Euglena* species. *Nannochloropsis*-associated viruses are part of *Duplornaviricota* and *Pisuviricota* phylum, whereas Euglena-associated viruses are part of *Pisuviricota* phylum. However, there are still knowledge gaps on how these viruses can infect the plants and eukaryotic supergroups (Charon et al., 2022). Next, Le et al. (2020) reported the identification of different Vibrio strains in microalgae cultures used to feed oyster larvae. *Vibrio* spp. proved to be pathogenic to the oysters with some strains being resistant to antibiotics. In addition, oysters fed with microalgae having *Vibrio* concentrations of 2.0×10^6 CFU/mL resulted in diseased animals. With regards to contaminations, filamentous cyanobacteria have been isolated from commercial *Chlorella* dietary supplements, along with several non-pathogenic bacteria. Authors remarked that this is likely

TABLE 5 Summary of chemical and microbiological hazards found on aquatic-based protein sources.

	Elements and metals	Cyanobacterial toxins	Organic pollutants	Bacterial strains/Viruses	Nanoplastics
Microalgae	√ (u,t)	√ (p,t)	√ (u,t)	√	√ (u,t)
Dietary supplements	√ (c)	√ (c)	√ (c)		
Duckweeds	√ (u, c)	√ (u)	√ (u, c)	√ (c)	

Abbreviations: c, contamination; p, production; t, transfer to food or supplements; u, uptake.

because microalgae-based food production involves minimal heat treatments; therefore, removal of pathogens can be difficult and not always complete (Hadi & Brightwell, 2021). The hazards associated with the aquatic-based alternative protein sources, according to the findings of this review, are summarized in Table 5.

3.3 | Meat- and dairy-analogues

A total of 13 papers were included in this section, 1 of which is also included in the results for aquatic-based alternative protein sources. Multiple studies reported a high susceptibility to chemical and microbial contamination for meat analogues, which is mostly attributed to manufacturing processes applied as well as specific product characteristics (e.g., high moisture and low pH). Moreover, although production processes use high temperatures, these products are frequently subjected to recontamination, postproduction, and post-opening contamination (Caldwell & Mills, 2022; Dai et al., 2022; Hadi & Brightwell, 2021).

3.3.1 | Chemical contamination

Findings on commercial meat-analogues reported the occurrence of mycotoxins and processing contaminants such as Maillard reaction products. Mycotoxins occurrence and co-occurrence were reported in different kinds of commercial soy-, pea-, chickpea-, and lupine-based meat analogues such as burgers, sliced meat, steak, textured products and meatballs as well as in other preparations (i.e., flour and protein products) (Table 6). Apart from mycotoxins, Deng et al. (2022) found an increase in the accumulation of heterocyclic amines (HAs) and advanced glycation end products (AGEs) during processing steps from raw plant proteins to plant-based analogues (burger). In detail, concentrations of free HAs increased from 6.63 ng/g (maximum level; raw materials) to 20.23 ng/g (maximum level; textured vegetable proteins) and up to 33.91 and 35.21 ng/g in raw and cooked commercial analogues, respectively. The protein-bound HAs content was highest for all product types, reaching a maximum of 290.53 ng/g in cooked commercial meat-analogues. The

TABLE 6 Mycotoxins occurrence in commercial meat-analogues (μg/kg, upper levels).

	Soy	Pea	Chickpea	Lupin
AFB1	10.1 (m)		4.2 (f)	7.1 (m)
AFB2	0.89 (f)		0.4 (f)	
AFG1	1.76 (f)		0.4 (f), 1.8 (m)[2]	
AFG2	100.2 (m), 1.77 (f)[2]		0.4 (f)	1.9 (m)
FB1	260.5 (m)	39.4 (m)	53.8 (m)	40.3 (m)
FB2	5.1 (m)	39 (m)	19.2 (m)	1.4 (m)
AOH	184.4 (m; a)	11.3 (m)	5.6 (m)	
AME	207.5 (m; a)	5.4 (m)	2.1 (m)	0.34 (m)
TEN	10.2 (m)		5.2 (m)	3.1 (m)
ZEN	214 (pp)			
OTA	8.6 (m),2.26 (pp)[2]	4.9 (m)	7.9 (m)	
DON	367.5 (m)			
ENA	323.81 (m; a)			
T2	251.3 (f), 32 (pp)			
HT2	11 (pp)			
STO	25 (f)			
MAS	19.5 (pp)			
DAS	21 (pp)			

Abbreviations: a, average content; AFB1, AFB2, AFG1, AFG2, aflatoxin B1, B2, G1, G2; AME, alternariol monomethyl ether; AOH, alternariol; DAS, diacetoxyscirpenol; DON, deoxynivalenol; ENA, enniatin A; f, flour; FB1, FB2, fumonisin B1, B2; m, meat analogues; MAS, monoacetoxyscirpenol; OTA, ochratoxin A; pp, protein products (i.e., textured, concentrate, and isolate); STO, scirpentriol; T2, HT2, toxin T2, HT2; TEN, tentoxin; ZEN, zearalenone.
Source: References: Mihalache et al., 2023, 2022.

study pointed out that free HAs mainly accumulate during extrusion processing with large variations according to specific product formulation. With regards of AGEs formation, *Nε*-(carboxymethyl) lysine and *Nε*-(carboxyethyl) lysine analyses revealed high accumulation in all samples during the cooking stage of analogues (Deng et al., 2022). Other studies reported that production processes and temperatures applied may also lead to the formation of other carcinogenic compounds, such as PAHs and nitrosamines, which, however, are poorly studied in these products (Caldwell & Mills, 2022; Hadi & Brightwell, 2021). Findings on commercial dairy analogues reported the occurrence of mycotoxins, bisphenols, trace elements, and heavy metals in milk and yoghurt analogues (Table 7).

TABLE 7 Chemical contamination in commercial dairy-analogues (upper levels).

	Oat	Pea	Soy	Rice	Coconut	Almond	Millet
Mycotoxins (μg/L)							
AFB1	0.3 (m)						
AFB2	0.4 (m)						
AFG1	0.1 (m)						
OTA	0.2 (m)		0.1 (m)				
T2	1.3 (m)						
DON				19 (m)			
Bisphenols (ng/mL)							
BPA	18.17 (m)		2.37 (m)	1.85 (m)	3.7 (m)	2.6 (m)	
BPB				5.17 (m)			
Elements and metals (μg/L or μg/kg)							
As	0.53 (m)		0.42 (m), 4.33 (y)	2.34 (m)	1.27 (m), 10.61 (y)	0.02 (m)	0.73 (m)
Al		5844.72 (y)	9019.05 (y)		1537.15 (y)		
Cr		9.23 (y)	39.50 (y)		88.14 (y)		
Co		10.54 (y)	40.56 (y)		14.93 (y)		
Ni		76.03 (y)	255.85 (y)		700.46 (y)		
Mo		355.70 (y)	207.61 (y)		37.83 (y)		
Ba		250.45 (y)	580.98 (y)		1505.71 (y)		
Cd			4.20 (y)		4.37 (y)		
Pb					60.7 (y)		

Abbreviations: AFB1, AFB2, AFG1, aflatoxin B1, B2, G1; Al, aluminum; As, arsenic; Ba, barium; BPA, BPB, bisphenol A, B; Cd, cadmium; Co, cobalt; Cr, chromium; DON, deoxynivalenol; m, milk analogues; Mo, molibdenum; Ni, nickel; OTA, ochratoxin A; Pb, lead; T2, toxin T2; y, yoghurt.
Source: References: Miro-Abella et al., 2017; Rebellato et al., 2023; Ruzik & Jakubowska, 2022; Schiano et al., 2022.

3.3.2 | Microbial contamination

Findings on commercial meat analogues made from soy and pea show the ability of these products to support pathogenic microorganisms and spore survival and growth. Indeed, faster microbial growth was reported in dishes prepared with pea-based meat-analogues compared to the same dishes prepared with conventional meat products. Interestingly, raw materials per se did not pose a food safety risk in terms of microbial contamination; however, this increased with the production of analogues and analogues-based dishes (Liu et al., 2023; Tóth et al., 2021). In addition, commercial samples (with and without preservatives) exceeded the National Standard of China and the established plant-based microbial index for *E. coli* and *Staphylococcus aureus* microbial count (Dai et al., 2022). Caldwell and Mills (2022) pointed out that microbial communities occurrence in commercial vegetarian and vegan meat analogues was low but wide-ranging, that is, <2.0–8.7 log CFU/g. Specifically, the authors reported high levels of Enterococci, which may be a food safety concern as associated with virulence factors, antimicrobial resistance, and gene transfer. Findings on commercial milk analogues made from coconut, almond, and cashew also showed significantly higher growth and proliferation rates of *Listeria monocytogenes* and *Salmonella enterica* compared to bovine milk (Bartula et al., 2023). The hazards associated with meat- and dairy-analogues, according to the findings of this review, are summarized in Table 8.

4 | DISCUSSION

Findings from this systematic literature review indicate that trace elements, heavy metals, mycotoxins, PAHs, and nitrates are the most common chemical contaminants in soil-based alternative protein sources and analogues currently on the market. This is consistent throughout with the fact that most of the analogues already on the market are indeed soil-based. Trace elements, heavy metals, and mycotoxins are considered exogenous sources of risk that can contaminate raw materials and easily end up in foods. In contrast, PAHs and nitrates are considered endogenous sources of risk as they generally result from production and processing practices (Lin et al., 2023). Beyond our results, recent publications listed pesticide residues, disinfection byproducts, and foreign bodies among the potential food safety risk factors for soil-based ingredients (Banach et al., 2023; Lin et al., 2023). Besides chemical contamination, findings on microbial contamination have been of little

TABLE 8 Summary of chemical and microbiological hazards found on analogues made from "known" alternative protein sources.

	Elements and minerals	Mycotoxins	Processing contaminants	Bisphenols	Enterococci	Support microbial growth	(Re-) contamination
Meat analogues		√ (c)	√ (c)		√ (c)	√	√
Dairy analogues	√ (c)	√ (c)		√ (c)		√	√

Abbreviation: c, contamination.

relevance. Indeed, sprouts are likely to have no role in protein extraction and analogues production. Nevertheless, their ability to internalize pathogens (*Salmonella* spp. and *E. coli*) and transfer antibiotic resistance cannot be excluded from a comprehensive food safety evaluation. Moreover, sprouts consumption is another dominant (and rising) dietary trend of the time being also associated with the growing interest in less processed and plant-based foods (Aloo et al., 2021; FACT.MR, 2023). Beyond our results, a recently published study on microbial contamination of plant-based ingredients used in dairy analogues reported the presence of *Bacillus* spp., *Pantoea* spp., *Kosakonia* spp., *Paenicillium* spp., *Enterococcus* spp., *Micrococcus* spp., *Staphylococcus* spp., *Acinetobacter* spp., *Leuconostoc* spp., and *Erwinia* spp. in faba bean, *Chronobacter* spp., *Enterobacter* spp., *Acinetobacter* spp., *Bacillus* spp., *Paenicillium* spp., *Lysinibacillus* spp., and *Geobacillus* spp. in mung bean, and *Bacillus* spp. in quinoa (Kyrylenko et al., 2023). The contamination of raw materials from pathogenic bacteria mostly results from the environment and improper hygienic and manufacturing practices; hence, it is categorized as an exogenous source of risk (Lin et al., 2023). Limited information has been found on quinoa, hemp, and *Moringa* spp. Nevertheless, heavy metals, mycotoxins, pesticide residues, and pathogenic bacteria can compromise food safety also for those protein sources and derived foods (Adeyeye et al., 2020; Ćaćić et al., 2019; Walia et al., 2019). In considering the above findings, it should be noted that they refer to raw materials, which are then processed into protein isolates and concentrates for use in the production of analogues. Although processing has effects on these contaminants, these effects are not fully understood yet (Banach et al., 2023). Therefore, the effect that processes from "raw materials > protein extracts > analogues" have on such contaminants should be further explored to determine if these protein-rich materials are the source of contamination in the finished products. This review also reported the formation of HAs and AGEs in meat analogues made from known alternative protein sources. These compounds are formed during Maillard reaction and protein pyrolysis due to interactions between carbohydrates and proteins at temperatures above 140°C. Such temperatures are needed to texturize plant proteins and produce a fibrous structure that mimics that of meat. Focusing on analogues, highly processed foods—regardless of type and contamination of raw materials—are likely to be contaminated with processing contaminants. Processing contaminants are a wide category of chemical compounds whose formation occurs within the products during processing upon conditions applied; hence, they are considered an endogenous source of risk (Lin et al., 2023). Beyond our results, a recent publication also reported acrylamide formation in commercial plant-based meat analogues at concentrations from 31.81 to 186.70 μg/kg (Fu et al., 2023). Besides chemical contamination, our findings pointed out the high capacity to support microbial growth and (re-)contamination due to intrinsic properties, that is, high moisture and neutral pH. Microbial contamination of analogues is considered an exogenous sources of risk; however, unlike raw materials', this depends on improper handling and storage of finished products (Lin et al., 2023).

Together, this information indicates that analogues made from "known" alternative protein sources can be contaminated with both chemical and microbiological hazards. Nevertheless, it is difficult to establish the origin of the contamination as well as a direct correlation such as "contaminated protein-rich raw materials" > "contaminated analogues," as these are made up of several ingredients that could all be source of contaminations. At the same time, this information could be used to guide direction of what hazards to expect after processing.

Considering the above, even if the exact fate of contaminants during the production of analogues is not fully understood, it cannot be conclusively established that they are degraded.

Apart from soil-based protein sources and analogues, findings from this review indicated that microalgae and duckweeds behave very similarly, as both categories can uptake chemical contaminants from the environment. Microalgae produce cyanobacterial toxins and can uptake elements, metals, organic pollutants, and nanoplastics from the environment. Such contaminants can all be transferred through aquatic and terrestrial environment and end up in foods and supplements. As for biological hazard, microalgae usually grow in symbiosis with (pathogenic and nonpathogenic) bacteria, and multiple viral species are associated with them. Duckweeds can also contribute with the entry of contaminants into the food chain. Indeed, these plants can uptake elements, metals, organic pollutants as well as cyanobacterial toxins produced by microalgae. In addition, duckweeds can host IAA producing bacterial strains. Beyond our results, additional literature supported the primary and central role of culture media (water) in contamination and spread of microalgae-associated hazards (Ferreira de Oliveira & Bragotto, 2022; Testai et al., 2016). To further appraise risks posed by microalgae-derived products consumption, choice was made to include also findings on food supplements. Beyond our results, a recently published study pointed out the occurrences of processing contaminants such as PAHs, glycidol, and 2- and 3-monochloropropanediol and its esters (MCPDs, MCPDEs) in microalgae biomasses and derived products (i.e., supplements and oils) (Ferreira de Oliveira & Bragotto, 2022). Therefore, also the aquatic-based alternative protein sources risk endogenous (produced by them naturally or during processing) and exogenous (absorbed by the culture media and the environment) contamination. However, the lack of knowledge on cultivation conditions and subsequent processing prevents a complete risk assessment of such hazards resulting from the consumption of foods like the analogues. Indeed, although hazards' production, uptake, and transfer to fish, crops, and supplements have been researched, their fate during processing is still unknown.

This review aims to improve understanding and identify knowledge gaps related to the chemical and microbiological contamination of emerging alternative protein sources and derived analogues. Nevertheless, for a comprehensive food safety assessment, consideration must also be given to the toxicological effects as well as the potential to generate allergic reactions associated with the protein sources and foods. Further studies are needed to explore these effects in order to complete the picture on the safety of emerging protein sources. The overall purpose was to provide knowledge to ensure "foresight" and safe-by-design approaches to food safety in the protein transition. To this end, multiple strategies exist to protect the health of consumers and ensure food safety. In Europe, MLs are established by the EC for some "contaminant-food" pairs; the latest regulation on this subject is the Commission Regulation (EU) 2023/915 of 25 April 2023. As regards the soil-based "contaminant-food" pairs identified in this review, the regulation only targets the amounts of lead and cadmium in legumes and pulses, cadmium in quinoa, and ochratoxin A in hempseeds. As regards the aquatic-based sources, these are addressed only as supplements. MLs have been established for total PAHs in *Spirulina* spp.—containing supplements and algae—containing botanical products; the latter are also subjected to MLs for pyrrolizidine alkaloids. With regards to MC-LR, MLs have been established for drinking water (European Commission, 2023; Testai et al., 2016). Instead, other microalgae (e.g., *Chlorella* spp.) and duckweeds are not addressed. Therefore, several "contaminant-food" pairs identified in this review are unregulated, with analogues being completely overlooked. To capture the current food safety scenario in Europe, a search was conducted in the Rapid Alert System for Food and Feed (RASFF tool) to track relevant notifications from 2020 onward. This resulted in a total of 147 and 14 notifications for emerging protein sources and analogues, respectively (Figure S1a and S1b). These results support our findings and further highlight how such ingredients and foods can undermine food safety in several ways.

5 | CONCLUSION

This study applied a systematic literature review to investigate the presence of chemical and microbial contaminants in emerging plant-based alternative protein sources used in commercial protein products and analogues formulations. The key findings are summarized as follows:

1. Chemical and microbial contaminations, both endogenous and exogenous, can occur in the emerging alternative protein sources included in this review, which are faba beans, mung beans, lentils, black gram, cowpea, quinoa, hemp, leaf proteins, microalgae, and duckweeds.
2. Chemical and microbial hazards also occur in commercial meat and dairy analogues made from known alternative protein sources, that is, soy, peas, chickpeas, lupins, oats, rice, coconut, almonds, and millet.
3. Further investigations are needed to establish whether contamination of the protein-rich raw materials is attributable to the contamination of the derived finished products. In detail, the effectiveness of the processing conditions applied in reducing such contaminations must be thoroughly established to ensure the food is safe for consumers.
4. In many cases, European legislations on MLs do not cover "contaminant-food" pairs identified (also) in this review. This particularly applies when it comes to newly introduced foods sources (e.g., microalgae, duckweeds, and leaves).
5. Microalgae as protein food, which constitute a separate fast-growing industry, are highly under-researched with respect to food safety. This results in limited knowledge on any potential dietary transfer to humans of taken-up compounds and toxins and increased consumers exposure to risk when using microalgae-derived foods and supplements.

These points highlighted non "foresight" and safe by design approaches toward food safety in the protein transition. In detail, in assessing food safety aspects of emerging food sources, it is of paramount importance to employ holistic and cross-cutting approaches. Scientific and technological knowledge, consumption trends, and regulatory frameworks should all be integrated and aligned. From a wider perspective, these aspects represent loopholes whereby academia, business, and policymakers are mutually dependent on each other. Indeed, to ensure safety and shape science-based legislation, it is necessary to be knowledgeable about foods (raw materials and analogues) before they enter the market. However, it is increasingly difficult to keep up with market and industry developments. Overall, these linkages increase the complexity in ensuring "foresight" and safe-by-design approaches to food safety, especially when it comes to dietary trends such as the consumption of analogues. In addition, in Europe, there is no legislative framework specially developed for analogues; therefore, the lack of either risk assessment and up-to-date regulatory frameworks prevents their spread among the general public, as they may fail in ensuring food safety. Nevertheless, the dietary transition is already in place and supported by institutions worldwide as one of the pathways to boost sustainability. Therefore, it would be advisable to align the different actors involved in such transition to facilitate the safe introduction of meat and dairy replacers.

AUTHOR CONTRIBUTIONS

M. Milana: Investigation; writing—original draft; methodology; writing—review and editing; formal analysis. **E. D. van Asselt**: Supervision; writing—review and editing; methodology. **H. J. van der Fels-Klerx**: Supervision; writing—review and editing; methodology.

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CONFLICTS OF INTEREST STATEMENT

The authors declare to have no conflicts of interest.

Websites

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Good Catch Foods: https://goodcatchfoods.com/
Zero Egg: https://zeroegg.com/
Violife Foods: https://www.violife.com/
The Bridge Bio: https://thebridgebio.com/
Good Hemp: https://www.goodhemp.com/
Rubisco Foods: https://rubiscofoods.com/
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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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