Taxonomy and risk classification of algae

Informing the risk classification of a dynamic taxonomic group

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On the cover: Photobioreactor for the production of microalgae (Ivar Pel, Wageningen)

Foreword

Algae are a large and diverse group of eukaryotic organisms, usually photoautotrophic but also mixotrophic in several cases, and with a wide range of properties. For practical reasons some prokaryotic organisms (cyanobacteria) are also considered algae. Exploiting their physiological versatility, algae are often used to produce feed ingredients, biofuel, etc., and as cell factories for the production of valuable carbon compounds from atmospheric CO_2. Their potential can be enhanced by engineering the metabolic pathways towards the desired product. In 2012, COGEM commissioned a research project to gain insight in the use of genetically modified (GM) algae as production organisms, paying special attention to the risks posed by GM algae when these would escape production enclosures and reach the environment. This has resulted in the research report "Algae and genetic modification. Research, production and risks"(CGM2012-05).

In the last couple of years, COGEM has been asked repeatedly to advice on the (a)pathogenicity of algal species as hosts for genetic modification. To assign algae used as GM hosts to pathogenicity classes, questions arise on the biological properties of these organisms and their possible harmful effects in the environment after escape. As COGEM expects the number of algal species used as GM hosts to increase, it has commissioned a research project on the taxonomy and characteristics of algae (including cyanobacteria). The information gathered in this research project will be used to examine whether it is possible to classify algal species on the basis of properties that indicate possible harmful effects in the environment.

The authors of the report enthusiastically dived into the fascinating world of algae and their (biotechnological) applications. They shed light on the dynamic taxonomic landscape of the algae and selected five hundred algal species that were studied in more detail. These species were the subject of a literature study that focused on potential pathogenicity and adverse environmental effects. The information obtained was used to compare the number of species with and without harmful properties within algal families. The steering committee welcomes the abundance of information retrieved which provides valuable input for future advices of COGEM.

Nico M. van Straalen
Chair of the Advisory Committee.

Summary

When an application for genetic modification of a specific algae species is submitted, COGEM is asked to determine the pathogenicity classification, if not already done. This study aimed to **review available risk-related information** that can inform this classification and provide references for the classification of wild-type algae according to their potential adverse effects on human and animal health and the environment. Furthermore, the question was raised if whole families or genera could be grouped or higher taxonomic levels could be identified that are recognised as safe or conversely known to be hazardous. The second aim of this study was therefore to examine the **feasibility of a more general risk classification** and to attempt to cluster as far as possible algae into groups with hazardous and non-hazardous properties. A set of algae was selected because of their potential of being applied in research, development and/or production.

Algae are a tremendously diverse group of organisms, and many algal groups belong to evolutionary unrelated lineages. Unicellular algae or microalgae as well as multicellular algae or macroalgae are known. The group has been assigned into numerous families and genera. As our knowledge on algal phylogeny increases, the taxonomic classification continues to evolve. In this report ‘algae’ refers to the algal species in the botanical sense as well as cyanobacteria.

While some species have been historically used as a food, the algae gained interest in the past decades not only for human and animal nutrition (food, feed and additives), but also as source of biomolecules as well as production systems for valuable pharmaceuticals and biofuels.

Production efficiency of algae and bio-manufacturing of a wide range of molecules of interest may be enhanced, among others by genetic modification. While algae are able to fix enormous amounts of CO_2, photosynthesis can still be improved. Also, the natural capacity of some algae to produce interesting biochemicals might not yet achieve economically relevant levels and can be enhanced. Furthermore, specific compounds, naturally not occurring in algae, may be produced by using algae as production systems. Moreover, algae as production systems are a sustainable way to manufacture chemicals and oils for food, feed and fuel. Hence, efforts have been made to adapt microalgae via genetic modification to obtain excellent production strains.

The ambiguity of algae taxonomy is an important challenge for this type of endeavour. In order to attribute characteristics to a specific species or strain, one must be able to refer to this species or strain in a traceable and fixed way. Yet, there is no unanimously accepted classification of algae. As the backbone for this project, we compiled a classification into a main reference framework. It includes 7191 genera, distributed over 800 families, 267 orders, 60 classes and 13 phyla/divisions (the **Algal Taxonomic Landscape** is provided as a separate Supplementary Document).

At the same time, the dynamic nature of the algal taxonomy must be stressed. During the initial research phase of this project, we observed changes at family level within the Cyanobacteria. Modifications at family, genus and species can be limited, but in other cases, for example when dealing with cryptic species complexes, may have a broad impact. This dynamic status of taxonomy impacts clustering and making any risk classification based on higher level taxa.

Addressing this enormous diversity, we further focussed this study on species selected based on relevance (algal genera/species already being applied or expected to be applied in the near future, *e.g.* for the production of biofuels (oil, diesel, hydrogen gas) or high value components); representativeness (aiming to include a broad range of taxa) and documented risk/safety profile. Based on these criteria, information from literature and valuable input from the consulted experts resulted in a selection of approx. 500 species.

For the selected species, elements of importance to inform the risk assessment relating to its impact on human and animal health and ecological effects were described in a table **Algal Risk Classification Features** in separate Supplementary Document. Species were classified as showing harmful properties (red), being confirmed as safe (green) and for which no hazard/safety data could be readily retrieved (grey). About 290 ‘red’ species were identified, whereas only about 80 species could be considered as safe. This information can serve as reference for developers as well as the

COGEM in considering an application for genetic modification of one of the selected and/or closely related species. **Table 5** of this report gives a summary of taxa with harmful (red) species, safe (green) species or undocumented (grey) species.

The information obtained in this study indicates that the choice to use an organism is predominantly determined by its capacity to perform a certain function, rather than by its safety profile. *E.g.* The fact that a species or derived product is used in food and/or feed is not an indication *per se* of the safety of the organism. Conversely, “safe” uses, can be based on hazardous species and species for which no safety information can be readily retrieved. In some cases, harmful and beneficial aspects go hand in hand: a species may produce a compound that presents a harm to humans and animals (*e.g.* by toxicity), but when produced and extracted can be used for industrial or medicinal use.

Specific algal species are known for their beneficial aspects, different uses and/or for harmful effects. Species were considered harmful when at least one of the main hazardous features (pathogenicity, toxicity, allergenicity) was reported. Since about 60% of cyanobacterial samples investigated worldwide contain toxins, it is prudent to presume a toxic potential in any cyanobacterial population. However, most species comprise toxic and nontoxic strains. While species are identified for which information is available, hardly any higher taxon can be clustered based on safety profiles: even when some species are safe, there are usually also some species that can be problematic, possibly under specific conditions.

Part of the challenge of determining the relevance of hazard factors is the fact that the expression seems to be often determined by the environment. Harmful algal blooms as well as expression of toxins or other deleterious compounds can be highly dependent on external factors, a “reaction” to specific external conditions (*e.g.* temperature, grazing, …) and therefore unpredictable in an uncontrolled environment. A case-by-case approach will remain the most appropriate.

Nevertheless, taxonomic relationship may provide indications on which (type of) compounds (*e.g.* metabolites, toxins) must be verified. Molecular techniques can be used to verify if sequences similar to those of genes known to code for already identified noxious compounds are present in yet undocumented species of the same taxon. In some cases biological assays, biochemical tests, chromatographic methods and/or test kits can be used to monitor the presence of noxious substances. Nevertheless, such test panel will be challenging since it should be specific for each of the major algal groups and continuously adapted to new and evolving knowledge.

A list of elements was presented that can be used to evaluate health and environmental impact for algae. This information is likely not available in an early R&D phase, and therefore an initial classification based on information from taxon-related species may be the only precautionary way forward until more information is collected.

Within the remit of this project, information on a selection of species was compiled and reported in a static manner. This report must be considered as a ‘snapshot’ of our current understanding up to February 2021. Changes in taxonomy and insights published since then were not considered. The compiled information can inform risk assessors. Yet, there is a risk that with further refined identification such as discerning so-called cryptic species, species that are subject of future applications may not be easily retrievable. Similarly, any attempt to cluster based on taxonomy is in jeopardy as soon as the taxonomic classification is modified. Recognising the dynamic nature of algal taxonomy, a flexible approach must be established that allows tracing the diverging and changing classification of algal taxa. An automated expert system would be more appropriate to consolidate this effort, expand the scope and ensure future relevance. Such an expert system, combining information on taxonomic changes with risk/safety information, will also be relevant for other regularly modified taxa like the Bacteria.

Samenvatting

Wanneer een toelating voor genetische modificatie van een bepaalde alg wordt aangevraagd, wordt COGEM gevraagd de pathogeniteitsklasse vast te stellen indien deze nog niet eerder werd vastgesteld. Deze studie had tot doel de **beschikbare informatie te verzamelen** die de risico-evaluatie kan ondersteunen en referenties aan te leveren voor de classificatie van wild-type algen volgens hun vermogen om schadelijke effecten te creëren aan mens, dier en milieu. Verder werd de vraag gesteld of het mogelijk is om hele families of genera te groeperen of hogere taxonomische niveaus aan te wijzen die algemeen als veilig worden beschouwd of juist gekend zijn als schadelijk. Het tweede oogpunt van deze studie was daarom de **haalbaarheid van een meer algemene risicoclassificatie** te onderzoeken en te trachten algen te groeperen in groepen met schadelijke en niet-schadelijke eigenschappen. Er werd een selectie gemaakt van algensoorten met potentieel om toegepast te worden in onderzoek en ontwikkeling en in productie.

Algen zijn een erg diverse groep van organismen, die evolutionair vaak niet verwant zijn. Zowel eencellige algen of microalgen als meercellige algen of macroalgen zijn bekend. De groep is ingedeeld in talloze families en genera. Naarmate onze kennis over de algenfylogenie toeneemt, blijft de taxonomische classificatie evolueren. De benaming ‘algen’ omvat in dit rapport zowel de algensoorten in de botanische zin als de cyanobacteria.

Waar sommige soorten historisch al werden gebruikt als voedselbron voor de mens, kregen algen in de laatste tientallen jaren hernieuwde belangstelling niet alleen voor de voeding van mens en dier (voeding, diervoeder en additieven), maar ook als bron van biomoleculen en als productiesystemen voor waardevolle farmaceutica en biobrandstoffen.

De productie-efficiëntie van algen en de bioproductie van een breed scala aan moleculen die van belang zijn, kunnen worden verbeterd door onder meer genetische modificatie. Hoewel algen in staat zijn enorme hoeveelheden CO_2 te fixeren, kan de fotosynthese nog steeds beter. Ook de natuurlijke aanleg om interessante biomoleculen te produceren is economische gezien misschien nog niet op peil en kan verder worden verbeterd. Meer nog, specifieke bestanddelen die niet van nature in algen voorkomen kunnen worden geproduceerd gebruikmakend van algen als productiesysteem. Daarenboven zijn algen als productiesysteem een duurzame manier om chemische stoffen en olie voor de voeding, voeder en biobrandstoffen te vervaardigen. Bijgevolg zijn er inspanningen geleverd om microalgen aan te passen via genetische modificatie om zo verbeterde productiestammen te verkrijgen.

De dubbelzinnigheid van de algentaxonomie is een belangrijke uitdaging voor dit opzet. Om karakteristieken toe te kennen aan een specifieke soort of stam, moet men in staat zijn naspeurbaar en coherent te refereren naar deze soort of stam. Er is echter geen unaniem geaccepteerde classificatie van algen. Als ruggengraat van dit project hebben wij een classificatie samengesteld als referentiekader. Die omvat 7191 genera, verdeeld over 800 families, 267 orden, 60 klassen en 13 fyla/divisies (het “**Algal Taxonomic Landscape”** is beschikbaar als afzonderlijk supplementair document).

Terzelfdertijd moet de dynamische natuur van de algentaxonomie worden benadrukt. Gedurende de beginfase van dit project hebben wij wijzigingen op familieniveau binnen de cyanobacteriën opgemerkt. Wijzigingen op niveau van family, genus en soort kunnen beperkt zijn, maar kunnen in andere gevallen, bijvoorbeeld als het gaat om cryptische soortencomplexen, een brede impact hebben. Deze dynamische status heeft gevolgen voor het groeperen en het maken van elke risicoclassificatie op taxa van een hoger niveau.

Om deze enorme diversiteit aan te pakken, werden soorten geselecteerd op basis van relevantie (algengenera/soorten die al worden toegepast of worden verwacht te worden toegepast in de nabije toekomst, bv. voor de productie van biobrandstoffen (olie, diesel, waterstofgas) of hoogwaardige componenten); representativiteit (met als doel een brede waaier aan taxa in te sluiten) en gedocumenteerd risico/veiligheidsprofiel. Volgens deze criteria resulteerde literatuurinformatie en de waardevolle inbreng van geraadpleegde experten in een selectie van ongeveer 500 soorten.

Voor deze geselecteerde soorten werden belangrijke elementen voor de risico-evaluatie in verband met milieublootstelling en het effect op de gezondheid van mens en dier en ecologische effecten beschreven in een tabel "**Algal Risk Classification Features"** in een afzonderlijk supplementair document. Soorten werden geclassificeerd volgens hun schadelijke eigenschappen (rood), zijnde bevestigd als veilig (groen) en waarvoor geen gevaar-/veiligheidsgegevens gemakkelijk konden worden opgehaald (grijs). Ongeveer 290 'rode' soorten werden geïdentificeerd, terwijl slechts ongeveer 80 soorten als veilig konden worden beschouwd. Deze informatie kan dienen als referentie voor de COGEM wanneer zij een van de geselecteerde en/of nauwe verwante soorten behandelen. **Table 5** van dit rapport geeft een overzicht van taxa met schadelijke (rode) soorten, veilige (groene) soorten of niet gedocumenteerde (grijze) soorten.

De informatie samengebracht in deze studie toont aan dat de keuze van een organisme voornamelijk wordt bepaald door zijn vermogen om een bepaalde functie uit te oefenen, eerder dan door zijn veiligheidsprofiel. Bv. het feit dat een soort of afgeleid product wordt gebruikt in voeding en/of voeder is op zich geen indicatie voor de veiligheid van een organisme. In tegendeel, "veilig" gebruik kan gebaseerd zijn op een schadelijke soort of soort waarvoor niet gemakkelijk veiligheidsinformatie kan worden gevonden. In sommige gevallen gaan schadelijkheid en veiligheid hand in hand: een soort kan een component produceren die in de alg gevaar voor mens en dier (bv. door toxiciteit) meebrengt, maar wanneer geproduceerd en geëxtraheerd een industrieel of medisch gebruik kent.

Specifieke algensoorten zijn bekend voor hun gunstige eigenschappen, diverse toepassingen en/of schadelijk effecten. Soorten werden als schadelijk beschouwd wanneer ten minste een van de belangrijkste gevaarlijke kenmerken (pathogeniteit, toxiciteit, allergeniciteit) werd gemeld. Omdat ongeveer 60% van de cyanobacteriestalen die wereldwijd werden bestudeerd toxines bevatten, is het verstandig om toxiciteit te veronderstellen in elke cyanobacteriepopulatie. Niettegenstaande dat kennen de meeste soorten toxische ent niet-toxische stammen. Hoewel informatie aanwezig kan zijn voor een species, kan nagenoeg geen enkel hoger taxon worden gegroepeerd op basis van veiligheidsprofielen: zelfs wanneer sommige soorten veilig zijn, zijn er gewoonlijk ook andere soorten die problematisch zijn, mogelijk alleen onder specifieke voorwaarden.

De uitdaging om relevante schadelijke factoren te bepalen is deels te wijten aan het feit dat expressie ervan vaak wordt bepaald door het milieu. Schadelijke algenbloei evenals expressie van toxinen of andere schadelijke componenten kunnen in hoge mate afhankelijk zijn van externe factoren, een "reactie" op specifieke externe condities (bv. temperatuur, begrazing), en daardoor onvoorspelbaar in een ongecontroleerd milieu. Een geval-per-geval benadering zal het meest geschikt blijven.

Daarentegen kan taxonomische verwantschap een indicatie geven welk (type van) bestanddelen (bv. metabolieten, toxines) moet worden geverifieerd. Moleculaire technieken kunnen worden gebruikt om sequenties na te gaan gelijkend op deze van genen waarvan al geweten is dat ze coderen voor schadelijke stoffen en dat voor nog niet gedocumenteerde species van hetzelfde taxon. In sommige gevallen kunnen biologische of biochemische testen, chromatografische methoden en/of testkits worden gebruikt om schadelijke componenten op te sporen. Niettemin is zo'n testpanel moeilijk omdat het specifiek moet zijn voor elk van de voornaamste algengroepen en voortdurend moet aangepast worden aan het voortschrijdend inzicht.

Een lijst van elementen werd voorgesteld die kunnen worden gebruikt om de impact op gezondheid en milieu in te schatten. Deze informatie is mogelijk nog niet beschikbaar in de vroege O&O-fase. Daarom kan een initiële classificatie gebaseerd op de informatie van verwante soorten binnen een taxon de enige voorzichtige benadering zijn totdat meer informatie is vergaard.

Binnen de opdracht van dit project, werd informatie voor een selectie van soorten opgesteld en gerapporteerd op statische wijze. Dit rapport moet worden beschouwd als een 'momentopname' van ons huidige inzicht (tot en met februari 2021). Wijzigingen in taxonomie en sindsdien gepubliceerde inzichten werden niet in overweging genomen. Deze informatie kan de risicobeoordelaars tot nut zijn. Toch is er een risico dat met het verder verfijnen van de identificatie zoals het onderscheiden van zogenaamde cryptische soorten, toekomstige toepassingen moeilijk traceerbaar zijn. Evenzo is elke poging om te groeperen op basis van taxonomie in gevaar zodra de taxonomische classificatie wordt gewijzigd. Rekening houdend met de dynamiek van de taxonomie, is een flexibele opstelling beter om de divergerende en zich wijzigende classificatie van algentaxa op te kunnen sporen. Een geautomatiseerd expertsysteem zou meer geschikt zijn om dit te verwezenlijken, het toepassingsgebied te verbreden en relevantie in de toekomst te verzekeren. Zo'n expertsysteem dat taxonomische wijzigingen en informatie over schadelijkheid/veiligheid combineert, zou ook relevant kunnen zijn voor andere zich regelmatig wijzigende taxa zoals de bacteriën.

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Abbreviations

ATCC	American Type Culture Collection
BCCM/DCG	Belgian Coordinated Collections of Microorganisms/ Diatoms Collection Ghent
BIOHAZ	EFSA's Panel on Biological Hazards
BMAA	β-N-methylamino-L-alanine
COGEM	Netherlands Commission on Genetic Modification *Commissie Genetische Modificatie*
DHA	docosahexaenoic acid
DNA	Deoxyribonucleic acid
DSMZ	German Collection of Microorganisms and Cell Cultures
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked Immuno Sorbent Assay
EPA	Eicosapentaenoic acid
EU	European Union
FDA	United States Food and Drug Administration
ggo	*Genetisch gemodificeerd organisme*
GM	Genetically modified
GMO	Genetically modified organism
GRAS	Generally recognised as safe
HAB	Harmful algal blooms
HGT	Horizontal gene transfer
IOC-UNESCO	Intergovernmental Oceanographic Commission - United Nations Educational, Scientific and Cultural Organization
LC/MS	Liquid Chromatography/Mass Spectrometry
MALDI-TOF	Matrix Assisted Laser Desorption Ionisation Time of Flight Mass Spectrometry
NCBI	National Centre for Biotechnology Information
OECD	Organisation for Economic Co-operation and Development
PAE	Lab for Protistology and Aquatic Ecology, Ghent University
PCC	Pasteur Culture Collection of Cyanobacteria
PCR	Polymerase chain reaction
PUFA	Polyunsaturated fatty acids
qPCR	Quantitative real-time PCR
QPS	Qualified presumption of safety
RG	Risk group
RP-HPLC	Reverse Phase High Performance Liquid Chromatography
RuBisCO	Ribulose-1,5-bisphosphate carboxylase/oxygenase
TSAR	Taxonomic 'supergroup' including telonemids, stramenopiles, alveolates, and *Rhizaria*
US EPA	United States Environmental Protection Agency
WUR	Wageningen University & Research
ZKBS	*Zentrale Kommission für die Biologische Sicherheit*

Introduction

Algae are a tremendously diverse group of organisms; many algal groups belonging to evolutionary unrelated lineages. They are encountered all over the world, in marine and freshwater environments as well as on land, and in extreme environments such as hot water springs and on glaciers. The group has been assigned into numerous families and genera, of which the taxonomic classification continues to evolve as our knowledge on the algal phylogeny increases. Unicellular algae or microalgae as well as multicellular algae or macroalgae are known. All these algae are eukaryotes. Commonly the blue-green cyanobacteria, although prokaryotes, are also considered microalgae.

While some species have been historically used as food, the algae gained interest in the past decades not only for human and animal nutrition (food, feed and additives), but also as source of yet undiscovered biomolecules as well as production systems for valuable pharmaceuticals and biofuels (Borowitzka, 2018b; Dewi *et al.*, 2018; Enzing *et al.*, 2012; Kay and Barton, 1991; Klamczynska and Mooney, 2017; Rahpeyma and Raheb, 2019; Tafreshi and Shariati, 2006). Many species and strains are studied in detail up to whole genome sequencing (Liang *et al.*, 2019). Furthermore, production efficiency of algae and bio-manufacturing of a wide range of molecules of interest may be enhanced by for example adapting growth media (*e.g.* use of additives), by selective breeding of algae, or genetic modification (Enzing *et al.*, 2012; Feng *et al.*, 2014; Liang *et al.*, 2019). Indeed, while algae are able to fix enormous amounts of CO_2, photosynthesis can still be improved. Biotechnology efforts are focused on obtaining genetic engineered algal strains with an improved light to biomass conversion efficiency. This can be achieved for example, by targeting the light harvesting antennae to induce resistance to photo-inhibition at high light intensities or by targeting RuBisCO, the most crucial enzyme in photosynthesis, to increase carbon assimilation efficiencies (see *e.g.* Vecchi *et al.* (2020)). Also, the natural capacity of some algae to produce interesting biochemicals might not yet result in economically relevant levels and can be enhanced. Furthermore, specific compounds, naturally not occurring in algae, may be produced by using algae as production systems. This in analogy with the bacterium *Escherichia coli* and yeast *Saccharomyces cerevisiae* that are currently deployed for the microbial synthesis of almost all natural products of interest (for an overview, see *e.g.* Jeandet *et al.* (2013)). However, the main advantage of algae is that most of them are photoautotrophic; using energy from sunlight, they convert CO_2 into organic compounds. As such, algae as production systems are a sustainable way to manufacture chemicals and oils for food, feed and fuel. Hence, efforts have been made to adapt microalgae via genetic modification to obtain excellent production strains (Eggink *et al.*, 2013).

Nevertheless, important challenges remain: in general cyanobacteria are, with the current knowledge, more easy to modify compared to eukaryotic algae. Cyanobacteria are used for small molecules to be excreted (ethanol, butanol, fatty acids and other organic acids), while eukaryotic algae are preferred for products stored within the cell such as oils (Eggink *et al.*, 2013).

At the same time, there is growing number of reports of adverse effects to human and animal health, and the environment (Caruana and Amzil, 2018; Cheney, 2016; Lassus *et al.*, 2016; Reddy and Mastan, 2011; Van Dolah, 2000). On one hand, toxic algal blooms are famous for *inter alia* poisoning people that consume affected fish and shellfish (cfr. dinoflagellate-associated human poisonings). On the other hand, any algae that form extensive blooms may, when dying off and when organic matter is broken down, lead to oxygen depletion, anoxia, and thereby killing all oxygen requiring organisms in the water (cfr. problematics of the ‘nuisance’ species *Phaeocystis* in the North Sea (Lassus *et al.*, 2016)). Such inherent characteristics must be taken into account when considering biotechnology applications of algae, in particular when potentially being released in the environment (Henley *et al.*, 2013; Kumar, 2015; Liang *et al.*, 2019; OECD, 2015b).

Purpose of this study

The starting point for the risk assessment process of an activity with a genetically modified organism is the identification of its (potentially) harmful properties. The characteristics of the wild-type recipient organism provide the baseline. The first aim of this study was to **review available information** that can inform this risk assessment and provide references for the classification of wild-type algae according to their potential adverse effects on human and animal health and the environment.

This study was therefore set up to collect references for classifying algal species according to hazards in view of their application in research and development using genetic modification. Two types of hazards are important to consider:

- Pathogenicity towards humans, animals and plants, and
- Adverse effects to the environment in case of an unintentional escape (*e.g.* invasiveness, toxicity, gene transfer).

Facing the large and diverse group of organisms commonly named algae, the question was raised if whole families or genera could be grouped or higher taxonomic levels could be identified that are generally recognised as safe or conversely known to be hazardous. The second aim of this study was therefore to examine **the feasibility of a more general risk classification** and to attempt to cluster as far as possible algae into groups with hazardous and non-hazardous properties. A set of algae was selected because of their potential of being applied in research, development and/or production.

The research approach was structured as follows:

1. Grouping of algal species according to the most recent, accepted classification (following Burki *et al.* (2020) and Algaebase[1], a global algal database of taxonomic, nomenclatural and distributional information (Guiry, 2021)) (a so-called taxonomic landscape);
2. Identifying the characteristics of species of interest;
3. Attempting to identify and make clusters consisting solely of species with adverse effects and clusters consisting solely of species that may be considered safe.

The information was used to explore if a tool could be established to support applicants and risk assessors with recognising taxonomic groups or clusters with and without species of concern. This background could then be used to guide further data gathering for risk determination: if a species is targeted belonging to a group of concern, detailed information may be requested; whereas for species belonging to a group with no negative indications a reduced data set may be needed. However, due to the developing scientific insights in algal phylogeny, taxonomy and biology, this study is merely a snapshot of the knowledge status.

As determined in the remit of the study, the scope includes phototrophic (photoautotrophic or heteroautotrophic) and mixotrophic eukaryotic algae as well as prokaryotic cyanobacteria.

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[1] Available at: http://www.algaebase.org/

1 Methods

1.1 Taxonomy of algae

The algae encompasses both members of the Prokaryota (limited to the Cyanobacteria) and Eukaryota. An overview of the current algal taxonomy, spanning from empire to genus level, was elaborated, to establish a ‘taxonomic landscape’ of algae. AlgaeBase[1] was used as a main reference framework. In case of doubt or conflict, the NCBI Taxonomy Browser[2] (Schoch et al., 2020) was consulted. Designation of the major lineages within the eukaryotic algae (so-called supergroups) is according to Adl *et al.* (2019) and Burki *et al.* (2020). Within the Eukaryota, algae can be found in the supergroups TSAR (telonemids, stramenopiles, alveolates, and *Rhizaria*), Haptista, Cryptista, Excavata (Euglenida) and Archaeplastida encompassing Chloroplastida (green algae), Rhodophyta (red algae), and Glaucophyta.

Where information was available on trophic status, only photoautotrophic (algae using light to convert CO_2 to organic carbon compounds), photoheterotrophic (algae using light for energy, but not able to use CO_2 as sole carbon source and thus requiring organic compounds from the environment) and mixotrophic algae (all combinations between photoautotrophic, photoheterotrophic and phagotrophic) were included. Within the Euglenozoa phototrophy is limited to the orders *Euglenida* and *Eutreptiida*. In addition, *Rapaza viridis*, the only phototrophic member of the otherwise phagotrophic order Rapazida was included. The publications of Bicudo and Menezes (2016); Cavalier-Smith (2016); Yamaguchi *et al.* (2012), served as guideline.

Some exceptions to this delineation were made. While Thraustochytrids (unicellular fungus-like organisms found within the TSAR supergroup, Stramenopiles clade) are heterotrophic, they were included in this study. This is due to the fact that, being of biotechnological importance, the Thraustochytrids have already been subject of a risk classification by COGEM. Although *Prototheca* lacks chlorophyll, it was included in this study as it contains both pathogenic representatives as a safe representative which has been granted GRAS status (see 2.1.2; *Prototheca moriformis*).

1.2 Information searches

A literature search was conducted to inventory harmful and beneficial effects caused by algae. Publications giving an overview of harmful algae or algae used for food or feed were the most important sources. For individual species Web of Knowledge, Scopus or Google Scholar were searched with appropriate key words such as hazard, pathogen, disease, toxin, toxic, poisoning, metabolite, infection, HAB, bloom, anoxia, invasive, safety, allergy, haemolytic, gill lesion, gill obstruction, food, feed, additive, biofuel, medicinal, cyst, horizontal gene transfer, nuisance and pest.

In addition, catalogues of algal culture collections (*e.g.* ATCC, DSMZ, PCC) were screened for data on risks identified for the wild-type parent organisms (*e.g.* toxin production, safe use for biofuel production, risk group).

Some authorities and risk assessment bodies have already issued risk assessments and opinions on specific cases. These were retrieved and analysed as they provide indications on relevant features. In particular information available from the following sources was verified:

- COGEM (the Netherlands),
- Zentrale Kommission für die Biologische Sicherheit (ZKBS; Germany),
- European Food Safety Authority (EFSA, EU),
- American Type Culture Collection (ATCC, USA),
- Intergovernmental Oceanographic Commission of UNESCO (IOC-UNESCO),
- Organisation for Economic Co-operation and Development (OECD),
- World Health Organization (WHO),

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[2] Available at: https://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/

- United States Environmental Protection Agency (US EPA),
- United States Food and Drug Administration (FDA).

Since different organisations may assess hazards differently (*e.g.* pathogenicity only towards humans, not to animals or plants) care was taken when compiling the information.

Information collection also included regulatory documents regarding dual use (*e.g.* Council Regulation (EC) No 428/2009 [3]). Dual-use items are goods, software and technology that can be used for both civilian and military applications. Although few biologicals are dual-use items, some toxin-producing algae are: *e.g.* several cyanobacterial species belonging to *Microcystis, Anabaena* produce microcystin which is considered as a dual use item.

Finally, in order to get an insight for relevance for the environment in the Netherlands, specific searches were conducted to evaluate if the species is endemic and/or can survive in local conditions. This information is based on distribution data provided in AlgaeBase[1] and the Global Biodiversity Information Facility (GBIF[4]), including Taxa Watermanagement the Netherlands[5].

Information published up to February 2021 was considered.

1.3 Expert consultations

The study was further supported by scientific guidance and critical review by academic experts, Dr. Peter Chaerle, Prof. Dr. Koen Sabbe and Prof. Dr. Wim Vyverman, of the Belgian Coordinated Collections of Micro-organisms/Diatoms Collection Ghent (BCCM/DCG). This unique collection of diatoms and microalgae is part of the Lab for Protistology and Aquatic Ecology (PAE, Ghent University).

In consultation with the Advisory Committee, several experts were identified and interviewed. In addition, contributors to the session “Biosafety and ERA of GM algae” at the 14th International Symposium on the Biosafety of Genetically Modified Organisms (ISBGMO, 2017[6]) were contacted. The consulted experts include Dr. Peter Chaerle, Em. Prof. Dr. Lucas Stal, Prof. Dr. Ir. René Wijffels, Dr. Sijtsma Lolke and Em. Prof. Dr. Joost Teixeira de Mattos.

Online interviews were conducted addressing:

- Which algal genera/species are of interest for the production of biofuels?
- Are there algal genera/species that might be interesting for other applications and/or production of high valuable products (other than biofuel production) and which must be included in our analysis?
- Are there algal genera/species that might become more prominent in the near future (*e.g.* for production of bioplastics)?
- Which genetic modifications are currently done to improve the production capacity of algae?
- Is it any way possible to attribute a risk group to entire algal genera/species, or to consider entire algal genera/species as safe? If this is the case, which algal genera/species are concerned?

In addition, the Advisory Committee provided useful suggestions on studies and publications.

1.4 Clustering

A catalogue of species of interest was constructed, where next to the taxonomic classification, data were reported concerning adverse effects (pathogenicity, toxicity, allergenicity, anoxia caused by blooming species, mechanical effects) and beneficial properties (food/feed source, medicinal

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[3] Council Regulation (EC) No 428/2009 of 5 May 2009 setting up a Community regime for the control of exports, transfer, brokering and transit of dual-use items. OJ L 134, 29.5.2009, p. 1–269

[4] https://www.gbif.org

[5] Available at: https://www.gbif.org/dataset/79f243f4-9c38-4305-997f-fa8ac3089dba or http://ipt.nlbif.nl/resource?r=checklist-twn

[6] Available at: http://isbr.info/files/tinymce/uploaded/BOA%20-%2020062017%20VA.pdf

applications). Furthermore, data on the natural habitat, ways of reproduction and survival potential in temperate climate regions were included.
Relevant species were described according to their biological characteristics and the prevalence or potential to survive in the Netherlands.

As literature data were collected on harmful and beneficial properties of algae, the respective species were indicated with a colour-code:

- Red: at least one of the main hazardous features (pathogenicity, toxicity, allergenicity) was reported
- Green: safety has been documented *e.g.* in toxicological assessment or in regulatory opinions for the organism
- Grey: no data on either hazards or risks could be retrieved from our sources

This allows on one hand identification of taxonomic groups with similar characteristics (harmful groups versus safe/beneficial groups), and on the other hand, taxonomic groups for which no or little information is available, requiring a more in-depth literature search.

2 Algal research and development

Algae, wild-type as well as genetically modified, are used to produce food, feed, medicines, cosmetics and biofuel. Useful compounds are synthesised by wild-type strains, but the production can be optimised and further diversified by selective crossing/breeding, induced mutation with UV or chemicals or by genetic modification.

In 2012, a preceding COGEM report (Enzing *et al.*, 2012) addressed research, production and risks of algae and genetic modification. It included an outlook on an increase in GM-algal research in the Netherlands and globally. Now, nearly a decade later, important progress has been made. Yet the (r)evolution which was expected to be imminent then, is only slowly becoming reality. Technical as well as economic factors influenced the large-scale deployment.

A brief by the Joint Research Centre (JRC) for the European Commission's Knowledge Centre for Bioeconomy confirms that the annual macroalgae biomass production has increased worldwide since 1950, reaching 32.67 Mt in 2016 and is mainly based on aquaculture cultivation (97% in 2016)(JRC, 2019). Data on microalgae biomass production are very fragmented and information is difficult to obtain.

Micro-algae are targeted predominantly for contained productions, macroalgae can be grown and harvested in large land-based tanks or in the open sea. While projects in the open environment can be a reality for wild-type strains, deployment of GM algae will require a thorough environmental risk assessment.

In this section, we provide a succinct overview, underlining that the GM applications in algae deserve the necessary attention to allow their realisation.

2.1 Applications involving wild-type algae

2.1.1 Medicinal use

The pharmaceutical industry is showing a growing interest for compounds found in microalgae with anticancer, antiviral, antibacterial, and antifungal properties, especially since these microorganisms can be cultured in large-scale production facilities (Borowitzka, 2018b; Dewi *et al.*, 2018).

- Anticancer
  Concerning anticancer compounds, significant anticancer activities have been reported for five carotenoids: β-carotene, lutein, astaxanthin, violaxanthin, and fucoxanthin. They are produced in *e.g.* the algae *Dunaliella* for β-carotene and *Haematococcus* for astaxanthin. Lutein is produced mainly in *Dunaliella salina*, *Chlorella sorokiniana* and *Chlorella protothecoides* while violaxanthin is isolated from *Dunaliella salina* and *Chlorella ellipsoidea*. Furthermore, some glycolipids, polysaccharides and proteins may be interesting.

  The cyanobacteria *Lyngbya majuscula, Geitlerinema* sp.*, Symploca* sp.*, Symploca hydnoides, Calothrix* sp.*, Phormidium* sp.*, Leptolyngbya* sp.*, Nostoc* sp.*,* and *Oscillatoria margaritifera.* are of interest for the production of polysaccharides, pigments, proteins, peptides, amides, and quinones with anticancer activities.

- Antiviral
  Several algal polysaccharides, proteins, lipids, pigments were shown to have antiviral properties (Borowitzka, 2018b; Dewi *et al.*, 2018). *Porphyridium* sp. polysaccharides have been shown to have a direct effect on the HSV-1 cycle, thereby significantly inhibited viral infection. The diatom *Navicula directa* produces an extracellular sulphated polysaccharide, naviculan, also effective against HSV-1 and HSV-2, influenza and HIV-1 by inhibiting the initial stages of viral replication. Calcium spirulan produced by *Arthrospira platensis* prevents virion adsorption and penetration into the host cells.

The lectins, cyanovirin-N, produced by the cyanobacterium *Nostoc ellipsosporum*, scytovirin from the cyanobacterium *Scytonema varium* and agglutinin from the cyanobacterium *Oscillatoria agardhii* have a strong potential for antiviral therapy against HIV (Dewi *et al.*, 2018).

Among the algal antiviral lipids are sulfolipids and glycolipids, such as sulfoquinovosyldiacylglycerides, monogalactosyldiacylglycerides and digalactosyldiacylglycerides produced by *e.g. Lyngbya lagerheimii* and *Phormidium tenue* (Dewi *et al.*, 2018). Marennine, a pigment produced by *Haslea ostrearia* is active against HSV-1 and HIV-1.

- Antibacterial

  Among the antibacterial substances isolated from algae and cyanobacteria are fatty acids, polyphenols, biphenyls etc. (Dewi *et al.*, 2018). Examples are the algae *Chaetoceros muelleri, Chlorella* spp., *Dunaliella* spp., *Porphyridium* spp., *Phaeodactylum tricornutum, Rhodella reticulata*, and the cyanobacteria *Nostoc insulare* and C*yanobacterium* spp. The latter produce compounds like aeruginosin, cyanopeptolin, microcystin, and cylindrospermopsin.

- Antifungal

  Extracts from algae and cyanobacteria may show more or less antifungal activity depending on the species and strain, and solvent (Borowitzka, 2018b; Dewi *et al.*, 2018). Among the studied microalgae are *Asterionella glacialis, Chaetoceros* spp., *Chlorella pyrenoidosa*, and *Desmodesmus communis* (formerly *Scenedesmus quadricauda*).

- Antiallergic

  Chlorella vulgaris or *Chlorella pyrenoidosa* (Chlorophyta) have been described for their antiallergic properties reducing IgE production in mice (Fleurence and Levine, 2018). *Dunalliella salina* (Chlorophyta) extracts show an inhibition activity to the hyaluronidase enzyme.

  The filamentous cyanobacterium *Arthrospira platensis*, consumed since ancient times and renowned for its high protein levels, is also known to provide a protective effect against the release of histamine and allergic rhinitis (Borowitzka, 2018b; Fleurence and Levine, 2018). *Arthrospira platensis* contains pigments belonging to the family of phycobiliproteins (phycocyanins). *Arthrospira maxima* phycocyanin was also reported as an antiarthritic compound.

  Polysaccharides of *Porphyridium cruentum* (Rhodophyta) are often cited for their antioxidant and anti-inflammatory activities (Fleurence and Levine, 2018).

2.1.2 Food and Food additives

Historically mostly macroalgae are known to be used for food: *Cladophora* (kai), *Laminaria* (kombu), *Porphyra* (nori), *Pyropia*, *Undaria* (wakame) (Guiry, 2021; Wells *et al.*, 2017). Concerning microalgae only the cyanobacteria *Nostoc* and *Arthrospira*, and lichens (fungus/algae associations) have been consumed since centuries (Borowitzka, 2018b). Also, extracts such as the hydrocolloids agars, alginates (*e.g.* from *Saccharina latissima*, which is a popular and commonly used source of alginates) and carrageenans have been used as thickening agents in foods (*e.g.* ice cream) and beverages.

More recently microalgae have become a source for the production of substances such as β-carotene, vitamins, minerals, proteins, pigments, γ-linolenic acid, long chain PUFAs (*e.g.* docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) oil), etc. (Enzing *et al.*, 2014; García *et al.*, 2017). The species and genera of interest are *e.g. Aphanizomenon flos-aquae*, *Chlorella* spp., *Arthrospira* spp., and *Scenedesmus* spp. and *Desmosdesmus* spp., *Porphyridium* spp. and *Dunaliella* spp. (Kay and Barton, 1991). Commercial microalgae production plants were established for *Chlorella, Arthrospira, Dunaliella* and *Haematococcus lacustris* (formerly *Haematococcus pluvialis*) first as a protein source, later for high-value molecules (Borowitzka, 2018b). *Dunaliella* spp. are cultured for their β-carotene, glycerol and protein content and used in dietary supplements (Kay and Barton, 1991). However, one needs to be cautious for *Aphanizomenon flos-aquae* as some strains are known to produce saxitoxins (Borowitzka, 2018b; Kay and Barton, 1991).

β-Carotene extracted from *Dunaliella salina* is authorised in the EU as food additive[7]

EFSA provided positive scientific opinions in the context of the Novel Food legislation[8]:

- For *Ecklonia cava* phlorotannins (marketed as SeaPolynol™) as a novel food[9]. The novel food is a phlorotannin-rich alcohol extract of *Ecklonia cava*, which is an edible marine brown alga species, and it was intended to market the novel food as a food supplement for healthy individuals over the age of 12 years.
- For *Schizochytrium* sp. oil as a novel food[10]. *Schizochytrium* sp. is a microalga. The novel food, an oil rich in DHA, is isolated from the microalgae by mechanical extraction.

In addition, EFSA's Panel on Biological Hazards (BIOHAZ) delivers scientific opinions on the maintenance of the list of qualified presumption of safety (QPS) biological agents intentionally added to food or feed. The QPS process was developed to provide a harmonised generic pre-evaluation procedure to support safety risk assessments of biological agents performed by EFSA's scientific Panels and Units. The following algae have been added in recent years:

- *Aurantiochytrium limacinum* SR21 (formerly *Schizochytrium limacinum* SR21) [11]
- *Euglena gracilis*[12]
- *Tetraselmis chuii* [13]

In these cases, QPS applies for production purposes only.

Addressing another perspective, EFSA investigated[14] the issue that seaweeds may present a high concentration potential for minerals and trace elements present in the surrounding waters, including noxious substances like mercury, lead, cadmium, and inorganic arsenic. This work aimed to obtain levels of these elements in species of seaweed (*e.g. Fucus vesiculosus, Saccharina latissima, Ulva lactuca and Cladophora* sp.) cultivated and harvested in Denmark.

Arthrospira platensis has received the GRAS status (generally recognised as safe) by the FDA in the United States[15] and is sold in tablets as a food supplement (Borowitzka, 2018b; Fleurence and Levine, 2018). Other microalgae with GRAS status are *Chlorella protothecoides* strain S106[16], *Chlamydomonas reinhardtii*[17], *Crypthecodinium cohnii*[18], *Prototheca moriformis*[19] and the seaweeds *Laminaria japonica*[20] and *Undaria pinnatifida*[21].

The human diet is often short on EPA. Fish (oil) is currently the major source of EPA, but fish get EPA from their feed *i.e.* mainly via zooplankton feeding on microalgae. Microalgae culture is therefore explored to deliver EPA, DHA and other fatty acids as a food supplement (Ma *et al.*, 2016; Winwood, 2015). *Crypthecodinium cohnii* is an example of a dinoflagellate used by DSM to produce DHA for infant formula. *Nannochloropsis oculata* is used by Aurora Algae and Quantas Health resp. to produce EPA.

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[7] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council Text with EEA relevance OJ L 83, 22.3.2012, p. 1–295

[8] Regulation (EU) 2015/2283 of the European Parliament and of the Council of 25 November 2015 on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (Text with EEA relevance) OJ L 327, 11.12.2015, p. 1–22.

[9] https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2017.5003

[10] https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2020.6242

[11] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2020.5965

[12] https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2019.5753%4010.1002/%28ISSN%291831-4732.QPS

[13] https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2020.5965

[14] https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2019.e170915

[15] FDA GRN No 127 Spirulina, the dried biomass of *Arthrospira platensis,* and
FDA GRN No 394 Dried biomass of Arthrospira platensis, also known as Spirulina platensis (Spirulina)

[16] FDA GRN No 384 Algal oil derived from *Chlorella protothecoides* strain S106 (Cp algal oil)
FDA GRN No 469 *Chlorella protothecoides* strain S106 flour with 40-70% lipid (algal flour)
FDA GRN No 519 *Chlorella protothecoides* strain S106 flour with 40-75% protein

[17] FDA GRN No. 773 Dried biomass of *Chlamydomonas reinhardtii*

[18] FDA GRN No. 41 DHASCO (docosahexaenoic acid-rich single-cell oil) and ARASCO (arachidonic acid-rich single-cell oil)

[19] FDA GRN No. 527 Algal oil (87% oleic acid) derived from *Prototheca moriformis* strain S2532
FDA GRN No. 673 Algal fat derived from Prototheca moriformis (S7737)
FDA GRN No.754 Algal oil (87% oleic acid) derived from Prototheca moriformis strain S6697

[20] FDA GRN No. 123 *Laminaria japonica* broth and extract powder

[21] FDA GRN No. 565 Fucoidan from *Undaria pinnatifida*

Phycobiliproteins are major photosynthetic accessory pigments in cyanobacteria (*Arthrospira* spp.) and red algae, used as natural dyes in food industry and pharmaceuticals products (Dewi *et al.*, 2018).

2.1.3 Feed and Feed additives

Microalgae are produced to serve as aquaculture feed, especially for shellfish larvae and juvenile stages of fish in hatcheries. But they are also promising alternatives as feed additive or supplement that can replace fishmeal and fish oil and to ensure sustainable aquaculture (Shah *et al.*, 2018). Species of interest are *Amphiprora hyalina*, *Amphora coffeaeformis*, *Chaetoceros muelleri*, *Chaetoceros calcitrans, Chlorella* spp*., Isochrysis galbana, Nannochloropsis* spp*., Pavlova lutheri, Phaeodactylum tricornutum, Skeletonema costatum, Tetraselmis* spp. and *Thalassiosira* spp. (Enzing *et al.*, 2014; García *et al.*, 2017; Muller-Feuga, 2000). *Haematococcus lacustris* is added as a source of astaxanthin to render salmon flesh pink.

Cladophora species are used as feed additives for aquaculture, as a fish meal replacement or protein supplement (Michalak and Messyasz, 2021). Also, *Desmodesmus* sp. biomass has been trialled as fish meal substitute (Kiy *et al.*, 2005).

In addition, *Chlorella* and *Arthrospira* are added to feed for cats, dogs, aquarium fish, ornamental birds, horses, poultry, cows and breeding bulls (García *et al.*, 2017).

2.1.4 Other uses

The potential of algae for biofuel production has been explored in many countries. Although technically feasible, many projects are economically not viable, except for the very large installations in the USA (pers. commun.)(Kumar, 2015). Most experts see Europe more as a place for algae-based production of food and feed ingredients.

Microalgae have been studied for the generation of biofuel, including biodiesel, oil, syngas, and hydrogen (Rahpeyma and Raheb, 2019; Tiwari and Kiran, 2018).

Many algal strains like *Chlamydomonas*, *Chlorella*, *Scenedesmus*, *Botryococcus braunii*, etc. have been reported to produce biofuels (Tiwari and Kiran, 2018). Among the candidate eukaryotic algae are *Emiliania huxleyi*, *Cyclotella* spp., *Nannochloropsis* spp., *Nannochloris* spp., *Neochloris* spp. and *Picochlorum* spp. (pers. commun.). Their low nutrient requirements make it possible to combine biofuel production with phycoremediation of agricultural, industrial and municipal wastewater (Tiwari and Kiran, 2018).

Cyanobacteria are of interest because of their high growth rate, simple and cheap nutritional requirements, their oil compositional similarity to fossil fuels and the easy genetic manipulation (Farrokh *et al.*, 2019). Many species have been studied among which *Anabaena sphaerica*, *Anabaena variabilis*, *Lyngbya* sp., *Microcystis aeruginosa*, *Oscillatoria* sp., *Phormidium tenue*, *Synechococcus elongatus*, *Synechocystis* sp. etc. However, the large-scale cultivation of cyanobacteria for biofuel production has not been economical, so far.

Algae may be used as biofertiliser. In general, when applied to seed, plant surfaces, or soil, a biofertiliser will colonise the rhizosphere or the interior of the plant and promote growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilisers can stimulate soil microbial activity, thereby recycling the carbon, nitrogen and other nutrients that they acquired via photosynthesis and nitrogen fixation (cyanobacteria) (Michalak and Messyasz, 2021; Renuka *et al.*, 2018). Via their heterocysts cyanobacteria fix atmospheric nitrogen that provide for the nitrogen needs of other water and soil organisms. Nitrogen fixing cyanobacteria (mainly *Nostoc* spp.) are also known to symbiotically associate with plants (*Gunnera*), ferns (*Azolla*), liverworts and hornworts (Santi *et al.*, 2013), These photosynthetic organisms also aid in the mineralisation and solubilisation of primary macro- and micronutrients in soil. Algae even produce plant hormones, *i.e.* indole-3-acetic acid. Algae are either directly collected from coasts or lakes, dried and applied onto the field, or are available as commercial products consisting of an inert carrier, such as peat, inoculated with viable microalgae (Chittora *et al.*, 2020; Schneider *et al.*, 2015). In the 20th century, it was common practice (*e.g.* in Japan, Alps) to use dried Charophytes as fertiliser on the field (Komuro *et al.*, 2017; Schmieder, 2004). Cyanobacterial inoculation in the soil, as seed dressings or broadcasting in fields, increase seed germination rate, plant growth and yield in variety of crops, such as rice, wheat, maize, and other arable and horticultural crops, either alone or in consortia with other algae, bacteria or fungi. Examples are consortia of *Anabaena* and *Nostoc* strains in rice, maize, wheat, beetroot and beans, *Chlorella vulgaris* and *Nannochloropsis* in maize, wheat and tomato, etc.

Furthermore, algae can even act as biocontrol agents combating plant and animal pathogens by releasing hydrolytic enzymes and biocidal compounds, such as benzoic acid, majusculonic acid, ambigol A, carbamidocyclophane A, etc. Cyanobacteria also prompt the plant defence response by eliciting the antioxidant and pathogenesis related machinery of the plant, including the activity of β-1, 3 endoglucanase, chitinase, catalase, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase (Renuka *et al.*, 2018).

Microalgae can survive and proliferate in hostile environments, such as oil and heavy-metal contaminated land. They may help in reclamation of these wastelands (Renuka *et al.*, 2018). Green algae inoculants further improve soil structure and aggregate stability and may prevent erosion. Microalgae offer an elegant solution to tertiary treatment of wastewater and sewage (*i.e.* removal and recycling of ammonium, nitrogen and phosphate) and quaternary treatment (*i.e.* removal of heavy metals, toxic organic compounds) as they use inorganic nitrogen and phosphorus (or other elements) for their growth (Abdel-Raouf *et al.*, 2012). Also Charophytes are used in phycoremediation as they may effectively remove organic chemicals such as hexachlorobenzene (Schneider and Nizzetto, 2012) and heavy metals (Herbst *et al.*, 2019; John, 2003).

Many algal species are model systems for studying *e.g.* metabolic pathways, transport processes, cell wall formation, the uptake of persistent organic chemicals into cells and photosynthesis. In particular Charophytes, with their extraordinarily large internode cells, their structure and ultrastructure similar to that of many plant cells, are excellent models for studying plant cell biology (Domozych *et al.*, 2016; Schneider *et al.*, 2015).

2.2 Genetic modification

Fundamental research aims to acquire a better understanding of how algae function. Biotechnology offers important tools to tackle the research questions. Yet, the lack of suitable techniques for genetically modifying algae presents a bottleneck for both fundamental and applied research (see *e.g.* Faktorová *et al.* (2020)). In particular dinoflagellates (*e.g. Symbiodinium microadriaticum*) and Prymnesiophyceae, also called coccolithophores (*e.g. Emiliania. huxleyi*) are difficult to transform; even if DNA appeared to be delivered, expression of the transformed genes cannot always be confirmed (Faktorová *et al.*, 2020).

Successful genetic transformation has been reported for mainly cyanobacteria, Chlorophyta, Rhodophyta, Phaeophyta, diatoms, euglenids and dinoflagellates (Radakovits *et al.*, 2010) but also representatives of other algal lineages are currently explored (see *e.g.* Faktorová *et al.* (2020)). Candidates for transgenesis are *Dunaliella salina* for the production of high-value biomolecules, *Chlorella*, and the cyanobacteria *Synechococcus elongatus* and *Synechocystis* sp. (Farrokh *et al.*, 2019; Feng *et al.*, 2014; Kumar, 2015). Genetic modification protocols have been established for several species, among which *Anabaena* sp., *Arthrospira platensis*, *Chlamydomonas reinhardtii*, *Chlorella* spp., *Euglena gracilis*, *Laminaria japonica*, *Nannochloropsis* sp., *Ostreococcus tauri*, *Phaeodactylum tricornutum*, *Porphyridium* sp., *Symbiodinium microadriaticum*, *Thalassiosira pseudonana*, *Ostreococcus tauri*, etc. (Butler *et al.*, 2020; Enzing *et al.*, 2012; Kroth *et al.*, 2018; OECD, 2015b). However, green algae (Chlorophyta) and cyanobacteria remain the most popular species for genetic modification (Kumar, 2015).

Applied research and development is performed to improve, modify or add to their biological characteristics. For genetic modification two directions can be discerned:

- Optimising the production capacity of the algal cell (strain improvement)

  This relates to the optimisation of photosynthesis or light to biomass conversion (*e.g.* avoidance of light inhibition in excess of light photons, increase carbon assimilation efficiencies), increasing stress tolerance (*e.g.* avoidance of growth inhibition due to unfavourable salt, pH, temperature conditions) or enhancing the metabolic routes (*e.g.* optimise lipid quality and accumulation inside the algal cell, decrease lipid or starch catabolism, increase the production of antibacterial toxins) in algal strains cultivated in bioreactors (Huang and Daboussi, 2017; Radakovits *et al.*, 2010; Vecchi *et al.*, 2020).

But also, trophic conversion, for example from photoautotrophy to heterotrophy as was done in *Phaeodactylum tricornutum*, can be achieved by introducing a single gene (glucose transporter) allowing growth on exogenous glucose in the absence of light. Such conversions may open new perspectives on large-scale light-independent exploitation of algae (*e.g.* fermentation) (Zaslavskaia *et al.*, 2001).

- Production of new compounds

  Metabolic pathways can be introduced to produce new chemicals which are not naturally produced by algae. Current applications include the production of energy related products such as ethanol, hydrogen, fatty acids and lactic acid (among others used for the production of bioplastics and polyesters with valuable medical properties)(Enzing *et al.*, 2012; Enzing *et al.*, 2014). For example, production of ethanol will require insertion of genes coding for pyruvate decarboxylase and alcohol dehydrogenase from ethanologenic bacteria.
  Likewise, microalgae can be used as 'biofactories' for the production of medicinal proteins (*e.g.* interferon, proinsulin, human growth hormone, monoclonal antibodies). However, currently, such microalgal biopharmaceuticals are still absent from the market, mainly due to the low yields of recombinant proteins obtained.
  Also, glyco-engineering has a great potential to produce non-immunogenic biopharmaceuticals in microalgae. This technique has already proven successful in classic 'biofactories' (*e.g. E. coli* and yeasts) to manipulate and 'humanise' glycans to produce active and safe biopharmaceuticals. So far, this has not yet been applied to microalgae, as their glycosylation pathways remain to be characterised to more detail (Barolo *et al.*, 2020).

2.3 Examples from the Netherlands

Combining a historical and geographical relationship to water and the sea with a pioneering and entrepreneurial strive for alternative and sustainable resources, the Netherlands have been at the forefront of exploring applications of algae.

Wageningen University & Research (WUR) has coordinated several European research projects dealing with algae, including:
- SPLASH[22] (1 September 2012 - 28 February 2017): sustainable polymers from algae sugars and hydrocarbons (*e.g.* bioplastics; using genetic modification);
- FLUEL4ME[23] (1 January 2013 - 31 December 2016): integrated and sustainable process for continuous biofuel production from microalgae;
- MIRACLES[24] (1 November 2013 - 31 October 2017): industry-driven R&D and innovation project aimed at developing integrated, multiple-product biorefinery technologies for the production of specialties from microalgae for application in food, aquaculture and non-food products.

Other projects with Dutch partners include:
- InteSusAl[25] (1 May 2011 - 31 July 2016): demonstration of integrated and sustainable microalgal cultivation with biodiesel validation;
- EnAlgae[26] (December 2011 – December 2015): Energetic Algae aims to reduce CO_2 emissions and dependency on unsustainable energy sources in North West Europe, by accelerating the development and use of algal-based bioenergy and GHG mitigation technologies.

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[22] http://eu-splash.eu/
[23] http://www.fuel4me.eu/index.php
[24] http://miraclesproject.eu/
[25] https://eurec.be/projects/intesusal/
[26] www.enalgae.eu

11 Organisations participating in MacroFuels[27], including TNO and the Dutch catalysis firm Avantium, are testing biofuel production processes on brown, red, and green seaweeds, including sugar kelp. Their projects include the development of both bio- and thermochemical routes to bioethanol, biobutanol, and other fuels. The MacroFuels partners are also advancing low-cost methods for cultivating seaweed, such as by rotating crops on various growing substrates anchored in the sea. The partners are due to complete their projects at the end of this year.

Another EU-funded project, Macro Cascade[28], seeks to use compounds present in seaweed to make specialty chemicals. It features 13 organisations, including WUR, TNO, and Hortimare.

The WUR also constructed AlgaePARC (Algae Production And Research Centre)[29] at the Wageningen Campus, with the goal to fill the gap between fundamental research on algae and full-scale algae production facilities. The main challenge for biofuels remains the costs of biomass production AlgaePARC investigates how it could be produced more cheaply, making it economically viable.

Algae may also provide missing pieces to complete the circular economy: it is explored if algae can grow in wastewater from *e.g.* breweries[30] or as the planned project IMARES[31], where algae cultivated in wastewater from greenhouse horticulture will be used as feed for oysters.

According to the latest available data (Emodnet database[32]), 12 algae-producing/harvesting companies are located in the Netherlands using wild-type and bred strains.

- AlgaeBiotech[33] - *Haematococcus pluvialis, Isochrysis sp., Nannochloropsis sp., Tetraselmis sp.*
- AlgaeLink - *Isochrisys galbana, Nannochloropsis oculata, Pavlova pinguis, Tetraselmis suecica*
- AlgaSpring B.V.[34] - *Chlorella* sp., *Nannochloropsis* sp., *Tetraselmis* sp.
- Algreen B.V.[35] is focussed on supporting algal producers
- Hortimare[36] - *Alaria esculenta, Palmaria palmata, Saccharina latissima*
- Olijck[37] - *Palmaria palmata, Undaria* sp.
- Photanol[38] uses cyanobacteria (including genetically modified *Synechocystis* sp.) fixing CO_2 to produce chemicals (*e.g.* lactic acid for the production of bioplastics).
- Phycom B.V.[39] produces algal paste, pellets, powder and flakes for food and feed application starting from *Chlorella vulgaris* and *Chlorella sorokiniana*.
- SeaFlavours B.V.[40] – Wakame production (*Undaria pinnatifida)*
- Seaweed Harvest Holland[41] - *Saccharina latissima*
- The Algae Factory[42] - *Spirulina sp.*
- Zeewaar[43] - *Alaria esculenta, Saccharina latissima, Ulva sp., Undaria sp., Caulerpa lentillifera, Caulerpa racemosa*

The following indicates companies in the Netherlands with a broader portfolio that are moving on the path to use algae:

- DSM produces algae-based omega-3 oil as a sustainable substitute for fish oil[44] (Veramaris®, DHAgold™, life'sDHA®, life's™OMEGA).
- Unilever will work together with Algenuity to investigate the potential of algae as a new protein source[45]. *Chlorella vulgaris* is the focus species.

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[27] https://www.macrofuels.eu/
[28] https://www.macrocascade.eu/
[29] http://www.algaeparc.com/
[30] https://www.wur.nl/nl/project/Algen-zuiveren-afvalwater-van-bierbrouwerij-1.htm
[31] https://www.wur.nl/en/show/Algae-cultivation-using-waste-water-from-greenhouse-horticulture-and-used-as-feed-for-oysters.htm
[32] https://www.emodnet-humanactivities.eu/view-data.php
[33] http://www.algae-biotech.com/
[34] https://www.algaspring.nl/
[35] https://www.algreen.eu/
[36] https://www.hortimare.com/
[37] https://www.olijckfoods.com/
[38] https://photanol.com/#technology
[39] https://phycom.eu/
[40] https://www.seaflavours.nl/
[41] https://www.dutchseaweedgroup.com/nl/
[42] http://thealgaefactory.com/
[43] https://www.zeewaar.nl/nl/
[44] https://www.dsm.com/corporate/solutions/resources-circularity/replacing-fish-with-algae-with-veramaris.html
[45] https://www.unilever.com/news/press-releases/2020/unilever-and-algenuity-partner-to-explore-use-of-microalgae-protein.html
https://www.unilever.com/news/news-and-features/Feature-article/2020/microalgae-partnership-marks-move-to-fairer-food.html

- Sustenso[46] pursues the development of a sustainable, environmentally friendly treatment of agricultural waste, whereby CO_2 released during waste treatment is used by microalgae to produce biomass for animal feed and human food.

Genetic modification of algae is still in an early phase, yet COGEM is often asked to advise on such applications in the Netherlands:

- Advies CGM/990308-11 (COGEM, 1999b),
- Advies CGM/990429-09 (COGEM, 1999a),
- CGM/011214-03. Biotechnologie in lagere planten (COGEM, 2001),
- CGM/110418-03. Grootschalige productie van melkzuur door genetisch gemodificeerde cyanobacteriën in een kweeksysteem voor eenmalig gebruik (COGEM, 2011b).
- CGM/110706-01. Classificatie van negen algensoorten (COGEM, 2011a),
- CGM/150821-01. Classificatie van cyanobacterie *Synechococcus* sp. stam PCC7002 (COGEM, 2015),
- CGM/160816-01. Classificatie cyanobacterie *Anabaena variabilis* stam ATCC 29413 (COGEM, 2016a),
- CGM/160504-0101. Pathogeniteitsclassificatie algensoorten *Nannochloropsis gaditana* en *Nannochloropsis oceanica* (COGEM, 2016b),
- CGM/200520-02. Pathogeniteitsclassificatie *Anabaena azollae* en inschaling van werkzaamheden met (gg-)*Azolla filiculoides* in associatie met (gg-)*A. azollae* (COGEM, 2020a),
- CGM/200225-01 Pathogeniteitsclassificatie van de cyanobacteriestam *Anabaena* sp. PCC 7120 (COGEM, 2020b),
- CGM/200128-02. Pathogeniteitsclassificatie van de micro-alg *Aurantiochytrium limacinum* (COGEM, 2020c),
- CGM/200421-01. Pathogeniteitsclassificatie van de micro-alg *Tetraselmis striata* (COGEM, 2020d).

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[46] http://www.sustenso.nl/

3 Components for risk classification

This section provides background on the information that was collected and analysed in the project aiming to support risk classification of species and higher taxa. The topics provide a very general indication to assist the reader in the delineation of the different aspects. It was not the intention to provide a complete review of each component, but rather describe and illustrate with a few examples the essential aspects for this approach.

When compiling this overview, we considered elements of importance to inform the risk assessment relating to impact on human and animal health and ecological effects. **Table 1** reflects elements reported in literature (Kumar, 2015; OECD, 2015a; Snow and Smith, 2012) as well as indicated by the US EPA (2020) as topics that applicants must provide for a TSCA Biotechnology Submission for algae. Furthermore, it is indicated which elements are included in the Supplementary Document **Algal Risk Classification Features** as a result of this study and which criteria were used to identify hazardous species.

Table 1 Elements relating to impact on human and animal health and ecological effects, with an indication of information included in the Supplementary Document **Algal Risk Classification Features** (X) and criteria used to identify hazardous species (red boxes).

▪	Biology	○ Natural growth forms or patterns (*e.g.*, unicellular, colonies/coenobia, chains, filamentous, mats)	X
		○ Photosynthetic ability	
		○ Nitrogen fixation ability	
		○ pH range and optimum for growth	
		○ Temperature range and optimum for growth	X
		○ Illumination conditions optimal for growth (intensity, photoperiod)	
▪	Habitat	○ Compatibility with specific habitats (e.g. soils, fresh or marine waters or sediment, wastewater, desert soils)	X
		○ Salinity tolerance (e.g., marine water, freshwater, brackish water, euryhaline)	X
		○ Position in water body or water column (e.g., planktonic, benthic, periphytic)	
		○ Prevalence/distribution in the environment	X
▪	Pathogenicity	○ Ability to cause harm or disease to human, animals ((marine) mammals, (sea) birds, fish, aquatic invertebrates) and plants	🟥 X
		○ Mechanical effects (e.g. lesions, obstruction of gills)	🟥 X
▪	Production of specific compounds of interest/concern	○ Composition (protein, carbohydrate, oil content, lipid yields, % ash-free dry weight, specific fatty acids produced)	X
		○ Toxin production (by the algae or symbionts)	🟥 X
		○ Haemolytic secretions	🟥 X
		○ Allergen production	🟥 X
▪	Survival and persistence	○ Can the organism survive in a dormant or resting state?	X
		○ Dormancy structures/strategies (e.g., spores, cysts, viable but nonculturable state)	X
		○ History of invasiveness and persistence	X
▪	Competition with indigenous species (invasiveness)	○ Propensity for blooming/anoxia	X
▪	Ways of dispersal in the environment	○ Dispersal by aerosols	X
		○ Dispersal by wildlife (birds, insects, reptiles, terrestrial animals)	
▪	Food web	○ Importance in aquatic food web/trophic interactions	
▪	Potential for gene transfer	○ Reproductive methods	X
		○ Horizontal gene transfer (HGT)	X
▪	Beneficial properties	○ Safe use as food, feed, additive or others	X
		○ Medicinal use	X

3.1 Taxonomic landscape

Strain identity is an important parameter for determining the potential risk. In order to identify taxa with safe and/or hazardous species, it is essential to have a clear and stable view on the taxonomy. “Algae” is a general name for a multitude of organisms (as illustrated by **Table 2**).

Table 2 Taxonomic diversity of the Algae.

	Empire	Supergroup*/Clade	Division*/Phylum
Algae	Prokaryota	Eubacteria	Cyanophyta/Cyanobacteria
	Eukaryota	Archaeplastida (former Plantae)	Glaucophyta
			Rhodophyta
			Chloroplastida/Chlorophyta
			Chloroplastida/Charophyta
		Cryptista (former Chromista)	Cryptophyta
		Haptista (former Chromista)	Haptophyta
		TSAR/Alveolata	Dinoflagellata
			Colpodellida (genus *Chromera*)
		TSAR/Rhizaria	Chlorachniophyceae
		TSAR/Stramenopiles	Ochrophyta/Diatomista
			Ochrophyta/Chrysista
		Excavates/Discoba	Euglenozoa

* sensu Adl *et al.* (2019); Burki *et al.* (2020); Guiry (2021)
TSAR: Taxonomic ‘supergroup’ including telonemids, stramenopiles, alveolates, and *Rhizaria.*

Microalgae can be determined as microscopic eukaryotic, unicellular, colonial, and filamentous algae, as well as the oxygenic photosynthetic bacteria (prokaryotes), the cyanobacteria. Macroalgae or so-called seaweeds, are multicellular photosynthetic eukaryotes (excluding the green land plant lineage).

There is no unanimously accepted classification of algae. Classification is subject of several types of changes. In first instance, simple name changes may occur (*e.g.* a species is assigned to another genus based on new insights). Secondly, more complex changes may occur (*e.g.* when a species is split into different ((semi)cryptic species). Finally, the most complex changes are those in which the delineation of a species may be revised, meaning that the old concept of the species will change.

There is discussion especially at the level of division (phylum) and class. However, algae naming is regulated according to the International Code of Nomenclature for algae, fungi, and plants (ICN) (Turland *et al.*, 2018). The Nomenclature Section meets every 5 years - the last time in Shenzhen in 2017 (19th International Botanical Congress) - , discusses proposals and then publishes them in the Code. Names can be verified in Appendices I to VII of the Code through a search function[47]. Other sources include the journal Taxon[48], which is entirely devoted to the systematics, phylogeny and taxonomy of algae, fungi and plants, and AlgaeBase[1] including Notulae Algarum[49], a journal that specifically discusses the nomenclature and taxonomy of algae. But also general phycological

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[47] https://naturalhistory2.si.edu/botany/codes-proposals/
[48] https://onlinelibrary.wiley.com/journal/19968175.
[49] http://www.notulaealgarum.com/.

journals such as Phycologia, Journal of Phycology, Diatom Research, Phytotaxa, etc. publish probably the majority of taxonomic papers on algae.

For classifications one can consult the Catalogue of Life[50] (an authoritative online taxonomy index) and NCBI Taxonomy browser[51] (Schoch *et al.*, 2020) (an online index with classifications of all organisms whose DNA and RNA sequences are publicly available). Specific databases such as Cyanotype [52] can be consulted for cyanobacteria, but also or AlgaeBase[1] including all algae.

As the backbone for this project, we compiled a classification mainly based on AlgaeBase[1] into a main reference framework. On March 1st 2021 when the data collection for this study was closed, it included 7191 genera, distributed over 800 families, 267 orders, 60 classes and 13 phyla/divisions. The complete **Algae Taxonomic Landscape** is provided as a table in a separate Supplementary Document.

The dynamic nature of the algae taxonomy must be stressed. During the initial research phase of this project (approx. 2 months), we observed changes at family level within the Cyanobacteria. These modifications can be limited, but in other case may have a broad impact: *e.g.* when reshuffling the eukaryote 'Tree of Life' (Burki *et al.*, 2020). It must be highlighted that this dynamic status of taxonomy impacts clustering and making any risk classification based on higher level taxa (mainly at kingdom, division, phylum and subphylum level).

3.2 Selection of species for this study

Our taxonomic landscape was used for the next steps in the analysis (*i.e.* a literature search of adverse effects, precedents and risk assessment) and for attempting clustering groups. With the enormous diversity as exemplified in the previous section, it was essential to make a selection of species for this study. **Table 3** lists the selection criteria that were used.

Table 3 Criteria used for selecting species for this study

▪ Relevance	Specific algal genera/species are already being applied or are expected to be applied in the near future, *e.g.* for the production of biofuels (oil, diesel, hydrogen gas) or high value components.
▪ Representativeness	The selection tried to include a broad range of taxa, rather than achieving clusters based on a biased selection.
▪ Documented risk/ safety profile.	Species for which risk/safety information was retrieved during searches were included. Even if these are not necessarily of immediate relevance they can serve as indicators for other species with the same taxon (an overview of the consulted source is provided in References Supp Doc Risk Classification Features)

In addition, essential information on relevant species and features to be considered was retrieved from the online interviews with the experts. Although a lot of research on biofuels is being conducted and the number of publications examining the potential of algal species as source of biofuels is extensive, only a limited number of algal species is effectively grown for biofuel production (*e.g. Dunaliella salina, Emiliania huxleyi, Nannochloropsis* spp., *Nannochloris* spp., *Neochloris* spp., *Pichochlorum* spp.). The algae of highest interest are those with a high lipid content (*e.g.* 60-90% of their respective dry weight). Apparently, the importance of biofuel production, at least in Europe, is limited. Main reasons were indicated as:

- algae must be grown in large shallow ponds to prevent self-shadowing, while space in Europe is limited,
- inability of biofuel to compete with the low-cost mainstream of fossil fuels.

Also, the consultations did not exclude the use of toxic algae for biofuel production, since several refining steps are required to obtain biofuel out of algal biomass and the final biofuel product is not

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[50] http://www.catalogueoflife.org/annual-checklist/2017

[51] https://www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html/.

[52] http://lege.ciimar.up.pt/cyanotype/

automatically triggering new concerns over toxicity compared with existing fuel solutions. However, when growing algal species on large scale that are toxic upon skin contact, ingestion or contact with aerosols (*e.g.* during cleaning of installations with high pressure cleaners), the necessary safety measures (*e.g.* personal protective equipment) must be implemented. In addition, appropriate waste flows for residual (toxic) algal biomass must be developed.

In contrast with biofuels, algal production for food and animal feed, production of natural pigments (*e.g.* carotenes, astaxanthin, phycobiliproteins), and bioplastics production from microalgae (producing *e.g.* polylactides, polyalkanoates), are expected to be of greater significance in Europe.

Based on these criteria, information from literature and valuable input from the consulted experts resulted in a selection of approx. 500 species, that were further described in detail. The selected species and the results are compiled in a table **Algal Risk Classification Features** in a separate Supplementary Document.

3.3 Ecology

The knowledge of the ecology of the different species serves the assessment of whether a species has the potential to disturb the environment in case of an accidental release. Introduction of non-native species is a potential threat arising from production activities with algae. Indeed, exotic algae might be more interesting candidates for production of useful compounds than indigenous algal species. Fast growth of algal species for biofuel production (which is the case for *e.g. Picochlorum*) is certainly an advantage, however, several experts emphasised that growth must be controllable to prevent proliferation and accidental release of GM algae. Therefore, it is important to know the temperature, pH, salinity range and optimum, light intensity, photoperiod, etc. to determine its chances to survive in the Netherlands. However, one has to bear in mind that in view of the current climate change, the environment may become more suitable for warm temperate and subtropical species.

In this view, the use of native or cosmopolitan species is absolutely recommended to limit consequences in case accidental environmental release should occur. However, previously cosmopolitan species, such as *Pseudo-nitzschia pungens,* may in fact be a complex of cryptic species (*i.e.* morphologically indistinguishable taxa for which there is genetic evidence that they belong to species complexes or different evolutionary lineages), of which each actual species has its own preferred climatic growing zone (Casteleyn *et al.*, 2008; De Luca *et al.*, 2021). One could also consider using species that are not able to survive and grow in the local environment. The prevalence in the Netherlands and the specific ecological niches already gives a certain indication, but even exotic species adapted to the natural conditions in the Netherlands may become invasive.

Algae are ubiquitous (Borowitzka, 2018a). They can be found from the poles to the equator, on land and in water, both salt and fresh, on ice and in hot springs. Species distribution may be restricted to specific zones, such as temperate or (sub)tropical zones. Within a specific biogeographical range species may be widespread. Many species are thought to be cosmopolitan (see previous remark on the increasing discovery of cryptic species). They can be free-living in the sunlit parts of lakes and seas (planktonic), attached to a substrate like rocks or sediment (benthic), seaweeds or plants (epiphytic), or in soils.

Distribution data retrieved from AlgaeBase[1] and GBIF[4] was indicative for the potential to survive in a temperate climate like in the Netherlands. Many species were found to be cosmopolitan (but see remark in the previous section). Few species seem to be limited to specific regions *e.g.* the tropics. Examples of the latter are *Acanthophora spicifera*, *Caulerpa racemosa* and *Dinophysis mitra.* Probably these species are not able to survive in the Netherlands, at least not year-round. Some are restricted to extreme conditions like *Cyanidioschyzon merolae* that lives in thermal acidic environments.

Several species were identified as invasive (*i.e.* non-native to an ecosystem and characterised by a fast growth, quick reproduction and aggressive spread, with potential to cause harm) and/or persistent (*i.e.* able to remain in a particular setting for a period of time after initial introduction, whether or not difficult to eradicate). Examples are *Caulerpa taxifolia* and *Grateloupia turuturu.* In particular, *Sargassum muticum* (Japanese wireweed) is notorious as invasive seaweed; originating from Japan, this *S. muticum* has spread to the US and Europe. Its efficient spread via aerocysts (gass-filled floats) and its limited number of natural predators are key to its successful establishment.

Also important to note is that several microalgal species grow in association with specific symbiotic bacteria which stimulate growth, offer competitive advantages and exchange of nutrients such as vitamin B12 or nitrogen(Stock *et al.*, 2019; Yao *et al.*, 2019). This has also been observed for the biofuel producing microalgae *Dunaliella salina* and *Emiliania huxleyi* (see *e.g.* Vardi *et al.* (2017); Yamane *et al.* (2013)) Such symbiotic microalgae–bacteria consortia may be used to improve microalgal biomass production and to enrich the biomass with valuable chemical and energy compounds.

3.4 Horizontal gene transfer

Horizontal gene transfer (HGT) is non-sexual transmission of genetic material between unrelated genomes. As specified in Directive 2001/18/EC[53] and further defined in EFSA guidance documents, HGT is not a hazard *per se* but rather a mechanism through which adverse effects may occur. COGEM takes the potential for HGT into account during the risk assessment. Whether a HGT event entails risks, will depend largely on the type of gene transferred (*e.g.* genes involved in toxin production).

HGT has been well documented among prokaryotes including cyanobacteria. Although less frequently encountered in eukaryotes, HGT events have been described in extremophilic red algae (*e.g. Galdieria sulphuraria*), diatoms (*e.g. Thalassiosira pseudonana, Phaeodactylum tricornutum*), Cryptophytes, Alveolata (*Chromera velia*), Ochrophytes/Chrysista and Haptophytes (Chan *et al.*, 2012; Dorrell *et al.*, 2021; Fan *et al.*, 2020; Soucy *et al.*, 2015; Vancaester *et al.*, 2020).

In this way, genes have been acquired from bacteria and it is thought that this has played a crucial role in expanding the adaptive capabilities of these algae (*e.g.* metabolite management, perception of environmental signals, polysaccharide biosynthesis). Up to 5% of diatom genes are thought to have arisen via HGT (Bowler *et al.*, 2008).

In the Cryptophyte *Teleaulax* a special form of HGT (kleptoplasty) is observed where its plastids are sequestered by host organisms. *Teleaulax* donates plastids (containing bacterial polymerase genes) to the ciliate *Mesodinium rubrum* and subsequently to the dinoflagellates *Dinophysis caudata* and *Amylax triacantha*. While most of the genetic information required for the activity and maintenance of the kleptoplastids disappears with the digestion of the algal nuclei, the photosynthetic organelles remain active during extended period of time (Kim *et al.*, 2015; Pillet, 2013).

3.5 Hazardous properties

3.5.1 Pathogenicity

Pathogenicity is a direct threat towards the general public. This characteristic and the availability of prophylaxis and/or treatment will define the protective measures that need to be taken. Not only people but also animals (and perhaps in first instance fish and aquatic animals) and plants need to be protected.

Some species are known as pathogenic. Examples include:

- *Desmodesmus armatus* can infect humans via open wounds (Westblade *et al.*, 2015).
- The human and animal pathogens *Prototheca* spp. (*Prototheca wickerhami, Prototheca cutis*) are causing protothecosis, a disease found in dogs, cats, cattle, and humans (Enzing *et al.*, 2012; Lass-Flörl and Mayr, 2007).
- *Chlorochytrium* spp. have been reported to cause algal dermatitis in ornamental cichlid fish; zoospores germinate into a tubular protrusion penetrating into host tissues (Yanong *et al.*, 2002).

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[53] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC. OJ L 106, 17.4.2001, p. 1–39.

- An example of plant pathogenic algae are *Cephaleuros* spp. causing “red rust” or “algal spot” on tea, mango, coffee, citrus, and guava leaves and fruits (Brooks *et al.*, 2015).

Nevertheless, pathogenesis towards humans and animals, directly caused by algae, is not common. On the other hand, illness due to contact, ingestion or inhalation of toxic substances occurs more often (see 3.5.2).

3.5.2 Toxicity

The potential of toxin production is a second hazard that may influence protective measures to be taken. Although the main danger of toxic algae is via ingestion (via food or drinking; *e.g.* cattle drinking from water reservoirs with toxic algal blooms; human consumption of shell fish in which algal toxins have accumulated), of special concern in a research and development environment are toxins that harm via skin contact and inhalation. However, often the conditions under which toxins are produced are not clear. This makes it difficult to estimate to what extent this entails risks.

Many algae produce a diverse collection of toxins that are harmful for humans, (marine) mammals, (sea) birds, fish and a broad range of aquatic invertebrates (*e.g.* zooplankton, molluscs, crustaceans). They are most commonly known from harmful algae blooms (HAB) seasonally found in coastal waters, as well as in brackish and freshwater ecosystems (Lassus *et al.*, 2016). Sometimes they also affect more offshore oceanic areas.

Blooms are described as a rapid increase or accumulation in the population of microscopic, unicellular algae in water. Because of the pigments in the alga species the water becomes coloured giving names such as “red tide” to certain blooms. Excessive growth of algae is usually the result of a sudden increase in nutrients, especially nitrogen and phosphorous. Not all blooms produce toxins, but they may be harmful in other ways (see 3.5.3, 3.5.4 and 3.5.5).

The class of dinoflagellates are most famous, but toxic blooms also occur in species belonging to the diatoms, Ochrophyta/Chrysista and cyanobacteria (OECD, 2015b). The main effects on human and animal health are direct due to contact toxicity (exotoxins) and indirect toxicity via ingestion of food in which endotoxins have accumulated (*e.g.* in shellfish). Other effects may include ichthyotoxicity and cytotoxicity (demonstrated *in vitro*), etc. Some microalgae (*e.g. Chattonella* spp., *Alexandrium* spp.) excrete compounds such as reactive oxygen species (ROS), mucopolysaccharides, or polyunsaturated fatty acids (PUFAs) (Anderson *et al.*, 2012). As such they are not toxic, but can be harmful or even lethal to fish kept caged in aquaculture farms, when blooming (Hallegraeff *et al.*, 2017).

For people, the toxins ingested via food can cause neurological or gastrointestinal symptoms, such as (Lassus *et al.*, 2016; World Health Organization, 2003a):

- Amnesic shellfish poisoning (ASP))
- Ciguatera fish poisoning (CFP)
- Diarrhetic shellfish poisoning (DSP)
- Neurotoxic shellfish poisoning (NSP)
- Paralytic shellfish poisoning (PSP), and
- Cyanotoxin poisoning.

Clearly, these potential indirect intoxications are no concern for co-workers manipulating algae in research or production facilities. However, an accidental release into the environment may lead eventually to food poisoning. Also, (sea)birds and (marine) mammals may suffer from toxins accumulated in lower trophic levels.

HAB species, causing harm due to their ability to produce potent toxins, or through impacts associated with their sheer biomass, cover many phytoplankton classes: diatoms, dinoflagellates, Raphidophyceae, Coccolithophyceae, Cyanobacteria, Dictyochophyceae, and Chrysophyceae (Lassus et al., 2016). Notorious are the dinoflagellates, *e.g.* the genera of *Alexandrium*, *Azadinium, Dinophysis*, *Fukuyoa*, *Gambierdiscus*, *Karenia*, *Phalacroma* and *Protoceratium*, and for the diatoms the *Pseudo-nitzschia* genus (Caruana and Amzil, 2018).

Many compounds have been identified, such as brevetoxins, ciguatoxins, domoic acid, Dinophysis toxins, mycosporine-like amino acids, microcystins, okadaic acid, saxitoxins, etc. (Berdalet *et al.*, 2016; Caruana and Amzil, 2018; Klisch and Häder, 2008; Lassus *et al.*, 2016). Microcystins are the most frequently occurring and widespread of the cyanotoxins (World Health Organization, 2003b).

Macroalgae, frequently consumed in some parts of the world, sporadically cause illness or even death. Reports have emerged for the *Caulerpa*, *Gracilaria*, *Acanthophora* genera (Cheney, 2016). However, the toxin often is produced by epiphytic cyanobacteria contaminants, instead of the seaweed itself.

Besides ingestion also inhalation of air-borne toxic algae (aerosols) may affect *e.g.* people walking on the seashore (Caruana and Amzil, 2018; World Health Organization, 2003a). Marine aerosols may be harmful especially during active blooms of *e.g. Karenia brevis* and *Ostreopsis* spp. (Berdalet *et al.*, 2016).

Although not as common as in marine environments, also in freshwater algal toxic blooms occur, and the incidence is increasing worldwide. Freshwater blooms are most often caused by cyanobacteria (Huisman *et al.*, 2018; World Health Organization, 2003b). The cyanobacterial blooms are a major threat for drinking water, recreation, etc. The most common toxic cyanobacteria in freshwater are *Microcystis* spp., *Cylindrospermopsis raciborskii*, *Planktothrix* (syn. *Oscillatoria*) *rubescens*, *Synechococcus* spp., *Planktothrix* (syn. *Oscillatoria*) *agardhii*, *Gloeotrichia* spp., *Anabaena* spp., *Lyngbya* spp., *Aphanizomenon* spp., *Nostoc* spp., some *Oscillatoria* spp. and *Synechocystis* spp. (World Health Organization, 2003b). Toxicity has also been shown for freshwater dinoflagellates and an ichthyotoxic species, *Peridinium polonicum*, has been detected in European lakes (World Health Organization, 2003b).

In recreational freshwaters humans may suffer from toxicity by contact, ingestion of water and inhalation (World Health Organization, 2003b).

Production of toxins is often induced under stress situations such as excess/depletion of nutrients, ocean acidification, high water temperature, grazing, extreme weather events, or when grown in culture.

As already mentioned in § 3.3 several microalgal species grow in association with specific symbiotic bacteria which stimulate growth, offer competitive advantages and exchange of nutrients. These symbiotic bacteria may also produce toxins which add to the overall toxicity of the algal host (see *e.g.* Zan *et al.* (2019)). When considering production/modification of such algal species, it is important to also include the bacterial symbiont in the risk assessment (not only from a toxicity point of view).

3.5.3 Allergenicity

Cyanobacteria are most frequently the cause of allergic reactions. Eukaryotic algae usually do not occur in densities high enough to cause allergic effects. Allergic or irritative dermal reactions of varying severity have been reported from a number of freshwater cyanobacterial genera (*Anabaena*, *Aphanizomenon*, *Nodularia*, *Oscillatoria*, *Gloeotrichia*) (World Health Organization, 2003b) and are linked to phycocyanin (Bernstein *et al.*, 2011; Geh *et al.*, 2015). Cyanobacteria inhabiting marine environments such as *Lyngbya majuscula*, present in temperate and tropical waters, also may induce dermatitis (Fleurence and Levine, 2018).

Airborne algae like *Prototheca* sp. may provoke allergic reactions in people frequenting beaches (Fleurence and Levine, 2018). House dust may contain the algal genera *Chlorococcum* and *Chlorella* causing allergic skin reactions. The airborne cyanobacteria *Phormidium fragile* and *Nostoc muscorum* are known to exhibit allergenic properties.

Arthrospira ‘(Spirulina’) tablets (cyanobacteria) used as nutritional supplements in human consumption have been reported to induce food allergy (Fleurence and Levine, 2018).

3.5.4 Mechanical effects

Algal blooms can hurt sea life, especially fish in a non-toxic way by obstructing gills or causing lesions (Lassus *et al.*, 2016). Often diatoms are the causal agents of fish and shellfish mortalities. For example diatoms bearing barbed setae, like *Chaetoceros* spp. cause bleeding and suffocation due to the mucus production as a result from the broken setae stuck into the gills (Lassus *et al.*, 2016) and may represent a threat to aquaculture.

These characteristics may become a concern in case of unintentional release.

3.5.5 Algal bloom / Anoxia

Algal blooms are maybe best known to cause anoxia. The term eutrophication is used to indicate the excessive enrichment of ecosystems with dissolved nutrients (for example, nitrate and phosphate), usually through human activity. Eutrophication can lead to algal blooms and oxygen depletion or anoxia due to algae growth (Huisman *et al.*, 2018)

Several mechanisms may cause anoxia or hypoxia. Some examples:

- Algal blooms both reduce water clarity thereby preventing sunlight from penetrating into the water and reaching submerged aquatic vegetation and benthic microalgae and induce selfshading (or shadow effect). Reduced photosynthesis releases less oxygen that would normally replenish the water's oxygen supply, thereby causing hypoxia. Also, in dense algal blooms, light availability may become limited to the bloom species itself; this so called self-shading or shadow plays an important role in the dynamics of algal blooms: it limits photosynthesis, limits growth of the algal blooms species and reduces the availability of oxygen (see *e.g.* Shigesada and Okubo (1981)).
- In shallow waters algal respiration at night may lower oxygen levels to close to zero.
- The algal blooms are either consumed leaving faecal pellets or die naturally (often through viral lysis) and sink. Decaying algae and their remains are mineralised by bacteria, an activity that consumes oxygen and deprives the water from oxygen. If the rate of oxygen consumption exceeds resupply, all oxygen-requiring organisms are at risk.

3.5.6 Other

Fish gills can be damaged by contact with extracellular haemolytic substances produced by some algae (Lassus *et al.*, 2016). These haemolysins destroy blood cells and the epithelium of gill tissues. These haemolytic secretions may consist of substances similar to PUFAs like in *Chattonella* spp. or mono- and digalactoglycerolipid type of molecules, amphidinols, maitotoxin-like substances, and ROS etc. Other species producing haemolysins are *e.g. Alexandrium* spp., *Gambierdiscus* spp., *Heterosigma akashiwo*, *Karenia mikimotoi* and *Karenia digitata*, *Prymnesium polylepis* and *Prymnesium parvum.*

Other species produce mucus sheets that hamper efficient filter feeding in bivalves (*e.g. Aureoumbra*).

4 Clustering

Within the context to facilitate risk assessments, it was the intention to explore options clustering whole families or genera or identifying higher taxonomic levels that are generally recognised as safe or conversely known to be hazardous, on the basis of risk/safety profiles as identified in the previous sections. The risk characterisation of the wild-type parental or recipient organism (baseline considerations) is a basic element for the risk classification of a GMO.

A selection was made of approx. 500 species according to relevance, representativeness and documented risk/ safety profile and as explained in Section 3.1. The distribution of these selected species over the taxonomic landscape is provided in **Table 4**.

Table 4 Comparison of taxa identified in the taxonomic landscape and the distribution of the selected species.

	Taxonomic landscape	Selection
phyla/divisions	13	13
classes	60	35
orders	267	80
families	800	139
genera	7191	211

For some phyla or classes, *e.g.* the Glaucophyta (1 species covered), Euglenophyceae (5 species covered), Bigyra (4 species covered) and Cryptophyta (2 species covered) only a limited number of data is available. Also, the phylum Rhodophyta, comprising approximately 1500 species, is to a limited extent represented (8 harmful species identified). The lack of information may indicate a bias in our selection to species with known safety profiles and being applied but may also indicate a genuine information gap on risks/safety.

The results of the specific reviews of selected species are compiled in the table **Algal Risk Classification Features** provided in a separate Supplementary Document. In this listing, taxonomic information on selected species is combined with indications on pathogenicity, toxicity, algal bloom/anoxia, allergenicity, mechanical effects (e.g. lesions, obstruction gills), other (e.g. haemolytic secretions), safe use, medicinal use, habitat, and proliferation potential in temperate climate.

Table 5 provides a summary of this elaborate listing and gives a glance on
1) algal taxa in which hazardous species are more frequently encountered (red) (n = 293),
2) algal taxa in which species are generally considered to be safe (green) (n = 83) and
3) algal taxa for which no indications of either hazardous properties or confirmation of safe use could be retrieved for the consulted literature sources (grey) (n = 134).

It is important to note that this latter category includes species that are currently used for multiple purposes such as use as food, feed, source of food additives (*e.g.* vitamins, pigments, alginates, carrageenan), for biofuel or glycerol production, model species for research or a source of medicinal compounds (*e.g.* antioxidants or compounds with an antimicrobial action).

The numbers in the columns hazardous/no hazard/no data represent all individual species and subspecies of a specific family for which relevant information was found. A species was considered hazardous if at least one reference on one or a combination of the following features was found: pathogenicity, toxicity, allergenicity and haemolytic secretions. The potential to cause blooms, mechanical effects and invasiveness potential were not considered, as these features are less important when considering production of algae under contained use conditions. A species was considered to pose no hazard when an official risk classification was available or when test had been conducted demonstrating safety (e.g. by *in vivo* feeding experiments in experimental animals).

Table 5 Overview of algal taxa to which the selected species belong with an indication of identified features for the selected species. The number in a cell indicates how many species were classified as showing harmful properties (red), as having some confirmation of safety (green) and for which no hazard/safety data could be retrieved (grey).

Division / Phylum	Class	Order	Family	Hazardous	No Hazard	No Data
-	-	Colpodellida	Chromeraceae			1
Bigyra	Blastocystea	Blastocystida	Blastocystidae	1		
	Labyrinthulea	Thraustochytriidia	Thraustochytriaceae		1	2
Charophyta	Charophyceae	Charales	Characeae	1		1
	Zygnematophyceae	Zygnematales	Zygnemataceae		3	
Chlorophyta	Chlorodendrophyceae	Chlorodendrales	Chlorodendraceae		2	4
	Chlorophyceae	Chaetophorales	Chaetophoraceae			1
		Chlamydomonadales	Chlamydomonadaceae		3	1
			Chlamydomonadales incertae sedis		1	
			Chlorochytriacaea	1		
			Chlorococcaceae	3		1
			Dunaliellaceae		4	6
			Haematococcaceae			1
			Volvocaceae			2
		Sphaeropleales	Scenedesmaceae	3	2	3
			Microsporaceae			1
			Neochloridaceae		1	
	Mamiellophyceae	Mamiellales	Bathycoccaceae			2
	Trebouxiophyceae	Chlorellales	Chlorellaceae	6	20	6
			Chlorellales incertae sedis			7
		Prasiolales	Koliellaceae			1
		Trebouxiales	Botryococcaceae	1		
			Trebouxiaceae		1	1
	Ulvophyceae	Bryopsidales	Caulerpaceae	5		
			Codiaceae		3	
		Cladophorales	Cladophoraceae	2		
		Trentpohliales	Trentepohliaceae	1		
		Ulvales	Ulvaceae	2		

Division / Phylum	Class	Order	Family	Hazardous	No Hazard	No Data
Cryptophyta	Cryptophyceae	Pyrenomonadeles	Geminigeraceae	1		1
Cyanobacteria	Cyanophyceae	Chroococcales	Aphanothecaceae	1		
			Chroococcaceae			1
			Microcystaceae	9		
		Nostocales	Aphanizomenonaceae	11		1
			Gloeotrichaceae	2		
			Hapalosiphonaceae	2		1
			Nostocaceae	10	6	3
			Rivulariaceae	1		
			Scytonemataceae			3
		Oscillatoriales	Microcoleaceae	7	1	1
			Oscillatoriaceae	9		3
			Coleofasciculaceae	1		
			Cyanothecaceae		1	
		Pleurocapsales	Hyellaceae	1		
		Synechococcales	Acaryochloidaceae	1		
			Coelosphaeriaceae	1		
			Leptolyngbyaceae	1		
			Merismopediaceae	1	1	
			Synechococcaceae	4		1
			Synechococcales familia incertae sedis	2		
			Prochloraceae			2
			Prochlorotrichaceae			2
	Oscillatoriophycidae	Chroococcales	Cyanobacteriaceae		1	1
Dinoflagellata	Dinophyceae	Amphidiniales	Amphidiniaceae	5		
		Dinophyceae ordo incertae sedis	Amphidomataceae	1		
		Dinophysales	Dinophysaceae	13		
			Oxyphysaceae	2		
		Gonyaulacales	Ceratiaceae		1	
			Goniodomataceae	1		
			Gonyaulacaceae	3		
			Lingulodininiaceae	1		
			Ostreopsidaceae	28		

Division / Phylum	Class	Order	Family	Hazardous	No Hazard	No Data
			Protoceratiaceae	1		
			Pyrophacaceae	21		
		Gymnodiniales	Brachidiniaceae	3		
			Gymnodiniaceae	7		1
			Kareniaceae	15	1	
			Polykrikaceae	1		
		Gyrodiniaceae	Gyrodiniaceae			1
		Peridiniales	Ensiculiferaceae			1
			Kryptoperidiniaceae			2
			Peridiniaceae	1		
			Crypthecodiniaceae		2	
		Prorocentrales	Prorocentraceae	18		1
		Suessiales	Symbiodiniaceae		1	
		Thoracosphaerales	Pfiesteriaceae	2		
			Thoracosphaeraceae		1	1
	Noctilucophyceae	Noctilucales	Noctilucaceae			1
Euglenozoa	Euglenophyceae	Euglenida	Euglenidae	7	1	
			Phacidae	1		
Glaucophyta	Glaucophyceae	Glaucocystales	Glaucocystaceae		1	
Haptophyta	Coccolithophyceae	Isochrysidales	Isochrysidaceae		1	1
			Noelaerhabdaceae			1
		Phaeocystales	Phaeocystaceae	2		1
		Prymnesiales	Chrysochromulinaceae	2		
			Prymnesiaceae	7		
	Pavlovophyceae	Pavlovales	Pavlovaceae			1
Ochrophyta/Chrysista	Chrysophyceae (golden brown algae)	Chromulinales	Chromulinaceae	1		
			Dinobryaceae	1		
	Dictyochophyceae	Florenciellales	Florenciellales incertae sedis	2		
	Eustigmatophyceae	Eustigmatales	Monodopsidaceae		3	6
		Goniochloridales	Goniochloridaceae			1
	Pelagophyceae	Pelagomonadales	Pelagomonadaceae	1		
		Sarcinochrysidales	Sarcinochrysidaceae	1		
	Phaeophyceae (brown algae)	Ectocarpales	Chordariaceae	1		1
		Fucales	Sargassaceae			5

Division / Phylum	Class	Order	Family	Hazardous	No Hazard	No Data
			Fucaceae	2	1	
			Himanthaliaceae		1	
		Laminariales	Alariaceae		1	1
			Laminariaceae	1		8
			Lessoniaceae			1
		Tilopteridales	Phyllariaceae			1
	Raphidophyceae	Chattonellales	Chattonellaceae	7		
			Fibrocapsaceae	1		
			Vacuolariaceae	1		
	Xanthophyceae	Mischococcales	Pleurochloridaceae			1
Ochrophyta/Diatomista (former Bacilllariophyta)	Bacillariophyceae	Bacillariales	Bacillariaceae	22	1	3
		Fragilariales	Staurosiraceae	1		
		Mastogloiales	Achnanthaceae	1		
		Naviculales	Naviculaceae	2	1	4
		Naviculales	Amphipleuraceae		1	
		Rhopalodiales	Rhopalodiaceae			1
		Tabellariales	Tabellariacea			1
		Thalassionematales	Thalassionemataceae			1
		Thalassiophysales	Catenulaceae	2		1
	Bacillariophyta classis incertae sedis	Bacillariophyta ordo incertae sedis	Phaeodactylaceae		1	
	Coscinodiscophyceae	Aulacoseirales	Aulacoseiraceae			1
		Melosirales	Melosiraceae			1
		Rhizosoleniales	Probosciaceae	1		
		Stephanopyxales	Stephanopyxidaceae			1
	Mediophyceae	Chaetocerotales	Chaetocerotaceae		5	
		Eupodiscales	Odontellaceae			1
		Stephanodiscales	Stephanodiscaceae		1	1
		Thalassiosirales	Skeletonemataceae	2		1
			Thalassiosiraceae	2		1
Rhodophyta	Bangiophyceae	Bangiales	Bangiaceae			6
	Cynanidiophyceae	Cyanidiales	Cyanidiaceae		1	
	Cynanidiophyceae	Cyanidiales	Galdieriaceae			1
	Florideophyceae	Bonnemaisoniales	Bonnemaisoniaceae	1		
		Ceramiales	Rhodomelaceae	4		

Division / Phylum	Class	Order	Family	Hazardous	No Hazard	No Data
		Corallinales	Lithothamniaceae			1
			Phymatolithae			1
		Gigartinales	Furcellariaceae		1	
			Gigartinaceae		2	
			Phyllophoraceae			1
			Solieriaceae		1	2
		Gracilariales	Gracilariaceae	3		3
		Halymeniales	Halimeniaceae			1
		Palmariales	Palmariaceae		1	
	Porphyridiophyceae	Porphyridiales	Porphyridiaceae		1	1
	Rhodellophyceae	Glaucosphaerales	Glaucosphaeraceae			1

Hazardous species most frequently relate to toxicity and are, as expected, relatively well represented in the Cyanobacteria (mainly *Microcystis, Anabaenopsis, Aphanizomenon, Nostoc, Oscillatoria, Lyngbya*), dinoflagellates (mainly *Dinophysis, Alexandrium, Gambierdiscus, Ostreopsis, Karenia* and *Prorocentrum*). In the diatoms, emphasis is placed on *Pseudo-nitzschia* spp. as main hazardous species.

The members of the Chlorellaceae (including *Chlorella*) outnumber all other algal families regarding safe use.

While **Table 5** indicates that higher taxa can include hazardous as well as non-hazardous species, we also identified species for which both toxic and non-toxic isolates have been isolated (*e.g. Pseudo-nitzschia*, *Amphora coffeaeformis, Aphanizomenon flos-aquae*). Consequently, for such species risk assessment will require a case-by-case approach for each isolate.

Taxa, for which relevant information could be retrieved, are further briefly discussed.

4.1 Discussion of specific taxa

4.1.1 Bigyra

These unicellular microalgae are part of the supergroup TSAR (Stramenopiles clade). As shown in **Table 5** only specific members of the families Blastocystidae and Thraustochytriaceae were included and highlighted in this study.

In first instance, *Blastocytis* was included as hazardous species given its pathogenicity. *Blastocystis* is an anaerobic, eukaryotic protist with a multitude of morphological forms (vacuolar, granular, amoeboid, avacuolar, multi-vacuolar and cysts). It is a zoonotic enteric parasite residing in the intestinal tract of diverse hosts causing diarrhoea in humans and infection in mammals and non-human primates and is therefore designated RG2. The life cycle of *Blastocystis* has not yet been fully elucidated due to the lack of a proper animal model.

Secondly, the heterotrophic Thraustochytrids were included in this study given their biotechnological importance (*e.g.* production of DHA oil, natural colourants (betacyanins), squalene (adjuvant in vaccines)) and the fact that one member (*Aurantiochytrium limacinum*) already has been subject of a risk classification by COGEM (COGEM, 2020c) and was designated RG1. Traustochytrids are unicellular fungus-like gliding flagellates producing a network of filaments.

DHA oils derived from *Schizochytrium* spp. and Ulkenia spp. have a GRAS statute[54]. Both species are also produced as aquaculture feed or novel food ingredient. However, insufficient data were available to consider them as safe.

4.1.2 Charophyta

Charophytes are freshwater green algae. Their morphology may be simple and filamentous or more complex with a main stem and branching (‘macrophytic algae’, almost resembling aquatic plants).

Charophytes play multiple roles in an aquatic ecosystem as part of the food web (important food source for *e.g.* waterfowl, amphibians, snails), in providing habitat for aquatic fauna (offering protection against predators and currents), in increasing water quality, and in nutrient cycling (important phosphorus and nitrogen sink) (de M. Bicudo and Bueno, 2013; Noordhuis *et al.*, 2002). Several species are known to produce repellent (allelopathic) compounds that exclude certain limnetic species of invertebrates and phytoplankton and in addition mitigate blooms of potentially toxic blue green algae (*e.g. Microcystis, Synecchococcus*) (Złoch *et al.*, 2018).

As indicated in **Table 5,** limited data were available for this phylum. *Mougeotia, Spirogyra* and *Zygnema* are excellent models for studying plant cell biology and have a history of safe use. No particular hazards were noted for this phylum.

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[54] FDA GRAS No. 677, DHA Algal Oil

Although Charophytes usually are considered advantageous in an ecosystem, it must be noted that they may be considered a nuisance. Charophytes can build up large biomasses (dense mats, so-called 'charophyte meadows') in brackish and freshwater ecosystems, due to presence of excessive nutrients (phosphorus & nitrogen) and sped up by vegetative propagation (see *e.g.* Mincheva *et al.* (2020)). Charophyte meadows may be a nuisance for boaters, anglers and swimmers who may become entangled. Charophytes can clog channels, reservoirs, clog the inlet to the farm ponds (Evans, 2016; Schneider *et al.*, 2015; Winter *et al.*, 2011; Zaneveld, 1940). No Charophyte species have been recorded to produced toxins; however, the spectacular Charophyte blooms can cause beach fouling and/or odour issues (COHPOA, 2015).

4.1.3 Chlorarachniophyceae

The Chlorarachniophyceae are a small group of unicellular, eukaryotic algae that was discovered in the nineties. These algae are characterised by an amoeboid morphology that may be the result of secondary endosymbiosis of a green alga by a non-photosynthetic amoeba (AlgaeBase[1], WoRMS[55]). They are exclusively marine and widely distributed in tropical and temperate waters. Sexual reproduction has only been reported in two species.

No particularities on this phylum were found in literature. So far, no harmful properties nor safe use have been reported for this group.

4.1.4 Chlorophyta

Chlorophyta are commonly called green algae. They are unicellular or multicellular, photoautotrophic algae with an enormously wide variability of shape and size. Most of the species live in freshwater. Some species are adapted to extreme habitats (deserts, arctic, hypersaline, acidic and alkaline habitats, marine deep waters). Many species have motile flagellated swimming cells.

Chlorophyta reproduce sexually and/or asexually.

As shown in **Table 5**, the members of the Chlorellaceae outnumber all other algal families regarding safe use. Many species and strains of *Chlamydomonas*, *Chlorella* and *Dunaliella* are determined Risk Group 1 according to ATCC[56], as these strains are not known to consistently cause disease in healthy adults and present minimal potential hazard to lab technicians and the environment.

For only a limited number of species, relevant data were gathered to classify the species as hazardous. These include *Chlorochytrium* sp., a fish pathogen, plant pathogens (*e.g. Chlorochytrium* spp., *Cephaleuros parasiticus*), species producing free fatty acids toxic to fish (e.g. *Botryococcus braunii*, *Chaetomorpha minima*), species for which allergic reactions upon inhalation have been reported (*e.g. Tetracystis* sp., *Chlorococcum botryoides*, *Scenedesmus basiliensis*, *Chlorella pyrenoidosa*) or species causing (opportunistic) wound infection in humans or vertebrates (*Desmodesmus* sp., *Chlorella* spp., *Prototheca* spp.).

The Chlorophyta also include *Caulerpa*, a genus that is notorious for its rapid growth, production of alkaloid toxins (caulerpicin, caulerpin, caulerpenyne) and its ability to alter marine habitats and affect biodiversity. In the past, *Caulerpa* has been widely used as a decorative plant in marine aquaria. As such a cold-water strain of *Caulerpa taxifolia* was accidentally released into the Mediterranean Sea by the Oceanographic Museum of Monaco where it subsequently has established. The invasive strain of *C. taxifolia* outcompetes other algal species, seagrasses and sessile invertebrate communities for food and light or due to the effects of the toxins contained in its foliage on aquatic herbivores (so leaving *Caulerpa* with virtually no natural predators).

4.1.5 *Chromera velia*

This unicellular photosynthetic organism is part of the supergroup TSAR (Alveolata clade) and is a bit of an oddity within the algae.

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[55] World Register of Marine Species. https://www.marinespecies.org/about.php

[56] https://www.lgcstandards-atcc.org/search?title=Algae%20(Alphanumeric)#q=%40productline%3DG031&sort=relevancy&f:contentTypeFacetATCC=[Products]

As so-called chromerids are related to both apicomplexan parasites (*e.g. Plasmodium*, causal agent of malaria) and dinoflagellates, they are excellent model for elucidating the biochemistry, physiology and evolution of apicomplexans and dinoflagellates and for developing antimalarial drugs.

As indicated in **Table 5** insufficient data were available to conclude on safety or hazards related to this particular species.

4.1.6 Cyanobacteria

Cyanobacteria are prokaryotic photosynthetic bacteria. They are unicellular but can also occur as paired cells or colonies that are filamentous, spherical or elongated in shape (Xiao *et al.*, 2018). They have a gram-negative cell wall that is often surrounded by a gelatinous or mucilaginous sheath. They acquire energy through photosynthesis, and some assimilate nitrogen from the air. Nitrogen fixation occurs in specialised thick-walled cells called heterocysts. Many species produce thick-walled dormant cells (akinetes) that serve a perennating role (storage of nutrients to sustain the cyanobacterium during unfavourable conditions). Cyanobacteria can be found in almost every terrestrial and aquatic habitat, or as symbiont in *e.g.* plant roots.

As shown in **Table 5** many hazardous cyanobacterial taxa were identified. Indeed, many cyanobacteria produce a range of toxins known as cyanotoxins (aphantoxin, anabaena-a anatoxin-a, cylindrospermopsin, deoxcylindrospermopsin, debromoaplysiatoxin, lyngbyatoxin A, microcystin, nodularin R, neosaxitoxin, and saxitoxin). Especially microcystins and saxitoxins are subject to the dual use legislation[57]. Aquatic cyanobacteria are notorious for their blooms and pose a danger to humans and animals, especially if the cyanobacteria involved produce toxins.

Some species are used as nutritional supplements for humans and animals (*e.g. Arthrospira*).

Cyanobacteria only know asexual reproduction (binary fission, spore production, or fragmentation). As such transgene transfer via reproduction to wild-type species is unlikely (Kumar, 2015). However, a particular point of attention when assessing the risks related to cyanobacteria is the possibility of HGT.

This phenomenon may occur either among cyanobacteria or between cyanobacteria and other organisms (Farrokh *et al.*, 2019). About 50% of extended gene families putatively have a history of HGT (either between cyanobacteria and other phyla, or within cyanobacteria, or both) (Kumar, 2015; OECD, 2015b). Often the genes that are at stake directly affect the competition and adaptation of cyanobacteria (Snow and Smith, 2012). HGT has played an important role in evolution and is a mechanism in real-time adaptation (Kumar, 2015; OECD, 2015b).

Moreover, there is evidence that HGT can equally take place via phages (in analogy with bacteria) (see *e.g.* Ou *et al.* (2015)). Cyanophages are viruses that infect cyanobacteria; the interaction between cyanophage and cyanobacterial host is always species specific. They play an important role in regulating the cyanobacteria biomass and community structures.

Furthermore, a limited number of cyanobacteria is known to be naturally competent, *i.e.* able to actively transport environmental DNA fragments across their cell envelope and into their cytoplasm (see *e.g.* Nies *et al.* (2020)). Natural transformation in cyanobacteria was initially considered a rare characteristic of a few unicellular species but seems more widespread among cyanobacteria than previously thought.

4.1.7 Diatoms

The Ochrophyta/Diatomista (former Bacillariophyta), or commonly called diatoms, is a species-rich phylum. Diatoms are mainly photoautotrophic; only a minority is heterotrophic or mixotrophic. Diatoms are unicellular eukaryotic organisms occurring either as solitary cells or in colonies. The diatom anatomy is characterised by a cell wall mainly made of silica (hydrated silicon dioxide), called a frustule and consists of two overlapping halves (thecae). Diatoms have two distinct shapes: centric diatoms are radially symmetric, while pennate diatoms are broadly bilaterally symmetric. Most species are benthic, but there is also a huge diversity of planktonic species

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[57] Council Regulation (EC) No 428/2009 of 5 May 2009 setting up a Community regime for the control of exports, transfer, brokering and transit of dual-use items. OJ L 134, 29.5.2009, p. 1–269

(including *Pseudo-nitzschia*). They reproduce asexually (primarily) and sexually. Diatoms are diploid in all stages of the life cycle other than the gametes. The sperm cells in planktonic (centric) diatoms are flagellated.

As shown in **Table 5** emphasis is placed on the Bacillariaceae (including *Pseudo-nitzschia* and *Nitzshia* spp.) as main diatom family grouping hazardous representatives. Indeed, especially *Pseudo-nitzschia* spp. are notorious as producers of toxins, in particular domoic acid which is associated with shellfish poisoning in humans and animals. Eighteen toxin producing *Pseudo-nitzschia* spp. were recorded and documented in this study. Also *Amphora coffeaeformis*, and *Nitzschia* spp. are equally known to produce toxins.

Several species were found to produce a non-protein amino acid, β-N-methylamino-L-alanine (BMAA). These include *Navicula pelliculosa*, *Proboscia inermis*, *Skeletonema marinoi* and *Thalassiosira* spp. Environmental exposure to BMAA, which is also produced by cyanobacteria, may cause amyotrophic lateral sclerosis (ALS) or Lou Gehrig's disease, a neurological disorder.

4.1.8 Dinoflagellates

These unicellular microalgae constitute the superclass Dinoflagellata, part of the supergroup TSAR (Alveolata clade). They live in all aquatic environments, including ice and snow. They are characterised by two dissimilar flagella. About 50% of the dinoflagellate species are photosynthetic often combined with phagocytose or myzocytosis which makes them actually mixotroph. Cells can be naked or covered with thecae, close-fitting cellulosic plates.

The life cycle usually involves asexual reproduction with a few species known to also reproduce sexually. The cells are haploid except for the zygote. Resting cysts are helpful to survive unfavourable conditions. The dinoflagellate genomes are among the largest of any organism and contain the unusual nucleobase hydroxymethyluracil (Borowitzka, 2018a).

Also, in this group species exist that induce a visible discolouration of the water, *i.e.* a harmful algal bloom commonly known as red tide. As indicated by **Table 5**, the dinoflagellates contain many hazardous representatives. Indeed, many species produce toxins, such as azaspiracids, brevetoxins, cooliatoxin, ciguatoxins goniodomin A, gonyautoxins, maitotoxin, okadaic acid, saxitoxins, spirolides and yessotoxin. Some of them are haemolytic. Their toxins are killing fish and accumulate in filter feeders such as shellfish, in turn causing several types of shellfish poisoning in animals and humans. Furthermore, blooms may irritate the respiratory tract.

4.1.9 Euglenozoa

Euglenozoa are best known of the genus *Euglena*, the most studied member (Borowitzka, 2018a). They are commonly found in freshwater, especially if rich in organic materials, with a few marine and endosymbiotic members. They are flagellates that can be photoautotrophic, mixotrophic, or heterotrophic. One or two flagella are present. They do not have a cell wall but a pellicle (pellicula) consisting of proteinaceous strips underneath the cell membrane, supported by microtubules. They only reproduce asexually in the form of longitudinal binary fission.

Euglena species living in fresh and brackish water habitats are unicellular and biflagellate. Only vegetative reproduction by longitudinal binary fission is known.

Euglena gracilis is used as source of α-tocopherol, paramylon or biofuels. It is also used in bioassays to determine vitamin B_{12} content in serum.

As shown in Table 5 several members of the the Euglenidae and Phacidae are considered (potentially) hazardous due to their ability produce an alkaloid toxin, euglenophycin. Hitherto, only for *Euglena sanguinea,* blooms have been linked to fish mortality and euglenophycin (Zimba et al., 2017).

4.1.10 Glaucophyta

The Glaucophyta are small group of unicellular freshwater algae. This group is a key lineage for the study of both the origin of primary plastids and the evolution of algae and plants. As in all members of the Archaeplastida, the chloroplast of glaucophytes, or 'cyanelle', is surrounded by 2 membranes as it has evolved from endosymbiosis with a cyanobacterium. Typically for glaucophytes, the chloroplast has a peptidoglycan layer (Birbeck *et al.*, 2019; Jackson *et al.*, 2015; Sanseverino *et al.*, 2017; Shoemaker *et al.*, 2015; Yang *et al.*, 2011; Zaffiro *et al.*, 2016). In particular, *Cyanophora paradoxa* is a well-studied evolutionary model as it is the most basal member of the Archaeplastida kingdom and is designated RG1.

As indicated in **Table 5**, no harmful properties have been reported for this group so far.

4.1.11 Haptophyta

Haptophytes have flagella and a haptonema, an organelle to capture and transport food (phagocytosis).

The Coccolithophyceae have an exoskeleton of calcareous plates called coccoliths. As shown in **Table 5** this class includes several hazardous species. *Chrysochromulina*, *Phaeocystis* and *Prymnesium* can cause harmful algal blooms. *Chrysochromulina* and *Prymnesium* produce toxins killing fish, (sea) birds, bivalves and other invertebrates, while *Phaeocystis* is a nuisance species forming extensive foamy blooms that may lead to oxygen depletion and anoxia.

Species of the genera *Pavlova* and *Isochrysis* are widely used in the aquaculture industry to feed oyster and shrimp larvae. They are rich in polyunsaturated fatty acids such DHA, stearidonic acid and alpha-linolenic acid. While there is sufficient data supporting the safe use of *Isochrysis galbana*, this is currently lacking for *Pavlova*.

4.1.12 Ochrophyta/Chrysista

The brown algae within this phylum are multicellular, mostly living in salt water, and are abundant in temperate-coastal zones in cold water. Both asexual and sexual reproduction is known.
All Eustigmatophytes are unicellular, with coccoid cells and polysaccharide cell walls. Eustigmatophytes reproduce via autosporogenesis or the formation of zoospores. Sexual reproduction has not been observed yet (Borowitzka, 2018a).

Species of the genera *Fucus*, *Himanthalia*, and *Laminaria* are used as human food. In particular, *Saccharina latissimi*a is at the moment a popular source of alginates used to thicken or gellify food (*e.g.* ice cream). Its common name, sugar kelp, refers to the whitish, sweet-tasting powder (mannose) used as sweetener which forms on the dried algal blades. While *Fucus vesiculosus* has a GRAS status[58] and safety of extracts used as food supplements has been demonstrated, it contains significant amounts of iodine which could cause an allergic reaction in sensitive people. As such this species was considered hazardous.

Ascophyllum nodosum is processed into health food tablets and alginates, used as fertiliser, or applied for the production of packaging material and biofuels. *Alaria esculenta* is consumed in northern and north-western Europe or used for production of biofuels or antioxidants. Both species can be considered as safe. Microalgal species *Nannochloropsis* spp. *Nannochloropsis gaditana* and *N. oceanica* were designated RG1 (COGEM, 2016b) given their use as feed in aquaculture, or as source of EPA and omega-3 fatty acids and their history of safe laboratory use.

As shown in **Table 5**, also in the phylum a fair number of species with hazardous properties could be identified. These mainly relate to the production of toxic compounds and toxicity to fish, shellfish, copepods or sea mammals (*e.g.* representatives of *Aureococcus, Aureoumbra*, *Dinobryon*, *Chattonella*, *Heterosigma, Fibrocapsa*, *Pseudochattonella* and *Uroglena*).

Gonyostomum semen is known to cause skin irritation; irritation is caused by ejections of slime threads from ejectosomes (trichocysts) lining the *Gonyostomum* cells.

Several *Laminaria* spp. are apparently used in gynaecology as osmotic dilator to stimulate cervical dilatation. *Laminaria* sticks dilate the uterine cervix by swelling as they absorb fluid from surrounding tissue. Anaphylaxis after medical insertion of *Laminaria ochroleuca* has been reported.

4.1.13 Rhodophyta

Most of the red algae are macroalgae, although a very small number of microalgal genera exist. Red algae are abundant in marine habitats and are relatively rare in freshwaters. They have double

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[58] FDA GRAS No. 661, Fucoidan Concentrate from *Fucus vesiculosus*

cell walls; the outer layers contain the polysaccharides agarose and agaropectin, the internal walls are mostly cellulose. Most red algae reproduce sexually as well as asexually.

Although the phylum Rhodophyta comprised approximately 1500 species, limited data was found on hazardous properties or safe use (see **Table 5**). Some species are used for food, often since ancient times (*e.g. Pyropia* spp., *Porphyra* spp., *Chondrus crispus*, *Eucheuma* spp.). The genus *Porphyridium* consists of microalgal species that are important sources of proteins, sulphated polysaccharides; the PUFAs arachidonic acid and EPA, and the phycobiliprotein phycoerythrin (Borowitzka, 2018a).

So far, only 8 harmful species were identified. Some *Gracilaria* spp. are poisonous due to the epiphytic presence of toxic cyanobacteria (*e.g. Lyngbya* spp.). For the same genus, poisoning incidents due to consumption of *Gracilaria* spp. producing toxic prostaglandins have been described in Japan (Fusetani and Hashimoto, 1984). Also release of toxic halogenated compounds (*e.g.* brominated phenols) from decaying rhodophyte biomasses that wash up on beaches is known (see *e.g.* Eklund *et al.* (2005)).

5 Conclusions

This study was set up to collect information and relevant references for classifying algal species in view of their application in research and development using genetic modification. In spite of promising prospects for sustainable production of essential products, genetic modification of algae is still relatively limited. Several research projects and industrial initiatives illustrate that the sector is maturing and that -when technical and economical hurdles are tackled- the applications can cover a broad range of products. It is speculated that notwithstanding the biofuel hype, production of high value and/or complex compounds may be more attractive.

Both micro and macroalgae are already being targeted. For algae likely to be cultivated in open systems, GM applications will require a thorough environmental risk assessment. On the other hand, for algae cultivated in containment the risk classification may be more focussed on ensuring, based on pathogenicity of the algal strains, that the containment features are adequate to protect employees and on worst case scenarios for public health and environment in case of an accidental release from containment.

The information obtained in this study indicates that the choice of organism is predominantly determined by its capacity to perform a certain function, rather than by its safety profile. **Table 6** compares the number of species for different applications and different safety profiles. The fact that a species or derived product is used in food and/or feed is not an indication *per se* of the safety of the organism. In fact, “safe” uses, including products with FDA GRAS status, can be based on species with a demonstrated safety profile as well as on species either known to be hazardous or for which no safety information can be readily retrieved.

Table 6 Summary table indicating the number of species that were identified with harmful properties, having some type of safety confirmation and for which no hazard/safety data could be retrieved for different types of applications.

Application	**Derived from algal species with safety profile**		
	Hazard	**No Hazard**	**No data**
Food/ Feed	27	34	49
Food/ Feed additive	12	23	41
Medicinal	13	20	14
Biofuel	23	46	42

In some cases, risks and beneficial aspects go hand in hand: a species may produce a compound that presents a risk (*e.g.* toxins or cytotoxic compounds), but when produced and extracted can be used for industrial or medicinal use. When classifying cyanobacteria, ZKBS[59] indicated that all cyanobacteria, including toxigenic species, are consistently classified in Risk Group 1, as they do not cause any infectious diseases. Nevertheless, when working with toxin-forming cyanobacteria, ZKBS recommends to carry out a risk assessment considering the protection of the employees and to take suitable measures to prevent the employees from being at risk.

① **Algae remain of high interest as possible sources for biomass and for the production of specific compounds. However, the straightforward approach that hazardous features determine risk class, in turn leading to standard containment measures seems to be less applicable. Instead, less stringent containment measures may apply when performing certain activities.**

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[59] https://www.zkbs-online.de/ZKBS/SharedDocs/Downloads/01_Allgemeine%20Stellungnahmen/02_Bakterien/Cyanobakterien_2011.pdf?__blob=publicationFile&v=3

Addressing this enormous diversity, this study researched in detail information on species selected based on relevance (algal genera/species already being applied or expected to be applied in the near future, *e.g.* for the production of biofuels (oil, diesel, hydrogen gas) or high value components); representativeness (aiming to include a broad range of taxa) and documented risk/safety profile.

Based on these criteria, information from literature and input from the consulted experts resulted in a selection of approx. 500 species. For the selected species taxonomic information was linked with indications on pathogenicity, toxicity, algal bloom/anoxia, allergenicity, mechanical effects (*e.g.* lesions, obstruction gills), other (*e.g.* haemolytic secretions), safe use, medicinal use, habitat, and proliferation potential in temperate climate.

These elements of importance to inform the risk assessment relating to its impact on human and animal health and ecological effects were described in a table Algal Risk Classification Features provided as a separate Supplementary Document. The information that was collected and supporting references can support risk assessors in classifying these specific species. It also provides an insight on the way to structure the information and an approach for future additions.

②

The information and references collected in this project for a selection of approx. 500 species can be used in support of the risk classification of specific algal species.

In order to organise the information and possibly clustering based on safety profiles a taxonomic landscape was established. The huge diversity of algae as well as the dynamic evolution of algae taxonomy may hinder a fixed risk classification. The main issues are depicted in **Figure 1**.

Names and taxa may change but in most cases the species entities remain the same. *E.g.* during the project several taxonomic changes at family level were observed within the Cyanobacteria. These changes can be easily tracked using databases such as Algaebase[1] that link any old name to new names or higher classes. Likely more changes will occur between conclusion of the data collection of this project and the publication of the report. Whereas a name change does not influence the safety characteristics of a taxonomic cluster, a taxonomic change potentially results in a modification.

In particular the ongoing increasing discovery and description of semicryptic and cryptic species will complicate a fixed classification considerably, as well-known species might fall apart into species complexes. Semicryptic species are taxa with only very subtle morphological difference or growth preferences for which there is genetic evidence that they belong to species complexes or different evolutionary lineages, while cryptic species are taxa which are morphologically indistinguishable but genetically distant. Cryptic species are likely one of the reasons why within a same species (as defined at the moment) both toxic and non-toxic isolates can be found, but based on genetic grounds and supported by biogeography the species is split up into different toxic and non-toxic species (see for example Bjørnholt Binzer *et al.* (2019) and Dai *et al.* (2017)). Taxonomic efforts could be focused on resolving such issues in economically relevant algal groups as this will yield more detailed information on which species are toxic or not.

Therefore, it must be stressed that the ‘taxonomic landscape’ presented together with this report must be considered as a transient ‘snapshot’, of a taxonomy that is constantly changing.

Taxa listings ideally include a function that allows tracing changes. Database tools exist that allow mapping such changes and this would provide an up-to-date reference. For algae, Algaebase[1] is a relatively complete inventory of all names. For many species “old” names can be entered, after which the correct recent name is given. This system already goes a long way towards dealing with taxonomic change, at least the most straightforward changes. Within the remit of this project, information on a selection of species was compiled and reported in a static manner. While this information can inform risk assessors, there is a risk that with changes in taxonomy, new species for which COGEM is asked advice may not be easily retrievable. Similarly, any attempt to cluster based on taxonomy is in jeopardy as soon as the taxonomic classification is modified.

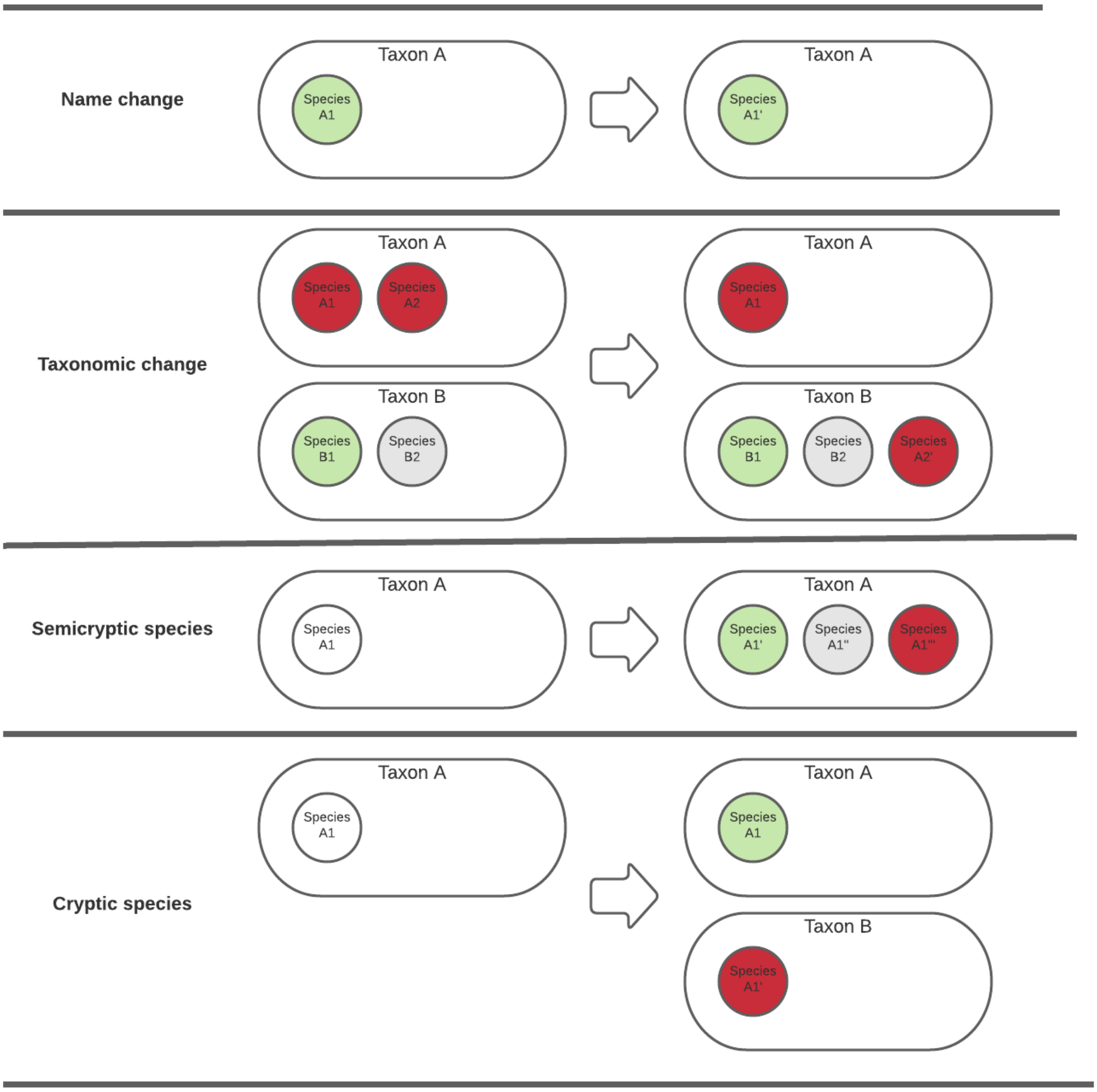

Figure 1 Schematic representation of issues arising from taxonomic modifications. Colours refer to known hazard (red), demonstrated to be safe (green), without safety indications (grey) or with mixed safety indications (white)

③ **Recognising the dynamic nature of algal taxonomy, a flexible approach must be established that allows tracing the diverging and changing classification of algal taxa. At the same time, further insights revealing semicryptic and cryptic species will necessitate assessing lower level taxa.**

Specific algal species are known for beneficial aspects, different uses and/or for risks. Since about 60% of cyanobacterial samples investigated worldwide contain toxins, it is prudent to presume a toxic potential in any cyanobacterial population (World Health Organization, 2003b). However, most species comprise toxic and non-toxic strains (see previous section regarding cryptic species). Incidents with macroalgae, as part of the diet of some populations, causing illness or even death, are in most cases the result of the presence of cyanobacterial epiphytes.

While species are identified for which information is available, hardly any higher taxon can be clustered based on safety profiles: even when some species are safe, there are usually also some species that can be problematic, possibly under specific conditions. Even this may be variable within a single species: *e.g. Alexandrium tamarense* (dinoflagellates) produces saxitoxins, gonyautoxins and neosaxitoxin; however, also non-toxic isolates have been described.

Part of the challenge of determining the relevance of hazard factors is the fact that the expression seems to be largely determined by the environment. It was pointed out that given the right conditions, many algae are capable of forming intense blooms. Similarly, expression of toxins or other deleterious compounds can be highly dependent on external factors and therefore unpredictable in an uncontrolled environment.

The consulted experts confirmed that - perhaps with the exception of Cyanobacteria and dinoflagellates - specific taxonomic groups with harmful or beneficial features cannot be readily delineated. Instead, a case-by-case approach (considering a single species or isolate) will be most appropriate. As demonstrated by the table '**Algal Risk Classification Features**' (provided as a separate Supplementary Document), several genera can be identified for which caution is urged such as *e.g. Pseudo-nitzschia*.

④ **At higher taxonomic levels no groups can be identified that are generally recognised as safe or conversely known to be hazardous. Moreover, the diversity of safety profiles seems to be largely influenced by external factors.**

Nevertheless, taxonomic clustering may provide indications on which (type of) elements or aspects must be verified. Molecular techniques can be used to verify if sequences similar to those of genes known to code for already identified noxious compounds are present in yet undocumented species of the same taxon. Also, in view of so-called (semi)cryptic species, it is highly recommended to request applicants, that submit an authorisation dossier for genetic modifications, to characterise the strains of interest rigorously. It is the responsibility of the applicants to ensure the identity of the strains described in the application dossier as this must reflect effectively the strains they have acquired for genetic modification. This can be done by providing a concise but correct strain description, optionally supported by barcoding or full genome sequence information for strains that lack information or are not well-defined. It must be noted that even when toxin genes are present in the genome, one cannot predict if these genes will be expressed (production of toxin is often induced under stress situations such as shortage of nutrients (in a bloom) or when grown in culture).

In view of the case-by-case approach, it was suggested by the consulted experts to impose a mandatory test panel to applicants in order to assess the hazardous potential of a receptor species. A plethora of methods is available for the detection of well-known algal toxins (mainly cyanotoxins and well-known toxins such as domoic acid, okadaic acid, saxitoxin) in water and food samples. These test methods range from biological assays (*e.g.* mouse bio-assays), biochemical tests (*e.g.* ELISA, ADDA-ELISA and protein phosphatase inhibition assays) over chromatographic methods (*e.g.* RP-HPLC, LC/MS, MALDI-TOF) and molecular methods (*e.g.* PCR, qPCR and microarrays/DNA chips) (Birbeck *et al.*, 2019; Medlin, 2018; Sanseverino *et al.*, 2017; Shoemaker *et al.*, 2015; Yang *et al.*, 2011; Zaffiro *et al.*, 2016). Some of these methods are expensive, time-consuming, or require sophisticated instruments and/or highly trained personnel. In contrast, commercially available rapid test kits, based on immunological detection, are easy to use and render fast preliminary results, but need confirmation by ELISA, HPLC or conventional analytical methods and do not allow detection of toxin congeners (Jellett *et al.*, 2002; Martinez *et al.*, 2005). Also, rapid multiplex PCR tests are nowadays used to detect, for example, contamination of 'spirulina' food supplements by microcystins coming from other harmful cyanobacteria that grow along with 'safe'

Arthrospira spp, with a detection limit for microcystin B encoding genes up to 250 fg/µL (Manali *et al.*, 2017).
Nevertheless, such test panel will be challenging since it should be specific for each of the major algal groups and continuously adapted to new and evolving knowledge.

In Section 3 a list of elements was presented that can be used to evaluate health and environmental impact for algae. This information is likely not available in an early R&D phase, and therefore an initial classification based on information from taxon-related species may be the only cautious way forward until more information is collected.

⑤ **Applicants need to ascertain an unequivocal determination of the identity of the algal species. This can be integrated in a clear, ideally internationally harmonised set of criteria to document the hazard / safety profile of not yet documented algae.**

The COGEM has experience in handling progressing insights in risk classification of a broad diversity of organisms. *E.g.* the classification of Bacteria is regularly expanded and revised. The algae may very well be handled in a similar way, acknowledging the higher complexity of the taxonomic dynamic. Furthermore, since likely only a fraction of the vast algal species diversity will be used, a more selective approach may be necessary. Following Conclusion 1, it can be contemplated to implement an expert system that combines taxonomic changes with automated searches on risk/safety information. Such a system will proof to be relevant not only for algae.

⑥ **An automated expert system would be more appropriate to consolidate this effort, expand the scope and ensure future relevance.**

6 Supplementary documents

The following supplementary documents are provided as Excel spreadsheets

- Algal Taxonomic Landscape
  A classification mainly based on AlgaeBase[1] and used as the backbone for this project. It includes 7191 genera, distributed over 800 families, 267 orders, 60 classes and 13 phyla/divisions.
  A summary indication of identified features is included for taxa for which species have been further documented in detail. If at least one species was identified with harmful, safe or medicinal properties the relevant cell was coloured respectively red, green or blue. The number in a cell indicates how many species were classified as such.

- Algal Risk Classification Features
  Listing taxonomic information on selected species linked with indications on pathogenicity, toxicity, algal bloom/anoxia, allergenicity, mechanical effects (*e.g.* lesions, obstruction gills), other (*e.g.* haemolytic secretions), safe use, medicinal use, habitat, and proliferation potential in temperate climate.

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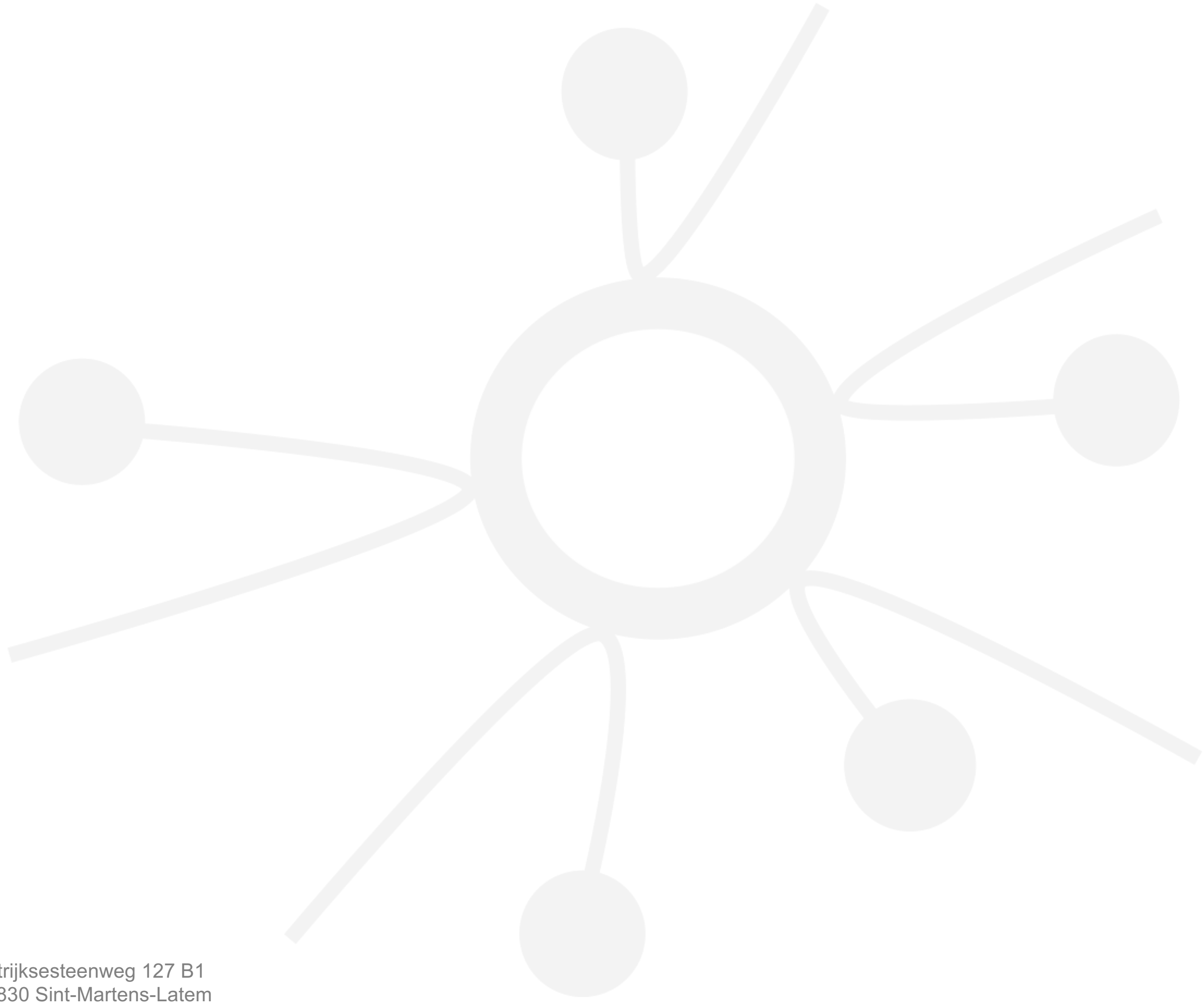

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