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Review

Reviewing the Potential of Algae Species as a Green Alternative to Produce Nanoparticles: Findings from a Database Analysis

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Abstract: Nanotechnology has seen increased research and implementation in recent decades in numerous applications. Based on the information in the papers, we built a database that included algae species used, biomass pre-processing, main precursors, solvents, production approaches, final size, and possible uses. An analysis of this data revealed a great diversity of algae species investigated for this purpose (68 species of algae and 45 families). The analysis of the strategy plot shows that there are four clusters of themes that are different from each other. Still, some patterns are recognizable, e.g., the general cluster with general methodologies and concepts is in the intersection zone. The second cluster is related to the use of macroalgae, which has had a strong development in the past, but now seems to be less attractive, and the third cluster is on the use of nanoparticles to control bacteria, which seems to be a topic that, although not currently driving the field, could become a major driver if current trends continue. New technological developments should be expected in the near future as NPs synthesis from green renewable sources such as algae poses an alternative to the traditional means in the coming years.

Keywords: nanoparticles; NPs; biosynthesis; microalgae; green synthesis; algae; macroalgae; bibliometric network

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Citation: Lamilla-Tamayo, L.; Escobar-Calderón, F.; Skalický, M. Reviewing the Potential of Algae Species as a Green Alternative to Produce Nanoparticles: Findings from a Database Analysis. *Water* **2023**, *15*, 2208. https://doi.org/10.3390/w15122208

Academic Editor: Guangyi Wang

Received: 3 April 2023
Revised: 4 June 2023
Accepted: 8 June 2023
Published: 12 June 2023

1. Introduction

Nanotechnology, according to the U.S. Environmental Protection Agency (EPA) [1], is defined as "research and technology development … [and] the creation and use of structures, devices, and systems that have novel properties and functions because of their small size … ". In recent decades, the use of numerous nanoscale materials has seen an increase in both research and implementation in wide-ranging applications [2]. This includes nanotechnology-produced nanoparticles (NPs), a varied class of materials with lengths in two or three dimensions between 1 nm and 100 nm that may exhibit size-related intensive properties (ASTM 2456-06 cited by Sajid and Płotka-Wasylka, 2020 [3]). In general, NPs are characterized by a high surface area to volume ratio, which is a gradual progression as the particle gets smaller [4], and for being highly mobile in the free state, which provokes slow sedimentation rates [5]. NPs can be classified according to their size, shape, and material properties [6], and the synthesis strategy influences their attributes, the experimental conditions [3], the catalyst and reaction media used as well as the production conditions [7]. Furthermore, an attractive factor about NPs relates to the fact that many characteristics and properties, such as color, bioactivity, solubility, and uses (e.g., antimicrobial effects), are affected by their size and differ from the ones displayed by larger particles of the same composition [8].

The uses and applications of NPs are very diverse and range from wastewater treatment [9], microorganisms inhibition [10], pathogen treatment [11], several branches of medicine [12,13], and dentistry [14] as well as aquacultural [15,16] and agricultural production [17]. NPs have also been added to algal cultures aiming, for instance, to promote greater biomass (a topic extensively reviewed by Vargas-Estrada et al., 2020) [18] as stimulants for the production of exciting by-products such as lipids [19,20] or polyphenols [21]. Additionally, some studies have also found adverse effects of NPs in algae cultures, including decreased carbohydrates and protein production [22] and diminished growth and photosynthetic capacity [23].

Broadly speaking, nanomaterials can be manufactured through two main methods: either by a size reduction from a larger mass to the nanoscale through the use of specialized mechanical methods, called the "top-down" approach A, or through a process involving the formation of larger nanostructures from atoms and small molecules with the help of a catalyst, a process known as "bottom-up" synthesis [24]. The "bottom-up" production of NPs can be conducted by two means: green synthesis or chemical synthesis; the first kind uses renewable raw and natural resources, mild reaction conditions, and avoids the use of potentially toxic solvents and reagents, emphasizing environmental sustainability. The chemical synthesis might use toxic solvents and chemical reductants [25]. Given the increased popularity of NPs and the interest of many industries in their application in diverse problems, many researchers have turned to the algae-based green synthesis of NPs to develop highly efficient production protocols at low cost and minimal environmental impact. NPs can be produced using whole living algal cultures or by pre-processing the biomass; centrifuging, filtering, or drying the algae; and then adding the NPs precursor in, most commonly, an aqueous Milli-Q water or ethanol solution. However, a wide range of variations of this general approach exists in the literature that uses different algae species from different taxonomic groups. On top of this, the algae are used to synthesize different kinds of NPs (Au, Ag, Cu, and others.) using various precursors with different intended uses. The considerable variation of approaches present in the field makes it hard to extract general trends of research or insights that can help guide further research.

In the following study, we intend to produce a report describing the evidence available on the green synthesis of NPs using algae in order to find trends, knowledge gaps, and research clusters. The analysis focuses on exploring the variation in the type of NPs produced, the characteristics of the NPs, and the intended use as well as the features of the algae used during the synthesis. The review aims to be as visual as possible, summarizing our findings in tables and figures. We have also complemented the analysis with a bibliometric network analysis using the popular research visualization tool VOSviewer alongside a quantitative description of the obtained networks.

2. Materials and Methods

2.1. Literature Search

The systematic review was produced from the primary literature published in peer-reviewed journals indexed in the Scopus bibliographic database. It focuses on the polyphyletic group known as "algae" and encompasses the existing literature discussing details related to the algae-mediated production of several types of NPs. Digital media was examined, and the interlibrary loan and document delivery program of the Czech University of Life Sciences, Prague was used to acquire hard or digital full-text copies of any document not available online.

2.2. Search Terms and Languages

The review team generated a list of potentially pertinent search terms that could broadly include the targeted articles. The general terms denominating each of the categories of algae, outcome, and type of synthesis were combined using the Boolean operator "OR" and "AND". The asterisk (*) was used as a wildcard to represent any characters (e.g., microalga * includes microalgae, microalgal, microalga) as suggested in the search guidelines. The entire query with the complete list of search terms used can be found in Supplementary File S1.

No language restrictions were placed on the search strings. However, during the screening and selection of the retrieved articles, language constraints were conducted following the recommendations of Pieper and Puljak, (2021) [26]. In this case, the omission of documents was conducted at the study selection stage, and the excluded articles are listed in the pre-processed database, allowing transparency regarding the number of eligible publications in addition to a written record for future authors to investigate these studies when checking information on the topic.

2.3. Article Screening and Study Eligibility Criteria

2.3.1. Screening Process and Eligibility Criteria

Articles found using the search strategy were screened at two distinct stages: (1) title and abstract and (2) full text after pre-selection. The following eligibility criteria were used to screen articles at title/abstract and later full-text screening:

2.3.2. Eligible Organisms

The organisms' inclusion results from a compromise between the conventional (artificial) system and the phylogenetic system; all the organisms incorporated are primary producers, using sunlight energy to convert inorganic substances into simple organic compounds. The term algae, in this context, includes an assemblage with no formal taxonomic cohesiveness encompassing oxygen-evolving photosynthetic organisms, incorporating prokaryote and eukaryote species.

2.3.3. Eligible Type of Synthesis

Eligibility consisted of any synthesis in which NPs were produced using whole living cell cultures, dry algae biomass, dry commercial (or in situ produced) algae powder, cellular lixiviates, lyophilized algae biomass, filtrates, supernatants or precipitates of algal origin, fresh fine pieces (macroalgae), ground pieces, or other types of algal biomass during the reaction that produced the NPs.

2.3.4. Date Range

No date restrictions were placed in the search strategy. All articles published until September 2022 are included in the analyses.

2.3.5. Reasons for Exclusion

A list of articles excluded after the title and abstract screening and the reason(s) for exclusion is provided as an additional txt file (Supplementary File S1). In short, the publications were omitted if (1) the authors biosynthesized NPs using other types of organisms (e.g., non -photosynthetic prokaryotes, yeasts, flowering plants). (2) The NPs were produced by other means (e.g., chemical) and then used to expose algae cultures aiming to obtain other bioproducts or to enhance or affect in any sense the algae culture in toxicity or bio-absorption tests.

2.3.6. Study Validity Assessment

No formal study validity assessment was performed on the articles included in this systematic review.

2.4. Post-Selection Information Retrieval and Summary of the Basic Features of the Dataset

Once we applied the inclusion criteria to select the papers from the Scopus search, we performed a structured information retrieval by checking each of the articles, including abstract, methods, and results; the following information was extracted from the selected papers:

1. Bibliographic information—title, authors, year, cited sources.

2. Organisms—algae—(micro, macro, eukaryote, prokaryote) scientific name, and any other relevant phylogenetic information, algae pre-process (dying, centrifuging, filtering, lyophilizing, whole living cultures, grounding, and cutting).
3. Final nanoparticle—nanoparticle characteristics—the type of NPs produced using the algae, chemical compound(s) used to produce the NPs, size of the produced NPs (measured and stated in the study), if the color of the reaction, shape, and surface of the NPs were investigated in the research and clearly mentioned.
4. Use of the synthesized nanoparticle—(in the specific article), categorized following the classes in Table 1.

Table 1. Uses of the synthesized NPs identified in the research papers.

Uses	Criteria
Antibacterial	Used to destroy bacteria or suppresses their growth or their ability to reproduce
Anticancerous	Used against or tending to arrest or prevent cancer
Antifungal	(also called antimycotic): Kill or stop the growth of fungi that cause infections
Antioxidant	Lessen or prevent the effects of free radicals
Cytotoxic	Potency to bring variations in cellular functions and results in cell death
Optimization	Improvement of the conditions to produce NPs using algae
Plant improvement	(and protection): Increased agricultural productivity is the primary concern
Other	Different industrial or technical applications from the stated before

Note: For missing or unclear information, the corresponding authors for each article were contacted by email when time and resources allowed.

2.5. Study Presentation, Visualization, and Descriptive Statistics

After extracting the information, we tabulated the data in a series of data frames, and this information was used to perform a descriptive analysis focusing specifically on items 2, 3, and 4 of the previous section: this includes (i) the most used algae species if they are micro, macro, eukaryote, or prokaryote, (ii) how was the biomass processed, (iii) the type of NPs produced, (iv) precursor, (v) size of the produced NPs, and (vi) main use. The remaining information that is mentioned in the post-selection information retrieval section and is not presented in the main Results and Discussion part (e.g., shape, color change, the detailed list of species, bibliographic information—title, authors, year, cited sources) is tabulated and can be found as a txt data frame in Supplementary File S4, Supplementary File S2, and Supplementary File S5, respectively. In addition to the written synthesis and .txt data frame, we used additional descriptive statistics to explore the overall number of papers per relevant topic (e.g., the number of articles and studies).

2.6. Semantic and Citation Network Building

On top of the descriptive analysis, we also wanted to explore the different relationships between the selected articles' research topics and the structure of the citation network. We tried to find a method to visually present under-represented topics that could warrant further research and well-represented approaches or issues that could potentially be knowledge clusters. For the relationships between research topics, we used the text-mining functionality of the software VOSviewer version 1.6.18 [27] to construct a co-occurrence network of terms by extracting the abstracts in the selected scientific literature; we will refer to this network as a semantic network in the following chapters. A minimum occurrence threshold of four was used to prevent unnecessary noise and show only the most relevant terms (an appropriate word is considered if it appears at least four times). Before generating the network, we produced a thesaurus to control for the acronyms and synonyms in the data. Moreover, this thesaurus helped to eliminate the terms of little significance in the documents.

Using the same software, we analyzed bibliographic data based on citation links. A citation link is a link between two items where one cites the other. Citation links are treated as undirected by VOSviewer. For this reason, no distinction is made between a citation from one thing to the other and a citation in the opposite direction [28]. The unit of analysis was the document; in this case, we also used a minimum occurrence threshold of four. We refer to this second network as the citation network.

One of the main features of VOSviewer is that it can produce a map of the research field that is amenable to qualitative interpretation; we took advantage of this aspect and performed a qualitative description of the obtained networks.

2.7. Bibliometric and Network Analysis (SNA)

Apart from the qualitative description of the network visualization, we also performed a quantitative description based on network metrics and statistics. First, we performed a node importance analysis based on centrality measures for both networks; these types of standards are designed to describe the level of importance of the nodes in the network under different criteria. For our study, we focused on two centralities measures: Strength (defined as the total number of edges connecting to a specific node multiplied by the weight of those edges) and betweenness (defined as the number of times a specific node lies in the shortest path between two other nodes). These two measures express the importance of the nodes in a network in different ways; strength is a way to find very popular or commonly used concepts (in the case of the semantic network) or papers (for the citation network). At the same time, betweenness is better to describe those nodes that serve as bridges between different nodes in the network.

In the second step, we performed a cohesion and clustering analysis to detect essential groups of concepts and words used in the semantic network. The clustering was performed using the method developed by Waltman et al. (2010) [29] and used progressively larger values of the resolution parameter γ, with ten random start points and 100 iterations. The resulting clusters were compared using the modularity statistic that varies from -0.5 for poorly supported clusters that can be explained by randomness to 1 for clusters that are meaningful [30]. The best-scored clusterization was then selected, and a strategic diagram was constructed out of them by plotting each cluster's scaled and centered measures of density and centrality. The resulting graph provides four different quadrants that can help to explain the role of a cluster (also called a theme) in the research field [31].

Additional to the automatic cluster generation, we also classified the words in the semantic network into six different groups: Topic, Use, Taxon-Use, Taxon, NP, and Method. The full criteria for assigning the words to categories can be found in Table 2. In the case of the citation network, the nodes were assigned attributes based on the characteristics of the papers; the characteristics used were "year of publication," "country of affiliation of the first author," and "region of origin of the first author."

To study the effects of these node attributes in the structure of both networks, we used the exponential random graph models for both networks using the package ERGM [32–34]. For the semantic network, we used the class of the word as a node level as well as a dyad-level predictor [35]. Node-level predictors model the probability of a node forming a connection with any other node based on one of its attributes, while dyad-level predictors model the probability of homophyly in the network; this is the probability that two or more nodes with the same characteristics are more likely to bound together.

A similar approach was used for the citation network to study the influence of the year of publication, the county of origin, and the region of origin on the characteristics of the network and the importance of the papers in it. An ERGM model was fitted in which we tested the effect of year, country, and region as a node-level predictor. Additionally, the country and region-induced homophily in the network was tested by including these attributes as dyad-level predictors.

All the analyses were performed in R (R Core Team, 2023) [36], using the packages igraph [37], ergm [32–34], and integraph [38].

Table 2. Criteria used to classify the words obtained from the text-mining process to construct the semantic network with a minimum occurrence threshold of four.

Categories	Criteria
Topic	A general word that refers to a comprehensive concept or group, e.g., temperature, biosynthesis, algae
Use	Specific uses of the NPs in the paper or methods related to the study of the benefits, e.g., antibacterial, inhibition zone
Taxon-Use	Name of a taxon (at any taxonomic level) that was used for testing the uses of the NPs, e.g., *Escherichia coli*, *Pseudomonas* sp.
Taxon	Name of a taxon of algae (at any taxonomic level) that was used for the synthesis of the NPs
NP	Characteristics related to the structure or class of NP, e.g., gold NPs, particle size, shape, color
Method	Analytical approaches used for the characterization of the NPs

3. Results and Discussion

3.1. Relevant Species and the Importance of Species Characterization

We retrieved a total of 118 articles from Scopus using the search strategy; after the title and abstract screening, 74 documents passed the eligibility criteria for a full-text assessment. The majority of published articles (73%) are studies performed on a single species of algae, followed by experiments conducted on two species (12.16%). Interestingly, one study focused on the production of NPs (specifically silver and AgNPs) using seven different species of algae (Chlorophyta, Charophyta, and Cyanobacteria) [39]. In detail, 68 other species of algae belonging to 45 families are reported in the documents corpus. Of those 68 different species, 18 correspond to macroalgae (26.47%), 42 are eukaryotic microalgae (61.76%), and the remaining amount is prokaryotic microalgae (eight, 11.76%), showing a large diversity in the number of taxa explored in this approach, Figure 1. Displays the frequency of use of algae species from different groups. The most used species is the green algae *Chlorella vulgaris*, reported in eight studies, followed by *Chlamydomonas reinhardtii* (also Chlorophyta), the macroalgae *Sargassum muticum* (Ochrophyta), the microalgae *Euglena gracilis* (Euglenozoa), and the Cyanobacteria *Spirulina* platensis—all of them used a total of five times. Additionally, *Dunaliella salina* (Chlorophyta) was used in four of the studies that passed the eligibility criteria.

With the exception of three publications, most of the algae in the studies were identified to the species or at least genus level (83.76% and 16.68%, respectively). Regarding the publications without specific information about the algae used, one was centered on the bioproduction of nickel oxide NPs (NiO NPs), which presents the particular taxon as "Red macroalgae" [40]. A second one tested the antibacterial properties of AgNPs biosynthesized by means of a "natural consortium of microalgae," in which no direct description of the number of taxa used or itemization about the consortium's taxonomic information is discussed in addition to the mention of different proportions of a diverse arrangement of phytoplankton phyla such as Bacillariophyta, Chlorophyta, Cyanophyta, Euglenophyta, Dinophyta, and Cryptophyta [41]. A third study remarks the use of "three unidentified strains" to produce zinc oxide NPs (ZnO NPs); nonetheless, two of them are identified to the genus levels *Phormidium* sp. and *Cosmarium* sp. in addition to one strain categorized up to the subkingdom Viridiplantae [42].

Identifying the species might not apply in all cases. For example, the previously mentioned study by Marella et al. (2022) [41] aimed to use an in situ consortium of algae as a way to remediate and recycle NPs' pollutants of agricultural origin in addition to simultaneously recovering them in a sort of circular economy approach. It might be less relevant to study this aspect since one of the key points was to benefit from a natural community within the area.

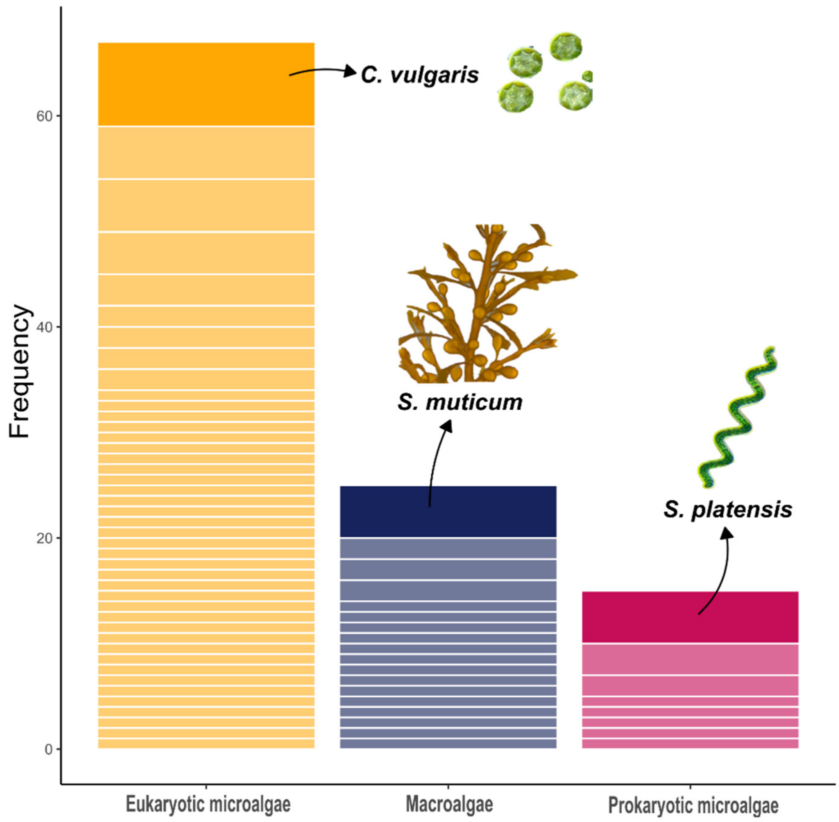


Figure 1. Frequency of use of algae species from different groups in the studies; the most used species in each group is highlighted and depicted (algae illustrations credit: www.protisten.de, Wikimedia commons).

3.2. Green Synthesis and Particle Size

The mean particle size by type of nanoparticle can be seen in Table 3; as previously mentioned, the AgNPs were the most commonly produced NPs in the literature reviewed, with a mean particle size of 36.84 nm. Other types of NPs, such as AuNPs and Cu, were much less common, with an average size of 21.13 and 32.50 nm, respectively, Figure 2. Shows the distribution of NPs for the two most common algae-produced NPs AgNPs and AuNPs differentiating between NPs synthesized using live algae cells and dead cells/extracts. In general, 91.78% of studies reported the size of the produced NPs.

Our survey found four papers in which the characteristics, precisely the size of the produced NPs, changed depending on the species despite utilizing the same conditions to pre-process the algae and to synthesize and extract the NPs.

In the first of these studies, the researchers obtained AgNPs from the extracts of *Ulva lactuca* and *Halopteris scoparia*; in this case, the sizes of the NPs changed with the reaction pH and the species used to produce the macroalgal extract. For instance, when using *U. lactuca* extract, the size of the nanoparticles fluctuated between 48.71 and 82.54 nm, while the NPs synthesized using *H. scoparia* extract tended to be smaller, ranging from 39.15 to 69.19 nm [43]. Another example of this was observed in a study that tested the production of AgNPs and their possible effectiveness against the bloom-forming cyanobacterium *Microcystis aeruginosa* using five different species of algae, i.e., the macroalgae *Sargassum cinereum*, *Turbinaria decurrens*, *Jania rubens*, *Caulerpa racemosa* and the cyanobacterium *Nostoc careneum*. In this case, the mean sizes corresponded to 45, 25, 133, 84, and

52 nm, respectively [44]. Likewise, Kashyap et al. (2019) [45] observed differences in the mean size depending on the species; the extracts were produced using *Chlorella* sp., *Lyngbya* sp., and *Scenedesmus vacuolatus*, and the mean size of the AgNPs was 90.6, 241.8, and 136.2 nm in that order. In this experiment, the authors also report an unsuccessful attempt to produce NPs using the Chlorophyta *Oocystis* sp. Lastly, El-Kassas and Ghobrial (2017) [46] also conclude that the species used influences the mean size of biosynthesized AgNPs *Tetraselmis tetrathele*, 17.7 nm, and *Nanochloropsis oculata*, 19 nm, although admittedly less conspicuous than the previous examples.

Table 3. Minimum and maximum sizes reported in the revised literature. Mean sizes (*) are presented when the articles clearly state that information only. Supplementary File S4 details the range of sizes obtained on each publication.

	NP Type	Minimum Size Obtained (nm)		Maximum Size Obtained (nm)
Using death cellsor extracts	Ag	1.8		100
	Au–Ag	5		45
	Al_2O_3			
	Au	2		67
	Cu	35		45
	$CuFe_2O_4$@Ag			
	CuO/Perlite	13		24
	FeO	4.8		9.07
	NiO *		30.2	
	Zn (nanoflowers) *		4000	
	ZnO	30		57
Using living cells	Ag	3		60
	Au	5		80
	Cd, Se *		7.0	
	Cu	15		65
	FeO *		45.0	
	ferrihydrite	0.6		1
	Se	36		190

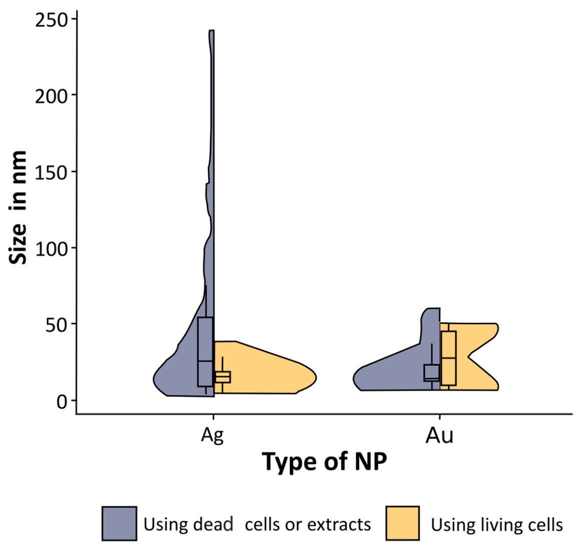


Figure 2. Distribution of NPs for the two most common NPs synthesized and comparison of NPs size between NPs produced using live algae cells and dead cells/extracts.

This is supported by research conducted on chemically produced NPs. In these studies, particle size has shown to be relevant for several applications, including biomedical purposes, since it plays a fundamental role in NP-cellular interactions altering the therapeutic efficiency [47]. For instance, in an experiment aiming to quantify the cellular uptake of silica NPs (SNPs) using human epithelial cervical cancer (HeLa) incubated while exposed to single or mixed-sized SNPs at different concentrations (average sizes shown in TEM images of the three treatments were 51, 104, and 152 nm), revealing a competition in the cellular uptake when small and bigger-sized SNPs were used simultaneously. Specifically, the bigger SNPs promoted the uptake of the smaller ones, while the smaller SNPs slightly inhibited the uptake of the bigger ones. NPs' cellular uptake is controlled by the size and surface chemistry of the nanomaterial, and depending on those properties, a material can have different uptake mechanisms and, therefore, a different intracellular response as well as a different metabolic outcome in the biological system [6].

For a deeper analysis of the effectivity of green NPs against different tumor types, De Matteis et al. (2020) [48] present a review with detailed information on the topic focused on the use of plant extracts, remarking on the advantage of the non-toxic effects of green NPs on healthy cells.

Other research has shown that the successful end-use of NPs depends on finding the right combination of characteristics; for example, the antimicrobial activity of AgNPs is affected by the size and shape of the NPs. To illustrate this, Osonga et al. (2020) [49] tested the antifungal and antibacterial activities of seven different combinations of sizes (9, 16, 21, 30, 35, and 37 nm) and shapes (spherical and quasi-spherical) of AgNPs on an ample assortment of plant and human pathogens that included the fungi Aspergillus nidulans, *Trichaptum biforme, Penicillium italicum, Fusarium oxysporum*, and *Colletotrichum gloeosporioides* and the Gram (−) bacteria *Pseudomonas aeruginosa, Aeromonas hydrophila, Escherichia coli*, and *Citrobacter freundii* in addition to the Gram (+) bacteria *Listeria monocytogenes* and *Staphylococcus epidermidis*. This demonstrated the selective size and shape-dependent capabilities in which smaller-sized spherical (9 nm) and quasi-spherical (21 nm) AgNPs exhibited 100% inhibition of the tested fungi and bacteria.

Continuing with that line, some other examples of the size-dependent properties of NPs can apply to agriculture; iron (III) oxide (Fe_2O_3) NPs between the 20–40 nm range, applied to wheat plants had a significant positive effect in terms of the fresh and dry weight of roots and leaves compared to non-treated seedlings and those treated with NPs between 8 and 10 nm and 30 and 50 nm. Additionally, the length of the roots was significantly longer in plants treated with NPs whose size was between 8 and 10 nm than in other individuals, evidencing not only a possible application of iron (III) oxide in agriculture but an influence of the particle size on the plants' biomass [50]. During the past century, most crop production improvements were achieved through adjustments in agricultural practices that focused on irrigation and the use of fertilizer as well as the development of a diverse set of tools to control pests and diseases [51]. However, some estimates show that in the following decades, the global population will grow to a point where the current yield and production rates cannot keep up with the demand [52]. The importance of finding alternatives to conventional agricultural approaches relies upon the necessity of reducing the amount of spread chemicals, minimizing nutrient losses in fertilization, and increasing yield through pest and nutrient management—specific issues that nanoparticles could help to tackle.

It is relevant to remark that the instances mentioned before were tested using commercial, chemically produced NPs. However, we selected these examples to highlight the importance of the final characteristics over the NPs' properties. During our whole-text screening, the main possible uses the researchers gave to the algae-produced NPs included but were not limited to antimicrobial, cytotoxic, and crop improvement results that will be presented in depth in the Leading Types of Nanoparticles and Possible Uses section. Cementing a reliable source of these types of nanomaterials using algae via green synthesis will need optimized and replicable procedures when specific characteristics are central for the final use of the NPs, in which case accurately identifying the organism(s) utilized to biosynthesize them could be a substantial part of the process.

In addition to changes in size resulting from the selection of algae species, we account for at least six articles in which the differences were linked to other factors. We divided these into two groups: (i) factors related to the algae culture conditions and (ii) changes related to the synthesis conditions:

- (i) Factors related to the algae culture conditions:

Given the diversity of ecosystems where microalgae can thrive, one could not pinpoint a single set of ideal conditions for these organisms to grow; the optimal parameters and tolerated ranges are species-specific. However, the factors controlling algal growth can be summarized as light, nutrients, pH, aeration, temperature, and salinity [53]. In the body of literature that concerns this bibliographic review, we found at least three instances in which the culture conditions somehow affected the characteristics of the produced nanoparticles and, therefore, might affect their final use. These cases are as follows: (i) the combination of light and salinity treatments during algae cultivation (in addition to if they were synthesized intracellularly or extracellularly) [54], (ii) the use of different nitrogen sources during the algae cultivation period [55], and (iii) changes due to the culture media pH [43,56].

(i) The study by Salas-Herrera et al. (2019) [54] detected the formation of CuO NPs with *Chlorella kessleri* in treatments with cells grown in low light and salinity (L − S-) as well as high light but low salinity (L + S-), showing that the formation of CuO NPs changed depending on those conditions. The authors focused on the production of NPs and did not test any possible use. For a comprehensive summary of the use of green-produced CuNPs in diverse fields, we suggest Chand Mali et al. (2023) [57]. Though, in the case of Salas-Herrera and collaborators (2019), they observed that cellularly produced NPs ranged between 15 and 25 nm (L − S-) and 55 and 65 nm (L + S-), and extracellularly produced (only the medium in which the algae were cultured) ranged between 35 and 45 nm (L − S-). This suggests that illumination, combined with culture media salinity, logically affects the microalgae but, more interestingly, influences the biosynthesis of NPs in terms of their size and conformation and the possibility of obtaining them. Prokaryotic microalgae are known to accumulate sucrose or α-glucosyl- glycerol as a response to stress conditions. In contrast, eukaryotic microalgae approach this challenge with several different strategies, such as glycerol production, sucrose production, or amino acid accumulation [58]. Most species grow best at a salinity slightly lower than their native habitat, and most marine microalgae are very tolerant to changes in salt concentration [59]; this factor should be taken into account when culturing algae to produce NPs.

In this subsection of algae culture conditions that can affect the particle size, we also include using (ii) different nitrogen sources. Darwesh et al. (2019) [55] observed that filtrates of *Scenedesmus obliquus* cultured with potassium and sodium nitrate-produced AgNPs of smaller size (5–10 and 4–10 nm, respectively) than those produced by the filtrates of algae cultured with ammonium sulfate and ammonium nitrate (25–50 and 30–50 nm, in that order). Most importantly, in this case, the NPs' final use was tested and shown to be affected by the algae culture conditions. The smaller-sized particles were the most effective against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Saccharomyces cerevisiae*, which is attributable to the relationship between the antimicrobial activity of the produced AgNPs as a function of the size, in this case, influenced by the nitrogen source. Usually, nitrogen concentration affects microalgal growth and their biochemical composition, playing an essential role in protein, lipid, and carbohydrate synthesis. Algae can assimilate different forms of nitrogen (especially nitrate, ammonia, or urea); however, nitrate is widely used for cultures as it is more stable and less likely to produce a pH shift, a topic discussed in great detail by Yaakob et al. (2021) [60].

The impact of (iii) medium pH on particle size has also been investigated. The pH range for most cultured algal species is between 7 and 9, with the optimum range being 8.2–8.7 [53]. Some consequences of maintaining a culture under pH stress conditions are the disruption of many cellular processes and the direct physiological effects that can result in a complete culture collapse. Variations in pH can affect algal growth in several ways: changing the distribution of carbon dioxide species and carbon disponibility and altering the availability of trace metals and essential nutrients [61]. Alipour et al. (2021) [56] observed fluctuations due to differences in the pH in the sizes of selenium nanoparticles (SeNPs) biosynthesized by the Cyanobacterium *Spirulina platensis*. In order to evaluate the effect of medium pH on the production of SeNPs, the cyanobacterial samples were incubated at room temperature under continuous lighting and treated with the NPs' precursor, and the pH of the media was adjusted at 5, 6, 7, and 8. The resulting SeNPs ranged between 136 and 190 nm. However, the smallest particles were obtained at pH7 136 $\pm$ 6 nm, while the largest particle size was achieved at pH 5 (185 $\pm$ 4 nm). In a comprehensive approach, the authors also tested the influence of lighting conditions (exposure time) and precursor concentration (sodium selenite), aiming to optimize the production not only focusing on the size but on the potential antioxidant activity of the NPs. Their results denote a significative lower polydispersity index in the NPs produced in cultures grown at pH 7 and an illumination cycle of 24 h dark/24 h light. In this case, the antioxidant activity was enhanced in homogeneous NPs, demonstrating that the light exposure time and pH of a medium are crucial factors to have in mind depending on the final use of the NPs.

- (ii) Changes related to the synthesis conditions:

In this section, we include the articles that found differences in size depending on the NPs precursor's initial concentration [62]. This is in addition to articles in which the changes are due to NPs being produced via intracellular or extracellular synthesis [63,64].

Concretely, Rahman et al. (2019) [62] synthesized silver nanoparticles using $AgNO_3$ as a precursor and observed that the resulting NPs size varied depending on the precursor concentration and algae pre-process, an aspect to which we will dedicate a part of the discussion in the following paragraphs: the average size of AgNPs produced by using whole life cultures of the Bacillariophyta *Chlamydomonas reinhardtii* at 1.250 mM $AgNO_3$ concentration ranged from 5.6 $\pm$ 2.3 to 19.2 $\pm$ 5.0 nm, whereas, at 0.625 mM $AgNO_3$, the range was 3.0 $\pm$ 1.3 nm to 11.3 $\pm$ 3.1 nm, respectively.

The intracellular process involves the initial electrostatic attraction of metal ions by carboxyl groups of the cell wall, resulting in the passage of metal ions through the cells and posterior reduction by intracellular proteins and cofactors to produce NPs. In juxtaposition, the extracellular process involves the reduction of the metal ions by the microorganism, in this case, algal enzymes and proteins, cell wall components, or organic molecules present in the culture medium [65]. The distinction in the event of size changes presented due to the mechanism of synthesis could be related to the interactions between bio-compounds such as polysaccharides, proteins, polyphenols and phenolic compounds, and metal atoms. Algal pigments, such as fucoxanthins, rich in hydroxyl groups, could also participate in the reduction. These pigments have reductive properties and could act as capping agents, preventing the aggregation of nanoparticles in a solution, and they can be released into the solution by diffusion, playing a relevant role in their extracellular synthesis and shaping [66].

Specifically, in the formerly mentioned paper by Y. Li et al. (2015) [64], the size of intracellularly synthesized AgNPs ranged from 6–24 nm compared to 15–60 nm produced extracellularly; the authors obtained the cell-free filtrate by the centrifugation of *E. gracilis* and *E. intermedia* cultures, while the intracellular process was cultured at room temperature under optimum light conditions for 12 h and a cell resuspension after centrifugation in the presence of the precursor $AgNO_3$. Contrasting with the observations by Jena et al. (2014) [63], who reported bigger (15–20 nm) AgNPs using resuspended biomass of *Scenedesmus* sp. in a similar fashion as described before, this time incubating the live culture for 72 h, the extracellularly obtained particles were smaller (5–10 nm) when using boiled algal (supernatant) extract. Regarding the resulting particle size of intracellularly or extracellularly produced NPs, the papers cited in the previous subsection, in addition to the currently discussed one, have contrasting results raising the point that more research is needed to determine the factors that truly affect the size during green synthesis.

3.3. Algae Pre-Process Dominant Methodologies and Solvents

Previously, we mentioned that the algal biomass was processed employing different techniques. In the studies we analyzed, only once did we encounter an article using commercially available, already-dried biomass (specifically *Spirulina* sp.). Most researchers report using algae either cultured by them, in a fellow lab, or collected directly from the field. In the commercial *Spirulina* sp. powder study, the authors tested different solvents to obtain the NPs by thermal decomposition (chloroform, aqueous extract, acetone, and ethanol). However, no further report on the resulting characteristics of NPs is discussed [67]. In the corpus of selected studies, the three most used methodologies to process the biomass were dried algae powder. In 22.73% of the publications, micro and macro species were desiccated, either in shade or using an oven, and grounded using tools such as mortar and pestle. The second most used approach was using whole living cultures (WLC), 21.21%, employing the algae in vivo with the NPs' precursor integrated into the culture media from the beginning in a period ranging from hours to a couple of days to produce the desired NPs. Finally, in the third place, we found that centrifugation and precipitate resuspension in the NPs' precursor was the algae pre-process methodology selected in nearly 10% of the studies. In this case, the resuspension was carried out in an aqueous solution, see Supplementary File S7. When the NPs were not produced using WLC, they were often obtained via thermolysis. In this method, heat is required to break chemical bonds in the compounds undergoing decomposition [68].

Some papers also report using physical methods, i.e., microwave irradiation to produce the NPs, for example, Merin et al. (2010) [69]; coincidentally, this technique was used in the earliest published article that passed the full screening process. The article focuses on the inhibitory properties of the produced NPs against four Gram (−) bacteria; in the methodology, the authors present two different approaches to producing AgNPs. In the first method, the algae were cultivated along with the precursor $AgNO_3$ (WLC), and the formation of NPs was confirmed by a change of color in the solution and a peak in the UV-vis spectrometry reading. In many cases, the UV–Vis spectrum of reaction at different wavelengths centering on the absorption peak indicates the formation of specific NPs; however, many report the change in the color of the solution as a sign of NP formation. The authors mention a microwave irradiation type of production, although no direct comparison between the efficiency of the different approaches or detailing of the characteristics of the resulting NPs is discussed. Regarding the change in color in the analyzed studies, 47 reported an alteration in this characteristic and linked it directly as a sign of the formation of the desired NPs. In general, the varied methodologies in algae processing and solution preparation, as well as the generous number of species selected to biosynthesize the NPs, hints at the stage of exploration of the research conducted on the topic.

From a green chemistry perspective, three main steps must be evaluated: the selection of the solvent medium, the selection of an environmentally benign reducing agent, and the selection of nontoxic substances for the stability of the nanoparticles [70]. In our review, the most used solvent was water (75% of the cases). The NP precursor was added either into a cell-free solution (after filtering the pre-processed biomass, for example) or into a solution containing intact cells and cellular lixiviates. Ethanol (7.03%) and Milli-Q water (6.25%) were the second and third most frequently used solvents.

3.4. Leading Types of Nanoparticles and Possible Uses

Figure 3 illustrates the diversity of NPs produced in relation to the most studied species of algae., additionally Figure 4 shows the relationship between three of the main factors during the biosynthesis of NPs, the type of algae used, the main element in the structure of the nanoparticles, and the intended use presented in each one of the articles.

As discussed in greater detail during the Relevant Species and the Importance of Species characterization section, the figure visually presents that the research is heavily dominated by the use of eukaryotic algae (such as *Chlorella* sp.) and macroalgae, with considerably fewer contributions using prokaryotic algae (i.e., Cyanobacteria). Furthermore, it depicts the constant pattern that appears regardless of the type of algae used: most papers focused on the production of silver nanoparticles (half of the papers), followed distantly by gold (19.17%), with the remaining elements having a small contribution and only appearing in a handful of papers.

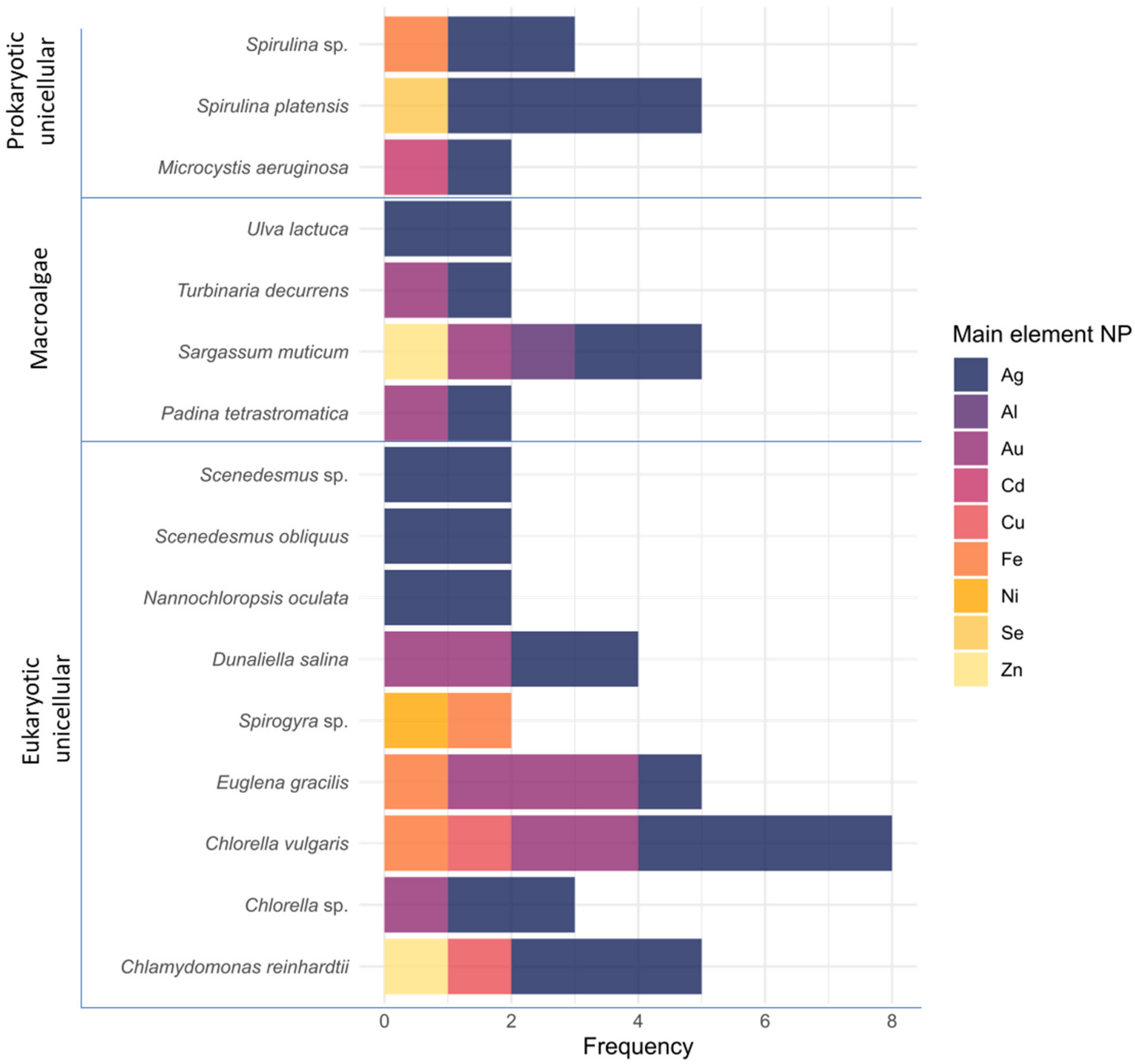


Figure 3. Diversity of NPs produced in relation to the most studied species of algae.

This tendency reflects the leading character of silver NPs in research in general, not only in phycology-related uses but also in nanoparticles in daily life applications. In their review discussing various environmental impacts, toxicities, and entry routes to the body of engineered NPs, Gupta and Xie (2018) [71] recognize AgNPs, AuNPs, zinc oxide nanoparticles (ZnO NPs) (detected in three algae biosynthesis papers in this review), and polymeric nanoparticles (PNPs) (in one algae biosynthesis paper) as some of the most frequently encountered nanomaterials in our daily life in addition to titanium dioxide nanoparticles (TiO_2NPs) and silica nanoparticles (SiO_2NPs), which we did not find any examples of in our sampled group of papers.

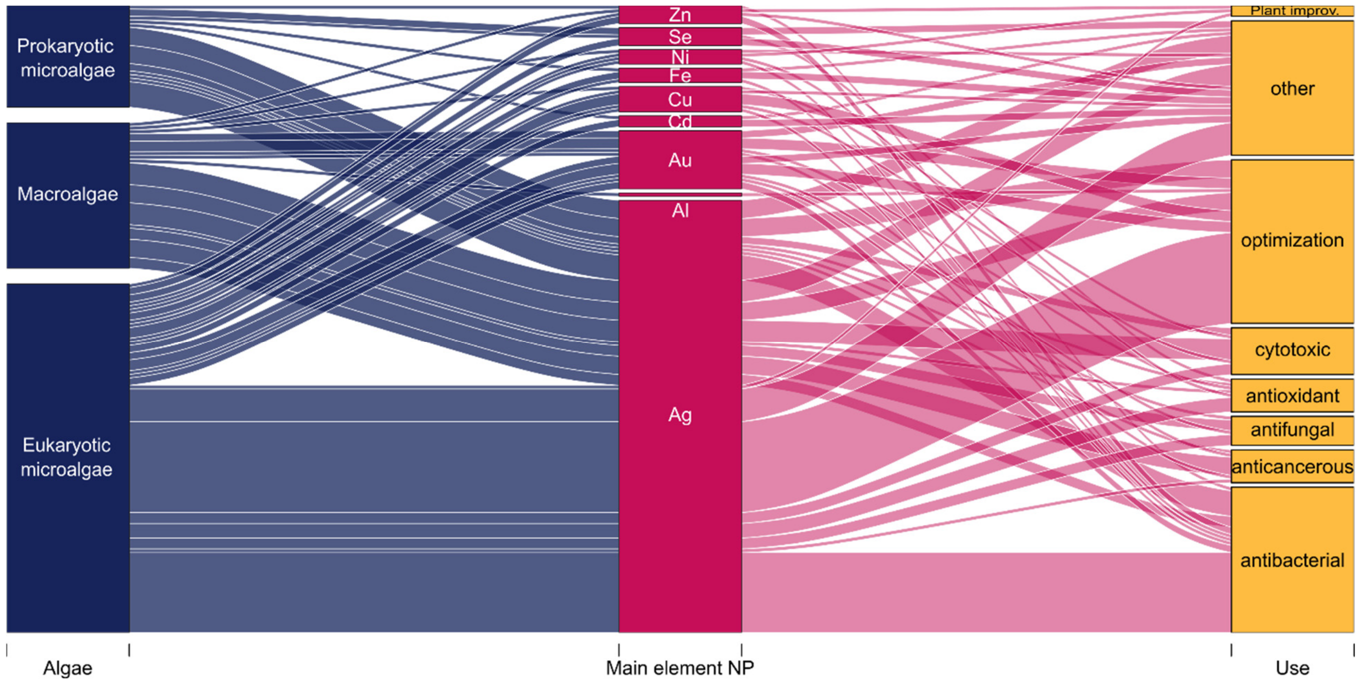


Figure 4. Alluvial plot exploring the relationship between the type of algae used, the main element of the nanoparticles produced, and the tested use.

The prevalence of AgNPs as the most common type of NP can be explained by the relative simplicity of the preparation method, which can be compared to silver nanoparticles of plant origin that, according to Hano and Abbasi (2021) [72], are one of the easiest and least expensive to produce among the different plant-derived NPs. A solution of silver metal ions and a reducing agent, in this case, a biomolecule present in the plants, and in our case, algae such as polysaccharides, vitamins, amino acids, proteins, phenols, saponins, alkaloids or terpenes is required to stabilize the Ag ions. Our study found that all articles producing AgNPs used $AgNO_3$ as the precursor. The dominant use of $AgNO_3$ might be due to its low cost and chemical stability compared to other silver salts [73]. However, other types of precursors exist, such as $AgC_2H_3O_2$, $AgClO_4$, or Ag_2SO_4. For a complete assessment focused on salt precursors and agents utilized in synthesizing silver nanoparticles, characteristics of AgNPs currently in use in the scientific literature, morphology, and an environmental perspective of the evidence is presented in the review article cited before by Tolaymat et al. (2010) [73], which gives an excellent overview. See Table 4 for a complete list of the possible precursors used to biosynthesize NPs using algae divided by the NP type.

In their editorial note, Hano and Abbasi (2021) [72] also discuss algae as ideal candidates for the green synthesis of NPs since they are rich in secondary metabolites that act as reducing and capping agents and mention that, unlike plants, algae usage is still in its onset, and commercial applications are still challenging. It is more relevant to identify the species of algae, methodologies (including algae pre-process, solvents, reducing and capping agents), and NPs characteristics (size, surface) in order to develop more robust and more reliable possible uses.

We identified eight major uses for the nanoparticles in the literature, with the antibacterial and optimization categories being the two most popular uses (25.96% and 24.04%, respectively) followed by the general category "others" (20.19%), antioxidant (8.65%), cytotoxic (7.69%), antifungal (5.77%), anticancerous (4.81%), and plant improvement (2.88%). The optimization group includes articles in which the authors tested the effect of several factors on the production of NPs. These factors are, for example, the effect of algal suspension volumes, pH, optimum reaction temperatures, timing, precursors concentration, differences in the algae pre-process conditions, and effects of cultivation salinity and light. The category "others" groups all the uses that were tested in the papers but that did not fit into categories defined previously, including things such as removal of contaminants from water, algaecide, flocculant agent in algae production, and photocatalysis of toxic dyes. For the complete list of uses and references, see Supplementary File S3.

Table 4. List of primary nanoparticles (NPs) produced mediated by algae and the precursor used in the process.

Type of NPs	Precursor	Number of Experiments
Ag	$AgNO_3$	42
Au	$HAuCl_4$	13
	Gold (III) chloride solution	1
	$HAuCl_5$	1
	$KAuCl_4$	1
Au–Ag	$AgNO_3$ + Gold (III) chloride solution	1
Al_2O_3	$Al_2(SO_4)_3$	1
Cd	$CdCl_2$	1
Se	Na_2SeO_3	2
Cu	$CuCl_2$	1
	$CuSO_4$	1
$CuFe_2O_4$@Ag	$CuCl_2$, $FeCl_3$, $AgNO_3$	1
CuO/Perlite	$CuCl_2$ + Perlite powder	1
FeO	$FeCl_2$ + $FeCl_3$	2
Ferrihydrite	equimolar Fe(II)/Fe(III) ions	1
NiO	$Ni(NO_3)_2$	1
	$NiCl_2$	1
Zn (nanoflowers)	$ZnC_4H_6O_4$	1
ZnO	Zinc acetate	1
	$Zn(NO_3)_2$	1
	$ZnSO_4$	1

It is also interesting to mention that, in contrast to the microalgae-focused research where the main paths concentrate on the use of the produced nanoparticles as antibacterial agents, in the studies carried out using macroalgae, the research is directed much more on the cytotoxic characteristics of the nanoparticles, and also the potential use as anticancerous agents. The main tendencies in this regard are correspondingly depicted in Figure 4.

3.5. Network Analysis

3.5.1. Qualitative Network Analysis

To provide insight into the main research trends, type of NPs produced, methods, and applications, we used a software tool with a text mining functionality that can be employed to analyze large amounts of text data aiming to create a term map based on the abstracts of all the selected documents [28]. We obtained two networks using the tool and parameters described before (A and B). For the text mining network, the resulting visualization corresponds to a distance-based map, in which the space between items reflects the strength of the relationship between them. A shorter distance indicates a stronger relationship, making identifying clusters of correlated elements easier—likewise, the stronger the link between two terms, the thicker the line that connects them. Additionally, the relative importance is indicated by the size of each circle, and the color of each circle is established by the cluster it belongs to. The output includes all the results obtained from all the articles that passed the screening process. The software is precious for displaying large bibliometric maps in an easy-to-interpret way. It has been previously used to represent the cognitive structure and main research lines in nanoscience and nanotechnology at a worldwide level [74] to identify major nanotechnology research fields in Turkey on the basis of the co-occurrence of words in the titles of papers [75]; for the mapping of nanosafety research including publication trends, authors, their geographical distribution, and leading journals on the topic [76]; and to identify and describe the research involving nanotechnology in the agro-food sector in Brazil [77]; among others. The results produced from the text-mining functionality of the software and the final characteristics are presented in the following paragraphs.

The visualization produced from text mining the abstracts of the selected articles depicts four clusters, Figure 5, formed by 46 words and 544 links between them, and lists each of the words belonging to the final clusters, Table 5 contains the clusters obtained in the semantic network. The first cluster is composed of *Chlorella vulgaris*, which is, as was mentioned earlier, the most-used algae. It also contains words related to Ag (such as silver nitrate) and Au nanoparticles (the most frequently produced). Additionally, this cluster has several words related to the most important aspects to characterize once the NPs are synthesized (shape, spherical, crystalline nature, and surface).

Table 5. Clusters with the highest modularity obtained for the semantic network.

Cluster 1	Cluster 2	Cluster 3	Cluster 4
algae	biomass	antibacterial	bioactive compound
antimicrobial activity	chemical method	bacteria	carbohydrates
antioxidant	*Chlamydomonas reinhardtii*	biomedical application	cytotoxicity
Chlorella vulgaris	ed x-ray spectroscopy	biomolecule	DPPH (antioxidant)
crystalline nature	electron microscopy	*Escherichia coli*	HRTEM
gold	green extract	inhibition	macroalgae
mechanism	intracellular biosynthesis	morphology	metal nps
polysaccharide	optimization	Spirulina	MIC
protein	SEM	*Staphylococcus aureus*	
reduction	synthesized nanoparticle	TEM analysis	
shape	temperature		
silver nitrate			
spherical shape			
stabilization			
surface			
toxicity			
XRD pattern			

All the clusters include at least one method used to characterize the NPs produced using algae, such as energy-dispersive X-ray spectroscopy (cluster 2), an analytical technique used for the elemental analysis or chemical characterization of a sample, high-resolution transmission electron microscopy (HRTEM) (cluster 4), and transmission electron microscopy (TEM) (cluster 3). Scanning electron microscope (SEM) (cluster 2) is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. XRD (cluster 1) is used extensively to examine materials and thin films, and its use depends upon having a crystalline material. After the synthesis, a precise particle characterization is necessary; the morphological and topographical features of NPs are of great interest since they influence most of the biological properties. These features include the size, shape, dispersity, localization, agglomeration/aggregation, surface morphology, surface area, and porosity of the NPs, and it is recommended to evaluate these characteristics before assessing toxicity or biocompatibility. Usually, to evaluate the synthesized nanomaterials, many analytical techniques have been used, including ultraviolet-visible spectroscopy (UV-vis spectroscopy), atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), dynamic light scattering (DLS), the already mentioned scanning electron microscopy (SEM), X-ray diffractometry (XRD), and transmission electron microscopy (TEM) [78]. For further information on the topic, Joudeh and Linke (2022) [79] present an excellent summary of the common techniques and methods used for NP characterization detailing which of the physicochemical properties and features can be resolved by each technique or method shown together with examples of experimental research.

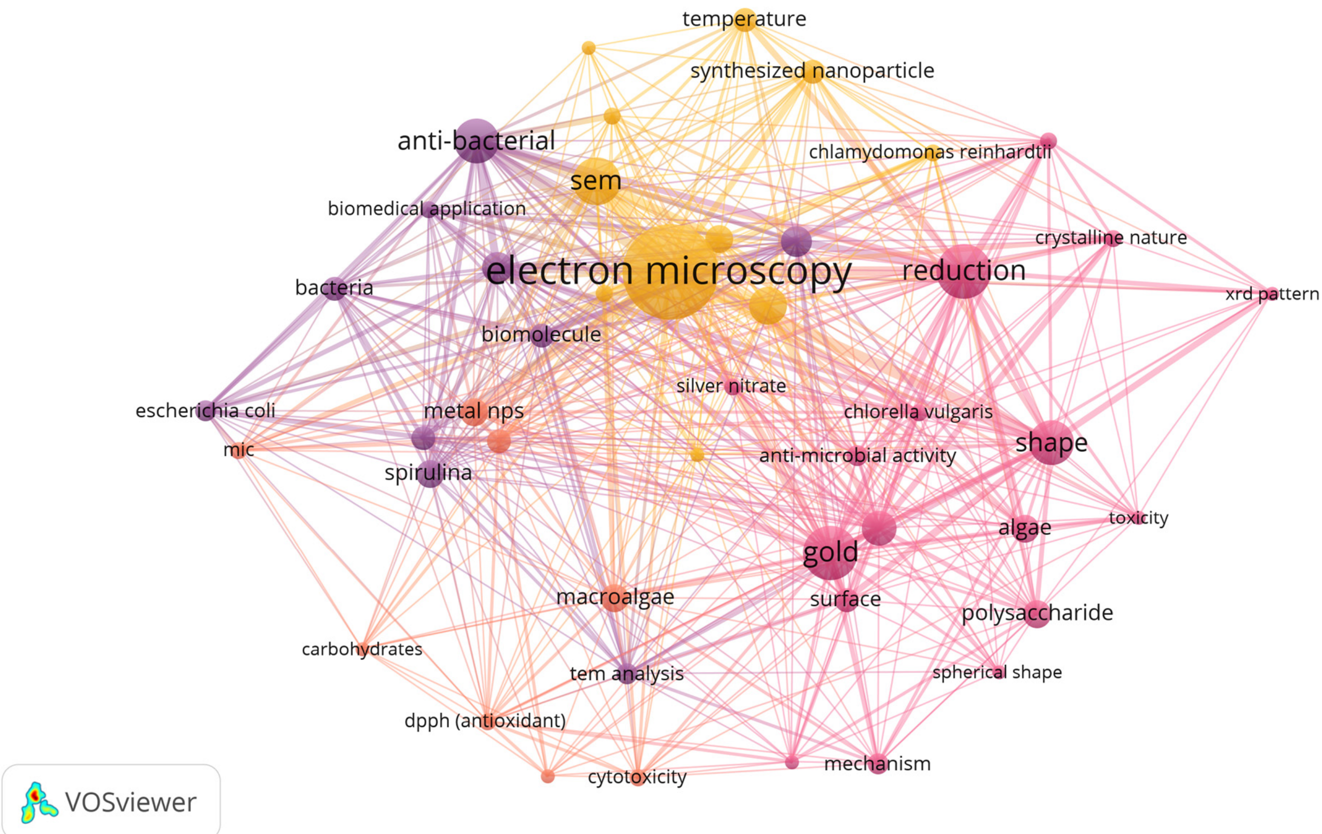
Cluster 3 is attractive because it includes several words related to the main use tested in the compiled articles: green-produced NPs in antibacterial uses, terms such as antibacterial, bacteria, biomedical application, Escherichia coli, inhibition, and Staphylococcus aureus comprise this cluster. This group appears to be the most cohesive of the four.

Words related to testing the effectivity of the produced NPs in possible uses or applications are in cluster 4: terminologies such as DPPH, a free radical method that is an antioxidant assay based on electron transfer that produces a violet solution in ethanol, and minimum inhibitory concentration (MIC), which is an assay that determines the lowest concentration of an antimicrobial agent that prevents the visible growth of a microorganism.

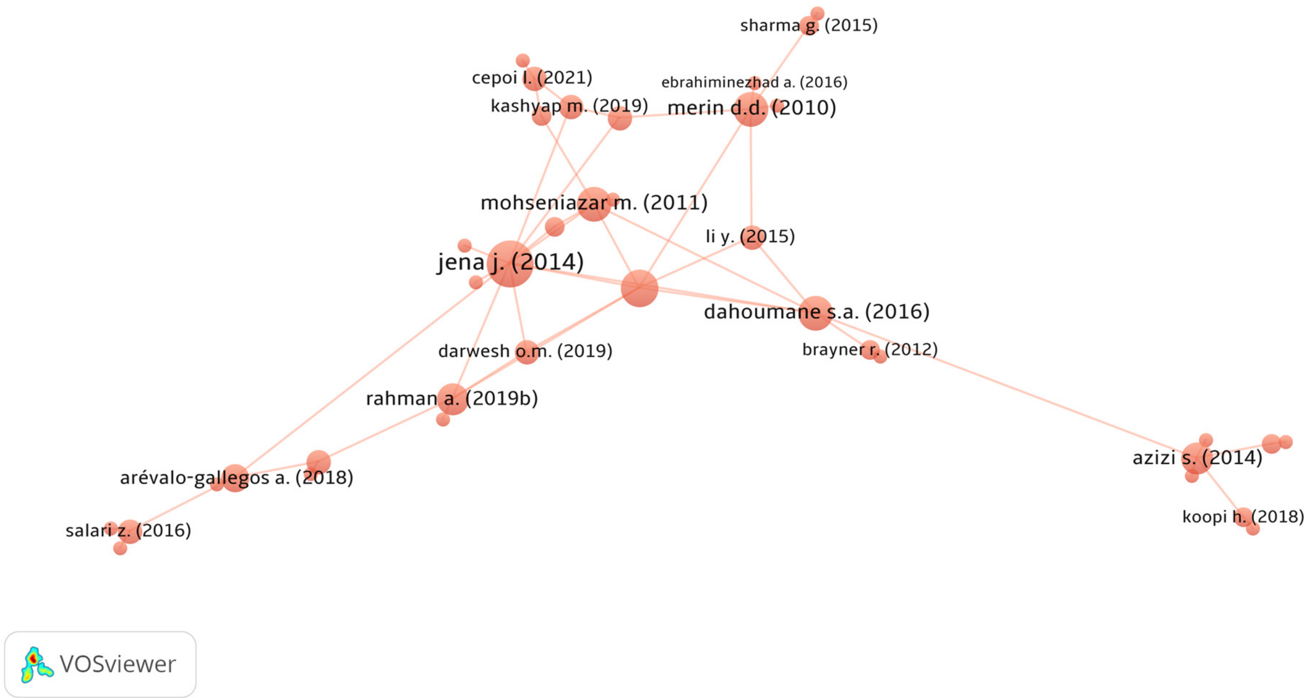
The produced bibliometric network can provide critical insights into the most important topics, main methods, potential uses of the green production of NPs using algae, and changes in research topics with respect to time.

3.5.2. Quantitative Networks Analysis

The visualization obtained from VOSviewer is useful for generating qualitative information; however, the networks generated by the software can also be analyzed quantitatively using metrics and statistics from network theory. For this section, we extracted the networks' structure generated by VOSviewer and analyzed them, focusing on three aspects: centrality measures, clustering analysis, and the importance of attributes. The final networks consisted of 46 and 38 semantic and citation network nodes, respectively. In the case of the semantic network, the 42 words were connected by 544 edges, the graph was well connected (meaning that there existed at least one path between all node pairs), and it had an edge density of 0.52. On the other hand, the citation network had a much smaller density of 0.07 and only 50 edges. Despite this, the graph was also well-connected. The degree distribution of both networks shows a strong skewness that suggests a high frequency of nodes with a low number of connections and less frequent but well-connected nodes or hubs. Table 6 shows the degree of the nodes in both networks for the 15 better-connected nodes. For the semantic network, the best-connected node was one referring to a methodology, electron microscopy, followed by the words "gold," "reduction," "protein," and "SEM." For this case, it is interesting to notice that the difference between the number of nodes is not so steep between the better-connected and the worst-connected nodes. For the semantic network, the word with a lower degree was "intracellular biosynthesis," which had 11 edges, one-fourth of the number of edges of the better-connected node. On the other hand, the less dense citation network showed a stronger variation, with the highest degree found for the paper of Jena J. (2014) with 11 edges and then 17 papers being connected only to a single other paper. Finally, it is important to mention that the fact that the citation network contained only 38 nodes when the whole dataset was composed of 74 papers indicates that a high proportion of the papers are not cited nor cite the other papers included in this study.



(**A**)



(**B**)

Figure 5. (**A**) Semantic network, the size of the nodes represents their degree, while the colors symbolize the four clusters. (**B**) Citation network [25,40,45,55,62–64,67,69,80–104].

Table 6. Degree of the 15 better-connected nodes on the semantic (left column) and citation network (right column).

Semantic Networks Nodes	Degree	Citation Networks Nodes	Degree
electron microscopy	40	Jena J. (2014) [63]	11
gold	39	Rahman A. (2019a) [62]	7
reduction	37	Dahoumane S.A. (2016) [80]	6
protein	36	Mohseniazar M. (2011) [81]	6
SEM	33	Merin D.D. (2010) [69]	6
antibacterial	31	Rahman A. (2019b) [98]	5
morphology	31	Azizi S. (2014) [99]	5
shape	31	Arévalo-Gallegos A. (2018) [100]	4
biomass	30	Kashyap M. (2021) [101]	3
antimicrobial activity	29	Cepoi L. (2021) [102]	3
Staphylococcus aureus	29	Fathy W. (2020) [103]	3
biomolecule	28	Darwesh O.M. (2019) [55]	3
HRTEM	28	Kashyap M. (2019) [45]	3
chemical method	27	Salari Z. (2016) [104]	3
inhibition	27	Li Y. (2015) [64]	3

Continuing with the centrality analysis, Figure 6 shows the strength and in-betweenness of the nodes in the semantic network. We see that the highest in-betweenness is reached by the node "antimicrobial activity." This is caused by the fact that this node provides the shortest paths between 65 pairs of nodes, working as a communication hub between concepts in the network that would not have a connection otherwise. This suggests a central role of antimicrobial activity in the field of research, appearing as an underlying topic that serves a common ground and a high-interest topic regardless of other characteristics of the investigation. The role of antimicrobial activity as a hub concept in the network is not surprising. However, previous research on NPs and applications shows a strong focus on their potential use to target bacteria as an alternative to antibiotics, stimulated by the appearance of drug-resistant bacteria and the increasing rate of hospital infection outbreaks. The prospective uses include the utilization of NPs in antibacterial coatings for implantable devices and medicinal materials to prevent infection and promote wound healing, in antibiotic delivery systems to treat disease, in bacterial detection systems to generate microbial diagnostics, and in antibacterial vaccines to control bacterial infections [105]. This increased interest, coupled with the complications of NPs synthesis through other methods, can explain why the use of algae for green synthesis has awakened so much interest in the field. On the other hand, regarding the strength of the nodes, we see that the node with the highest strength corresponds to a methodological approach, electron microscopy. During nanoparticle production, an electron microscope performs qualitative characterizations of the nanoparticles, including general shape and size. This is the first characterization step for many studies, followed by other procedures such as the FT-IR spectra used to identify the possible biomolecules responsible for reducing the metal ions and capping the formed NPs, for example. The electron microscope image (especially TEM) can show particle size and dispersion. In our case, though, we see that the other methods do not share this importance in the network, suggesting that many studies stop on this first characterization. Many of the papers analyzed focused mainly on synthesizing the NPs using different combinations of precursors and algae, with electron microscopy being used to confirm the presence of the nanoparticles. This could speak of the exploratory phase in which the field appears to be, with many investigations approaching the problems from very different perspectives, using different algae species and different depths of analysis of the produced NPs. The high level of homogeneity further confirms this in the strength and betweenness of the rest of the nodes.

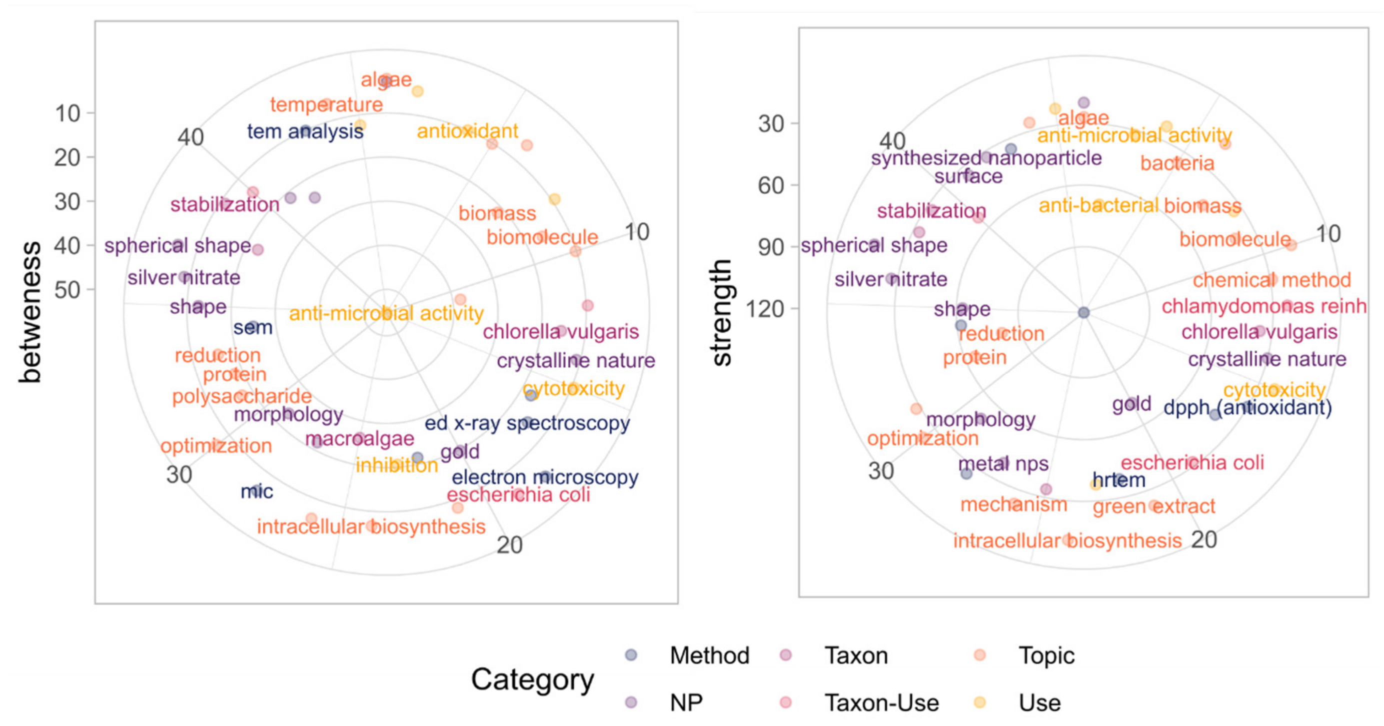


Figure 6. Betweenness and strength of the nodes in the semantic network; nodes closer to the center are more relevant in the network; colors represent the different classes to which the words were assigned.

Regarding the citation network, two papers were found with high in-betweenness: Jena et al. 2014 [64] and Dahoumane et al. 2016 [80], Figure 7. These papers stand as communication hubs of the literature in the network. In the case of the paper by Jena et al. 2014, the study investigates the use of *Scenedesmus* sp. microalga for intracellular and extracellular biosynthesis of silver nanoparticles. The synthesized AgNPs were confirmed to be spherical crystalline particles of sizes ranging from 5–20 nm and showed high antimicrobial activity against gram-negative and gram-positive bacteria. The study provides a simple and cost-effective method for large-scale synthesis using a microalgal system in addition to laying the ground for many methodological approaches of the produced NP since the authors characterized the synthesized NPs by AAS, UV-Vis spectroscopy, TEM, XRD, FTIR, DLS, TGA studies and finally checked for antibacterial activity. On the other hand, the Dahoumane et al. paper (2016) focuses in greater detail on the optimization part of the research. The paper presents a study on the biosynthesis of gold nanoparticles using living cells of *Euglena gracilis*. The researchers examined the impact of growth conditions on the features of the bio-produced AuNPs. The study found that growth under mixotrophic conditions, i.e., exposed to light and grown in an organic carbon-enriched culture medium, resulted in a significant increase in the growth rate of the microorganism, which was seven to eight times higher compared to growth under autotrophic conditions. This improvement resulted in higher yields, enhanced kinetics, and colloidal stability of the biosynthesized nanoparticles. Moreover, the shape and size were also affected, with new shapes such as triangles and hexagons appearing. This contributes to developing scalable and sustainable methods for producing nanomaterials using photobioreactors.

3.6. Clustering Analysis of the Semantic Network

We ran ten different clusterization with different resolution parameters, and the total number of clusters obtained varied from 4 to 12. The modularity showed that the network was better divided into four clusters (modularity = 0.11), coinciding with the results obtained using the VOSviewer software. We maintained the generated clusters equal to the ones resulting from the VOSviewer for this analysis, and they are presented in Figure 5 and Table 5. Generally, the words in the clusters are quite dissimilar and represent a mix of general topics with methods and uses. Only in the case of Cluster 3 can a cohesive pattern be recognized, with 6 of the 10 words in the clusters having a relationship with the use of nanoparticles as antimicrobial agents. The general lack of strong clusters in the networks is an indication of a highly interconnected research field with low sub-specialization. One possible cause for this might be the relative newness of the research and the abundance of papers with an exploratory approach.

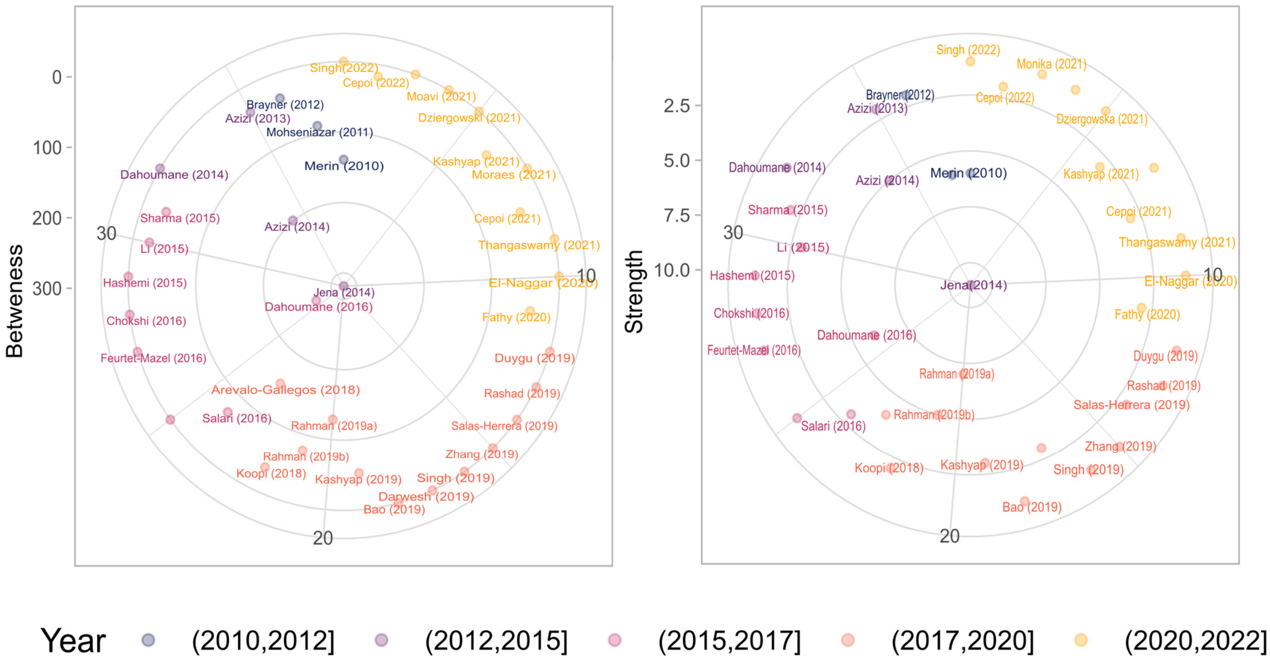


Figure 7. Betweenness and strength of the nodes in the semantic network; nodes closer to the center are more relevant in the network, and colors represent the countries of affiliation of the first [25,40,45,55,62–64,67,69,80–104].

Figure 8 shows the strategic diagram of the clusters. The largest clusters, Cluster 1 and Cluster 2, are located in the fourth quadrant of the plot. This quadrant indicates topics that are central in the research field but not densely interconnected. Typically, this refers to basic and transversal themes that are common across the field. Additionally, the low densities but high centrality could indicate a high level of interest in these topics but with a lower level of development, indicating topics that are transitioning to a mature well-developed state as research continues. Finally, these themes can also signify transition themes, connecting the observed network with topics outside of it [31]. Most of the words in the general category "Topic" are located in these two clusters, alongside most words related to the nanoparticles' characteristics, such as size, shape, and surface.

Cluster 3, composed of words related to using the NPs as antimicrobials, is located on the edge between quadrants 2 and 1. Quadrant 2 refers to themes that are highly developed but isolated, while themes in quadrant 1 are themes that are both significant and well-developed (also called motor themes). The fact that Cluster 3 is located so close to the edge could indicate that the research on antimicrobial uses of algae-generated NPs is transitioning from a well-developed topic in isolation to a topic that is becoming central in the field. This conclusion seems feasible given that in at least 27 of the 74 studies, the authors tested the effect of the biosynthesized nanoparticles as an antibacterial agent and in 6 as an antifungal. The fact that nanoparticles act by bypassing drug resistance mechanisms in bacteria and inhibiting biofilm formation or other important processes related to their virulence potential, for example, penetrating the cell wall and membrane or disrupting important molecular mechanisms [106], this seems promising as bacteria are now more resistant to natural and synthetic antibiotics.

Figure 8. Strategic diagram of the clusters in the semantic network, clusters (themes) in Q1 are considered motor themes. Q2 is considered isolated but developed themes. Q3 is considered emergent and isolated themes, and Q4 groups themes that are basic and transversal.

Lastly, Cluster 4, composed of a set of varied words from all classes, is located in quadrant 2; this quadrant indicates isolated but well-developed themes. The cluster density put is just above the edge between Q2 and Q3, indicating that the theme is not completely developed. We think that this cluster is driven by the presence of the word "macroalgae." Research on this topic is quite isolated, with the majority of research papers focusing on the use of microalgae.

3.7. Importance of Node Attributes

An earlier text-mining analysis of the world's open nanotechnology literature using data from 1994 to 2004 focused on the development and growth of nanoscience and nanotechnology on a global scale, especially authors, institutions, countries, and other bibliometric data, found that the USA, P.R. China, Japan, Germany, and France were the most productive countries in terms of the number of publications [107]. Compared with our results regarding the county of origin of the papers, our network reveals the high importance of papers from Asia, mainly India and Iran. Outside Asia, the highest importance is found for the papers published in France and the United States (Figure 9). The cited review by Kostoff and collaborators was not limited to nanoparticles. It includes an extensive list of authors (50,969), laying a comprehensive background of the seminal works in nanotechnology that could help to elucidate the main tendencies to compare the main trends in terms of the country of affiliation of the first author.

The exponential random graph model for both networks is summarized in Figure 10. For the semantic network, the model found a significant effect on the class of the word at the node level. The nodes that were in the class "Method" had a higher probability of forming edges, followed by the class "NP" (Odds ratio vs. Methods: 0.75, p-value: 0.06), "Taxon" (OR: 0.73, p-value: 0.1), "use" (OR:0.70, p-value: 0.03), and the class "Taxon-Use" (OR: 0.61, p-value: 0.02). The class with the lowest probability of edge formation was the class "Topic." The words in this class were 41% less likely to form edges than words in the class "Methods" (p-value: <0.01). Furthermore, the model did not find a significant effect of the dyadic level predictors, meaning that nodes of the same class were as likely to form as nodes between words of different classes. The left panel of Figure 10B summarizes the probability of pairs of nodes forming edges according to their class. The most probable pairings are the ones that include a word from the class "Methods," while the lowest probabilities are for pairs that contain the words from the class "Topic." The fact that nodes in the class "Methods" are the most likely to form edges make sense under the structure of the network. This class includes some of the best-connected nodes in the network, such as electron microscopy and high-resolution transmission electron microscopy, methods that are standard across the field, regardless of other factors such as intended use of the NPs, or species of algae or bacteria used (in case of antimicrobial effects studies).

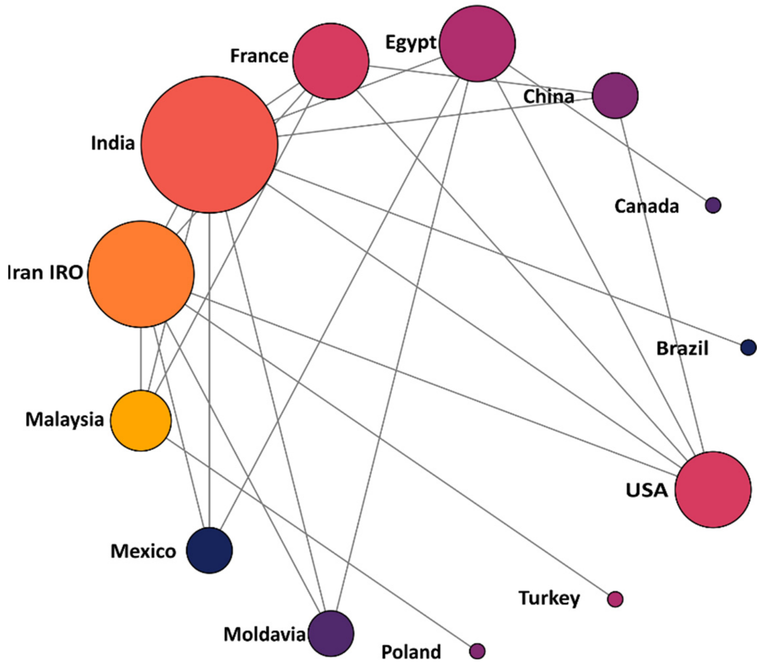


Figure 9. Who cites who: collapsed citation networks between countries in the field in our sample.

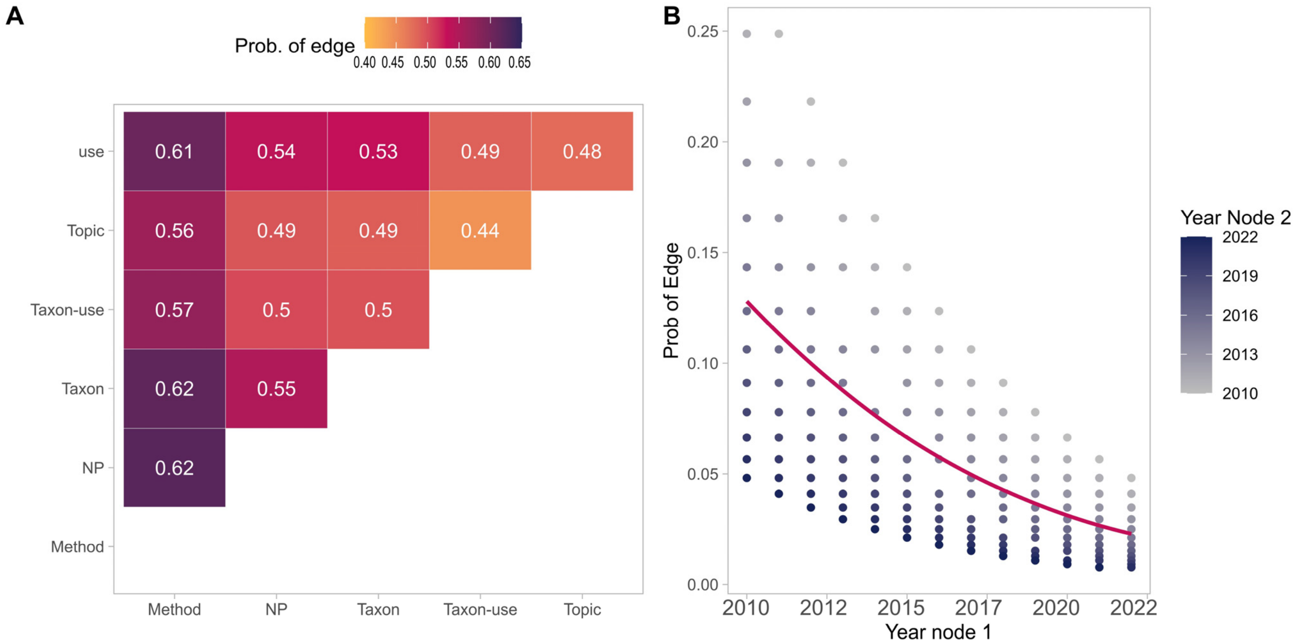


Figure 10. (**A**) Probability of pairings between nodes in the semantic network according to the exponential graph model. (**B**) Year-dependent probabilities of edge formation between two nodes in the citation network. Older papers have a higher probability of being cited.

On the other hand, the low probability of connection values for words in class "Topic" can be explained by the heterogeneity of the class. Apart from being the largest class with 15 members, this class groups together general words that are transversal across the field but that are very dissimilar between them. Moreover, the average strength and betweenness of the words in this class are among the lowest on the graph (average strength = 32.4, average betweenness = 12.5).

In the citation network model, edges represent citations between the papers. The exponential graph model found a significant node-level effect for the year of publication, while the main author's country and region of affiliation had no significant effect on the number of citations. Similarly, no evidence of homophily was detected in any of the dyadic predictors, which means that authors from the same countries and regions do not tend to prefer papers from the same area when citing.

The right panel (B) of Figure 10 shows the effect of the year of publication on the citation probability of a paper; in general, the model found a linear effect of the year of publication, with older papers having more probability of being cited than more recent papers. This behavior is observed in other fields of research: citation patterns for a single paper tend to follow an S-shape. Generally, during its first years of existence, a paper receives a low number of citations, followed by a period in which the number of citations increases exponentially before declining and stabilizing again [108]. The speed at which this happens and the length of each one of these phases are very dependent on the size of the community of researchers potentially interested in the paper and the possible lateral "paths" of diffusion that the paper might have while it disperses through the target audience. The field of biosynthesis of NPs using algae is relatively recent and is still consolidating, which we believe could go together with the worldwide expansion of algae cultivation due to these organisms' potential in various applications, including direct uses such as food and feed, plus interesting bioproducts namely medicines, vitamin supplements, inks, and others) [109]. Parallel to the algal-based bioenergy, the limitations or constraints of algal mass cultivation, and, therefore, of alga—derived NPs, such as large water requirements and nutrients cost, are some of the largest operating expenses that large-scale production might face [110]. The review article by Rai et al. (2018) [111] on nanoparticle-plant interaction and their implications in energy, environment, and agriculture could pose as a simile to the costs and constraints this type of production could encounter using algal biomass.

3.8. Comments on the Methodology Used to Compile the Data

Our results aggregate a written synthesis, several searchable.txt data frames, and descriptive statistics exploring the overall number of papers per pertinent topic (e.g., species, important types of NPs, main solvents, dominant algae pre-process trends), summarized by relevance based on the most frequent observations, and methodologies found in our sample of publications. To our knowledge, this is the first effort encapsulating the topic of algae-produced NPs that tries to present the results quantitatively, complementing previously published works conducted on the topic.

Articles such as the study conducted by El-Sheekh et al. (2022) [112] consciously register, for example, different applications of algal-mediated nanoparticles, many of them being the same as the ones found in this report (antibacterial, antifungal, anticancer, among others) in addition to outlining the strategies of algal mediated nanoparticles biosynthesis (using algal living cells, culture-free cells) and coincide with some of the factors we identify as influencers of the characteristics of the nanoparticles biosynthesized using algae (pH and illumination). However, El-Sheekh and collaborators add the effect of reactant concentration, and temperature that we slightly mention embedded in the "optimization category" and dedicate a lengthier description to the effect of reactant concentration as one of the five factors influencing the desired shape and crystal size [112]. More concise reviews such as Mukherjee et al. (2021) [113] agree TEM and SEM are used by researchers to characterize NPs, terms that the network analysis pinpoints as relevant and transversal across the field.

During the process of planning, compiling, and analyzing the articles, we had in mind to include the greatest amount of information, yet the search focused on ensuring that the whole methodology was as replicable and efficient as possible. For that, we selected the Scopus database and used a query exclusively targeting certain words that were tested before and that produced the least number of irrelevant results (Supplementary File S1); in addition to planning a strategy based on preset inclusion rules and logging the information to be assessed against the same underlying criteria, we would like to reflect on the results and suggest some points to take into account in future opportunities.

The main advantage of retrieving the documents using the information in the title, abstract, and keywords mainly relies on avoiding any subjective selection of research to support a particular line of argument. However, this methodology is not error-free, and the prompt used to retrieve the articles will limit the outcome and is open to inadvertently omitting sections of the literature. Fine-tuning the query should be a priority not only to optimize the pre-selection time for the reviewing team but also to produce a meaningful summary of the trends, knowledge gaps, and information clusters. We encourage future revisits of the methodology adding other terms or different perspectives to the search strategy that we anticipate will strengthen the bibliometric network analysis and the citation network building.

4. Conclusions

We highlight the benefits of using algae in the green synthesis of nanoparticles, given their characteristics, such as their diversity, ability to survive in different environments, and technological advancements in algae cultivation practices. Our findings revealed that eukaryotic microalgae were the most commonly studied algae for nanoparticle synthesis, and dried algae powder and whole living cultures were the most common methodologies used. The semantic network showed that methodologies and general concepts were the most central themes, and methodologies were the most important themes for creating connections in the field. The bibliometric network showed that older publications had more citations. We also used a strategic plot to analyze the clusters of topics in the field and found four distinct clusters. Our study suggests that although certain themes have been more prominent in the past, the field is still maturing, and new technological developments are expected in the near future. Overall, our study hopes to provide a comprehensive overview of the latest trends and developments in the bio-production of nanoparticles from algae from what we consider could pose a promising alternative to traditional means.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15122208/s1, Supplementary File S1: Exclusion criteria for the selection of papers; Supplementary File S2: Taxonomic information of algae species used in the papers studied; Supplementary File S3: Intended uses of the biosynthesized NPs in the papers studied; Supplementary File S4: Studied characteristics of the biosynthesized NPs in the papers studied; Supplementary File S5: Final list of papers selected for study with bibliographic information; Supplementary File S6: Algae sources and preprocessing steps used for biosynthesis of NPs in the papers studied.

Author Contributions: Conceptualization, L.L.-T.; methodology, L.L.-T.; software, L.L.-T. and F.E.-C.; data curation, F.E.-C.; writing—original draft preparation, L.L.-T.; writing—review and editing, F.E.-C. and M.S.; visualization, L.L.-T.; supervision, M.S.; funding acquisition, M.S. All authors have read and agreed to the published version of the manuscript.

Funding: The study was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic (S grant of MSMT CR—SV23-15-21110), and the project Improvement in Quality of the Internal Grant Scheme at CZU, Reg. No. CZ.02.2.69/0.0/0.0/19_073/0016944, financed from the funds of Operational Program Research, Development, and Education, in the framework of ESF Call no. 02_19_073 for Improving the Quality of Internal Grant Schemes at Higher Educational Institutions in priority Axis 2 OP.

Data Availability Statement: All data produced and used in the analyses is provided as txt files as Supplementary Materials for further use.

Conflicts of Interest: The authors declare no conflict of interest.

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