Review

Remediation of Pesticides by Microalgae as Feasible Approach in Agriculture: Bibliometric Strategies

Gayathiri Verasoundarapandian ¹ , Zheng Syuen Lim ¹ , Syahirah Batrisyia Mohamed Radziff ¹, Siti Hajar Taufik ¹ , Nurul Aini Puasa ¹, Noor Azmi Shaharuddin ^{1,2}, Faradina Merican ³, Chiew-Yen Wong ⁴ , Japareng Lalung ⁵ and Siti Aqlima Ahmad ^{1,6,*}

- ¹ Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia; gayathiri1802@gmail.com (G.V.); syuenylim@gmail.com (Z.S.L.); syahirahbatrisyia@gmail.com (S.B.M.R.); hajartaufik21@gmail.com (S.H.T.); nurulainipuasa@gmail.com (N.A.P.); noorazmi@upm.edu.my (N.A.S.)
- ² Institute of Plantation Studies, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia
- ³ School of Biological Sciences, Universiti Sains Malaysia, Minden 11800, Pulau Pinang, Malaysia; faradina@usm.my
- ⁴ School of Health Sciences, International Medical University, Bukit Jalil, Kuala Lumpur 57000, Selangor, Malaysia; WongChiewYen@imu.edu.my
- ⁵ School of Industrial Technology, Universiti Sains Malaysia, Minden 11800, Pulau Pinang, Malaysia; japareng@usm.my
- ⁶ Laboratory of Bioresource Management, Institute of Tropical Forestry and Forest Products (INTROP), Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia
- * Correspondence: aqlima@upm.edu.my

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Abstract: Pesticide treatment dramatically reduces crop loss and enhances agricultural productivity, promoting global food security and economic growth. However, owing to high accrual and persistent tendency, pesticides could create significant ecological consequences when used often. Lately, the perspective has transitioned to implementing biological material, environmentally sustainable, and economical strategies via bioremediation approaches to eradicate pesticides contaminations. Microalgae were regarded as a prominent option for the detoxification of such hazardous contaminants. Sustainable application and remediation strategies of pesticides pollutants in the agriculture system by microalgae from the past studies, and recent advancements were integrated into this review. Bibliometric strategies to enhance the research advancements in pesticide bioremediation by microalgae between 2010 and 2020 were implemented through critical comparative analysis of documents from Scopus and PubMed databases. As a result, this study identified a growing annual research trend from 1994 to 2020 ($n_{\text{Scopus}} > n_{\text{PubMed}}$). Global production of pesticide remediation by microalgae demonstrated significant contributions from India (23.8%) and China (16.7%). The author's keyword clustering was visualized using bibliometric software (VOSviewer), which revealed the strongest network formed by “microalgae”, “bioremediation”, “biodegradation”, “cyanobacteria”, “wastewater”, and “pesticide” as significant to the research topic. Hence, this bibliometric review will facilitate the future roadmap for many scholars and authors who were drawing attention to the burgeoning research on bioremediation of pesticides to counteract environmental impacts while maintaining food sustainability.

Keywords: bioremediation; pesticides; microalgae; agriculture; bibliometric analysis

1. Introduction

The use of pesticides is expanding daily in tandem with increased urbanization and concomitant agricultural intensification. According to the World Health Organization, pesticides are chemical compounds that control pests. Pesticide formulations typically include both active and inert components. The active compound targets and kills the pest, whilst the inert component boosts the active component's efficacy [1]. The Green Revolution

of the 1960s and 1970s, which remarkably increased agricultural yields, backfired with detrimental environmental issues from the poorly managed irrigation and overuse of pesticides [2,3]. Although pesticides have high potential, excessive use of these pesticides created grave concerns about human and environmental health. Pesticides could be released into the air, absorbed into the soil, or ingested by plants and animals. The environmental conditions as well as the physical and chemical characteristics determines the fate of pesticide; soil mobility, solubility in water, and volatility in the air [4]. Direct application of pesticides on agri-crops due to their rapid bioaccumulation in nontarget organisms can lead to soil fertility and water system contamination through soil leaching. Furthermore, water pollution is by far the most critical issue contributed by agricultural run-off. Nitrate was recognized as one of the most common sources of agricultural pollution leading to eutrophication [5]. Since then, the European Drinking Water Directive 98/83/EC established a single pesticide threshold of 0.1 µg/L and total pesticides of 0.5 µg/L in human drinking water [6]. Therefore, agricultural pollutants need to be remediated to prevent adverse effects on humans and animals.

Bioremediation can be a feasible alternative in the instance of pesticide contamination. Microorganisms are adapted in bioremediation to eliminate or convert hazardous chemicals into nontoxic compounds [7–9]. It is suggested to be an economical and reliable alternative. The current review attempts to explore the emerging pesticide contaminations and their usage and environmental implications, as well as applications of microalgae as remediating agents and their mechanisms of action for effective removal and treatment of pesticides or derivatives in agriculture. To date, the contributions of microalgae in their entirety were only examined systematically and visualized in very limited number of studies [9–11]. Even so, an aspiring researcher could not go through a handful of studies to comprehend the advances in a topic or field. There is still a scarcity of thorough assessments concentrating on the fundamental background on bioremediation of pesticides by microalgae research throughout the world. Therefore, bibliometric analysis was performed as a comprehensive tool for accomplishing this goal.

The Scopus and PubMed scholarly databases were used to extract research documents and assess the present our outlook on utilizing microalgae as a feasible approach for remediating pesticides pollution. The data will uncover the core knowledge in this field, as well as statistically visualize (VOSviewer and DataWrapper) and evaluate the most relevant study pathways. Systematic literature reviews centered on bibliometric analyses offer additional insight into annual publication trends, national contributions, and keyword cooccurrence cluster mapping. The significance of the work emphasizes the link between keywords and related terms, forming clusters that draw the key research interest, and thus highlighting topics that are emerging and require additional investigation.

2. Application of Pesticides in Agriculture

Agriculture underpins the livelihoods of over 2.5 billion people, and the demands for agricultural production are constantly growing. By 2030, China would require a total of almost 100 billion kilograms more grain than the current production [12]. The agricultural sector is particularly vulnerable to natural disasters and hazards. In response to that, the economy of low-income countries (LIC) and lower-middle-income countries (LMIC) that rely heavily on agriculture were hit the hardest, especially since 2020 in the aftermath of COVID-19. The International Food Policy Research predicts a 25% reduction in agriculture- and food-related commodity exports due to the underlying food insecurity issue, which heightened since the onset of COVID-19 [13]. While the loss in crop production due to natural disasters is inevitable, the impact from crop and livestock pests, diseases, and infestation can be alleviated with the help of pesticides. Pesticides were used chiefly to improve the quality and quantity of crops and livestock and increasing yield and revenue. World annual pesticide consumption in 2019 recorded a high at 4.19 million tons [14]. China alone contributed 1.41 million tons of consumption, followed by the United States, Brazil, Argentina, and Canada. The advances in pesticide application long replaced the

traditional mechanical control of weeds using mule- or horse-drawn and hand hoeing, thus boosting the overall farming efficiency. Weed and pest management generates both primary and secondary benefits. The primary benefit is the direct gains from use, such as higher crop yield and better crop quality. Secondary benefits are less immediate or less obvious, which is a cumulative result from many primary benefits relative to food security and national and global economic benefits [15].

Pesticides can be classified by the target organisms (the types of pests they control), and the chemical structures or physical states (solid, liquid and gas). Pesticides are most recognized as insecticides, herbicides, rodenticides and fungicides. However, according to US Environmental Protection Agency (EPA), there are 24 types of pesticides on the market based on their target organisms. Amongst the wide range of pesticides, the herbicide is the most commonly used, with a consumption percentage of 40–50%, which signifies its extensive application to kill weeds and unwanted plant species [14]. Herbicides can be categorized into contact and systemic herbicides, determined by their toxic mode of action and their application method. Contact herbicides usually provide an instant effect on the application site upon contact, whereas systemic herbicides transport internally to other parts of the plant through absorption of plant tissues [16]. Based on their modes of action, herbicides can be grouped into growth regulators, amino acid synthesis inhibitors, photosynthetic inhibitors, lipid synthesis inhibitors, seedling root or shoot growth inhibitors, cell membrane disrupters, and pigment inhibitors [17].

An example of a widely used herbicide, namely, 2,4-dichlorophenoxyacetic acid (2,4-D), is the first herbicide developed commercially for broadleaf control. It is sometimes referred to as synthetic auxin, and thus acts as a plant growth regulator herbicide [18]. At low doses, 2,4-D functions as a plant growth promoter, whereas at high doses, it induces uncontrollable growth, leads to stems curling, cupped leaves, and ultimately, plant death. Jyothi [19] reported the positive effect of the application of 2,4-D in stimulating the growth and synthesis of cellular metabolites at low doses (200 µg/mL) in the cyanobacterium, *Synechococcus aeruginosus*. In contrast, a high dose (i.e., 500, 800, or 1000 µg/mL) poses a lethal effect on growth of *S. aeruginosus*. Another main toxic effect caused by 2,4-D is the production of reactive oxygen species (ROS) due to the activation of specific enzymes such as xanthine oxidoreductase and acyl-CoA oxidase [20]. In response to the overproduction of ROS, young leaves will proceed into the epinasty development while the adult tissues will enter the senescence process. Pre- and postharvest application of 2,4-D in the citrus fruit industry are also evident in reducing premature fruit drop, stem-end rot and prolonging the fruit shelf life when in cooperating with other fungicides [21,22].

Aside from the major classes of herbicide aforementioned, organic arsenicals herbicide (e.g., DSMA and MSMA) is poorly understood regarding its mode of action, but it is known to exhibit a similar cell membrane disruption effect on weeds. An antagonistic effect was reported on several broadleaves and grass weeds in the combined application of MSMA with glyphosate and glufosinate herbicides [23]. This is due to the disruption of cell membrane, which impedes the absorption and translocation of systemic herbicides. Thus, the application of organic arsenicals with systemic herbicides in a mixture tank is not recommended. A few more commonly used pesticides with their application and effects on target organisms are listed in Table 1. The use of pesticides in modern agriculture ranges widely from crops production to livestock rearing, marking its indispensable role in the industry. Using an estimation model, the impact of pesticide reduction was computed and quantified in crop yield loss. A 50% reduction in pesticide use would result in the winter wheat yield loss of 5–13% of the yield obtained compared to the current pesticide use in France (0.4–0.9 t ha^{−1}). In comparison, the potential grain yield loss for zero-pesticide systems varied from 2–2.3 t ha^{−1} [24].

To counteract to global warming issue, the application of pesticides in the future climate condition has gained global attention because even the slightest increase in herbicide tolerance will be a future liability. Most of the previous simulation studies report a reduced efficacy of herbicides under high CO₂ concentration [25–27]. At the same time,

a neutral effect of elevated CO₂ concentration on herbicide efficacy against weeds has also been reported in rare cases [25,28–30]. Jabran and Doğan [28] showed that the activity of glyphosate was not impacted despite the positively stimulated weed growth at 450 ppm CO₂ treatment. The result is corroborated by Bajwa et al. [29], who further demonstrated this finding. The overall efficacy of glyphosate was not significantly impacted at the end of the 21 days of their observation period. The recommended dosage of glyphosate application (1034 g ae ha^{−1}) gave 100% biomass reduction under ambient and elevated CO₂ environments for *S. cannabina* weed [30]. On the other hand, in the experiment testing on an extensive range of weeds (vine, herb, shrub and grass species) under elevated atmospheric CO₂ concentration (550 ppm), weed species demonstrated different responses and tolerance levels towards glyphosate application [25]. Fast-growing plants and vines showed the highest resistance, while C3 grasses tended to be the most sensitive towards herbicide application. These experiments proved an equally effective glyphosate activity at CO₂ concentration slightly higher than the ambient atmospheric level (380 ppm). However, further enrichment of CO₂ (above 600 ppm) was reported to diminish the glyphosate efficacy [26,27]. No generalization of the effect of elevated CO₂ levels on herbicide efficacy was concluded, but the susceptibility of weeds towards herbicide application is species-specific. Although the underlying causal mechanism of the decline is unclear, various studies attribute the phenomenon to the increase of weeds growth (biomass) and morphological adaptation (leaf thickness and wax deposition), which lead to slowed translocation of herbicide [31,32]. Nevertheless, glyphosate application is still highly damaging, reducing the growth and flowering of plants across all levels of CO₂ treatments, albeit at a reduced rate.

Table 1. Example of pesticides from three main categories—herbicides, insecticides, and fungicide with their target organism and mode of action.

Active Ingredient	Type of Pesticide	Target Organism	Mode Action	Agricultural Application	Availability Status	References
Herbicides						
2,4-D (2,4-Dichloro-phenoxyacetic acid)	Chlorophenoxy herbicide (systemic herbicide)	Broadleaf weeds and evasive weeds in the aquatic environment	Acts as a growth regulator. Induce uncontrolled cell division in vascular tissue due to abnormal increases in cell wall plasticity, biosynthesis of proteins, and production of ethylene	Field corn, soybean, spring wheat, hazelnuts, sugarcane, cereals	Current	[33,34]
Glyphosate	Organophosphate herbicide and desiccant (systemic herbicide)	Annual and perennial weeds, broadleaf weeds and grasses	Disrupts the shikimic acid pathway via inhibition of 5-enolpyruvylshikimate-3-phosphate synthase.	Fruits, vegetables, grains, legumes, herbs, and spices	Current	[35]
Dicamba (3,6-dichloro-2-methoxy-benzoic acid)	Benzoic acid herbicide (systemic herbicide)	Annual and perennial broadleaf weeds and woody plants (e.g., bedstraw, buttercup, pigweed)	Acts as a growth regulator. Induces abnormal and uncontrollable growth at high dicamba concentrations.	Corn, cereals, dicamba tolerant (DT) soybeans and cotton	Current	[36,37]

Table 1. Cont.

Active Ingredient	Type of Pesticide	Target Organism	Mode Action	Agricultural Application	Availability Status	References
Atrazine	Triazine herbicide (systemic herbicide)	Broadleaf weeds and grasses (e.g., morning glory, crabgrass, barnyard grass)	Competitive inhibition for plastoquinone binding leads to inhibition of photosynthesis.	Corn, sorghum, sugarcane	Current	[38]
Paraquat/ paraquat dichloride	Bipyridinium herbicide and desiccant (contact herbicide)	Broadleaf weeds (e.g., cocksfoot) and grasses (e.g., ryegrass, wild oats, fescue Yorkshire fog grass)	Inhibits photosynthesis and disrupt cell membranes, which allows water to escape leads to rapid desiccation.	Beans and peas, cotton, maize, rubber, tomato, citrus orchards, vineyards	Banned in the European Union nations, China, and Thailand	[39]
Monosodium methanearsonate (MSMA)	Organic arsenical herbicide (contact herbicide)	Broadleaf weed, grasses and sedges	Mode of action is not known. The rapid desiccation of plant suggests cell membrane destruction.	Cotton	Severely restricted. Not permitted for other agricultural crops	[33,40]
Insecticides						
Chlorpyrifos	Organophosphate insecticide, acaricide and miticide	Foliage and soil-borne insect pests (e.g., scale, armyworm, flea beetles, fire ants)	Disrupts the nervous system by inhibiting acetylcholinesterase.	Food crops (e.g., cereals, cotton, fruits, tomatoes, nuts, vegetables) and livestock	Current	[33,40]
Malathion	Organophosphate insecticide and acaricide	Insect pests (e.g., aphids, leafhoppers, Japanese beetles)	Disrupts the nervous system by inhibiting acetylcholinesterase.	Food, feed, and ornamental crops	Current	[41]
Tetrachlorvinphos	Organophosphate insecticide and acaricide	Insect pests (e.g., fleas, ticks, flies, lice, insect larvae)	Disrupts the nervous system by inhibiting acetylcholinesterase.	Poultry (applied dermal), horses, cattle, goats, and swine (oral feed-through)	Not permitted for food crop use in U.S. and banned for all uses in the European Union	[42]
Methomyl	Carbamate insecticide	Foliage and soil-borne insect pests (e.g., lepidoptera, coleoptera, diptera)	Disrupts the nervous system by reversibly inhibiting acetylcholinesterase.	Field vegetables, orchard crops, cotton, sugar beet	Certain uses in U.S. are cancelled (barley, oat and rye) or reduced (wheat, corn and lettuce)	[33,40]

Table 1. Cont.

Active Ingredient	Type of Pesticide	Target Organism	Mode Action	Agricultural Application	Availability Status	References
Carbofuran	Carbamate insecticide, nematocide and miticide	Soil insect and nematode (e.g., corn rootworms, spider mites, nematodes,)	Disrupts the nervous system by reversibly inhibiting acetylcholinesterase.	Potatoes, rice, citrus fruits, vegetables, cotton, alfalfa	Banned in many countries	[40]
Cypermethrin	Synthetic pyrethroid insecticide	Insect pests (e.g., pod midge, yellow cereal fly, blossom beetles)	Disrupts the nervous system, which prolongs the opening of the sodium channel leading to hyperexcitation.	Cereals, peas and beans, oilseed rape, potatoes	Current	[43]
Imidacloprid	neonicotinoid insecticide	Sucking and soil insects (e.g., plant hoppers, aphids, termites, crane flies, crickets)	Interferes neurotransmission by postsynaptic antagonism of nicotinic acetylcholine receptors.	Rice, cereals, maize, potatoes, sugar beet	Current	[44]
			Fungicide			
Mancozeb	Dithiocarbamate fungicide (contact fungicide)	Fungal pathogens (potato blight, leaf spot, scab)	Prohibits chelating properties, which interferes with enzymes containing sulfhydryl group in fungi.	Potatoes, fruit, cotton, corn, ornamental shrubs	Current	[40,45]

3. Environmental Impacts of Pesticides in Agriculture

Extensive utilization of pesticides is needed to maximize crop production. Pesticides are known to be a chemical control for pests and also aid in preventing disease carriers. Generally, there are several mechanisms of pesticides degradation in the environment involving biological, chemical, and physical processes. Ideally, pesticides would be poisonous solely to the target organism, biodegradable, and leave no unpleasant residues on in the environment [46]. However, numerous reports on the negative impacts of the usage of pesticides on the environment are relatively significant [47–51]. The contamination of soil, ground, and surface water by various pesticides brought high toxicity effects to nontarget organisms, including humans. This is due to their low sorption and high period of degradation. Naturally, soil, microorganisms, plants, and animals are the biotic and abiotic elements directly in contact with pesticides.

Soil is the sole medium for agricultural production, which makes it crucially important to maintain. The soil functions that allow the soil to receive, retain, and recycle water, nutrients, and energy are referred to as soil quality [46]. Some pesticides promote microbial growth, while others have either a depressing effect or no effect at all [52]. Pesticides that alter the activities of soil microorganisms are likely to have a negative effect on soil nutritional quality and, as a result, have major ecological repercussions [46]. Fertilizers and pesticides have prolonged durability in the soil. Therefore, they are guaranteed to disrupt soil health by affecting the soil microflora and the livelihood of soil invertebrates [53,54]. In addition, soil fertility is affected not only by chemical and physical properties, but also biological properties of soil [55]. Soil bacteria converted or processed indigenous and added nutrients in soil to be readily available for plants [56]. However, the molecular signals that allow nitrogen-fixing bacteria to function are blocked by the usage of common insecticides, reducing microbes' ability to work properly. In the long run, the soils around

treated plants might become depleted in nitrogen compounds. Hence, more fertilizer is required to obtain the same yield in which can be costly.

One of the subclasses of pesticides is insecticide. There is a type of insecticide known as a systemic insecticide which travels in the plant parts through water upstream [57]. Bees and other pollinators may be killed by systemic pesticides that poison pollen and nectar in the bloom [56]. Insects are important pollinators of flowering plants, with around 75% of all agricultural plant species being entomophilous. Although many flowering plants may self-fertilize, most of them still rely on insect pollination to produce fertile seeds and sustain genetic variety [50]. Furthermore, the deposition of organic compounds or pesticides in soil exposes soil inhabitants directly. It raises the risk of exposure for other higher organisms through ingestion, which can have serious consequences for the soil ecology, waterways, plants, and human life [51]. Pesticides are currently posing a threat to human health and the environment, which has aroused concerns. Data from the last two decades revealed that certain pesticides induce neurological disorders and degenerative diseases, some affect prenatal growth and create congenital malformations, and others are carcinogenic to humans [52,58–60].

4. Mechanism of Action by Microalgae Remediation

Microalgae are best suited for contaminant removal due to their high biosorption capabilities. The carbohydrate structure of the microalgal cell wall helps in the biosorption of toxic contaminants [9,61]. A study conducted by Hussein et al. [62], reported that biosorption occurs in removing pesticides such as atrazine, carbofuran, dimethoate, and simazine by living *Chlorella vulgaris*. Since the ouster removal occurs within a short time, it impossible for any action for active uptake and metabolization process to take place. In addition, physicochemical factors in such molecular size and structures also affect the biosorption process. The larger the algal particles, the greater the surface area available for pesticides to be biosorbed [63]. Hence, microalgae can effectively remove pesticides through biosorption. Microalgae are also able to accumulate toxic compounds. Their bioaccumulation ability depends on algal cell lipid content, which is affected by the growth condition and cell distribution [64,65]. Microorganisms can take up and remove pesticides either through the active or passive process. The active process requires energy-driven externally, whereas the passive process, involves direct interaction of physicochemical at chemical structures of the microorganism cell wall. The efficacy of pesticides removal by microalgae are heavily reliant on the chemical structure of the pesticide and the strain of microalgae employed. Microalgae can exploit pesticides, hydrocarbon, and cyanide as their carbon and nitrogen sources [9]. Bioaccumulation, biodegradation and biosorption are strategies in eliminating organic pollutants by microalgae (Table 2) [66].

4.1. Biosorption

In the biosorption process, the solid and liquid phases containing the dissolved or suspended species to be sorbed are involved [62]. Thus, it is defined as the attachment of potentially toxic pesticides elements to the surface of microalgae. Biosorption of pesticides by microalgae is considered a passive and metabolic-independent process that occurs faster than the bioaccumulation process. The unique cellular walls of microalgae are composed of sulfated polysaccharides that can increase the efficiency of pesticide adsorption from polluted water. In addition, the presence of polysaccharides, proteins, or some lipid-containing functional groups such as amino, hydroxyl, carboxyl, and sulfate, can act as binding sites [63,64]. Properties of microalgae and surface-active groups are the factors that contribute to the adsorption efficiency [65]. Other factors that affect the removal adsorption of pesticide are related to structure of pesticide, pH, temperature, salinity, nutrients, and light quality and strength [11].

Table 2. Remediation of pesticides by microalgae.

Pesticides	Species of Algae	Mode of Action	Concentration of Pesticides Tested	Removal Efficiency (%)	References
α -endosulfan	<i>Chlorococcum</i> sp. <i>Scenedesmus</i> sp.	Biodegradation	1000 $\mu\text{g}/\mu\text{L}$	65–75	[67]
Atrazine	<i>Chlorella vulgaris</i>	Biosorption	10 $\mu\text{g}/\text{L}$	96.29	[6]
Bifenthrin	<i>Chlamydomonas mexicana</i>	Biodegradation	10 $\mu\text{g}/\text{L}$	36	[68]
Chlorpyrifos	<i>Parachlorella kessleri</i>	Biodegradation	5 mg/L	85	[48]
Diazinon	<i>Chlorella sorokiniana</i>	Biosorption	(100, 300, 500) ppm	(99.18, 99.85, 97.86)	[50]
Dimethomorph	<i>Chlorella vulgaris</i>	Biodegradation	20 mg/L	94	[69]
	<i>Scenedesmus obliquus</i>	Biodegradation	600 $\mu\text{g}/\text{L}$	24	
	<i>Scenedesmus quadricauda</i>	Biodegradation		15	[70]
Fluroxypyr	<i>Chlamydomonas reinhardtii</i>	Biodegradation	0.5 mg/L	57	[58]
Imidacloprid	<i>Nannochloropsis</i> sp.	Biodegradation	9.59 $\mu\text{g}/\text{L}$	50	[71]
Isoproturon	<i>Chlamydomonas reinhardtii</i>	Bioaccumulation, biodegradation	50 $\mu\text{g}/\text{L}$	15.1	[65]
Lindane	<i>Nannochloris oculata</i>	Bioaccumulation	0.1 mg/L	73	[72]
Malathion	<i>Chlorella sorokiniana</i>	Biodegradation	25, 100 ppm	(90, 70)	[73]
Mesotrione	<i>Scenedesmus quadricauda</i>	Biodegradation	5 mg/L	15.2	[74]
Metalaxyl	<i>Chlorella vulgaris</i>	Biodegradation	4 ppm	100	[75]
Prometryne	<i>Chlamydomonas reinhardtii</i>	Biodegradation	7.5 $\mu\text{g}/\text{L}$	32	[76]
Trichlorfon	<i>Chlamydomonas reinhardtii</i>	Biodegradation	200 mg/L	96.2	[59]

4.2. Bioaccumulation

Bioaccumulation is a metabolism-dependent process known as active biosorption. It involves two mechanisms: the first is similar to biosorption, and the second step is the active transportation of ions into cells [77]. It is defined as the transfer process of pollutants into the interior of living cells. This process is driven by energy which requires the microalgae to transfer pesticides compounds across the cell membrane for accumulation and metabolization [68]. Studies also show that microalgae can undergo the metabolic mechanism of bioaccumulation [72,78].

4.3. Biodegradation

Biodegradation of pesticides by microalgae into smaller molecules acts as a nutrient source to supplement microalgae growth [79]. The biodegradation of pesticides depends on the metabolic activity of various enzymes such as hydrolase, phosphatase, phosphotriesterase, oxygenase, esterase, transferase, and oxidoreductases [80,81]. There are three methods involved in the degradation of the pesticide involving enzyme metabolisms [82–84]. Firstly, via oxidation, reduction, and hydroxylation reactions of the activation of pesticides in the absence of functional groups by cytochrome P450. This is to gain more degradable, hydrophilic, soluble, and more intoxicant compounds. Secondly, the conjugation with glutathione, glucose, and malonate is formed by transferring the enzymes in the cytosol to pesticides that have functional groups. Thirdly, via glutathione transporters, the conjugates are transported into vacuoles [85]. The importance of biosorption, bioaccumulation, and biodegradation process in the microalgae removal of pesticides was postulated; the mechanism of action by microalgae remediation for diverse pesticides needs further investigation.

5. Microalgae as Biological Remediating Agent for Pesticides: Bibliometric Trend Analysis

5.1. Bibliometric Data and Methodology

Bibliometrics is the systematic review of articles, books, and other academic papers using quantitative tools in scientific data and collection libraries [86,87]. Methodologies such as keyword co-occurrences analysis, publications, and worldwide research trends in a decade were used to detect the development of research themes. Hence, the current biblio-

metric study is required to revise and assess the available research on bioremediation of pesticides by microalgae, which involves synthesizing the core literature work, identifying critical points, and modeling potential research paths.

The first step in performing a bibliometric analysis is to select the ideal datasets that complements the scientific scope of a study area. Although there are several bibliographic databases, such as Scopus, Web of Science (WoS), PubMed, Google Scholar (GS) and Dimensions, most of them do not include relevant data that permit bibliometric analysis to be performed readily [88]. In addition, 84% of WoS titles are likewise indexed in Scopus, however only 54% of Scopus titles are indexed in WoS [89].

An Elsevier based abstract and citation database, Scopus is the only database that integrates a copious, carefully managed abstract and citation repository with augmented data and associated academic papers from a broad spectrum of fields. The database recognizes professionals, locates essential and credible work, and offers substantial algorithms and statistical analysis [90,91]. Meanwhile, PubMed is a journal search engine that allows users access to the MEDLINE database of biomedical and life science publications. Multiple bibliometric reviews have used Scopus or PubMed to assist bibliometric assessments [92–94] (Table 3).

Table 3. Features and accessibilities of both Scopus and PubMed scholarly databases for bibliometric studies.

Element	Scopus [86,95,96]	PubMed [86,94,97]
Coverage	~82 million items	~32 million items
Discipline	Multidisciplinary	Medicine or biological sciences
Bibliographic	1.7 billion	Not available
Provider	Elsevier	National Institute of Science (NIH)
Accessibility	<ul style="list-style-type: none"> • Accessible only to subscribed institutions • Limited-free preview 	Free
Features	<ul style="list-style-type: none"> • Author profiles • Bibliometric trend analysis • h-index calculator • Journal rankings • Easy to navigate 	<ul style="list-style-type: none"> • Access to citations and abstracts • Full text links to free PDFs or publisher sites

Bibliometric search strategies were applied as shown in Figure 1. From 2010 to 2020, the subscription-based Scopus and free search engine PubMed databases were used to search, retrieve, and perform research pattern analysis on the utilization of microalgae as pesticide bioremediation agents. Specific query keywords “((pesticide OR pesticides) AND microalgae AND (bioremediation OR remediation))” related to the main idea were applied through “Title, Abstract, Keywords” in both databases. Between the publishing years of 1994–2020 (PubMed) and 1996–2020 (Scopus), all single categories of documents were included and sorted into the English language. In our primary screening, 46 and 43 papers, were discovered, respectively, from the Scopus and PubMed databases between 1994 and 2020. A limited number of documents was collected through the databases due to the specific of keywords used in the search engine. Following that, a spotlight on publications between 2010 and 2020 was explored to analyze the most recent research evolution on integrating microalgae as a bioremediation technique for pesticides. Scopus and PubMed detected 42 and 35 documents respectively corresponding to each database. The annual publishing output trends were organized through Excel Version 2017 (Microsoft, Redmond, DC, USA). All bibliometric investigations were conducted using Scopus, which provided 9.9% more hits than PubMed. The eligibility of datasets was thoroughly checked prior to content visualization.

Figure 1. Bibliometric protocol for identifying and processing scholarly documents based on research topic.

The Visualization of Similarities Viewer or VOSviewer (version 1.6.15., Leiden University, Leiden, The Netherlands) and Datawrapper were used to visualize the processed Scopus datasets. VOSviewer is a program that allows users to create and visualize systematic mapping networks [98,99]. Collaborative filtering capabilities are included in VOSviewer, which may be used to create and visualize cluster networks of relevant terms retrieved from a collection of published studies [99]. Datawrapper (Datawrapper GmbH) is an online charting tool that collects data from a specific data source and converts it into well-designed charts, maps, and tables that can be interpreted relatively quickly [100]. The outlook for the author's keyword co-occurrences and publication development by global nations was presented and discussed.

5.2. Bibliometric Evolution Analysis

Annually published documents on the bioremediation of pesticides by microalgae and their literature pattern were presented in Figure 2. A total of 42 and 35 documents were identified from the Scopus and PubMed databases respectively. There was a substantial gap in the publishing trend between 1994 and 2020 for both databases, indicating that the publication pattern had an inconsistent evolution. Scopus and PubMed revealed that no research or publications on pesticide bioremediation by microalgae were undertaken in 1997, 1999, 2000, 2003, 2005, or 2009. However, according to both databases, after a stagnant publication period from 1994 to 2009, an exponential trend was observed from 2010 forward. The development of research trends from 2017 to 2020 was noticeably constant for PubMed articles, whereas a significant growth in publication production was identified from Scopus database. Amidst the inconsistencies, the linear modelling resulted in a good r^2 values of 0.9023 (Scopus) and 0.9921 (PubMed). The calculated trend was 1.6

and 1.7 publications per year, respectively to PubMed and Scopus search outputs, which indicates the rise in publication rate. The following bibliometric analyses centered on the latest research shift on bioremediation of pesticides by microalgae between 2010 and 2020 from Scopus database ($n = 42$ documents) (Table 4) [10,101–105].

Figure 2. Annual research publication trends from 1994–2020 for both PubMed and Scopus database.

Table 4. List of documents retrieved from the Scopus database between 2010 and 2020.

No.	Year	Document Type	Title	Author Keywords	Reference
1	2020	Article	Permeable reactive surface-biobarriers. Testing and evaluation of an ecotechnology for the removal of agrototoxic compounds carried by agricultural runoffs	Ecotechnology, nonpoint source pollution; <i>Micractinium</i> sp.; microbial community; sustainable remediation	[106]
2	2020	Article	Microalgae-based bioremediation of water contaminated by pesticides in peri-urban agricultural areas	Agriculture; contaminants of emerging concern; ecotoxicity; low-cost treatment; photobioreactor	[107]
3	2020	Article	Potential for bifenthrin removal using microalgae from a natural source	Bifenthrin; bioremediation; microalgae; pesticide; phycoremediation; residual waters	[69]
4	2020	Article	Reactive Oxygen Species (ROS)-mediated degradation of organophosphate pesticides by the green microalgae <i>Coccomyxa subellipsoidea</i>	Algae; bioremediation; organophosphate pesticide; reactive oxygen species; water quality	[108]
5	2020	Review	Applications of microalgal and cyanobacterial biomass on a way to safe, cleaner, and a sustainable environment	Biofuel; cyanobacteria; microalgae; secondary metabolites; value-added products; wastewater	[109]
6	2020	Article	Bioremediation of water containing pesticides by microalgae: mechanisms, methods, and prospects for future research	Biodiesel; green remediation; microalgae; pesticide; water pollution	[11]

Table 4. Cont.

No.	Year	Document Type	Title	Author Keywords	Reference
7	2020	Article	Kinetic study of nutrients removal from municipal wastewater by <i>Chlorella vulgaris</i> in photobioreactor supplied with CO ₂ -enriched air	<i>Chlorella vulgaris</i> ; kinetic study; municipal wastewater; nutrient bioremediation; photobioreactor	[110]
8	2020	Article	Toxicity, Biodegradation, and Metabolic Fate of Organophosphorus Pesticide Trichlorfon on the Freshwater Algae <i>Chlamydomonas reinhardtii</i>	biodegradation; metabolic fate; microalgae; toxicity; trichlorfon	[111]
9	2020	Review	Macro and Micro Algae in Pollution Control and Biofuel Production—A Review	Algae; biofuel; biosorption; CO ₂ fixation; wastewater	[112]
10	2020	Article	Developments in enzyme and microalgae based biotechniques to remediate micropollutants from aqueous systems—a review	Bioremediation; laccase; microalgae; peroxidase; wastewater treatment	[113]
11	2020	Article	The significance of tropical microalgae <i>Chlorella sorokiniana</i> as a remediate of polluted water caused by chlorpyrifos	Bioremediation; <i>Chlorella sorokiniana</i> ; chlorpyrifos; contact time; pesticide	[114]
12	2019	Review	Biological contamination and its chemical control in microalgal mass cultures	Algal parasites; contamination; control; mass cultures; microalgae	[115]
13	2019	Review	Microalgal bioremediation of emerging contaminants—Opportunities and challenges	Aquatic pollution; bioadsorption; Biodegradation; emerging contaminants; microalgal treatment systems	[9]
14	2019	Article	Sensitivity of selected tropical microalgae isolated from a farmland and a eutrophic lake to atrazine and endosulfan	Atrazine; chlorella; endosulfan; microalgae; oxidative stress; <i>Scenedesmus</i>	[116]
15	2019	Article	Evaluation of amicarbazone toxicity removal through degradation processes based on hydroxyl and sulfate radicals	amicarbazone degradation products; pesticide; sulfate and hydroxyl radicals-mediated degradation; toxicity assays	[117]
16	2019	Article	Chemical mixtures and fluorescence in situ hybridization analysis of natural microbial community in the Tiber river	Anthropogenic pollution and stressors; bioindicators; freshwater; microbial populations; water quality	[118]
17	2019	Article	The light-dependent lethal effects of 1,2-benzisothiazol-3(2H)-one and its biodegradation by freshwater microalgae	1,2-Benzisothiazol-3(2H)-one; biodegradation; environmental risk estimation; light-dependent lethal effects; microalgae	[119]
18	2019	Review	Acute hazard of biocides for the aquatic environmental compartment from a life-cycle perspective	Aquatic compartment; biocide; metabolite; toxicity category	[120]
19	2019	Article	Bioremediation of a pesticide and selected heavy metals in wastewater from various sources using a consortium of microalgae and cyanobacteria	Bioremediation; heavy metals; microalgae; microorganisms; pesticides; wastewater	[105]

Table 4. Cont.

No.	Year	Document Type	Title	Author Keywords	Reference
20	2018	Book Chapter	Phytoremediation for the elimination of metals, pesticides, PAHs, and other pollutants from wastewater and soil	-	[121]
21	2018	Article	Effects of fungal-assisted algal harvesting through biopellet formation on pesticides in water	Aspergillus niger; bioremediation; chlorella vulgaris; emerging pollutants; water quality	[122]
22	2018	Article	Influence of bacteria on the response of microalgae to contaminant mixtures	Metallic and pesticide contaminants; Microbial interactions; sediments	[123]
23	2018	Book Chapter	Phycotechnological approaches toward wastewater management	Microalgae; phycoremediation; pollutant; wastewater	[124]
24	2018	Book Chapter	Bioremediation by microalgae	Biosorption; contamination; environment; industrialization; microalgae	[125]
25	2018	Book Chapter	Bioremediation of pesticides residues: A psychological approach	Bioremediation; cyanobacteria; pesticides residue	[126]
26	2017	Article	Toxicity assessment of pesticide triclosan by aquatic organisms and degradation studies	2,4-Dichlorophenol; biodegradation; cyanobacteria; microalgae; toxicity; triclosan	[127]
27	2017	Article	Phytoremediation of organochlorine and pyrethroid pesticides by aquatic macrophytes and algae in freshwater systems	organochlorine; phytoremediation; pyrethroids	[128]
28	2017	Review	Interaction of chiral herbicides with soil microorganisms, algae, and vascular plants	Biodegradation; chiral herbicide; enantiomer; enantioselective toxicology; herbicide toxicity; plant	[129]
29	2017	Book Chapter	Technological approach of bioremediation using microbial tools: bacteria, fungi, and algae	-	[130]
30	2016	Review	Growth regime and environmental remediation of microalgae	Bioremediation; microalgae; nutrient removal; wastewater	[10]
31	2016	Article	Landfill leachate treatment using bacto-algal coculture: an integrated approach using chemical analyses and toxicological assessment	Coculture; detoxification; landfill leachate; lysimeter; treatment	[131]
32	2016	Article	Batch vs. continuous-feeding operational mode for the removal of pesticides from agricultural run-off by microalgae systems: a laboratory scale study	Agricultural run-off; biodegradation; microalgae; pesticides; removal	[132]
33	2016	Article	Effect of microalgal treatments on pesticides in water	Bioremediation; biosorption; <i>Chlorella vulgaris</i> ; organic pollutants; water treatment	[133]
34	2016	Article	Effect of the pesticide lindane on the biomass of the microalgae <i>Nannochloris oculata</i>	chronic toxicity; microalgae; organochloride pesticide; phycoremediation; removal	[81]
35	2016	Review	Substratum-associated microbiota	Algae; bacteria; biofilm; cyanobacteria; diatoms; microbes; periphyton	[134]

Table 4. Cont.

No.	Year	Document Type	Title	Author Keywords	Reference
36	2015	Article	Capability of microalgae-based wastewater treatment systems to remove emerging organic contaminants: A pilot-scale study	Biodegradation; emerging organic contaminants; high-rate algal pond; microalgae; photodegradation; volatilization	[135]
37	2013	Book Chapter	Toxicity and removal of organic pollutants by microalgae: A review	-	[136]
38	2013	Review	Mixotrophic cyanobacteria and microalgae as distinctive biological agents for organic pollutant degradation	Biodegradation; biological agents; cyanobacteria; microalgae; mixotrophy; organic pollutants	[137]
39	2011	Article	Bioaccumulation and degradation of pesticide fluroxypyr are associated with toxic tolerance in green alga <i>Chlamydomonas reinhardtii</i>	Bioaccumulation; biodegradation; <i>Chlamydomonas reinhardtii</i> ; fluroxypyr; oxidation	[138]
40	2010	Conference Paper	Microalgal remediation of sewage effluent	Heavy metals; microalgae; nitrogen; phosphorous; sewage; wastewater	[139]
41	2010	Article	Cleanup of atrazine-contaminated soils: Ecotoxicological study on the efficacy of a bioremediation tool with <i>Pseudomonas</i> sp. ADP	Atrazine; bioremediation; ecotoxicology; pesticides; soil contamination	[140]
42	2010	Article	Fungicides and herbicide removal in <i>Scenedesmus</i> cell suspensions	Bioremediation; chlorophyll fluorescence; <i>Scenedesmus</i> ; toxicity; uptake	[73]

5.3. Global Publications Contribution

The worldwide map in Figure 3 displayed the general distribution of publications associated with 28 countries between 2010 and 2020. India provided the most scientific contributions on the research topic (23.8%), followed by China (16.7%). The United States, Sweden, and Spain each provided 9.5% of the publications; the United Kingdom, Brazil, France, and Egypt contributed 7.1% of the publications; and Australia, Hong Kong, Malaysia, Mexico, and Portugal contributed 4.8%. Between 2010 and 2020, the remaining 50% of the nations generated at least one article (2.4%).

5.4. Author's Keywords Cluster Analysis and Literature Review

The relatedness of items is defined by the number of documents that appeared together in a co-occurrence analysis. When it comes to analyzing and controlling research interests and development patterns, keywords might drive the limitations of a study [110,119]. Figure 4 was constructed on the co-occurrences of the author's keywords, generating 17 clusters with 348 links and the total link strength (TLS) of 377 assigned in different colors. Nodes of identical colors were designated to a cluster, and the outcomes accurately portrayed the research's concepts and trends in a discipline area. In the network visualization, the minimum number of occurrences of a keyword was set to one, and 108 keywords met the threshold. The nodes reflected the author's keywords that related to bioremediation of pesticides by microalgae. The size of the nodes, which denoted keywords, was relative to the number of occurrences in which the author's keyword exists. The strength of the keywords co-occurrences can be seen in the lines that connected these nodes. The number of links connecting them indicated the intensity of node-to-node interactions. The thickness of the connecting lines denoted by the degrees of keyword co-occurrences linked to the

nodes [116,133]. The similarity (S_{ab}), between two items a and b is estimated using the correlation strength as follows:

$$S_{ab} = \frac{C_{ab}}{W_a W_b}$$

Figure 3. Globally distributed publications on research topic, bioremediation of pesticides by microalgae from 2010–2020.

Figure 4. Network cluster visualization based on all authors' keyword co-occurrences generated based on Scopus dataset produced 17 clusters ($n = 108$ keywords).

C_{ab} is the co-occurrences quantity of items a and b , whereas W_a and W_b are the overall frequency of occurrences of items a and b or the sum of both co-occurrences [89].

In the dynamic field of bioremediation or biodegradation, publication keywords offer practical evidence of authors' research themes and objectives, as visualized in Figure 4. The cluster network on this topic built based on the top 10 author's keywords, were

“microalgae” (TLS 78) with strongest research network, followed by “bioremediation” (TLS 43), “biodegradation” (TLS 40), “cyanobacteria” (TLS 23), “wastewater” (TLS 20), “pesticide” (TLS 16), and the rest are listed in Table 5.

Table 5. List of author’s keywords as central of top 10 clusters in co-occurrence’s analysis.

Cluster	Author Keyword	Links	TLS	Occurrences
1	Bioremediation	35	43	11
2	Pesticide	14	16	4
3	<i>Chlorella vulgaris</i>	11	12	3
4	Water quality	11	12	3
5	Microalgae	57	78	18
6	Cyanobacteria	20	23	5
7	Biosorption	12	12	3
8	Wastewater	15	20	5
9	Heavy metals	9	10	2
10	Biodegradation	33	40	9

The cluster network showed a profound number of diverse clusters (top 5 clusters), indicating the heterogeneity of topics amongst the main researchers. Cluster 1 refers to “bioremediation” of “pesticides” by common green microalgae from the genus “*Chlorella*” and “*Scenedesmus*”. Microalgae are fundamental in the food web as primary producers and have a great extent of ecological tolerance. As a result, microalgae with their great value as commercialized biomass, were effectively employed in mitigating pollutants (oil, heavy metals, pharmaceutical chemicals, dyes, wastewater treatment etc.) [141]. Algae can digest contaminants enzymatically and utilize the substrates as a source of nutrients. One of their bioremediation techniques is the bio-uptake of pesticides compounds into the cells’ compartments through three pathways of diffusions (passive diffusion, active transport, and passive-facilitated diffusion) across the cell membrane [117]. Some of their notable applications as a bioremediating agents are *Chlorella vulgaris* (amicarbazone) [142]; consortium of microalgae and cyanobacteria *Chlorella vulgaris*, *Scenedesmus quadricuda*, and *Spirulina platensis* (organophosphate malathion) [100]; *Scenedesmus arcuatus* (atrazine and endosulfan) [101] and *Chlorella vulgaris* (1,2-benzisothiazol-3(2H)-one (BIT)) [119].

Cluster 2 is centralized by the keywords “pesticide”, “amicarbazone degradation products”, “biodiesel”, and “*Chlorella sorokiniana*”. The most effective removal of organophosphate chlorpyrifos by microalgae *Chlorella sorokiniana* was proposed by Habibah et al. [142] at various concentrations of 99.85% (300 ppm), 99.18% (100 ppm), and 97.86% (500 ppm) in polluted water bodies. Algal biomass generated after wastewater treatment is recycled and potentially used for biochar generation and biodiesel production [9].

Several authors’ keywords identified in Cluster 3 were “*Chlorella vulgaris*”, “kinetic study”, “photobioreactor”, and “agriculture”. In a study by García-Galán et al. [107,114], the removal efficiency of several pesticides from agricultural run-off by mix microalgae culture in a microalgae based-photobioreactor found the promising treatment to mitigate diverse arrays of pesticides. The application of microalgae in the mentioned system proved to be remarkably release treated effluent and produce microalgae biomass, which may subsequently transform into renewable energy [122].

Apart from that, in Cluster 4, the keywords “*Aspergillus niger*”, “organophosphate pesticide”, “bioindicators” were interconnected with the largest node, “water quality”. The application of fungus (*Aspergillus niger*) for harvesting microalgae (*Chlorella vulgaris*) via biopellet formation has a great potential for sustainable development. Reduced level of pesticides were observed after the harvesting procedure [108]. In another study, a unicellular green microalga, *Coccomyxa subellipsoidea*, demonstrated the ability to metabolize organophosphates from agricultural run-off with minimal toxicity to the cell by producing ROS [143].

From Cluster 5, the term “microalgae” has the greatest number of connecting (78 links) co-occurring keywords with a TLS of 78 in the network cluster. The term “phycoremedia-

tion" relates to the "microalgae" distinct potential to metabolize and sequester carbon sources in comparison with that of other microorganisms [111,144]. Fluroxypyr is an herbicide commonly used to manage weeds and insects, but its extensive usage has led to substantial damage to the soil and surrounding waters. The rapid bioaccumulation, degradation (57% cellular breakdown), and biological adaptation of fluroxypyr in *Chlamydomonas reinhardtii* were stimulated through ROS formation and the actions of antioxidative enzymes [108,145]. The keywords cluster revealed 5 out of a total of 17 clusters focusing on topics involving bioremediation of pesticides and their byproducts, wastewater treatment, bioindicators, and phycoremediation meanwhile, the remaining clusters, contributed to the overall research trend's development. Remediation of pollutants by microalgae or macroalgae in a water system is described as phycoremediation. Photosynthesis allows algae to fix carbon dioxide and eliminate excess nutrients at a low cost. It cleans wastewater of harmful microbes and toxins [85]. Moreover, microalgal biomass produced through the remediation process is more beneficial than conventional biomass generation. Recycled microalgae biomass produced in large quantities could be used for a wide range of applications (for example, biodiesel production, feed, cosmetics, pharmaceuticals, and electricity) [11]. Frequent pesticide application decreases crop production because of their long-lasting and accumulative nature, which could potentially create substantial environmental effects.

6. Conclusions

The environmentally destructive application of pesticides in agriculture severely influences the ecosystem, inflicting damage on biodiversity and aquatic systems. This work demonstrated the research progress in recent times through bibliometric analytical review. In this case, bibliometric analysis can aid researchers in comprehending the global trend and serving as a model for future study. The evolution of the present research trend on bioremediation of pesticides by microalgae from 2010 to 2016 appeared to fluctuate. However, between 2017 and 2020, the trend was noticeably consistent for PubMed articles, and the Scopus dataset demonstrated a significant increase in publication productivity. Economically developed countries such as India and China progressively contributed to the scientific communities in investigating sustainable remediation strategies for pesticide-related pollutions. The study's topic of investigation was guided by the author's keyword clustering networks, which included "microalgae," "bioremediation," "biodegradation," "cyanobacteria," "wastewater", and "pesticide." Microalgae are a naturally occurring biological agent that was described as one of the most suitable techniques for pollution control and efficient at eliminating pesticide contaminants from agricultural run-off and polluted water effluents. Indeed, a proactive approach such as bioremediation could help improve the quality of the environment, and it must be developed by acknowledging the ability of microalgae and their metabolic functions.

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