



An Overview of Light-Mediated Impact of Graphene Oxide on Algae: Photo-Transform, Toxicity and Mechanism

Yang Gao ^{1,2,*}, Li Chen ^{1,2}, Shenghua Cheng ¹, Ling Zhu ¹, Lijuan Liu ¹, Peihuan Wen ^{1,2}, Letao Zhou ^{1,2}, Wenjing Xue ³, Songhua Lu ⁴, Wei Zhang ^{1,2}, Lean Zhou ^{1,2} and Shiquan Sun ^{1,2}

- School of Hydraulic and Environmental Engineering, Changsha University of Science and Technology, Changsha 410114, China
- ² Key Laboratory of Dongting Lake Aquatic Eco-Environmental Control and Restoration of Hunan Province, Changsha 410114, China
- ³ College of Environmental Science and Engineering, Yangzhou University, Yangzhou 225009, China
- ⁴ College of Environmental Science and Engineering, Changzhou University, Jiangsu 213164, China
- * Correspondence: gaoyang@csust.edu.cn; Tel./Fax: +86-0731-8525-8438

Abstract: Due to the unique chemical and physical properties, graphene-based nanomaterials are increasingly being introduced into various scientific fields. They all play very important roles in different fields and are widely used. Graphene oxide (GO) is one of the most popular and representative carbon nanomaterials; scientists have great research interest in it. When carbon nanomaterials such as GO are released into the aquatic environment, their physicochemical properties will be influenced by natural light, resulting in the potential change in toxic effects on aquatic organisms. Algae, as a typical aquatic organism, is extensively regarded as a model microorganism to assess the biotoxicity of nanomaterials. In this review, we overview the light-mediated impact of GO on algae. We summarize the photo-transformation of GO under different illumination conditions and the effect of illumination on the physicochemical properties of GO. Then, we combined metabolomics, genotoxicity, and proteomics with standard toxicity assays (cell division, membrane permeability, oxidative stress, photosynthesis, cellular ultrastructure, and so on) to compare native and environmentally transformed GO induction toxicological mechanisms. By correlating lights, physicochemical properties, and biotoxicity, this review is valuable for environmental fate assessments on graphene-based nanoparticles, providing a theoretical basis and support for evaluating the potential ecological health and environmental risks of graphene-based nanoparticles in real natural water environments.

Keywords: graphene oxide; light; photo-transform; toxicity; mechanism

1. Introduction

Microorganisms in the water environment are an important force driving the earth cycle of important life elements, such as carbon, nitrogen and sulfur in the hydrosphere and the degradation and metabolism of organic pollutants. A variety of inorganic or organic substances are dissolved or suspended in the water environment, which can provide the nutrients needed for microbial growth and reproduction. However, the natural surface water system inevitably receives pollutants from human activities, industrialization and urbanization [1]. The migration and transformation of these pollutants in the process of the water cycle pose a potential threat to water microorganisms, ecological safety and human health.

The excellent physicochemical properties of nanomaterials mean that they are widely used in electrical, optical, energy, biomedical, environmental protection and water treatment fields [2–5]. Among them, graphene and its derivatives have attracted widespread attention due to their unique structure and excellent properties, and have become one of the hot spots in the research of new nanomaterials. Graphene is a layer of carbon atoms (sp²-hybridized) arranged in the honeycomb lattice composed of six-membered rings, and



Citation: Gao, Y.; Chen, L.; Cheng, S.; Zhu, L.; Liu, L.; Wen, P.; Zhou, L.; Xue, W.; Lu, S.; Zhang, W.; et al. An Overview of Light-Mediated Impact of Graphene Oxide on Algae: Photo-Transform, Toxicity and Mechanism. *Water* **2022**, *14*, 2997. https://doi.org/10.3390/w14192997

Academic Editor: Dongmei Zhou

Received: 14 August 2022 Accepted: 19 September 2022 Published: 23 September 2022

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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). its specific surface area can theoretically reach $2620 \text{ m}^2/\text{g}$ [6], which means that it has excellent physicochemical properties. In addition, graphene oxide (GO) is currently the most representative graphene derivative, which mainly contains the following three elements: carbon (C), hydrogen (H), and oxygen (O). Two-dimensional flake GO has a monolayered structure, abundant oxygen-containing functional groups and excellent specific surface area. GO has many oxygen-containing functional groups, such as hydroxyl, carbonyl, carboxyl, and epoxycarbonyl, on the surface [5], which endow it with hydrophilicity. In the continuous production and application, GO will inevitably be exposed to the natural aquatic environment during its life cycle [4,7]. Therefore, it is particularly important to evaluate the environmental behavior and biotoxicity effects of GO after exposure to the natural environment, especially the interaction and mechanism between GO and microorganisms such as algae under natural environment factors (sunlight) [8,9]. GO mainly contains the following two hybridized regions, the hydrophilic sp³ hybridized region with oxygen-containing functional groups and the hydrophobic π -conjugated sp² hybridized region [10,11]. The hydrophilic sp³-hybridized region makes GO negatively charged, with high reactivity and affinity for water components and microorganisms. The hydrophobic π -conjugated sp² hybrid region enables GO to absorb sunlight efficiently, so GO is photoactive [11–13]. Several studies have shown that the light-induced transformation process can alter the physicochemical properties of GO, directly affecting its environmental behavior and toxicity [14–16]. The change in various defects (destruction of the basal plane, oxygen-containing groups, and so on) provides many active sites, promoting the interaction between graphene materials and other materials, microorganism, ions, or molecules [17]. Once the GOs enter natural surface water, due to their specific interaction with microorganisms, they may become a new pollutant and bring potential harm to the microbiology in natural surface water, and then threaten human health. Therefore, how to accurately understand the environmental behavior and biological effects of nanoparticles, and how to prevent and control their environmental risks are the core challenges and urgent needs for scientific solutions to the current and future major environmental and ecological problems faced by the development of nanotechnology.

In recent years, due to the inflow of a large number of nutrients, such as nitrogen and phosphorus, in domestic sewage and industrial and agricultural water into water bodies, the phenomenon of eutrophication in water bodies has become increasingly serious, resulting in the overgrowth of algae [18,19]. Algae cells usually grow on the surface of natural water bodies, reducing the light transmittance of water bodies, thereby inhibiting the photosynthesis of aquatic plants and hindering the normal growth of aquatic organisms [20]. Meanwhile, algae are extensively regarded as the model microalgae organism to assess the biotoxicity of pollution (traditional and emerging) due to their extensive existence, rapid propagation, small size, and sensitivity to toxicants [21]. At the same time, multiple studies have shown that GO can adversely affect animals, plants, microorganisms, cells and biological macromolecules [22,23]. Among them, the research on the bio-toxicology of graphene-based nanomaterial in the natural aquatic environment is relatively small or lacking [24]. There is limited literature on the environmental behavior and biotoxicity effects of GO and its derivatives on aquatic organisms under the influence of light. Among the aquatic organisms, algae are the main producers of the aquatic food chain in aquatic ecosystems and have the characteristics of being light-sensitive and restrictive, so they are widely used in aquatic toxicology research [25–28]. The rapid growth of algae poses enormous risks to the water environment and the health of aquatic organisms and humans. Therefore, it is very necessary to carry out the biotoxicological study of GO on algae. Algae are also good test subjects for toxicology studies of nanoparticles, organic pollutants and heavy metals [29]. However, compared with traditional pollutants (organic pollutants or heavy metals), the toxicological endpoints of nanoparticles are not well defined, especially in the natural surface water, where the effect of light should not be ignored [30]. The commercial production and application of nanoparticles will bring potential environmental risks to natural surface water and especially the interaction and environmental

behavior between nanoparticles and microorganisms under natural environmental factors (light) will further affect the structure and diversity of microbial communities in natural water [31–33]. Meanwhile, the selection of toxicological endpoints will also affect the experimental conclusions [34–36]. Indicators such as cell division, cell membrane damage, cell ultrastructural damage and oxidative stress are common toxicological endpoints in nanomaterial toxicology [4]. In recent years, the development of microbial high-throughput omics technology and the integration of multi-disciplinary methods have made it possible to study related complex and deep biotoxicity mechanisms. Furthermore, the monitoring of targeted or minority metabolites possibly neglect other or even primary reactions caused by nanoparticles. Metabolomics provides information on how biological processes actually take place, and its main advantage lies in providing links to cell/tissue phenotypes under specific environmental conditions [37-39]. However, without other supporting and comprehensive analyses, the individual metabolomics analysis is inadequate and one-sided. Therefore, we need to combine metabolomics with standard assays (cell reproduction, membrane permeability, oxidative stress, photosynthesis, and cellular ultrastructure) to study GO-induced bio-toxicological effects. In addition, both proteomics and metabolomics are effective methods to reveal the toxicological mechanisms of pollutants [40,41]. However, the current proposed mechanisms of biological toxicity are mostly based on a particular model put forward by the microbial specific mechanism of action, without considering the effect of light on the nanomaterial and the interaction.

Under the light conditions, the photo-transformation and encapsulation or capture effect of GO after it enters natural surface water, as well as the further interfacial interaction, will have a certain impact on the biotoxicity of GO in natural surface water. This review summarizes the effects of light-mediated GO on its physicochemical properties, as well as the biotoxicity and mechanism. First of all, we summarized the influence of the physical and chemical properties of GO under light conditions, and then summarized the impact of the corresponding changes in physical and chemical properties on the biological toxicity of GO to algae. Finally, we explored the deep biotoxicity mechanism from metabolomics, genotoxicity, and proteomics, combined with standard toxicity assays. Once GO is released into the aquatic environment, it will naturally be affected by natural environmental factors, such as light, and it will also bring many unknown effects to aquatic organisms. This paper summarizes the related nanomaterials, such as GO, which will provide help for the research on the biological toxicity and effects of nanomaterials on algae and other aquatic organisms in the natural surface water. Through this review, the risk and application safety of GO in the natural surface water environment can be evaluated and predicted. Then, we can provide a theoretical basis and support for the comprehensive estimate of the potential health risks of graphene nanoparticles that enter natural surface water under a real light environment.

2. Effects of Different Light Conditions on Physicochemical Properties of Graphene Oxide and Photo-Transform Mechanism

With global warming, the impact of light on the natural environment is increasing, especially for natural surface water bodies. Sunlight mainly includes ultraviolet (UV) light and visible light (VL), while different light has different energy and influence. At present, there are a few studies on the changes in physicochemical properties of graphene oxide (GO) mediated by UV, VL and simulated sunlight [14,31,42–45]. However, there are few reports on light-mediated GO-natural water microbial interactions, toxicity and mechanisms. So, carrying out the biotoxicity and interaction mechanism study of nanoparticles (GO) to microorganisms in natural water under natural environment factors (light) helps to evaluate the application security and environment risk of nanoparticles. The environmental process of nanoparticles in the Earth system can be made transparent, the environmental trend can be predicted and the environmental risk can be prevented. Finally, it will serve the national nanotechnology strategy and policy, and ensure the green application and sustainable development of nanotechnology. This section mainly reviews the changes in physical

and chemical properties of GO under three different light sources, focusing on the visual appearance, morphology, size, functional groups, and surface charge of GO (Figure 1). In addition, the photo-transform changes the physicochemical properties of GO, such as morphology, layer number, lateral size, dispersibility, and so on [14,31,43]. Many studies have investigated the relationship between the physicochemical properties of GO and its biotoxicity [46,47]. Therefore, correlating the biotoxicity of GO with its physicochemical properties under light conditions is important to investigate its real environmental risk.

2.1. UV Irradiation

UV irradiation is widely used in water disinfection treatment and is a common disinfection method. In addition, it has a great influence on the light conversion of GO and can affect the physicochemical properties of GO. In previous studies, there are four kinds of possible reactions in the photo-transformation of GO, including production of OH, oxidation, reduction and degradation that induces the change in the physicochemical properties of GO [31]. The changes in the morphology and visual appearance of GO were very obvious when exposed to UV light (Figure 1A). The color of the GO solution deepens at first, gradually fades after 60 h of UV irradiation, and becomes colorless after 84 h of UV irradiation [31]. The initial darkening of the solution is due to the photochemical reaction of GO and the formation of photoproducts that absorb light [44]. After 60 h of UV exposure, GO began to fade, which may be on account of the destruction of C=O and C=C chromophore groups by UV exposure [31,48]. After 84 h of UV irradiation, the suspension supernatant of GO became colorless, indicating that GO underwent significant photo-transformation and degradation [31,45]. As shown in the Figure 1E,F, the GO nanosheets will break, and will be further broken into small fragments after UV exposure [43,49]. Using dynamic light scattering spectroscopy, the researchers investigated the changes in the hydrodynamic size of GO during UV photochemical reduction and initially found that the increase in GO size was due to the reduction of oxygen-containing functional groups and the fragmentation of hydrogen bonds [50]. At deeper reaction stages, the size of GO decreases because of the π - π stacking interaction and the wrinkling of the GO sheet [50,51]. The oxygen-containing functional group gives GO unique optical characteristics [52], and the change in functional groups has a great impact on the physicochemical properties of GO. After a few hours of UV irradiation, GO nanosheets form nanopores in the oxygen-containing region, and a light reaction occurs to generate reduced GO (rGO), CO_2 and O_2 [49]. Through XPS analysis, it can be observed that epoxide and carbonyl decreased, while hydroxide and carboxyl increased, and the increase in the number of carboxyls promoted the formation of O₂ [51]. Schwenzer et al. found that hierarchically stacked GO films degrade rapidly under UV irradiation [53]. Longer UV irradiation will destroy the benzene ring structure of GO and induce the formation of fragments, which is beneficial to increasing the stability of the colloid [43]. The surface of GO is negatively charged due to the dissociation of carboxyl, enol and phenolic groups [54]. It is shown that the oxidation of -C-OH/-C=Ofunctional groups to -COOH functional groups leads to an increase in the electronegativity of GO, whereas the reduction of oxygen-containing functional groups reduces the negative charge [55].

Yuan et al. [56] found a green and effective method to remove GO. By irradiating GO solution with UV light, the removal rate reached 99.1% after 32 h [56]. A recent study showed that the UV band plays a crucial role in the photoconversion process of GO, and UV-A and UV-B dominate the photoreduction of GO, which can be confirmed by the reduction of O/C [57]. The photoconversion of O_2 under UV light plays an important role, and UV-C causes significant oxidation of GO under aerobic conditions [57].



Figure 1. Photograph of GO suspensions as a function of UV (**A**) and VL (**B**) exposure time [31]; the photograph of GO air-equilibrated samples before and after sunlight exposure (**C**) [44]; phototransformation of GO containing 100 mM H₂O₂ in sunlight (**D**) [45]; TEM images of GO before and after UV exposure (**E**) [43]; AFM images of the GO nanosheet after the photoreaction in O₂ under UV irradiation (**F**) [49]; AFM images of GO containing 100 mM H₂O₂ in sunlight (**G**) [45]; AFM images of GO in air-equilibrated sunlight (**H**) [44].

2.2. Visible Light

In recent years, most of the research on visible light is usually related to the degradation of environmental pollutants in GO composites [51]. Due to the low photon energy of visible light, GO does not have excellent pollutant degradation ability [51,58]. UV irradiation can cause the change in carboxyl groups, affecting the photoluminescence property of GO, while VL irradiation cannot [31]. As shown in the Figure 1B, some studies have reported the light transformation of VL [31]. For example, Gao et al. compared the ways in which UV and VL irradiation changed the environmental fate and risk of GO, and VL irradiation was significantly weaker than UV irradiation in changing the physicochemical properties and toxicity of GO [31]. In previous studies, it was found that graphene-based nanomaterials can be modified, oxidized, and degraded under VL irradiation [59,60]. GO will produce ROS (including hydroxyl radical –OH, superoxide O_2^- and singlet oxygen 1O_2) under UV and visible light irradiation [61], but in different proportions [62]. Hydroxyl radicals (-OH) are thought to play a very important role in the oxidative degradation of GO [14,55,63]. Among all the reactive oxygen species, -OH hydroxylated GO, which mainly led to the decomposition of GO [62]. Wang et al. found that in the presence of hypochlorite, visible light irradiation could promote the decomposition of the sp^2 structure of GO, produce alkanes and aromatics with short carbon skeletons, and provide GO with biological toxicity and inhibit the growth of algae [39].

2.3. Simulated Sunlight

Numerous studies have shown that sunlight exposure can change the physicochemical properties of GO, thereby affecting the aggregation/deposition and photoreaction of GO [14]. As shown in the Figure 1C,D, GO easily undergoes photoreactions under simulated sunlight conditions, and electron–hole pairs may be generated [44,45]. It has been confirmed that GO can be photo-disproportionated into CO₂ [44]. After simulated sunlight irradiation, GO was reduced to rGO, the oxygen-containing functional groups were reduced, the size of the intermediate photoproducts was smaller (Figure 1G,H), and polycyclic aromatic hydrocarbons with small molecular weight were generated [44,45]. The relative abundance of C-C/C=C increased compared to pristine GO [64]. Shams et al. extensively characterized the physicochemical properties of GO before and after simulated sunlight irradiation and found that hydroxyl and epoxy functional groups were the most easily photodegraded, and the number of oxygen-containing functional groups decreased with irradiation time [65]. Simulating solar radiation can stimulate the generation of reactive oxygen species (ROS), and GO acts as an electron donor, transferring electrons to molecular oxygen to form ROS [66]. Furthermore, GO is often used in photocatalytic reactions, and the CO yield under simulated sunlight irradiation is higher than that of UV-irradiated GO, indicating that simulated sunlight irradiation is more favorable for GO activation [67]. In addition, Zhao and Wang found that dissolved O_2 in water plays an important role in photoconversion, while superoxide (O_2^-) is not involved [68]. One study showed that GO produces only singlet oxygen $({}^{1}O_{2})$ under simulated sunlight because electron-hole pairs are generated on the surface of GO, which is resistant to the slight oxidation of biomolecules [69]. Recently, Zhao et al. summarized the potential pathways of GO to generate ROS according to the current field [51]. We mentioned the conversion pathway in the photoreaction process above. GO is mainly converted into CO₂, rGO and small molecular weight polycyclic aromatic hydrocarbons after sunlight irradiation. In this process, GO acts as an electron donor, transferring electrons to oxygen molecules to generate a large amount of dissolved oxygen and H_2O_2 .

As shown in Figure 2A, Koinuma et al. establishes the probable models of the pore production by the photoreaction of GO and proton conduction at epoxide groups [49]. Matsumoto et al. builds the structural models of GO nanosheet samples before and after the Xe lamp photoreaction (Figure 2B). The oxygen-containing functional groups of GO were reduced to produce holes (defects) with zigzag edges [11]. The reaction scheme in Figure 2C shows that direct and indirect photolysis occur concurrently in the initial stage of GO photo-transformation under sunlight conditions [45]. Figure 2D shows the schematic diagram of the photo-transformation mechanism of GO after irradiation by UV lights under aerobic conditions [57]. The probable photo-transform process of GO contains electron-hole pairs, reduction, decarboxylation, and oxidation. In conclusion, the different photo-transform mechanisms of GO were predicted in different conditions (Figure 2). The main purpose of irradiating GO under a single light condition is to study the mechanism, and the mechanism under a single light will also be clearer. In order to provide a theoretical and research basis for studying the mechanism in the natural environment or real environment, researchers first summarized the changes in the physicochemical properties of GO and the mechanism of light transformation under UV and VL lighting conditions, and then explored the influence and mechanism under simulated solar lighting conditions, in addition to sunlight in the natural surface water. The research on the influence of light on the physicochemical properties of GO can provide the scientific basis and basic data for the establishment of the material migration and transformation and biological effect theory of GO in natural water systems.



Figure 2. Models of the pore production by the photoreaction of GO in N₂ and O₂, and the model of proton conduction at epoxide groups (**A**) [49]; structural models of nanosheet samples before and after the photoreaction (**B**) [11]; schematic illustrating the pathway of indirect photolysis in solar transformation of GO (**C**) [45]; schematic diagram of the photo-transformation mechanism of GO after irradiation by UV lights under aerobic conditions (**D**) [57].

3. Biotoxicity and Mechanism of Light-Mediated GO to Algae

After GO enters the natural surface water, GO will undergo interface interaction with different kinds of microorganisms in natural water. In particular, the photo-transformation and aggregation behavior of GO will further affect the diversity distribution, toxic effects and biological effects of microorganisms in natural surface water. However, researchers mainly focus on the specific toxicity mechanism of GO to a single individual model organism, with a high exposure dose and short exposure period. The short-term toxic effects of high concentrations of GO on single individual model organisms (erythrocytes, fibrocytes, Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Paecilomyces lilac, Chlorella and zebrafish) were studied [17,34,70-72]. The following three main mechanisms of biotoxicity of GO were proposed: the nanoknife effect caused by the sharp edge effect, oxidative stress effect mediated or not mediated by ROS, and bacterial membrane encapsulation or capture effect caused by the membrane structure [17]. Due to the sharp edges of GO, GO can penetrate the cell membrane and enter the cell, causing direct cell membrane damage through cleavage. After entering cells, GO can cause oxidative stress, mitochondrial dysfunction and DNA damage [73]. Wrapping or coating of cell membranes was regarded as the mechanism that contributes to the biotoxicity of graphene materials, as well as nanoknives and oxidative stress [17]. The sp³-hybrid region of GO (oxygen-containing functional group) can produce strong interfacial interaction with the microbial surface and be coated on the microbial surface, showing a high affinity for microbiology. Meanwhile, GO is one of the thinnest films, consisting of a layer of carbon atoms (sp²-hybrid) arranged in the hexagonal crystal structure. Its unique two-dimensional transverse structure can provide a flexible and unique barrier to isolate a microorganism from its ambient medium. So, GO will form a surface coating on the cell surface or start to aggregate and attach to the cell surface, resulting in a shading effect. Zhao et al. summarized the cytotoxicity mechanism at the cellular level and proposed several cytotoxicity mechanisms of graphene nanomaterials, as shown in Figure 3 [73].

When GO enters the environment, its physical and chemical properties are changed by the influence of light, and the change in physical and chemical properties and its migration, transformation and interaction in environmental media will further change its biotoxicity effects and mechanisms, affecting environmental ecological security. So, it is urgent to study the biotoxicity and mechanisms of GO after it enters the natural surface water environment system under light. Thus, the environmental risks and application safety of increasingly widely produced and applied commercial GO can be evaluated, the environmental behavior and toxicity mechanism of graphene nanoparticles can be analyzed, risk diagnosis and early warning will be realized, and scientific basis and methods will be provided for the prevention and control of graphene nanoparticles.

3.1. Cell Division

The physicochemical characteristics of GO (e.g., morphology, structure, functional groups, size, etc.) are related to its toxicity. The size of GO decreased after irradiation, which inhibited the growth and reproduction of algae. Experiments have shown that graphene oxide quantum dots (GOQDs) have a stronger ability to inhibit cell division than GO nanosheets [34]. Carbon nanomaterials also have a significant effect on the cell division of algae. For example, the carbon nanotube suspension stored for three days has a more obvious growth inhibition effect on *Chlorella vulgaris* than the freshly prepared carbon nanotube suspension [74]. Algae can survive in lower concentrations of GO solutions, and their presence may also lead to lateral size changes in GO [75]. Evidently, the physicochemical properties of GO changed after light exposure. As shown in Figure 3A, after exposure to GO at a concentration of 50 mg/L for 96 h, the growth of algae was significantly inhibited [76]. It was found that the dead algae settled on the bottom of the bottle and turned black [76]. As shown in Figure 3B, the exposure to GO first promotes and then inhibits Chlorella vulgaris cell division. The consistent changes in cell division that occur over 24 h are probably linked to the GO coating phenomenon [77]. As the probable photo-transform products (rGO), the growth of green alga Scenedesmus obliquus cells were suppressed remarkably after rGO treatment, and the suppression level increased with increasing levels of RGO (Figure 3B) [78].



Figure 3. Growth inhibition of algal cells as a function of graphene-family material concentrations after 96 h of exposure (A) [76]. Growth curves of *Chlorella vulgaris* under graphene oxide exposure

for 96 h (**B**) [77]; growth curves of *S. obliquus*. exposure to 0–300 mg/L of rGO for 72 h (**C**) [78]; the contents of chlorophyll a of five different algal cell types (*C. vulgaris, S. obliquus, C. reinhardtii, M. aeruginosa, Cyclotella* sp.) exposed to 1/10 mg/L of GO (**D**) [79]; change in chlorophyll a concentration for *M. aeruginosa* exposed to different concentrations of GO (**E**) [80]. Letters (a, b, c, d) above the bars denote significant differences compared to the control at p < 0.05.

3.2. Cell Membrane Damage and Shadowing Effects

In recent years, there have been more and more studies on the toxicology of algae by graphene-based nanomaterials, but the related reports are still very limited. Currently, toxicological data on algae mainly focus on GO [81,82]. As shown in Figure 4A, GO will produce blister-like nanostructures on the surface of microalgae and GO as a coating will envelop microalgae cells [81]. Other graphene-like nanomaterials, such as rGO, monolayer graphene, and multilayer graphene, may have different surface features (e.g., oxygencontaining groups) and structural properties (e.g., sp^2/sp^3 ratio) from GO, so that they show different toxic effects on algae [76]. As shown in Figure 4B, the interactions between GO and the surface of cells were mainly found to be involved in cell cutting, penetration and envelopment [79,83]. Yin et al. found that the ruffles and grooves in C. vulgaris, M. aeruginosa and C. reinhardtii cells disappeared and were replaced by GO when exposed to GO, the cell wall of *S. obliquus* was punctured by GO and the cell wall of *Cyclotella* sp. was destroyed and fragmented [79]. As shown in Figure 4C, Zhao et al. hypothesized that graphene-like nanomaterials modulate their toxicity to algal cells through direct contact with algae (e.g., membrane damage) and indirect toxicity (e.g., shading effect and nutrient depletion) [76]. GO has an obvious two-dimensional nanosheet structure [84,85], and GO will form a coating after being exposed to *Chlorella* for 24 h [77], and attach to the surface of algal cells, resulting in a shielding effect, that is, nanotoxicity. The interaction of GO with algae will trigger the direct toxicity of GO, and the process of GO attaching to algae may cause cell membrane damage to algae. Nakabayash et al. observed significant differences in the levels of oxidative stress and membrane damage by fluorescence analysis, where a positive correlation between oxidative stress and membrane damage was observed for GO concentrations above 10 μ g/mL [86]. It is worth noting that the indirect nanoeffect of GO may work by reducing the light source (shading effect) and depleting algal nutrients, but there are relatively few nanotoxicological studies in this regard [87]. Other carbon nanomaterials also have indirect toxic effects on algae, such as multilayer carbon nanotubes, which have a shielding effect on algal growth [88]. GO is converted to rGO during environmental transformation, and rGO adheres to the surface of algal cells as a translucent coating that may prevent chlorophyll from absorbing light, thereby inhibiting algal growth [74,78]. It has been reported that the inhibition of algal cell division is caused by shading and agglomeration of carbon nanotubes [74], and GO-induced DNA fragmentation and chromosomal aberrations can also inhibit cell division [89].



Figure 4. SEM images of microalgae cells exposed to GO (**A**) [81]; SEM images of *C. vulgaris, S. obliquus, M. aeruginosa, C. reinhardtii,* and *Cyclotella* sp. exposed to GO (**B**) [79]; SEM images of algal cells that were physically penetrated by rGO and multi-layer graphene (**C**) [76].

3.3. ROS Generation and Oxidative Stress

The generation of reactive oxygen species (ROS) is a common toxicity indicator for carbon nanomaterials and other nanomaterials. In addition to the ability of GO to generate ROS by itself [90], ROS may be mainly generated by the interaction of GO with mitochondria, chloroplasts (plant and algal cells), peroxisomes, and oxidases [91]. Excessive ROS may lead to subsequent cell death through multiple pathways, such as organelle dysfunction [73]. It has been reported that carbon nanoparticles can lead to the production of ROS, and the concentration of GO has a certain effect on the production of ROS [86]. Other nanomaterials have similar conclusions. Li et al. found through experimental exploration that the site where nano-TiO₂ induces ROS production in algal cells is the chloroplast, and confirmed that ROS will be generated and will accumulate in algal cells [92]. Zhang et al. used DCFH-DA staining to measure ROS levels and found that low concentrations of GO, i.e., GO below 500 ppm, did not cause cell death, while when the GO concentration was above 1000 ppm, GO caused a significant increase in intra-cellular ROS levels, causing oxidative damage [93]. The research object was expanded to graphene-based nanomaterials. After algae were exposed to rGO, GO, and multilayer graphene for 96 h, it was found that the above three materials significantly increased the intracellular ROS level of algal cells, in the order of rGO > GO > MG [76]. After GO was exposed to light, a large number of its functional groups were reduced to form rGO. The higher the oxidation degree of GO, the more obvious the change in the generated rGO, and the higher the number of reactive oxygen species generated [94]. In addition, the biotoxicity of saxitoxin (STX) to algae is similar to that of GO, and STX also causes ROS formation, as well as oxidative stress [95].

3.4. Effects of Photosynthesis and Chlorophyll Content

In recent years, autofluorescence (chlorophyll a fluorescence) has been assessed by fluorescent staining to observe the biotoxicity of GO to algae. The degree of effect of GO on green algae mainly depends on the concentration of GO and the time of exposure to GO. The toxic effect of GO on algae begins when the concentration of GO is greater than 10 μ g/mL, and when the algae are exposed to GO for more than 96 h, the percentage of chlorophyll autofluorescence intensity decreased at this time, and this toxicity may be caused by oxidative stress and membrane damage (cell viability) [86]. A similar conclusion was obtained for *M. aeruginosa*. When the exposure time was extended to 96 h and the concentration exceeded 10 μ g/mL, the autofluorescence of chlorophyll a changed significantly (as shown in Figure 3E), and GO demonstrated significant growth inhibition of *M. aeruginosa* [80]. Elisa Banchi et al. also assessed the effects of 4-week exposure of terrestrial green microalgae to graphene-based nanomaterials by analyzing chlorophyll-a fluorescence (Chl-a) and photosynthetic pigments. Microalgae are not toxic because their thick cell walls effectively hinder internalization, preventing interference with the cytoplasm [96]. In addition, Tang et al. measured the content of chlorophyll a by UV spectrophotometry and found that with the increase in GO concentration, the growth inhibition of algal cells increased significantly, and there was no significant difference in chlorophyll a content between the control group and 1 mg/LGO exposed cells, while as shown in Figure 3D, a significant difference was observed when the GO concentration was 10 mg/L, and the chlorophyll a content of cells exposed to GO was significantly reduced [79,97]. When exploring the combined biotoxicity of GO and cadmium on the survival and photosynthetic capacity of *M. aeruginosa*, it was further found that low concentrations of GO did not show obvious toxicity and would not damage the algal oxygen-producing photosynthetic system, but significantly enhanced the toxicity of Cd²⁺ [97]. The GO-containing Cd²⁺ easily entered the algae, demonstrated by scanning electron microscopy and transmission electron microscopy [97]. When exploring the biotoxicity of GO and copper on freshwater *Scenedesmus*, we found a slightly different conclusion from the previous one [98]. At an environmentally relevant concentration of 1 mg/L, GO would not have any adverse effect, but could significantly inhibit the effect of copper on the toxicity of algae [98].

GOQDs have also been widely used and compared with GO, GOQDs can induce higher reactive oxygen species (ROS) and malondialdehyde (MDA), and destroy antioxidant enzymes, resulting in chlorophyll a and proteins, etc. Cell content was suppressed [99]. In addition, GO quantum dots exhibited persistent toxic effects on chlorophyll a, which was due to the persistent effects of GO quantum dots on chloroplast ultrastructures, and the biosynthesis of chlorophyll a in algae could not be restored to previous levels [100]. In addition to GO quantum dots, rGO also inhibited the growth of the green alga *Scenedesmus obliquus*, and by fluorescence analysis, it was observed that GO could significantly down-regulate the activity of photosystem II, inhibiting algal growth and photosynthesis [78].

3.5. Cellular Ultrastructural Damage and Membrane Permeability

With sharp edges and excellent mechanical properties, GO nanosheets may act as "nano-knives", causing damage to the cell membrane, thereby entering the cell and causing damage to the ultrastructure of the cell [71]. Researchers generally observe the ultrastructure of algal cells by transmission electron microscopy, and found that after exposure to GO, *Chlorella* showed obvious plasmolysis and slight rupture of cell membranes, and the starch granules and lysozyme in *Chlorella* appeared. In general, the increase in the number of intracellular starch granules and lysosomes is considered to be a manifestation of the

cell's self-defense mechanism [101,102]. Hu et al. compared the cellular ultrastructure damage of GO and C-SWCNT to Chlorella vulgaris (Figure 5A) and they confirmed that the C-SWCNT cellular uptake occurs more readily than GO uptake [82]. As shown in Figure 5B, GO nanosheets and GO quantum dots induce significant plasmolysis, and the shrinkage of the plasma membrane after GO nanosheets exposure was more obvious than that after GO quantum dots exposure [34]. As shown in Figure 5C, Hu et al. found that thylakoids could not be discerned after GO exposure to Chlorella for 96 h, indicating that the ultrastructure of chloroplasts was disrupted, while the control group without GO exposure showed intact ultrastructural morphology (including cell wall, plasma membrane, chloroplast, nucleus and other cytoplasmic compartments) [77]. Yan et al. found that GO quantum dots induce stronger upregulation of *Microcystis aeruginosa* cell lipid bodies, plasmolysis and permeability compared to GO (Figure 5D), and the higher concentrations of GO and GO quantum dots induce the disintegration of cell infrastructure, disappearance of nucleoids, and cracking of thylakoid layers [99]. Ouyang et al. found that GO and GO-nanocolloids have envelopment effects on Chlorella vulgaris cells, and could enter cell walls, inducing damage and plasmolysis (Figure 5E) [21]. Figure 5F shows that variations in green algae cell wall composition led to different extents of mechanical damage and that the Cyclotella sp. silica frustules and S. obliquus autosporine division are prone to damage by GO [79]. When exploring the combined toxicity of GO and wastewater to Chlamydomonas reinhardtii, the researchers found that with GO, much fewer cells are affected and less plasmolysis occurs in wastewater mixtures than GO alone and combined wastewater and the cell wall damage is low, but the cytoplasmic contraction of C. reinhardtii cells can still be observed under the transmission electron microscope, with the irregular plasma membrane and plasma membrane separation morphology [103]. Also exploring the combined toxicity, Ouyang et al. studied the toxicity of GO to freshwater algae mediated by natural nanocolloids and found that when the concentration was 0.1 mg/L, the permeability of GO and nanocolloids alone to algal cells had no significant effect, while GO-manocolloids had a significant effect [21].

The toxicity of graphene-based nanomaterials to freshwater algae can be compared by flow cytometry. Zhao et al. found that the membrane damage caused by multilayer graphene and rGO was greater, GO did not directly damage algal cells in the experiment and the agglomeration of algal cells with GO is weak and the contact probability is low [76]. The researchers further confirmed the above conclusion by proposing that graphene microflakes penetrate into cells through spontaneous membrane penetration through mathematical modeling [104]. Another study found that GO quantum dots induced stronger cell permeability, plasmolysis, and liposome upregulation than GO [99]. As shown in Figure 5D, the rupture of the thylakoid layer, disappearance of nucleoids and disintegration of the cellular infrastructure were observed at higher concentrations [99].



Figure 5. Damage to the cellular ultrastructure and uptake of 10 mg/L GO at 96 h (**A**) [82]; envelopment of the cells by10.0 mg/L GO nanosheets/GO quantum dots at 96 h (**B**) [34]; cell permeability and plasmolysis image of cells after 1 mg/L GO exposure for 96 h and recovery for 96 h (**C**) [77]; the effects of nanomaterials on ultrastructure of *M. aeruginosa* cells (**D**) [99]; the algal ultrastructure and nanoparticle uptake (**E**) [21]; TEM images of algae cells in *C. vulgaris, S. obliquus, M. aeruginosa, C. reinhardtii,* and *Cyclotella* sp. after GO exposure (**F**) [79].

4. Biotoxicity Mechanism of Light-Mediated GO to Algae

Over the past few years, scientists have gained significant knowledge about nanotoxicology and have learnt that nanoparticles such as GO can penetrate cell membranes and then induce biological responses [30,105,106]. These biological responses are also fundamental indicators of toxicity, including growth inhibition, structural damage, oxidative stress, genotoxicity, protein modification, and metabolic disturbances [107,108]. However, many environmental processes and interaction mechanisms of nanoparticles determined by the complexity of natural environmental systems and interdisciplinary nature are still unclear. In particular, the effect mechanism of light-mediated GO on microorganisms in real natural surface water bodies with complexity and diversity is still unclear and urgently needs multidisciplinary and continuous in-depth research. In recent years, the development of high-throughput omics technology and the integration of multi-disciplinary methods have made it possible to study the related scientific problems. Metabolomics is an omics technique that elucidates metabolism-related processes by examining the overall dynamic changes in a large number of metabolites before and after stimulation or disturbance. In recent years, this method has been applied to the health risk assessment of environmental pollutants, especially nano particles, and has become one of the key research methods in environmental science, especially environmental ecotoxicology. The new generation of high throughput sequencing omics technology breakthroughs in biology provides opportunities for the environment, breaks through the traditional individual biology research limits, faces the complicated water microbes in natural water bodies, has realized the change from single biological process research to the biological community level and greatly expanded the understanding of the environmental microbial community structure and function. This enables researchers to study the microbial ecological effects of GO at the level of a more complex real water environment. Most environmental studies test a few metabolites or genes as biological endpoints to study toxicological mechanisms [30,109]. In most studies, Chlorella is usually used as a model organism for toxicity assessment [110], with a few genes, proteins or metabolites as toxicological endpoints and here, we review the existing related studies.

4.1. Metabolomics

In recent years, the development and application of single-cell genomics, transcriptomics, proteomics and metabolomics have increased [111]. Among them, metabolism is a direct indicator of cytotoxicity and can quickly reflect how cells affect the environment [30,111]. Decreased chlorophyll a content after exposure of nanomaterials to algal cells has been demonstrated, but the associated toxicity mechanism is unclear [34,112,113]. Serine is involved in the biosynthesis of purines and pyrimidines of chlorophyll a, which are the precursors of chlorophyll a, and the level of serine decreases after exposure to GO of different sizes, resulting in a decrease in chlorophyll a content. Serine is involved in the biosynthesis of purines and pyrimidines of chlorophyll a, which are the precursors of chlorophyll a [114], and the level of serine decreases after exposure to GO of different sizes, resulting in a decrease in the content of chlorophyll a [34]. Oxidative stress is generally considered to be the main mechanism of nanotoxicology [115,116]. Ouyang et al. found that after exposure of nanocolloids in natural water to Chlorella, their metabolomic analysis found that amino acid down-regulation and fatty acid up-regulation resulted in increased ROS, and decreased chlorophyll a content, and plasmolysis [28]. As shown in Figure 6A, the down-regulated pathways of amino acids are closely related to the inhibition of algal blooms [39]. Hexadecanoic acid, octadecanoic acid and their unsaturated structures are the main fatty acids in algae [77]. At the same time, Hu et al. found that after GO exposure to Chlorella, saturated fatty acids were converted to unsaturated fatty acids, resulting in a decrease in chlorophyll a levels, and an increase in ROS levels, triggering oxidative stress, while carbohydrate metabolism affected plasmolysis (Figure 6B) [77]. Wang et al. showed that both hypochlorite and VL regulate GO-induced metabolic perturbations, and their metabolomic analysis demonstrated that increased membrane permeability and enhanced oxidative stress were associated with the down-regulation of proline, alanine, asparagine. Metabolomics is a new tool. At present, most scientists focus on metabolites and use them as toxicological endpoints. However, there are few studies on genotoxicity. Next, comprehensive research is needed to establish a more comprehensive toxicity mechanism.



Figure 6. Perturbation of the metabolic profile by pristine and treated GO (**A**) [39]; relationships between the disturbance of metabolic pathways and the alterations of biological endpoints (**B**) [77]; proteomics and metabolomics analysis of algae exposed to 1 mg/L nanocolloids, GO, and GO-nanocolloids at 96 h (**C**) [21]; protein–protein interaction analysis of proteins and metabolites involved in amino acid metabolism (**D**) [117].

4.2. Proteomics

Multifarious proteins are indispensable components of microorganism cells and are distributed in the cell plasma, cell membrane and wall. The proteins (carrier proteins, enzymes, and so on) that regulate and control the metabolism of microbiology can be easily disturbed in the natural environment, where various pollutants exist. Proteins are an important component of biological media, and when introduced into a physiological environment, nanomaterials readily bind to proteins by forming protein coronas on their surfaces [30]. This protein corona has an important effect on the surface properties of nanomaterials and may affect their interaction with cells [118]. Ribosomes are cellular structures that synthesize proteins [119], and some common proteins play a crucial role in the fate of nanomaterials. You et al. investigated the single and combined toxicity of GO and four antibiotics to *Synechocystis* sp. [120]. Proteomic analysis showed that exposure to antibiotics upregulated ribosomal pathways but downregulated oxidative phosphorylation pathways [120]. Furthermore, the proteins involved in ribosomal pathways and nitrate and phosphate transport were up-regulated by antibiotics, but were down-regulated by the addition of GO in the co-exposed group [120]. Ouyang et al. found that GO exposure

in Chlorella induced hundreds of differentially expressed proteins (DEPs), identifying approximately 983 DEPs and 46 metabolites (e.g., fatty acids, amino acids, carbohydrate metabolites, and small molecules acids) in each sample (Figure 6C) [21]. In addition, they found that nine significantly altered proteins were involved in photosynthesis, and that changes in these proteins resulted in the down-regulation of chlorophyll b biosynthesis, reduced light capture, and inhibition of electron transport in photoreactions [21]. Li et al. found that there are differences in the toxicity mechanism of GO with different sizes, and GO quantum dots down-regulate more proteins related to endocytosis and transmembrane transporter activity than GO. Through the protein-protein interaction network analysis (Figure 6D), they found that the perturbed proteins and metabolites are related to carbohydrate and amino acid metabolism, indicating that the experimental group with the addition of GO quantum dots has a more strong defense mechanism [117]. Proteomics can provide direct information on plant responses to abiotic stresses and elucidate the main toxicity mechanisms of nanomaterials [121,122]. We usually link proteomics and metabolomics to study toxicological mechanisms, and then we summarize the related research of metabolomics.

4.3. Genotoxicity

Microorganism genes typically consist of plasmids (extrachromosomal DNAs) or DNA (single continuous stretch) that may involve genes for virulence factors and antibiotic resistance. If the gene is destroyed or a deviation occurs during the process of replication, microorganisms will mutate and even die. Once the nanomaterial invades the cell, DNA probably interacts with the graphene nanomaterial through electrostatic adsorption, hydrogen bonding, and $\pi - \pi$ stacking on account of the existence of oxygen-containing functional groups and π -conjugated structure. As shown in Figure 7A, the nanomaterial can physically interact with the DNA molecule and cellular proteins (those involved in the cell division process) induce the damage of DNA. In addition, the nanomaterial can induce other cellular responses that, in turn, lead to genotoxicity (aberrant signaling responses, inflammation and oxidative stress) [123]. Therefore, genotoxicity is a unique and important type of biological toxicity, but the genotoxicity of GO to algal cells is rarely reported, and the research on the genotoxicity of graphene nanomaterials to DNA is still largely unknown. Therefore, we summarize the mechanism of genotoxicity induced by graphene-based nanomaterials. Graphene-based nanomaterials are one of the most popular nanomaterials at present, which have good application prospects and have received extensive attention. In general, genotoxicity can be subdivided into direct and indirect genotoxicity in the cell or nucleus [124–127]. Wu et al. proposed the genotoxic effects for the graphene family of nanomaterials, as shown in Figure 7B, and reported that oxidative stress induced by the graphene family of nanomaterials causes DNA damage, which has been well established and studied [124]. Samadian et al. provided an overview of the potential genotoxicity of carbon-based nanomaterials and explored the risk assessment (Figure 7C), in which the genotoxicity of each part of the carbon-based nanomaterials family must be evaluated and considered together with other parameters [128]. As shown in Figure 7D, Kong et al. employed density functional theory and molecular dynamics simulation to evaluate the DNA genotoxicity of graphene quantum dots theoretically. They demonstrated that the DNA damaging mechanism of graphene quantum dots depends on the size of graphene quantum dots [129]. Direct genotoxicity refers to the ability of GO and rGO to penetrate the cell membrane or cell wall of plant cells after exposure to graphene-based nanomaterials, thereby directly causing physical membrane damage [83,130]. Graphene nanomaterials interact with DNA mainly through hydrogen bonding and π - π bond stacking. The damage mechanism of GO quantum dots to DNA depends on the size. The smaller the size of GO quantum dots, the easier it is to enter DNA molecules, resulting in base mismatch in DNA [129]. The larger GO quantum dots are more likely to attach to the ends of DNA molecules, which causes the DNA to unfold [129]. Indirect nanogenotoxicity is often described in terms of oxidative stress, epigenotoxicity, DNA replication, DNA repair and

transcription, inflammation, and autophagy [124]. Evidently, genotoxicity is closely related to the physical and chemical properties of graphene-based nanomaterials, such as the surface properties, size structure, dose, and test organisms of graphene-based nanomaterials [123,131]. The most commonly used genotoxicity tests by scientists today are the following: the Ames test, comet test (single-cell gel electrophoresis), chromosomal aberration (CHA), and micronucleus (MN) [132].



Figure 7. Indirect genotoxicity mechanisms of nanomaterials (**A**) [123]; direct and indirect effects of graphene family nanomaterials on DNA (**B**) [124]; genotoxicity of carbon-based nanomaterials (**C**) [128]; the process of DNA fragment interaction with graphene quantum dots as a function of simulation time (**D**) [129].

5. Challenges and Perspectives

The colloidal behavior, biological effects and toxicity of carbon nanomaterials are the focus of attention at present. Among them, GO, as an important member of the carbon nanomaterial family, has attracted extensive research interest from scientists, due to its various excellent properties. The unique physicochemical properties of GO not only mean that it is widely produced and applied and inevitably enters the environment, but that it is also naturally affected by environmental factors, such as light. Here, for aquatic organisms such as algae, we explore the photo-transformation of the physical and chemical properties of GO after it enters the aquatic environment and what type of biological toxicity these physical and chemical properties of GO will bring to algae, and further study the underlying toxicological mechanism. This article summarizes the above content by citing some specific experimental data. At present, the research on related content is relatively limited and faces many challenges, such as the genotoxicity study of GO on aquatic organisms such as algae. More studies should focus on the effects of GO on microbiology in natural surface water systems under light conditions and explore its biotoxicity mechanism, the real environmental risk and application safety. Researchers should analyze the environmental behavior and toxicity mechanism of graphene nanoparticles in a natural environment. Only in this way can the risk diagnosis and early warning be realized, and scientific basis and methods for the prevention and control of graphene nanoparticles can be provided. Thus, the environmental chemical process and biological response mechanism of graphene nanoparticles in the Earth system can be clarified, and the environmental process of nanoparticles can be made transparent, so as to achieve the goal of understanding the environmental trend and risk. This review can provide a theoretical basis and support for evaluating the potential ecological health and environmental risks of graphene-based nanomaterials in real aquatic environments. More researchers are suggested to focus on the environment-related concentration research of GO in the real environment and evaluate the possible ecological risks of GO in the real environmental system. In addition, in view of the complex and diverse aquatic microorganisms in natural water bodies with different spatial and temporal differences, the impact of GO on microbiology should be explored to clarify the light-mediated GO microbial interface and its biotoxicity and molecular mechanism. Future works are suggested to focus on the following points:

- (1) Based on the unique physicochemical properties of GO, researchers have carried out extensive experimental research work. After GO is exposed to the natural environment, it is easily affected by natural environment factors, thereby affecting its physical and chemical properties. In future work, we should first study the photo-transformation mechanism under single illumination conditions, because the mechanism under single illumination will be clearer. In order to provide a theoretical foundation and research basis for studying the mechanism in natural or real environments, we must first explore the change in physical and chemical properties of GO and the mechanism of light transformation under UV and VL illumination, and then explore the influence and mechanism under simulated solar illumination.
- (2) The current research status is that researchers mainly focus on the biotoxicity of GO, without considering the changes in the biotoxicity of GO to aquatic organisms, such as algae, and the mechanism of interaction under light conditions. To solve this, we can set different light conditions for experimental exploration. The laboratory-related studies all include high-concentration and short-term exposure experiments. The concentration of GO used in laboratory experiments is generally high and the exposure time is short. The concentration and time difference between the actual situation and the actual situation will affect the study of its toxicity. Low-dose and long-term exposure experiments should be carried out, taking into account the complex natural environment and low-intensity light effects in the actual environment. Most of the relevant studies are carried out on algae in medium conditions, and in the follow-up study, we can add a simulated natural surface water experimental group, which can better evaluate various toxicity indicators in the real aquatic environment.

(3) Proteomics, metabolomics and genotoxicity are very important elements in the study of the mechanisms of toxicity, but there are not many reports on the application of aquatic organisms such as algae. So far, there are still many shortcomings in this field, and there is a lack of detection and tracking technology specifically for studying the interaction of graphene-like nanomaterials with DNA. In addition, the evaluation database on the types of graphene-based nanomaterials, applied doses, and exposure times is incomplete. In conclusion, continued research is needed to address the above issues, combining several omics to explore the mechanism of toxicity.

Author Contributions: Conceptualization, Y.G. and L.C.; validation, W.Z., L.Z. (Lean Zhou) and S.S.; formal analysis, W.X. and S.L.; resources, P.W. and L.Z. (Letao Zhou); writing—original draft preparation, L.C., S.C., L.Z. (Ling Zhu) and L.L.; writing—review and editing, Y.G.; supervision, S.S.; project administration, Y.G. and S.S. All authors have read and agreed to the published version of the manuscript.

Funding: Financial support from the National Key Research and Development Program of China (2022YFE0105600); National Natural Science Foundation of China (42207337 and 52200185); Natural Science Foundation of Hunan Province (2021JJ40562, 2022JJ40507, 2022JJ40482, 2021JJ40606, 2020JJ4612 and 2020JJ4613); Programs for Science and Technology Innovation, Department of Transportation of Hunan Province (202034); Hunan Key R&D Program Project (2019SK2191); Shanghai Tongji Gao Tingyao Environmental Science and Technology Development Foundation (STGEF) is acknowledged.

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

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