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Application of Clay-Polymer Nanocomposites for the Removal of Toxic Cyanobacteria and Other Phytoplankton from Water – A Laboratory Scale Study

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Abstract

The increasing global frequency of harmful cyanobacterial blooms (CyanoHABs), driven by nutrient enrichment and climate change, poses a severe threat to aquatic ecosystems and public health. This study evaluates the effectiveness of novel clay-polymer nanocomposites—combining the charge-neutralizing capabilities of polydiallyldimethylammonium chloride (PolyDADMAC) with the high density of clay minerals (kaolinite and sepiolite) for the rapid removal of toxic cyanobacteria from water. Laboratory-scale experiments were conducted using *Microcystis aeruginosa*, *Aphanizomenon ovalisporum*, and *Chlorella sp.*, with treatment doses determined by particle charge detector (PCD) measurements to identify the "nominal dose" required for full charge neutralization. Results demonstrate that clay-polymer nanocomposites achieve over 95% removal of turbidity and chlorophyll in *M. aeruginosa* at doses significantly lower (15–20%) than the calculated nominal dose, likely due to specific physical bridging interactions with the cyanobacteria's external exopolysaccharide fibers. In contrast, *A. ovalisporum* and *Chlorella sp.* required doses closer to full charge neutralization for optimal removal. Among the materials tested, kaolinite-based nanocomposites (DKG24) showed slightly superior and more stable performance than sepiolite-based versions. Notably, application at or above the nominal dose was associated with increased soluble microcystin levels, suggesting that excessive polymer concentrations may compromise cell integrity and lead to toxin leakage. These findings suggest that engineered nanocomposites offer highly efficient, scalable technology for CyanoHAB management, provided that operational doses are carefully optimized to maximize biomass removal while minimizing toxin release.

Keywords: clay-polymer nanocomposites; PolyDADMAC; cyanobacteria; clarification; *Microcystis aeruginosa*

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1. Introduction

The escalating global prevalence of harmful cyanobacterial blooms (CyanoHABs), driven by nutrient enrichment and climate change, poses a significant threat to aquatic ecosystems and human health [1–3]. These blooms lead to water quality deterioration through the production of potent toxins (e.g., microcystins, saxitoxins) that contaminate drinking water sources. Addressing this contamination requires efficient, cost-effective, and environmentally sound water treatment technologies [4].

Traditional methods for removing suspended particles including cyanobacteria during the treatment of natural water sources are based on coagulation/flocculation processes. Traditional coagulants as aluminum or iron-based compounds are commonly used, providing up to 90%

reduction of cyanobacteria biomass (e.g., using poly aluminum chloride)[5,6], however the removal efficiency is highly sensitive to pH and alkalinity variations in the source water.

The use of synthetic or natural polymers like polyDADMAC (PD) and chitosan has been investigated to improve microalgae flocculation efficiency, including its interaction with extracellular organic matter[7]. Magnetic polymer-oxide nanoparticles (e.g., polyethylenimine-coated iron oxide) were applied to sediment *Microcystis* colonies[8]. Clay/polymer composites based on clay-enriched local soil modified with cationic agents like starch, proteins, or chitosan were examined, where positively charged agents neutralize the cells, and long polymers (like chitosan) link the small particles into large, rapidly settling flocs[9].

In recent years, nanotechnology has emerged as a promising avenue for advanced water purification. Materials structured at the nanoscale exhibit unique physical, chemical, and adsorption properties, including exceptionally high surface area-to-mass ratios, which enhance their reactivity and binding capacity towards organic and inorganic pollutants [10,11]. Specifically, the development of nanocomposites—materials combining two or more distinct components, often a natural or synthetic polymer embedded nanoparticles—offers a powerful synergy in clarification of water and wastewater[12,13]. These composites combine the neutralizing charge of the polymers with the relatively high density of the clay minerals yielding a combined, one-step coagulation-flocculation process defined as "coago-flocculation". However, a major concern for all agglomeration methods is the potential for cell damage and the resulting leakage of toxins into the water.

This study focuses on the application of novel clay-polymer nanocomposite materials for the effective and selective removal of whole phytoplankton cells, with a particular emphasis on toxic cyanobacteria, from water under controlled laboratory conditions. Following advanced water treatment practices, and cyanoHAB management and mitigation technologies[3,14] this investigation aims to assess the performance, kinetics, and mechanism of action of engineered nanocomposites.

Specifically, we utilize custom-designed clay-polymer nanocomposites to evaluate:

1. Their efficiency in physically removing intact cyanobacteria and other common phytoplankton species from water samples.
2. The influence of key operational parameters (e.g., contact time, nanocomposite dose) on the removal process.

The findings contribute to the ongoing efforts to develop sustainable, highly efficient, and scalable technologies for managing the pervasive threat of toxic cyanobacterial in potable waters.

2. Materials and Methods

2.1. Cyanobacteria and algae

The following species were used in this study: The cyanobacterium *Microcystis aeruginosa* strain INCCA C-1004 (Isolated from Lake Kinneret as reported by (Hadas et al., 2009), the filamentous cyanobacterium *Aphanizomenon (Chrysosporum) ovalisporum* (Forti) strain ILC-164 [15] and the chlorophyte *Chlorella* sp. (INCCA-G1009). These species are maintained at the Israel National Culture Collection of Algae [16]. These strains were cultivated in BG-11 [17] under laboratory conditions using 3 L Erlenmeyer flasks. Cultures were maintained at 20°C and illuminated by warm white light LEDs at intensity of 35 μmol photons m^{-2} s^{-1}. Cultures were monitored for their growth using a standard hemocytometer for tracking cell densities over time. Chlorophyll concentrations of the cultures were determined fluorometry in 90 % acetone extraction using the method of Holm-Hansen et al. [18]. As cultures reached the density of $8{\cdot}10^6$ cell/ml (chlorophyll concentration of ca. 0.1 ug/ml) or higher density as required, they were used to estimate cell colloidal charge density and were further subjected to coago-flocculation experiments.

2.2. Clays and Polymers

Analytical 40% polydiallyl dimethylammonium chloride (PD; medium molecular weight, 200.000 to 350.000) was purchased from Sigma-Aldrich (Israel). Commercial grade coagulant with

40% PD as an active ingredient (FLOQUAT® FL-45) manufactured by SNF Ltd. was purchased from Amgal-Depotchem Ltd. (Beer Tuvia, Israel). Commercial NC24 based on 3% sepiolite clay and 5.4% PD was obtained from BlueGen Water Co. (Tirat Hacarmel, Israel). NC20 clay polymer nanocomposite suspension was prepared as described by [19,20] using 10 g/L sepiolite S9 provided by Tolsa S.A. (Madrid, Spain), and 15 g/L analytical 40% PD solution. DKG24 kaolinite clay polymer nanocomposites[21] were prepared in a similar way, using 10 g/L MPO kaolinite provided by Agat Minerals and Yehu Clays Ltd (Israel) and 45 g/L FL-45. Table 1 presents the different nanocomposites used in this study and their properties.

Table 1. A list of clay-polymer nanocomposites that were used in this study and their properties. .

Name	properties	Clay type	Polymer type	Polymer to clay mass ratio	Reference
NC20	nanocomposite	Sepiolite	PolyDADMAC	0.6:1	[20]
NC24	nanocomposite	Sepiolite	PolyDADMAC	1.8:1	[19]
DKG24	nanocomposite	Kaolinite	PolyDADMAC	1.8:1	[21]
PolyDADMAC	polymer	N/A[2]		N/A[2]	

[1] PolyDADMAC - a homopolymer of diallyl dimethyl ammonium chloride. [2] not applicable.

2.3. Particle charge detector (PCD) measurements

A Mütek PCD 05 instrument (BTG Mütek, Eclépens, Switzerland) was used to quantify electrokinetic colloidal charge of algae and cyanobacteria by measuring the streaming current potential. The technique is used to measure the amount of electrokinetic surface charge directly by combining an electrokinetic streaming current detector probe with titration of a charge-compensating polyelectrolyte[22]. An algae or cyanobacteria suspension was placed in a measuring cell containing a moving piston. As the piston oscillates, it creates a high-shear flow of liquid against the cell walls. Since algae cells typically carry a negative surface charge due to functional groups like carboxyl and hydroxyl, they attract a cloud of positive counter-ions from the surrounding medium. The movement of the liquid by the piston strips these counter-ions away from the cell surface, generating a "streaming potential" [23], which is detected by two gold electrodes. To determine the colloidal charge density, polyelectrolyte titration is performed. A titrant of the opposite charge (in this case- cationic polyDADMAC) was added to the sample. The titration continues until the measured potential current reaches zero, representing the point of zero charge (PZC) or the isoelectric point. Representative results of titration experiments with suspensions of *Microcystis*, *Chlorella* and *Aphanizomenon* are presented in Figure S1.

The volume of titrant consumed at the endpoint was used to calculate the charge demand (measured in mmol$_c$ / L). This value provides a more comprehensive "charge distribution" value than ξ potential alone, as it is correlated to the actual number of available colloidal charges. It should be emphasized that electrokinetic colloidal charge is not equal to the total surface charge due to double layer effects, however- clear correlation between both can be observed [24,25]. This measurement reflects the colloids stability and used to estimate the flocculant concentration required for optimal flocculation [20]. Lower doses than optimal concentration might be ineffective whereas higher doses may lead to "charge reversal" and turbid suspensions. In this study the measured dose by this method is denoted as "*nominal dose*" and is calculated by equation 1:

$$[1]\ ND = \frac{V(PD) \times C(PD)}{V} X\ \frac{1}{D}$$

Where ND is the nominal dose [mg/L], V(PD) is the volume [mL] of the PD solution used to neutralize charges of a given culture, C(PD) is the charge concentration of the PD solution [mmol$_c$ /L], V is the volume of culture used for the measurement [mL] and D is the charge density of the clay-polymer nanocomposite [mmol$_c$ /L].

2.4. Experimental Set Up

Prior any coago-flocculation experiment of algae or cyanobacteria suspension, the colloidal charge density was measured using the Mütek PCD 05 instrument. A 10 ml sample collected from a laboratory culture was inserted in the measuring chamber and titrated with a polyDADMAC solution its charge concentration was predetermined until the instrument reached the point of zero charge. As mentioned in section 2.3, this dose was defined as the ″*nominal dose*″ and was used to estimate the nanocomposite dose required to achieve full neutralization that might yield optimal flocculation of the studied cultures.

Measurements of the optimal dose of a nanocomposite for removing phytoplankton from the suspension, were performed in a series of 250 ml Pyrex beakers filled with 200 ml culture. A predetermined volume of nanocomposites suspension or polyDADMAC solution were applied to each beaker to set a linear gradient of nanocomposite concentration below and above the "*nominal dose*". No nanocomposite or polymer was applied at one beaker which was used as a control. Immediately after the application, the suspension in each beaker was manually stirred with a glass stick for 15 seconds. It should be emphasized that one of the advantages in the use of nanocomposites for coago-flocculation is the very fast interaction that makes the need for two tanks (one for coagulant and another for flocculant) with different mixing speeds unnecessary. The treated suspensions were left to stabilize and sampled after 1, 2 and 3 hours. Samples were withdrawn with a pipet from halfway between the surface of the suspension and the bottom of the beaker or from the apparently most clarified layer. Algae and cyanobacteria removal efficiency were estimated in the collected samples by measuring turbidity using a LaMotte 2020i turbidimeter (Chesterton, MD, USA) and chlorophyll concentration measured as mentioned in section 2.1. Prior each sampling event the beakers were photographed to provide a visual effect of the flocculation process.

During the last sampling event (3 hrs) from the *Microcystis* cultures an additional 50 ml sample was withdrawn from each beaker from the clarified layer. Samples were filtrated on 0.45-micron Whatman, and the filtrate was frozen and freeze dried until subjected to quantitative analysis of microcystins using HPLC- DAD system (see below). Unfiltered samples were collected from untreated *Microcystis* suspension and freeze dried prior to the analysis for total microcystins concentration in the sample.

2.5. Analysis of Microcystins

Both the whole (unfiltered) and filtered freeze-dried samples were extracted in methanol and their toxin content determined by High Performance Liquid Chromatography and diode array detector (DAD) (Lawton et al., 1994) for microcystin concentration using MC-LR, MCRR and MC-YR as standards. Microcystin concentration was expressed in microgram per litter (μg/L).

3. Results

3.1. Cell Surface Charge Density and Nominal Dose

Two cyanobacteria species and a single chlorophyte species were used in this study as cultures with different biomass concentrations. The cultures properties measured by turbidity and chlorophyll concentration are presented in Table 2. For each culture a charge concentration was estimated based on PCD measurement and charge density was calculated as the ratio of charge concentration and chlorophyll concentration. A nominal dose was estimated for each culture and the corresponding nanocomposite (Table 2). Interestingly, a linear correlation was observed between the chlorophyll concentration and the measured charge concentration (CC) for the entire data set presented in Table 2. ($CC = 22.4xChl$ $R^2 = 0.835$ (Figure S2). Based on the nominal dose, a set of linear gradients of nanocomposite concentration was used to optimize the removal of the biomass in coago-flocculation experiments.

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3.2. Algae and Cyanobacteria Removal by Nanocomposites

3.2.1 *Microcystis* – All three nanocomposites and raw PD were highly effective in removal of *Microcystis*, based on both turbidity and chlorophyll (Figure 1). DKG24 and PD demonstrated 90-95% removal even at a dose ranged between 15 and 20% of the calculated nominal dose, whereas NC24 and NC20 required higher doses, but still below the nominal dose, to achieve 90% removal. Based on both parameters the application of PD near or above the nominal dose imposed significant reduction of the removal rates of both parameters, turbidity and chlorophyll. Much higher stability of the high removal rate was documented with the nanocomposites at doses higher than the nominal dose (Figure 1). Visual observation of optimal clarification of the *Microcystis* suspension at concentrations significantly lower than the calculated nominal dose is presented in Figure 2A for NC24, where samples exposed to different concentrations of NC24 and settled for 1 hr before photographed. Note that suspension clarification and biomass flotation were recorded with a dose of 4.2 mg/L whereas the calculated nominal dose was estimated at almost 2-fold higher concentration (8.2 mg/L, circled value in Figure 2A).

In an attempt to further clarify the discrepancy between the calculated nominal dose for *Microcystis* culture and the effective, much lower dose, that imposed suspension clarification, the PCD titration procedure was visually followed as presented in Figure 2B. *Microcystis* suspensions were titrated with cationic polymer (PD) or NC24 nanocomposite at amounts equivalent to 0, 25, 50, 75, 100 or 150% of the amount required to neutralize the charge of the culture (1.249±0.187 $\mathrm{mmol_c}$ /L). After 1 hr settling, cell aggregation was observed already at 25% neutralization, while complete aggregation and turbidity removal is achieved already at 50% neutralization (Figure 2B).

Table 2. Basic properties of the algae and cyanobacteria cultures used in the study: Turbidity, chlorophyll concentration, charge density (calculated based on charge concentration derived from PCD measurements and normalized to chlorophyll concentration) and nominal dose estimated based on PCD measurements to predict the dose of a given nanocomposite (or PD) required to achieve optimal particle removal.

Organism	Turbidity [NTU]	Chlorophyll [μmol/L]	Charge density[1] [$\mathrm{mol_c}$ /mol Chl]	Nominal dose[2] [mg/L]
Microcystis	54	0.9	41.9	5.6 (DKG24)
	100	1.6	69.1	10.8 (PD)
	157	1.8	21.8	14.8 (NC20)
	264	2.9	13.0	8.2 (NC24)
	379	6.1	18.0	15.4 PD)
Aphanizomenon	121	1.5	24.6	5.7 (GKG24)
	275	3.2	15.5	10.8 (NC24)
	1224	2972	36.3	12.1 (PD)
	3055	6193	31.8	31.1 (PD)
Chlorella	55	3347	12.0	29.3 (NC20)
	99	3420	13.8	8.2 (DKG24)
	104	4424	16.1	17.4NC24)

1 Charge density of a given culture was estimated from PCD measurements of charge concentration and chlorophyll concentration. 2 Nominal dose was calculated for a given suspension based on the PCD measurement and a given nanocomposite to be used, as described in Eq 1. The nanocomposites (NC24, DKG24, NC20) or polyDADMAC (PD) used for the determination of nominal concentration are indicated in parentheses.

.3.2.2 *Aphanizomenon* – A similar removal patterns of *Aphanizomenon* filaments based on both turbidity and chlorophyll were recorded for all three nanocomposites and PD (Figure 3). Maximal removal rate based on both suspension turbidity and chlorophyll concentration was achieved at a dose near the estimated nominal dose. All three examined nanocomposites performed slightly better than PD, with NC24 and DKG24 achieved 80-90% removal rate at lower relative dose than NC20. High removal rate was maintained even when nanocomposites were applied at doses higher than the nominal dose, whereas increased turbidity and reduction of the removal rate was observed when PD applied at a dose 4 times higher than the nominal dose (Figure 3). Visual observation of dose effect on clarification of *Aphanizomenon* suspensions is presented in Figure 4 for DKG24. Suspension clarification and biomass settling at ca 90% were recorded with a dose of 4.2 mg/L whereas the calculated nominal dose was 5.7 mg/L (circled value in Figure 4).

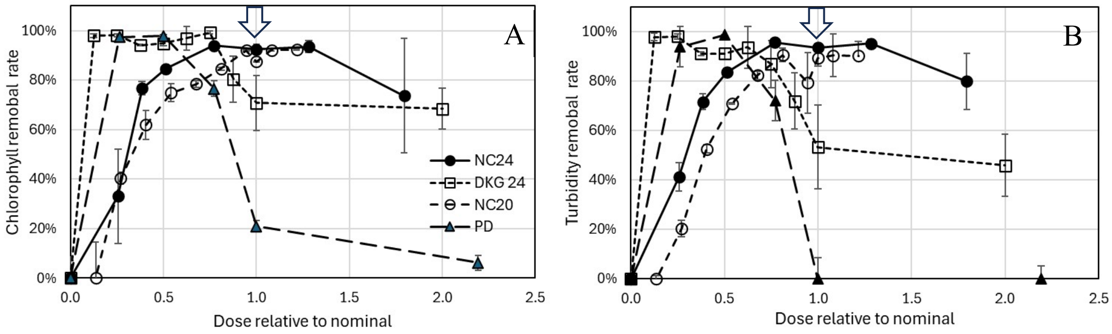

Figure 1. Removal rate of chlorophyll (A) and turbidity (B) following exposure of *Microcystis* culture to different doses of nanocomposites (NC24, DKG24, NC20) or polyDADMAC (PD). Removal rate is calculated relative to initial concentration, and the applied dose is normalized to the nominal dose, estimated based on PCD measurements for each culture. The vertical arrow indicates an applied dose equal to nominal dose. Values present the average of 3 independent measurements and ± STD.

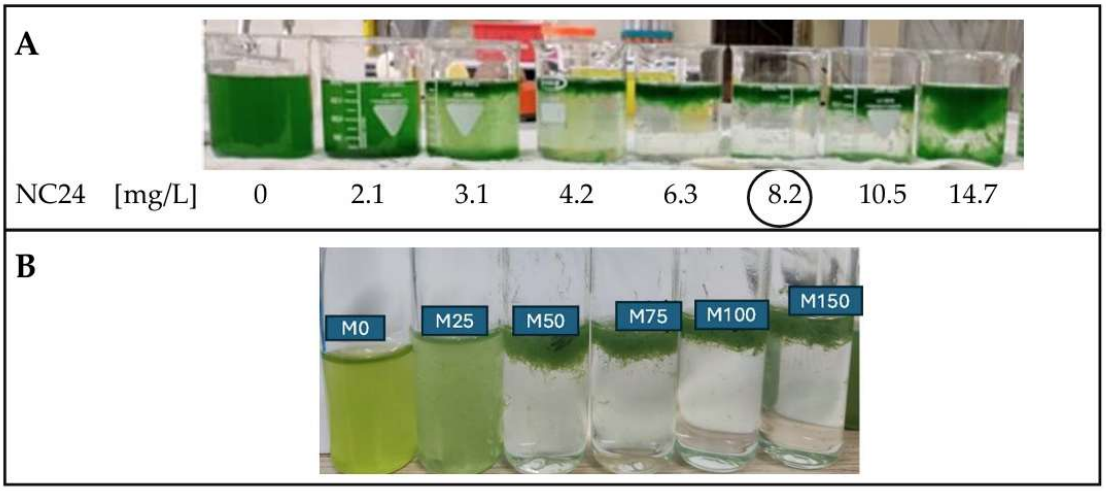

Figure 2. A - Visual demonstration of *Microcystis* culture exposed to different doses of NC24 after 1 hr. The nominal dose [8.2 mg/L] is indicated by a circle. B – Clarification observed in *Microcystis* suspension 1 hour after addition of different doses of PD. Numbers indicate the percentage of the PD solution used relative to the volume required to achieve charge neutralization. The letter M stands for *Microcystis*.

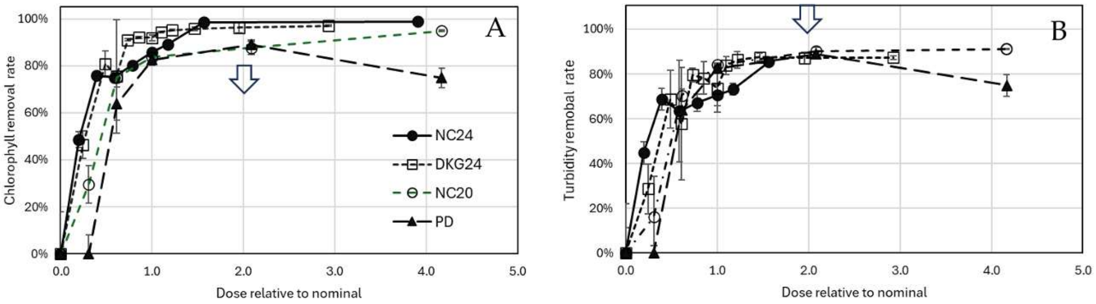

Figure 3. Removal rate of chlorophyll (A) and turbidity (B) following exposure of *Aphanizomenon* culture to different doses of nanocomposites (NC24, DKG24, NC20) or polyDADMAC (PD). Removal rate is calculated relative to initial concentration, and the applied dose is normalized to the nominal dose estimated based on PCD measurements for each culture. The vertical arrow indicates an applied dose equal to nominal dose. Values present the average of 3 independent measurements and ± STD. .

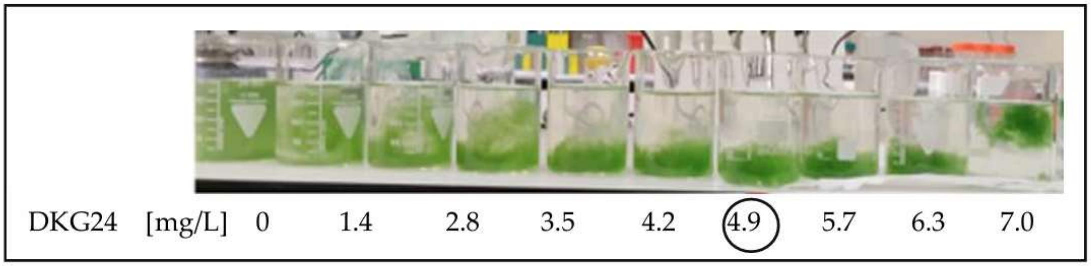

Figure 4. Visual demonstration of Aphanizomenon culture exposed to different doses of DKG24 after 1 hr. The nominal dose (5.6 mg/L) is indicated by a circle.

3.2.3 Chlorella – Above 90% removal rate of chlorophyll was recorded with a nominal dose of DKG24. This high removal rate was maintaned at higher doses, 2.5 and 3.5 times the nominal dose. Similar pattern of the DKG24 effeciency was recorded for tubidity removal (Figure 5). Maximal chlorophyll removal in Chlorella cultures was obtained by NC24 around the nominal dose but significantly reduced at higher doses. Similar pattern but at much lower removal rates was recorded for turbidity. The response of the Chlorella culture to NC20 suybstentially deviated from the two other nanocomposites as maximal removal rates were recorded at doses much lower than the nominal concentration and slightly reduced as the NC20 dose exceeded the nominal dose. Visual observation of dose effect on clarification of Chlorella suspensions is presented in Figure 6 for DKG24. Suspension clarification and biomass settling at ca 90% was recorded with a dose of around the calculated nominal dose, 8.2 mg/L (circled value in Figure 6A).

The PCD titration procedure for Chlorella cultures was visualized to confirm that optimal removal occured at charge neutralization as opposed to Microcystis cultures as presented in Figure 6B. Chlorella suspensions were titrated with cationic polymer (PD) at amounts equivalent to 0, 25, 50, 75, 100 or 150% of the amount required to neutralize the charge of the culture (0.482±0.070 mmol$_c$ /L). After 1 hr settling, aggregation and culture clarification was observed only at 100% neutralization (Figure 6B). Unlike in Figure 2, a tight sedimented aggregates are observed rather then biomass flotation..

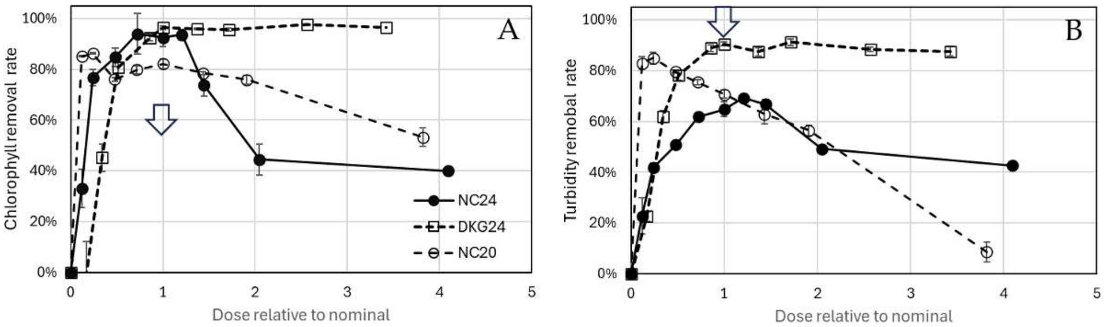

Figure 5. Removal rate of chlorophyll (A) and turbidity (B) following exposure of *Chlorella* culture to different doses of nanocomposites (NC24, DKG24, NC20). Removal rate is calculated relative to initial concentration, and the applied dose is normalized to the nominal dose estimated based on PCD measurements for each culture. Values present the average of 3 independent measurements and ± STD.

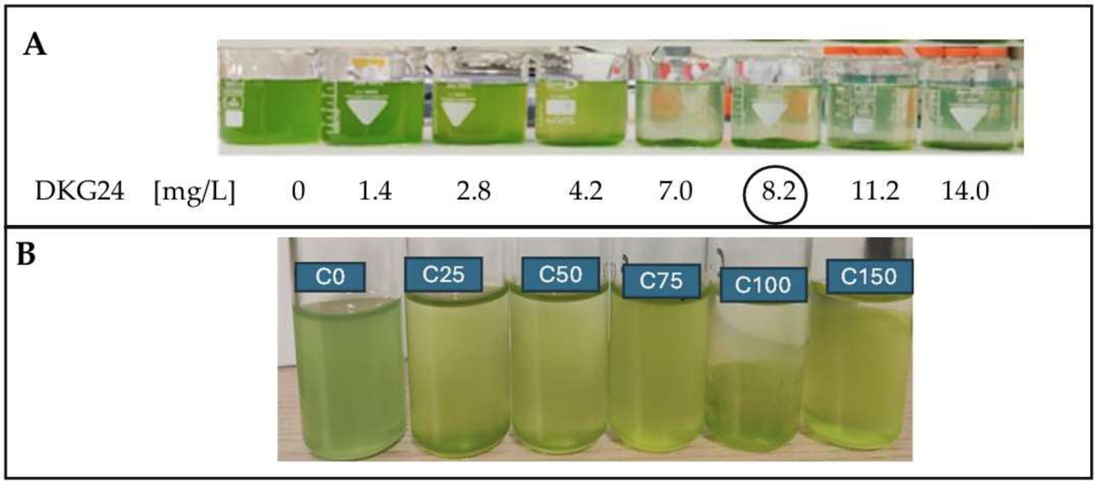

Figure 6. A - Visual demonstration of *Chlorella* culture exposed to different doses of DKG24 for 1 hr. The nominal dose [8.2 mg/L] is indicated by a circle. B – Clarification observed in *Chlorella* suspension 1 hour after addition of different doses of PD. Numbers indicate the percentage of the PD solution used relative to the volume required to achieve charge neutralization. The letter C stands for *Chlorella*.

3.3. Charge neutralization and biomass aggregation

Differences in the aggregation pattern between the different phytoplankton species tested were recorded. The chlorophyte *Chlorella* and filamentous cyanobacterium *Aphanizomenon* present the coago-flocculation pattern described in previous studies [13,20], where efficient clarification occurs as 80-120% of the particles' surface charge is neutralized (Figures 4 and 6). The cyanobacterium *Microcystis aeruginosa* presents a different pattern where efficient coago-flocculation occurs as 50% of the particles' surface charge is neutralized (Figure 2) or even less. A clear demonstration of these different patterns is depicted in Figure 7 where clear aggregates and clarification of the suspension were observed already 5 minutes after the addition of PD at only 25% of the amount needed for charge neutralized of *Microcystis* culture, but not in *Chlorella* or *Aphanizomenon* cultures.

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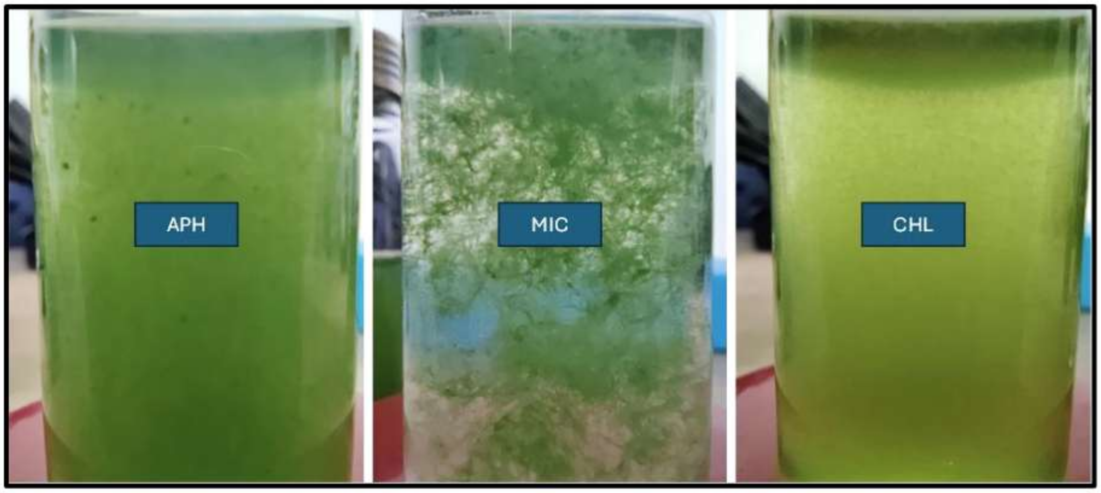

Figure 7. Clarification observed in *Aphanizomenon (APH). Microcystis (MIC) or Chlorella (CHL*) "young" suspensions 5 minutes after addition of PD at doses equivalent to the dose required to neutralize only 25% of the relevant charges.

It is interesting to note that the *Microcystis* culture presented in Figure 7 was relatively fresh culture whereas the culture presented in Figure 2B was older. Culture aging may explain the observed differences, 25% charge neutralization in the "fresh" culture, whereas 50% charge neutralization was required in the “old” culture for efficient culture clarification. Stationary phase cultures release organic substances that can inhibit flocculant performance [26,27]. Still a low dose, much lower than the estimated nominal dose, efficiently impose coago-flocculation of *Microcystis* cells as opposite to the other cultures. This observation may be attributed to the unique external structure of *Microcystis* cells and specifically to the large number of external fibers, exopolysaccharides that the cells secrete. These fibers are extremely long up to several millimeters in length [28]. Furthermore, comparison between doses of NC24 and corresponding doses of alum in terms of the percentage of charge neutralization applied on a young suspension of *Microcystis* (Figure S3), showed that nanocomposites imposed the formation of large aggregates at a dose as low as 20% of the neutralization dose, and complete clarification at 40%. During the same period in alum treatment, very small aggregates began to form and minor clarification was observed only at a dose of 100% neutralization.

Effect of nanocomposites on the removal of microcystina

The interaction between high-charge density cationic polymers such as PD and the cyanobacterial cells can lead to leakage of intracellular metabolite, including microcystins (MCs) from the cytoplasm into the surrounding water. Consequently, extracellular microcystin concentration may increase during the flocculation process. Measurements of the soluble MC in the coago-flocculation experiments indicated that the MC soluble pool remained relatively constant with doses lower than the nominal dose, however application of nominal dose or higher imposed substantial increase in the soluble pool (Figure 8).

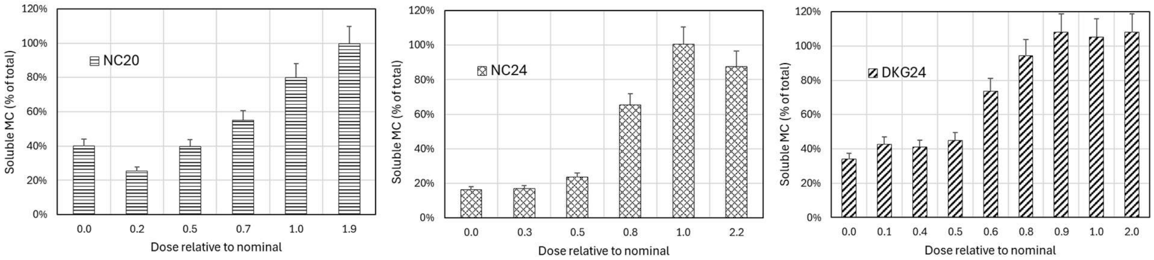

Figure 8. **S**oluble microcystins (MC) normalized to total, in *Microcystis* cultures treated with different doses of nanocomposites NC 20, NC24, and DKG24. Histograms represent the sum of all MC congeners and std. The applied dose was normalized to the nominal dose estimated based on PCD measurements for each culture.

These results indicate that in the case of *Microcystis*, the added nanocomposites first interact with the cell surface complexes, namely the external fibers, to bridge between cells at a dose considerably lower than the nominal dose, forming large aggregates. Only at higher doses, the positively charged polymer penetrates the outer cell layer and interacts directly with the cytoplasmic membrane. This leads to membrane stretching, and pore formation and leakage on the internal metabolites as reflected by the increase concentration of the soluble MC. Further analysis of the soluble MC congeners in the soluble fractions prior and after application of nanocomposites indicates minor effects of the dose applied on their relative abundance. The *Microcystis* strain used in this study produces four MC congeners (MC-RR, MC [D-Asp3]-RR, MC-WR, and MC [D-Asp3]-WR) with some variations documented under different culture age [29]. The relative abundance of these MC congeners found in the soluble fraction of *Microcystis* cultures exposed to different doses on nanocomposites is presented in Figure 8. Only minor changes were recorded in the relative abundance of MC congeners when NC20 nanocomposite was used at a wide range of doses. Applying NC24 and DKG24 nanocomposites imposed some variations in MC RR, MC WR and MC 3ASP WR congeners with doses presenting 50% of the nominal dose and higher (Figure 8).

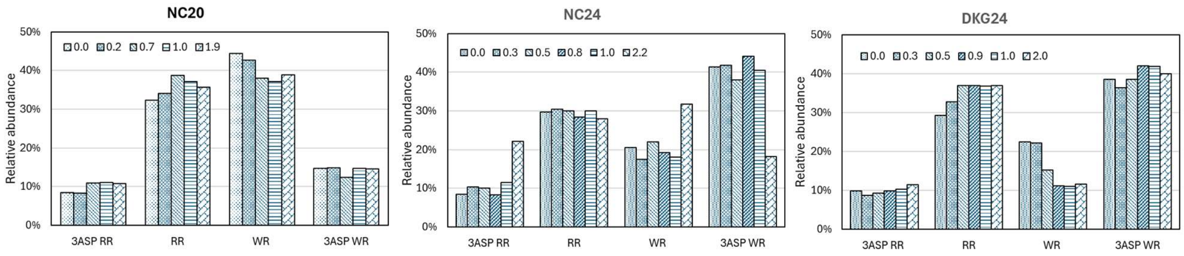

Figure 9. The relative abundance of major microcystin congeners found in the soluble fraction of *Microcystis* cultures exposed to different doses on nanocomposites. The data is presented for different nanocomposites doses normalized to the nominal dose estimated based on PCD measurements for each culture.

4. Discussion

This study demonstrates that clay-polymer nanocomposites are effective agents for the removal of phytoplankton (algae and cyanobacteria) from water, although their efficiency and mechanism of action vary significantly among species. The core hypothesis—that these materials combine the charge-neutralization capabilities of polymers with the ballast-increasing density of clay minerals—was supported by the rapid sedimentation and high removal rates observed across multiple taxa.

An interesting finding is the unique behavior of *Microcystis aeruginosa* during the coago-flocculation process. Unlike *Chlorella* sp. and *Aphanizomenon ovalisporum*, which followed standard patterns requiring near 100% charge neutralization for optimal removal, *Microcystis* achieved over 95% removal at doses as low as 15–20% of the calculated nominal dose. This high sensitivity likely stems from the extensive exopolysaccharide (EPS) fibers secreted by *Microcystis*. These long external fibers probably act as "bridges", allowing the nanocomposites to aggregate cells through inter-particle bridging even before the surface charge of the individual cells is fully neutralized.

While the nanocomposites proved effective for biomass removal, the study highlights a significant operational risk: the release of intracellular toxins. At or above the nominal dose required for full charge neutralization, a substantial increase in soluble microcystins (MCs) was documented. This suggests that high concentrations of the cationic polymer (PD) can penetrate the outer cell layers

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and interact with the cytoplasmic membrane, leading to pore formation and the subsequent leakage of metabolites. Interestingly, the relative abundance of MC congeners remained largely stable across different doses, indicating that the leakage is a physical breach of the membrane rather than a selective biochemical response.

Among the tested materials, the kaolinite-based DKG24 showed slightly superior performance and higher stability at supra-optimal doses compared to the sepiolite-based NC24 and NC20. Furthermore, all nanocomposites exhibited a "buffering" effect not seen with raw polyDADMAC. While high doses of raw PD led to "charge reversal" and a subsequent decline in removal efficiency (re-stabilization of the suspension), the clay-based composites maintained high removal rates even when applied significantly above the nominal dose.

5. Conclusions

This research confirms that clay-polymer nanocomposites may be implemented as potent agents for managing harmful cyanobacterial blooms. They present exceptional efficiency as providing over 95% removal of turbidity and chlorophyll, with *Microcystis aeruginosa* being particularly susceptible due to its EPS fiber-mediated bridging. Mechanistically, the removal process is driven by a combination of charge neutralization and physical bridging. However, the presence of external fibers allows for effective treatment at doses significantly lower than those predicted by standard particle charge detector (PCD) measurements. Treatment conducted at sub-nominal doses also minimizes the risk of microcystin release, since high doses of cationic polymers can damage cell membranes, leading to toxin leakage. Unlike raw polymers, clay-based nanocomposites (especially DKG24) maintain high removal efficiency even at supra-optimal doses, reducing the risk of treatment failure due to overdosing. Finaly the rapid interaction of these materials simplifies water treatment by potentially combining coagulation and flocculation into a single, high-speed step. Future development should focus on testing these materials in natural water bodies containing dissolved organic matter (DOM) and investigating the long-term stability of the resulting sediment to ensure that captured toxins are not re-released into the environment.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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Abbreviations

The following abbreviations are used in this manuscript:

PD	PolyDADMAC
PCD	Particle charge detector
MC	Microcystin
CC	Charge concentration
HPLC- DAD	High Performance Liquid Chromatography and Diode array detector
CyanoHABs	Harmful cyanobacterial blooms

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