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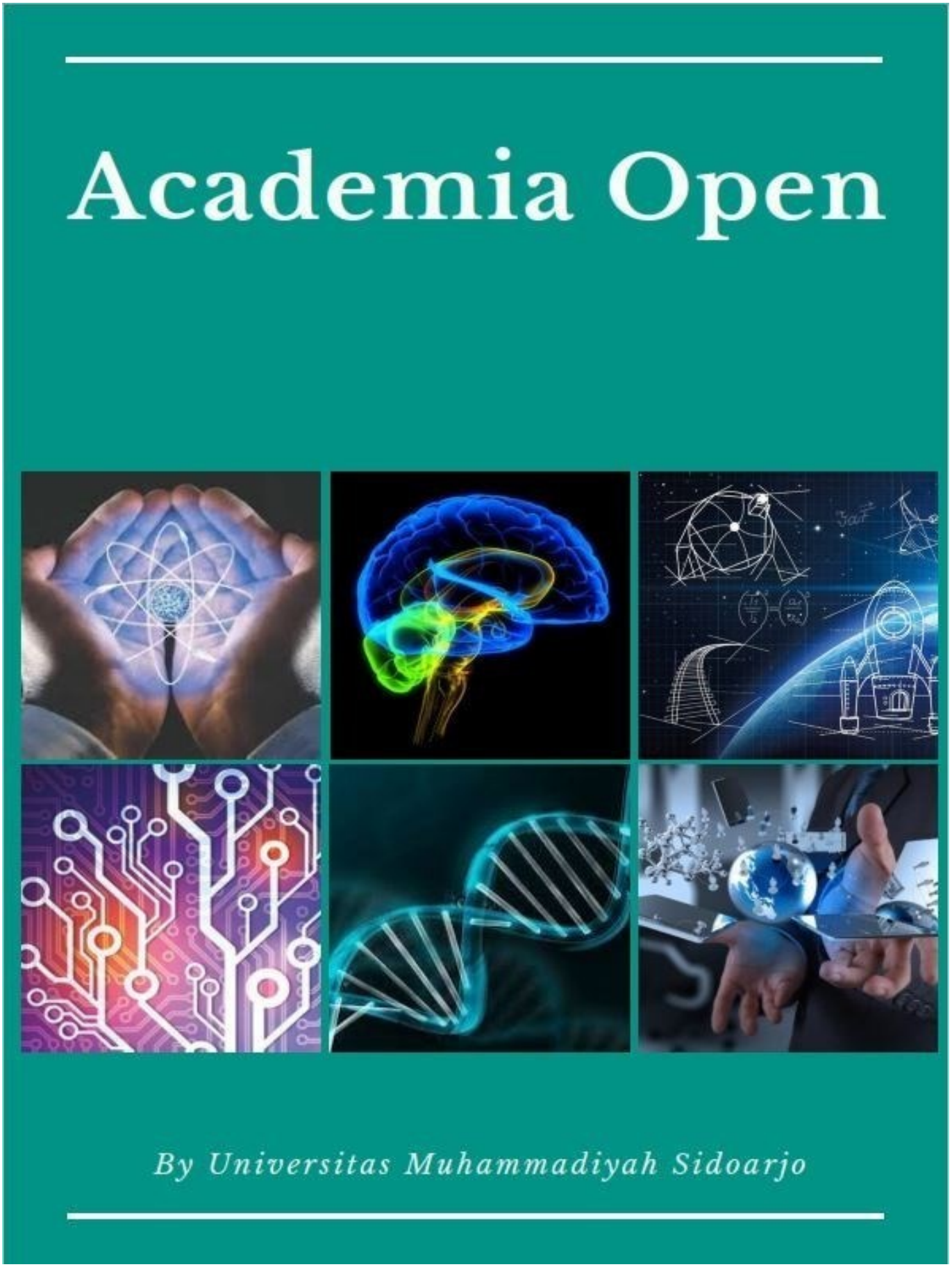

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Microalgae as Biosensors for Heavy Metal Detection Using Spectroscopic Techniques

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Abstract

General Background: Heavy metal pollution in aquatic ecosystems represents a persistent environmental challenge due to their non-biodegradable nature and bioaccumulation in the food chain, posing severe risks to human and ecological health. Specific Background: Traditional detection techniques like atomic absorption and ICP-MS are effective but expensive and time-consuming, driving interest toward biological alternatives such as microalgae-based biosensors that can detect and respond to pollutants through physiological and biochemical changes. Knowledge Gap: Limited studies have compared the biosensing capacities of Ankistrodesmus falcatus and Scenedesmus obliquus using integrative spectroscopic and metabolomic analyses under realistic contamination conditions. Aims: This study investigates the biosensing and bioremediation abilities of these two microalgal species in detecting lead, cadmium, and mercury in contaminated water. Results: Both species showed high metal removal efficiency (up to 97% for Hg^{2+}), with Ankistrodesmus exhibiting faster biosorption and stronger sensitivity, while Scenedesmus demonstrated higher resilience to lead. Spectroscopic analyses revealed distinct functional group shifts, reduced chlorophyll fluorescence, and metabolic reprogramming under metal stress. Novelty: The integration of FTIR, fluorescence, and GC-MS analyses provided a comprehensive biochemical understanding of algal stress responses. Implications: These findings support the development of cost-effective, eco-friendly biosensing systems for monitoring and remediating heavy metal pollution in aquatic environments.

Highlight :

- Two algal species showed strong potential as biosensors for detecting heavy metal contamination in water.
- Spectroscopic analyses confirmed biochemical and physiological changes under metal exposure.
- Ankistrodesmus responded faster to mercury, while Scenedesmus showed better tolerance to lead.

Keywords : Microalgae, Heavy Metal Pollution, Biosensing, Spectroscopic Techniques, Water Quality

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Introduction

Among the microscopic organisms in the water, plankton are the most interesting, and they combat toxic heavy metals. These organisms have also evolved to help them survive in this toxic world. Due to the strength and effectiveness of environmental pollutants, various algae have been highlighted for their effectiveness in combating environmental pollutants [1].

Algae absorb metals and reduce their effects. The reason for their limited cultivation is their slow growth [2]. To reduce the impact of heavy metals, algae perform photosynthesis. Their simple structure and rapid reproductive cycle enable them to reduce the effects of metals [3].

Algae are characterized by their high and distinct ability to treat the effects of heavy metals and reduce toxicity. We have witnessed the emergence of foreign pollutants in our world, and the quantities of dangerous heavy metals have increased.With the ever-growing population, shelves are filled with goods, resulting in the constant production of waste and industrial wastewater. This waste contains massive quantities of heavy metals, and many of these foreign materials invade the ecosystem, both on land and in water. Even more dangerous is the phenomenon of biomagnification, where heavy metals increase in the food chain, posing a significant threat to human life. Although some heavy metals are essential for vital cellular functions in various life forms, their replacement by toxic heavy metals can be a time bomb, leading to mutations, immune and neurological defects, and even contributing to the development of cancer and other devastating health risks. Once these toxic heavy metals become a threat to humans and all living organisms, animals require an effective strategy and effective measures to clean the environment of these pollutants [4].

The pollution of ecosystems with Heavy metals such as lead (Pb^{2+}), cadmium (Cd^{2+}) and mercury (Hg^{2+}) also pose a threat because these metals do not biodegrade and accumulate in food. The reason for this water pollution is mining, dumping industrial waste, painting, battery manufacturing, etc., and as a result, it causes oxidative stress, damage to cell membranes, and inhibition of enzymes [5].

Biosensing methods such as algae have emerged due to their high ability to detect toxic substances and reflect the physiological effect at the cellular level instead of the known methods such as atomic absorption spectroscopy (AAS), and Methods inductively coupled plasma mass spectrometry (ICP-MS), and electrochemical sensors [6]. An abiosensor is essentially a fascinating analytical device designed to detect specific chemical substances. This device uniquely combines a biological component with a physicochemical reagent. Essentially, this device relies on a sensitive biological element, such as tissues and microorganisms, as well as organelles, cell receptors, enzymes, antibodies, and finally nucleic acids. These components, whether derived from natural biological sources or crafted to mimic them, engage with the analyst being studied. They interact, bind, or recognize the target substance, making the biosensor an intriguing intersection of biology and chemistry.

Human activities have also been responsible for the increased presence of heavy metals, such as the increased use of fertilizers in agriculture and the increased discharge of hazardous waste into water. Heavy metals persist and accumulate in the aquatic environment because they are not decomposed, causing increased pollution. They also lead to toxicity to living organisms [7]. Therefore, they must be treated before being discharged into water. Lead, mercury, and cadmium ions in the aquatic environment at low concentrations affect physiological functions due to their toxicity, and they also impact the metabolic and chemical processes of organisms exposed to polluted water. Furthermore, they affect human health and pose risks to humans, whether through direct exposure to persistent organic pollutants or when consuming food or drinking contaminated water [8].

Heavy metals, when present in large quantities, are a cause of serious health problems that can negatively impact human life. They are known to be associated with muscle deterioration, complicated neurological conditions, and destructive effects on physical structure. Prolonged exposure to these toxic metals can lead to muscle atrophy and serious diseases such as Alzheimer's disease, various types of cancer, and even multiple sclerosis [9]. But the damage doesn't stop there; their negative effects on lung, liver, kidney, and even blood components have also been documented, not to mention disturbances to the central nervous system and mental processes [10]. When these heavy metals are discharged into water sources, the problem escalates to manifest itself in physical, chemical, and biological changes, leading to fluctuations in density, species diversity, population composition, and community management. The concentration of heavy metal ions is a crucial factor influencing this transformation, as these metals can disrupt enzyme function. Surprisingly, there are no recyclable organic pollutants, and various metals remain trapped within the ecosystem for long periods. In fact, relying on physical and chemical remediation methods is not only uneconomical but also produces a huge amount of hazardous waste. Given these challenges, interest is growing significantly toward restoring the balance of hazardous metals, capitalizing on the potential of biosensitive microbes [11]. These efforts indicate steps toward a positive reversal that requires research and development.

Bioremediation is a technology that uses algae and other organisms to degrade pollutants. It is preferred because it is inexpensive compared to other analytical methods, environmentally friendly, and harmless compared to some other analytical methods. These organisms utilize changes in ion permeability and cellular chelation processes to remove and absorb metals [12].

The purpose of using the algae Ankistrodesmus falcatus and Scenedesmus obliquus is due to the limited research on them and their high importance. They can absorb harmful metals and reduce their impact on water. They also have a unique ability to detect harmful metabolic and physiological indicators. This will be explored and their capabilities determined through a variety of measurements, such as ultraviolet-visible spectroscopy, including Fourier transform infrared (FTIR) and chlorophyll fluorescence (IR). For more accurate results, gas chromatography-mass spectrometry (GC-MS) analysis is used [13].

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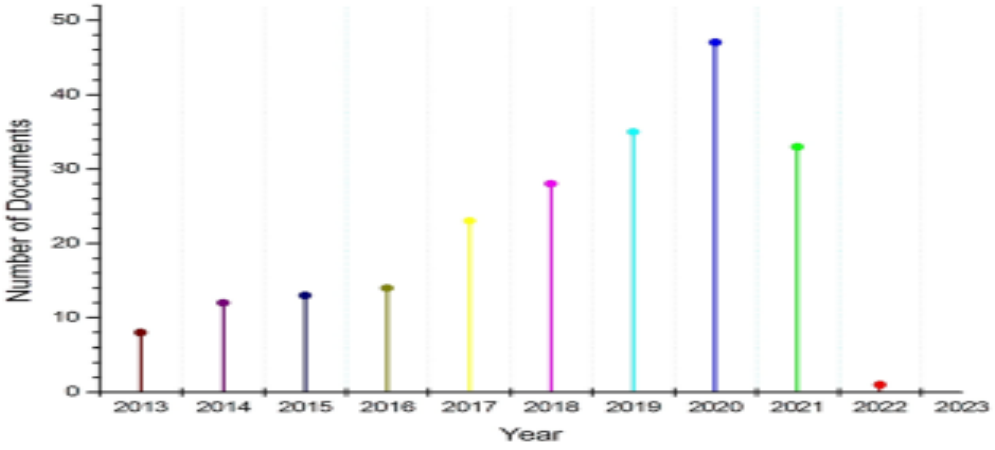

Figure 1 shows the data ({microalgae}, {heavy metals}, and {detoxification}) from 2013 to 2020, compiled from Scopus.

Materials and Methods

A. Sampling and Storage

The sources of this experiment were collected from agricultural and industrial wastewater, at a depth of 10 to 50 cm, during the period from May to September **[14].** After that, to transport the water to the laboratory, the samples were stored in containers free from any contaminants. To ensure this, certain measures were taken, such as sterilizing the container, deionizing it, made of polyethylene, and finally, it was tightly closed, so as to prevent the entry of any air, at a temperature of 4 ± 1 °C, and transferred to the laboratory. This maintains the physical and chemical conditions and prevents the introduction of any bacterial contamination. This was done according to the American Public Health Association **[15].**

B. Isolation and Cultivation of Microalgae

The sample was transferred to blue-green 11 (BG11) medium, as described **by [16] and [17].**It was maintained in optimal cultures at pH 7.8 and 28°C, under constant illumination of 800 lux. After two weeks, the cultures were cultured. Fifteen days later, we removed another single colony and examined it under a light microscope. We followed the criteria established by **[18],** we then performed further rigorous examinations using polymerase chain reaction (PCR) to confirm the accurate classification of the algal genera, utilizing the technology to ensure that each step was carried out accurately and professionally.

Stock solutions (1000 mg/L) were prepared, dissolved in high-purity deionized water, using analytical compounds, such as $Pb(NO_3)_2$ for lead, $HgCl_2$ for mercury, and $CdCl_2$ for cadmium. All solutions It was filtered through 0.22 µm syringe filters to ensure proper sterility and then stored in acid-cleaned amber bottles at 4°C **[19].**

C. In-situ Measurement and Environmental Context

Physical and chemical properties were measured the environment surrounding the water was determined at the beginning of the experiment, through several measurements such as temperature, pH, and electrical conductivity. Dissolved oxygen must be measured using a calibrated portable probe (such as Hach HQ40d or equivalent). These measurements reflect the actual conditions in the contaminated environment used for the experiment. To conduct the experiment, suspended particles must be removed. Therefore, the collected the samples are filtered through 0.45 µm cellulose nitrate membrane filters. The samples were acidified to a pH of less than 2 using ultrapure nitric acid (HNO_3). The analytical salts of lead nitrate [$Pb(NO_3)_2$], cadmium chloride [$CdCl_2$ 2.5H_2O], and mercury (II) chloride [$HgCl_2$] were used. All solutions and through 0.22 micrometer syringe filters it was filtered. to ensure sterility. Working solutions were diluted from stock to the desired concentrations before each experiment and stored in acid-cleaned amber bottles at 4°C until use **[20].**

D. Exposure Design and Experimental Setup

Exponentially growing Ankistrodesmus falcatus and Scenedesmus obliquus cultures were exposed to increasing concentrations of heavy metal ions. A solution containing specific ratios of heavy metal ions, at concentrations of 0.5 mg/L to 5 mg/L, was prepared in sterile 250 ml conical flasks, each containing 100 ml of BG-11 growth medium. Each flask was injected with 10 ml of the medium. A metal-free control group was placed under identical conditions.

Each treatment was performed in triplicate (n = 3) to ensure statistical validity. During the 14-day exposure period, the flasks were placed under the same environmental conditions used during cultivation, as previously described. The pH of the medium was recorded and maintained within the optimal range (6.8–7.2) to minimize associated stress. To measure growth, oxidative stress indicators, pigment content, and biochemical changes occurring, it was necessary to ensure that the water was carefully stored at an optimal temperature (-20°C) for analyses that required this, or dried for other analyses that required a dried sample **[21].** To ensure the accuracy and reliability of the results, the sample had to be collected and centrifuged at 500 rpm for only 10 minutes.

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E. Spectroscopic Detection and Biosensing Evaluation

UV-Vis Spectroscopy for Growth and Pigment Analysis

Ultraviolet-visible (UV-Vis) spectroscopy is an analytical technique developed to provide more precise and complex details, which are necessary for molecular structure, concentration, and behavior measurements. This is achieved by using visible light, which measures and analyzes molecular interactions through several steps: pigment analysis, quantification, and monitoring the absorption and transmission of light at specific wavelengths.

Dose-time interactions were studied using multiple different responses to understand the basis for inhibition of photosynthetic efficiency and microalgal growth. Using spectrophotometry and chlorophyll content, optical density (OD) was measured at a wavelength of 680 nm, and growth was recorded. Measurements were performed using a Shimadzu UV-1800 spectrometer, and data were expressed in absorbance units (AU) **[22].**

Fourier transform infrared (FTIR) spectroscopy for the identification of functional groups

This method is used when we want to identify and synthesize chemicals, as well as complex materials that are extremely difficult to analyze. It also helps measure infrared absorption and emission. When measuring algae, we grind and dry them, and use a Bruker Alpha II spectrometer in the 400-400 cm^1 range to record the spectra. was used in the 4000-400 cm^1 range to record the spectra. Metal reactions result in chemical or biological changes in sugars, proteins, and lipids. We use the changes corresponding to these four groups to determine these changes: first, hydroxyl groups (–OH), second, carboxyl groups (–COOH), third, amide groups (–NH), and finally, phosphate groups (PO_4^{3-}).

Fluorescence Spectroscopy to Detect Oxidative Stress

Chlorophyll fluorescence patterns and the amazing ability of chlorophyll molecules to emit light after absorption can be useful. This demonstrates how these molecules lose energy in multiple, different ways, adding depth and mystery to their interactions. This analysis is important when studying plant physiology in photosystem II (PSII). It can also reveal the efficiency of photosynthesis and reveal plant responses to stress caused by metal exposure Chlorophyll-a emits a fluorescent signal that peaks at a wavelength of approximately 680 nm, and measuring this emission provides a rapid and non-invasive indicator of photosynthetic efficiency and algal health **[23].**

Gas Chromatography-Mass Spectrometry (GC-MS) for Metabolite Analysis

Metabolic analysis using Gas Chromatography–Mass Spectrometry (GC-MS) is defined as an analytical technique used to separate and identify different substances chemical compounds present in biological samples such as tissues. Using gas chromatography (GC), compounds are separated and then identified using mass spectrometry (MS).

Using gas chromatography-mass spectrometry (GC-MS), we identify changes in the algae after their metal uptake. To identify changes using this technique, we first dry the algae thoroughly with nitrogen, then extract them with methanol and chloroform at a ratio of 2:1 (vol/vol). In the next step, we derivatize the compounds using N-methyl-N-(trimethylsilyl) (MSTFA). We then compared the results of this analysis with metabolites identified from comprehensive spectra libraries from the National Institute of Standards and Technology (NIST) on biochemical pathways affected by metal stress to confirm the validity of the results **[24].**

Table 1 shows the operational specifications of the equipment and describes the spectroscopic techniques used in the experiment.

Technique	Equipment Model	Parameter Measured	Wavelength / Range	Notes
UV-Vis Spectroscopy	Shimadzu UV-1800	Optical Density (OD 680 nm)	680 nm	Proxy for growth/chlorophyll
FTIR Spectroscopy	Bruker Alpha II	Functional Group Profiles	4000–400 cm^{-1}	KBr pellet method
Fluorescence Spectroscopy	PerkinElmer LS 55	Chlorophyll autofluorescence	Excitation: 435 nm, Emission: 685 nm	ROS indicator
GC-MS	Agilent 7890A/5975C	Metabolites	Programmed temperature ramp	MSTFA derivatization

F. Statistical Analysis

To conduct statistical analysis for the experiment, SPSS was used. One-way analysis of variance (ANOVA) was used for each type of algae used in the experiment to evaluate and measure the significance of differences between the different treatments and concentrations. Results are presented

as mean ± standard deviation (SD), with statistical significance considered significant at a p-value of ≤ 0.05.

Table 2. shows the experimental parameters and the number of replicates (n = 3) for each heavy metal concentration.

Treatment	Heavy Metal	Concentration (mg/L)	Replicates (n)
Control	—	0.0	3
Pb Treatment	Pb^{2+}	0.5 : 5.0	3
Cd Treatment	Cd^{2+}	0.5 : 5.0	3
Hg Treatment	Hg^{2+}	5 : 5.0	3

Results and Discussion

A. Physicochemical Characteristics of Surface Water Samples

For physical and chemical parameters, water was measured from three sites after being collected for sampling, environmental conditions, and heavy metal contamination. As shown in Table 3, the pH ranged between 6.2 and 6.7 at the time of sampling, indicating a slightly acidic or near-neutral pH. The solubility and dispersion of metals were also affected, as was their bioavailability in aquatic environments. It is noted that their transport through water affects their accessibility to microalgae. This movement plays a role in the bioavailability of these metals and their interactions.Table 3. Results of physical and chemical properties of water samples collected from three industrially impacted sites.

As for the concentration of dissolved ions, i.e., electrical conductivity (EC), values increased at all sites: 820 µS/cm at Site A, 1100 µS/cm at Site B, and 1350 µS/cm at Site C. The high levels of conductivity are due to industrial discharges and the presence of various salts and pollutants. indicating elevated ion concentrations typically associated with wastewater discharges.

Dissolved oxygen levels reached 4.1 mg/L at Site 1, 3.8 mg/L at Site 2, and 3.5 mg/L at Site 3, indicating deteriorating water quality with increasing proximity to discharge points and industrial sources. This likely reflects higher organic loads or higher chemical oxygen demand at the collection sites.

Elemental analysis revealed the consistent presence of lead (Pb^{2+}), cadmium (Cd^{2+}), and mercury (Hg^{2+}), with the highest concentrations recorded at Site 3 (Pb^{2}: 0.38 mg/L; Cd^{2+}: 0.16 mg/L; Hg^{2+}: 0.09 mg/L), exceeding the safety limits set by the United States Environmental Protection Agency **[20]** and the World Health Organization **[25].** These environmental parameters provide realistic conditions for assessing the physiological responses of microalgae to metal exposure under field stress levels.

Table 3. Physicochemical characteristics and heavy metal concentrations in surface water samples from three industrially affected sites

Sampling Site	pH	Electrical Conductivity (µS/cm)	Dissolved Oxygen (mg/L)	Pb^{2+} (mg/L)	Cd^{2+} (mg/L)	Hg^{2+} (mg/L)
Site A	6.2	820	4.1	0.15	0.07	0.02
Site B	6.7	1100	3.8	0.28	0.11	0.05
Site C	7.1	1350	3.5	0.38	0.16	0.09

The high electrical conductivity (EC = 820-1350 µS/cm), low dissolved oxygen (DO = 3.5-4.8 mg/L), and slightly acidic pH (6.2-7.1) at the three sampling sites indicate favorable conditions for increased mobility and bioavailability of heavy metals such as Pb^{2+}, Cd^{2+}, and Hg^{2+}. These physicochemical parameters significantly influence metal speciation, solubility, and potential uptake by microalgae. Notably, low pH enhances metal solubility and biosorption, while increased ion concentration (EC) and dissolved oxygen depletion reflect anthropogenic pollution and facilitate metal release from sediments (e.g., migration to the aqueous phase). **[26]** noted that as water pH decreases, more hydrogen competes for bonds with dissolved metals, enhancing metal release. They also mentioned that temperature and salinity were positively associated with metal release, and that industrial conditions may alter these parameters and indirectly enhance metal mobility. They also examined the effect of organic matter on metal binding in sediments, suggesting that low-oxygen conditions stimulate the release of associated metals, which is consistent with the results of my study.

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Figure 2. Concentrations of Pb^{2+}, Cd^{2+}, and Hg^{2+} in surface water samples from three industrial sites

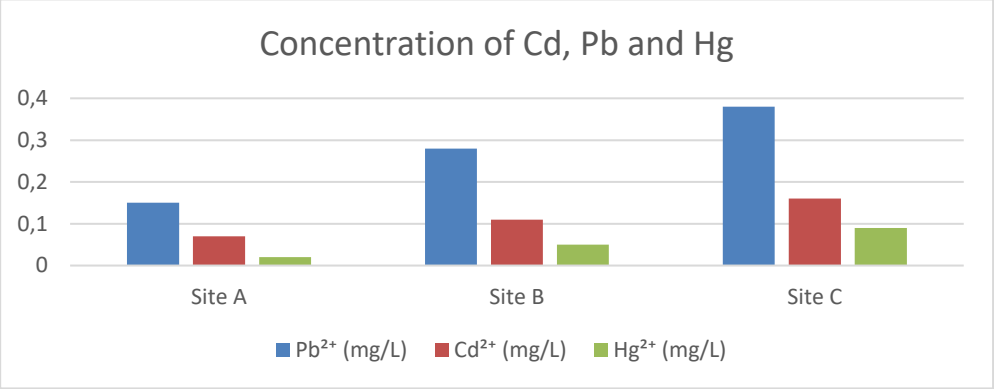


Figure 3. Electrical conductivity of surface water at different locations

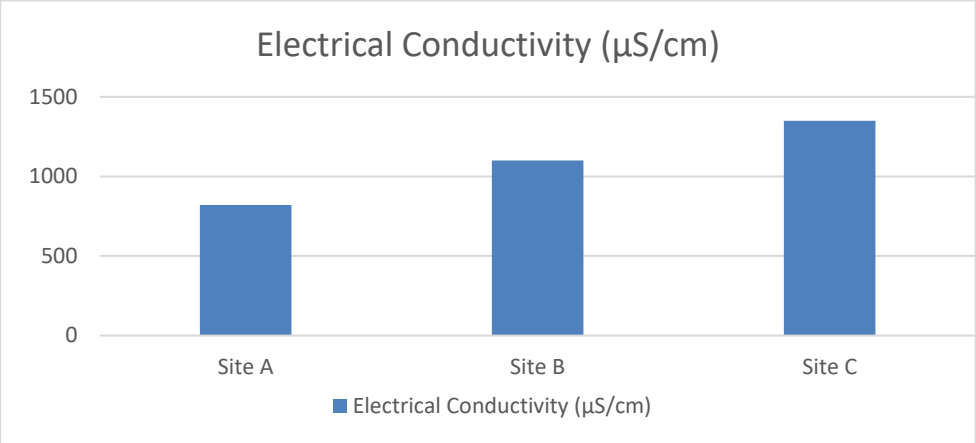


Figure 4. PH of surface water at different locations

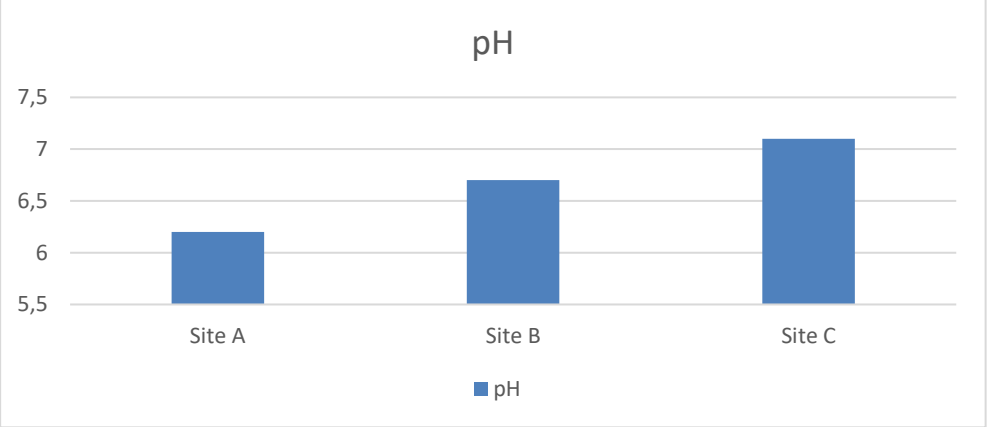
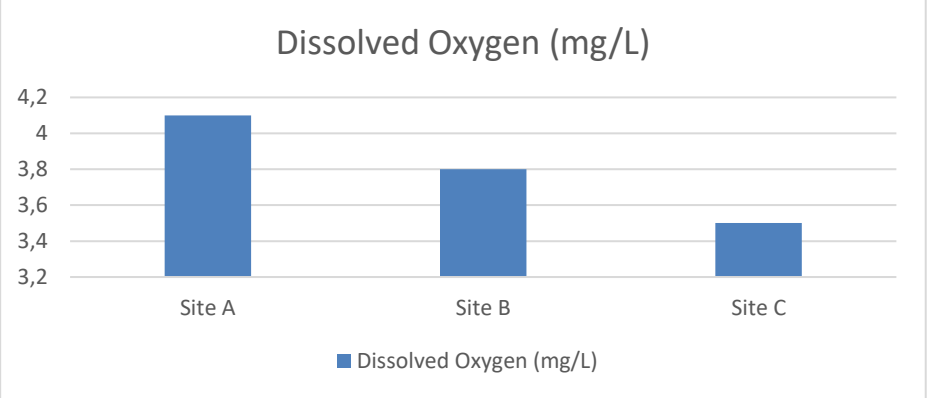


Figure 5. Dissolved oxygen concentration in surface water at different locations



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B. Physiological Response of Microalgae to Heavy Metals

Scenedesmus algae showed tolerance to Pb^{2+} toxicity at low concentrations (5 mg/l), resulting in enhanced chlorophyll a and protein synthesis. This result suggests that the efficiency of the photosynthetic system appeared to be less affected by Pb than significantly altered by Hg^{2+}.

Hg^{2+} also showed strong inhibition of chlorophyll a synthesis even at low concentrations (5 mg/L). Cadmium toxicity was mostly intermediate (between Hg^{2+} and Pb^{2+}), and its stimulating effect on algal growth (chlorophyll a and protein content) was evident at low concentrations (5 mg/L) in Scenedesmus algae.

However, in Scenedesmus algae treated with a lower concentration of Hg (5 mg/L), chlorophyll a content was severely affected, indicating that chlorophyll a content and total protein accumulation are not always correlated under heavy metal stress.

These findings indicate that the increased protein content of Scenedesmus treated with 5 mg/ml mercury, coupled with a significant reduction in growth, suggests that this organism may have redirected its protein synthesis from a growth-oriented function to a survival strategy. This is likely achieved through the production of stress-related peptides to a survival state **[27].**

For Ankistrodesmus algae growth was affected, decreasing with increasing cadmium concentration in the medium. Concentrations ranging from 1 to 5 mg/L were used and compared to the control group. The control group reached a maximum biomass density of 4.00×10^5 cells/mL at the onset of the death phase, after 12 days of cultivation. Cultures exposed to 0.5 mg/L cadmium reached a maximum density of 3.75×10^5 cells/mL after 9 days, respectively. This behavior may be attributed to a slight prolongation of the initial acclimatization phase. The second group, exposed to a cadmium concentration of 5.0 mg/L, reached a maximum biomass density of 1.50×10^5 cells/mL after 3 days of cultivation, then stabilized and growth stopped.

Exposure to cadmium concentrations exceeding 5.0 mg/L led to a marked decline in algal growth, indicating a breakdown of physiological functions triggered by heavy metal stress **[28].**

The maximum biosorption rate for Cd^{2+} and Pb^{2+} was reached after half an hour, reaching 86% for Cd^{2+} and 70% for Pb^{2+}. The removal rate of Hg^{2+} remained constant for two days, reaching 97% for Hg^{2+} after one day of the experiment. This result suggests that this is due to the balance between the cell and the algae, or to the breakdown of the cell-algae bonds by microorganisms. When the concentration of ions absorbed by the particles increased, there was greater degradation of those particles. However, this degradation was greater in cells exposed to mercury and cadmium concentrations than in those exposed to lead concentrations.

Significant changes occur in the environment exposed to high concentrations of heavy metals. These changes have been observed to include both the physical and chemical environment. When the concentration of cadmium or mercury increased, there was a decrease in the acidity of the environment containing the heavy metals. This may be due to increased metabolic stress.

[29] explains that the presence of metals in algae is due to the distribution of metals among living organisms. Selective absorption is observed in the environment containing zinc, with plant tissues containing zinc concentrations outperforming aquatic media. This phenomenon is similar to the selective absorption of Scenedesmus and Ankistrodesmus algae. When pH decreases understresses of Cd^{2+} and Hg^{2+}, the bioavailability of metals to microalgae increases. [28] supported these findings, stating that when pH decreased and organic matter increased, there was an increase in metal mobility, which, as a result, also increased metal uptake through chelation and ion exchange.

In a study conducted by **[30]** they observed diverse responses in algae when exposed to high concentrations of heavy metals. They also observed that pH, cell density, and microalgal age were significantly affected. Due to the limited research on the two algae used in this study, C. vulgaris was used because it has similar characteristics to the algae used in my research. It is sensitive to copper at pH 7.5 and has an optimal biological response at a cell density of 7×10^6 cells/ml in culture for 3 days. In a study published in IEEE Sensors in 2017, it was demonstrated that the conditions surrounding the algae, and the chemical and biological changes that result from low chlorophyll and protein content, increase the uptake of metals such as cadmium and lead. These results were consistent with my research, as exposure to cadmium^{2+} Cd^{2+} or mercury^{2+} Hg^{2+} increased, pH decreased, and physiological disturbances increased in algae cultures. Therefore, it is important to understand the interacting environmental factors, as this is essential to understanding the biological capacity of microalgae to absorb metals and reduce stress resulting from heavy metal pollution.

Tabel 4: "Bioabsorption rates of heavy metals by microalgae over specific time periods"

Metal Type	Absorption rate (%)	Notes
Cd^{2+}	86%	Rapid absorption
Pb^{2+}	70%	Less than Cd^{2+}
Hg^{2+}	97%	High and stable absorption

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Figure 6. *Biosorption Percentage (%) of Cd^{2+}, Pb^{2+}, and Hg^{2+} by Microalgae*

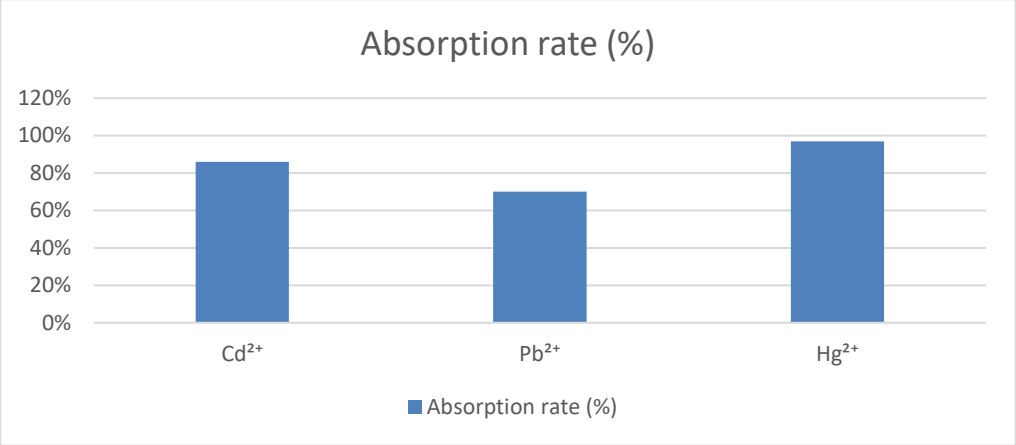
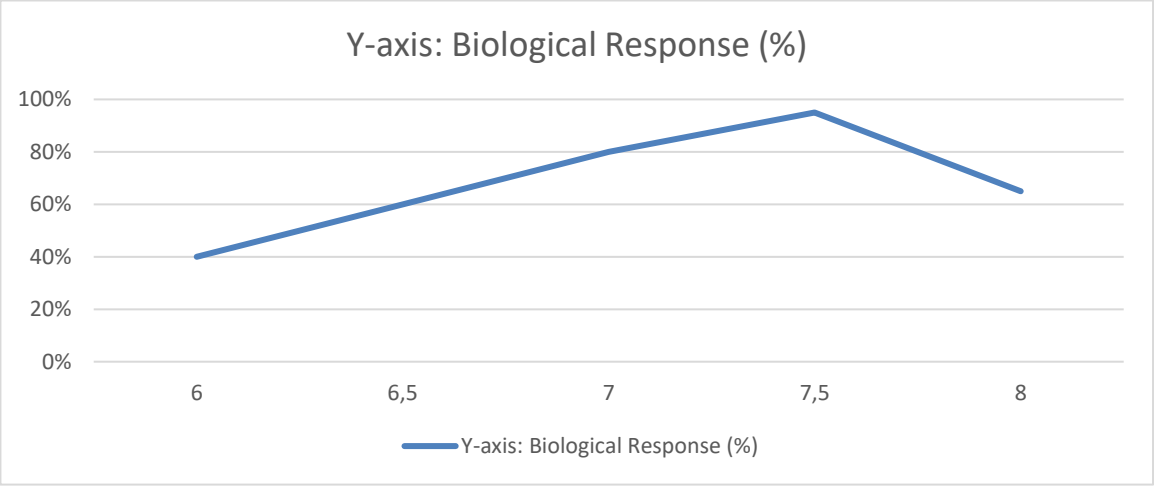


Table 5. Effect of pH on the Biological Response of *C. vulgaris* to Copper (Cu^{2+}) Exposure

pH Level	Biological Response (%)	Remarks
6.0	Low	Initial response, weak effect
6.5	Moderate	Noticeable improvement
7.0	High	Strong response, near optimal level
7.5	Highest	Peak response recorded
8.0	Moderate	Slight decline after peak

Figure 7. Effect of pH on the Biological Response (%) of *C. vulgaris* to Copper (Cu^{2+}) Exposure



C. Spectroscopic Detection and Biosensing Evaluation

UV-Visible Spectroscopy for Growth and Pigment Analysis

We observed that the algae used in the experiment showed significant growth during the first three days. However, we observed that Ankistrodesmus achieved a high optical density during this period, reaching 0.91±0.04, faster than Scenedesmus, which increased its optical density to 0.78±0.03. This is due to the surface volume to surface area ratio-to-cell-diameter ratio of algae, which increases their ability to measure metals **.[31]** In the end the experiment, the density reached 0.32 g/L, higher than Scenedesmus's density of 0.27 g/L, at 25±2°C and a 8:16 h dark:light cycle.

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This density was used to estimate biomass accumulation during the experiment **[32].** However, exposure to increasing concentrations of heavy metals resulted in dose-dependent growth inhibition in both species. The algae demonstrated efficient metal uptake. During the experiment, the biomass and color of Anastrodismus algae decreased, as did dry weight and turbidity. Cell proliferation increased, particularly in the more concentrated algae group. The control group also showed changes in color, turbidity, and sedimentation. Analysis of variance (ANOVA) showed that the observed decreases in OD were statistically significant ($p<0.01$), particularly at concentrations ≥2.0 mg/L for all three metals.

Fourier Transform Infrared Spectroscopy: Functional Group Changes

Both Ankistrodesmus falcatus and Scenedesmus obliquus showed significant changes in specific functional groups. The decrease in O–H stretching (approximately 3400 cm^1) and amide I/II peaks (1650–1540 cm^1) indicates the degradation or modification of proteins and polysaccharides. It was observed that the biosorption of Ankistrodesmus algae was evident, with the C=O (approximately 1730 cm^1) and P–O (approximately 1060 cm^1) shifts, indicating the accumulation of heavy metal molecules with these groups.

In a study by **[33],** using FTIR, we found that the carboxyl (–COOH) and amino ($–NH_2$) groups in the algae were essential for the algae's interaction with cadmium ions (Cd), which helped create suitable conditions for Scenedesmus obliquus to absorb.

These results are consistent with those of **[34],** who observed that when Scenedesmus sp. was exposed to heavy metals, the O-H shift shifted from 2935 cm^{-1} to 2944 cm^{-1} when exposed to lead, and stabilized at 2918 cm^{-1} for cadmium. N-H also moved from 1625 cm^{-1} to 1629 cm^{-1} in the case of lead, and to 1627 cm^{-1} in cadmium. These shifts indicate a

Profound interaction between these functional groups and metal ions, and also indicate the complex interaction taking place, the chemical and biological dynamics of which may be revealed (using Fourier transform infrared). This agrees with and confirms our hypothesis that similar groups participate in similar biosorption processes in many microalgal species.

[35], analyzed the interaction of Hg^{2+} ions with extracellular polymers (EPS) produced by mercury-resistant bacteria using FTIR, UV-Vis, and 1H-NMR techniques. The results demonstrated that functional groups such as carboxyl, phosphate, hydroxyl, amino, and thiol were involved in the interaction. This indicates a stronger binding force and, possibly, a higher biosorption capacity. It also suggests that A. falcatus may act as a more efficient biosorbent in polluted aquatic systems. The differences in Fourier transform infrared patterns between treatments were statistically significant ($P < 0.05$), supporting the occurrence of metal-induced modifications in functional group. Functional groups refer to the chemically reactive parts of molecules involved in metal binding (e.g., -OH, -COOH, $-NH_2$, $-PO_4$). Their changes indicate biochemical stress or detoxification mechanisms.

Tabel 6. FTIR Spectral Shifts Indicating Functional Group Changes in ***Ankistrodesmus falcatus*** and ***Scenedesmus obliquus*** Under Heavy Metal Exposure (Cd^{2+}, Pb^{2+}, Hg^{2+})"

Functional range	Normal status (without metals)	Cd^{2+} (cm^{-1})	Pb^{2+} (cm^{-1})	Hg^{2+} (cm^{-1})	Affected group
O–H / N–H Stretch	3400	3385	3390	3370	Hydroxyl / Amine
C=O (Carboxyl)	1730	1722	1725	1718	Carboxyl group
Amide I (C=O stretch)	1650	1642	1645	1638	Protein backbone
Amide II (N–H bend)	1540	1530	1532	1525	Proteins
P–O (Phosphate)	1060	1045	1050	1035	Phosphates (DNA/ATP)
S–H (Thiols)	—	—	—	2550	Only seen with Hg^{2+} binding

Figure 8. FTIR Analysis of Functional Group Changes in Algae Exposed to Heavy Metals

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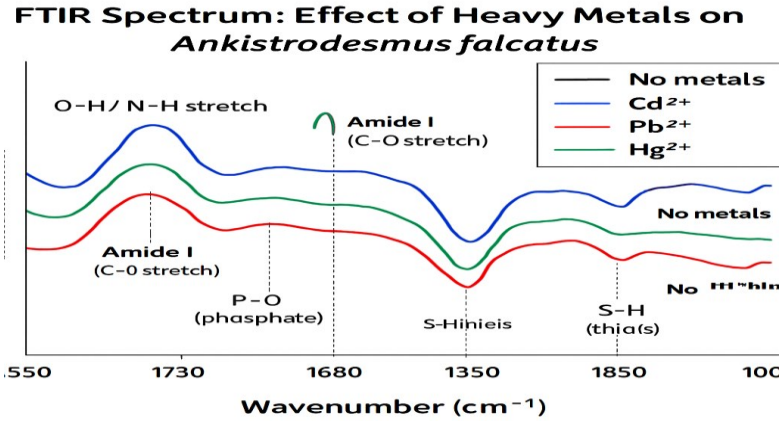


Fluorescence Spectroscopy: Chlorophyll Quenching and Reactive Oxygen Species (ROS) Response

Fluorescence emission at a wavelength of 685 nm decreased significantly with high concentration heavy metal. In Ankistrodesmus, chlorophyll-a fluorescence intensity decreased by 54% when treated with 5.0 mg/L of mercury^{2+}, while S. obliquus showed a 40% decrease. The production of reactive oxygen species (ROS) is also increased **[36],[37]** confirming that fluorescence is a sensitive indicator for real-time biosensing. The stronger response in A. falcatus indicates a higher sensitivity to oxidative stress and greater potential for use as an early warning biosensor. These results are similar to previous studies demonstrating that fluorescence inhibition is a reliable biomarker of environmental toxicity. Fluorescence measurements showed significant differences ($P < 0.05$) across metal concentrations, confirming their diagnostic value.

Heavy metal exposure can elevate intracellular reactive oxygen species (ROS), disrupt the photosynthetic electron transport chain, and damage photosystem II (PSII), thereby reducing fluorescence yield **[38].** Algae are known to activate protective mechanisms such as NPQ and ROS-fighting systems to protect PSII from chronic photodamage.

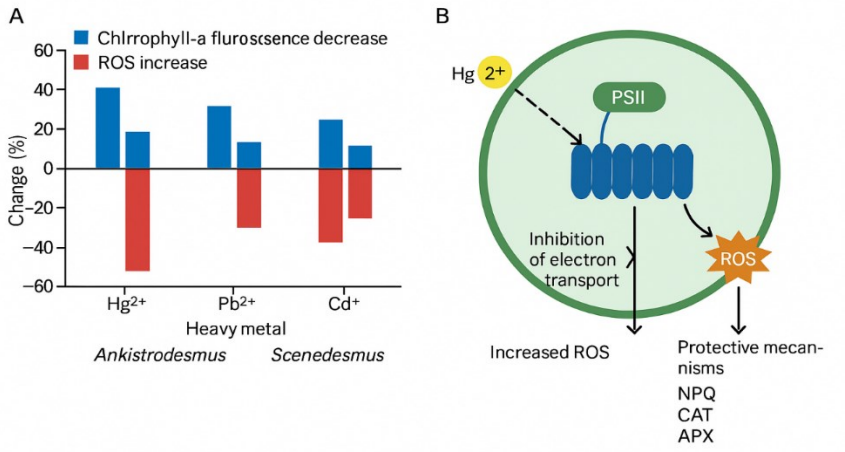
[39] results showed that the metal in question, cadmium, significantly inhibited PS II photochemistry, as demonstrated by the values of F_0, F_v/F_m, qN, and qP after 12 hours of exposure. The variables F_m, $F_v/2$, and F_0/F_v showed significant changes after only one hour of treatment. The results indicate that the effect of metals is not limited to initial and maximum fluorescence or maximum quantum yield, and chemical and non-chemical suppression, but also extends to significant changes in the water-splitting system within the oxidizing side of PS II. The study concluded that the F_0/F_v ratio may be an effective tool for metal stress research and environmental impact assessment.

In Scenedesmus acutus, exposure to very low concentrations of lead ($0.03–0.87 \times 10^{-9}$ M) inhibited plant growth, accompanied by a significant 4.5-fold increase in intracellular reactive oxygen species (ROS) levels, while photosynthetic performance was unaffected. These results suggest that elevated ROS levels may represent an earlier and more sensitive indicator of heavy metal stress than their direct impact on the photosynthetic machinery.

In Chlamydomonas reinhardtii, **[40]** reported that exposure to increasing mercury concentrations (0–8 μM) resulted in ROS and peroxidase product accumulation, alongside increased activity and gene expression of antioxidant enzymes such as SOD, CAT, and APX. These results highlight the close relationship between oxidative stress and biological antioxidant responses.

In Ankistrodesmus sp. B-11, Cd^{2+} ions were shown to inhibit electron transport in PSII, manifested by a decrease in φE_o and PI_ABS, a delay in photoreduction of P_{700}, a significant increase in energy dissipation (DI_0/RC), and a ΔpH-dependent non-photochemical quenching (qE). Importantly, PI_ABS emerged as the most sensitive chlorophyll fluorescence parameter for the early detection of Cd^{2+} toxicity **[41].**

Figure 9. Effect of heavy metals (Cd, Pb, Hg) on chlorophyll fluorescence intensity and reactive oxygen species (ROS) formation in the algae Ankistrodesmus and Scenedesmus



Effects of Cd^{2+}, Pb^{2+} and Hg^{2+} on chlorophyll ffluorescence and ROS production in *Ankistrodesmus falcatus* and *Scenedesmus obliquus*, and the proposed mechanism of PSII damage and oxidative stress response

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Metabolomics Analysis Using Gas Chromatography and Mass Spectrometry

To reprogram the metabolism caused by stress in microalgae, gas chromatography-mass spectrometry (GC-MS) was used in experiments on the algae Synodontia and Anaxtrodismus. This method facilitated the detection of changes in organic acids, lipids, and pigments. It is known to identify a wide range of volatile and semi-volatile metabolites in samples. It also reveals the chemical and biological changes resulting from exposure to heavy metals.

An increase in malic acid and succinic acid was observed in Anaxtrodismus exposed to mercury, along with increased lipid levels. While chlorophyll-derived compounds and fatty acid methyl esters decreased, this has implications for oxidative membrane degradation and stress-induced reprogramming. These results also demonstrate stress-related metabolic shifts in algae, and are consistent with previous studies by **[42].**

Metabolic reprogramming of microalgae is important when exposed to metals. Receptor reanalysis is also important because it measures the algal response and reveals the presence of biomolecules involved in increased energy production. Algal accumulation in the environment is due to several reasons, such as detoxification, protection from osmotic stress, and structural maintenance. When Anchodesmus and Pseudodesmus were exposed to mercury, lipid degradation products increased and chlorophyll levels decreased. Due to the inefficiency of antioxidant systems, Anchodesmus is more susceptible than Pseudodesmus. Oxidative damage to cell membranes during metabolic reorganization is an effective means of adapting to oxidative stress. Using global chromatography-mass spectrometry (GC-MS), a study by **[43]** demonstrated that Scenedesmus obliquus algae was affected by lead (Pb^{2+}) ions, and experienced numerous changes in its intracellular metabolism, including increased concentrations of alkanes, aromatics, and sterols, as well as changes in FAME ratios. This indicates that mineral stress varies with changes in mineral stress. As lead levels increased, chlorophyll content decreased, along with protein and carbohydrate content. Conversely, carotenoids and lipids increased. Therefore, we must redirect metabolic pathways to eliminate lead from within cells.

This indicates that it has a defensive response to mitigate the oxidative stress caused by excess lead. As a result, the production of alkanes, aromatic compounds, and sterols, components that enhance membrane stability, increased.Despite the limited research on Ankistrodesmus, the overall response is similar, but with varying degrees of severity. This suggests that Scenedesmus algae is more tolerant to lead. Metabolic analysis revealed increased organic acid levels in Scenedesmus obliquus when cadmium concentrations increased. These results are consistent with those of **[44],** who analyzed Scenedesmus and Ankistrodesmus exposed to different heavy metal concentrations using gas chromatography-mass spectrometry. The study results showed that increasing cadmium concentrations increased organic acids and decreased chlorophyll-related compounds and some fatty acid methyl esters. This suggests that cadmium is a cause of oxidative membrane damage. Consequently, the significant increase in the trichloroacetic acid cycle and the decrease in chlorophyll and lipid metabolism in Ankistrodesmus indicate that cadmium causes oxidative stress. Scenedesmus also possesses more effective photoprotective and detoxification capabilities.

Table 7. Comparative metabolomic and physiological responses of Scenedesmus and Ankistrodesmus algae to heavy metal (Hg^{2+}, Pb^{2+}, Cd^{2+}) exposure as revealed by GC–MS analysis.

Mineral	Scenedesmus	Ankistrodesmus	Similarities/Differences
Hg^{2+}	↑ Organic acids, ↑ lipid breakdown products, ↓ chlorophyll-derived compounds	↑↑ Organic acids, marked ↓ in FAMEs, strong adaptive metabolic reprogramming	Both show oxidative stress–induced metabolic reprogramming; Ankistrodesmus shows greater sensitivity
Pb^{2+}	↑ Carotenoids, ↑ lipids, ↓ chlorophyll, ↓ carbohydrates & proteins, ↑ alkanes, aromatics & sterols, ↓ alkenes & MUFAs	Limited data; general trend similar with variable intensity	Defensive metabolic shift in both; Scenedesmus displays higher resilience
Cd^{2+}	↑ TCA cycle intermediates, ↑ organic acids (malic, succinic), ↓ some membrane lipids & chlorophyll compounds	Similar increases in organic acids, more pronounced ↓ in chlorophyll-related metabolites	Both undergo metabolic recalibration; Ankistrodesmus shows stronger chlorophyll degradation

Legend:

↑ = increase; ↓ = decrease; MUFAs = monounsaturated fatty acids; TCA = tricarboxylic acid cycle; FAMEs = fatty acid methyl esters

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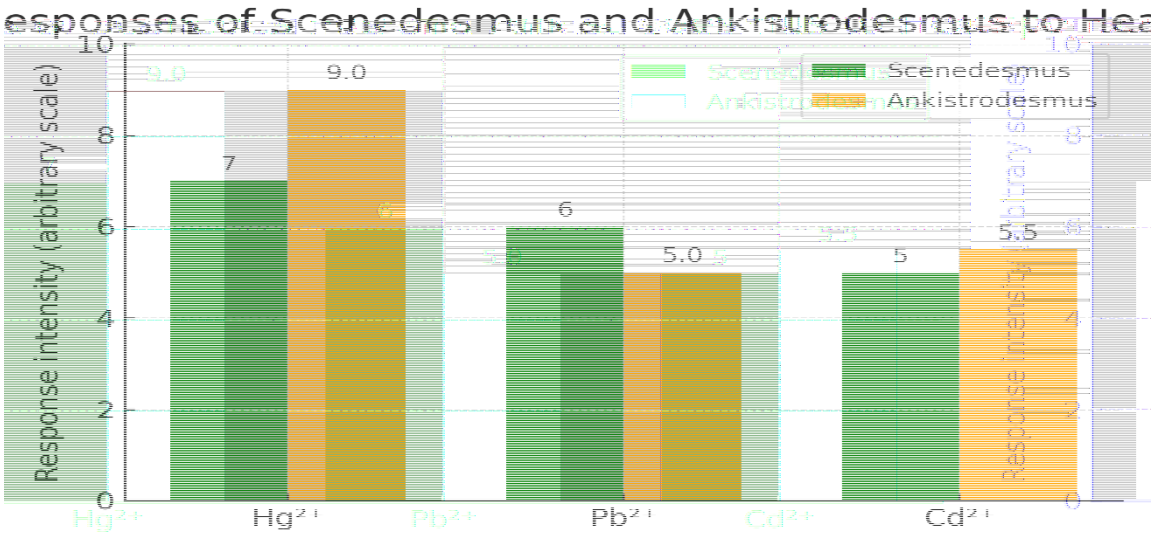
Figure 9. Comparative metabolomic and physiological responses of Scenedesmus and Ankistrodesmus algae to heavy metal exposure (Hg^{2+}, Pb^{2+}, Cd^{2+}) based on GC–MS analysis.



Conclusion

Based on the above study, several strategic recommendations can be made to enhance the practical use of microalgae in environmental biosensing and bioremediation.

The results of this study demonstrated that the algae Ankistrodesmus falcatus and Scenedesmus obliquus in biosensing systems equipped with spectral detectors, such as UV-visible and chlorophyll-fluorescent sensors, enable us to identify water contaminated with heavy metals, whether in industrial wastewater or freshwater. This will enhance the responsiveness and efficiency of water quality assessment systems and support field applications. Efforts should be made to increase the cultivation of these microalgae in dedicated, scalable, and inexpensive laboratories and facilities, such as photobioreactors or algal biofilm systems. These cultivation methods provide high biomass yields and physiological sensitivity under changing environmental conditions.

These results confirm that for field applications, we must expand future experiments to include more than one metal to understand how these microalgal species react when exposed to different types of pollutants. Furthermore, we must leverage advances in genomic technologies, particularly transcriptional and metabolic profiling, to further understand the pathways involved in metal uptake, detoxification, and identification. This will enable us to generate new, genetically enhanced algal strains with superior sensitivity and specificity for biosensing.

Working with regulatory and environmental bodies is also crucial. We must improve and develop biosensing methods, exposure conditions, and optimization techniques to produce better strains. We must also validate analytical endpoints to ensure consistency, regulatory acceptance, and reproducibility. These steps are particularly important in areas where access to chemical monitoring equipment is limited. We also recommend further comparative experiments in environments Using diverse carbon patterns and sources, we aim to achieve higher cell densities, whether during light or dark periods regulated by the farms. should be varied, as should the pH and temperature, and the microalgal concentrations of cadmium, mercury, and lead. The removal rates should also be compared Based on the results we obtained in this experiment or study, we found that the microalgae used in the experiments, which had previously been exposed to cadmium, mercury, and lead should be used, as these strains have developed a greater tolerance Large amounts of heavy metal concentrations and exhibit higher removal rates.

Recommendations for Future Applications

To improve practical applications, the integration of A. falcatus and S. obliquus into biosensing systems equipped with spectral detectors, such as UV-visible and chlorophyll fluorescence sensors, is highly recommended. These systems can facilitate the rapid detection of heavy metal contamination in industrial wastewater and freshwater sources. Efforts should focus on large-scale, cost-efficient cultivation using photobioreactors or algal biofilm systems to achieve high biomass yields and maintain consistent sensitivity under varying environmental conditions.

Future research should include exposure to multiple heavy metals simultaneously to better replicate real-world scenarios and evaluate the combined effects. Advances in genomic and metabolomic profiling offer a promising approach to uncover the molecular mechanisms behind metal uptake, detoxification, and sensing, paving the way for genetically enhanced strains with improved performance. Collaboration with regulatory and environmental organizations will be crucial for optimizing biosensing methodologies, standardizing analytical endpoints, and ensuring replicable results, especially in areas where sophisticated chemical monitoring infrastructure is limited.

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