



Aquatic toxicity of particulate matter emitted by five electroplating processes in two marine microalgae species

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ABSTRACT

Electroplating is a widely used group of industrial processes that make a metal coating on a solid substrate. Our previous research studied the concentrations, characteristics, and chemical composition of nano- and micro-particles emitted during different electroplating processes. The objective of this study was to evaluate the environmental toxicity of particulate matter obtained from five different electrochemical processes. We collected airborne particle samples formed during aluminum cleaning, aluminum etching, chemical degreasing, nonferrous metals etching, and nickel plating. The toxicity of the particles was evaluated by the standard microalgae growth rate inhibition test. Additionally, we evaluated membrane potential and cell size changes in the microalgae *H. akashiwo* and *P. purpureum* exposed to the obtained suspensions of electroplating particles. The findings of this research demonstrate that the aquatic toxicity of electroplating emissions significantly varies between different industrial processes and mostly depends on particle chemical composition and solubility rather than the number of insoluble particles. The sample from an aluminum cleaning workshop was significantly more toxic for both microalgae species compared to the other samples and demonstrated dose and time-dependent toxicity. The samples obtained during chemical degreasing and nonferrous metals etching processes induced depolarization of microalgal cell membranes, demonstrated the potential of chronic toxicity, and stimulated the growth rate of microalgae after 72 h of exposure. Moreover, the sample from a nonferrous metals etching workshop revealed hormetic dose-response toxicity in *H. akashiwo*, which can lead to harmful algal blooms in the environment.

1. Introduction

Electrochemical processes are widely used in making coatings for industry, electronics, decoration, etc. [1–3]. Recent studies have shown that particulate matter (PM) containing metal components even at low concentrations may cause adverse health effects [4,5]. Continuous occupational exposure to harmful substances used in electroplating production, such as nickel, hexavalent chromium, and other toxic

metals, can cause serious health effects, including cancer, asthma, and other diseases of respiratory, cardiovascular, and musculoskeletal system [6–8]. In the environment, industrial PM accumulated in soil and water bodies can be a significant source of metallic contamination [9], which will lead to the degradation of ecosystems and the potential implementation of toxic components in trophic chains [10]. A known fact that low concentrations of PM, metals, metalloids, and ions can induce positive effects on algae and higher plants stimulating their

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growth [11], which can upset the balance of the environment. It is therefore important to evaluate the impact of emissions from the electroplating industry on the environment and human health.

The aerodynamic size is one of the most important parameters in determining the atmospheric lifetime of particles and their deposition onto soil or water [12,13]. PM has been classified by particle size as PM₁₀ (particles ≤10 μm in diameter), also called coarse particles, PM_{2.5} (particles ≤2.5 μm in diameter), also called fine particles, and PM_{0.1} (particles ≤0.1 μm in diameter), also called ultrafine particles [13]. Our previous work has shown that the number of PM of median aerodynamic diameter less than 0.3 μm (PM_{0.3}), also called quasi-ultrafine particles, is over 10,000 times larger than the number of PM₁₀ particles in the air of industrial electroplating workshop [14]. This fraction of atmospheric particles has recently attracted scientists' attention due to their abundance in the air of urban areas [15] and potential toxicity to human health [16,17]. The comprehensive study of Sicard et al. [18] demonstrated that despite the existing air quality standards and limitations, PM levels in urban areas of the EU widely exceeding the WHO limit values for the protection of human health.

Longer residence in the atmosphere allows fine, quasi-ultrafine, and ultrafine PM a large geographic distribution [19]. These particles can travel far beyond the working area of an electroplating production line, workshop, or protection zone of an enterprise. The smallest industrial particles have been found at considerable distances from manufacturing buildings [20]. The precipitation of industrial PM through rain, production of industrial wastewater, or electroplating sludge disposal can introduce toxic electroplating products into soil and water bodies [21–23]. Intensified human activities in coastal regions [24,25] lead to progressive degradation of coastal and marine ecosystems [26] and can facilitate the transfer of toxic metals and persistent organic pollutants through food chains to seafood, fish, and eventually to humans [27,28]. The regulation of the negative impact of pollutant emissions on the aquatic environment requires the consideration of standards for different types of pollutants.

The current US Environmental Protection Agency (EPA) regulation called National Emission Standards for Hazardous Air Pollutants (NESHAP) includes a category of Plating and Polishing Operations, which requires minimizing, control, and capture of exhaust emissions. The EU Industrial Emissions Directive (2010/75/EU, IED) requires an application and regular update of the best available techniques in its Surface Treatment of Metals and Plastics by Electrolytic and Chemical Processes. The existing standards and regulations are generally based on mass concentrations of PM, and do not take into account particle characteristics, composition, the toxicity of different components, or their combination [12] which may be crucial for electroplating emissions.

Several studies have shown that the smaller the size, the greater the solubility of PM is because of the increased surface-to-volume ratio [29,30]. Other studies have shown that most toxic metals accumulate in fractions of PM_{2.5} or less [31–33], and the solubility of trace metals from PM mostly depend on chemical composition rather than particle size [34]. In practice, the population and the environment are never exposed to one single chemical but are subject to constant multi-chemical exposure from many different sources, usually below regulatory limits, and may exert synergistic, combined, or competitive action [35–37]. Therefore, even small emissions of the electroplating industry present a potential risk to the environment and human health, because of the combined toxic action of multi-component pollution by PM and metals with different characteristics and compositions [38–40].

The wastes generated in different electroplating procedures vary in characteristics and possible environmental impact [41]. Electroplating includes different steps, such as degreasing, rinsing, etching, etc. [42]. Despite the existing body of research on galvanic wastewater treatment [43–45], there is little published data on the toxic properties of different electroplating aerosols [46,47]. Considering the possible release of industrial exhaust gases and the need for regulation development, the properties of galvanic aerosols required investigation in terms of

environmental toxicity.

This study aims to assess the aquatic toxicity of airborne particles emitted by five common electrochemical processes, namely aluminum cleaning (AC), aluminum etching (AE), chemical degreasing (CDG), nonferrous metals etching (NME), and nickel plating (NP). PM toxicity was evaluated by the standard microalgae growth-rate inhibition test using flow cytometry. Moreover, we evaluated membrane potential and cell size changes in the marine microalgae *Heterosigma akashiwo* and *Porphyridium purpureum* exposed to PM emitted by five electroplating processes. This study examined the general differences in acute toxic effects of different electroplating emissions in microalgae cells. The establishing of chronic exposure was beyond the scope of this study. The findings of this work provide a basic level for further environmental risk assessment of electroplating emissions in real-life conditions, and they should make an important contribution to the field of the environmental regulation of the electroplating industry.

2. Materials and methods

2.1. Collection of electrochemical exhaust particles

To study the toxicity of airborne particles formed during different electrochemical processes, we collected samples in real workshop conditions. The chemical composition and quantitative distribution of aerosol particles having an average diameter between 0.3 and 10 μm in the air of an electroplating workshop are described in our previous work [14]. The sampling procedure was performed according to the previously reported method by [48,49]: 2.7-liter sterile plastic containers with distilled water were placed on the floor of the workshop during its operation. Before the experiment, the containers were thoroughly washed, namely one time with running water and two times with distilled water. After that, the containers were filled with 800 mL of distilled water obtained with the DE-4–02-EMO water distiller (Electromedoborudovanie, Russia).

The duration of sample collection was eight hours in line with the operating shift of the workshop. The containers were placed near the still baths and opened at the beginning of the work shift. At the end of the work shift, the containers were tightly closed, marked, and transported to the laboratory for further analyses, and were stored at room temperature for 24 h. The samples obtained during the operation of five electrochemical processes were allocated for the toxicity bioassay (Table 1).

The negative impact of aerosol particles on organisms and human health is often associated with particle size [50,51]. In this study, we only used quasi-ultrafine particles. To obtain the required size fractions, the samples were filtered through 0.45 μm Whatman PVDF syringe filters (Sigma-Aldrich, USA).

Additionally, we carried out particle size analysis of the filtered samples by a CytoFLEX flow cytometer (Beckman Coulter, USA) using the data of forward scattering of a violet laser (405 nm). The cytometer was calibrated with a mixture of fluorescent particles Megamix-Plus SSC and Megamix-Plus FSC (BioCytex, France). Each sample was measured in triplicate at a flow rate of 25 μL/min for 60 s. The registration settings, including acquisition mode, threshold, and cleaning between the samples, were performed according to [52], and flow rate was adjusted empirically to achieve stable acquisition.

2.2. Microalgae cultures

We assessed the toxicity and morphological and biochemical changes in microalgae cells with the microalgae growth-rate inhibition test. Two species of marine microalgae, namely a golden-brown algae *Heterosigma akashiwo* (Y.Hada) Y.Hada ex Y.Hara & M.Chihara, 1987. (Ochrophyta) and a red alga *Porphyridium purpureum* (Bory de Saint-Vincent) Drew et Ross, 1965 (Rhodophyta) originally isolated from the Peter the Great Bay (Sea of Japan, Far Eastern Russia) were provided by the Resource

Table 1
Sampling information and particle characteristics.

Coded name	Sampling point (electrochemical process)	Electrolyte composition	Chemical composition of the particles ^a	Morphology of the particles ^a
AC	Aluminum cleaning	HNO ₃	high content of potassium compounds: Al, Ca, Na, Mg, Fe, Zn (chemical salts); Zn particles.	spheroidal and lamellar structure
AE	Aluminum etching	NaOH	high content of magnesium and potassium compounds: Al, Ca, Na, Cl, Fe with inclusions of Br and S. High content of Ba particles.	acicular
CDG	Chemical degreasing	Detergent Labomid 203 (Chempack, Russia)	compounds of Na, S, and K; Zn, Cu and Cr, oxides of Fe and Al, aluminosilicates, and NaCl.	agglomerates and drop-shaped clusters
NME	Nonferrous metals etching	HNO ₃ ; H ₂ SO ₄ ; HCl	inclusions of Cu, Zn, Pb, Ni, Fe, Al, SnO, and PbS.	dendritic aggregates, spherical particles of tin (8–24 %)
NP	Nickel plating	NiSO ₄ ; MgSO ₄ ; Na ₂ SO ₄ ; NaCl; H ₃ BO ₃	the bulk was represented by fakes of Na, S, Al, Cu, Na, and Cl; inclusions of Ni, Co, Cu, Zn, Ag, Fe, Cr, W, S.	polygons with relatively smooth surface

^a Results of electron microscopy and energy dispersive X-ray analysis were reported in previous work [14].

Collection *Marine Biobank* of the National Scientific Center of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences (NSCMB FEB RAS). The microalgae model was chosen as a sensitive bioindicator [53], and as a crucial element of all aquatic trophic chains which is the main producer of organic matter in the aquatic environment [54]. The microalgae species were selected based on their abundance among microalgae in the Sea of Japan [55] and their suitability as test-organisms in ecotoxicology [56,57]. Their relevance as test-organisms in toxicity bioassay of welding fume particles has been previously confirmed [48].

Microalgae culturing and bioassay conditions were according to OECD No. 201 guidance [58], with minor modifications as previously described [59]. Microalgae cultures were cultivated in f/2 medium [60] under a temperature of 20 ± 2 °C, with 12:12 h light:dark irradiation cycle. For bioassays, microalgae cultures were used in the exponential growth phase.

2.3. Bioassays

Toxicity bioassays were performed in 24-well plates. Each well was filled with 1 mL of microalgal culture and 1 mL of sample suspension diluted by distilled water to obtain the final concentrations of 10, 25, and 50 % of the tested sample in microalgae culturing media. The same volume of distilled water was added to the control groups. All the experiments were performed in quadruplicate.

A CytoFLEX flow cytometer with CytExpert v.2.4 software package (Beckman Coulter, USA) was used to determine the number of microalgal cells and their morphological and biochemical changes. During the growth-rate inhibition measurement, dead cells were excluded from the

count by propidium iodide (PI) staining, according to the standard procedure [61]. The membrane potential of microalgal cells was assessed by a lipophilic, positively charged fluorescent dye 3,3'-dihexyloxycarbocyanine iodide (DiOC₆) [62]. The emission filters were selected according to the characteristics of the maximum emission of dyes provided by the manufacturer (Molecular Probes, USA). A blue laser (488 nm) of the CytoFLEX flow cytometer was chosen as the source of excitation light. To determine the size of microalgal cells, a size calibration kit (batch F13838, Molecular probes, USA) with a certified size distribution of 1, 2, 4, 6, 10, 15 μm was used for the FSC (forward scattering of a blue laser) channel. The toxicity bioassay endpoints used in this work and conditions for their registration are shown in Table 2. Each sample was measured at a flow rate of 100 μL/min for 30 s.

To determine the optimal concentration of fluorescent dyes and the optimal duration of staining, a series of preliminary measurements were made for each microalgae species before the assessment of growth-inhibition and membrane potential of cells [59]. The exposure periods of 24 and 72 h were selected according to the standard methods commonly used to assess the toxicity of test substances and materials in aqueous systems with microalgae [63,64].

2.4. Statistical analysis

Statistical analyses were performed using the software package GraphPad Prism 8.0.2 (GraphPad Software, USA). Normality was checked using the Shapiro-Wilk test. The one-way ANOVA test was used for analysis. A value of $p \leq 0.05$ was considered statistically significant.

3. Results

The results of particle size analysis performed by flow cytometer CytoFLEX are presented in Fig. 1 as a number of particles per one mL in three different size ranges.

Among the tested samples, a similarity in particle size distribution can be observed for AC, NME, and NP samples. The particles obtained from these galvanic processes had about one million particles of an average diameter less than 100 nm per one mL. Sample AE had 2.5 and 4.5 million particles per mL in the size ranges less than 100 nm and 100–200 nm, respectively. Sample CDG had 3.2 and 3.4 million particles per mL in the size ranges less than 100 nm and 100–200 nm, respectively. Moreover, samples AE and CDG had 2.8 and 1.3 thousand particles per mL in the size range of 200–300 nm, respectively.

The changes in the growth rate of microalgae *H. akashiwo* and *P. purpureum* exposed to particle suspensions representing five electroplating processes are presented in Fig. 2. Statistical analysis demonstrated that all datasets have passed the Shapiro-Wilk normality test. The statistical significance of each treatment was computed by One-way ANOVA with multiple comparisons of the mean data of each treatment to the mean data of a control group.

The most significant growth rate inhibition and death of both microalgae species was caused by sample AC, obtained at the workshop of an aluminum cleaning process. At a concentration of 50 %, this sample caused the death of all *H. akashiwo* cells and about 80 % of *P. purpureum* cells after 24 h of exposure (Fig. 2a, b). The negative effect slightly increased over time between 24 and 72 h of exposure (Fig. 2c,

Table 2
Toxicity bioassay endpoints and their registration conditions.

Endpoint	Registration time, h	Biomarker	CytoFLEX emission filter, nm
Growth-rate inhibition	24, 72	PI	ECD, 610
Membrane potential	24, 72	DiOC ₆	FITC, 525
Cell size	72	Forward scattering intensity	FSC

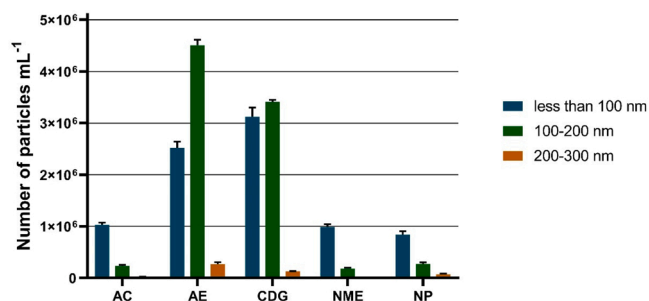


Fig. 1. Particle size distribution in galvanic particle suspensions. AC, Aluminum cleaning; AE, Aluminum etching; CDG, Chemical degreasing; NME, Nonferrous metals etching; NP, Nickel plating.

d). For the red alga *P. purpureum*, only the AC sample caused a growth rate inhibition of more than 20 % after 24 h of exposure (Fig. 2b).

Samples AE and CDG had no significant effect on the growth rate of both microalgae species after 24 h of exposure (Fig. 2a, b). However, after 72 h of exposure, sample CDG stimulated the growth rate of *H. akashiwo* (Fig. 1c) and caused about 40 % growth rate inhibition in *P. purpureum*. Sample AE demonstrated did not show a dose-dependent growth rate inhibition for either microalgae species after 72 h of exposure (Fig. 2c, d).

Sample NME caused significant growth rate inhibition and death of *H. akashiwo* cells after 72 h of exposure at the highest used concentration. Interestingly, after 72 h of exposure to the low concentration (10 %) of this sample (Fig. 2c), the growth rate of *H. akashiwo* was stimulated up to 60 %, which indicates biphasic dose-response relationship. All concentrations of the NME sample stimulated the growth rate of red microalgae *P. purpureum* after 72 h of exposure (Fig. 2d).

Sample NP inhibited the growth rate of *H. akashiwo* at the highest

concentration (about 60 %) after 24 h of exposure (Fig. 2a), and after 72 h the negative effect was not observed (Fig. 1c). On the contrary, this sample inhibited the growth rate of *P. purpureum* (about 40 %) only after 72 h of exposure (Fig. 2d).

The membrane polarization changes in the cells of *H. akashiwo* and *P. purpureum* exposed to particle suspensions obtained from five electroplating workshops are presented in Fig. 3.

After 24 h of exposure, the CDG sample had almost no effect on membrane polarization of both microalgae species (Fig. 3a, b). NP demonstrated membrane depolarization at concentrations 10 and 50 % in both microalgae species after 24 h (Fig. 3a, b). AC, AE, and NME caused depolarization of membranes of the red microalgae *P. purpureum* only at the 10 % concentrations (Fig. 3b), but for *H. akashiwo*, AC caused the highest membrane depolarization at a concentration of 25 %, and NME caused membrane hyperpolarization after 24 h of the exposure (Fig. 3a). The influence of AC at the concentration of 50 % on membrane polarization of *H. akashiwo* (Fig. 3a) is not shown because almost 100 % of the cells were dead, according to the results of growth rate inhibition (Fig. 1a).

After 72 h of the exposure, almost all samples caused depolarization of *H. akashiwo* cell membranes (Fig. 3c). However, the membrane hyperpolarization of *H. akashiwo* under the influence of NME increased after 72 h compared to 24 h of exposure. Membrane hyperpolarization was also observed in *H. akashiwo* cells after 72 h of exposure to AE at the highest concentration. At the same time, the red microalgae *P. purpureum* responded with membrane hyperpolarization after 72 h of exposure to AC and CDG, and with dose-dependent depolarization after 72 h of exposure to NP (Fig. 3d). NME had no effect on membrane polarization of *P. purpureum* after 72 h of exposure.

The changes in the size of *H. akashiwo* and *P. purpureum* cells after 72 h of exposure to galvanic particle suspensions are presented in Fig. 4.

In the control group, *H. akashiwo* had 20 % of 6–10 μm cells and 80 % of 10–15 μm cells. All the tested samples caused enlargement of

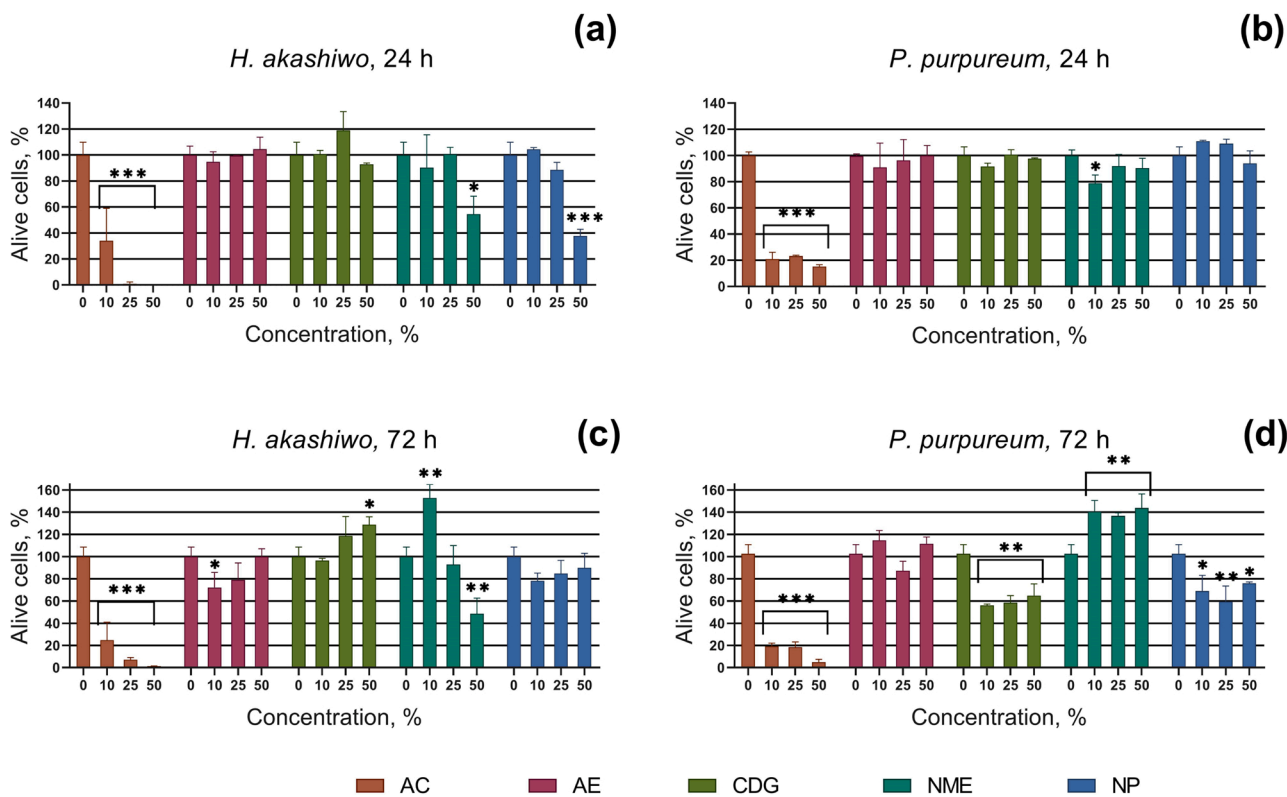


Fig. 2. Growth inhibition of microalgae *H. akashiwo* and *P. purpureum* exposed to galvanic particle suspensions. (a) *H. akashiwo* after 24 h of exposure, (b) *P. purpureum* after 24 h of exposure, (c) *H. akashiwo* after 72 h of exposure, (d) *P. purpureum* after 72 h of exposure. AC, Aluminum cleaning; AE, Aluminum etching; CDG, Chemical degreasing; NME, Nonferrous metals etching; NP, Nickel plating. * p < 0.05, ** p < 0.001, *** p < 0.0001.

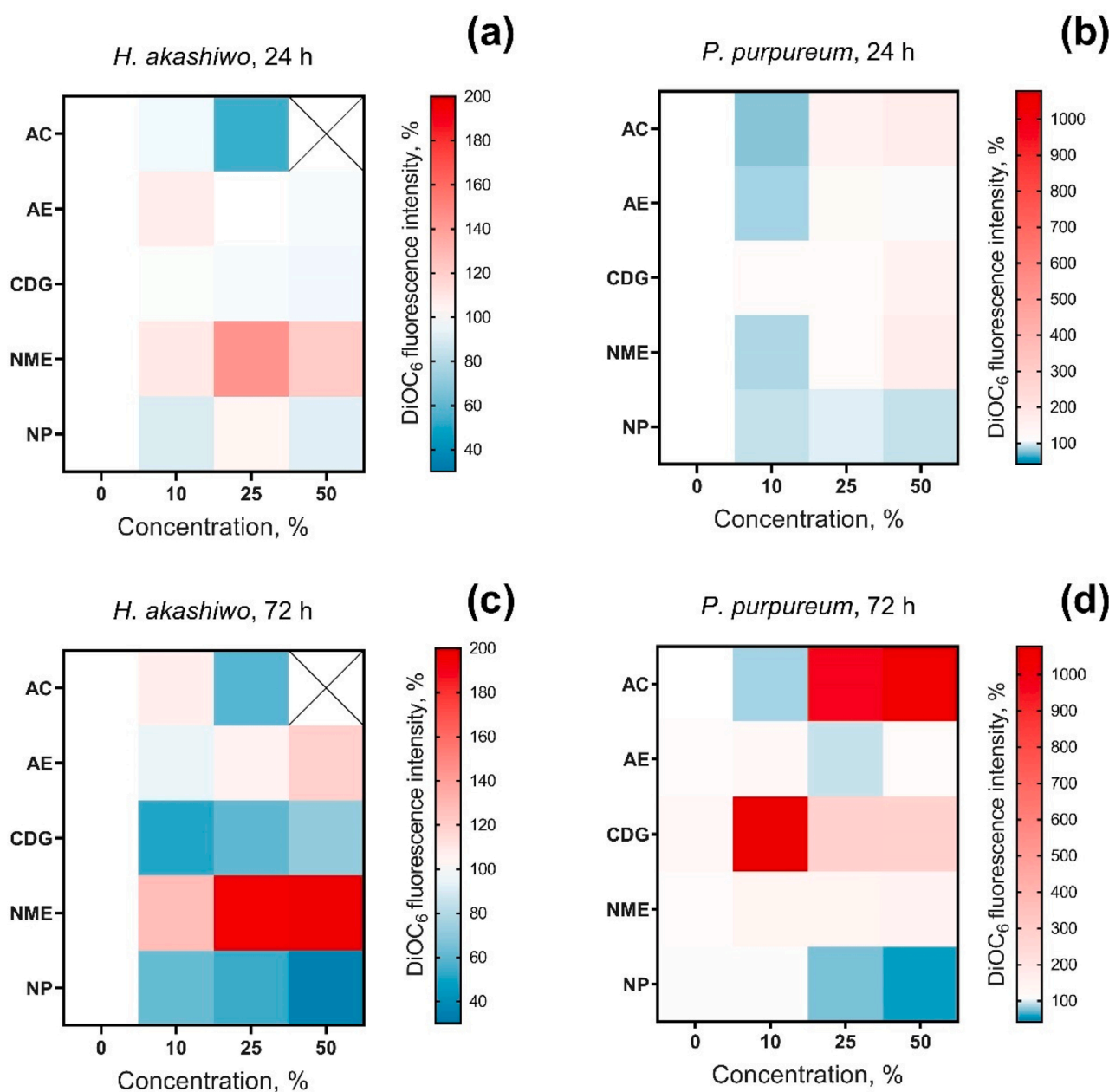


Fig. 3. Changes of microalgae membrane potential after 24 h and 72 h of exposure to galvanic particle suspensions (a) *H. akashiwo* after 24 h of exposure, (b) *P. purpureum* after 24 h of exposure, (c) *H. akashiwo* after 72 h of exposure, (d) *P. purpureum* after 72 h of exposure. AC, Aluminum cleaning; AE, Aluminum etching; CDG, Chemical degreasing; NME, Nonferrous metals etching; NP, Nickel plating.

H. akashiwo cells after 72 h of exposure (Fig. 4a). The most pronounced effect was observed for the cells of *H. akashiwo* that were exposed to the AC and NME samples, where 17 % and 29 % of the cells, respectively, increased by more than 15 μm at the highest used concentration (the data of *H. akashiwo* exposed to AC at the concentration of 50 % are not shown, because almost all the cells were dead).

In the control group, the red alga *P. purpureum* had 62 % of 2–4 μm cells, 36 % of 4–6 μm cells, and 2% of 6–10 μm cells. The enlargement of the algal cells was evident after 72 h of exposure to the AC, AE, and NME samples (Fig. 4b). In the case of *P. purpureum*, the NP sample caused cell size mitigation at the highest used concentration, where 71 % of the cells were in the size range 2–4 μm (Fig. 4b).

4. Discussion

Aquatic toxicity studies are an important part of environmental risk

assessment [59,65]. However, the results of galvanic particle size and quantity distribution in prepared suspensions (Fig. 1) are not correlated with the number of particles in the air near the same electrochemical processes compared to our previous study [14]. In that study, the quantity of PM_{0.3} particles measured by the AeroTrak Handheld Particle Counter 9306 (TSI Incorporated, USA) in the air near the five workshops studied in current work was in the following (descending) order: NP ($8.4 \cdot 10^8$) > AC ($5.1 \cdot 10^8$) > AE ($5.0 \cdot 10^8$) > CDG ($4.5 \cdot 10^8$) > NME ($3.0 \cdot 10^8$). However, the results from this study (Fig. 1) showed that the highest number of particles of this size range were in the AE and CDG sample suspensions. This observation leads us to assume that ultrafine particles emitted from NP and AC processes tend to dissociate in water more than the particles from the other processes under study. Therefore, the higher amount of toxic and bioavailable metal ions from dissolved particles may be the reason behind the significantly higher toxicity of sample AC compared to the other samples (Fig. 2). Several studies

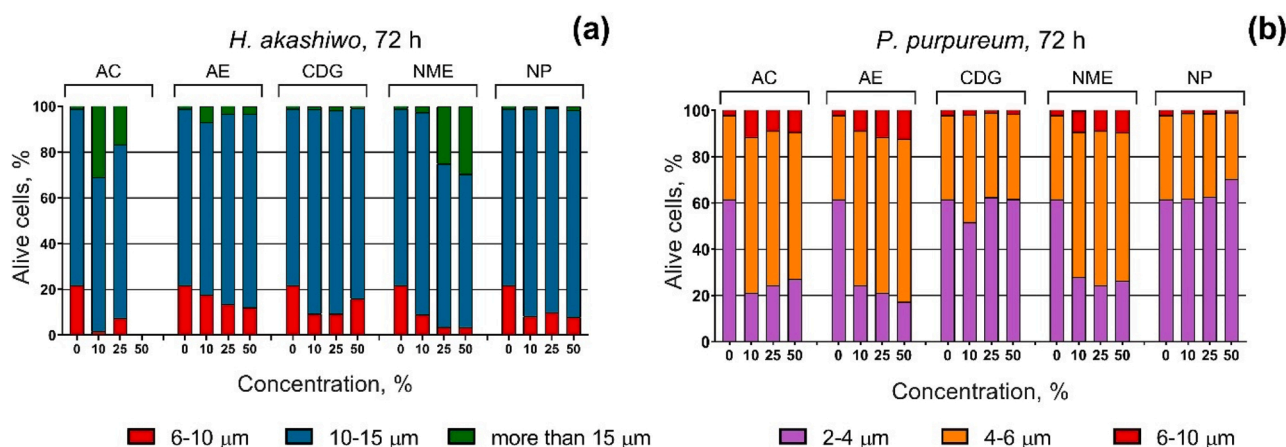


Fig. 4. Changes in microalgae cell size 72 h of exposure to galvanic particle suspensions (a) *H. akashiwo* after 72 h of exposure, (b) *P. purpureum* after 72 h of exposure. AC, Aluminum cleaning; AE, Aluminum etching; CDG, Chemical degreasing; NME, Nonferrous metals etching; NP, Nickel plating.

[66–68] have demonstrated that apart from the chemical composition of particulate matter, the type of interaction between metals and organic compounds plays a significant role in their toxicity, which is synergistic at the lower concentrations of metals.

The influence of industrial particulate matter on membrane polarization and the size of microalgae cells is also often associated with the toxicity of metal-containing particles and released metal ions [69,70]. The increase in cell size of both microalgae species after 72 h of the exposure to samples AC, AE, and NME (Fig. 4) was most probably caused by increased intracellular and extracellular ROS production and further metabolic disorders in microalgal cells affected by toxic metals and metal ions. Thus, an increase in the number of large cells of both microalgae species indicates either the adaptation of microalgae to toxic influence or the disruption of metabolic processes associated with carbon sequestration and cell division [3].

Growth inhibition and change in cell size of microalgae should be assessed as an endpoint of direct cytotoxic action. At the same time, changes of membrane potential can indicate either the initial stage of toxic action or activation of the adaptive capacity of organisms [71,72]. Damage, deformation, and violation of the cell wall integrity are very dangerous for cell life. In animal cells, the intact cell membrane is a vital factor because the membranes accomplish the barrier, mechanical, and matrix properties of organisms [73,74]. Decreased membrane potential (depolarization) may be accompanied by changes in membrane elasticity, loss of lipid microdomains, and changes in ion permeability [75]. Increased polarization (hyperpolarization) of microalgal membranes can lead to membrane dysfunction, disruption of metabolic processes in cells, and subsequent cell death [73].

It should be noted that the *H. akashiwo* and *P. purpureum* cells responded differently to the galvanic suspensions with regards to the membrane polarization alterations (Fig. 3). Microalgal cell-wall is composed of lipids, polysaccharides, and glycoproteins, and can form different chemical bonds with metal-containing particulate matter depend on the nature of the metal ion and donor atoms in biomolecules [76]. Moreover, *P. purpureum* has a higher adherence to more hydrophobic components due to a mucous covering of the cells [77].

A stronger cell hyperpolarization of red microalgae *P. purpureum* (Fig. 3d) was caused by the most toxic sample AC, according to the growth rate inhibition data (Fig. 2). This indicates a significant time and dose-dependent toxicity of this sample, and the possibility of chronic toxicity, which may represent a risk for aquatic organisms even at relatively low concentrations after long-term exposure. The high membrane hyperpolarization of *P. purpureum* after 72 h of exposure to the low concentration of CDG sample (Fig. 3d) correlates with the growth rate inhibition data (Fig. 2d). Therefore, decreased toxicity and decreased cell hyperpolarization with increased concentration of sample

CDG may indicate the activation of algal cell adaptation mechanisms. A similar effect was observed earlier for *P. purpureum* when exposed to metal-based nanoparticles [59]. For *H. akashiwo*, cell membrane hyperpolarization and, therefore, potential chronic toxicity can be highlighted for sample NME (Fig. 3c). Moreover, pronounced biphasic dose-response toxicity of this sample with a low dose stimulation and a high dose inhibition (Fig. 2c) indicates hormesis commonly observed in algae and higher plants [11,78]. The hormetic effect can lead to algal bloom and to further death of living organisms by the hypoxic aquatic condition and cyanotoxin poisoning [79,80]. This observation directly applicable to *H. akashiwo* which can form harmful blooms [81].

The observed toxic effects of electroplating emissions on microalgae confirm their serious threat to aquatic organisms. Long term low dose exposure of multi-component pollutants such as galvanic wastes combined with existing pollution sources can be dramatic for coastal and marine ecosystems. The fact that the effects of toxic stimuli combinations are not taken into account in setting regulatory exposure limits warrants a more detailed approach for further toxicity studies [82]. The application of a real-life risk simulation framework [83–85] is a good opportunity for further environmental toxicity assessment of electroplating exhausts with long-term low dose experiments.

5. Conclusions

In general, the toxicity of PM suspensions obtained in galvanic workshop significantly varies between different electroplating processes. The most hazardous effect and direct cytotoxicity in both microalgae species were caused by the sample collected during the aluminum cleaning process. The toxic effects of galvanic suspensions were expressed in a considerable growth rate inhibition and death of microalgae cells, hyperpolarization of cell membranes, and increase of microalgae cell size. Sample CDG collected during the chemical degreasing process demonstrated chronic toxicity potential at lower concentrations on the red algae *P. purpureum* and stimulated growth-rate of bloom-forming golden-brown algae *H. akashiwo*. Sample NME collected during nonferrous metals etching stimulated the growth-rate of *H. akashiwo* at low concentration and inhibited at high concentration, revealing the hormetic concentration-response relationship. Based on the results of the current study we can conclude that the toxicity of tested suspensions does not correlate with the number of insoluble PM and is associated with the influence of metal ions released in water, which can be a reason for high toxicity to aquatic organisms. These findings highlight the need for more stringent regulation of electroplating exhausts not only in terms of human health but also in the context of environmental safety. Therefore, further research with different test-organisms, a broader set of toxicity biomarkers, and the

application of a real-life risk simulation approach should be performed to address the problem of galvanic exhaust environmental regulation. Further studies in this area will facilitate the regulation of emissions produced by the electroplating industry and prevent the negative impact on human health and the environment.

CRedit authorship contribution statement

Konstantin Pikula: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - review & editing. **Konstantin Kirichenko:** Resources, Writing - original draft. **Igor Vakhniuk:** Resources, Writing - original draft. **Olga-Ioanna Kalantzi:** Writing - review & editing. **Aleksei Kholodov:** Resources, Writing - original draft. **Tatiana Orlova:** Resources. **Zhanna Markina:** Resources. **Aristidis Tsatsakis:** Supervision, Writing - review & editing. **Kirill Golokhvast:** Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

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