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ИнБЮМ – IBSS

Морской биологический журнал
Marine Biological Journal
2025, vol. 10, no. 3, pp. 120–124
https://marine-biology.ru

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UDC 582.26/.27-114.328:549

THE EFFECT OF HEAVY METALS ON THE GROWTH RATE OF MICROALGAE *HETEROSIGMA AKASHIWO, ALEXANDRIUM AFFINE,* AND *PROROCENTRUM FORAMINOSUM*

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Received 18.11.2022; revised 14.04.2023; accepted 12.08.2025.

A rise in the frequency of harmful algal blooms is associated with increasing environmental pollution, in particular with a gain in heavy metal content in waters. The aim of the study was to assess the effect of heavy metals on the growth rate of microalgae causing such blooms. The effect of heavy metals on the growth rate of microalgae *Heterosigma akashiwo*, *Alexandrium affine*, and *Prorocentrum foraminosum* was investigated: cadmium Cd^{2+}, nickel Ni^{2+}, and lead Pb^{2+} at concentrations of 10 and 20 $\mu g \cdot L^{-1}$, as well as zinc Zn^{2+} and iron Fe^{3+} at 50 and 100 $\mu g \cdot L^{-1}$. The evaluation was carried out on the third and seventh days of the experiment. These heavy metals were found to affect all investigated algae species. On the third day, the growth rate of *H. akashiwo* increased with the addition of Cd^{2+}, Pb^{2+}, Ni^{2+}, and Fe^{3+}; the growth was suppressed with the addition of Zn^{2+}. On the seventh day, the microalga inhibition was recorded at 10 and 20 $\mu g \cdot L^{-1}$ of Cd^{2+}, 10 $\mu g \cdot L^{-1}$ of Pb^{2+} and Ni^{2+}, and 100 $\mu g \cdot L^{-1}$ of Zn^{2+}. *H. akashiwo* stimulation occurred at 20 $\mu g \cdot L^{-1}$ of Pb^{2+} and Ni^{2+}, as well as at 50 and 100 $\mu g \cdot L^{-1}$ of Fe^{3+}. The growth rate of *A. affine* on the third day rose at 20 $\mu g \cdot L^{-1}$ of Cd^{2+}, 10 and 20 $\mu g \cdot L^{-1}$ of Pb^{2+} and Ni^{2+}, and 100 $\mu g \cdot L^{-1}$ of Fe^{2+} and Zn^{2+}. On the seventh day, the growth rate dropped because of the negative effect of 10 and 20 $\mu g \cdot L^{-1}$ of Cd^{2+} and Pb^{2+}, as well as 20 $\mu g \cdot L^{-1}$ of Ni^{2+}. Stimulation of *A. affine* growth was registered at 10 $\mu g \cdot L^{-1}$ of Ni^{2+} and at both concentrations of Fe^{3+} and Zn^{2+}. *P. foraminosum* was the least resistant to heavy metals. Its growth rate decreased when exposed to all toxicants, except for Fe^{3+}: in this case, stimulation of the microalga growth occurred on the seventh day of the experiment.

Keywords: *Heterosigma akashiwo*, *Alexandrium affine*, *Prorocentrum foraminosum*, cadmium, nickel, lead, zinc, iron, heavy metals

In recent decades, in various areas of the World Ocean, a considerable rise in harmful blooms of algae has been registered caused by a raphidophyte *Heterosigma akashiwo* (Y. Hada) Y. Hada ex Y. Hara, M. Chihara [Dursun et al., 2016; Heisler et al., 2008] and by dinoflagellates of the genera *Alexandrium* Halim, 1960 [Anderson et al., 2012] and *Prorocentrum* Ehrenberg, 1834 [Li et al., 2021; Shin et al., 2019]. Some researchers relate a gain in frequency of such events with increasing pollution of the environment [Heisler et al., 2008]. Heavy metals are regularly recorded in waters of Russian seas [Marine Water Pollution, 2020], and they are more and more often being revealed in other areas of the World Ocean. Effects of these toxicants on plant organisms have been studied for a long time, but most investigations are focused on sublethal and lethal concentrations, while metal content corresponding to a natural one is analyzed less and less [Nagajoti et al., 2010].

Due to the above, the aim of this work was to assess the effect of cadmium, nickel, lead, zinc, and iron on the growth rate of three microalgae: *Heterosigma akashiwo*, *Alexandrium affine* H. Inoue & Y. Fukuyo Balech, and *Prorocentrum foraminosum* Faust.

MATERIAL AND METHODS

Plant material. The objects of the study were cultures of unicellular algae *H. akashiwo* (strain MBRU_HAK-SR11) (Raphidophyceae), *A. affine* (strain AFRU-12) (Dinophyta), and *P. foraminosum* (strain MBRU_PrRUS_16) (Dinophyta). All the algae were provided by the "Marine Biobank" core facility of the A. V. Zhirmunsky National Scientific Center of Marine Biology, FEB RAS (https://marbank.dvo.ru/).

Experimental conditions. The algae were cultured on *f* medium [Guillard, Ryther, 1962] in 250-mL Erlenmeyer flasks. A culture medium volume was 100 mL; temperature was +18 °C; light intensity was 70 $\mu mol \cdot m^{-2} \cdot s^{-1}$; and a light/dark cycle was 14 h : 10 h (light : dark). There was no air bubbling. A culture at the exponential growth stage was used as an inoculum. The initial cell concentration was 20,000 $cells \cdot mL^{-1}$ for *H. akashiwo*, 300 $cells \cdot mL^{-1}$ for *P. foraminosum*, and 1,000 $cells \cdot mL^{-1}$ for *A. affine*. Experiments lasted for seven days.

Samples of 1 mL were taken with a pipette dispenser (Tomanalit, Russia). Cells were counted on the third and seventh days in a 50-mL Nageotte chamber under an EVOS M5000 microscope (Thermo Fisher Scientific, the USA) at 10× magnification. A total of six replicates were counted for each variant of the experiment. The investigations were carried out in three biological replicates [Rukovodstvo, 2002].

Toxicants and their concentrations. Cd^{2+} was added as $3CdSO_4 \times 8H_2O$; Ni^{2+}, $NiSO_4 \times 7H_2O$; Pb^{2+}, $PbCl_2$; Zn^{2+}, $ZnSO_4 \times 7H_2O$; and Fe^{3+}, $FeCl_3 \times 6H_2O$, with recalculation into metal ions on the day of the experiment. Concentrations were chosen based on data on the content of these heavy metals in coastal waters of Russia and their maximum permissible concentrations (MPC). Analyzed values correspond to MPC and 2MPC [Marine Water Pollution, 2020].

The growth rate was calculated by the standard formula [Guillard, Ryther, 1962] The reliability of differences between samples was assessed using the Mann–Whitney test at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

The growth rate of *H. akashiwo* was higher on the third day than the control when exposed to Pb^{2+} and Ni^{2+} at a concentration of 10 $\mu g \cdot L^{-1}$ and Fe^{2+} at 50 and 100 $\mu g \cdot L^{-1}$ (Table 1). In other cases, the value did not differ significantly from the control. On the seventh day, the growth rate at 10 $\mu g \cdot L^{-1}$ of Cd^{2+} was noticeably lower than that in the control.

The growth rate of *A. affine* increased significantly on the third day under the effect of Cd^{2+} at a concentration of 20 $\mu g \cdot L^{-1}$, as well as Pb^{2+} and Ni^{2+} (Table 2). The addition of 50 $\mu g \cdot L^{-1}$ of Fe^{2+} and Zn^{2+} governed a decrease in the studied parameter. On the seventh day, with 10 and 20 $\mu g \cdot L^{-1}$ of Cd^{2+} and Pb^{2+} in a medium, the alga growth was inhibited. At the same time, 10 $\mu g \cdot L^{-1}$ of Ni^{2+}, 50 $\mu g \cdot L^{-1}$ of Fe^{2+}, and 100 $\mu g \cdot L^{-1}$ of Zn^{2+} stimulated the growth.

Table 1. Mean values of the growth rate (div.·day^{-1}) of *Heterosigma akashiwo* when exposed to heavy metals at different concentrations (μg·L^{-1})

Day	0	Cd^{2+}		Pb^{2+}		Ni^{2+}		Fe^{2+}		Zn^{2+}	
		10	20	10	20	10	20	50	100	50	100
The third	0.31	0.34	0.31	**0.39**	0.35	**0.39**	0.35	**0.42**	**0.40**	0.27	0.28
The seventh	0.23	*0.17*	0.20	0.21	0.26	0.21	0.26	0.24	0.25	0.23	0.22

Note: values significantly higher than the control level ($p < 0.05$) are highlighted in bold; a value significantly lower than the control level is highlighted in italics.

Table 2. Mean values of the growth rate (div.·day^{-1}) of *Alexandrium affine* when exposed to heavy metals at different concentrations (μg·L^{-1})

Day	0	Cd^{2+}		Pb^{2+}		Ni^{2+}		Fe^{2+}		Zn^{2+}	
		10	20	10	20	10	20	50	100	50	100
The third	0.21	0.18	**0.33**	**0.36**	**0.36**	**0.45**	**0.37**	*0.03*	0.24	*0.11*	**0.41**
The seventh	0.13	*−0.16*	*−0.29*	*−0.01*	*0.05*	**0.21**	0.12	**0.27**	0.14	0.13	**0.23**

Note: values significantly higher than the control level ($p < 0.05$) are highlighted in bold; values significantly lower than the control level are highlighted in italics.

The growth rate of *P. foraminosum* at all analyzed metal concentrations was lower both on the third and seventh days compared to the control. However, on the seventh day of the experiment, with the addition of Fe^{2+}, stimulation of the microalga growth was recorded (Table 3).

Table 3. Mean values of the growth rate (div.·day^{-1}) of *Prorocentrum foraminosum* when exposed to heavy metals at different concentrations (μg·L^{-1})

Day	0	Cd^{2+}		Pb^{2+}		Ni^{2+}		Fe^{2+}		Zn^{2+}	
		10	20	10	20	10	20	50	100	50	100
The third	0.24	*0.05*	0.21	*0.12*	*0.10*	*0.08*	0.20	*−0.20*	*−0.33*	*−0.11*	*0.10*
The seventh	0.13	*0.05*	*0.01*	0.12	0.10	*0.01*	*−0.03*	0.16	0.24	0.13	0.06

Note: values significantly lower than the control level ($p < 0.05$) are highlighted in italics.

The toxicity of heavy metals for microalgae of different divisions has been shown repeatedly. It is related mostly to the fact that they cause oxidative stress resulting from an increase in abundance of free radicals. Due to damage to molecules by free radicals, physiological processes are violated, and this ultimately affects the viability of cells [Nagajoti et al., 2010].

Heavy metals are non-degradable; accordingly, their interaction with organisms remains constant throughout an experiment. In this case, a toxic effect may occur, and it was registered for *P. foraminosum* in our study. However, we recorded stimulation of growth with a further decrease in its intensity, *i. e.*, hormesis effect, for *H. akashiwo* in a medium containing lead, nickel, and iron and for *A. affine* in a medium containing cadmium, lead, nickel, and zinc. Hormesis is a two-phase, sometimes multiphase, dose-dependent response of an organism to the effect of a chemical substance; it is characterized by periods of stimulation and suppression of various biological functions (growth intensity of the population of organisms changes as well). The manifestation of hormesis is driven by physiological characteristics of an organism. Usually, it occurs under the effect of concentrations lower than lethal ones [Calabrese, Mattson, 2011; Cedergreen et al., 2007].

In general, the studied species were more sensitive to the effect of all metals than other representatives of microalgae. Thus, inhibition of the growth rate in *Phaeocystis antarctica* population by 10% was revealed at a concentration of 135 μg·L^{-1} of Cd^{2+} and 260 μg·L^{-1} of Pb^{2+} [Gissi et al., 2015]. The growth rate of *Isochrysis galbana* rose at 50–100 μg·L^{-1} of Pb^{2+} [Ahmadi et al., 2021]. At the same time, *Ankistrodesmus falcatus* turned out to be a species close in its resistance to the effect of Ni^{2+}: its cell abundance decreased at metal concentrations of 15–30 μg·L^{-1} after just 24 h of the experiment [Martínez-Ruiz, Martínez-Jeronimo, 2015].

Conclusion. Heavy metals we tested affected all analyzed microalgae species. The most sensitive one was *Prorocentrum foraminosum*, and the least sensitive one was *Heterosigma akashiwo*. The growth rate of *H. akashiwo* increased with the addition of Cd^{2+}, Pb^{2+}, Ni^{2+}, and Fe^{2+}; the growth rate of *Alexandrium affine* rose in a medium containing Cd^{2+}, Pb^{2+}, Ni^{2+}, Fe^{2+}, and Zn^{2+}. Therefore, it can be assumed that the listed metals can affect the forming of blooms of these species.

This work was financed by the A. V. Zhirmunsky National Scientific Center of Marine Biology, FEB RAS, state research assignment FWFE-2024-0004 "Dynamics of marine ecosystems, adaptation of marine organisms and communities to environmental factors."

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ДЕЙСТВИЕ ТЯЖЁЛЫХ МЕТАЛЛОВ НА СКОРОСТЬ РОСТА МИКРОВОДОРОСЛЕЙ *HETEROSIGMA AKASHIWO*, *ALEXANDRIUM AFFINE* И *PROROCENTRUM FORAMINOSUM*

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Увеличение частоты вредоносных цветений водорослей связывают с растущим загрязнением окружающей среды, в частности с повышением содержания тяжёлых металлов в водах. Цель работы — оценить влияние тяжёлых металлов на скорость роста микроводорослей, вызывающих такие цветения. Изучено действие ионов кадмия Cd^{2+}, свинца Pb^{2+} и никеля Ni^{2+} в концентрациях 10 и 20 мкг·л$^{-1}$, а также цинка Zn^{2+} и железа Fe^{3+} в концентрациях 50 и 100 мкг·л$^{-1}$ на скорость роста микроводорослей *Heterosigma akashiwo*, *Alexandrium affine* и *Prorocentrum foraminosum*. Оценка выполнена на третьи и седьмые сутки опыта. Выявлено, что эти тяжёлые металлы оказывали влияние на все изученные виды водорослей. На третьи сутки скорость роста *H. akashiwo* увеличивалась при добавлении Cd^{2+}, Pb^{2+}, Ni^{2+} и Fe^{3+}; подавление роста обнаружено при внесении Zn^{2+}. На седьмые сутки ингибирование микроводоросли выявлено при содержании 10 и 20 мкг·л$^{-1}$ Cd^{2+}, 10 мкг·л$^{-1}$ Pb^{2+} и Ni^{2+}, а также 100 мкг·л$^{-1}$ Zn^{2+}. Стимуляция *H. akashiwo* происходила при 20 мкг·л$^{-1}$ Pb^{2+} и Ni^{2+}, 50 и 100 мкг·л$^{-1}$ Fe^{3+}. Скорость роста *A. affine* на третьи сутки увеличивалась при 20 мкг·л$^{-1}$ Cd^{2+}, 10 и 20 мкг·л$^{-1}$ Pb^{2+} и Ni^{2+}, а также 100 мкг·л$^{-1}$ Fe^{2+} и Zn^{2+}. На седьмые сутки рост уменьшался в результате негативного влияния 10 и 20 мкг·л$^{-1}$ Cd^{2+} и Pb^{2+} и 20 мкг·л$^{-1}$ Ni^{2+}. Стимуляция роста водоросли зафиксирована при 10 мкг·л$^{-1}$ Ni^{2+}, а также при обеих концентрациях Fe^{3+} и Zn^{2+}. Наименее устойчивым к тяжёлым металлам оказался вид *P. foraminosum*. Его скорость роста снижалась при воздействии всех токсикантов, за исключением Fe^{3+}: в данном случае происходила стимуляция роста микроводоросли на седьмой день эксперимента.

Ключевые слова: *Heterosigma akashiwo*, *Alexandrium affine*, *Prorocentrum foraminosum*, кадмий, никель, свинец, цинк, железо, тяжёлые металлы