

Protective Effect of Extracts from Some *Artemisia* L. Species at Plant Plasma Membrane Disturbances Caused by Heavy Metals

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Plant bioactive compounds are supposed to be the modifiers of structural-functional properties of cell plasma membrane under heavy metals contamination. To test a protective effect of extracts from *Artemisia* L. species, possessing high adaptive potential to different soil contaminations, on electrical properties of algae cells under HM treatments was the aim of the present work. Extracts from *Artemisia* plants were isolated from above-ground parts of *A.scoparia* and *A.szovitsiana* by acetone hydrolyzation. Microelectrode techniques were used to study the regularities of change of membrane potential and membrane resistance of *Chara gymnophylla* and *Nitella flexilis* plasmalemma under influence of Cd, Cu, Zn, Pb and the extracts from *Artemisia* species. Statistically reliable hyperpolarizing effect of *A.szovitsiana* and *A.scoparia* extracts in relation to Zn and Pb was revealed, respectively. Elimination by extracts a decrease of membrane conductivity caused by Cd and Zn and also an increase of conductivity by Cu was determined. Though a high HM accumulation capacity of *Artemisia* L. species is noticed to not connect with protective effect of their extracts on membrane conductivity, they can be a potential source for the searching new chemical compounds which are capable to induce the plant tolerance to stress impacts.

Key words: heavy metals, extracts from *Artemisia* L., plasma membrane of *Chara gymnophylla* and *Nitella flexilis*, protective effect, accumulation capacity

INTRODUCTION

Biodiversity of flora and fauna is currently under considerable threat due to rapidly increasing number of pollution and irrational utilization of biological resources all over, in Azerbaijan as well. Azerbaijan enjoys an extremely rich diversity of natural species of valuable plants. However, arisen of ecological imbalance, in particular due to environmental pollution is a serious reason for vigilance for fragmentation of biocenosis and requires the acceptance of urgent actions, including appropriate monitoring system, necessary investigation and assessment of biodiversity. In spite of great significance is attached to the conservation of biodiversity of natural population here (National Strategy and Action Plan on Conservation and Sustainable Use of Biodiversity in the Republic of Azerbaijan, 2006) a reduction of the rare and endemic species of plants is still observed.

Among the key threats to ecosystems the pollution by heavy metals (HM) occupies one of the main places. Plants differ one from other by response reactions to HM-contaminations on various levels of their organization. The majority of them are the plants distinguished by their resistance strategy. *Artemisia* plants are one of genus species of

which show the different strategies of tolerance to HM (Toderich et al., 2002; Bashmakov and Lukatkin, 2002; Kim et al., 2003; Li et al., 2003; Takeda et al., 2005; Alirzayeva et al., 2006, 2008).

Previous work on monitoring of plant species growing on various contaminated regions of Azerbaijan made possible to find out the more adapted *Artemisia* L. plants on these areas (Alirzayeva et al., 2006). Some species/ecotypes of *Artemisia* L. are shown to display distinctive strategies of resistance to HM in dependence on their habitat, appearing as tolerant, either excluder or indicator or else accumulator plants (Alirzayeva et al., 2006, 2008). In spite of the developed various tolerance strategies all tested *Artemisia* species possessed the high adaptive potential to HM-pollution.

HM are known to be able to cause functional modifications in plant organism on plasmalemma (Yurin, 2003) changing the membrane electrical potential and conductivity of a cells. Plant extracts are shown to have a protective effect displaying an ability of interacting with cell membrane components (Adam et al., 1998; Ali-zade et al., 2003, 2006; Sokolik et al., 2005) and changing their potential and conductivity (Maffei et al., 2001).

Extracts of *Artemisia* plants with high resistance and accumulation capacity of HM containing

rather considerable contents of biological active substances were supposed to play a role in increasing of resistance of cells of other plants, i.e. in another level of plant organization. However, the biological active chemical compounds of *Artemisia* species, particularly from regions of Azerbaijan as modifiers of structural-functional properties of plant membrane are insufficiently studied.

The aim of the present work was a testing of protective effect of extracts from some *Artemisia* L. species on electrical membrane potential (MP) and conductivity of algae cells under HM treatments based on tolerance and accumulation capacity of *Artemisia* plants.

MATERIALS AND METHODS

Two *Artemisia* species *A.scoparia* and *A.szovitsiana* and freshwater algae *Chara gymnophylla* and *Nitella flexilis* were used as experimental objects.

Algae were grown in solution of Artificial Pond Water (APW) consisting of 10^{-4} mol/l KH_2PO_4 , $2 \cdot 10^{-4}$ mol/l $\text{Mg}(\text{NO}_3)_2$, $4 \cdot 10^{-4}$ mol/l CaCl_2 , 10^{-3} mol/l NaHCO_3 at room temperature under artificial illumination.

Extract stock solutions in concentration of 10 mg l^{-1} were successively diluted by APW up to concentrations used in experiments. In one part of experiment, extracts ($1 \text{ } \mu\text{g l}^{-1}$, $10 \text{ } \mu\text{g l}^{-1}$) were added to the flowing solution after 5-day keeping of plants in APW + extract in the same concentration and establishment of steady-state MP level. Plants exposed to one of the tested HM (concentration of 10^{-5} - 10^{-6} mol/l) for 5 days were then used in another part of experiment and after establishment of steady-state MP level the extracts were added to the flowing solution.

Parameter of electrogenic activity of cell plasmalemma was MP, and parameter of integral conductivity was membrane resistance. MP of *Chara* cells is defined on two-electrode Hogg methods (Hogg et al., 1968). Method of voltage clamp was also used when potential difference of plasmalemma of *Nitella flexilis* was fixed on certain level and ion current was registered.

Microelectrode techniques were used for the determination of regularities of change of MP (E_m) and membrane resistance (R_m) of plasma membranes of *Chara gymnophylla* and *Nitella flexilis* cells under influence of both HM (Cd, Cu, Zn, Pb) and the extracts containing the biologically active compounds from the shoots of *Artemisia* species.

To study the distinctions of membranotropic effects of extracts from *Artemisia* plants they were isolated from crushed air-dry above-ground parts of *A.scoparia* and *A.szovitsiana* by threefold acetone hydrolyzation; the hydrolyzates were then filtrated

and distilled in water bath (Serkerov and Aleskerova, 1985).

All analyses were run in several replications. Data were evaluated by analysis of variance using MS Excel.

RESULTS AND DISCUSSION

A studying of accumulation, transfer and action of heavy metals in plants is of high importance for understanding of mechanisms of their effects on different levels of plant organization. An importance of the electrophysiological approaches to the studying of influence peculiarities of xenobiotics (pesticides, heavy metals, etc.) and physiologically active compounds on mechanisms of ion transport in plant plasma membranes is somewhat reported (Demidchik et al., 1997; Musayev et al., 2001). Ion transport system of investigated *Chara gymnophylla* cells was observed to be in various functional states under HM effect. A dispersion of values of MP – E_m , and resistance – R_m of cells in wide ranges is hereof evidence (Musayev and Ismayilov, 2005).

Extracts of *Artemisia* plants are known to contain the valuable biological active compounds like sesquiterpene lactones, coumarins, glycosides and other secondary metabolites, (Serkerov and Aleskerova, 1981; Serkerov, 2005) which interacting with sterols of the cell membranes (Ali-zade et al., 2005; Yarnell and Abascal, 2009) lead to the destruction of their selective permeability. The leaves of *A.scoparia* were reported to contain 33 chemical constituents representing 99,83% essential oils rich in monoterpenes (71,6%) (Singh et al., 2009a). So, *A.scoparia* is considered as an important biore-source for extraction of monoterpenoid-rich oil (Singh et al., 2009b). Thereby *Artemisia* plants growing under the different strained environmental conditions possess the protective mechanisms enable them to withstand both osmotic and toxic actions. Hence a study of protective effect of extracts from resistant to HM *Artemisia* species on electrophysiological characteristics of plants arouses an interest. Extracts from tested *Artemisia* L. species had action on change of electrophysiological properties of algae cells in different ways in the presence of various metals (Cd, Cu, Zn, Pb).

A testing of their effects on change of MP of the *Chara* cells in Cd and Cu ions presence has revealed the statistically reliable differences (Fig. 1). Under these conditions, the extract from *A.scoparia* displayed a more positive effect on change of MP than extract from *A.szovitsiana*. The observed positive effect of both species is evidence for initial action of extracts on plasma membrane that correspondingly leads to change of balance of ion fluxes.

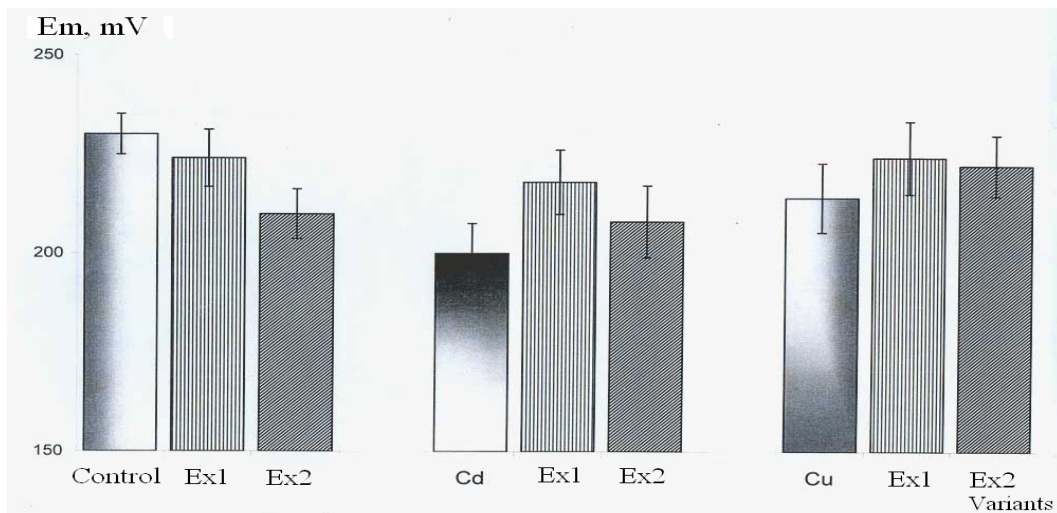


Figure 1. Effect of plant extracts from *Artemisia scoparia* (Ex1) and *Artemisia szovitsiana* (Ex2) on membrane potential value of cells in CdCl₂ and CuSO₄ presence. Salt solutions were used in 10⁻⁶ mol/l concentration.

Results obtained also confirm a fact of statistically reliable hyperpolarization of *Chara* membrane growth in the presence of both Cd and extract from *A.scoparia* in solution. The character of kinetic curves clearly demonstrates a hyperpolarizing effect of this extract against a background of depolarization of membrane potential by different Cd concentrations (Fig. 2).

A diverse effect of two extracts at inhibition of MP by Pb and Zn was found (Table 1). An adding of Ex1 from *A.scoparia* removes a depolarizing effect, but Ex2 from *A.szovitsiana* recovers the MP to the initial level at the Zn treatment.

At the same time, Ex1 leads to hyperpolarization of MP at the Pb treatment of plant. Data obtained distinctly reveal a hyperpolarizing effect of

A.szovitsiana in relation to Zn and *A.scoparia* in relation to Pb. It should be noted that extracts themselves induced the significantly less membrane effects than their using together with metals.

Extracts from *A.scoparia* and *A.szovitsiana* displayed a distinctive protective effect on the Zn-induced changes of plasmalemma conductivity of *Nitella flexilis* (Fig. 3). Extracts from *A.scoparia* collected from both HM-polluted (Ex3) and unpolluted soils (Ex1) did not possess the protective effect in relation to Zn, while extract from *A.szovitsiana* displayed a protective effect on Zn treated cells. It may be a result of modification of membrane lipid component or extract-induced growth of conductivity of nonselective cation channels.

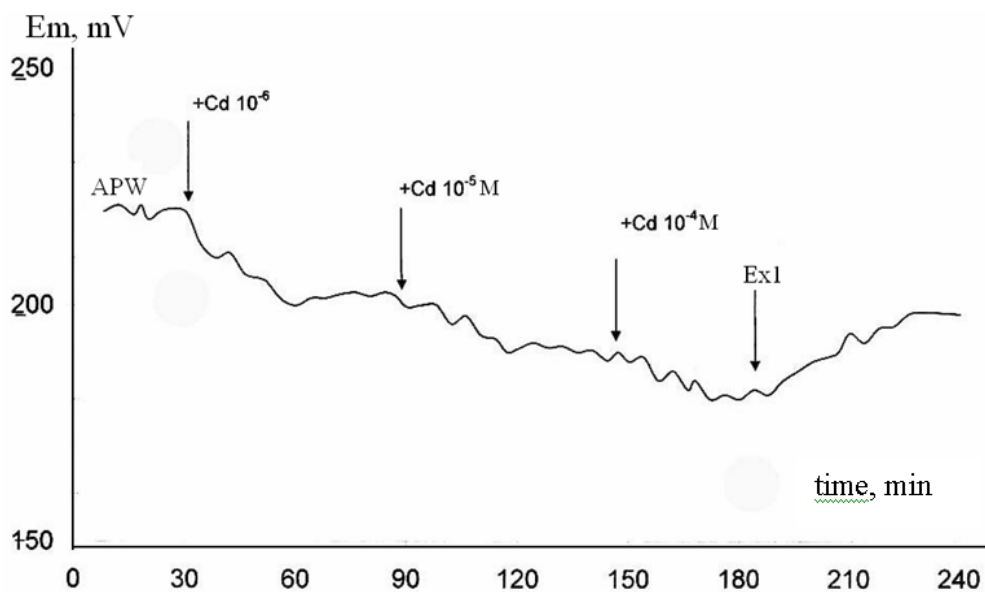


Figure 2. Kinetics of membrane potential change (E_m) of *Chara* cells at addition of rising concentrations of CdSO₄ to the medium and at its replacement by extract from *Artemisia scoparia* (Ex1).

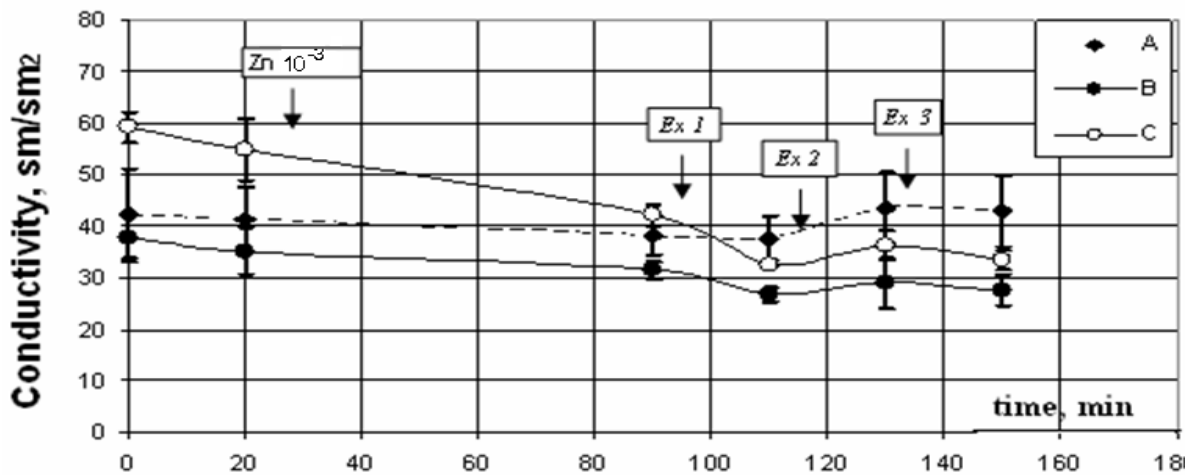
Table 1. Effect of plant extracts from *Artemisia scoparia* (Ex1) and *Artemisia szovitsiana* (Ex2) on membrane potential (E_m , mV) of *Chara gymnophylla* cells cultivated in $ZnSO_4$ and $Pb(NO_3)_2$ presence.

Control (APW)	10^{-7} mol/l $ZnSO_4$ (5-day exposition)	10^{-5} mg l^{-1} Ex1 (in experiment)	10^{-5} mg l^{-1} Ex2 (in experiment)
-213 ± 17 (5)	-172 ± 9 (5)	-189 ± 10 (5)	-215 ± 8 (5)
Control (APW)	10^{-6} mol/l $Pb(NO_3)_2$ (5-day exposition)	10^{-5} mg/l Ex1 (in experiment)	10^{-5} mg/l Ex2 (in experiment)
-190 ± 9 (5)	-187 ± 6 (5)	-202 ± 12 (5)	-195 ± 11 (5)

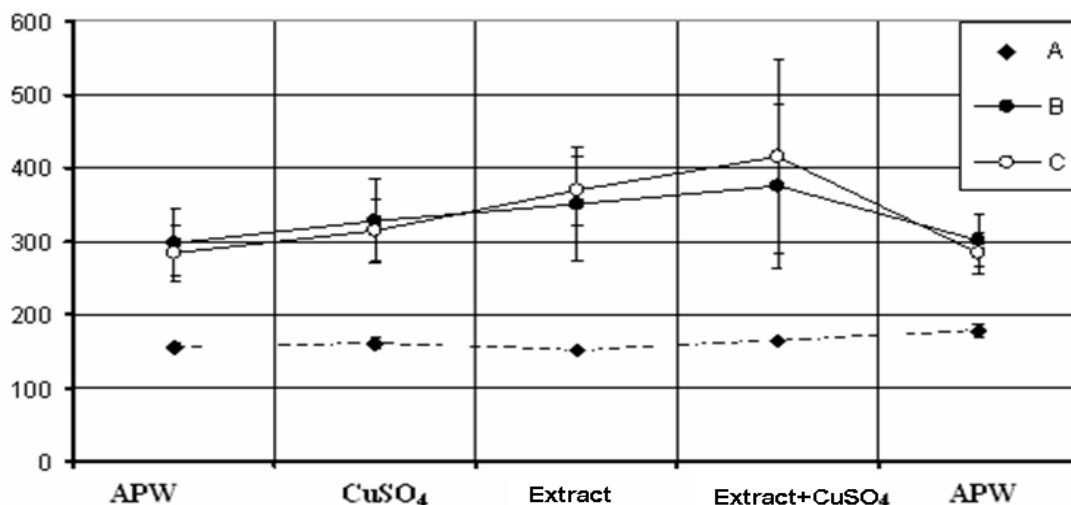
Cu increased the conductivity of outward-rectified K^+ -channels (ORKC) for one and a half time, while the change in relation to inward-rectified channels (IRKC) was not observed. But *A.szovitsiana* extract increased the conductivity of IRKC and fast activated ORKC for a 10%. In the combined presence of Cu and extracts the sharp rise of membrane conductivity and cells destruction were then observed (Fig. 4). Thus it is supposed that the extract unambiguously delays the Cu-

induced growth of membrane conductivity that can be interpreted as its protective effect.

It is known that coumarins are the dominating compounds in *A.scoparia* extracts in magnitude relation, while in *A.szovitsiana* extracts lactones are prevailing components (Serkerov and Aleskerova, 1981; Serkerov, 2005). Hence, either coumarins or lactones as the main components of these species are supposed to induce a protective effect on cell plasma membrane under HM contamination.

**Figure 3.** Effect of extracts from *A.scoparia* (Ex1) and *A.szovitsiana* (Ex2) on Zn-induced change of plasmalemma conductivity of *Nitella flexilis*.

A - inward-rectified K^+ -channels, B - outward-rectified K^+ -channels, inward current, C – outward-rectified K^+ -channels, outward current.

**Figure 4.** Effect of *A.szovitsiana* extracts on Cu-induced changes of plasmalemma conductivity of *Nitella flexilis*.

A - inward-rectified K^+ -channels, B – fast activated outward-rectified K^+ -channels, C – slow activated outward-rectified K^+ -channels.

Table 2. Comparison of accumulating capacity of *Artemisia* L. species and elimination of HM effect by their extracts in relation to membrane potential of plant cells

Metals	Plants	<i>A.scoparia</i>		<i>A.szovitsiana</i>	
		Accumulation capacity, $\mu\text{g g}^{-1}$	MP changes, mV	Accumulation capacity, $\mu\text{g g}^{-1}$	MP changes, mV
Cd		4,02±0,9	18±2	0,11±0,03	8±2
Cu		34,01±8,3	10±2	17,5±1,5	8±2
Pb		160,08±42,4	15±2	1,79±0,3	5±2
Zn		735,93±129,6	17±2	106,5±13,1	42±2

Among the *Artemisia* species tested the *A.scoparia* showed the highest capacity to accumulate heavy metals, mainly Zn, Pb, Cu in shoots (Alirzayeva et al., 2006) and can be referred to accumulator group plants. *A.szovitsiana* also distinguished by its adaptive potential to various types of contaminations and is widespread in all polluted areas of Azerbaijan. As other *Artemisia* species (*A.scoparia*, *A.fragrans*, *A.caucasica*) tested (Alirzayeva et al., 2006) this species also mostly accumulated Zn and Cu in its shoots. *A.szovitsiana* growing on sites with different levels of HM-contamination demonstrated different resistance strategies. On relatively low contaminated sites where the soil Cu and Zn concentrations were revealed to be about 6 mg kg⁻¹ and 20 mg kg⁻¹, respectively, plants from these sites storing in their shoots about Cu 20 mg kg⁻¹ and Zn 100 mg kg⁻¹ were proved as accumulator species. *A.szovitsiana* growing on soils with 60 mg kg⁻¹ of Cu concentration and 100 mg kg⁻¹ of Zn not displaying high uptake capacity accumulated Cu and Zn in amounts about 15 and 70 mg kg⁻¹, respectively.

The efforts to compare the data on HM accumulation by both *Artemisia* species tested (Alirzayeva et al., 2006) and elimination of HM effect by extracts from these species revealed their distinctive strategies also in these characteristics (Table 2). While *A.scoparia* characterized by its highest accumulation capacity for Zn, the largest elimination effect with highest value of MP was observed for this metal under the influence of *A.szovitsiana* extract. But the higher elimination effect for Cd, Cu, Pb was observed in the presence of *A.scoparia* extract. At the same time, *A.scoparia* plants accumulated in its shoots the higher amounts of these metals than *A.szovitsiana*. With respect to the ratio of plant accumulation capacity to change of MP this value was found to be several times higher in case of *A.scoparia* for all HM tested.

CONCLUSIONS

A revelation of protective role of biological active substances from *Artemisia* L. plants in increasing of resistance of algae cells plasma membrane

under HM-contamination has revealed a statistically reliable hyperpolarizing effect of *A. szovitsiana* and *A. scoparia* extracts in relation to Zn and Pb influence, respectively. Elimination by extracts a decrease of membrane conductivity caused by Cd and Zn and also an increase of conductivity caused by Cu was also determined.

Though it is noticed that the high HM accumulation capacity of some investigated *Artemisia* L. species is not connected with protective effect of their extracts in relation to membrane conductivity. But the finding data suggests that some compounds in *Artemisia* extracts are able to induce the tolerance to toxic influence of heavy metals on plasma membrane level. Thus, *Artemisia* can be the potential source for searching new substances which are capable to induce in plants the tolerance to stress impacts.

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Аğır Metalların Тәсириндән Bitkilәrin Плазmatик Membranında Baғ Verән Zәdәlәнмәләр Zamanı Bәzi Artemisia Növlәri Ekstraktlarının Qoruyucu Тәsiri

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Әvvәлләр müәyyән olunmuş tәcrübә nәticәләр әsasında fәrz olunmuşdur ki, ağır metallarla (AM) çirklәнmә zamanı bitkilәrin biofәal birlәşmәləri hüceyrәnin plazmatik membranının struktur-funksional xüsusiyyətlәrinin modifikatoru rolunu oynayır. Tәqdim edilән tәdqiqat işinin mәqsәdi müxtәlif torpaq çirklәнmәlərinә yüksәk adaptasiya potensialına malik *Artemisia L.* növlәrinin ekstraktlarının AM-la işlәнmiş yosun hüceyrәlərinin elektrik xüsusiyyətlәrinә qoruyucu tәsirinин öyrәnilmәsi olmuşdur. Ekstraktlar *A.scoparia* vә *A.szovitsiana* bitkilәrinin yerüstü hissәlərindән asetonla hidrodистilyasiya metodu ilə alınmışdır. Cd, Cu, Zn, Pb vә *Artemisia* növlәrinin ekstraktlarının iştirakı ilə *Chara gymnophylla* vә *Nitella flexilis*-in plazmalemmasının potensialı vә müqavimәtinin dәyişmә qanunauyğunluqlarının tәdqiqi üçün mikroelektrod texnikasından istifadә edilmişdir. Zn vә Pb-la işlәнmiş hüceyrәlərdә, müvafiq olaraq, *A.szovitsiana* vә *A.scoparia* ekstraktlarının tәsiri plazmatik membranın statistik yәqin hyperpolyarlaşmasını törәtmışdir. Ekstraktların vasitәsilә Cd vә Zn-ın tәsirindән membran keçiriciliyindә baғ verән azalmanın, vә Cu-un tәsirindән isә artımın aradan qaldırılması müәyyән edilmişdir. Baxmayaraq ki, *Artemisia L.* növlәrinin AM-ı yüksәk toplama qabiliyyәtinin onların ekstraktlarının hüceyrәlərin membran keçiriciliyinә qoruyucu tәsiri ilə әlaqәli deyil, onlar bitkilәrin stress tәsirlәrә davamlılığını artıra bilәcәk yeni kimyәvi birlәşmәlərin axtarışı üçün potensial mәнбә ola bilәр.

Протекторное Действие Экстрактов Некоторых Видов *Artemisia L.* При Повреждениях Плазматических Мембран Растений Тяжелыми Металлами

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На основе ранее установленных экспериментальных данных было предположено, что при загрязнении тяжелыми металлами (ТМ) биоактивные соединения растений оказываются модификаторами структурно-функциональных свойств плазматических мембран клеток. Целью настоящей работы явилась проверка протекторного действия экстрактов различных видов *Artemisia*, обладающих высоким адаптивным потенциалом к почвенным загрязнениям, на электрические свойства клеток водорослей при обработке их ТМ. Растительные экстракты были получены из надземных частей *A. scoparia* и *A. szovitsiana*, методом ацетоновой гидродистилляции. Для изучения закономерностей изменения потенциала и сопротивления плазмалеммы *Chara gymnophylla*, *Nitella flexilis* в присутствии Cd, Cu, Zn, Pb и экстрактов различных видов *Artemisia* использована микроэлектродная техника. Обнаружен статистически достоверный гиперполяризующий эффект экстрактов из *A. szovitsiana* и *A. scoparia* в клетках, обработанных цинком и свинцом, соответственно. Выявлено устранение экстрактами снижения мембранной проводимости, вызванного Cd и Zn, а также роста проводимости, вызванного Cu. Несмотря на то, что отмеченная высокая аккумуляционная способность к ТМ различных видов *Artemisia* не связана с протекторным действием их экстрактов на мембранную проводимость клеток, они могут быть потенциальным источником для поиска новых химических соединений, способных индуцировать устойчивость растений к стрессовым воздействиям.