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Influence of copper, cadmium and zinc ions on the composition and metabolism of lipids of chloroplast membranes in *Hydrilla verticillata*

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ABSTRACT

The *Hydrilla verticillata* was incubated for a period of 3 days in the presence of 100 μM copper (Cu), cadmium (Cd) or zinc (Zn) in the forms of nitrate salts. Lipids were extracted from chloroplast-enriched fractions of the *H. verticillata*. The HM stress resulted in the changes of the lipid composition of chloroplast membranes, decrease of fatty acid unsaturation. It was revealed that the greater were the damages caused by HM, the more intensive synthesis of lipids occurred. It was found that Cu, Cd and Zn caused an increased synthesis of phosphatidylglycerols (PG) and monogalactosyldiacylglycerols (MGDG), PG and digalactosyldiacylglycerols (DGDG), and MGDG, respectively. It was concluded that changes in the lipid metabolism compensate the negative effect of HM.

KEYWORDS

Hydrilla verticillata;
Heavy metals;
Chloroplasts;
Pigments;
Lipid metabolism;
Fatty acids.

INTRODUCTION

Environmental exposure to heavy metals (HM) is one of the main critical issues on environmental and public health[15]. Aquatic ecosystems are particularly susceptible as they often act as a final receptor for these contaminants. Plants can accumulate HM in tissues and organs. Of a special researcher's interest is a reaction of water plants to HM pollution as it is known that many kinds of these plants are capable to accumulate HM in quantities exceeding the natural content typical for their habitat[20]. Once having penetrated a cell, HM produce a toxic effect on many processes including growth, photosynthesis and respiration, they deteriorate mineral nutrition and water exchange and change the hormonal balance[3,17,36,38].

Cu and Zn is known to be an essential micronutrients for higher plants, however, when present in excess, it causes severe damage to plant organelles through inhibitory effects to photosynthetic electron transport and degradation of the chloroplast inner structure and pigment content, but Cd is toxic even in trace amounts[13].

Acting as a natural barrier, membranes are the primary target and provide the first line of protection of a living cell from any polluting substances[9]. The function of a membrane largely depends on the composition of lipids which are the main structure-forming components of membranes[11]. It has been shown that plant lipids are sensitive to HM in many organ-

isms, including marine algae, moss, lichens, higher and lower plants[6,7,22,28].

Chloroplast represents one of the forms of cells-specific organelles for plants. By changing the structure of chloroplast membranes the photosynthetic plants apparatus reacts to the changes of environmental conditions (illumination, temperature, humidity, mineral nutrition)[34]. Phospholipids (PL) and glycolipids (GL) represent major building blocks for biological membranes. In membrane of chloroplasts of higher plants monogalactosyldiacylglycerols (MGDG), digalactosyldiacylglycerols (DGDG) and sulfoquinovosyldiacylglycerols (SQDG) and phosphatidylglycerols (PG) are the predominating lipid classes[12].

Plants have a range of potential mechanisms at the cellular level that might be involved in the detoxification and thus tolerance to HM stress. However, very little was known about the lipid metabolism of chloroplast membranes.

The objective of this work is the studying of the change of composition, content and metabolism of lipids of chloroplast membranes of the submersed plant *Hydrilla verticillata* under the influence of Cu, Cd and Zn in the concentration of 100 μM.

HM concentration is matched proceeding from high accumulative ability of submersed plants in whole and *Hydrilla verticillata* in particular.

MATERIALS AND METHODS

Experimental

An aquatic vascular plant *Hydrilla verticillata* (L. fil.) Royle (family *Hydrocharitaceae*, order *Hydrocharitales*, class *Liliopsida*) was used in the experimental work.

The *Hydrilla verticillata* plants were cultivated under laboratory conditions in 5% Hoagland-Arnon medium. Before the experiment, plants were cut to 4 g fragments (beginning from top) and placed in vegetative 1-litre vessels. HM were added to the medium as nitrate salts $Cu(NO_3)_2$, $Zn(NO_3)_2$, $Cd(NO_3)_2$ at the concentration of 100 μM. Plants were incubated for a period of 3 days at 20°Ñ under photosynthetic photon flux having the density equal to 1200 ± 100 μM m^{-2} s^{-1} (10-h light day). After the end of influence of HM the plant was washed in the distilled water and used for analysis.

Pigments

Plants (200-300 mg of fresh weight) were homogenized on ice with mortar and pestle in 90% of cold acetone. After centrifugation for 10 min at 5000g, the absorbance of the pigment extract was measured at wavelengths of 662, 645 and 470 nm using an SF-46 LOMO spectrophotometer (Russia). The contents of pigments (chlorophyll a, chlorophyll b and carotenoids) were estimated according to Lichtenthaler[18].

Isolation of chloroplasts

Experimental plants (4 g of fresh weight) were homogenized on ice with mortar and pestle in cold buffer (0.5 M saccharose, 5 mM EDTA, 50mM tris-HCl pH 7.8, 10 mM 2-$HSCH_2CH_2OH$). The homogenate was filtered and centrifuged at 3000g for 10 min (fraction of chloroplasts)[32].

Extraction and analysis of lipids

Lipids were extracted from chloroplast-enriched fractions[14]. Individual lipids were separated by thin-layer chromatography on silica gel microplates (6×6 or 10×10 cm; Laene Kalur, Estonia). GL were separated in the acetone/benzene/distilled water system (91/30/8, v/v/v); PL were separated first in the chloroform-methanol-benzene-ammonia system (130/60/20/12, v/v/v/v; Direction 1) and then in the chloroform/methanol/benzene/acetone/acetic acid system (140/60/20/10/8, v/v/v/v/v; Direction 2). Specific lipids were identified using the following reagents: anthrone (GL), malachite-green reagent (PL), Dragendorff's reagent (choline-containing lipids) acetone solution of ninhydrine (primary amine-containing lipids)[14].

The quantities of total and individual PL were estimated by phosphorus assay[37], whereas GL were quantified using a DenSkan-04 densitometer (Russia) versus an MGDG (Larodan, Sweden) calibration curve.

Fatty acid analysis

The analysis of fatty acids (FA) was made by gas chromatography. Dried samples of the total lipid extract were underwent methanolysis in a methanol/sulphuric acid mixture (50/1, v/v) in the presence of benzene at 85°C for 1 h. After methanolysis, double volumes of water were added into the flasks, and methyl esters of FA were extracted from the reaction mixture with three portions of hexane.

FA methyl esters were analyzed with a Kristall-

5000.1 gas chromatograph (Russia) using a RESTEK capillary column (length, 105 m; diameter, 0.25 mm) under the following conditions: injector temperature, 180°C; detector temperature, 260°C; gas (helium) flow rate, 20 ml min^{-1}.

Lipid metabolism

The intensity of lipid metabolism was assessed by the level of ^{14}C incorporation into lipids. Plant samples were incubated for 1 h in 2-[^{14}C]-sodium acetate (185 KBq) under illumination (photosynthetic photon flux density 1200 μM m^{-2} s^{-1}) at 20°C. After separation of lipids on silica gel plates, single lipid spots were transferred into 5 ml of 0.7% (w/v) butyl-PBD in toluene[32], and their radioactivity was measured with a Beckman LS-100 liquid scintillation counter (USA).

Statistical analysis

The differences between the control and HM-incubated samples were verified on the basis of Student's *t*-test at the confidence interval $P \leq 0.05$. The values given in the tables are means ± SE of three independent experiments.

RESULTS

H. verticillata treated with HM in concentration up to 100 μM for 3 d efficiently accumulated these metals in its tissue. The results of measurements showed that *H. verticillata* placed in the solutions containing HM accumulated them in quantity from 0.2 (Zn) to 11.0 (Cd) mg g^{-1} of dry weight (Figure). The maximum level of HM accumulation occurred on the third day.

The content of main photosynthetic pigments (chlorophyll *a*, *b* and total carotenoids) was estimated quantitatively in acetone extracts from untreated and HM treated plants and recalculated per gram of fresh weight.

The contents of chlorophyll *a* and *b* in the tissues of control plant samples amounted to 0.45 and 0.20 mg g^{-1} of fresh weight, respectively (TABLE 1). In the copper-treated plants, the content of chlorophyll *a* and *b* dropped by 55 and 40%, respectively. A similar tendency, although not so apparent as in the case of Cu, was observed with Zn and Cd. As a result, the total chlorophyll *a* and *b* content decreased by a factor of 2.0 in the Cu-affected plants and by a factor of 1.3-1.4 in the Cd- and Zn-treated ones. Chlorophyll *b* turned out to be more resistant than chlorophyll *a* to the effect

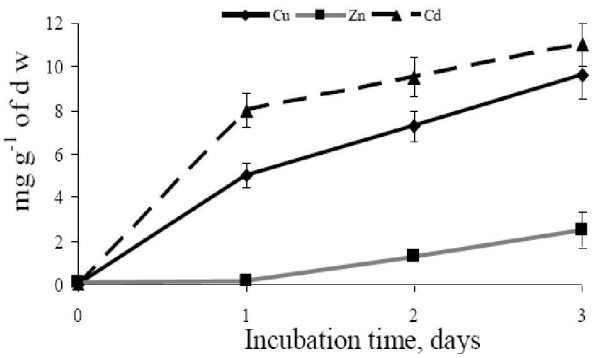

Figure 1 : Concentration of Cu, Cd and Zn in tissues *H. verticillata*

of Cu and Cd, whereas Zn equally reduced the content of both *a* and *b* chlorophylls.

In the control plant samples, the content of carotenoids was 0.15 mg g^{-1} of fresh weight. Cu caused more than 45% fall of the carotenoids level; in the presence of Cd, carotenoids dropped by 20% depending; Zn did not cause any changes in the carotenoid content.

Effect of Cu, Cd and Zn ions on the lipid composition was estimated in chloroplast-enriched fractions of *H. verticillata*. Chloroplasts were isolated from *H. verticillata* plants after 3 days of exposition with HM.

Incubation of plants with Cu and Cd ions resulted in a sharp decrease in the content of GL (TABLE 2). For example, Cd excess caused a remarkable decrease by 75 and 85 % in the amounts of two principal GL molecules in particular MGDG and DGDG, respectively. In addition, in the Cd-treated plants the content of PG decreased 3.5 times. Similar changes were observed in the Cu-treated plants (decreased 1.5 times). In contrast to Cd and Cu, Zn caused an increase in the content of GL, mainly by raising the MGDG level. Also, in the Zn-treated plants the content of PG increased by 29 %.

Changes in FA composition of lipids serve as the indicator of modification of biological membranes. Increased share of unsaturated FA gives considerable fluidity to membranes and, as a result, the synthesis of structural components of lipids is renewed, membranes acquire plasticity characteristic of adaptive restructure[10]. In its turn, the increased share of saturated FA stabilizes cell membranes due to the formation of a more densely packed bi-layer making the membranes less permeable and capable to provide better protection in various stressful situations including HM effects[26].

Results of FA analysis of the lipids of chloroplast-

TABLE 1 : Influence of Cu, Cd and Zn on the maintenance of lipids and pigments in chloroplast of *H. verticillata*, mg g^{-1} of f w

	Control	Cu	Cd	Zn
Lipids+ pigments	1.7±0.15	1.0±0.06	1.1±0.03	1.6±0.14
Chl *a*	0.45±0.08	0.20±0.02	0.30±0.01	0.31±0.02
Chl *b*	0.20±0.01	0.12±0.01	0.18±0.01	0.14±0.01
Car	0.15±0.01	0.08±0.00	0.12±0.01	0.15±0.01
Lipids	0.90±0.05	0.60±0.03	0.50±0.00	1.00±0.10

TABLE 2 : Influence of Cu, Cd and Zn on the content of lipids in chloroplast membranes of *H. verticillata*, mg g^{-1} of f w

Lipid	Control	Cu	Cd	Zn
MGDG	0.32±0.02 (100)	0.06±0.00 (19)	0.08±0.00 (25)	0.42±0.05 (131)
DGDG	0.13±0.01 (100)	0.02±0.00 (15)	0.02±0.00 (15)	0.12±0.01 (92)
SQDG	0.04±0.00 (100)	0.01±0.00 (25)	0.01±0.00 (25)	0.04±0.00 (100)
PG	0.14±0.01 (100)	0.09±0.01 (64)	0.04±0.00 (29)	0.18±0.01 (129)

The note: in brackets the values of % from control are resulted

TABLE 3 : Influence of Cu, Cd and Zn on the content of FA of lipids in chloroplast membranes of *H. verticillata*, % from the sum of FA

FA	Control	Cu	Cd	Zn
< C 16:0	1.1±0.1	5.3±0.3	5.1±0.3	2.9±0.2
C 16:0	18.5±1.5	42.3±3.1	46.2±4.5	32.7±2.5
C 16:1	2.2±0.0	4.4±0.2	5.1±0.5	3.1±0.1
C 18:0	2.0±0.2	13.0±0.3	13.5±1.1	4.9±0.3
C 18:1	4.5±0.1	5.6±0.4	3.6±0.2	5.1±0.2
C 18:2	13.2±1.1	7.1±0.6	8.0±0.5	12.2±1.1
C 18:3	55.3±4.9	8.3±0.8	6.4±0.2	26.7±2.0
> C 20:0	1.2±0.0	8.8±0.8	6.0±0.6	6.6±0.3
Others	2.0±0.2	5.2±0.5	6.1±0.4	5.8±0.1
IUS	201.6	55.3	47.4	115.7

TABLE 4 : Influence of Cu, Cd and Zn on the incorporation of 2-[^{14}C]-sodium acetate into the major lipids of chloroplast membranes of *H. verticillata*, % from the control

Lipid	Control	Cu	Cd	Zn
MGDG	100±3	184±8	136±7	133±3
DGDG	100±5	164±5	220±10	56±2
PG	100±2	348±14	483±21	91±5
PL+GL	100±5	286±6	262±3	146±3

enriched fractions of *H. verticillata* are given in table 3. The main, more than 90% of sum, group of FA in control plants consisted of acids with chain length 16 - 18 atomic of carbon. $C_{16:0}$ dominated the saturated FA. Among unsaturated FA, the following acids prevailed: $C_{18:2}$ and $C_{18:3}$. The content major FA varied in the presence of HM: the content of the saturated acid $C_{16:0}$ increased while that of $C_{18:3}$ and $C_{18:2}$ decreased (TABLE 3).

Metabolism of chloroplast lipids

In order to monitor rapidly any disturbances to metabolism, radiolabelling from 2-[^{14}C] acetate was used. This precursor has been well justified for lipid labeling in plant tissues[27].

In the control samples, total incorporation into polar chloroplast lipids was equal to 869 counts per min mg^{-1} of lipid. In the Cu- and Cd-treated plants, this value increased by a factor of 2.0 or even more; the effect of Zn was not so pronounced (1.5-fold increase in radioactivity). In the case of Cu or Cd, most of the label was incorporated in PG (threefold compared to the control plants); in the case of Zn, the primary target was MGDG (a 33 % increase compared to the control level). At the same time, specific radioactivity of DGDG in the Zn-affected plants amounted only to 56 % of that in the control samples (TABLE 4).

DISCUSSION

The results of measurements showed that, tissues of *H. verticillata* efficiently accumulated Cu, Cd and Zn at the concentration 100 μM in the culture medium after 3 days of incubation. These results were comparable to those for other aquatic plants[20,25].

All the HM examined reduced the content of major pigments in the tissues of *H. verticillata*. However, chlorophyll *b* turned out more resistant to Cu and Cd than chlorophyll *a*, whereas Zn exerted equal negative effect on both *a* and *b* chlorophylls. It should be noted that in terrestrial plants, chlorophyll *b* is more sensitive to HM than chlorophyll *a*[1,16,39]. The different effect of HM on pigments of aquatic plants may relate to the specific anatomy of their photoassimilating organs (absence of stomata, reduction of mechanical tissues etc.) or specificity of their pigment apparatus (a lower chlorophyll *a*+*b*/carotenoids ratio as compared to that of terrestrial plants). Besides, one should take into account that in terrestrial plants, the first coming into contact with HM will be the root, in case of water species; it will be the entire plant.

Many authors note that pigment reduction may result from the direct effect of HM on the enzymes of chlorophyll biosynthesis[24,31]. The primary targets of HM are the photoactive chlorophyll reductase complex and synthesis of δ-aminolevulinic acid[35]. As shown in a number of works, HM can suppress chlorophyll biosynthesis by inhibiting protochlorophyllide oxidoreductase[21]. This assumption is in agreement with data of many authors, who observed reduction of the grana number and chloroplast size at low HM concentrations and the decreased number of plastids per cell at high HM concentrations[19,29].

Our data on the HM-induced changes in the content and composition of chloroplast lipids indicate probable structural variations in the photosynthetic membranes. The decrease in total GL and PL, especially evident in the Cu- and Cd-treated plants, may reflect the reduction of the number of plastids. Our data prove that the number of chloroplasts decreases and the architecture of thylakoid membranes changes, for example, to more densely packed grans, limiting mobility of proteins. These changes result from a decrease in the content of GL and PL, especially vivid in the cases of Cu and Cd and an increase in the level of saturated FA[5,19,23,30].

Attention should also be paid to the role of PG, which is necessary for stabilization of membranes and light-harvesting complexes of photosystem[12]. In the study by Hagio et al.[8], the low-PG *Arabidopsis thaliana* mutants were shown to have anomalous leaf morphology and underdeveloped thylakoid membranes. Based on literature and our results, we conclude, that the HM-induced changes in the content and composition of *H. verticillata* chloroplast lipids should contribute to the overall inhibition of the photosynthetic function[2,4,22,23,33]. Indeed, while chlorophyll is responsible for absorption and primary transformation of light energy, chloroplast lipids support the highly organized membrane structures and provide optimal conditions for the operation of membrane proteins and energy conversion[5,29,35].

The experiments on the incorporation of 2-[^{14}C]-sodium acetate into polar chloroplast lipids showed that the *de novo* synthesis of lipids in the HM-affected plants intensified. The stronger were effects of an HM at the biochemical levels, the more intensive the lipid synthesis was. In our experiments Cu exerted the most negative effect on the condition of plants and intensity of photosynthesis, and the incorporation of the label into lipids increased by a factor of 2.0 or even more compared to control samples. Zn was the least toxic, and the label incorporation increased only by a factor of 1.5.

In addition, the HM tested showed a certain specific effect on the intensity of lipid exchange. That is, in the Cu-treated plants, most of the label was incorporated in PG followed by MGDG and DGDG; in the Cd-treated ones, the label was first found in PG and then in DGDG and MGDG. Upon the Zn treatment, MGDG revealed an increase in specific radioactivity, but that of DGDG and PG dropped compared to the control samples. Evidently, the changes in the content, composition and metabolism of lipids are aimed to compensate for the negative effect of HM.

Thus, the data presented show that the tested HM ions have a negative effect on lipids and pigments of chloroplasts of the aquatic plant *H. verticillata*. The specificity of lipid changes depended on the HM present, the amplitude of these changes correlated with the kind of HM. However, the synthesis of lipids in chloroplasts, necessary for the photosynthetic membranes to keep function, was obviously stimulated as a way to compensate for the negative HM effects.

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ABBREVIATIONS

DGDG : Digalactosyldiacylglycerols
FA : Fatty acids
GL : Glycolipids
HM : Heavy metals
IUS : Index of fatty acid unsaturation
MGDG : Monogalactosyldiacylglycerols
PG : Phosphatidylglycerols
PL : Phospholipids
SQDG : Sulphoquinovosyldiacylglycerols

REFERENCES

[1] F.Al-Qurainy; Australian Journal of Basic and Applied Sciences, **3(3)**, 3025-3035 **(2009)**.
[2] A.Arunachalam, K.Maithani, S.Pandiaraj; Indian Journal of Plant Physiology, **1(1)**, 49-51 **(1996)**.

[3] M.M.A.Boojar, F.Goodarzi; Chemosphere, **67(11)**, 2138-2147 **(2007)**.

[4] S.Bunluesin, M.Kruatrachue, P.Pokethitiyook, S.Upatham, G.R.Lanza; Journal of Bioscience and Bioengineering, **103(6)**, 509-513 **(2007)**.

[5] V.Demidchik, A.Sokolik, V.Yurin; Advances of Modern Biology, **121(5)**, 511-525 (in Russian) **(2001)**.

[6] I.A.Guschina, J.L.Harwood; Biochem.Soc.Trans, **28**, 910-912 **(2000)**.

[7] I.A.Guschina, J.L.Harwood; Journal of Experimental Botany, **53**, 445-463 **(2002)**.

[8] M.Hagio, I.Sakurai, S.Sato, T.Kato, S.Tabata, H.Wada; Plant Cell Physiology, **43**, 1456-1464 **(2002)**.

[9] J.L.Hall; Journal of Experimental Botany, **53**, 1-11 **(2002)**.

[10] J.L.Harwood; 'What's So Special About Plant Lipids?', In: J.L.Harwood, (Ed.); Plant Lipid Biosynthesis, Fundamentals and Agricultural Applications. Cambridge Univ.Press, Cambridge, 1-28 **(1998)**.

[11] J.L.Harwood; 'Environmental Effects on Plant Lipid Biochemistry', In: J.L.Harwood, (Ed.); Plant Lipid Biosynthesis, Fundamentals and Agricultural Applications, Cambridge Univ.Press, Cambridge, 305-363 **(1999)**.

[12] G.Holzl, P.Dorman; Progress in Lipid Research, **46**, 225-243 **(2007)**.

[13] A.Kabata-Pendiaz, H.Pendiaz; 'Microelements in Soils and Plants', Mir Publishers, Moscow, 439 **(1989)** (in Russian).

[14] M.Kates; 'Techniques of Lipidology', North Holland/American Elsevier, Amsterdam, New York, 323 **(1975)**.

[15] M.Kanoun-Boule, J.A.F.Vicente, C.Nabais, M.N.V.Prasad, H.Freitas; Aquatic Toxicology, **91**, 1-9 **(2009)**.

[16] V.A.Karavaev, A.M.Baulin, T.V.Gordienko, S.A.Dovyd'kov, A.N.Tikhonov; Plant Physiology (Moscow), **48**, 47-54 **(2001)** (in Russian).

[17] H.Kupper, P.M.H.Kroneck; Met.Ions.Biol.Syst., **44**, 97-142 **(2005)**.

[18] H.K.Lichtenthaler, R.R.Welburn; Biochem.Soc.Trans, **603**, 591-592 **(1983)**.

[19] W.Maksymiec; Photosynthetica, **34(3)**, 321-342 **(1997)**.

[20] M.G.Maleva, G.F.Nekrasova, P.Malec, M.N.V.Prasad, K.Strzalka; Chemosphere, **77**, 392-398 **(2009)**.

[21] B.Mysliwa-Kurdziel, K.Strzalka; Ag.Ecosys.Environ., **106**, 199-207 **(2004)**.

[22] I.Nouairi, W.Ben Ammar, N.Ben Youssef, D.Ben Miled Daoud, M.Habib Ghorbal, M.Zarrouk; Plant Science, **170**, 511-519 **(2006)**.

[23] O.Ouariti, N.Boussama, M.Zarrouk, A.Cherif, M.H.Ghorbal; Phytochemistry, **45(7)**, 1343-1350 **(1997)**.

[24] H.V.Perales-Vela, S.Gonzalez-Moreno, C.Montes-Horcasitas, R.O.Canizares-Villanueva; Chemosphere, **67**, 2274-2281 **(2007)**.

[25] M.N.V.Prasad; Analusis, **26(6)**, 25-28 **(1998)**.

[26] S.Rama Devi, M.N.V.Prasad; 'Membrane Lipid Alteration in Heavy Metal Exposed Plants', In: M.N.V.Prasad, J.Hagemeyer (Eds.); Heavy Metal Stress in Plants, From Molecules to Ecosystems. Springer, Berlin, 99-117 **(1999)**.

[27] P.G.Roughan, C.R.Slack; Annu.Rev.of Ðlant Physiol., **33**, 97-132 **(1982)**.

[28] O.A.Rozentsvet, S.V.Murzaeva, I.A.Gushchina; Proceedings of RAS, Biology Series, **2**, 232-239 **(2005)** (in Russian).

[29] A.Siedlecka, Z.Krupa; Photosynthetica, **36**, 321-331 **(1999)**.

[30] P.A.Siegenthaler; 'Molecular Organization of Acyl Lipids in Photosynthetic Membranes of Higher Plants', In: P.A.Siegenthaler, N.Ìurata (Eds.); Lipids in Photosynthesis, Structure, Function and Genetics, Kluwer Academic Publishers, Dordrecht, 119-144 **(1998)**.

[31] S.Sinha, K.Pandey; Bull.Environ.Cont.and Toxicol., **71**, 175-183 **(2003)**.

[32] N.F.Sinyutina, E.D.Kouzova; The role of lipids upon the effect of abscisic acid on maize coleoptiles. Herald of St. Petersburg University 3(1): 86-92 **(2005)** (in Russian).

[33] S.Srivastava, S.Mishra, R.D.Tripathi, S.Dwivedi, D.K.Gupta; Aquatic Toxicology, **80(4)**, 405-415 **(2006)**.

[34] A.N.Tikhonov; Sorosovsky Educational Journal, **11**, 16-21 **(1999)** (in Russian).

[35] A.F.Titov, V.V.Talanova, N.M.Kaznina, G.F.Laydinen; Karelian Scientific Center RAS, Petrozavodsk, 172 **(2007)** (in Russian).

[36] C.E.Umebese, O.M.Alebiosu; Environment and Conservation, **15(3)**, 421-425 **(2009)**.

[37] V.E.Vaskovsky, N.A.Latyshev; J.Chromatogr., **115**, 246-249 **(1975)**.

[38] I.Yruela; Brazilian Journal of Plant Physiology, **17(1)**, 145-156 **(2005)**.

[39] I.M.Zeid, H.M.A.El Ghate; Pakistan Journal of Biological Sciences, **10(6)**, 874-879 **(2007)**.