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Cadmium-induced ultrastructural changes in chloroplasts of the leaves and stems parenchyma in *Myriophyllum spicatum* L.

D.P. STOYANOVA* and E.S. TCHAKALOVA

Department of Botany, Faculty of Biology, Sofia University 'St. Kliment Ohridski', 8 Dragan Tzankov Str., BG-1421 Sofia, Bulgaria

Abstract

After a 5 d cultivation in solutions with different concentrations of cadmium, changes in chloroplasts of the leaf and stem parenchyma from *Myriophyllum spicatum* L. were observed: swelling and merging of the grana thylakoids in various degrees, coagulation of the stroma as well as the formation of crystal-like structures in it, accompanied by changes in the plastoglobuli and starch. Concentrations of 3.5 and 6.0 $g(Cd^{2+})$ m^{-3} caused a vast destruction of the whole plastid apparatus. Chloroplasts in the parenchyma of stems showed a reduction in the inner membrane system, and an increased number of plastoglobuli. A comparative analysis that we carried out indicated that the changes were not uniform and metal-specific.

Additional key words: crystal-like body; granum; membrane system; plastoglobuli; starch; stroma; thylakoid.

Introduction

The photosynthetic apparatus organization in *M. spicatum* L. has undergone an adaptation to aquatic environment and, like with most aquatic plants, its epidermis and parenchyma are the basic photosynthesizing tissues. Certain specific structural characteristics, mainly of the plastid apparatus of these tissues, have evolved in consequence to this process. Such characteristics have been studied in various aquatic plants (Zauralova 1980, Goryshina and Zauralova-Pepelyaeva 1983, Venanzi and Pasqualini 1984, Milashvili and Gamaleï 1985). Studies on the peripheral plastid reticulum of *M. spicatum* (Lunney *et al.* 1975) and thylakoid structure of *Nymphoides indica* (van Steveninck and van Steveninck 1980a,b) have also been performed. Yet, insufficient research has been carried out on the plastid apparatus of higher aquatic plants structurally changed due to toxic agents (Rebechini and Hanzely 1974,

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*Phone: 00 359 (2) 63 30 246; fax: 00 359 (2) 65 66 41.

Miroslavov *et al.* 1982, Stoyanova and Tchakalova 1990, 1992, 1993, Stoyanova 1993).

The aim of this study was to compare ultrastructural changes in the chloroplasts of leaves and stems of *M. spicatum* after treatment with increasing doses of cadmium.

Materials and methods

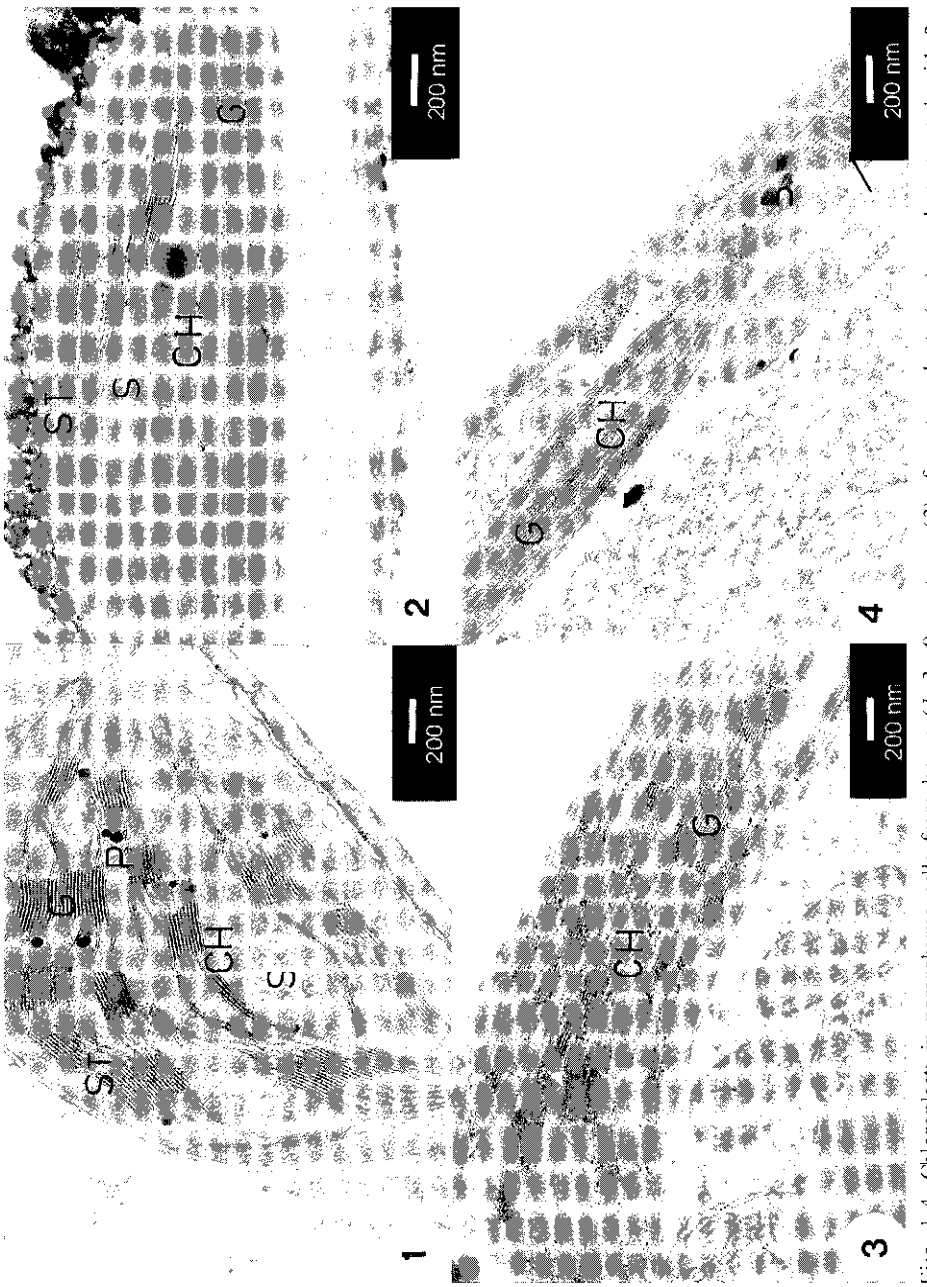
The investigations were carried out with *M. spicatum* L. after 5 d of cultivation in a phytostat. The plants were grown in tap water, at a neutral pH (7.0-7.5), water temperature 18 °C, irradiance 20-25 W m^{-2}, and natural photoperiod (16/8 h day/night). Cd was introduced as cadmium sulphate, 0.5, 1, 2, 3, 5, and 6 g(Cd^{2+}) m^{-3}. The highest concentration applied was the threshold concentration for survival of the species established by plasmolysis (Repp 1963). Plants cultivated under conditions of the experiment but without Cd were used as the controls. Samples for electron microscopy were collected from leaves and stems, from the same plants fixed in phosphate buffered (pH 7.4) OsO_4 for 12 h at 4 C, and postfixed in OsO_4 for 4 h at room temperature. After dehydration the material was embedded in *Durcupan*. Ultrathin sections were cut using a *Tesla* ultramicrotome. They were stained with lead citrate, and examined under a *JEOL 1200 EX* electron microscope.

Results

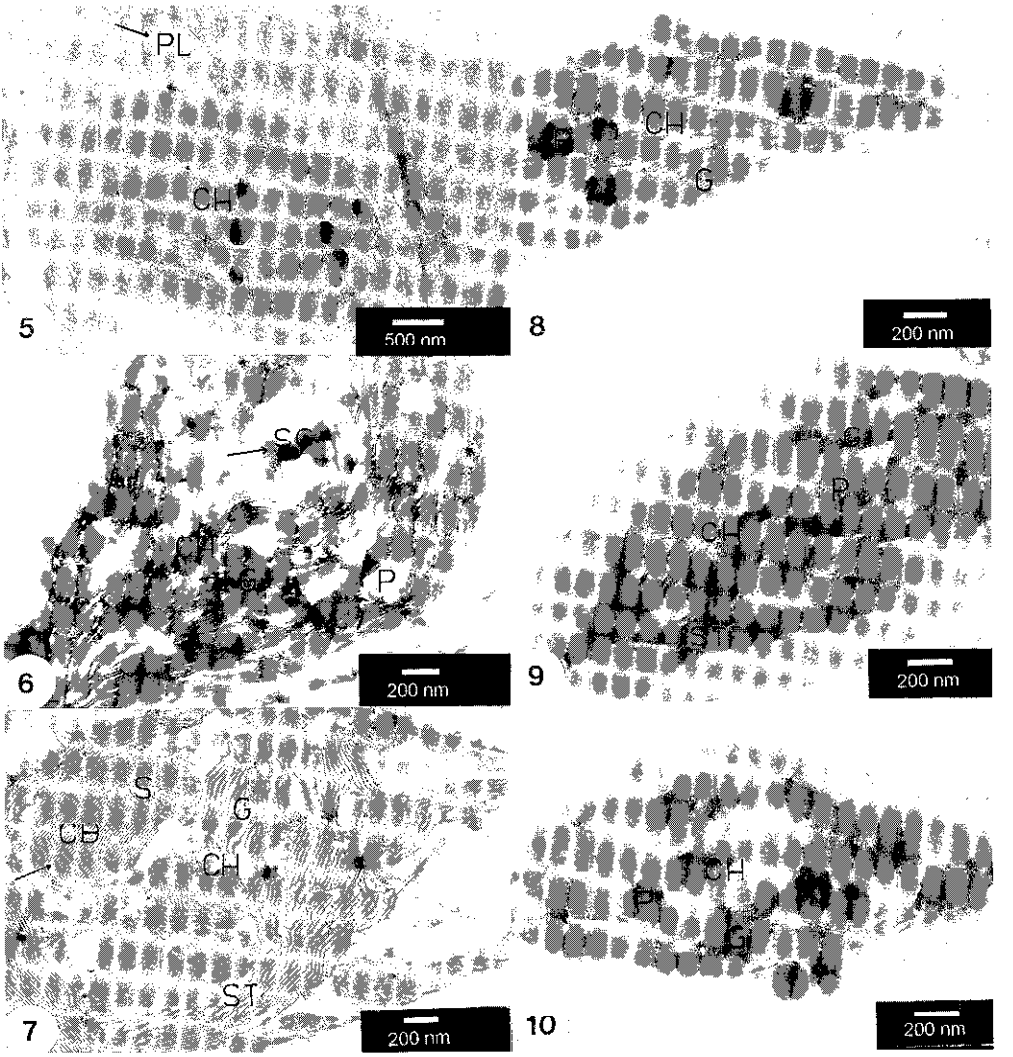
The parenchyma of leaves and stems of *M. spicatum* was characterised by a well-differentiated plastid apparatus. Chloroplasts in the mesophyll of leaves significantly outnumbered those in the parenchyma of stems. In both organs they possessed a well-developed inner membrane system (Figs. 1 and 2). In the leaves it consisted of grana in different lengths (from 5 to 25 thylakoids) and comparatively long stromal thylakoids (Fig. 1). There were but a few plastoglobuli in the stroma, with diameters up to 80 nm. The chloroplasts in the parenchyma cells of stems were more elongated than those in leaves (Fig. 2), with stroma forming a smaller part of their volume. The inner membrane system had fewer grana, though bigger in size (up to two fold when viewed in cross-section). In contrast to the leaf thylakoids, the thylakoids in stems possessed a pronounced electron density and an osmiophilic stroma.

In the photosynthetic apparatus of *Nymphoides indica,* van Steveninck and van Steveninck (1980a) found a plastid dimorphism, resulting from functional adaptation of the tissues to aquatic environment. Danilova and Kashina (1995) suggested that the thylakoid dimorphism resulted from the necessity of keeping the level of auxins and the regulation of photomorphogenesis in plants.

The treatment of parenchyma cells with increasing concentrations of Cd caused different transformations in the plastid apparatus of the two organs. When applied to leaves, the concentrations of 0.5, 1, and 2 g(Cd^{2+}) m^{-3} led to an elongation of the chloroplasts and to a greater number of shorter but wider grana (Figs. 3 and 4).



Figs. 1-4. Chloroplasts in parenchyma cells from leaves (*1*, *3*, *4*) or stems (*2*) of contro plants (*top*) or plants treated with 2 g(Cd^{2+}) m^{-3} (*bottom*) of *Myriophyllum spicatum*. CH - chloroplast, G - grauum, P - plastoglobu.i, S - stroma, ST - stroma thylakoids.



Figs. 5-10. Chloroplasts in parenchyma cells of *Myriophyllum spicatum* leaves (*5*, *6*, *7*) or stems (*8*, *9*, *10*) treated with 0.5 (*5*), 1 (*8*), 3 (*6*, *9*), 5 (*7*), or 6 (*10*) $g(Cd^{2+})\ m^{-3}$, respectively. CB - crystal-like body, G - granum, P - plastoglobuli, PL - proliferation, SG - starch grain, ST - stroma thylakoids.

Besides this type of structural organization, chloroplasts with underdeveloped inner membrane system, or almost lacking any discrete grana, could be detected in some cells. They had central structures similar to prolamellar bodies (Fig. 4, *arrow*). These cells contained many plastoglobuli, with diameters up to 100 nm. The structural diversity of the plastid apparatus was complemented by single oval chloroplasts with specific stromal outgrowths on them (proliferations; Fig. 5, *arrow*).

3 g(Cd^{2+}) m^{-3} caused a radical change in the ultrastructure of leaf mesophyll. The protoplasts were destroyed in a significant measure. The only protoplasmic component detectable, despite of their considerably altered structure, were the chloroplasts (Fig. 6). Their stroma and inner membrane system were largely destroyed. Most affected were the grana, since the destruction of thylakoids led to full merging. A part of the stromal thylakoids was slightly swollen and there were areas of a low electron density between them, probably at the locations of former plastoglobuli. The starch grains were almost entirely destroyed; their form was atypical and irregular (Fig. 6, *arrow*).

The application of highest concentrations of Cd, *i.e.*, 5 and 6 g(Cd^{2+}) m^{-3}, to the mesophyll of leaves led to various structural changes in the plastid apparatus. The membrane of the chloroplasts was entirely destroyed, and the stroma was coagulated (Fig. 7). The thylakoids were considerably swollen, and the volume of the thylakoid stroma increased uniformly. This was the reason why the inner membrane system of some of the chloroplasts was close to the agranal type. Crystal-like formations with central position very much recalling stroma centres could also be detected (Fig. 7, *arrow*).

Transformations in the structure of the chloroplasts in parenchyma cells of stems were different. Their character was uniform for all tested concentrations of Cd. With low Cd concentrations (0.5, 1.0, and 2.0 g m^{-3}) the chloroplast structure was close to the one in control plants. A part of the stroma thylakoids was demolished, and the number of plastoglobuli increased (Fig. 8). After the treatment with a high Cd concentration (3 g m^{-3}) the chloroplasts became oval. There was a reduction in the inner membrane system, accompanied again by an increase in the number of plastoglobuli (Fig. 9). Transformations in the inner membrane system were most pronounced for 6 g(Cd^{2+}) m^{-3} (Fig. 10). As a result of the destructive processes almost no stroma thylakoids remained, and merging of granal thylakoids was observed.

Discussion

Changes established in the plastid apparatus of assimilation parenchyma of leaves and stems due to the action of increasing concentrations of Cd were organ-specific. The character of the changes in chloroplasts of leaves differed depending on the concentration of Cd. Considerable transformations in the inner membrane system occurred under concentrations higher than 3 g(Cd^{2+}) m^{-3} in the form of a dissipation of the thylakoids inside grana. This was the most pronounced form of destruction of the constituent membranes of the grana. Nyomarkay *et al.* (1981) and Rak (1982)

also support such hypothesis. They observed similar changes under the influence of SO_2 as a stage in the chain of transformations following the swelling of thylakoids. The ions of toxic substances penetrate the chloroplast membranes, react with lipids, and thus alter the structure and permeability of the membranes (Heath 1980). Heavy metals are in the group of toxic substances destroying the lipids in chloroplast membranes (Baszyński *et al.* 1980, Baszyński 1986).

In this study, the highest Cd concentrations applied led to a partial reduction of the inner membrane system of chloroplasts, strong swelling of thylakoids and increased volume of the thylakoid stroma. A similar response was observed with the plastid apparatus of *Elodea canadensis* after treatment with copper (Stoyanova and Tchakalova 1993) as well as with the same range of Cd concentrations (Stoyanova and Chakalova 1990). The stress reactions typical of aquatic plants treated with heavy metals are not different from those found in terrestrial plants (Soikkeli 1981, Miyake *et al.* 1984, Ïordanov *et al.* 1986, Psaras and Christodoulakis 1987). Such type of destruction is often considered a symptom of the initial stage of pathological chloroplast aging (Vassilev *et al.* 1982, Ruetze and Schmitt 1988).

Structural changes of plastoglobuli under the action of 3 $g(Cd^{2+})\ m^{-3}$ may be considered as a process of dissolving of their lipid contents induced by Cd (Baszyński 1986). Changes in the structure of plastoglobuli, similar to changes in plastosomes, were established by Rebechini and Hanzely (1974) after treatment of *Ceratophyllum demersum* with lead. The transformations of starch grains were also comparable.

The changes in the crystal-like structures located in the middle of the stroma were distinctly different from the known transformations. Visually, they were similar to the stroma centres described by Gunning (1965), and could not be compared to the phytoferritin particles observed by Maksymiec *et al.* (1995). Nevertheless, they were similar to the crystal-like bodies found in the leaves of beans treated with ozone (Thomson *et al.* 1974) and to the pseudocrystal-like structures found in the mesophyll of *Nicotiana glutinosa* leaves grown at extreme nutrient and lighting regimes (Ragetli *et al.* 1970). According to Ascaso and Rapsch (1986), their appearance was related to the reduced permeability of chloroplast membranes and to the dehydration of the stroma in the spots where they were found. They were probably caused by stroma coagulation in the chloroplasts. The changes inflicted by low Cd concentrations [0.5 to 2.0 $g(Cd^{2+})\ m^{-3}$] could be viewed as primary stages in the transformations.

In stem parenchyma cells, Cd induced chloroplast changes of a different character: reduction in the number of thylakoids, and condensation and osmiophilization of thylakoid stroma and membranes. Conformational changes in the organization of the thylakoid membranes could be the most probable reason for the changes. This process was parallel with an increase in the number of plastoglobuli. We suppose that in the studied species the lipids released upon destruction of thylakoids were the most probable reason (for a similar opinion see Baszynski *et al.* 1980). On the other hand, according to Silaeva (1978) the increased number of plastoglobuli could be related to the functional state of the chloroplasts, and depended on external factors. Torgasheva (1984) points out that plastoglobuli are the localisers of toxicant in chloroplasts. We came to the same conclusion when studying the effects of Pb, Cd, and Cu on the

photosynthetic apparatus of *Potamogeton natans* (Stoyanova and Tchakalova 1992, Stoyanova 1993).

The variety of transformations in the structure of chloroplasts under different concentrations of Cd proved that the plastid apparatus of parenchyma of leaves was structurally more flexible than that in the fairly inert parenchyma cells of stems. The changes in them were uniform, and an increase in Cd concentration only deepened the effect.

References

Ascaso, C., Rapsch, S.: Ultrastructural changes in chloroplasts of *Quercus rotundifolia* Lam. in response to evernic acid. - Ann. Bot. **57**: 407-413, 1986.

Baszyński, T.: Interference of Cd^{2+} in functioning of the photosynthetic apparatus of higher plants. - Acta Soc. Bot. Pol. **55**: 291-304, 1986.

Baszyński, T., Wajda, L., Król, M., Wolińska, D., Krupa, Z., Tukendorf, A.: Photosynthetic activities of cadmium-treated tomato plants. - Physiol. Plant. **48**: 365-370, 1980.

Danilova, M., Kashina, T.K.: [Photoperiodism, leaf development and the dimorphism of chloroplast thylakoids in *Perilla ocymoides*.] - Fiziol. Rast. **42**: 14-22, 1995. [In Russ.]

Goryshina, T., Zauralova-Pepelyaeva, N.O.: [Plastid apparatus of leaves of aquatic macrophytes and littoral plants.] - Ekologiya **1983**(5): 25-32, 1983. [In Russ.]

Gunning, B.E.S.: The fine structure of chloroplast stroma following aldehyde osmium tetroxide fixation. - J. Cell Biol. **24**: 79-93, 1965.

Heath, R.L.: Initial events in injury to plants by air pollutants. - Annu. Rev. Plant Physiol. **31**: 395-431, 1980.

Ïordanov, I.T., Chakalova, E.S., Stoyanova, Ts.D.: [The influence of chloramphenicol, tetracycline and cycloheximide on the ultrastructure of bean chloroplasts.] - God. sofiï. Univ. "Kliment Okhridski", biol. Fak. **77**(2-Bot.): 44-54, 1986. [In Bulg.]

Lunney, C.A., Davis, G.J., Jones, M.N.: Unusual structure associated with peripheral reticulum in chloroplasts of *Myriophyllum spicatum* L. - J. Ultrastruct. Res. **50**: 293-296, 1975.

Maksymiec, W., Bednara, J., Baszyński, T.: Responses of runner bean plants to excess copper as a function of plant growth stages: Effects on morphology and structure of primary leaves and their chloroplast ultrastructure. - Photosynthetica **31**: 427-435, 1995.

Milashvili, T.R., Gamaleï, Yu.V.: [Variations of the plastid apparatus of the submerged and aerial leaves of *Batrachium eradicatum* and *B. peltatum*.] - Tsitologiya **27**: 511-517, 1985. [In Russ.]

Miroslavov, E., Kravkina, J., Lairand, N.: [The influence of surface-active substances on the ultrastructure of the plant cell.] - In: Vzaimodeïstvie Mezdu Lesnymi Ėkosistemami i Zagryaznitelyami. Pp. 81-83. Pervogo Sovetsko-Amerik. Symp. Pr. 02.03-21, Tallin 1982. [In Russ.]

Miyake, H., Furukawa, A., Totsuka, T., Maeda, E.: Differential effects of ozone and sulphur dioxide on the fine structure of spinach leaf cells. - New Phytol. **96**: 215-228, 1984.

Nyomárkay, M.K., Fridvalszky, L., Szász, J.: Zusammenhänge zwischen der Schwefeldioxydkonzentration und den Schäden der Nadelblätter der Kiefers (*Pinus silvestris* L.). - Ann. Univ. Sci. budapest. Rolando Eötvös nomin. **22-23**: 101-108, 1980-1.

Psaras, G.K., Christodoulakis, N.S.: Air pollution effects on the ultrastructure of *Phlomis fruticosa* mesophyll cells. - Bull. environ. Contamin. Toxicol. **38**: 610-617, 1987.

Ragetli, H.W.J., Veintraub, M., Lo, E.: Degeneration of leaf cells resulting from starvation after excision. I. Electron microscopic observations. - Can. J. Bot. **48**: 1913-1922, 1970.

Rak, L.D.: [Characteristics of the assimilation apparatus of fir-trees under air pollution.] - In: Vzaimodeïstvie Mezhdu Lesnymi Ėkosistemami i Zagryaznitelyami. Pp. 88-90. Pervogo Sovetsko-Amerik. Symp. Pr. 02.03-21, Tallin 1982. [In Russ.]

Rebechini, H.M., Hanzely, L.: Lead-induced ultrastructural changes in chloroplasts of the hydrophyte, *Ceratophyllum demersum*. - Z. Pflanzenphysiol. **73**: 377-386, 1974.

Repp, G.: Die Kupferresistenz des Protoplasmas höherer Pflanzen auf Kupfererzboden. - Protoplasma **57**: 643 659, 1963.

Ruetze, M., Schmitt, U.: Histologie der Alterung von Fichtennadeln (*Picea abies* (L.) Karst.). - Angew. Bot. **62**: 9-20, 1988.

Silaeva, A.M.: Struktura Khloroplastov i Faktory Sredy. [Structure of Chloroplasts and Factors of the Environment.] - Naukova Dumka, Kiev 1978. [In Russ.]

Soikkeli, S.: The types of ultrastructural injuries in conifer needles of northern industrial environments. - Silva fenn. **15**: 399-404, 1981.

Stoyanova, D.: Strukturelle Veränderungen der Palisadenzellchloroplasten in Schwimmblättern von *Potamogeton natans* L. unter Einwirkung von Blei. - Compt. rend. Acad. bulg. Sci. **46**(1): 129-132, 1993.

Stoyanova, D., Chakalova, E.: [Effect of cadmium on the photosynthetic apparatus in *Elodea canadensis* Rich.] - Fiziol. Rast. (Sofia) **16**(3): 18-26, 1990. [In Bulg.]

Stoyanova, D., Tchakalova, E.: Strukturelle Veränderungen der Epidermalchloroplasten von kormophyten Wasserpflanzen unter Schwermetalbeeinflussung. - Ann. Univ. Sofia "St. Kliment Ohridski" **83**(2): 39-51, 1992.

Stoyanova, D.P., Tchakalova, E.S.: The effect of lead and copper on the photosynthetic apparatus in *Elodea canadensis* Rich. - Photosynthetica **28**: 63-74, 1993.

Thomson, W.W., Nagahashi, J., Platt, K.: Further observation on the effect of ozone on the ultrastructure of leaf tissue. - ACS Symp. Ser. **3**[Air Pollution Effect on Plant Growth]: 83-93, 1974.

Torgasheva, E.G.: [Ultrastructural changes in the chloroplasts of leaf mesophyll of *Morus alba* (*Moraceae*) growing in vicinity of an aluminium plant.] - Bot. Zh. **69**: 921-925, 1984. [In Russ.]

van Steveninck, M.E., van Steveninck, R.F.M.: Plastids with densely staining thylakoid contents in *Nymphoides indica*. I. Plastid development. - Protoplasma **103**: 333-342, 1980a.

van Steveninck, M.E., van Steveninck, R.F.M.: Plastids with densely staining thylakoid contents in *Nymphoides indica*. II. Characterization of stainable substance. - Protoplasma **103**: 343-360, 1980b.

Vassilev, A.E., Danilova, M.F., Kravkina, J.M., Kreng, R.F.: [Ultrastructural changes in the mesophyll of poplar leaves during the autumn aging and under the influence of sulphuric gas.] - In: Vzaimodeïstvie mezhdu Lesnymi Ėkosistemami i Zagryaznitelyami. Pp. 72-76. Pervogo Sovetsko-Amerik. Symp. Pr. 02.03-21, Tallin 1982. [In Russ.]

Venanzi, G., Pasqualini, S.: [Characteristics of chloroplasts of some aquatic plants of the Trasimeno lake.] - Ann. Fac. Agr. Univ. Perugia **38**: 397-412, 1984. [In Ital.]

Zauralova, N.O.: [The assimilation apparatus of several aquatic plants species.] - Bot. Zh. **65**: 1439-1446, 1980. [In Russ.]