

ZDZISŁAWA BEATA ROMANOWSKA-DUDA*,
MIECZYŚLAW GRZESIK**, HAZEM M. KALAJI***

PHYTOTOXKIT TEST IN GROWTH ASSESSMENT OF CORN AS AN ENERGY PLANT FERTILIZED WITH SEWAGE SLUDGE

The aim of the research was to analyze and evaluate both growth and physiological features of corn, cultivated in various soils and fertilised with sewage sludge. The present study investigates the effect of manuring on energy plant development, especially those cultivated on low-quality soils, and the usefulness of Phytotoxkit test in predicting the efficiency of such a treatment. Sand as well as various types of soil, e.g. sandy, peat or clay, were enriched with 10–15% doses of sewage sludge which, to a large extent, influenced the growth of plants. Moreover, their biomass and chlorophyll *a* and *b* content increased, and photosynthesis, membrane stability and RNase activity in leaves were considerably improved. As far as Phytotoxkit is concerned, it appears that it might be useful when predicting the development of fertilized plants.

1. INTRODUCTION

Renewable energy is becoming very essential in energy balance in the world. It is to be of crucial importance to the energy safety improvement, economical development of societies and reduction of gases in the atmosphere. Nowadays, about 75% of the energy obtained from renewable sources comes from plant biomass. Due to an increasing demand for food supply, the production of energy biomass ought to be performed on low-quality soils and degraded areas. However, the main problem is lack of information on effective methods of particular energy plants cultivation on such soils. Not much is also known about the tests which could be helpful in predicting a given method efficiency without performing field experiments. Professional literature mentions that soil fertilization with sewage sludge may be helpful and bene-

* Department of Ecophysiology and Plant Development, University of Łódź, ul. Banacha 12/16, 90-237 Łódź, Poland. E-mail: romano@biol.uni.lodz.pl

** Research Institute of Pomology and Floriculture, ul. Pomologiczna 18, 96-100 Skierniewice, Poland. E-mail: mgrzesik@insad.pl

*** Warsaw University of Life Sciences SGGW, ul. Nowoursynowska 159, 02-776 Warsaw, Poland. E-mail: hazem@kalaji.pl

ficial in the production of some plant species [15], [1], [7], [10], [2], [9], [6]. Our preliminary research proves that sewage sludge can be used in cultivation of deciduous ornamentals and energy plants as well [5], [14].

According to recent reports, Phytotoxkit tests can be used in bio-detection of toxic pollutants in water. These tests show the sensitivity of germinated seeds to various substances [11], [4], [13]. It is not sure whether Phytotoxkit can be used for predicting the influence of sewage sludge fertilization on plant growth and its efficiency.

The aim of this study was to evaluate the growth and physiological characteristic of corn, cultivated in various soils fertilised with sewage sludge, and to develop the methods for increasing the energy plant biomass on low-quality soils. Simultaneously, the usefulness of Phytotoxkit test for predicting this fertilization efficiency was assessed.

2. MATERIAL AND METHODS

Plants obtained from commercial corn grains (*Zea mays* L.) were cultivated in Phytotoxkit disks and in 3-litre pots filled either with sand or with soil of various types (sand, peat and clay mixed with sewage sludge in various proportions, ranging from 0 (control), 5, 10 to 15%). Sewage sludge was obtained from a small town waste refinery and had the certification for fertilization of inedible plants.

The seeds of grain germinated and new plants grown on Phytotoxkit disks were placed in growth chamber (20 °C and 8-hour dark/16-hour light cycle – SON-T AGRO 400 W, 100 $\mu\text{molm}^{-2}\text{s}^{-1}$), while the pots with growing plants were placed in a container area for the whole period of vegetative season. They were watered with tap water. The optimal moisture content of the soils used was 32%. Each treatment was to be repeated three times, each including ten seeds or ten plants.

There were several factors taken into consideration when evaluating the influence of fertilized soils on plant growth in Phytotoxkit disks. Each day during the first 2 weeks germinated seeds were counted, and root length and seedling height measured. Germination is considered to be complete when the radicle penetrates the seed coat [4].

The development of plants growing in pots with fertilized soil was evaluated on the basis of the number of emerged seedlings, each day during the first 3 weeks as well as on the basis of their height measurements. Seedling fresh and dry biomass, the content of chlorophylls *a* and *b*, net photosynthesis, electrolyte leakage, and RNase activity in leaves, determined each month during the growing season, were also indicative of their development. Fresh biomass and dry biomass were respectively weighed before and after drying at 130 °C for a few days to constant weight. Chlorophylls *a* and *b* content was evaluated according to Minolta SPAD 502. Net photosynthesis was measured in infrared light using LCA-3 gas analyzer apparatus equipped with Parkinson camera [12]. Electrolyte leakage was investigated at 20 °C after plac-

ing leaves' segments in test-tubes and adding 3 cm³ of distilled water. Electrolyte leakage was measured after 2 and 4 hours, using CC-551 Elmetron microcomputer conductometer [3]. The activity of RNase was evaluated according to KNYPL [8].

3. RESULTS AND DISCUSSION

Soil fertilization with sewage sludge may be beneficial in the production of some plant species, even though their wide use in agriculture is still very limited [15], [1], [7], [10], [2], [9], [6]. Our preliminary research [5], [14] and the results presented in this study show that sewage sludge fertilization can also be used in the case of corn, cultivated as energy plant on low-quality soils and on degraded areas.

The results show that sewage sludge added in various doses (5–15%) to sand or sandy, peat and clay soils affects neither the number of germinated seeds significantly nor the dynamics of their germination in Phytotoxkit disks. What is more, the number of emerged seedlings and the dynamics of their emergence in the soils mixed with sewage sludge in pots are not significantly affected (data not shown). This is in conformity with our previous observations which prove that effective and very fast germination of corn grains is less sensitive to some harmless treatments (nonpublished data).

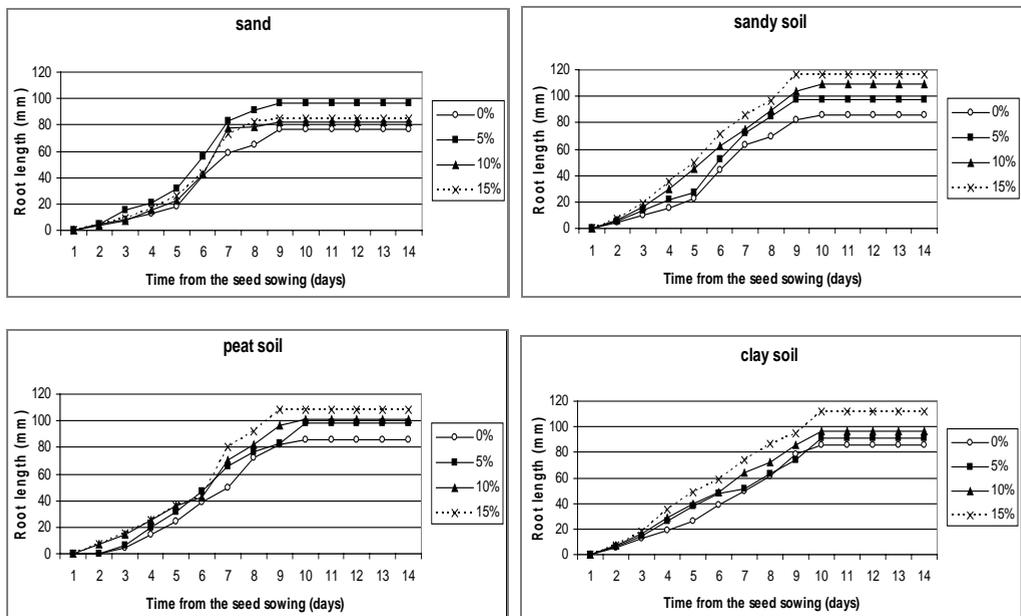


Fig. 1. Length of the corn roots grown in the Phytotoxkit disks on sand and sandy, peat and clay soils mixed with 0–15% dosages of sewage sludge

The type of soil had a great influence on the growth of plants developed in Phytotoxkit disks and those growing in the pots. Each experiment type proved that the growth of plants (taking into consideration their height) on the soil being not mixed with sewage sludge (control) was slow. A slightly faster plant growth was observed in sandy soil, in clay or peat soil, while in sand the growth was very limited (figures 1 and 2). The growth inhibition was caused by the very low amount of macroelements in these subsoils.

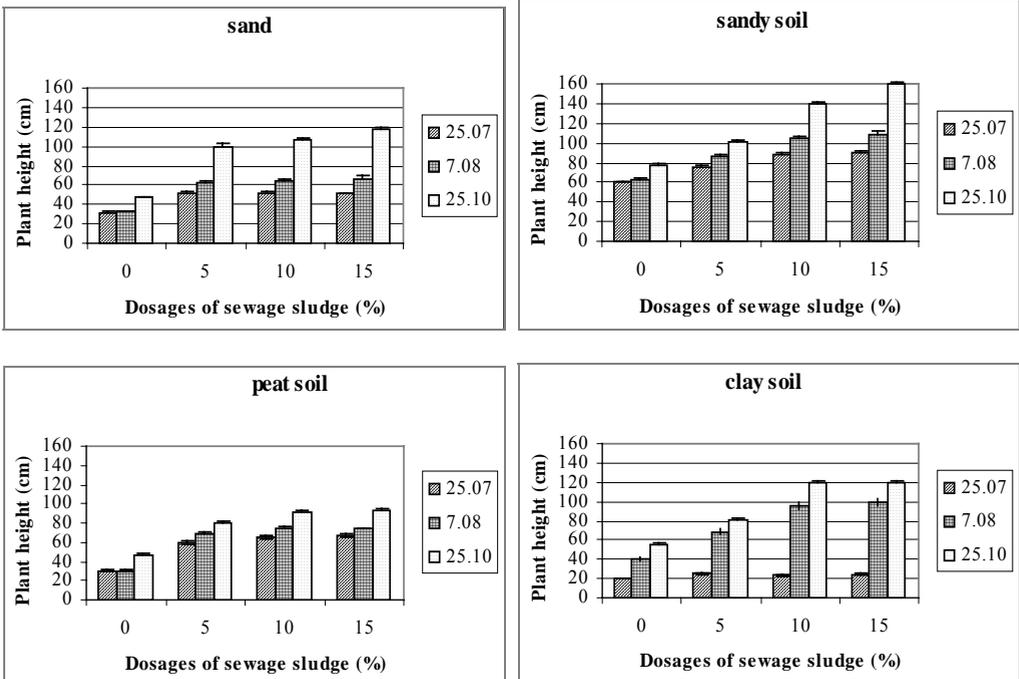


Fig. 2. Height of the corn plants grown in the pots on sand and sandy, peat and clay soils mixed with 0–15% dosages of sewage sludge. Vertical bars denote \pm SE

Sewage sludge addition to the soils stimulated and accelerated the plant development greatly, which could be exemplified by a dynamic root growth in Phytotoxkit disks as well as by the height of plants cultivated in pots, their fresh and dry biomass and the chlorophylls *a* and *b* content, photosynthesis, membrane stability and RNase activity in leaves. In all the types of soil amended with sewage sludge (10 and 15%), the fastest plant development was observed. Such a soil fertilization increased, to a large extent, the plant height. What is worth mentioning, the highest acceleration of the plant growth, resulting from fertilization, was observed in sand and sandy soil, and the plants grown in such media were about 2.5–3 times higher than in the control (figures 1–3).

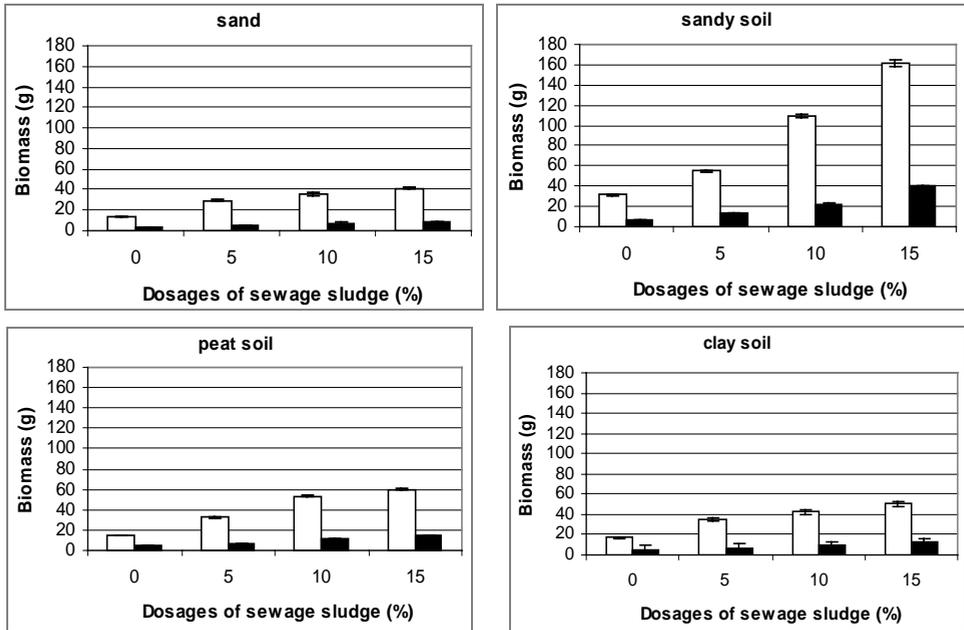


Fig. 3. Fresh (o) and (●) dry biomass of corn plants grown in the pots on sand and sandy, peat and clay soils mixed with 0–15% dosages of sewage sludge. Vertical bars denote \pm SE

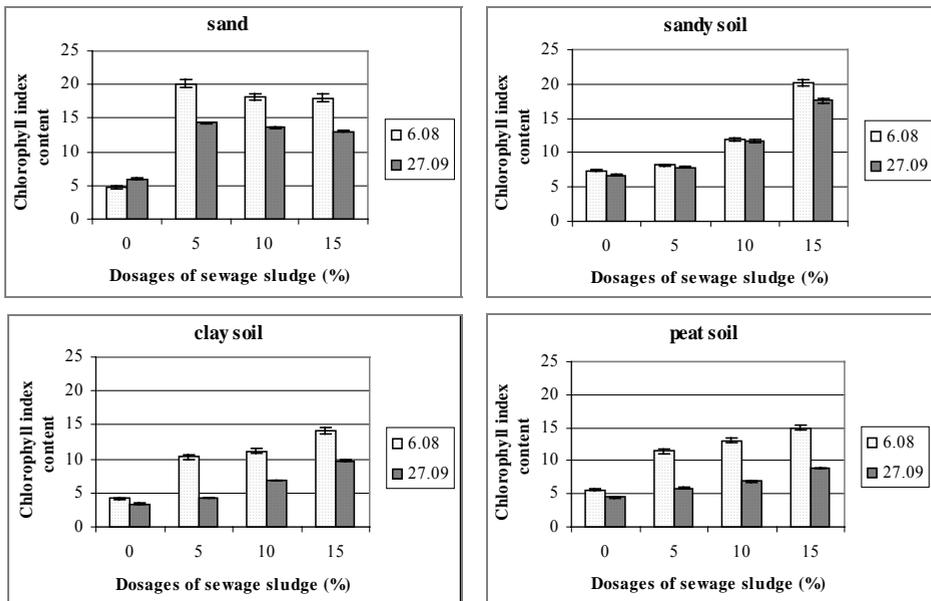


Fig. 4. Chlorophyll content in the leaves of corn plants grown in the pots on sand and sandy, peat and clay soils mixed with 0–15% dosages of sewage sludge. Vertical bars denote \pm S

The chlorophylls *a* and *b* content was the highest in the plants grown in sand fertilized with sewage sludge. In the case of peat and clay soils, these values were similar (figure 4). Net photosynthesis (given in $\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) in the case of corn was most efficient in soil with 5% sewage sludge content and peat soil with 10% sewage sludge content. Other values of all the variants were very similar (figure 5). Electrolyte leakage ($23.2 \mu\text{S seed}^{-1} \text{ water cm}^{-3}$) from corn leaves was higher in the plants grown on clay soil (figure 6). RNase activity (in $\text{U g}^{-1} \text{ f.w.}$) was noticed in the plants on clay soil with 10 and 15% sewage sludge content (figure 7).

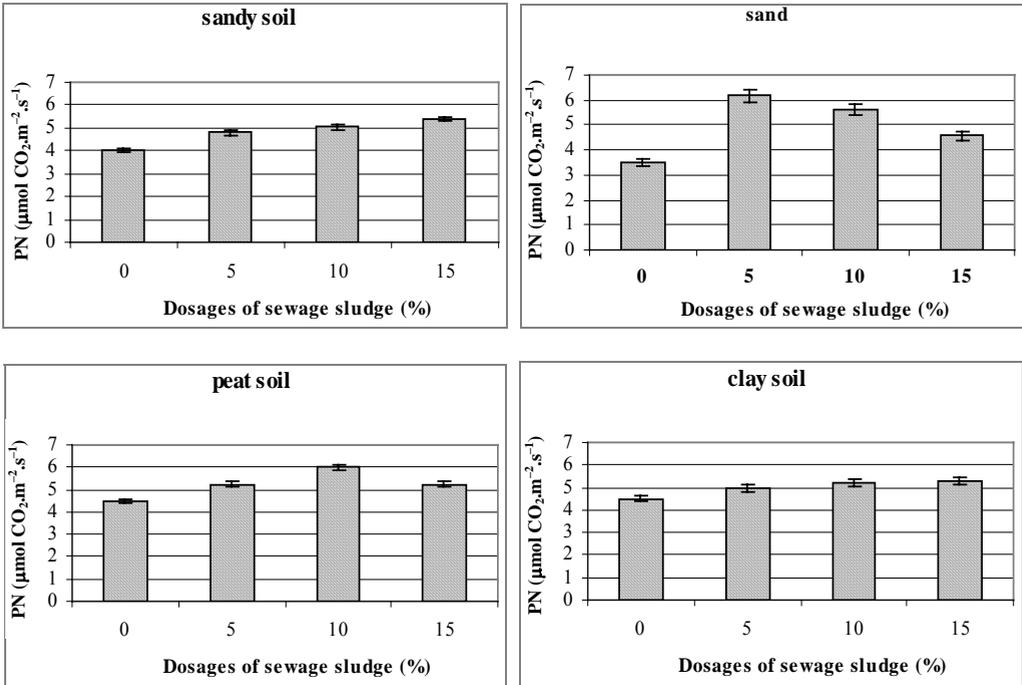


Fig. 5. Net photosynthesis in the leaves of corn plants grown in the pots on sand and sandy, peat and clay soils mixed with 0–15% dosages of sewage sludge. Vertical bars denote \pm SE

The above results indicate that the sewage sludge can hardly be used as a fertilizer of sandy and low-quality soils which usually are recommended for energy plants cultivation. Furthermore, the use of sewage sludge on large scale in the energy plant production resolves the ecological problems of their storage and reduces the pollution of the environment [13].

The results obtained show that the relationships between the planting conditions and the height of plants or other parameters of plant growth and metabolism are similar. Each treatment increased the plant height, their fresh and dry biomass, chlorophylls *a* and *b* content, photosynthesis, membrane stability and RNase activity in

leaves (figures 1–7). These observations prove that the characteristics mentioned can be the good indicators of plant development [3], [13], [14].

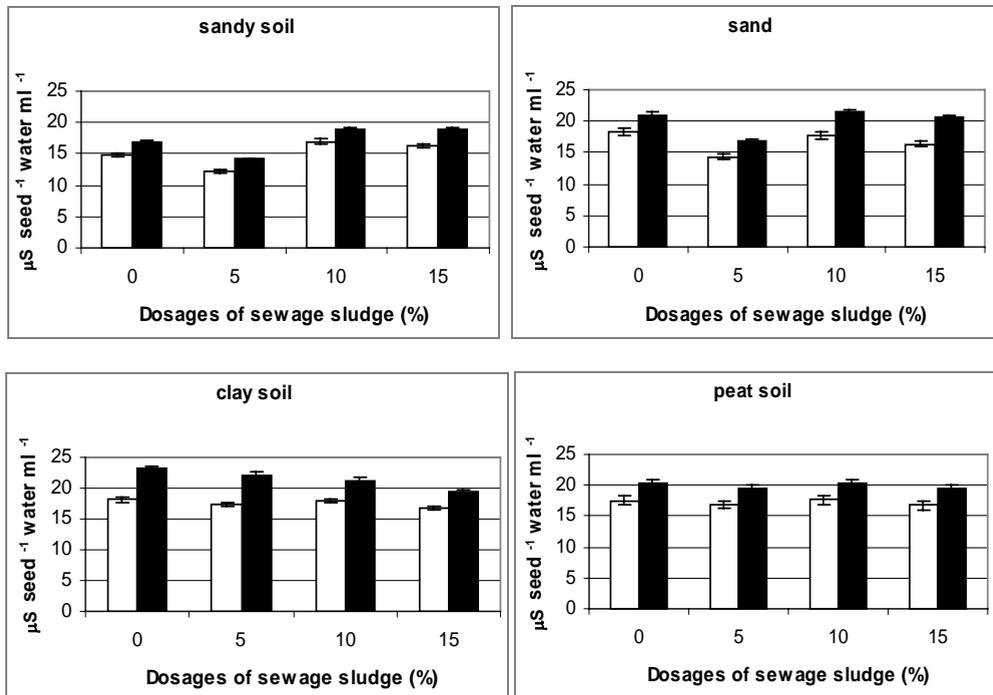


Fig. 6. Electrolyte leakage from leaves of corn plants grown in the pots on sand and sandy, peat and clay soils mixed with 0–15% dosages of sewage sludge, after 2 h (o) and after 4 h (●). Vertical bars denote \pm SE

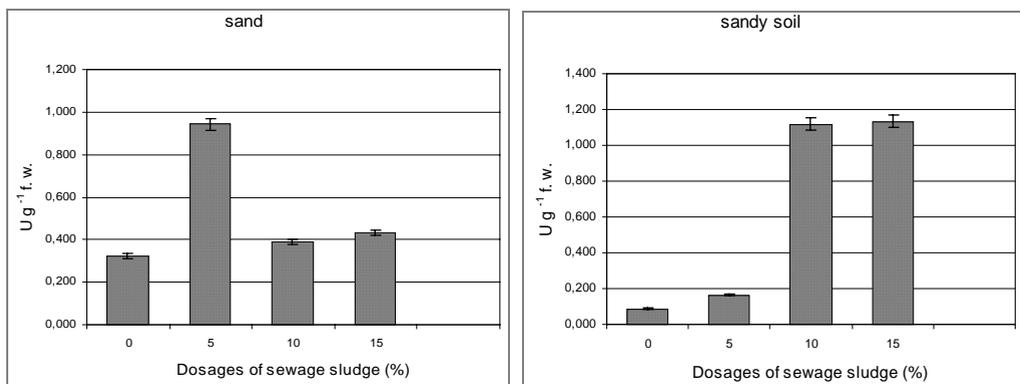


Fig. 7. RNase activity in leaves of corn plants grown in the pots on sand and sandy soil mixed with 0–15% dosages of sewage sludge. Vertical bars denote \pm SE

The present research also shows that the Phytotoxkit test can be useful in predicting the growth of plants, depending on the soil type. The results are based on the dynamics of 2-week root growth (measurement). The plant growth in the field was assessed over the whole vegetative season. The results obtained confirm that Phytotoxkits are fast and easy to use tests for evaluating the usefulness of various types of soil for the cultivation of particular plant species and toxicity monitoring [11], [4], [13].

ACKNOWLEDGEMENT

Research was sponsored by the Ministry of Science and Higher Education, Grant No. N 305 05 332/1931

LITERATURE

- [1] COGALIASTRO A., DOMON G., DAIGLE S., *Effects of wastewater sludge and woodchip combinations on soil properties and growth of planted hardwood trees and willows on a restored site*, Ecological Engineering, 2001, 16, 471–485.
- [2] CZYŻYK F., KOZDRAŚ M., *Wpływ nawożenia traw kompostem z osadów ściekowych na skład chemiczny odcieków z gleby*, Zeszyty Problemowe Postępów Nauk Rolniczych, Warszawa, 2003, 494, 85–92.
- [3] GÓRNIK K., GRZESIK M., *Effect of Asahi SL on China aster 'Aleksandra' seed yield, germination and some metabolic events*, Acta Physiologiae Plantarum, 2002, Vol. 24, No. 4, 379–383.
- [4] GRZESIK M., ROMANOWSKA-DUDA Z., *Sensitivity and monitoring response of Lactuca sativa germinated seeds to the toxicity of heavy metal salts in drinking water*, [in:] *Nutrient Management in Wastewater Treatment Processes and Recycle Streams*, 2005, 1305–1308.
- [5] GRZESIK M., ROMANOWSKA-DUDA Z., ANDRZEJCZAK M.E., WOŹNICKI P., WARZECHA D., *Application of sewage sludge to improve of soil quality by make use of model plant energy*, Acta Physiology Plantarum (supplement), 2007, 102.
- [6] HERNÁNDEZ-APAOLAZA L., GASCÓ A.M., GASCÓ J.M., GUERRERO F., *Reuse of waste materials as growing media for ornamental plants*, Bioresource Technology, 2005, 96, 125–131.
- [7] HICKLENTON P.R., RODD V., WARMAN P.R., *The effectiveness and consistency of source-separated municipal solid waste and bark compost as components of container growing media*, Scientia Horticulturae, 2001, 91, 365–378.
- [8] KNYPL J.S., *Growth, phosphatase and ribonuclease activity in phosphate deficient Spirodela oligorrhiza cultures*, Biochem. Physiol. Pflanzen, 1977, 17, 279–287.
- [9] KRZYWY E., IZEWSKA A., *Gospodarka ściekami i osadami ściekowymi*, Wydawnictwo Akademii Rolniczej w Szczecinie, 2004, 186.
- [10] MAĆKOWIAK Cz., *Wartość nawozowa osadów ściekowych*, Inżynieria Ekologiczna. Przyrodnicze użytkowanie osadów ściekowych. Ochrona i rekultywacja gruntów, Bydgoszcz, 4–6.06.2001, 3, 135–145.
- [11] PERSOONE G., *Recent new microbiotest for cost effective toxicity monitoring: The Rapidtoxkit and the Phytotoxkit*, 12th International Symposium on Toxicity Assessment, 12–17.06.2005, Skiathos Island, Greece, 2005, 112.
- [12] ROMANOWSKA-DUDA Z.B., KALAJI H., STRASSER R., *The use of PSII activity of Spirodela oligorrhiza plants as an indicator for water toxicity*, Fundamental Aspects to Global Perspectives. Photosynthesis Research., Spec. Issue, 585–587.

- [13] ROMANOWSKA-DUDA Z. B., GRZESIK M., MANKIEWICZ J., ZALEWSKI M., *Bioindication of microcystins toxicity by germinating seeds*, [in:] *Environmental Toxicology*, A.G. Kungolos, C.A. Brebbia, C.P. Samaras and V. Popov (editors), 2006, 243–252.
- [14] ROMANOWSKA-DUDA Z., GRZESIK M., ANDRZEJCZAK M.E., WOŹNICKI P., WARZECHA D., *Influence of stabilized sewage sludge on biomass growth of chosen species of energy plants*, *Acta Physiol. Plantarum* (supplement), 2007, 66–67.
- [15] WOŁOSZCZYK CZ., KRZYWY E., JAKUBOWSKI W., *Badania nad rolniczym wykorzystaniem odpadów komunalnych i przemysłowych*, *Folia Universitatis Agriculturae Stinensis*, 2000, 211, 84, 527–532.