Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2015.1043492

57 (2016) 1515–1524
January

Taylor & Francis
Taylor & Francis Group

Removal of mercury (II) from the aquatic environment by phytoremediation

Magdalena Sitarska*, Teodora Traczewska, Viktoriya Filyarovskaya

Politechnika Wrocławska, Wybrzeże S. Wyspiańskiego 27, Wrocław 50-370, Poland, Tel. +48 71 320 33 50; Fax: +48 71 328 29 80; email: magdalena.sitarska@pwr.edu.pl (M. Sitarska)

Received 18 July 2014; Accepted 9 March 2015

ABSTRACT

Phytoremediation may be an alternative to traditional methods of removing heavy metals from the aquatic environment. In order to remove mercury at a concentration of 0.3 mg/L from Hoagland medium, two species of pleustophytes were used, namely *Salvinia natans* and *Lemna minor*. Some homogeneous cultures and another one mixed in a weight ratio of 2:1 were used. The physiological condition of the plants was controlled after 7, 14 and 21 d by evaluating biomass growth, analysing the changes in the amount of total protein and assimilation dyes. At the same time, the level of the mercury content in the base and plant tissues was controlled. The increase in biomass during plant exposure to mercury was up to 190% compared to baseline. Accumulation of mercury in plant tissues to 238.34 $mg/kg_{d.m.}$ contributed to a significant reduction in its concentration in the medium. The bad condition of the plants led to an attempt to support phytoremediation by micro-organisms taking an active part in the transformation of mercury. For this purpose, epiphytic bacteria, accompanying *S. natans*, which are resistant to mercury at a concentrations of 0.3 mg/L, were isolated. Studies indicate the possibility of using the plants *S. natans* and *L. minor* to remove mercury compounds from the aquatic environment, while providing a basis to determine the principles of design and operation of *Lemna* ponds, especially when the process is simultaneously stimulated by those bacteria that are resistant to high concentrations of mercury.

Keywords: Pleustofits; *Salvinia natans*; *Lemna minor*; Epiphytic bacteria; *Lemna* ponds

1. Introduction

The progress of civilization has contributed to a significant increase in environmental pollution by heavy metals, including mercury. The exhaust gas, waste and excessive use of various types of chemicals applied in industry and agriculture lead to the contamination not only on a local scale, but more and more frequently on a global one [1,2]. Contamination of ecosystems with mercury adversely affects the metabolism of plant and animal organisms, and consequently the human health [3]. Particular attention should be paid to aquatic ecosystems since they still represent a major route of contamination entering the human body despite using increasingly sophisticated methods of treatment [4,5]. In an

*Corresponding author.

Presented at the 12th Scientific Conference on Microcontaminants in Human Environment 25–27 September 2014, Czestochowa, Poland

aqueous environment, mercury may be present in different chemical forms largely dependent on the oxidation–reduction conditions.

An alternative to traditional methods of removing heavy metals from the aquatic environment is phytoextraction [6–8], using the above-average natural ability of some plant species to accumulate contaminants [9,10]. The efficiency of the process largely depends on the plant, which should be primarily characterized by a rapid growth [1,11–13]. Its big advantage is the low cost [14].

Among hyperaccumulators, there are also pleustofits, i.e. *Salvinia natans* and *Lemna minor*, occurring in the territory of Poland [15–17].

The phytoremediation process may be supported by micro-organisms [18–20]. Microflora (bacteria and fungi) takes an active part in the transformation, and among other things, mercury detoxification [21]. There are a number of defence mechanisms against the heavy metals: formation of insoluble mercury sulphide, lowering the cell wall permeability, methylation of mercury compounds and conversion of non-volatile mercury compounds to volatile form at zero oxidation degree [22]. The mechanism of bacterial resistance to mercury ions is one of the best known regarding bacteria resistance to heavy metals [23,24].

The aim of the study was to determine the accumulation of mercury by *S. natans* and *L. minor*. The results were compared with the results of phytoremediation assisted by micro-organisms resistant to mercury (mercury transformation mechanisms). This process depends greatly on the condition of the plants and therefore is dependent on the size of their growth and some physiological parameters (total protein, chlorophyll).

2. Methods

2.1. Material

Homogenous cultures of *S. natans* (S.n.) and *L. minor* (L.m.), as well as a mixed culture of both species (S.n. + L.m.) in a weight ratio of 2:1, which resulted from the same water surface area occupied by the plants, were used for the tests. In the case of a homogeneous culture in 90 ml of liquid medium was placed 1 g of plants. The plants were taken from commercial farms.

Five strains from own collection obtained by selecting *S. natans* epiphytes were used.

2.2. Experimental procedure

The Hoagland medium [25] contaminated with mercury (II) nitrate, obtained a concentration of 0.3 mg/L (the maximum concentration for which no deadly effect for plants was observed), was used for the tests. The time of plant's exposure to mercury was 7, 14 and 21 d. In the case of microbiologically supported phytoremediation, the culture was fed with three (with five isolated) different strains of epiphytic bacteria as an inoculum in a quantity of 1 ml/100 ml of medium. The tests of microbiologically supported phytoextraction were carried out separately for each bacterial strain. In order to maintain constant physical conditions, the incubation was conducted in a phytotron FD 147 Inox manufactured by Biosell, fitted with 18 W/965 Biolux fluorescent lamps by OSRAM, in a day/night cycle (12 h/12 h) at relative humidity of 40% and a temperature of 22℃/15℃.

2.3. Analytical methods

The increase in plant biomass was evaluated using an analytical balance WAA 160/C/1 manufactured by Radwag.

Total protein was determined by the Lowry's method [26] modified by Eggstein and Kreutz [27]. The samples for the determination of protein were hydrolysates obtained by mechanical homogenization of 0.1 g of fresh plant mass in 1 M NaOH solution in an Ultra-Turrax Tube Driver. Subsequently, the hydrolysate was incubated in a water bath at a temperature of 100℃ for 10 min (physicial denaturation) and filtered, and the absorbance was measured at $\lambda = 750$ nm on the apparatus T80 + UV/VIS (PG Instruments Ltd).

For the determination of a and b chlorophyll, the plant extracts were prepared by mechanical homogenization of 0.1 g fresh plant mass at 90% acetone by means of the homogenizer-type Ultra-Turrax Tube Driver. The resultant homogenates were extracted for 22 h in the dark at a temperature of 5℃ and then filtered. The absorbance measurements were made on the apparatus T80 + UV/VIS (PG Instruments Ltd) at $\lambda = 663$ nm for chlorophyll a and $\lambda = 645$ nm for chlorophyll b. The total chlorophyll content C_{Chl} was calculated on the basis of the results of the analyses of chlorophyll a and b in plant material by the following formula:

$$C_{Chl} = 8.02 \times A_{(663)} + 20.2 \times A_{(645)}$$

The content of mercury in solutions and in the air-dry plant mass was determined using an atomic absorption spectrometer AMA 254 manufactured by Altem. The standard for AMA 254 was mercury (II) nitrate from Merck.

3. Results

The increase in plant biomass in the given environmental conditions can be indirectly a parameter for the evaluation of the effectiveness of the phytoextraction process, namely the ability of plants to accumulate contaminants in tissues, since along with its growth, the plant collects from the environment not only some necessary nutrients, but also the contaminants. Systematic growth of *S. natans* plant was observed for both the reference samples and incubated with mercury ones. For the reference samples, it was 58% on the 7th day, 103% on the 14th day and 158% at the 21st day. In relation to the baseline, the increase in biomass of *S. natans* after the 7 d of incubation with mercury was 122; 135% on the 14th day; and 190% on the 21st day, suggesting a stimulating influence of mercury as an effect of toxicity (Fig. 1). Different results were obtained for *L. minor*, whose growth in the reference was significantly more intense than in the case of exposure to mercury. For the reference samples, the 67% growth was achieved on the 7th day, which in the subsequent days was 134% (14th day) and 295% (21st day). The samples exposed to mercury showed a growth of 64% after the 7th day and it was similar to the reference, but in the subsequent days of exposure, the growth was significantly smaller than the reference one. On the 14th day, it was 71%, which is twice smaller than the reference one, and on the 21st day, it was even three times amounting to 97%. These results were obvious in view of its characteristics (high sensitivity), which led to the choice for the test organism in generally used toxicity tests. This was particularly evident on the 21st day of the experiment; the growth of the reference *L. minor* was as much as 295%, and one exposed to mercury was only 97%.

The tests were also performed with the participation of the mixed culture, for which, as in the case of *L. minor*, the reductions were observed in the growth of plant biomass as a result of exposure to the tested toxic substance. On the 7th day, it was only 51% and significantly increased up to 152% on 14th day, but it decreased to 123% on the 21st day. The biomass growth of reference samples in the mixed culture was the lowest in comparison to other cultures. Such a situation could result from ecological dependencies of the organisms occupying the same habitat, and it suggests probable antagonistic impacts. The results obtained for samples exposed to the metal were only 26% on the 7th day, 66% on the 14th day and 87% on the 21st day. Just like for the reference also for the plants exposed to mercury, the results obtained for the mixed culture were significantly lower than that for the monocultures. Within the first seven days of the experiment, the increase in biomass was 38–96%; on the 14th day was 5–69%; and even above 100% on the 21st day.

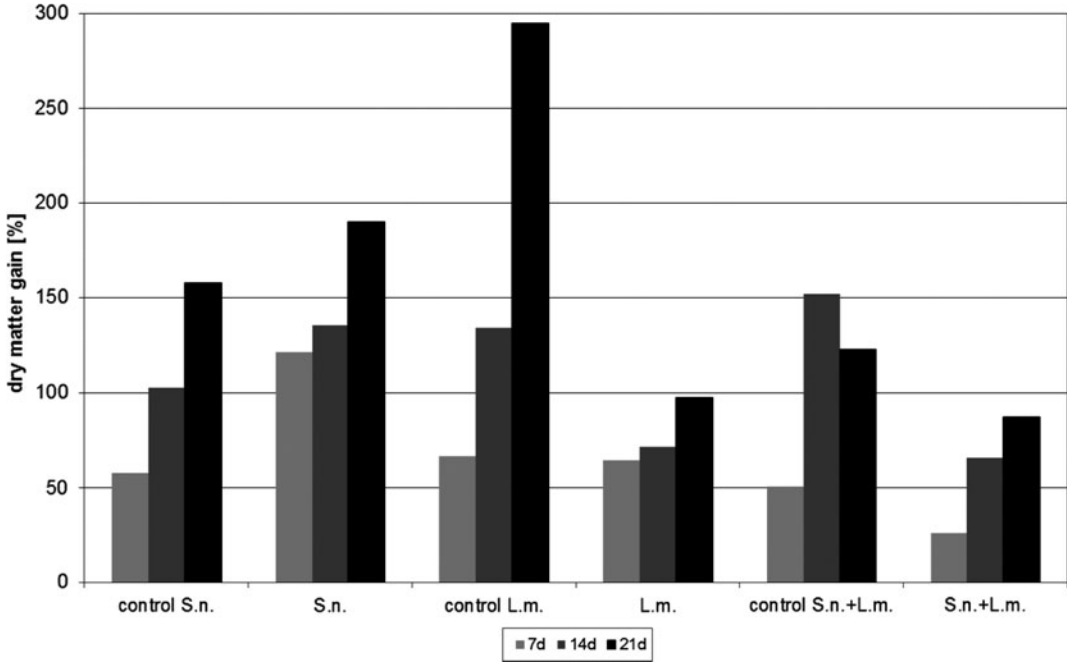

Fig. 1. Increase in plant dry mass after exposure to mercury at a concentration of 0.3 mg/L.

In the reference samples of *S. natans*, the quantity of protein was 457.64 mg/$g_{d.m.;}$ however, when exposed to mercury, it started to grow (Fig. 2). On the 7th day, it amounted to 555.86 mg/$g_{d.m.}$ (an increase by 21% relative to the reference); on the 14th day, 609.16 mg/$g_{d.m.}$ (an increase by 33% relative to the reference); and on the 21st day, even 802.08 mg/$g_{d.m.}$ (an increase by 75% relative to the reference). Similar correlations were observed also for *L. minor*, but the increase in the quantity of total protein did not exceed 34% (14 d) relative to the reference sample. In the mixed culture, the toxicity effect manifested by stimulation of protein synthesis was the greatest. Whereas, for the reference samples, it was 354.23 mg/$g_{d.m.,}$ and then, it rose to 666.57 mg/$g_{d.m.}$ on the 7th day, 544.08 mg/$g_{d.m.}$ on the 14th day, and up to 704.71 mg/$g_{d.m.}$ on the 21st day. The quantity of total protein in plant tissues was even 99% higher (21 d) than for the reference. The increased quantity of total protein in the presence of mercury suggests launching the tolerance mechanisms by pleustofits, through the synthesis of proteins, which can be primarily enzymatic proteins (transformations of mercury) or stabilizing one (binding the metal in the biomass).

In all tested cultures, the total chlorophyll content decreased when compared to the reference as the time of exposure to mercury lengthened (Fig. 3). In the case of *S. natans*, its quantity decreased even by 60%. The reference samples contained 60.29 mg/g of protein, but this quantity was reduced to 30.13 mg/g of protein on the 7th day of incubation with mercury and to 20.58 mg/g of protein on the 14th day. The analysis on the 21st day (34.64 mg/g of protein) showed a general increase in the quantity of total chlorophyll in relation to the 7th and 14th days; however, its quantity was still much below the reference samples, which did not affect the production of proteins. A similar situation was in the case of *L. minor*; however, the reduction of the quantity of dyes did not exceed 24%. The reference samples contained 43.28 mg/g of protein and the samples after incubation with mercury—37.75 mg/g of protein (7 d), 33.25 mg/g of protein (14 d) and 34.66 mg/g of protein (21 d). The mixed culture showed quite significant instability of the total chlorophyll content during incubation with mercury. For the reference samples, the quantity was stated as 52.85 mg/g of protein, whereas on the 7th day, its value decreased by 40% compared to the reference and amounted to 31.50 mg/g of protein. Then, the total chlorophyll content in tissues increased to the value of 53.64 mg/g of protein (14d), achieving a value close to the reference. On the 21st day, it decreased again achieving a value of 45.37 mg/g of protein.

In order to assess the effectiveness of phytoextraction, the changes in the levels of mercury content in plant biomass (Fig. 4) were analysed. The quantity of mercury for *S. natans* in the reference samples amounted to 0.24–0.28 mg/$kg_{d.m.;}$ much higher contents were observed in *L. minor* and in the mixed culture (0.26–0.53 mg/$kg_{d.m.}$). This proves its constant presence in the environment in a form of

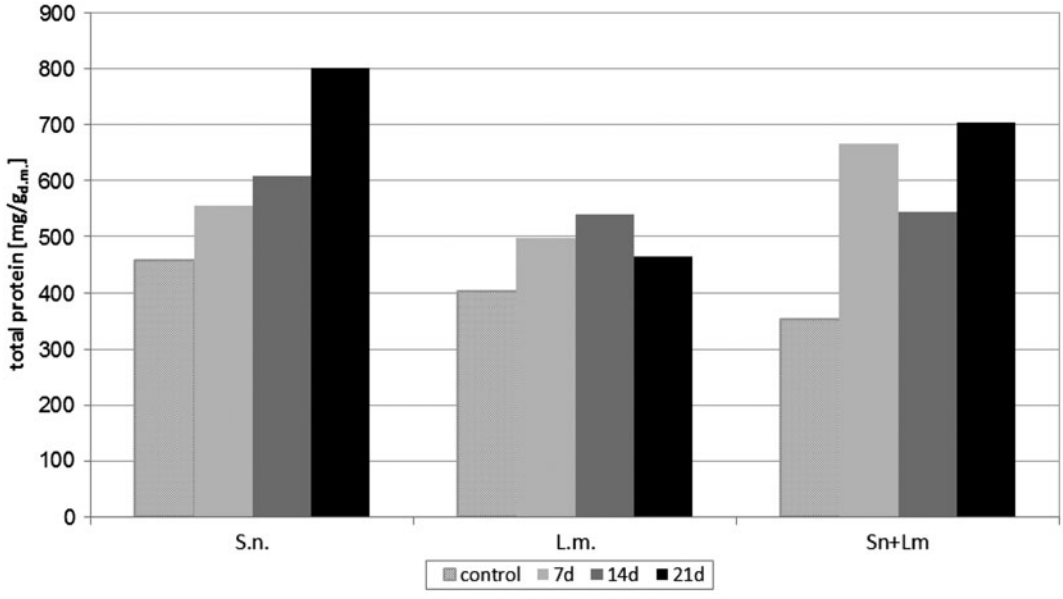

Fig. 2. Total protein content in plants exposed to mercury at a concentration of 0.3 mg/L.

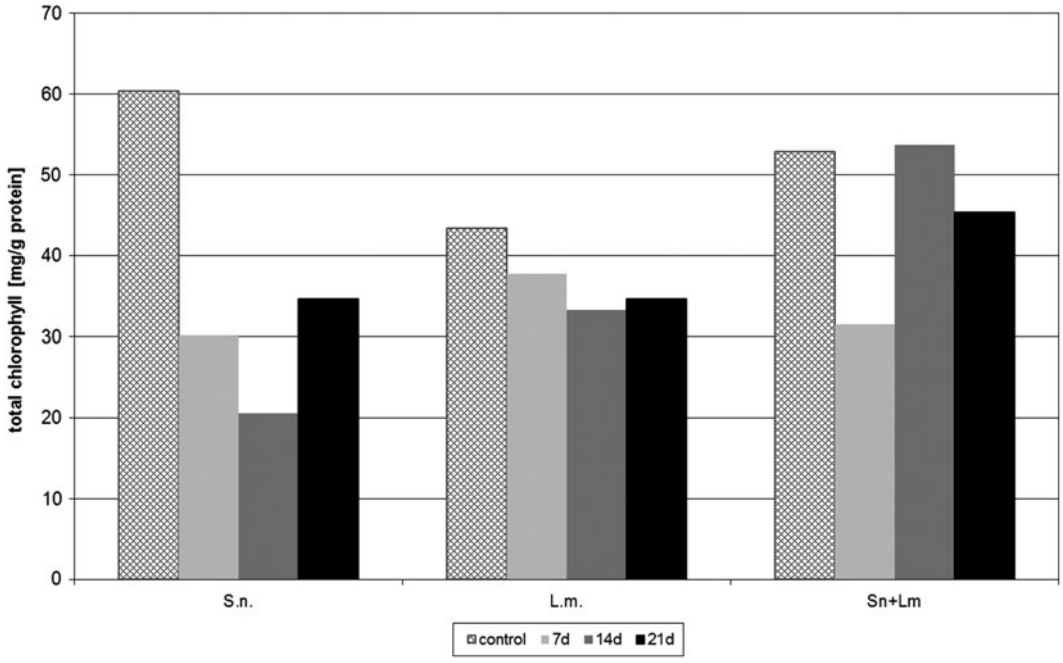

Fig. 3. Total chlorophyll content in the plants exposed to mercury at a concentration of 0.3 mg/L.

"background" and therefore its presence in the tissues of plants cultivated on the medium containing no mercury.

The analysis of the mercury content in the tissues of *S. natans* showed its continuous decrease with increasing incubation time. On the 7th day, it was 166.68 mg/$kg_{d.m.}$, on the 14th day and decreased to 152.03 mg/$kg_{d.m.}$, and on the 21st day, it decreased to 116.46 mg/$kg_{d.m.}$ Reducing the content of mercury in the biomass of *S. natans* by almost 9% on the 14th day and by more than 30% on the 21st day compared to the 7th day of the experiment proves that the element

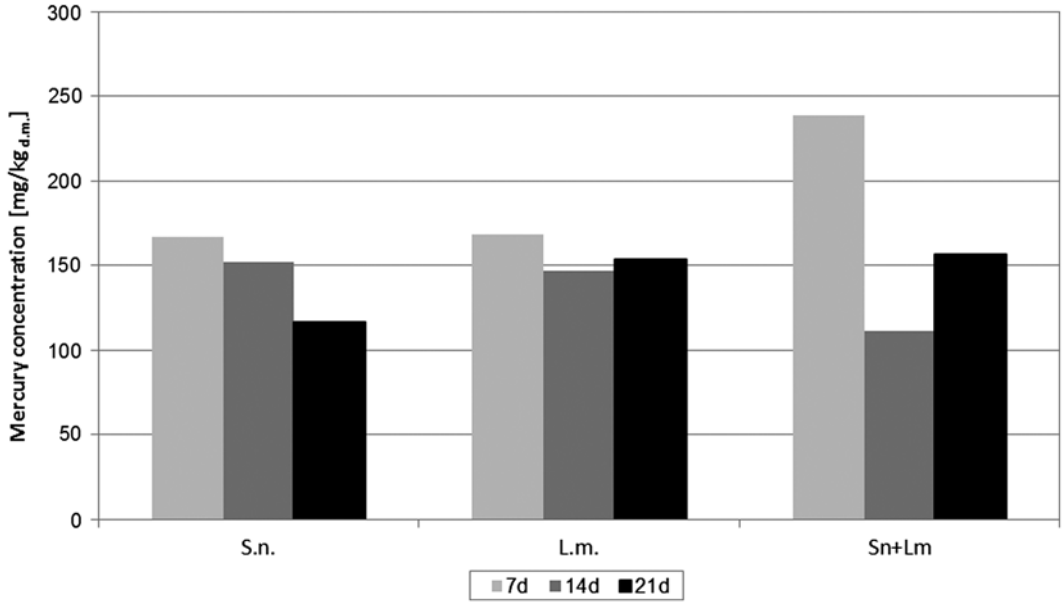

Fig. 4. Total mercury content in tissue of the plants exposed to mercury with a concentration 0.3 mg/L.

releases from plant tissues most likely as a result of volatilization. This is confirmed by the fact that the quantity of mercury in the medium did not increase along with the reduction in its quantity in the plant tissues. A similar phenomenon was observed in the case of *L. minor*. On the 7th day, the quantity of mercury in plants was 168.18 mg/$kg_{d.m.}$ and was similar to the values obtained for *S. natans*. In the following days of exposure, its quantity, as for the *S. natans*, decreased to 147.19 and 153.83 mg/$kg_{d.m.}$ on the 14th and 21st day, respectively. Particular attention should be paid to the mixed culture which, despite its much smaller increase, accumulated a larger quantity of mercury in the tissues. On the 7th day, it was up to 238.34 mg/$kg_{d.m.}$, and this quantity decreased significantly on the 14th day (111.39 mg/$kg_{d.m.}$) and then increased to 156.83 mg/$kg_{d.m.}$ on the 21st day.

Analysing the changes in the mercury contents in the tissues of plants, it is evident that its greatest content was stated on the 7th day of exposure in the case of all cultures, which proves the most intense accumulation of contaminants in the first days of exposure and, presumably, its emission into the atmosphere. This is confirmed by the analysis of mercury in the medium, where its quantity within the first seven days decreased by over 90% and remained at that level until the end of the experiment.

The plants used in this study are commercial ones, which in the case of the mixed culture increased the mercury content in tissues on the 21st day relative to the 14th day. This may suggest that the mechanism of tolerance was launched as a result of the stress associated with the presence of toxins with the concurrent ecological competition, which was not observed in the case of homogeneous cultures.

The deteriorating condition of plants engaged in the process of phytoextraction prompted to support the process by micro-organisms capable of transforming mercury.

The analysis of the phytoremediation process in the presence of 5 bacterial strains insensitive to mercury at a concentration of 0.3 mg/L from own collection was performed using *S. natans* under analogous conditions. In the presence of two of the five tested strains based on visual assessment of the condition of the plants, the poor condition of pleustofits (Fig. 5) was stated. The negative impact of bacteria may be related to the ability of these bacteria to methylation of mercury or the fact that tested micro-organisms belonged to plant pathogens, and therefore, they could not be used in further research.

The results obtained from the study of plant biomass growth (Fig. 6) show a very good plant growth in the presence of three types of micro-organisms tested in media with mercury.

Particularly good results were obtained for the floating fern in the presence of the strain 1, wherein the weight gain was 381% on the 7th day, 474% on the 14th day and 682% on the 21st day of the experiment. Compared to the plant biomass in the medium without micro-organisms, the percentage growth of biomass for the stain 1 was nine times higher on the 7th day, three times higher on the 14th and 13 times higher on the 21st day of the experiment. The plants from the medium without the addition of micro-organisms were characterized by the smallest increase in biomass as compared to *S. natans* containing bacterial cultures. On the 7th day of the experiment, the increment was 40%, and on the 14th day increased to 140%, but it decreased to 51% on the 21st day. In the case of the strain 2, the percentage increase in biomass on the 7th day was 184% and decreased to 159% on 14th day and then increased to 682% on the 21st day of the experiment. The observed phenomenon may be indicative of supporting plant life parameters by the use of bacteria.

For the strain 3, the reverse trend was observed. On the 7th day, it was 197%, and rapidly increased to 533% on the 14th day, but on the 21st day of the experiment, it decreased to 301%. However, the increase in biomass of plants in the case of the phytoremediation supported by the micro-organisms was higher by 250% when compared to the plants exposed to mercury without epiphytic micro-organisms resistant to mercury.

The analysis of the total protein content (Fig. 7) showed a similar trend for all plants. On the 14th day of the experiment, the quantity of protein increased when compared to the 7th day, and then decreased (21st day comparing to 14th day). For plants from the medium without the addition of micro-organisms, the value rapidly increases on the 14th day of experiment from 49.60 to 239.20 mg/$g_{d.m.}$ On the 21st day, the final content was 139.90 mg/$g_{d.m.}$. The process proceeded analogously in other cases; however, the quantity of protein in the test organisms from the media with micro-organisms was characterized by a higher value (166.02 mg/$g_{d.m.}$ for the medium with the strain 1, 173.79 mg/$g_{d.m.}$ for the medium with the strain of 2 and 194. 23 mg/$g_{d.m.}$ for the medium with the strain 3). The results indicate that the applied strains support and stimulate the plant life parameters.

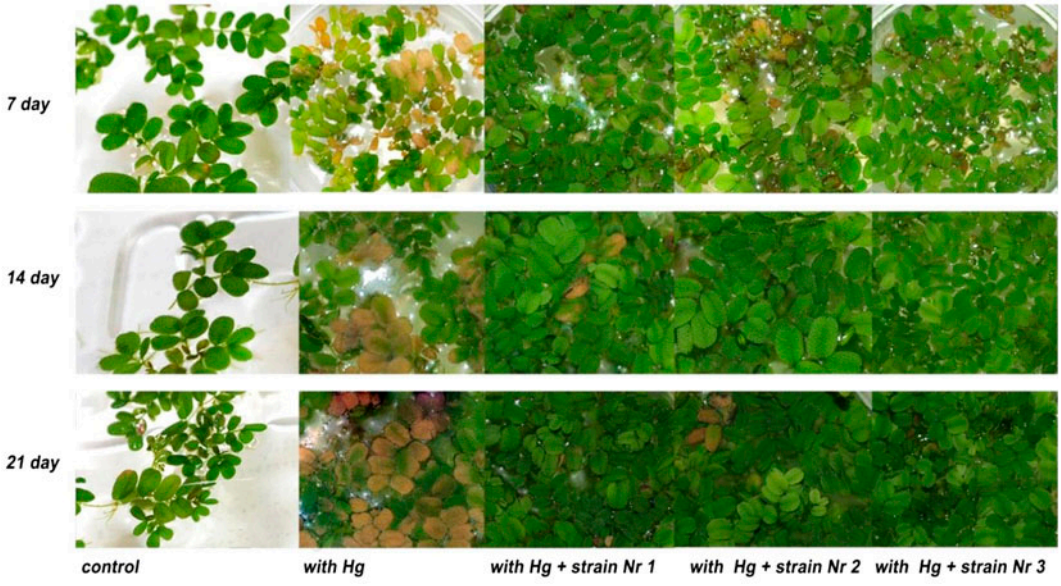

Fig. 5. Visual evaluation of the test organisms under the influence of the mercury on the 7, 14 and 21 d of the experiment.

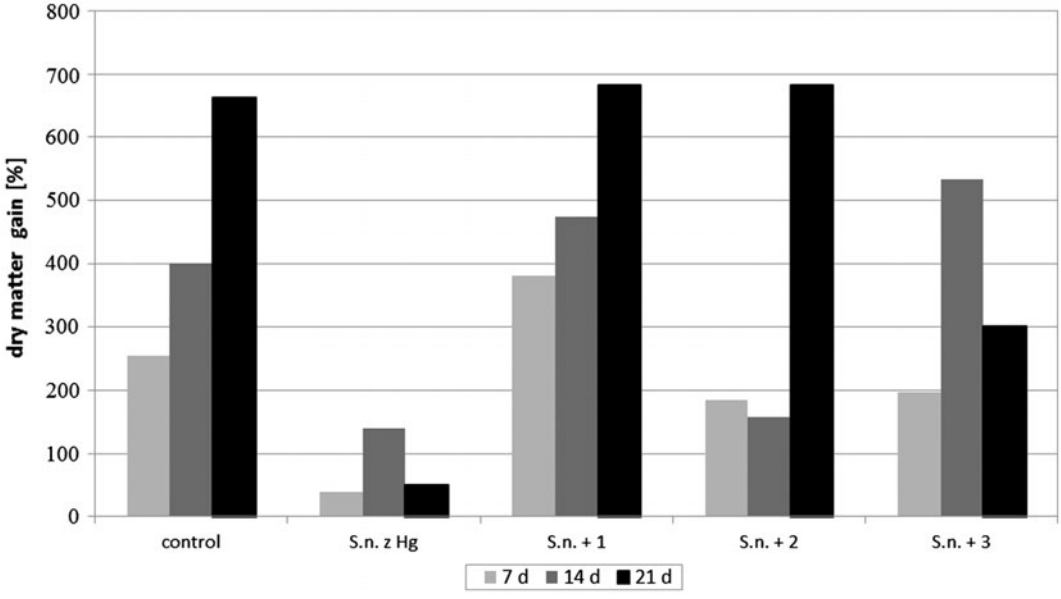

Fig. 6. Increase in the plant dry matter in the medium at a concentration of 0.3 mg/L.

In the case of total chlorophyll (Fig. 8), a downward trend for the amount of assimilation dye for the plants from the medium with mercury was also maintained since the value decreased from 7.43 to 6.98 (for the plants from the medium with the strain 2—from 11.79 on the 7th day to 8.14 on the 21st day, and for the plants from the medium with the strain 3—from 13.71 to 8.59). The smallest amount of dyes was characteristic for the test organisms from the medium with mercury and without bacterial cultures, which may indicate that the plants in the medium with the addition of micro-organisms were characterized by better physiological condition.

S. natans accumulated from 291.00 to 693.20 mg/$kg_{d.m.}$ (Fig. 9). The minimum capacity of mercury accumulation in the plants was characteristic for the

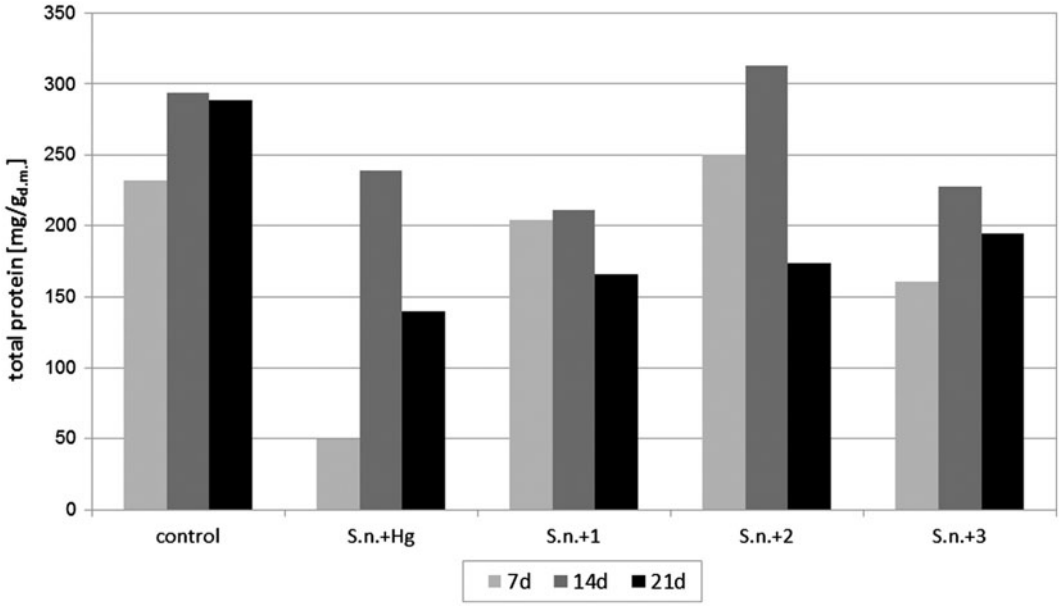

Fig. 7. The total protein content in plants exposed to mercury at a concentration of 0.3 mg/L in the presence of bacterial strains.

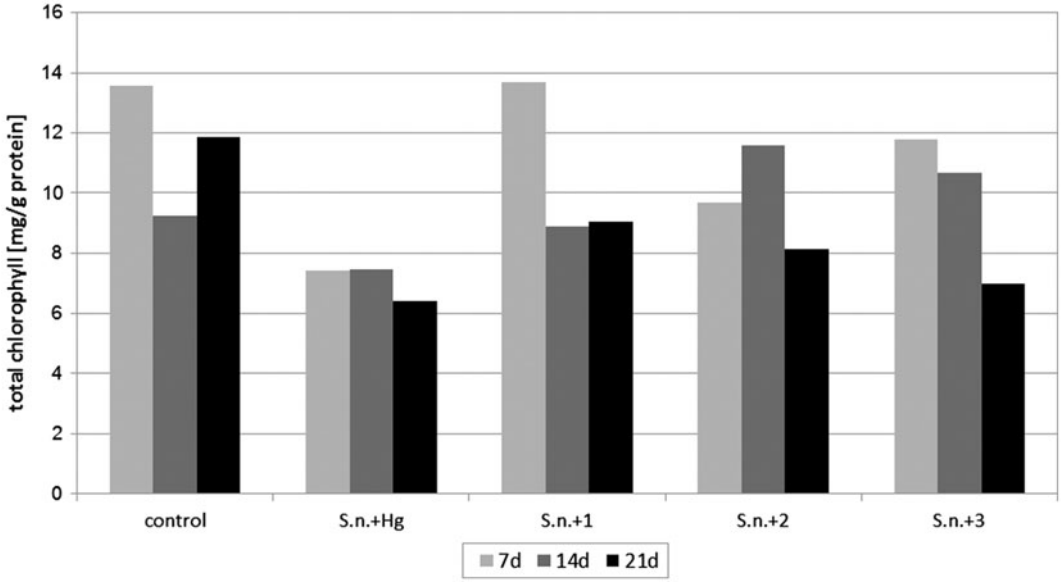

Fig. 8. The total chlorophyll content in the plants exposed to mercury at a concentration of 0.3 mg/L in the presence of bacterial strains.

plants in the medium with mercury and without micro-organisms resistant to mercury (from 225.10 to 567.30 mg/kg$_{d.m.}$).

For all analysed media, there was observed a substantial reduction in the mercury content compared to initial values (0.3 mg/L). The largest loss of mercury in the medium was observed for phytoremediation in a presence of the strain 1, where the values similar to those in reference samples were obtained on the 7th day. The smallest degree of mercury removal from the medium was observed for phytoremediation not supported by micro-organisms.

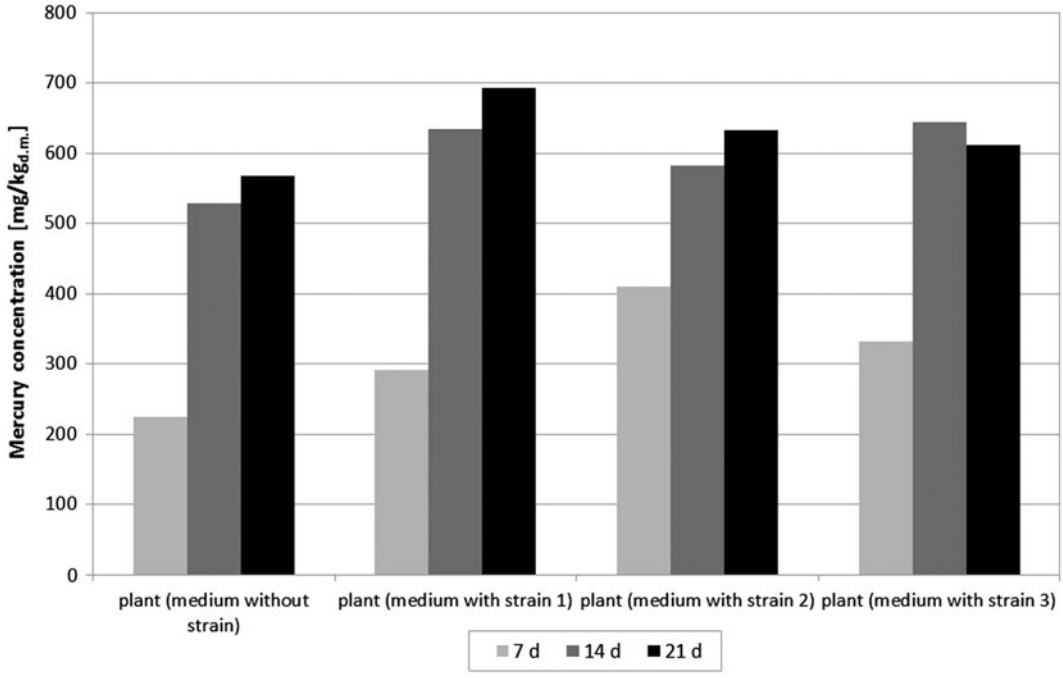

Fig. 9. The mercury content in plant tissues exposed to mercury with a concentration of 0.3 mg/L in the presence of bacterial strains.

4. Conclusion

(1) *S. natans* and *L. minor* pleustophytes belong to the species of plants tolerant to mercury in a concentration of 0.3 mg/L and hence can be used in the process of phytoremediation of water contaminated with it.
(2) Mercury has an important impact on the physiological processes of plants, especially the biosynthesis of proteins and assimilation dyes, which on the one hand confirms its toxicity, but on the other indicates a high degree of tolerance of the tested plants to mercury contamination.
(3) *L. minor* as an organism, which due to its sensitivity, is used to evaluate the toxicity of the environment, showed higher sensitivity to mercury contamination, as evidenced by its lower growth and changes in the content of total protein and assimilation dyes.
(4) The obtained results of physiological parameters of *S. natans* in the presence of micro-organisms insensitive to mercury may indicate that the used strains support the life processes of floating fern.
(5) The obtained results indicate an increased mercury removal efficiency in the presence of isolated micro-organisms resistant to mercury rather than merely involving plants.

Acknowledgement

The study was conducted under a Grant No. N N523 612139 entitled "Evaluation of the effectiveness of phytoremediation of water contaminated with mercury (II) by pleustophytes in Lower Silesia".

References

[1] D.W. Boening, Ecological effects, transport, and fate of mercury: A general review, Chemosphere 40 (2000) 1335–1351.
[2] A. Glodek, D. Panasiuk, J.M. Pacyna, Mercury emission from anthropogenic sources in Poland and their scenarios to the year 2020, Water Air Soil Pollut. 213 (2010) 227–236.
[3] R.L. Chaney, J.S. Angle, C.L. Broadhurst, C.A. Peters, R.V. Tappero, D.L. Sparks, Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies, J. Environ. Qual. 36 (2007) 1429–1443.
[4] S. Clemens, Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants, Biochimie 88 (2006) 1707–1719.
[5] S.A. Rahman, A.K. Wood, S. Sarmani, A.A. Majid, Determination of mercury and organic mercury contents in Malaysian seafood, J. Radioanal. Nucl. Chem. 217 (1997) 53–56.

[6] D.N. Dowling, S.L. Doty, Improving phytoremediation through biotechnology, Curr. Opin. Biotechnol. 20 (2009) 204–206.
[7] M.N.V. Prasad, Aquatic plants for phytotechnology, Environmental Bioremediation Technologies, Springer-Verlag, Berlin, Heidelberg, 2007, pp. 259–774.
[8] Q. Wang, D. Kim, D.D. Dionysiou, G.A. Sorial, D. Timberlake, Sources and remediation for mercury contamination in aquatic systems—A literature review, Environ. Pollut. 131 (2004) 323–336.
[9] H. Ali, E. Khan, M.A. Sajad, Phytoremediation of heavy metals—Concepts and applications, Chemosphere 91 (2013) 869–881.
[10] R.B. Meagher, Phytoremediation of toxic elemental and organic pollutants, Curr. Opin. Plant Biol. 3 (2000) 153–162.
[11] S.D. Cunningham, D.W. Ow, Promises and prospects of phytoremediation, Plant Physiol. 110 (1996) 715–719.
[12] M. Miransari, Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals, Biotechnol. Adv. 29 (2011) 645–653.
[13] G. Thapa, A. Sadhukhan, S.K. Panda, L. Sahoo, Molecular mechanistic model of plant heavy metal tolerance, BioMetals 25 (2012) 489–505.
[14] C. Garbisu, I. Alkorta, Phytoextraction: A cost-effective plant-based technology for the removal of metals from the environment, Bioresour. Technol. 77 (2001) 229–236.
[15] N. Haque, J.R. Peralta-Videa, M. Duarte-Gardea, J.L. Gardea-Torresdey, Differential effect of metals/metalloids on the growth and element uptake of mesquite plants obtained from plants grown at a copper mine tailing and commercial seeds, Bioresour. Technol. 100 (2009) 6177–6182.
[16] M. Sitarska, T. Traczewska, A. Stanicka-Łotocka, V. Filyarovskaya, D. Zamorska-Wojdyła, Accumulation of mercury in the biomas sof selected pleustophytes, Environ. Prot. Eng. 40 (2014) 165–174.
[17] L.M. Stout, E.N. Dodova, J.F. Tyson, K. Nüsslein, Phytoprotective influence of bacteria on growth and cadmium accumulation in the aquatic plant *Lemna minor*, Water Res. 44 (2010) 4970–4979.
[18] L.A. Licht, J.G. Isebrands, Linking phytoremediated pollutant removal to biomass economic opportunities, Biomass Bioenergy 28 (2005) 203–218.
[19] S. Mindlin, G. Kholodii, Z. Gorlenko, S. Minakhina, L. Minakhin, E. Kalyaeva, A. Kopteva, M. Petrova, O. Yurieva, V. Nikiforov, Mercury resistance transposons of gram-negative environmental bacteria and their classification, Res. Microbiol. 152 (2001) 811–822.
[20] L.M. Stout, K. Nüsslein, Biotechnological potential of aquatic plant–microbe interactions, Curr. Opin. Biotechnol. 21 (2010) 339–345.
[21] S. Silver, L.T. Phung, A bacterial view of the periodic table: Genes and proteins for toxic inorganic ions, J. Ind. Microbiol. Biotechnol. 32 (2005) 587–605.
[22] D.N. Ostrovskii, E.I. Lysak, G.R. Demina, V.I. Binyukov, Interaction of bacteria with mercuric compounds, Microbiology 69 (2000) 516–523.
[23] A. Nascimento, E. Chartone-Souza, Operon *mer*: Bacterial resistance to mercury and potential for bioremediation of contaminated environments, Gen. Mol. Res. 2 (2003) 92–101.
[24] J.L. Hall, Cellular mechanisms for heavy metal detoxification and tolerance, J. Exp. Botany 53 (2002) 1–11.
[25] D.R. Hoagland, D.J. Arnold, The water culture method of growing plants without soil, Cailf. Agric. Exp. Stn. Cir. 347 (1950) 1–32.
[26] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[27] M. Eggstein, F.H. Kreutz, Vergleichende Untersuchungen zur quantitativen Eiweissbestimmung im Liquor und eiweissarmen Lösungen (Comparative studies of quantitative determination of proteins in cerebrospinal fluid and low-protein solutions), Klin Wochenschiftr 33 (1955) 879–884.