GeoScience Engineering

VERIFICATION OF BIODEGRADATION EFFICIENCY OF TEXTILE WASTEWATER USING THE ECOTOXICITY TEST ON *SINAPIS ALBA*

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ABSTRACT

The impact of biodegradation of textile wastewater using the microorganism *Phoma* sp. was assessed and verified. The resulting ecotoxicity of biodegraded wastewater on seeds of *Sinapis alba* was evaluated in accordance with the Methodological Instruction of the Ministry of the Environment of the Czech Republic for the evaluation of toxicity of waste and aqueous extracts (Decree 94/2016 Coll.). Wastewater from the textile industry produced in connection with wool dyeing was tested, the first sample (A) was taken immediately after the dyeing process, the second sample (B) was taken from the first rinse with 20% dilution. The biodegradation efficiency of textile wastewater was confirmed on the length of the tested plants' seed roots. The root length of the sample 3A increased by 265.79 %, while 3B increased by more than 118 %, compared to the control sample. The obtained results indicate a significant impact of microbial biodegradation, with a direct effect on the length of the root of germinated seeds.

Keywords: Ecotoxicity; Microorganisms; Textile industry; Wastewater.

1 INTRODUCTION

The history of the textile industry in the Czech Republic dates back to the 18th century, when the first large-scale industrial plants for the processing of natural materials and dyes were established [1]. For technological and economic reasons, and with regard to the growing demand, natural textile dyes were gradually replaced by synthetic dyes, which are characterized by better mechanical stability, colour and light stability. However, a major problem of synthetic textile dyes is the presence of heavy metals and synthetic chemicals with possible toxic effects on aquatic ecosystems. It has previously been proven that it is not possible to capture these substances perfectly at wastewater treatment plants [2] with up to 20 % of these dyes not being fixed to the substance during dyeing [2, 3].

Wastewater from the wool textile industry is characterized by the content of organic and inorganic pollution, organic pollution comes from lanolin residues, pollution of inorganic origin is from metal-complex dyes, which are most often used when dyeing wool due to their high affinity for natural fibres [3]. Several studies have already demonstrated the positive effect of biodegradation of textile wastewater by various types of microorganisms [3, 4]. Microorganisms show the ability to adsorb heavy metals into cellular structures and metabolize them, at the same time they can reduce water toxicity by biodegradation mechanisms [5].

For this study, samples from a plant that primarily focuses on wool processing and dyeing were taken after staining with metal-complex dye Blu Medio (mix of Giallo Poliestere, Rosso Zetalene, Blu Marino). In this study, the biodegradation potential of the ascomycete *Phoma* sp. LA-9 was verified. Species of the coelomycetes genus *Phoma* are ubiquitously present in the environment and occupy numerous ecological niches. More than 220 species are currently recognised, but the actual number of taxa within this genus is probably much higher [6].

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2 METHODS

Wastewater samples were taken from the textile industry (Nová Mosilana, Brno, Czech Republic); when the dyeing process was finished (series A) and subsequently after the first rinsing with 20% dilution (series B).

2.1. Biodegradation experiments

Samples were taken in 1.5l containers and were stored without access to light at a temperature of 4 °C in accordance with the methodology of sampling and storage of samples [7]. After transport to the laboratory, the wastewater samples were divided into Erlenmeyer flasks with a volume of 100 ml, into which microorganisms *Phoma* sp., which were isolated from the Ostrava lagoons, from environments with high organic and inorganic pollution, were inoculated. Biodegradation experiments were performed in the form of static cultivation at 25 ° C for 28 days. All experiments were performed in triplicate, including controls: negative control microorganism-free wastewater and positive control microorganism growth in liquid medium M033 Sabouraud dextrose broth (HiMedia Laboratories, Mumbai, India). The experiments were evaluated in three stages: the first measurement was performed on 25. 5.–28. 5. 2020, the second one on 1. 6.–4. 6. 2020 and last one on 8. 6.–11. 6. 2020.

2.2. Ecotoxicological tests

Ecotoxicological tests were used to assess the ability of applied strain *Phoma* sp. LA-9 to reduce the amount of dyes and thus the toxicity of wastewater from the textile industry. The level of toxicity of biodegraded wastewater was verified by ecotoxicological tests on *Sinapis alba* seeds according to the Methodological Instruction of the Ministry of the Environment of the Czech Republic [7]. The subject of the Methodological Instruction is the procedure for determining and evaluating the ecotoxicity of waste in accordance with the requirements of Decree No. 338/1997 Coll. [7], and evaluation of ecotoxicity as hazardous properties of waste in accordance with the requirements of Decree No. 94/2016 Coll. [8]. As the assessment of ecotoxicity of waste in the sense of these decrees and as amended is not standardized in the Czech Republic, the above-mentioned Methodological Instruction of the Ministry of the Environment of the Czech Republic was followed within the recommended uniform procedure to achieve the highest possible comparability of results in the sense of the above decrees. With regard to the uniform methods prescribed in Annex No. 1 to Decree No. 94/2016 Coll. [8], to evaluate the ecotoxicity of the investigated textile wastewaters from the wool dyeing process, *Sinapis alba* seeds with a duration of action of 72 hours were selected as the test organism.

From each biodegraded textile wastewater sample, 5 ml of the solution were taken, which were applied on filter paper in Petri dishes, and 5 seeds of the *Sinapis alba* were subsequently added thereto. Next, the prepared Petri dishes were placed in a thermostat and cultured at a constant temperature of 20 °C without access to light for 72 hours. After 72 hours, the number of germinated seeds and the length of their root were evaluated. Subsequently, two further replicates were performed in accordance with the above-mentioned methodology.

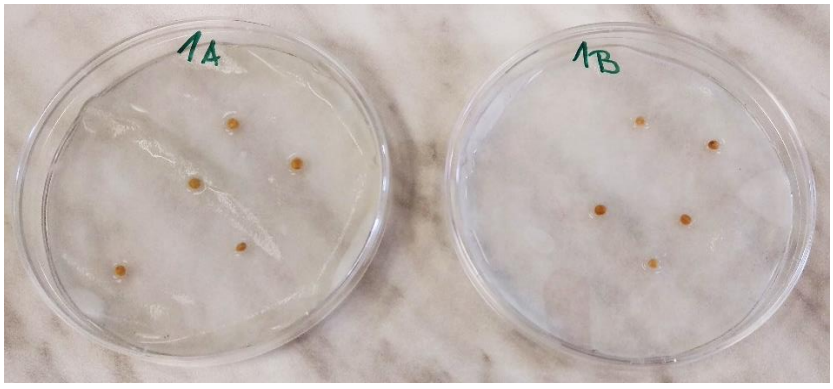

Figure 1. *Establishment of the experiment – Petri dishes with seeds*

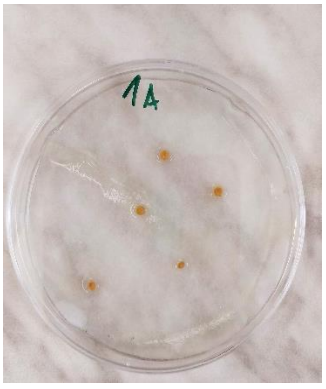

Figure 2. *Close-up*

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3 RESULTS

3.1. Biodegradation of textile industry wastewater by *Phoma* sp.

The obtained data, germination, and root length are described in Tables 1 and 2. The increase in the number of germinated seeds and a significant increase in root length show a significant reduction in toxicity during microbial biodegradation using the applied strain *Phoma* sp. The use of microorganisms for wastewater treatment has already been verified several times in previous experiments [6, 9]. Residues of synthetic textile dyes, which remained in the tested sample of textile wastewater, caused significant morphological changes in the growth of *Phoma* sp., which was reflected in the growth of biofilm up to fibrous structures (Fig. 3). The efficiency of biodegradation by a given microbial strain was primarily observed on the number of germinated seeds of *Sinapis alba* (Fig. 4) and at different root lengths compared to control samples (Fig. 5). On some seeds, it was possible to observe the initial growth of lateral roots and leaves. Significant differences were observed especially in the length of roots in germinated seeds of *Sinapis alba*. There were no significant differences in the number of germinated seeds.

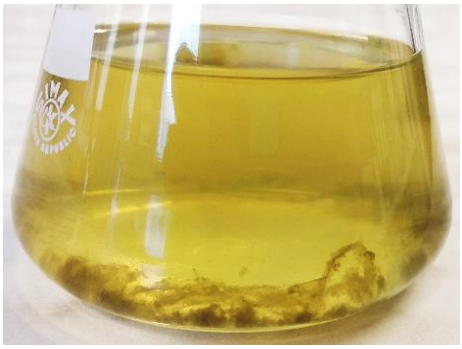

Figure 3. *Biofilm growth Phoma sp.*

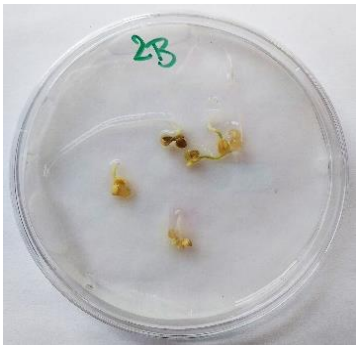

Figure 4. *Seed germination (Sinapis alba)*

Figure 5. *Root length (Sinapis alba)*

Table 1. *Measurement results for series 1A–3A*

	1A		2A		3A		KA	
	root length [cm]	germ. seeds [pcs]	root length [cm]	germ. seeds [pcs]	root length [cm]	germ. seeds [pcs]	root length [cm]	germ. seeds [pcs]
1st measure	1.9; 1.5; 0.7; 1.4; 0	4	0.8; 0.2; 1.1; 1; 0	4	1.1; 0.4; 1.5; 2.4; 0.4	5	1.5; 0.5; 0.4; 1.5; 2.2	5
	± 1.1		± 0.62		± 1.16		±1.22	
2nd measure	0.6; 1.5; 0; 0; 0	2	1.5; 1.0; 1.4; 0; 0	3	0,6; 1,3; 0,7; 0,8; 0,4	5	0.3; 0.6; 0.4; 0.5; 0.8	5
	± 0.42		± 0.78		± 0,76		± 0.45	
3rd measure	1.5; 1.9; 1.4; 1.8; 0	4	2.1; 2.8; 2.4; 1.6; 1.1	5	3,5; 3,4; 2,4; 1,5; 3,1	5	1; 0.5; 0.9; 0.4; 1	5
	± 1.32		± 2.0		± 2.78		± 0.76	

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Table 2. *Measurement results for series 1B–3B*

	1B		2B		3B		KB	
	root length [cm]	germ. seeds [pcs]	root length [cm]	germ. seeds [pcs]	root length [cm]	germ. seeds [pcs]	root length [cm]	germ. seeds [pcs]
1st measure	3.5; 4; 2; 1.5; 0	3	3; 1.8; 3.3; 1.5; 2.2	5	2.1; 0.5; 0.9; 2.6; 2	5	22.3; 1.3; 0.7; 1.4; 1.5	4
	± 1.42		± 2.38		± 1.8		± 1.2	
2nd measure	3.5; 4; 2; 1.5; 0	4	3; 1.8; 3.3; 1.5; 2.2	5	2.1; 0.5; 0.9; 2.6; 2	5	2.3; 1.3; 0.7; 1.4; 1.5	5
	± 2.2		±2.36		±1.62		± 1.44	
3rd measure	3.2; 1.8; 3.3; 0; 0	3	3.5; 3.8; 0; 0; 0	2	3.4; 3.8; 1.9; 1.6; 0	4	0.3; 0.4; 2; 1.8; 0.4	5
	± 1.66		± 1.46		± 2.14		± 0.98	

3.2. Ecotoxicological tests

Ecotoxicological tests are used to evaluate the effects of toxic substances contained in aqueous solutions, soil samples, etc., on the test organisms, both higher and lower plants and living organisms. Tests are standardized on the basis of several regulations. The test performed in this study was performed on the basis of a decree: Decree (94/2016 Coll.) (Replaced the previous one, Decree on the evaluation of hazardous properties of waste) [8]. The results of the experiment from the ecotoxicological point of view are shown in Figure 6 for series A, Figure 7 for series B. In both Figures 6 and 7, a gradual increase in root length can be observed compared to the control sample. This difference was up to 265.79% for the individual sets tested, as can be seen for sample 3 A. For series B, the most significant difference in root length was about 118.37 % compared to the control sample. Based on these data, the real positive effect of biodegradation of textile waste by microorganisms can be evaluated and leads directly to a reduction of the toxicity of aqueous ecosystems.

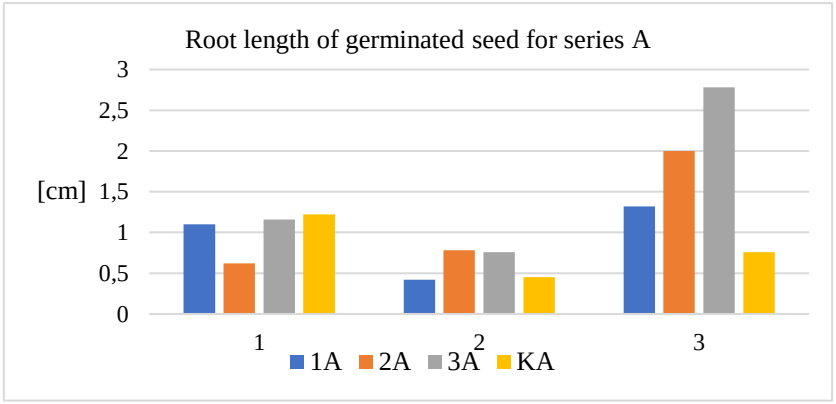

Figure 6. *Root length of germinated seed 1 A–3 A (average value)*

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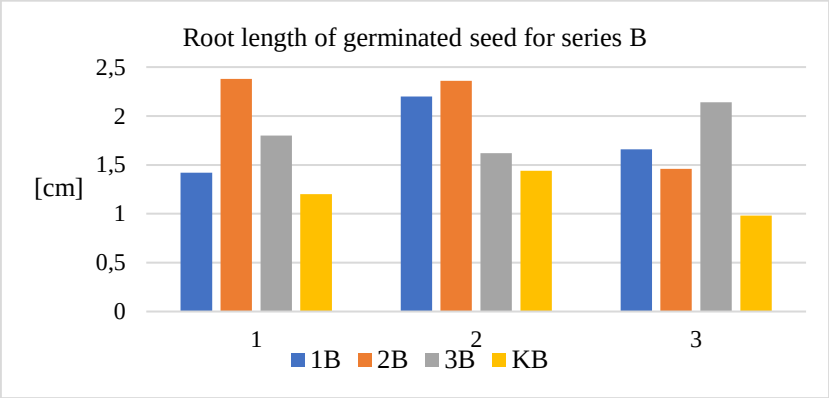

Figure 7. *Root length of germinated seed 1 B–3 B (average value)*

Figures 8 and 9 present the root length, where each measurement (three replicates of each sample, for example 3 x 1A) is summarized and expressed as a percentage, when the control sample, which was not subjected to biodegradation by microbial consortium, corresponds to 100 %.

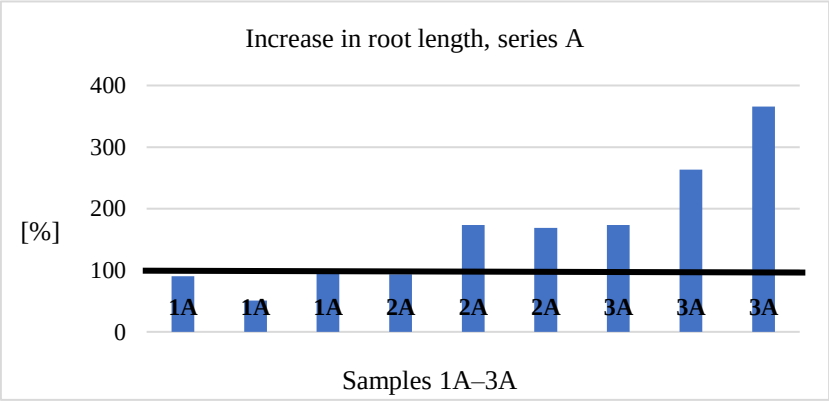

Figure 8. *Increase in root length compared to control sample, series A*

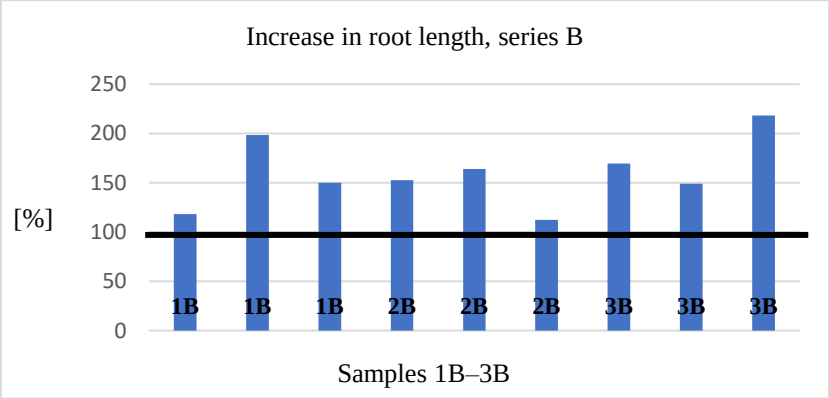

Figure 9. *Increase in root length compared to control sample, series B*

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4 DISCUSION

Industrial wastewater is becoming more complex with the increasing diversity of manufactured products. It often contains bio-recalcitrant pollutants the concentration and type of which vary to a great extent as a function of the consumer demands. This applies in particular to dyehouse wastewater. Dyes and especially the metal complex ones are extensively used in textile industry. During the dyeing of fibres, such as cellulosic ones, substantial amounts of dyes are not fixed on the fabric: dyehouse wastewater usually contains about 10-50 mg/l of dyes in solution. Such concentrations are high enough to induce a remarkable colouring of the receiving water bodies where they are discharged [10, 11].

It has been revealed that the microflora in heavy-metal-contaminated soils could develop abilities to be resistant to and to accumulate the metals in their cells. Species of the coelomycetes genus *Phoma* are ubiquitously present in the environment and occupy numerous ecological niches. More than 220 species are currently recognised, but the actual number of taxa within this genus is probably much higher, as only a fraction of the thousands of species described in literature have been verified in vitro. For as long as the genus exists, identification has posed problems to taxonomists due to the asexual nature of most species, the high morphological variability in vivo, and the vague generic circumscription according to the Saccardoan system. [6].

Many microbes, including bacteria, yeast, moulds, and algae have been reported to be able to remove metals efficiently from solution by biosorption. A cadmium (II)-resistant fungus, strain F2, isolated from blende soil was identified as *Phoma* sp. by morphological study and internal transcribed spacer sequencing. This strain could accumulate 280 mg of Cd (II)/g dry weight mycelium. In liquid medium containing 163.8 mg Cd (II)/L, 96 % of Cd (II) was removed by the actively growing mycelium [9, 12].

5 CONCLUSION

Testing the toxicity on *Sinapis alba*, the reduction of toxicity of textile wastewater from the wool industry due to biodegradation, was evaluated using *Phoma* sp. The wastewater contained residues of dye Blu Medio, which are toxic because they contain heavy metals (Pb, Cd. Cr). Within the experiment, the biodegradation potential of new isolated microorganisms *Phoma* sp. LA-9 were investigated on wastewater from textile industry and also the impact of water toxic substances residues on seed germination and root length of the model plant *Sinapis alba*. This experiment was based on a standardized ecotoxicological test according to the Methodical Instruction of the Ministry of the Environment of the Czech Republic for the evaluation of the toxicity of waste and aqueous. Based on the obtained results, a significant effect of residues on root length of germinated seeds can be evaluated. As the individual series were tested, the difference in root length between the biodegraded samples and the original control samples gradually increased with respect to the duration of the experiment. The difference in the 3 A set compared to the control sample was already about 265.79 %.

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