Testing the Toxicity of *Stachybotrys chartarum* in Indoor Environments—A Case Study

Marlena Piontek and Katarzyna Łuszczyńska *

Institute of Environmental Engineering, University of Zielona Góra, Licealna 9, PL 65-417 Zielona Góra, Poland; M.Piontek@iis.uz.zgora.pl
* Correspondence: K.Luszczynska@iis.uz.zgora.pl; Tel.: +48-683-282-679

Abstract: Infestation of interior walls of buildings with fungal mould is a reason for health concern which is exacerbated in energy-efficient buildings that limit air circulation. Both mycological and mycotoxicological studies are needed to determine the potential health hazards to residents. In this paper, a rare case of the occurrence of *Stachybotrys chartarum* in an apartment building in the Lubuskie Province in Poland has been described. Isolated as the major constituent of a mixed mycobiota, its specific health relevance still needs to be carefully analyzed as its biochemical aptitude for the synthesis of mycotoxins may be expressed at different levels. Therefore, ecotoxicological tests were performed using two bioindicators: *Dugesia tigrina* Girard and *Daphnia magna* Straus. *D. tigrina* was used for the first time to examine the toxicity of *S. chartarum*. The ecotoxicological tests showed that the analyzed strain belonged to the third and fourth toxicity classes according to Liebmann’s classification. The strain of *S. chartarum* was moderately toxic on Potato Dextrose Agar (PDA) as a culture medium (toxicity class III), and slightly toxic on Malt Extract Agar (MEA) (toxicity class IV). Toxicity was additionally tested by instrumental analytical methods (LC-MS/MS). This method allowed for the identification of 13 metabolites (five metabolites reported for *Stachybotrys* and eight for unspecific metabolites). Spirocyclic drimanes were detected in considerable quantities (ng/g); a higher concentration was observed for stachybotryamide (109,000 on PDA and 62,500 on MEA) and lower for stachybotrylactam (27,100 on PDA and 46,300 on MEA). Both may explain the result observed through the bioindicators. Highly toxic compounds such as satratoxins were not found in the sample. This confirms the applicability of the two bioindicators, which also show mutual compatibility, as suitable tools to assess the toxicity of moulds.

Keywords: biotests; moulds; indoor contamination; spirocyclic drimanes; stachybotryamide; stachybotrylactam

1. Introduction

The problem of moulds on partition walls in buildings occurs worldwide in all climate zones. Mycological studies in buildings point to two of the most dangerous mycotoxigenic species of moulds. These are *Stachybotrys chartarum* and *Aspergillus versicolor* [1–7]. Bloom et al. [8] demonstrated that several mycotoxins synthesized by *S. chartarum* (macro cyclic trichothecenes) and *A. versicolor* (sterigmatocystins) may be present in the majority of samples collected from the construction materials of damp apartments and from samples of dust deposits. Interest in *S. chartarum* in buildings increased when a relationship between the growth of this mould in residential buildings and primary idiopathic pulmonary hemosiderosis (IPH) was confirmed [9–12]. *S. chartarum* in buildings can always be found in areas characterized by excessive humidity. *S. chartarum* is a “hydrophilic” fungus with a preference for moist conditions [13]. It is a tertiary colonizer on partition walls in building interiors, occurring at water activity (aw) as high as 0.98. It grows on materials with a high content of cellulose, e.g., plasterboard, wood and wood panelling, natural fibre carpets, insulation pipe coverings, etc. A frequent cause of infestation of the partitions
is excessive humidity caused by flooding, leaks, or water condensation [14–17]. Lack of proper ventilation in energy-efficient buildings may contribute to the problem.

However, studies have shown that not all strains of *S. chartarum* are highly toxic [9]. *S. chartarum* is present in two chemotypes: S and A. In terms of morphology, these chemotypes do not demonstrate any differences; nevertheless, what differentiates them is the type of secondary metabolites produced [9,18]. *S. chartarum* synthesizes macrocyclic trichothecenes that are highly cytotoxic, such as satratoxin H, G, F, and iso-F, or roridin L-2. Additionally, several roridin E epimers have been identified: hydroxyroridin E and verrucarin J and B. However, not all strains from residential housing synthesize these harmful mycotoxins (only 30–40% of chemotype S strains: usually satratoxin H and roridin E and L-2), [9,19–24]. With other isolates, diterpenoid atranones have been found, as well as their dolabellane precursors [25] and simple (non-macroyclic) trichothecenes in small amounts. *S. chartarum* chemotype A does not produce macrocyclic trichothecenes (in 70–80% of strains) [9,20]. Both chemotypes synthesize many metabolites which belong to the family of spirocyclic drimanes (stachybotryamide, stachybotrylactam) in quantities much greater than trichothecenes and atranones [9,17,25,26].

There are about 140 known compounds coming from *Stachybotrys* sp. [26]. *S. chartarum* has been shown to produce large quantities of spirocyclic drimanes, of which up to 40 different species have been found [9,19,27]. Production pathways are through a terpenoid structure (generating two lower rings under the spiro bond) and from polyketides that produce the upper part of the molecule [9,27,28]. The harmful biological properties of spirocyclic drimanes include enzyme inhibition, disruption of the complement system, inhibition of TNF-α liberation, cytotoxicity and neurotoxicity, and stimulation of plasminogen, fibrinolysis, and thrombolyis [9,29–33].

In mycological research in the Lubuskie Province, 82 species of moulds were identified in more than 280 residential and public buildings. *S. chartarum* was found sporadically (only in 4 cases), [4,7,34,35]. In the presented studies tests were conducted on *S. chartarum*, which was isolated from an infested partition wall of a tenement house in Zielona Góra (Figure 1) from a site where residents complained of health problems such as allergic diseases, frequent eye and ear inflammations, headaches, and coughs. In another three cases, such mould occurred in buildings intended for repair after technological failures. Understanding occurrence and health relevance of moulds is critical, especially in the case of thermal modernization of buildings.

![Building partition of the tenement house (Zielona Góra, Poland) infested with *S. chartarum*.](image)

Research conducted by Gravesen and Flannigan in Danish residential buildings proved that *S. chartarum* belonged to a species which can be encountered most frequently on the walls in that country [36–38]. However, despite extensive research [18,21,22,39–41], the harmfulness of this mould has not been clearly established, and the strains found in residential buildings still need to be subjected to analytical–toxicological and ecotoxicological tests. The aim of this research was the application of ecotoxicological tests using *D. tigrina*
and *D. magna* to evaluate the toxicity of *S. chartarum* and assess the mycotoxic risks for the residents of water-damaged buildings or where there are other factors contributing to mould formation in homes where residents reported significant health problems.

*D. tigrina* is a sensitive bioindicator for mycotoxins. It has been used previously on *Aspergillus versicolor* Tiraboschi - sterigmatocystin and is a more sensitive organism than *D. magna* [4,7,42]. Ecotoxicological analyses of biomass of *A. versicolor* showed that less than 50% of strains can produce significant amounts of sterigmatocystin, whereby significant amounts were detected in only three out of 17 samples [42]. Therefore, the presence of toxic mould biomasses and mycotoxins could be detected by applying the *D. tigrina* bioassay. Here, the first report on its application on *S. chartarum* is presented. Introducing a different indicator allows for the strengthening of the evidence regarding the toxicity of the observed moulds.

2. Materials and Methods

2.1. Sample Collection and Cultivation

Samples were collected from the inner surfaces of partition walls with visible mould from 4 places: the Palace in Rakow; a building of the University of Zielona Góra (UZ); a tenement house in Zielona Góra; and the Scout’s house, Zielona Góra, in Poland. *S. chartarum* from the tenement house was selected for further research (Figure 1).

The walls were made of bricks covered with cement–lime plaster. Acrylic paint was used as the finishing material. The wall moisture content was measured at the sample collection site using a Trotec T650 hygrometer. The moisture content of the partition wall was assessed according to the operating instructions supplied with the device. Wall mass moistures (%) were 0–3: dry wall, 3–5: wall with low moisture content, 5–8: wall with medium moisture content, 8–12: wall with high moisture content.

For the mycological analysis, a methodology developed by the CBS (Centraalbureau voor Schimmelcultures) [13] was applied. In this methodology, a material containing moulds is disaggregated into small pieces and inserted to Petri dishes directly at the collection site that were pre-prepared with culture media [43]. Four replicates were taken from each sample: two using Malt Extract Agar (MEA) as the culture medium and two using Potato Dextrose Agar (PDA), Merck. Then the samples were transported to the laboratory of the Institute of Environmental Engineering, University of Zielona Góra, for further mycological analysis. They were covered with white linen and incubated at room temperatures between 18 and 22 °C. Day/night rhythms were maintained. From the mixed starter cultures pure (axenic) cultures were isolated and again transferred onto the PDA and MEA media, respectively. The total cultivation time until single isolated species were observed was 21 days [4,7,42]. For identification and taxonomic classification of isolated strains, Nikon light microscopy was used (Figure 2): [13,44–49].

![Figure 2. Spores and hyphae of *S. chartarum* (Nikon microscopy).](image-url)
2.2. Cultures of *Stachybotrys chartarum*

Based on the isolated strains, mass cultures of *S. chartarum* were established in order to obtain the mould biomass needed for ecotoxicological and physico-chemical tests using two different growth media (Figure 3a,b): MEA and PDA according to the CBS [13].

![Figure 3](image-link)  
*Figure 3. Growth of *S. chartarum* on different growth media after a three-month incubation (a) PDA, (b) MEA.*

A total of 5 mL of the respective media, PDA, MEA (Merck), were poured onto 30 Petri dishes with ø of 9 cm. Mould spores were applied centrally onto the growth media using a preparation needle. Again, samples were incubated after having been covered with white linen and cultivated at room temperature (18–22 °C) while keeping the circadian rhythm. Then, cultivation of the isolated culture lasted 3 months in order to allow growth, sporulation, and regrowth to extend over a significant part of the Petri dish. The strain aged and the mycelium was allowed to air-dry. The method of the cultivation of the mould according to Piontek [4,7,42] reflects the conditions prevalent on partition walls in residential housing. With a scalpel, the dried moulds (still containing remains of the culture medium) were scraped off the Petri dishes. The material extracted was weighed and transferred for storage to glass jars, which were closed with a ground stopper (Figure 4). Further analyses (see below) were performed using methanol extracts of these materials.

![Figure 4](image-link)  
*Figure 4. *Stachybotrys chartarum* mass culture after 3 months of incubation on the PDA medium (a) and dry biomasses of *S. chartarum* on PDA medium in a glass jar closed with a ground stopper (b).*

2.3. Methanol Extracts for Ecotoxicological Tests

The method developed and refined by Piontek [4,42,50] was applied to extract analytes from the samples for further mycotoxin testing. Prepared pure (single species) mould biomass samples (see Section 2.2 above) were collected in the form of 1 g of air-dry extracts.
and held in 100 mL of 80% methanol for 96 h (room temperature). Methanol extracts were prepared in duplicate. The extracts were then filtered through 47 mm fiberglass filter discs (Whatman GF/C), and the supernatant was collected for ecotoxicological analysis in which *D. tigrina* and *D. magna* were used as the indicator organisms. In this procedure, 1 mL of extract was obtained from 10.0 mg of an air-dried sample (moulds + medium).

### 2.4. Ecotoxicological Tests Using *Dugesia Tigrina*

Following the concepts of Piontek [51], planarians were cultivated and used in toxicological tests (Figure 5). Starting from the two methanol extracts, solutions were diluted to different concentrations, and 40 mL of a test solution was stored in beakers with a capacity of 50 mL. Each concentration level was prepared in three repetitions; furthermore, control tests were added. Ten cut organisms were inserted into each beaker so as to have thirty organisms per concentration level. The mortality of the planarians was determined after ten days (240 h) in order to establish the lethal concentration for half of the organisms (240 h LC 50). A graphical method (probit analysis) was applied in this interpretation step. The χ²-test was used to check whether the empirical results matched the normal distribution. Agreement was considered sufficient when the χ²-test resulted in probabilities higher than 0.7 [56]. The toxicity, according to the ecotoxicological tests, was established following Liebmann’s classification [57] (Table 1). According to the standard [58], the control tests ascertain whether any foreign factors, apart from the toxicity of the tested substances, interfere with the test or not, as evidenced by the mortality of the control organisms not exceeding 10%. This also applies when checking the condition of the bio-indicators used in the research.

![Figure 5](image_url)  
**Figure 5.** Breeding of *Dugesia tigrina* used for ecotoxicological tests.

**Table 1.** Toxicity classes of poison substances [57].

| Result of Toxicity Test–LC 50 Value (mg L⁻¹) | Toxicity Classes | Classes |
|-------------------------------------------|------------------|---------|
| <1                                        | highly toxic     | I       |
| 1–10                                      | potently toxic   | II      |
| 10–100                                    | moderately toxic | III     |
| 100–1000                                  | slightly toxic   | IV      |
| >1000                                     | barely toxic     | V       |

### 2.5. Ecotoxicological Tests Using *Daphnia Magna*

The daphnia used for ecotoxicological testing were bred in the laboratory of the Institute of Environmental Engineering at the Zielona Góra University. In order to conduct...
the toxicological tests, 3-day-old organisms of equal size and condition were collected. Two methanol extracts obtained from the dry biomass of *S. chartarum* cultivated on two different media were used. A total of 10 concentrations were prepared. Tubes with a volume of 50 mL were filled with the test solution (about 45 mL) at varying concentrations, and then the daphnia (10 organisms in each case) were added using a dropper. The test was performed in three repetitions including the control test. The test results are considered reliable if the percentage of daphnia mortality in the contaminated sample is 10% or less [58]. The prepared samples were left for 48 h, and then the mortality of the test organisms was checked. This was performed for the purpose of the calculation of the 48h LC 50. In order to calculate the values of the LC 50 concentrations, the method of graphic interpretation (probit analysis) was applied just as in the case of *Dugesia tigrina*.

### 2.6. Determination of Secondary Metabolites of *S. chartarum* Using the LC-MS/MS Method

The *S. chartarum* biomass samples (PDA and MEA basis) were analysed using the LC-MS/MS method [59, 60]. The samples were diluted with acetonitrile/water/acetic acid solvent (79:20:1, v/v/v), resulting in a sample-to-solvent ratio of 1:8, then centrifuged and transferred to autosampler vials in aliquots of 100 µL. Liquid chromatography was used for separation on a Gemini® C18-column, 150 × 4.6 mm i.d., 5 µm particle size, protected by a C18 guard cartridge, 4 × 3 mm i.d. (all from Phenomenex, Torrance, CA, USA). A binary gradient mode with 5 mM ammonium acetate in a methanol/water/acetic acid mixture was used for elution [60]. Mass spectrometric detection took advantage of the scheduled multiple reaction monitoring (sMRM) mode. Two separate runs were made for each sample, one in positive and one in negative polarity, by scanning two fragmentation reactions per analyte. Metabolites were identified unambiguously by comparing retention times and sMRM ion ratio of standards according to the criteria established for mycotoxins [61]. The tests were carried out in the Center for Analytical Chemistry, Department for Agrobiotechnology (IFA-Tulln), University of Natural Resources and Life Sciences, Vienna (BOKU).

### 2.7. Statistical Analysis

Statistical analysis was performed using Excel 2010. In order to show the significance of differences between the results of the metabolites of *S. chartarum* grown on various media, the PDA and MEA analysis of variance at a significance level of α = 0.01 was calculated.

### 3. Results

#### 3.1. Results of Mycological Analysis

Mycological analysis allowed for the determination of the species which accompanied *S. chartarum* on the infested building partitions (Table 2). Studies showed that *S. chartarum* always occurs in the presence of *Penicillium chrysogenum* and a large amount of bacteria due to high humidity. In the tenement house these were *Penicillium chrysogenum, Mucor hiemalis*, and bacteria. Results regarding the moisture content level of the partition wall and air humidity on the day during which samples were collected were as follows: partition wall—11% water content, hence this is a wall with a high moisture content. The air humidity was only 53%. The conditions prevailing at the sample collection site confirmed that the described mould grew well in damp locations (three other cases).

#### 3.2. Toxicological Tests

Ecotoxicological tests using two bioindicators were conducted in the laboratory of the Institute of Environmental Engineering at the Zielona Góra University (test methodology). The results obtained for the LC 50 are listed in Table 3.

Toxicological tests have confirmed a moderate to low total toxicity of samples. The LC 50 values from the respective bioindicators show good agreement, confirming that *D. tigrina* is a sensitive bioindicator for *Stachybotrys* metabolites (the calculations of LC 50 are included in Supplementary Materials).
Table 2. Mycological and moisture analysis of samples.

| No. | Sampling Place              | Coexisting Moulds and Organisms                                      | Wall Finishing Material | Wall Humidity [%] | Air Humidity [%] |
|-----|-----------------------------|---------------------------------------------------------------------|-------------------------|------------------|-----------------|
| 1   | Palace in Rakow, Poland     | *Stachybotrys chartarum*, *Ulocladium botrytis*, *Penicillium chrysogenum*, Bacteria | glue paint              | 8                | 72              |
| 2   | Building of UZ, Zielona Góra, Poland | *Stachybotrys chartarum*, *Penicillium chrysogenum*, Actinobacteria Bacteria | acrylic paint           | 10               | 65              |
| 3   | Tenement house, Poland      | *Stachybotrys chartarum*, *Penicillium chrysogenum*, Mucor hiemalis Bacteria | acrylic paint           | 11               | 53              |
| 4   | Scout’s house, Zielona Góra, Poland | *Stachybotrys chartarum*, *Penicillium chrysogenum*, Actinobacteria Bacteria | wallpaper               | 10               | 58              |

* S. chartarum tested.

Table 3. Results of the ecotoxicological tests using *D. tigrina* and *D. magna* for two methanol extracts prepared from biomasses of *S. chartarum* on different growth media.

| Type of Medium/Bioindicator | LC 50 [mg L\(^{-1}\)] | Toxicity Class According to Liebmann |
|-----------------------------|------------------------|--------------------------------------|
| PDA                         |                        |                                      |
| *D. tigrina*                | 67.6                   | class III (moderately toxic)         |
| *D. magna*                  | 75.9                   | class III (moderately toxic)         |
| MEA                         |                        |                                      |
| *D. tigrina*                | 169.8                  | class IV (slightly toxic)            |
| *D. magna*                  | 190.5                  | class IV (slightly toxic)            |

3.3. Chromatographic Analysis

In order to carry out the chromatographic analysis, two air-dried biomass surfaces of *S. chartarum* on PDA and MEA media were analysed as the background for the obtained results (Table 4). The MS/MS chromatograms of the identified analytes were included in the Supplementary Materials. According to [62], the expanded measurement uncertainty close to a 95% confidence interval for the method is 50%. From the variability of the results for all species, we derived a generally applicable relative standard deviation of the measurements of 20%.

*S. chartarum* produced secondary metabolites on PDA, in the following quantities: stachybotryamide—109,000 ng/g, stachybotrylactam—27,100 ng/g, antibiotic F 1839 A—6470 ng/g, aurantin—67.1 ng/g, orsellinic acid—21,500 ng/g. On the other hand, the quantity of stachybotryamide on MEA was smaller and amounted to 62,500 ng/g. The quantity of stachybotrylactam and antibiotic F 1839 A increased and amounted to 46,300 ng/g and 10,200 ng/g, respectively. *S. chartarum* on MEA: aurantine and orsellinic acid were below the detection limit. Regarding the occurrence of unspecific metabolites from *S. chartarum* on the PDA medium samples, eight metabolites were found, including five at concentrations below the limit of detection (LOD): brevianamid F, cyclo(L-Pro-L-Tyr), cyclo(L-Pro-L-Val), N-benzoyl-phenylalanine, and rugulusovin. In the samples on the MEA medium N-benzoyl-phenylalanine did not occur, instead tenuazonic acid was detected. Asperglaucide and tryptophol were not detected in either medium. The analysis of variance showed highly significant differences between the amounts of metabolites of *S. chartarum* grown on media PDA and MEA (\(F_{\text{calc}} 8.46, F_{\text{crit}} 4.16, p > 0.01\)).
Table 4. Results of chromatographic analyses performed by means of liquid chromatography combined with tandem mass spectrometry (LC-MS/MS).

| Metabolites of Stachybotrys chartarum | S. chartarum on PDA [ng/g] | S. chartarum on MEA [ng/g] |
|--------------------------------------|---------------------------|---------------------------|
| Stachybotryamide                     | 109,000                   | 62,500                    |
| Stachybotrylactam                    | 27,100                    | 46,300                    |
| Antibiotic F 1839A                   | 6470                      | 10,200                    |
| Aurantane                            | 67.1                      | <LOD                      |
| Orsellinic acid                      | 21,500                    | <LOD                      |

Metabolites Reported for Stachybotrys in Antibase

| Metabolites                         | S. chartarum on PDA [ng/g] | S. chartarum on MEA [ng/g] |
|-------------------------------------|----------------------------|---------------------------|
| Stachybotryamide                    | 109,000                    | 62,500                    |
| Stachybotrylactam                   | 27,100                     | 46,300                    |
| Antibiotic F 1839A                  | 6470                       | 10,200                    |
| Aurantane                           | 67.1                       | <LOD                      |
| Orsellinic acid                     | 21,500                     | <LOD                      |

Unspecific metabolites

| Metabolites                         | S. chartarum on PDA [ng/g] | S. chartarum on MEA [ng/g] |
|-------------------------------------|----------------------------|---------------------------|
| Asperglaucide                       | <LOD                       | <LOD                      |
| Brevianamid F                       | 62.7                       | 1697                      |
| Cyclo(L-Pro-L-Tyr)                  | 136                        | 252,200                   |
| Cyclo(L-Pro-L-Val)                  | 583                        | 66,420                    |
| N-benzoyl-Phenylalanine             | 4.07                       | <LOD                      |
| Rugulusovin                         | 21.8                       | 375                       |
| Tenuazonic acid                     | <LOD                       | 48.7                      |
| Tryptophol                          | <LOD                       | <LOD                      |

LOD—limit of detection.

4. Discussion

*S. chartarum* is a species which can be very dangerous to the health of the dwellers of infested premises; therefore, toxicity analysis of this species is necessary. In this case, the occupants of the buildings with infected partitions complained of various health problems associated with massive indoor moisture and mould problems. Two bioindicators were used to carry out the toxicological tests: *D. tigrina* and *D. magna*, and Liebmann’s classification [57] was used to allocate the toxicity class. The analysed mould strain was shown to be moderately toxic (toxicity class III) for the *S. chartarum* strain cultivated on the PDA medium and slightly toxic (toxicity class IV) for the *S. chartarum* strain cultivated on MEA. The results of the bioassays confirmed that the used organisms were sensitive to the presence of mycotoxins.

Studies by other authors [63] have shown the effect of the culture medium on the production of *Stachybotrys chartarum* mycotoxins. The highest concentrations of macrocyclic trichotheccenes were determined on the PDA medium; the MEA medium was the intermediate medium. The lowest amounts of mycotoxins were detected on moulds grown on glucose–yeast–peptone–agar and Sabouraud–dextrose–agar media. The research carried out with the use of *D. tigrina* and *D. magna* confirmed that growing *Stachybotrys chartarum* on PDA was more toxic than on the MEA medium.

Testing of the dry biomass of *S. chartarum* conducted by means of the liquid chromatography technique combined with tandem mass spectrometry (LC-MS/MS) demonstrated the presence of 13 of them in different quantities, depending on the medium on which the mould grew (PDA and MEA). Satratoxins are included in the method but were not detected. The quantity of stachybotryamide synthesized by *S. chartarum* on PDA was higher than on MEA, which indicates that this compound may be responsible for the medium toxicity of the sample (toxicity class III according to Liebmann). The lower quantity of stachybotryamide (on MEA, just half the concentration) caused the extract to be less toxic according to the bio-indicators (toxicity class IV according to Liebmann). Additionally, according to the chromatographic test, *S. chartarum* on MEA did not produce...
any orsellinic acid or aurantine. The tests carried out by Gaylarde et al. [64] on moulds of painted surfaces showed that S. chartarum synthesized on the substrate to an amount of MEA stachybotryamide (3167 ng/sample), while stachybotrylactam came to an amount of 914 ng/sample. Neither orsellinic acid nor aurantine were found.

Nielsen [9] distinguishes the important drimanes detected as coming from S. chartarum, which are stachybotryamide, stachybotrylactams, and di-aldehydes. These metabolites have been detected at significantly higher quantities in plasterboard samples of this species compared to those found in other moulds [3,18,22,65–67], using methods such as LC-UV, LC-MS, and bioassays. In the samples of S. chartarum on MEA, tenuazonic acid was also detected. Tenuazonic acid is known for its antitumor and antiviral activities. It inhibits protein synthesis in vivo and in vitro and protects in vitro cells against 1-β-D-arabinofuranosylcytosine. This protection has been ascribed to its protein synthesis inhibition properties [68]. Furthermore, tenuazonic acid has been linked to the hematologic disease onyalai [13,69].

Tests using the HPLC method conducted on cultures of S. chartarum isolated from apartment walls in Cleveland demonstrated that trichothecenes can be present in very different quantities. Additionally, the MTT (tetrazolim reduction assay) cytotoxicity tests were conducted on the cell lines of cat lung cells. Spirodrimanes in greater concentrations than trichothecenes were detected in all the samples. Additionally, in this case no relationship was found between their concentration and the cytotoxicity of the samples [21].

The results of the tests for mycotoxins in building materials conducted by Gutarowska [70] have confirmed the high quantity of the spirotirmenes produced on MEA by S. chartarum (stachybotrylactam at a quantity of 156,800 ng/g); on the other hand, the quantity of stachybotrylactam on building materials was one hundred times lower (plaster mortar at a quantity of 1312 ng/g, plasterboard—2584 ng/g). The cytotoxicity tests for S. chartarum conducted by the author using XTT (cell proliferation assay) demonstrated that S. chartarum was not toxic for mouse fibroblasts; neither was the genotoxicity of S. chartarum proven (MLA test) after 21 days of mould growth both on building materials and on MEA.

Pieckova et al. [71] analysed indoor-originated S. chartarum from an office. Tests conducted with male rats have shown that this mould can generate metabolites in extracellular products that can be associated with lung cytotoxicity. Some authors argue that several analytical techniques should be used to investigate building-related health hazards [3].

5. Conclusions

This study confirmed the scientific and practical usefulness of biotesting with D. tigrina for analysing the risks from moulds in the human residential environment. All biomass extracts were toxic to D. tigrina.

Toxicological studies of moulds from partition walls using test organisms from our own culture (a laboratory of the Institute of Environmental Engineering, University of Zielona Góra) and using our own published methods [6,65] were carried out to determine the toxicity of moulds from S. chartarum. The conducted studies are a continuation of studies on the toxicity of moulds in partition walls using Dugesia tigrina as a bioindicator. Studies on Aspergillus versicolor strains have shown that there are strains whose toxicity ranges from low to high [7,22,23,65]. The ecotoxicological tests using bioindicators such as planarians (D. tigrina) or daphnia (D. magna) demonstrate the sample toxicity through the total value. These tests allow for the assessment of the actual risk from moulds in buildings. The simplicity of the cultivation of the organisms and the method of performance of the test including the low financial outlay make them readily applicable for the evaluation of the mycotoxic hazard in residential housing. Every single case of the occurrence of S. chartarum is dangerous to the health of the residents. Instrumental analysis provides results that allow for the explanation of the toxicities observed using these bioindicators. Mycological and ecotoxicological testing in residential housing must still be continued.

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