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Remediating Carcinogenic Contamination Produced by Textile Waste Using Wood-decay Fungus

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***Abstract*—With 92 million tonnes of clothing discarded each year, the textile industry is responsible for 20% of global water pollution. Additionally, the manufacturing and disposal process, mostly occurring in developing nations, involves numerous toxins that may cause lung cancer as workers inhale them. However, wood-decay fungus, which secretes an enzyme capable of decomposing common soil pollutants, may offer a potential solution. Therefore, this research was conducted to study the feasibility of using wood-decay fungus as a bioremediator solution for fiber-related pollutants. In our experiment, contaminated water created by soaking worn-out T-shirts in distilled water revealed low dissolved oxygen (DO) and high dissolved carbon dioxide (COD) levels. In addition, when exposed to the contaminated water, lung cancer cell line A549 experienced increased cell growth and a long band of fragmented DNA. However, *Lentinula edodes* and *Trametes versicolor* (wood-decay fungi) placed in the contaminated water remediated the existing pollution. *L. edodes* was excluded from further experiments, as it suppressed the growth of other plants/biomass, revealing that it may be dangerous to be used as a solution in marine environments. Furthermore, A549 cells treated with *T. versicolor*-treated contaminated water had decreased lung cancer cell growth and less DNA damage. This data was collected by placing a net containing dry fungi next to running textile wastewater or by adding *T. versicolor* in dyeing and bleaching processes. In conclusion, *T. versicolor* remediates clothing waste pollutants in water, inhibits the proliferation of lung cancer cells, and prevents DNA damage. Understanding the effect of wood-decay fungi on clothing waste pollutants and may be significant in discovering new approaches to minimizing environmental impacts and protecting the health of garment workers in the textile industry.**

I. Introduction

With the rapid rise of fast fashion, consumers now purchase 60% more clothing than they did 15 years ago [1]. Each year, 92 million tonnes of clothing are wasted, and this waste is responsible for 20% of global water pollution [3,4]. The manufacturing of fabrics uses heavy metals such as cadmium, lead, arsenic, and benzene that contaminate local water supplies that act as resources for irrigation and drinking [5]. When these pollutants enter the human body, through drinking or inhalation in garment hubs, they can cause respiratory, skin, and gastrointestinal health complications, as well as lung cancer [6]. Continuation of current consumer habits will lead to greater water pollution as well as pose risks of cancer for millions of textile factory workers. For example, an estimated 11.4 million women and girls in developing nations, like Bangladesh and Kenya, are victims of forced labor and work up to 15 hours a day, making them highly susceptible to exposure to these toxins [2].

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Wood-decay fungus offers a potential solution, as it secretes laccase, which decomposes environmental hormones that are similar in structure to lignin, cellulose, and hemicellulose [7]. Often called mycoremediation, wood-decay fungus, specifically white-rot fungus, is being used on a pilot scale to remediate soil contamination, implemented in both *ex situ* (biopiling, composting, etc.) and *in situ* (aerobic bioremediation) technologies [8].

This paper investigates the possibility of using the wood-decay fungi *Trametes versicolor* and *Lentinula edodes* as a bioremediating method for fiber-related pollutants, therefore preventing the proliferation of lung cancer cells and DNA damage. This will be assessed through water quality measurements alongside cell proliferation and DNA fragmentation tests.

II. Materials and Methods

1. Water Quality Measurement of Contaminated Water and Synthetic Dye

Contaminated water conditions were imitated by submerging worn-out cotton t-shirts in Erlenmeyer flasks with distilled water (DW) (Fig. 1A). To represent clothing dye used in garment hubs, synthetic dye was created using powdered Rit Dye with a 3% NaCl salt solution (Fig. 1C & 1D) [1]. White cloth was dyed yellow and placed in an Erlenmeyer flask with DW to see possible differences in water quality with regard to dye migration. All flasks were placed on a magnetic stirrer for two weeks (Fig. 1B & 1E).

Figure 1. Contaminated water and synthetic dye solution preparation. (*A. Worn-out clothing; B. Clothing on magnetic stirrers; C. Powdered synthetic dye; D. Synthetic dye with 3% salt solution; E. Yellow-dyed cloth on magnetic stirrer*)

The water quality of each contaminated water was measured using a Water Monitoring Test Kit Set (ECOSAVER, Korea). Synthetic dyes were diluted by 0.1x until clear before their water quality was assessed using the most conventionally measured parameters: dissolved oxygen (DO), NO_2^-, NO_3, PO_4^{3-}, pH, and chemical oxygen demand (COD).

2. Remediation Effect of Wood-Decay Fungus on Waste Clothing Contaminated Water

The remediation effect was observed in two conditions: prevention (Pv) and purification (Pu). The former evaluated the effects of wood-decay fungus added at the same time as waste clothing while the latter measured the effects of wood-decay fungus on already contaminated water.

For Pv conditions, powdered and whole *Lentinula edodes (L. edodes)* and *Trametes versicolor (T. versicolor)* were separately placed in 500-mL glass bottles with white cloth and DW. One bottle only had white cloth and DW as a control, and each container was left sitting for 2 weeks (Fig. 2B). A sample from each bottle was collected, centrifuged, and measured in DO and COD using a Water Monitoring Test Kit. For Pu conditions, white cloth-contaminated water from method 1 was added to separate 50 mL conical tubes with blended *L. edodes* (BLe), whole *L. edodes* (WLe), blended *T. versicolor* (BTv), whole *T. versicolor* (WTv), as well as one tube with only contaminated water as a control (Fig. 2A). After 2 weeks, COD levels were measured.

Figure 2. *T. versicolor* and *L. edodes* placed with waste clothing. (*A. Water contaminated with waste clothing for 2 weeks; B. Waste clothing submerged in DW and wood-decay fungi for 2 weeks; BLe=blended* L. edodes*; BTv=blended* T. versicolor*; WTv=whole* T. versicolor*; WLe=whole* L. edodes*; WC=waste clothing contaminated water*)

3. Wood-decay Fungus' Effect on Plant Growth (Biotoxicity)

Hydroponics was performed by placing cabbage seeds on a gauze soaked with liquid samples from Fig. 2A and 2B to see if the selected wood-decay fungi would negatively impact aquatic plant growth once used underwater. Cabbage seeds treated with samples from fungi-treated contaminated water had growth levels assessed after nine days of growth.

Growth levels of hydroponics results were evaluated based on the number of germinated seedlings and the level of plant growth.

4. Lung Cancer Cell Proliferation Test

The lung cancer cell line, A549 (KCLB 10185, BSL 1) was cultured in RPMI 1640 with 1% penicillin/streptomycin (P/S) and 10% FBS. The cells were seeded in two 6-well plates and treated with 1 µL or 10 µL of filtered waste clothing-contaminated water.

After 48 hours of incubation at 37 C° and 5% CO_2, the cell mixture was seeded in a 96-well plate. EZ-Cytox (DoGenBio, Korea) was added in each well and measured in absorbance using a microplate reader (Diatek, India) at 450 nm.

5. DNA Fragmentation Test

From the waste clothing contaminated water-treated cells in the previous method, DNA was extracted using a Genomic DNA Extraction Kit (AccuPrep, Korea). Gel electrophoresis was conducted and the DNA band was observed under a UV transilluminator (Accuris Instruments, USA).

6. Statistical Analysis

All cell proliferation tests were repeated three or more times and the values obtained were presented as mean ± SE and analyzed using a student *t*-test. Data with p values of 0.01 to 0.05 were marked as '*' and ' 0.05 to 0.001 as '**.', indicating significance.

III. Results

1. Water Pollution of Waste Clothing and Synthetic Dye Contaminants

DO levels for all clothing were lower than the control while COD levels increased by at least 6 ppm, indicating high pollution (Table 1). PO_4^{3-} levels also increased for white- and yellow-dyed cloth, which may contribute to eutrophication. Therefore, this test revealed that all clothing, especially white and freshly dyed cloth, contaminates water.

	Control	White cloth	Blue cloth	Black cloth	Dyed cloth
DO (ppm)	8	5	2	5	5
NO_2^- (ppm)	0	0	0	0	0
NO_3 (ppm)	0.1	0.1	0.1	0.1	0
PO_4^{3-} (ppm)	0.045	0.09	0	0.015	0.33
pH	6	7	6	6	6
COD (ppm)	2	8	20	15	8

Table 1. Water Quality Test Result of Water Contaminated with Different Colored Waste Clothing *(Contaminated water=worn out T-shirts was stored with DW for 14 days; Dyed cloth=white T-shirt stained with yellow synthetic dye)*

All dyes had DO levels lower than the control and PO_4^- unexpectedly increased at a concentration of $1x10^{-7}$ for certain colors (Fig. 3). Therefore, it was determined that there is a negative correlation between DO and synthetic dye but no clear correlation between dye concentration and PO_4^{3-} due to original colors of synthetic dyes interfering with results.

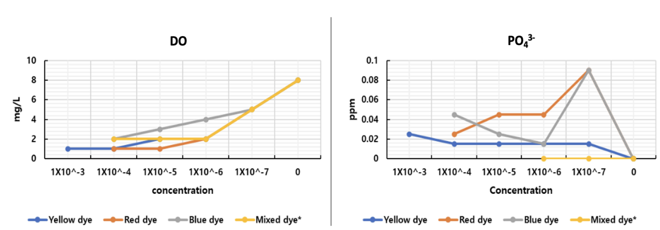

Figure 3. DO and PO_4^{3-} levels of synthetic dye at different concentrations.

2. Pollution Remediation Effect of Wood-Decay Fungus

For Pv conditions of wood-decay fungus, DO levels of *T. versicolor* and *L. edodes*-treated contaminated water showed a change in color but not a change to any indicator colors displayed on the monitoring kit. COD levels in both Pv and Pu conditions showed no change, revealing that wood-decay fungus was able to suppress further contamination from waste clothing.

3. Biotoxicity Results of Wood-decay Fungus Treated Contaminated Water

Contaminated water-exposed seeds had a growth level of 4 and contaminated water treated with whole and blended *T. versicolor* also had strong growth levels of 4 and 5. However, for *L. edodes*, the levels were at 1, meaning that it suppressed the growth of cabbage seeds. Therefore, *L. edodes* was excluded from the remaining experiments, as it would be harmful to plant life in aquatic biomes.

4. Effect of Waste Clothing Contaminant and Wood-decay on Lung Cancer Cell Growth

Cells that were exposed to contaminated water (Pv_control and Pu_control) had a positive trend in cell proliferation compared to DW-treated cells (Fig. 4). However, there was not much change for DC. As such, worn-out textile waste seems able to accelerate the growth of lung cancer cells.

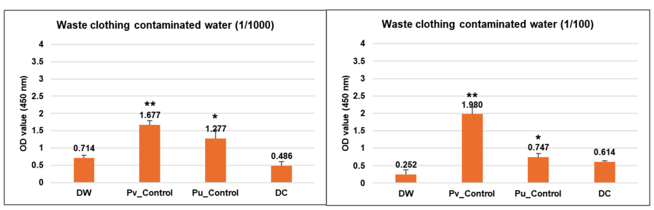

Figure 4. OD value of cells exposed to waste clothing contaminated water. (*Pv=prevention; Pu=purification; DC=dyed clothing, *=0.01<p<0.05; **=0.05<p<0.001*)

Second, for Pv conditions, though results for cells treated with blended *T. versicolor* were unclear, cells treated with whole *T. versicolor* showed a strong decrease in OD value by 91% and 93% in both 1/1000 and 1/100 concentrations. This result means that whole *T. versicolor* can effectively suppress the growth of lung cancer cells in preventive conditions.

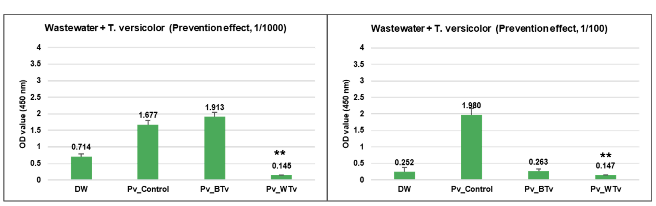

Figure 5. OD value of cells exposed to wood-decay fungus treated waste clothing contaminating water for Pv. (*Pv=prevention; BTv=blended T. versicolor; WTv=whole T. versicolor; **=0.05<p<0.001*)

Third, for Pu conditions, there was little to no change in OD value when cells were treated with *T. versicolor* at 1/100 concentration. Though cells treated at 1/1000 concentration of blended *T. versicolor* showed a 52.7% decrease and 25.2% decrease, for whole ones, a clear correlation was not determined due to the results' high p values.

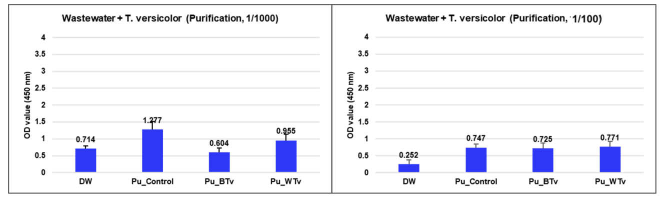

Figure 6. OD values of cells exposed to wood-decay fungus treated waste clothing contaminated water for Pu. (*Pv=prevention; BTv=blended T. versicolor; WTv=whole T. versicolor*)

5. DNA Fragmentation of Lung Cancer Cells Exposed to Waste Clothing Contaminated Water

Sample 1 (Pv_Control) showed a long band of DNA smearing with fragmentation lower than 100 bp, meaning that the waste clothing contaminants severely damaged the DNA. Comparing this to sample 2 (Pv_BTv), sample 2 was only fragmented until near 500 bp. This finding shows that blended *T. versicolor* was able to prevent DNA damage from waste clothing contaminants. For samples 3 and 6 (Pv_WTv and Pu_WTv), a single DNA band was present, meaning that there was no damage. These results show that waste clothing contaminants damage DNA, but whole *T. versicolor* prevents and stops this damage from occurring.

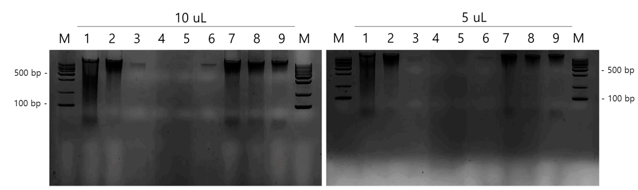

Figure 7. DNA fragmentation results of A549 cells exposed to waste cloth contaminated water with and without wood-decay fungus treatment. (*M=DNA size marker, 1-3=Pv of wood-decay fungus; 4-6=Pu of wood-decay fungus; 1 & 3=waste cloth contaminated water; 2 & 4=blended T. versicolor; 3 & 6=whole T. versicolor; 7=dyed cloth contaminated water; 8=distilled water; 9=control, No treatment; Left-hand side loaded with 10 uL of DNA product; Right-hand side loaded with 5 uL of DNA product*)

IV. Discussion and Conclusion

This research revealed *T. versicolor*'s ability to remediate water pollution caused by textile waste, suppress lung cancer cell growth, and prevent DNA damage from contaminants. Waste clothing contaminated water's water quality measurements were negative, with low DO and high COD levels, and the water proliferated the growth of lung cancer cells and damaged the cells' DNA. *T. versicolor* and *L. edodes* showed pollution remediation effects against the contaminants which can be due to the fungi's ligninolytic enzymes such as laccase [7]. Cell viability and DNA fragmentation tests showed that treating contaminated water with whole *T. versicolor* in preventive conditions suppresses lung cancer cell growth and protects those cells from further DNA damage. The results of this paper suggest that placing nets containing the dry fungi next to running clothing wastewater or incorporating the fungi into the dyeing or bleaching processes may help mitigate the effects of textile pollution. Toxins included in clothing manufacturing processes may also cause other respiratory, skin, and gastrointestinal health concerns. Limitations of this study include the lack of variety in wood-decay fungus used due to insufficient time available for research. Therefore, further research should explore the remediation effect of multiple types of wood-decay fungus available in developing nations on specific categories of waste clothing pollutants.

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