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Evaluation of Metal Content, Metal Release, Cytotoxicity and Antibacterial Efficiency Properties of Antibacterial Socks

Candan Akca[1*], Fatma Doyuk[2]

[1] Department of Fashion Design, Salihli Vocational School, Manisa Celal Bayar University, Manisa, Türkiye.
[2] DEFAM (Applied Science Research Center), Manisa Celal Bayar University, Manisa, Türkiye.
* candan.akca@cbu.edu.tr, candanakca@yahoo.com
* Orcid No: 0000-0001-5305-0296

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Abstract

Textiles may be exposed to metal contamination during their production, functionalization and storage processes. While some of these metals and metallic compounds cause contamination in the final textile product through fiber production, pre-treatment processes such as boiling and bleaching, dyeing and some finishing processes, some of them which provide specific functional properties to the final textile product (anti-microbial property, self-cleaning property, UV protection feature, electromagnetic wave shielding feature, etc.) cause contamination in the final textile product during the functionalization process. Although a wide variety of antimicrobial substances are used in the production of antibacterial textiles, metal/metallic compounds and nano metal particles are also widely used as antibacterial substances. Studies on antibacterial textiles generally focus on the synthesis of antibacterial material, its application to fabric and its effectiveness against pathogenic microorganisms. However, more studies are needed on the effects of the metal content of antibacterial textile products on the user's skin and health throughout their lifetime. Because textile products are in direct and long-term contact with human skin. In this study, ICP-MS metal content analysis, time-dependent metal release amount analysis, antibacterial activity analysis and cytotoxicity test with cell culture were performed on antibacterial socks and the results were evaluated.

Keywords: Antibacterial textiles, antibacterial socks, metal content, metal release, antibacterial efficiency, HaCaT toxicity test

1. Introduction

The textile industry is an industry where very diverse of chemicals are used in the production stages from fiber production to the final textile product, and the textile material/product can be contaminated with metals/metallic compounds at each production stage [1, 2]. Contamination of textile material/textile product with metals can continue during the storage phase after production [1].

When looking at the literature; it is observed that aluminum, iron, manganese, copper and zinc can come from raw cotton [3], antimony can come from fiber production [4], iron and manganese can come from pretreatment processes [5], chromium, vanadium, barium, lead, copper, cobalt, copper, nickel, zinc and trace concentrations of mercury, cadmium and arsenic can come from textile dyeing [6, 3], antimony can come from finishing processes (for flame retardant, etc.) and cause metal contamination of textile products [7].

While providing new functional properties to the textile material/product such as self-cleaning, hydrophobicity, antibacterial properties, UV blocking, electromagnetic shielding, zinc [8, 9], copper [10], silver [11, 12], titanium [13], aluminum [14], nickel [15], iron [16], cobalt [17] contamination may occur.

Increasing awareness of health and hygiene issues, epidemic diseases, the increase in the elderly population and the desire for an active life cause an increase in the interest and demand for antibacterial textiles. And as a result, the antibacterial textile market volume is gradually increasing [18]. In antibacterial textile production; Many types of antimicrobial substances of organic, inorganic and biological origin are used [11, 12, 18]. However, compared to other antimicrobial substances, metal/metal salts/nano metal particles have

an important place in terms of ratio and quantity [19]. Silver, on the other hand, stands out in both commercial products and scientific studies compared to other metals [20].

Many chemicals used in textile production have the potential to be toxic [21, 22]. Likewise, metal contamination from production stages can also cause toxic effects. Studies have reported that disorders such as asthma, rhinitis and dermatitis are observed in textile workers exposed to textile chemicals [21, 23, 24, 25]. Metals found on textiles are released into the environment during their use. These metals can be absorbed by the skin, accumulate in organs and tissues through the circulatory system, and negatively affect human health [26]. In addition, metals released from textile products during washing pose a risk for the environment [18].

In Oeko-Tex Standard 100, the limit values allowed on the fabric for some harmful chemicals have been determined and textile products complying with the limits in this standard can receive eco-labels [21, 27]. Because the textile product should protect both the textile product and the user from pathogenic microorganisms, prevent the formation of bad odor and color, as well as be non-toxic, not cause irritation and allergies on the skin [28].

The aim of this study is to evaluate the metal content, antibacterial efficiency and cytotoxic properties of different socks that are commercially available and claimed to be antibacterial (such as baby socks, sports socks, diabetes socks, etc.). ICP-MS analysis for metal content analysis, quantitative antibacterial efficiency test (according to AATCC 100) for antibacterial efficiency, and cytotoxicity test with human keratinocyte HaCaT cell line to evaluate cytotoxic properties were performed on six antibacterial socks obtained from the local market, and the results were evaluated.

2. Materials and Methods

2.1. Materials and Chemicals

In the trials, one non-antibacterial sock (negative control socks) obtained from the local market and five socks claimed to be antibacterial (silver content) by their manufacturers were used. The features of the socks used in the experiments are given in Table 1.

Sodium chloride, sodium hydroxide, ammonium chloride, acetic acid, lactic acid and urea used to prepare the artificial sweat solution were obtained from Merck. Silver nitrate used in calculating the IC50 value of ionic silver was obtained from Merck.

Table 1. Code, product type, composition, product features and colors of the socks used in the study

Code	Product Type	Composition	Product Features	Color
S1	Negative control socks	80% Cotton, 17% Polyamide, 3% Elastane	Non-antibacterial	Black
S2	Men's socks	85% Cotton, 7% Elastane, 8% Silver nanoparticles	Antibacterial declarated	Black
S3	Baby socks	82% Cotton, 16% Polyamide with silver ions, 2% Elastane	Antibacterial declareted	Dark navy blue
S4	Women's socks	75% Bamboo, 11% Poliamide, 4% Elastane, 10% Polyester with silver ions	Antibacterial declareted	Black
S5	Diabetes socks	70% Cotton, 30% Polyamide with silver ions	Antibacterial declareted	Black
S6	Men-women sport socks	76% Cotton, 21% Polyamide with silver ions, 3% Elastane	Antibacterial declareted	Black

2.2. Methods

2.2.1. Extraction Process

Artificial sweat solution for the extraction of sock samples was arranged according to the ISO 3160/2 standard (ISO 3160/2) [29]. 1.5 grams of each socks sample was taken and extraction was performed in artificial sweat solution at 37 °C for 24 hours. The amount of metal in the extracts obtained after the filtration process was analyzed by ICP-MS.

2.3. Analyzes

2.3.1. ICP-MS Metal Analysis

Antibacterial and non-antibacterial socks were decomposed by weighing 0.2 grams and completing 8 mL of 70% HNO_3 with 25 mL of ultrapure water with the microwave conditions in Table 2.

Table 2. Microwave-assisted acid degradation conditions

Time (min.)	Temperature (° C)
0-2	25-150
2-7	150
7-9	150-170
9-19	170
19-20	170-200
20-40	200

After microwave decomposition, the presence of As, Zn, Mo, Cd, Co, Ba, Cr, Li, Cu, Sb, Hg, Mn, Ni, Pb, Sn, Ti, Ag, Be, V, Fe metals were analyzed by ICP-MS. In addition, the extracts obtained after extraction at 37°C for 24 hours were analyzed by ICP-MS for the presence of the above metals. All metal analyzes were performed in triplicate. Both the total metal amounts analyzed by microwave degradation method and the metal amounts in the extracts were evaluated by comparing them with the Oeko-Tex Standard limit values.

2.3.2. Antibacterial Activity Analysis

The antibacterial efficiency analysis of antibacterial and non-antibacterial socks against *Staphylococcus aureus* and *Escherichia coli* was carried out according to the AATCC 100 Standard. Antibacterial efficiency tests were performed twice. The incubation time was 24 hours. The test result is the percentage of bacteria that decreases at the end of the incubation period.

2.3.3. Cytotoxicity Analysis

Toxicity analysis of antibacterial and non-antibacterial socks were carried out quantitatively with the human keratinocyte HaCaT cell line (monolayer) according to Cytotoxicity extraction method ISO 10993:5-2009 Standard for Tests for In Vitro Cytotoxicity.

Serum-free cell culture medium (Dulbecco's MEM High Glucose, DMEM-HXA, Capricorn Scientific, Germany) was used as solvent. Dulbecco's MEM High Glucose, 10% Fetal Bovine Serum (FBS, S181G, Biowest, France), 1% Sodium Pyruvate (S8636, Sigma, Germany), 0.1% Penicillin-Streptomycin (A221, Biochrom, Germany) was used as culture medium. Material known to have no cytotoxic effect on the cell, Polyethylene (PE) was used as control.

UV sterilization method was performed for test materials (30 minutes each side). Fabric test materials were extracted in serum-free Dulbecco's MEM High Glucose nutrient medium for 24 hours. Extracts of socks were prepared according to standard ISO 10993-12, with 0.1 g of sample per 1 mL^{-1} of cultivation medium.

Cytotoxicity testing was performed with HaCat cells. Material extracts (5% CO_2 at 37°C) applied for 24 hours at four different dilutions (1:1, 1:2, 1:4 and 1:8) on cells seeded at a concentration of 1×10^5 cells/mL on 96-well microplates. The cytotoxic effect was evaluated by reading the absorbance in the spectrophotometer (570 nm) with the MTT test. The results of the 4-hour MTT test performed as a result of incubation of cells with extracts of test materials for 24 hours were evaluated. All experiments were repeated three times and the results were analyzed statistically and Graphpad Prism 8 was used as a statistical program. The results were also analyzed with the One-Way ANOVA test ($p<0.0001$), and Dunnett was chosen as the post-doc method.

Additionally, cytotoxicity analysis of $AgNO_3$ solution was performed at 0-2.5 mg/l concentrations. A concentration of 1.31 mg/l was found to be the IC_{50} value.

3. Results and Discussion

3.1. ICP-MS Metal Analysis Results

After microwave decomposition, the presence of metals mentioned in ICP-MS Metal Analysis section were analyzed by ICP-MS. In addition, the amounts and percentages of metal released into the environment after 24 hours of extraction are given in Table 3.

Table 3. Total metal content, metal release amount of 24 hours extraction and percentage of extracted and total metal content ratio of studied antibacterial socks

Code	As		Cd		Co		Cr		Cu	
	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext.
S1	0.04±0.01	-	0.15±0.00	-	0.76±0.00	-	0.49±0.00	0,01±0,00	0.38±0.02	0.02±0,00
S2	0.03±0,00	0.01±0.00	0.16±0.01	-	0.76±0.02	-	0.44±0.03	-	0.42±0.02	-
S3	0.02±0.00	-	0.16±0.00	-	0.76±0.05	-	0.95±0.08	-	0.60±0.05	-
S4	0.05±0.00	-	0.20±0.01	-	0.81±0.06	-	0.72±0.06	-	2.46±0.18	0.08±0.00
S5	0.04±0.00	0.02±0.00	0.14±0.00	-	0.74±0.06	-	0.61±0.02	0,01±0,00	0.94±0.07	-
S6	0.02±0.00	-	0.15±0.00	-	0.88±0.02	-	0.43±0.00	-	29.69±0.10	-

* **MD:** Total metal content of microwave-assisted decomposed socks samples (mg/kg) * **24 hours ext:** Amount of metal extracted after 24 hours (mg/kg)

* **- :** Not detected

Table 3. Cont.

Code	Hg		Mn		Ni		Pb		Sn	
	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext.
S1	0.13±0.06	-	3.61±0.09	0.08±0.02	2.71±0.04	0.07±0.01	0.55±0.01	-	0.18±0.00	-
S2	0.06±0.02	-	2.54±0.14	0.13±0.00	1.03±0.07	0.03±0.00	0.95±0.03	0.01±0.00	0.24±0.01	-
S3	0.06±0.03	-	3.01±0.30	0.01±0.00	1.07±0.07	0.03±0.00	1.24±0.06	0.01±0.00	0.22±0.02	-
S4	ND	-	1.29±0.13	-	1.21±0.09	0.04±0.00	0.48±0.04	-	0.11±0.00	-
S5	ND	-	2.56±0.27	-	0.70±0.00	0.02±0.00	1.04±0.05	-	0.13±0.00	-
S6	ND	-	0.71±0.01	0.01±0.00	0.99±0.02	0.02±0.00	0.77±0.04	-	0.37±0.01	-

* **MD:** Total metal content of microwave-assisted decomposed socks samples (mg/kg) * **24 hours ext:** Amount of metal extracted after 24 hours (mg/kg)

* **- :** Not detected

Table 3. Cont.

Code	Ti		Zn		Ag		Mo		Sb	
	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext.
S1	317.17±36.13	0.25±0.08	0.57±0.09	-	-	-	0.12±0.04	0.01±0.00	0.82±0.01	-
S2	6.19±1.81	-	2.52±0.27	-	7.88±0.32	0.42±0.02	0.20±0.01	0.05±0.00	0.01±0.00	-
S3	237.66±9.49	0.13±0.05	1.68±0.11	-	1.62±0.70	0.05±0.00	-	-	0.75±0.02	-
S4	246.71±12.90	0.32±0.00	5.18±0.31	-	1.57±0.01	-	0.02±0.00	-	0.03±0.00	-
S5	10.79±0.10	0.08±0.05	4.13±0.21	-	3.04±0.02	0.13±0.01	0.04±0.00	0.01±0.00	0.05±0.00	0.01±0.00
S6	13.50±0.82	0.49±0.14	1.61±0.31	0.05±0.00	2.77±0.37	0.07±0.00	0.31±0.04	0.01±0.00	92.45±0.96	0.17±0.01

* **MD:** Total metal content of microwave-assisted decomposed socks samples (mg/kg) * **24 hours ext:** Amount of metal extracted after 24 hours (mg/kg)

* **- :** Not detected

Table 3. Cont.

Code	Ba		Li		Be		V		Fe	
	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext	MD (mg/kg)	24 h ext.	MD (mg/kg)	24 h ext.
S1	0.97±0.01	-	0.04±0.01	-	-	-	0.08±0.00	-	46.82±1.33	0.17±0.01
S2	1.39±0.09	-	0.08±0.01	0.01±0.00	-	-	0.05±0.00	-	8.18±1.07	-
S3	1.07±0.01	-	0.03±0.01	0.01±0.00	-	-	0.03±0.00	-	22.12±2.37	-
S4	0.68±0.00	-	-	-	-	-	-	-	15.81±1.60	-
S5	1.58±0.00	-	0.18±0.01	0.03±0.00	-	-	0.09±0.01	0.01±0.00	26.09±2.09	0.08±0.05
S6	1.32±0.00	-	0.04±0.00	-	-	-	0.04±0.00	-	12.01±0.18	-

* **MD:** Total metal content of microwave-assisted decomposed socks samples (mg/kg) * **24 hours ext:** Amount of metal extracted after 24 hours (mg/kg)

* **- :** Not detected

Table 4. Oeko-Tex limit values and fastness, part I (Oeko-Tex Standard 100)

Product Class	**Class I Baby**	**Class II in direct contact with skin**
pH value	**4.0-7.5**	**4.0-7.5**
Extractable heavy metals [mg/kg]		
Sb (Antimony)	30.0	30.0
As (Arsenic)	0.2	1.0
Pb (Lead)	0.2	1.0
Cd (Cadmium)	0.1	0.1
Cr (Chromium)	1.0	2.0
Cr (VI)	0.5	0.5
Co (Cobalt)	1.0	4.0
Cu (Copper)	25.0	50.0
Ni (Nickel)	1.0	4.0
Hg (Mercury)	0.02	0.02
Ba (Barium)	1000	1000
Se (Selenium)	100	100
Heavy metals total content [mg/kg]		
As (Arsenic)	100	100
Cd (Cadmium)	40.0	40.0
Hg (Mercury)	0.5	0.5
Pb (Lead)	90	90

Table 4 contains the total heavy metal amounts and limit values of extractable heavy metal amounts and fastnesses, Part I, for some heavy metals allowed in textile materials in the Oeko-Tex Standard. Total heavy metal amounts and extractable heavy metal amounts on the antibacterial socks used in the trial were evaluated according to the Oeko-Tex Standard.

The total amount of As (Arsenic) detected in the study varies between 0.02-0.05 mg/kg and does not exceed the Oeko-Tex Standard permissible As (Arsenic) limit. After 24 hours of extraction, As (Arsenic) concentration was detected between 0.01-0.02 mg/kg and is again within the allowed limits according to the standard. The amount of total Cd (Cadmium) detected in the study varies between 0.14-0.20 mg/kg and is within the limit values according to the standard. After 24 hours of extraction, Cd (Cadmium) was not detected. The amount of total Hg (Mercury) detected varies between 0.06-0.13 mg/kg and is below the limit value of 0.5 mg/kg (for both baby textiles and textiles in direct contact with the skin). Hg (Mercury) was not detected after 24 hours of extraction. The detected total Pb (Lead) amount varies between 0.48-1.24 mg/kg. According to the standard, the allowed total Pb (lead) amount is below 90 mg/kg. The highest amount of Pb (Lead) detected after 24 hours of extraction was 0.01 mg/kg. And it is below the limits allowed in the standard (0.2 mg/kg for Class I baby, 1.0 mg/kg Class II in direct contact with skin).

In the study, the amounts of Sb (Antimony), Cr (Chromium), Co (Cobalt), Cu (Copper), Ni (Nickel), Ba (Barium) detected after 24 hours of extraction are within the limits allowed in the standard. Be (Beryllium) was not detected in any sample in the study.

The amounts of total Mn (Manganese), Sn (Tin), Zn (Zinc), Mo (Molybdenum), Sb (Antimony), V (Vanadium) determined in the study are parallel to the total metal amounts determined by Rujido-Santos et al. in their study in 2022. It has been observed that . The total amount of Li (Lithium) determined in the study varies between 0.03-0.18 mg/kg. The total amount of Li (Lithium) determined by Rujido-Santos et al. in their study in 2022 varies between 0-6.999 mg/kg. The total amount of Fe (Iron) determined in the study varies between 8.18-46.82 mg/kg. The total amount of Fe (Iron) determined by Rujido-Santos et al. in their study in 2022 varies between 7.85-57.1 mg/kg. In the study carried through by Menezes et al. in 2010, the total amount of Fe (Iron) determined varied between 12.1-66.1 mg/kg. The total amount of Ti (Titanium) detected in the study varies between 6.19-317.17 mg/kg. In the study carried through by Rujido-Santos et al. in 2022, the total amount of Ti (Titanium) they detected in antibacterial socks ranged between 426-2946 mg/kg. The total amount of Zn (Zinc) detected varies between 0.57-5.18 mg/kg. In the study carried through by Rujido-Santos et al. in 2022, the total amount of Zn (Zinc) they detected in antibacterial socks ranged between 0.819-5.68 mg/kg.

The total amount of Ag (Silver) detected in the study varies between 1.57-7.88 mg/kg. In the study carried through by Rujido-Santos et al. in 2022, the total amount of Ag (Silver) they detected in antibacterial socks ranged between 5.83-6.40 mg/kg.

3.2. Antibacterial Efficiency Analysis Results

Table 5. % Antibacterial Efficiency of Studied Antibacterial Socks Against *S.Aureus* and *E.Coli*

Code	0. Hour		24. Hour			
	S.Aureus	***E.Coli***	***S.Aureus***		***E.Coli***	
	Bacterial count	**Bacterial count**	**Bacterial count**	**% Bacterial reduction**	**Bacterial count**	**% Bacterial reduction**
S1	365.10^3	420.10^3	310000	15.07	345000	17.86
S2	365.10^3	420.10^3	3200	99.12	1700	99.60
S3	365.10^3	420.10^3	125706	65.56	355000	15.27
S4	365.10^3	420.10^3	217832	59.68	373884	10.98
S5	365.10^3	420.10^3	61795	83.07	113988	72.86
S6	365.10^3	420.10^3	85556	75.56	166572	60.34

As seen in Table 5, commercially available non-antibacterial socks (control socks) with code S1 showed 15.07% antibacterial efficiency against *S.Aureus* and 17.86% against *E.Coli* after a 24-hour contact period. Men's socks coded S2 showed 99.12% antibacterial efficiency against *S.Aureus* and 99.60% against *E.Coli*

after a 24-hour contact period. Antibacterial diabetes socks coded S5 showed 83.07% antibacterial efficiency against *S.Aureus* and 72.86% antibacterial efficiency against *E.Coli* after a 24-hour contact period. Men's and women's sports socks coded S6 showed 75.56% antibacterial efficiency against *S.Aureus* and 60.34% against *E.Coli* after a 24-hour contact period. When the amount of Ag contained in the sock samples and the antibacterial activity values they show are examined, it is seen that the results support each other and are consistent.

3.3. Cytotoxicity Analysis Results

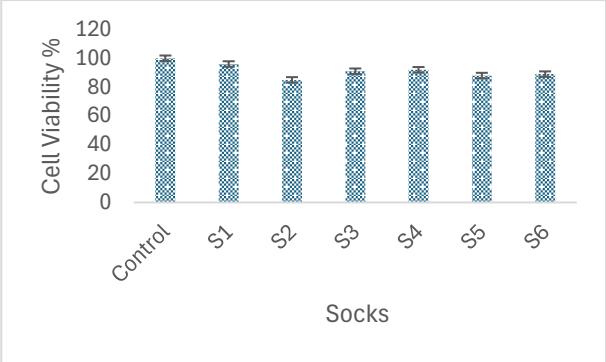

Figure 1. a) Cell viability % of antibacterial socks after 24 hours contact time

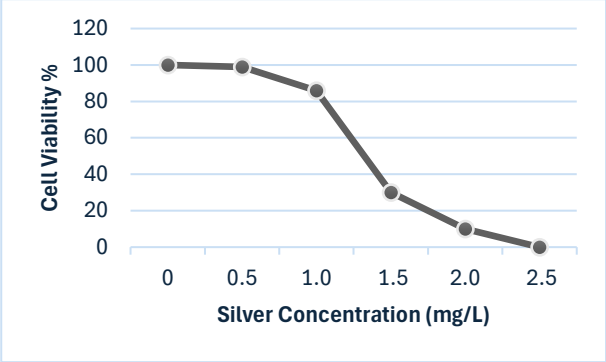

Figure 1. b) Cell viability % of silver concentration at 24 hours contact time

MTT results of test materials with reference to "Control" have been interpreted according to ISO 10993-5:2009 Tests for In Vitro Cytotoxicity. In the MTT analysis performed with the extracts of the fabric samples, it was determined that none of these samples had any cytotoxic effect, since the cell viability was above 70%.

As seen in Figure 1.a), the antibacterial men-women sports socks sample coded S2 has the lowest cell viability and is the sample containing the highest amount of silver (7.88 mg/kg). Antibacterial women's socks coded S4 was the sample with the lowest amount of silver (1.57 mg/kg) and was found to have the highest cell viability value among the antibacterial sock samples. When the cytotoxic effect of antibacterial socks, which are claimed to contain silver by their manufacturers, on HaCat cells is examined, it is seen that there is a consistent relationship between the cytotoxic effect and the amount of silver they contain. When all results were examined statistically, it was determined that there was a significant difference compared to the control in all cell groups that were applied the test material (extract of antibacterial socks) for 24 hours.

Figure 1.b) shows the cytotoxic effect of different silver concentrations (0.0-2.5 mg/L) on human keratinocyte HaCaT cells after 24 hours of contact time. IC50 value was found to be 1.31 mg/L.

4. Conclusion

Total metal content, metal release amount into artificial sweat solution (37 °C, 24 h), quantitative antibacterial activities and cytotoxic effects on human keratinocyte HaCaT cells of antibacterial socks procured from the domestic market and frequently and widely used in daily life were investigated.

When the total metal content values found as a result of ICP-MS analysis and the metal release values found after extraction are evaluated according to the limit values in Oeko-Tex Standard 100; It was observed that the total metal amounts of the sock samples and the metal amounts in the extracts obtained after extraction in artificial sweat solution at 37°C for 24 hours were within the Oeko-Tex Standard limits.

According to the antibacterial efficiency test results, the only sample coded S2 (men-women sports socks) showed more than 90% activity against both gram-positive and gram-negative bacteria. Other sock samples showed lower antibacterial efficiency. When the antibacterial effect of antibacterial socks, which are claimed to contain silver by their manufacturers, is examined; It has been observed that there is a consistent relationship between the antibacterial effect and the amount of silver they contain.

The HaCat cell line was used in the study because it represents the primary cell type of the epidermis in contact with textiles and has been used in the few textile toxicity studies in the literature. When human keratinocyte HaCaT toxicity test results were evaluated, it was seen that none of the samples with a contact time of 24 hours were toxic. When the cytotoxic effect of different silver concentrations (0.0-2.5 mg/L) on human keratinocyte HaCaT cells in 24 hours was examined, the IC50 value was found to be 1.31 mg/L

Author's Contributions

Candan Akca: Supervising, analytical analysis, writing, evaluating.

Fatma Doyuk: Assisting in analytical analysis, evaluating.

Ethics

There are no ethical issues after the publication of this manuscript.

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