Investigation of Toxicity in Textile Materials from Natural and Synthetic-based Polymers Utilizing Bioassay Performances

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Assessing the toxicity of textile samples in terms of risks to human well-being and health is a significant issue. In this study, 11 textile materials were tested using two procedures: the sperm motility inhibition test using bull spermatozoa and the acute immobility test using *Daphnia magna*. A comparative analysis was carried out considering the advantages of each toxicity assessment method. The bull sperm test was shown to be less sensitive and more complicated to carry out than the *Daphnia magna* immobility test. In addition, the inclusion of both dyes and synthetic fibres significantly influenced textile toxicity, with aqueous extracts from dyed textiles showing higher toxicity levels when tested alongside undyed textiles. The toxicity index for dyed textiles ranged from 37% to 62% in the motility inhibition test, while the *Daphnia magna* test showed an acute immobility parameter of 100% with the uncontaminated control medium.

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INTRODUCTION

Consumer safety is of paramount importance, especially with home textiles, as it has a direct impact on human well-being. Applying modified fibres, coatings, and textile auxiliaries to improve the operational and decorative characteristics of fabrics and nonwoven materials can pose a risk to human health. The shortcomings of fabric processing technology and the intricate formulations of fibre dressing and fabric finishing, which rely on synthetic resins together with various chemical compounds, both lead to the migration of chemicals into the environment and pose a threat to people.

Various chemicals are used during the processing of fibres. Toxic compounds most commonly found in textiles include pesticides, antimicrobial additives, and residual monomers in synthetic fibres, dyes and finishes, and pesticides in natural fibres (Ahn *et al*. 2008; Kemi 2016; Patti *et al*. 2020; Bour *et al*. 2023; Palanisamy *et al*. 2023a). Some of these substances can be hazardous, and they may be released into the environment through the production, consumption, and disposal of fabrics, negatively affecting the environment and people's health. Table 1 shows a set of toxic compounds most commonly found in textiles [Technical Regulations of the Customs Union "On the safety of light industry products" (TR CU 017/2011)].

Table 1. Toxic Compounds Most Commonly Found in Textiles

Textile Fibers	Name of the Substance
Natural raw materials from vegetables	Formaldehyde (finishing), Pentachlorophenol (from pesticides)
Artificial (viscose and acetate)	Formaldehyde (finishing), Carbon disulfide (production)
Polyester	Dimethyl Terephthalate (residual monomers), Acetaldehyde (residual monomers)
Polyamide	Caprolactam (residual monomers), Hexamethylenediamine (residual monomers)
Polyurethane (elastane)	Ethylene Glycol (residual monomers) Acetaldehyde (residual monomers)
Extractable chemical elements (depending on the dye)	Arsenic (As), Lead (Pb), Chromium (Cr), Cobalt (Co), Copper (Cu), Nickel (Ni)

For example, the following synthetic fibres can release their original monomers (substances used in their synthesis) into the environment: polyester, which contains acetaldehyde and dimethyl terephthalate; polyamide, which contains hexamethylene diamine and caprolactam; and polyacrylonitrile, which contains acrylonitrile and dimethyl formamide (Mather *et al.* 2023). These monomers can be toxic, allergenic or cause skin irritation (Armengol *et al.* 2022). The use of certain dyes in textile manufacturing may involve the presence of potentially harmful substances such as aromatic and ammonium compounds, metals and their compounds, alkali salts, *etc.* Certain dyes have been classified as carcinogenic and are banned in several countries (Starovoitova and Odido 2014). Some substances, such as chromium, can be highly toxic, while others have significant effects on the skin due to their use as colour fixatives in fabrics (Croce *et al*. 2017; Santulli *et al*. 2022).

The presence of impurities in textile materials made from natural fibres (such as cotton and linen) is a common occurrence, as these materials tend to acquire extraneous substances throughout the development of textile plants (Lusinyan *et al*. 2018; Ayrilmis *et*

al. 2024). Special antimicrobial additives are used to treat natural fibres, which can be harmful to live organisms to prevent microbiological damage.

Formaldehyde (CH_2O) is often found in the finishing compounds used to make textiles hydrophobic and dimensionally stable. CH_2O can break down and transform into free structures, as well as be released into the environment *via* the skin (Nair *et al*. 2013; Patti *et al*. 2020). Standards for the maximum quantity of CH_2O that can be present in fabric-based textiles are crucial and are taken into account throughout the approval process. Particularly high concentrations of free CH_2O may be present during the finishing process of fabrics containing pre-condensates of resins that are thermoset to provide durability, crush strength and low shrinkage. CH_2O amount in fabrics is limited by legislation in several countries, including Russia, due to the severe toxicity of CH_2O-containing finishes for textile treatments and the widespread use of these agents (Chubirko *et al*. 2019; Saidakhmet *et al*. 2022).

Bioassays are utilized to determine the effect of substances on live animals (in vivo) or tissue/cell systems of culture (*in vitro*) for a thorough evaluation of warning signs for safety in toxicology in conjunction with chemical methods of control. Different bioassay systems are used as test objects, including ciliates, bacteria, mammal sperm, mammal corneal preparations, *etc*. (Klemola 2008; Tabanca *et al*. 2018).

Given the rapid growth of new goods and materials, it is crucial to focus on developing efficient bioassay methods. The use of express methods is recommended in the following scenarios: a) during the initial stages of developing materials and products to select optimal laboratory samples; b) when assessing various methods for transforming substances into a product; c) when determining the most suitable sterilization technique for a product; d) when altering the material's composition; d) when expanding the application of a well-researched material (Brack *et al.* 2016; Mylsamy *et al*. 2024).

The toxicity evaluation of the detrimental effects of substances and the monitoring of toxicology in aquatic ecosystems are often assessed by using bioassays (Häder 2018). Some of these are as follows. Ecotoxicity tests for textile dyes; filter paper contact test with earthworms (*Eisenia foetida*); seed germination and root elongation toxicity test (*Cucumis sativus*, *Lactuca sativa* and *Lycopersicon esculentum*); acute immobilization test (*Daphnia magna* and *Artemia salina*); and the Comet assay with the rainbow trout gonad-2 cell fish line (RTG-2) and *D. magna* (Starovoitova and Odido 2014).

The use of bioassay techniques for detecting compounds along with toxic substances in the environment offers several advantages over chemical analysis methods. It is a more efficient, cost-effective, and straightforward approach (Hader 2018; Tišler and Zagorc-Kon 2008; Hybská *et al*. 2017; Padmanabhan *et al*. 2024).

Because of the complex nature of textiles and the variety of treatments used, it is essential to investigate the extent and character of adverse effects caused by aqueous extracts derived from textiles. Bioassay techniques detecting the combined effects of chemicals on test systems can provide valuable data for the development of materials with lower toxicity. On the other hand, bioassay methods are highly sensitive. Disadvantages of using these techniques to determine toxicity include false positive or inaccurate bioassay results due to the unique response of the test subject to exposure to toxicants and other environmental factors. It is preferable to integrate the application of multiple bioassays that complement each other in their responsiveness to different chemicals.

Today, a motility inhibition test, which involves a culture of mammal cells, usually bull spermatozoa, is often carried out to evaluate the toxicity of textiles and clothing (Klemola *et al*. 2006; Starovoitova and Odido 2014). This involves assessing changes in

sperm motility using special analysers, which adds complexity to this procedure (Yudina *et al*. 2023). The inhibition sperm motility test is used as a standard assay in the development of novel bioassay approaches. Klemola *et al*. (2007) assessed the potential toxicity of components and reactive dyes in textiles using the Hepa-1 cytotoxicity test in conjunction with the sperm test. Researchers have also developed and used cytotoxicity tests using mouse hepatoma cells (Klemola *et al*. 2007), tests using *Vibrio fischeri* bacteria (Birhanlı and Ozmen 2005), embryo teratogenesis assays, and other methods (Wang *et al*. 2002).

When studying the ecotoxicity and water quality of industrial effluents, protozoa such as *Daphnia magna* are often utilized as test subjects (Castro *et al*. 2019). Wastewater from the textile industry may be evaluated for possible environmental hazards using an acute toxic study that uses daphnids as a model organism for biotesting aqueous medium. Much research has investigated residual fluids along with leached extracts through textile materials to mimic the environmental impacts of chemicals produced by textiles (Dave and Aspegren 2010; Jemec *et al*. 2016). Leachate water analysis is useful for estimating the potential environmental toxicity associated with chemical additives leached from laundry detergents. However, it is not the best method for determining the skin toxicity of compounds. Additionally, the rates of leaching vary between fabrics made from various basic materials.

Many studies have focused on analysing the toxicity of chemicals in model environments using the *Daphnia magna* Straus (1820) procedure, as they are the most hazardous textile additives (Bae and Freeman 2007; Verma 2008; de Oliveira *et al*. 2018). Investigating the potential of textile materials to exert harmful ecotoxicological effects was the primary objective of this investigation. This study evaluated the toxicity of water extracts of several different textile specimens against a reference specimen without harmful compounds. This study aimed to compare the results of a new technique for the visual determination of daphnid immobility in response to toxic substances extracted in an aqueous medium with those of the traditional sperm movement inhibition test. The standard visual technique using *Daphnia magna* is less complicated than the sperm technique because it does not require any additional equipment or analysers. The advantages of the method include its cost-effectiveness and the high sensitivity of the test items. The dynamics of daphnia immobilisation could be tracked over time by studying aqueous extracts of textile materials.

EXPERIMENTAL

Two techniques of toxicity assessment were used to evaluate eleven different textile materials, each with its own unique composition and treatment type. Table 2 lists the substances researched, including their fundamental features. The study included the most common types of fabrics and knitted fabrics: natural (cotton, linen), synthetic (polyester, polyamide), and mixed (polyester with cotton, viscose fibres, elastane) (Hicks *et al.* 1971). A variety of material properties were defined for woven textiles (ISO 2959 2011; Değirmenci and Çelik 2016) and knitted fabrics (ISO 8388 2003-12; Malcolm-Davies *et al*. 2018), including fabric thickness (*T*) for woven and knitted fabrics (ISO 5084 1996; Rogina-Car *et al*. 2020), area density calculated as mass per unit area (BS EN 12127 1998; Gore *et al*. 2006), and warp/weft mass/area (ISO 7211-6 1984; Silva-Santos *et al.* 2019).

Table 2. Features of the Textiles Analyzed in this Research

Count of Diagnostic Samples	Material Kind / Ingredients in Raw Materials	Kind of Completion	Construction Type	Thickness (mm)	Area Density (g/m^2)	Weft and Warp Densities (tex)
1	100% cotton woven fabric	treated with bleach	simple	0.17	135	25.70 / 31.50
2	100% cotton woven fabric	black colour regularly dyed	sateen	0.7	256	34.64 / 19.50
3	100% cotton flannel woven fabric	printed	simple	0.6	174	7.86 / 23.92
4	100% cotton woven fabric	treated with bleach	simple	0.4	155	87.64 / 80.18
5	100% linen woven fabric	acidification treatment	simple	0.5	240	87.06 / 58.12
6	100% polyester woven fabric	bleached	simple	0.2	102	7.38 / 13.90
7	100% polyester woven fabric	printed	jacquard	0.2	101	6.34 / 13.18
8	100% polyamide woven fabric	bleached	simple	0.1	78	7.38 / 13.90
9	(65% of polyester / 35% of cotton) woven fabric	blue colour uniformly dyed	simple	0.2	109	14.34 / 14.82
10	Knitted fabric composed of 54% of polyester, 39% of viscose, and 7% of elastane	bleached	flat	0.3	145	-
11	Knitted fabric comprising 55% of polyester, 41% of viscose, and 4% of elastane	grey colour uniformly dyed	flat	0.3	129	-

All of the fabric samples were brand new and never washed before testing. Pieces weighing 1.0 ± 0.01 g were obtained for the CH_2O test and bull sperm test, whereas pieces weighing between 0.5 and 4.5 g± 0.01 g were obtained for the *Daphnia magna* test. These pieces were cut using a pair of stainless-steel scissors. After soaking the samples in 50 mL of distilled water, the aqueous extracts could be made. For 24 h, the extraction process was conducted in a thermostat at 40 ± 2 °C.

The residual CH_2O was determined by ISO 14184-1 (2011) (Rogina-Car *et al*. 2020). First, 10 mL of acetylacetone was combined with 5 to 10 mL of each sample extract. The mixture was incubated at 40 ± 2 °C for 30 min before being cooled to 18 to 25 °C. It was then moved to 100 mL volumetric flasks that had been adequately filled with distilled water. At the same time, a "blank experiment" was conducted using distilled water rather than the textile extracts. Sample No. 2, which was made of consistently coloured black cotton, was one example where distilled water was used in lieu of acetylacetone to colour the extract. An optical density of the obtained solutions was determined at a wavelength of 412 nm by photoelectric colorimeter device KFK-2 ZOMZ (Russia). This was followed by the determination of the CH_2O content by making use of calibration curves.

The toxicity of textile extracts in water was evaluated using a sperm test, which is a technique for inhibiting cell motility in a mammalian suspension culture. The tests were carried out in compliance with the GOST 32075 (2013) (Yudina *et al*. 2023) & GOST R 53485 (2009) (Skriabin *et al*. 2024; Yudina *et al*. 2023) criteria, the national standards in Russia. The test was performed with a specialized analyzer monitoring the mobility parameter of bull spermatozoa in water-based textile media. The goal was to halt the movement altogether. Frozen granular bull sperm in liquid nitrogen vapours were provided as the biological test item. Sperm was prepared from freshly obtained undiluted semen obtained from bulls that have been tested for the quality of their offspring by dilution with synthetic media and subsequent freezing in liquid nitrogen (Lach *et al*. 2022). The fertilizing ability of bull semen, tested by artificial insemination of cows and heifers with frozen-thawed semen, must be at least 50% within 60 to 90 days after the first insemination. The sperm, after thawing, met the requirements and standards specified in Table 3 according to organoleptic, physical, biological, and morphological indicators.

Table 3. Characteristics of Frozen Bull Semen after Thawing

Indicator	Characteristics and Norm
Appearance, consistency, color	Homogeneous, yellow or light-yellow liquid without foreign impurities
Number of sperm with rectilinear translational movement, %, not less than	40
Dose-volume for insemination, cm^3, not less than	0.2
Number of spermatozoa with rectilinear translational motion per dose, million, not less than	15
Survival of sperm at a temperature of 38 °C, h, not less than	5
Number of sperm with an intact acrosome, %, not less than	60
Number of sperm with abnormal morphology, %, no more than	18

In the sperm test, textile concentrates were prepared at a ratio of 0.02 g/mL and combined with glucose and sodium citrate. Distilled water was used as an extractant. To prepare the extract, one of the selected elementary samples weighing 1.0 ±0.01 g was used. The elementary sample was placed in a flask with a ground-in stopper, filled with distilled

water, and thoroughly mixed, ensuring complete wetting of the textile material with water. The experimental solution was an extract with the addition of the dry reagents glucose and sodium citrate (per 10 mL of test solution – glucose 0.4 g, sodium citrate 0.1 g). A glucose-citrate control solution was prepared as follows: 10 mL of distilled water; glucose, 0.4 g; and sodium citrate, 0.1 g.

To thaw frozen sperm, a diluent was taken into a test tube in the volume indicated in the passport for bull sperm, and it was placed in the thermostat of the analyzer at 40 ± 1.5 °C. Using anatomical tweezers, a sperm granule was removed from the Dewar flask and dropped into a test tube with a solution heated to 40 ± 1.5 °C. Immediately after defrosting, the contents of the test tube were thoroughly mixed by shaking the test tube and placed back in the thermostat for 5 to 6 min. A mixture of thawed diluted semen and textile extracts was prepared, resulting in a final sperm concentration of 3-5 million/mL.

The sperm motility was observed using the AT-05 Toxicity Analyzer (Russia), with examinations conducted every 15 min. The toxicity index was determined by matching the experimental data of the solution with the referent one. The test temperature was 40 ± 1.5 °C. The tested solutions (control and experimental) must be constantly held at the specified temperature during the experiment. The control and experimental solutions (0.4 mL of each) were taken into test tubes with ground-in stoppers and placed in the thermostat block of the AT-05 image analyzer at 40 ± 1.5 °C. A total of 0.1 mL of the resulting sperm suspension was placed in test tubes with control and experimental solutions. The sperm motility period was calculated as an average duration between double measurements, with the first measurement recording the presence of one motile cell and the second one indicating the complete stop of motion.

When sperm motility was approximately 10% of the initial activity in experimental capillaries, the process of accumulating experimental data was stopped. The toxicity index of textile water extracts was calculated based on a variance in cell motility between the experimental and referent media (Eq. 1). A toxicity index value falling within the 70% to 120% range indicated that the textile material was deemed non-toxic (Yudina *et al*. 2023).

$$I_m = \frac{t_{test}}{t_{control}} \times 100 \quad (1)$$

In Eq. 1, I_m is an index of toxicity, t_{test} is the duration of sperm motility within the experimental specimen, and $t_{control}$ is duration of sperm motility within the referent specimen.

An innovative approach to determining the toxicity of textiles has been developed based on the water quality bioassay technique as described in ISO 6341 (Subrero *et al*. 2019). Using *Daphnia magna* in an acute immobilisation test, acute toxicity is measured by comparing the survival and reproduction rates of the control sample with those of daphnids subjected to harmful chemicals for 2 days using a strain culture. The quantity of daphnids showing marks of movement under the experimental conditions is the main metric analysed. This metric is therefore influenced by reproductive success and longevity. Active filtrates include planktonic crustaceans of the genus *Daphnia*. In their process of naturally purifying water, they can absorb significant amounts of harmful compounds by circulating large amounts of water throughout their bodies. This group of creatures accumulates pollutants at an alarming rate. Daphnids are very sensitive to chemicals, even at low levels. In comparison to the uncontaminated control medium, the toxicity is ascertained through visual observation of the motor activity of daphnids, specifically the rate of movement and the overall count of deceased organisms. Testing functions may

therefore be motor activity or daphnid mortality (Terekhova *et al*. 2018)

The capacity of daphnids to respond to the existence of hazardous compounds in the water textile extracts that impact their immobilization formed the basis of the established toxicity determination technique. The following practical issues were resolved to conduct toxicant analysis using *Daphnia magna* acute immobility test (henceforth, daphnids test): determining the minimum necessary mass of the sample to evaluate the toxicant effect; and determining the optimal period to account for motor activity and mortality.

Young *Daphnia* crustaceans less than 24 h old at the beginning of the test were exposed to a standard test substance in a certain concentration range. Immobilization was defined as the inability of Daphnia to move within 15 seconds after the contents of the test vessel were gently agitated, even if they were still able to move their limbs. The standard substance potassium dichromate ($K_2Cr_2O_7$ with a concentration of 1 mg/L) was examined to verify the correctness of the test conditions. Test reliability criteria: in the control test, including the control test with a solvent, no more than 10% of daphnids were immobilized. The concentration of dissolved oxygen at the end of the test was 3 mg/L in the control and test samples. Testing was performed in glass test tubes, which were not tightly closed during the experiment to reduce water loss due to evaporation and to avoid dust getting into the tested solutions. Daphnids were obtained from a healthy population (without symptoms of stress, with low mortality, without the presence of males and ephippia, colorless specimens, *etc*.). Organisms used for a particular test were obtained from a culture of the same *Daphnia* population. *Daphnia* were kept under standard cultivation conditions, and a climatic camera was used for cultivation. For cultivation during the experiment, water that is constantly used for cultivating daphnia in the laboratory was used. The water quality was constant throughout the test period and the water hardness was 200 mg/dm^3 in terms of $CaCO_3$. The test was performed without pH adjustment.

The water extracts of textiles (experimental specimens) were used to create the samples (20 mL) in the following ratios: 0.01, 0.02, 0.03, 0.04, 0.05, 0.07, or 0.09 g of sample to 1 mL of distillate. The best test parameters were identified by experimenting with different concentrations of the test sample in the medium. Next, 20 mL of test medium with a particular concentration of the samples were subjected to 10 juvenile daphnids that were less than 24 h old for a period of 148 h. For every fabric sample, the experiment was repeated three times with different concentrations of extract. After 24, 48, 72, and 96 h of testing, the number of organisms that survived was analyzed and compared to the reference values. The free-moving and immobilized daphnids were counted in the volume of the test experimental medium. The average number of test individuals who made it through a certain time period in either the experimental or control conditions was used as a survival indicator.

Toxic effects were defined as a 50% mortality rate or higher in comparison to the control media after 96 h of exposure to the test medium. Using checkpoints at 24, 48, 72, and 96 h, the daphnids' survival was monitored for 96 h. Acute immobility is a useful metric for describing the level of toxicity. This metric is derived using the percentage of test organisms that die or remain immobile for every amount of aqueous extract relative to the control media, as shown in Equation (2) (Subrero *et al*. 2019).

$$A = \frac{\overline{X}_{control} - \overline{X}_{test}}{\overline{X}_{control}} \times 100 \quad (2)$$

where A is a parameter for acute immobility, $\overline{X}_{control}$ is the average quantity of organisms

that survived in the referent medium, and $\bar{X}_{test}$ is the average quantity of organisms that survived in the experimental medium for all concentrations of the sample. The explanation of these experimental findings is given in Table 4 (Subrero *et al*. 2019).

Table 4. The Analysis of Toxicity Level by A

Parameter for Acute Immobility (A, %)	Interpretation in General	Comprehensive Description
0-10	absence of toxicity	safe to use
10-25	absence of acute toxicity	slightly toxic
25-35		minimally toxic
35-50		moderately toxic
50-100	severe toxicity	extremely toxic

RESULTS AND DISCUSSION

Because formaldehyde (CH_2O) is often used to treat cotton or linen-based textiles, the quantity of residual CH_2O was measured in test samples No. 1–5 that were made of natural fibres. Table 5 displays the amounts of formaldehyde and optical densities at 412 nm for the water extracts of textile specimens that were established using calibration curves. Sample No.2 (cotton evenly coloured black) and No.5 (linen treated with acid) both had residual levels of free CH_2O, according to the performed analyses.

The sperm test was used to evaluate the toxicity of textile extracts. Purified water, dextrose, and sodium citrate formed a solution that served as the control medium. Figure 1 displays the time-dependent motor activity of spermatozoa. Each testing cycle lasted 15 min, for a total of 3 h. The motility parameters of all samples dropped as the exposure period progressed. Toxic indices (I_m) were computed by comparing the motility parameters of the experimental and reference specimens. Because the test medium has the potential to stimulate sperm, the toxicity index may go above 100%.

The bleached cotton sample 1 and the coloured cotton sample 2 are shown side by side in Fig. 1(a). The results showed that the water-based extract from black-dyed, 100% cotton significantly inhibited the growth of bull spermatozoa. Additionally, an acute toxicity was shown by the extract from sample No. 2. One h into the trial, sperm motility dropped significantly. Clothes may lose some of their sanitary qualities if they include dyes made from natural materials. No. 4 and No. 5 linen samples, which were tested, did not contain any harmful compounds that might be removed into water (Fig. 1(b)). In the early stages, the acidified linen sample extract stimulated spermatozoa, leading to an increase in motility.

The extract from specimen No. 8 (bleached polyamide) exhibited a moderate degree of toxicity for bull spermatozoa, as shown in Fig. 1(c). Simultaneously, sample No. 6 (bleached polyester) included an aqueous extract that was not hazardous; its motility parameters were similar to those of the reference medium. The two specimens taken from knit materials containing a mixture of chemicals were very poisonous (Fig. 1(d)). After thirty min, the sperm motility in these test mediums began to diminish.

Table 5. Concentration of Free CH_2O in the Water Extracts from Textile Samples

Count of Diagnostic Samples	Descriptive Information	Absorbance Optical Density (D, abs. units)	Solution of Free CH_2O (mg/L)	Sample of Free CH_2O (mg/g)
1	100% bleached cotton	0	0	0
2	100% uniformly dyed cotton	0.0050	0.0200	0.0799
3	100% pure cotton (“printed flannel”)	0	0	0
4	100% bleached linen	0	0	0
5	100% pure linen treated with acidification	0.0080	0.0400	0.1773

Table 6. The Sperm Test Measured the Toxicity Index of Textile Aqueous Extracts

Count of Diagnostic Samples	Sample Description	Index of Toxicity (I_m, %)	Process of Interpreting
1	100% bleached cotton	98.8	safe to use
2	100% uniformly dyed cotton	58.0	toxic
3	100% pure cotton ("printed flannel")	100.5	safe to use
4	100% bleached linen	102.1	safe to use
5	100% pure linen treated with acidification	99.8	safe to use
6	100% bleached polyester	101.5	safe to use
7	100% printed polyester	95.3	safe to use
8	100% bleached polyamide	83.3	safe to use
9	uniformly dyed (65% of polyester/35% of cotton)	37.2	extremely toxic
10	bleached 54% of polyester, 39% of viscose, and 7% of elastane	42.6	extremely toxic
11	uniformly dyed of 55% of polyester, 41% of viscose, and 4% of elastane	61.8	toxic

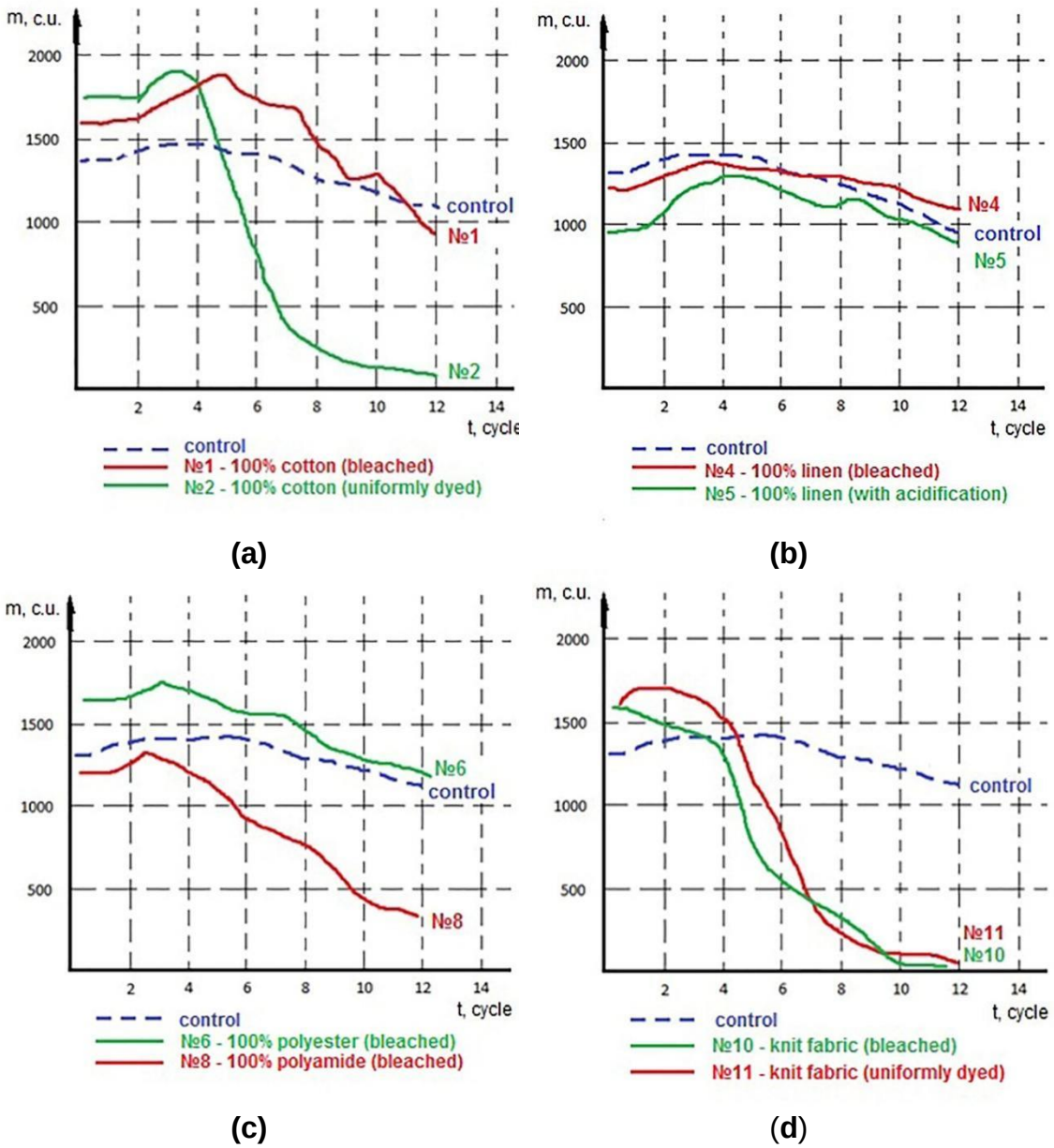

Fig. 1. Dependence of sperm motor activity (*m*, conventional units) on exposure time (*t*, cycle; one cycle = 900 sec): (**a**) for the specimens No.1 – woven fabric with 100% bleached cotton (red graph) and No.2 – woven fabric with 100% uniformly dyed in black colour cotton (green graph); (**b**) for the specimens No.4 – woven fabric with100% bleached linen (red graph) and No.5 – woven fabric with 100% acidification treated linen (green graph); (**c**) for the specimens No.6 – woven fabric with 100% bleached polyester (green graph) and No.8 – woven fabric with 100% bleached polyamide (red graph); (**d**) for the specimens No.10 – knit fabric along with bleached 54% of polyester, 39% of viscose and 7% of spandex (green graph) and No.11 – knit fabric along uniformly dyed in grey colour with 55% of polyester, 41% of viscose, 4% of spandex (red curve). Dependence for the reference (control) medium is the dotted graph.

Table 6 provides an overview of the test outcomes. According to GOST 32075 (2013) (Yudina *et al*. 2023) and GOST R 53485 (2009) (Skriabin *et al*. 2024), the following samples were determined to be toxic or extremely toxic: No. 2 (dyed cotton fabric), No. 9 (dyed cotton and polyester fabric), No. 10 (polyester and spandex bleached knit fabric), and No. 11 (dyed polyester and elastane knit fabric).

Aquatic extracts from textiles were used to block the movement of daphnids, which made it possible to calculate the acute immobility parameter. For the daphnid investigations, the ideal concentration of the textile specimen in the watery medium was determined using concentrations of the sample ranging from 0.01 to 0.09 g/mL. With each test case, three separate determinations were performed. To get an average value, the collected data were subjected to mathematical statistical processing.

Table 7. Daphnids Were Fully or Partially Immobilized in the Water-based Textile Extracts at 24-, 48-, 72-, and 96-h Post-experiment Commencement

Samples for Testing	Aqueous Extract Sample Concentration (g/mL)	Testing Period (h)				
		initial	24	48	72	96
		Quantity of surviving Daphnia				
No.1. Woven fabric with 100% bleached cotton	0.05	10	9.4±0.6	7.2±1.9	6.5±0.6	4.6±0.5
	0.07	10	8.6±0.5	7.2±1.8	5.3±0.7	3.2±1.1
	0.09	10	8.5±0.8	4.6±0.2	4.0±1.0	3.2±0.2
No.2. Woven fabric with 100% uniformly dyed in black colour cotton	0.05	10	0	-	-	-
	0.07	10	0	-	-	-
	0.09	10	0	-	-	-
No.3. Woven fabric with (Printed, “Flannel”) 100% cotton	0.05	10	9.0±0.5	9.2±0.2	8.6±0.7	8.6±0.8
	0.07	10	9.2±0.8	9.2±0.6	8.4±0.5	8.0±1.8
	0.09	10	9.2±0.5	8.6±0.3	8.0±0.4	6.6±0.9
No.4. Woven fabric with100% bleached linen	0.05	10	8.6±0.8	8.6±0.8	3.3±0.6	4.0±1.3
	0.07	10	8.0±1.2	6.6±2.3	4.6±1.8	2.6±0.8
	0.09	10	9.2±0.7	6.8±0.3	4.6±0.5	2.0±0.3
No.5. Woven fabric with 100% acidification treated linen	0.05	10	6.0±0.8	5.2±0.7	2.3±0.5	2.6±0.6
	0.07	10	8.6±1.9	4.6±1.1	0.3±0.7	0.6±0.5
	0.09	10	4.6±0.8	1.2±0.2	1.2±1.0	0.6±0.5
No.6. Woven fabric with 100% bleached polyester	0.05	10	8.6±1.1	6.6±1.2	4.6±0.6	2.0±0.6
	0.07	10	8.0±0.8	6.6±0.9	2.0±1.2	2.9±1.8
	0.09	10	7.2±0.6	5.2±0.5	2.0±1.5	2.0±0.3
No.7. Woven fabric with 100% printed polyester	0.05	10	8.6±0.8	8.6±0.5	6.5±0.6	4.0±1.4
	0.07	10	8.6±0.8	8.6±1.1	8.0±0.1	3.2±0.2
	0.09	10	8.0±0.9	6.6±1.7	4.6±1.6	2.6±1.3
No.8. Woven fabric with 100% bleached polyamide	0.05	10	9.2±0.9	5.2±0.7	2.6±0.8	1.2±0.7
	0.07	10	8.6±0.6	6.6±0.5	2.5±0.3	0.6±0.5
	0.09	10	6.5±0.5	6.0±0.5	0.6±0.5	0.6±0.6
No.9. Woven fabric along uniformly dyed in blue colour with 35% of cotton and 65% of polyester	0.05	10	0	-	-	-
	0.07	10	0	-	-	-
	0.09	10	0	-	-	-
No.10. Knit fabric along with bleached 54% of polyester, 39% of viscose and 7% of elastane	0.05	10	0	-	-	-
	0.07	10	0	-	-	-
	0.09	10	0	-	-	-
No.11. Knit fabric along uniformly dyed in grey colour with 55% of polyester, 41% of viscose, 4% of elastane	0.05	10	0	-	-	-
	0.07	10	0	-	-	-
	0.09	10	0	-	-	-

Doses between 0.01 to 0.04 g/mL considerably lengthen the test period; thus, these values are inappropriate for the experiment. Acute immobility testing with *Daphnia magna* was therefore conducted at cloth concentrations of 0.05, 0.07, and 0.09 g/mL. Table 7 compares the reference medium with aqueous extracts containing varying content of textile materials (0.05, 0.07, and 0.09 g/mL) to illustrate the signs of complete or incomplete immobilization of daphnids.

After trying out various doses, it was concluded that 0.07 g/mL was optimal for using the daphnids test to evaluate the toxicity of textile extracts. Equation (2) was used to figure out the severe immobility parameters (*A*, %) for a sample content of 0.07 g/mL in an aqueous extract. Table 8 presents the obtained results.

Table 8. Parameters of Acute Immobility for *Daphnia magna* in Water Extracts Derived from Textile Specimens (0.07 g/mL)

Samples for Testing	Specimen	Acute Immobility (*A*, %) (Δ±5%)				Process of Interpreting (after 24 h)
		24 h	48 h	72 h	96 h	
1	Woven fabric with 100% bleached cotton	14	28	47	68	slightly toxic
2	Woven fabric with 100% uniformly dyed in black colour cotton	100	100	100	100	extremely toxic
3	Woven fabric with (Printed, “Flannel”) 100% cotton	8	8	16	20	safe to use
4	Woven fabric with100% bleached linen	20	34	54	74	slightly toxic
5	Woven fabric with 100% acidification treated linen	14	54	97	94	slightly toxic
6	Woven fabric with 100% bleached polyester	20	34	80	71	slightly toxic
7	Woven fabric with 100% printed polyester	14	14	20	68	slightly toxic
8	Woven fabric with 100% bleached polyamide	14	34	75	94	slightly toxic
9	Woven fabric along uniformly dyed in blue colour with 35% of cotton and 65% of polyester	100	100	100	100	extremely toxic
10	Knit fabric along with bleached 54% of polyester, 39% of viscose and 7% of elastane	100	100	100	100	extremely toxic
11	Knit fabric along uniformly dyed in grey colour with 55% of polyester, 41% of viscose, 4% of elastane	100	100	100	100	extremely toxic

Note: The assessment of toxicity was conducted using the 24-h value

Notably, the hazardous specimens (No. 2 & 9–11) displayed significant acute immobility characteristics of daphnids, according to the sperm test. Although the sperm test provided a more general idea of toxicity levels (slightly-low-medium-highly), the degree of toxicity was quantified more precisely according to the parameters of water

toxicity provided in ISO 6341 (Subrero *et al*. 2019). After the 24-h period of testing, the *A* parameters for *Daphnia magna* were used to evaluate the toxicity.

Table 9. Contrastive Analysis of the Results: Free CH_2O Concentration, Toxicity as Assessed by a Test that Inhibits Motility with Bull Sperm (3 h) and an Acute Immobility Test with *Daphnia magna* (24 h)

Samples for Testing	Specimen	Concentration of Free CH_2O	Bull Sperm Motility test		*Daphnia magna Straus* Test	
			I_m (%)	description	*A* (%)	description
1	Woven fabric with 100% bleached cotton	0	99	safe to use	14	slightly toxic
2	Woven fabric with 100% uniformly dyed in black colour cotton	min amount	58	toxic	100	extremely toxic
3	Woven fabric with (Printed, “Flannel”) 100% cotton	0	101	safe to use	8	safe to use
4	Woven fabric with100% bleached linen	0	102	safe to use	20	slightly toxic
5	Woven fabric with 100% acidification treated linen	min amount	100	safe to use	14	slightly toxic
6	Woven fabric with 100% bleached polyester	not determined *	102	safe to use	20	slightly toxic
7	Woven fabric with 100% printed polyester	not determined *	95	safe to use	14	slightly toxic
8	Woven fabric with 100% bleached polyamide	not determined *	83	safe to use	14	slightly toxic
9	Woven fabric along uniformly dyed in blue colour with 35% of cotton and 65% of polyester	not determined *	37	extremely toxic	100	extremely toxic
10	Knit fabric along with bleached 54% of polyester, 39% of viscose and 7% of elastane	not determined *	43	extremely toxic	100	extremely toxic
11	Knit fabric along uniformly dyed in grey colour with 55% of polyester, 41% of viscose, 4% of elastane	not determined *	62	toxic	100	extremely toxic

Note: As formaldehyde is often used to finish natural fibres textiles, the quantity of residual CH_2O in specimens No. 1 through 5 was measured.

A comparison of the outcomes acquired *via* the use of three distinct techniques is shown in Table 9. These techniques include the free CH_2O content test, the motility inhibition test using spermatozoa, and the acute immobility test using *Daphnia magna*. Each of these tests produced findings that were very consistent with each other. Toxic effects were amplified when free formaldehyde was present in the substance. In addition, the sensitivity of the acute daphnid test was higher than that of the sperm test.

Pearson correlation analysis was used to understand how the sperm and daphnids tests for toxicity related to one another. It is a number between -1 (perfect negative correlation) and 1 (perfect positive correlation) that measures the strength and direction of the relationship between two variables. Pearson correlation analysis is essential to take notice of the fact that the acute immobility parameter produced by the daphnids test and the toxicity index derived by the sperm test have an inverse connection. This is shown by the fact that the Pearson correlation coefficient (r) which is a parametric statistic has a negative sign (Table 10).

Table 10. Toxicity Index (Im) and Acute Immobility Parameter (A) Measured by the Bull Sperm and the Daphnids Techniques According to Pearson's Correlation Coefficient

Parameter	Index of Toxicity (for inhibition of motility using bull sperm test)	Acute Immobility Parameter (acute immobility test with *Daphnia magna Straus*)			
		1 day	2 days	3 days	4 days
Pearson correlation coefficient	1	-0.940	-0.898	-0.667	-0.654

Discussion

Using bioassay methods, this research focused on studying the toxicity of 11 different textile materials. Analyzing water-based extracts of textile samples mimicking bodily fluids allowed for the prediction of the sanitary and hazardous characteristics of textiles coming into contact with human skin. The main task was to analyse the toxicity of the studied textile materials for humans and the primary elements influencing it. The impact of raw materials and the inclusion of harmful additives were the main points of discussion. The development of guidelines for the implementation of a novel approach to determine the toxicity of textiles (daphnids test) was another objective of this project. This test is often used to determine the purity of water. A comparison of the obtained results was performed with those of the gold standard for determining the toxicity of textiles, which is the sperm test.

The primary goal of the first experiment was to identify the amount of residual formaldehyde in the water-based extracts of the textile samples. Clothes and first-layer materials may not contain more than 0.075 mg/g of free formaldehyde, under the Technical Regulation Customs Union (TR CU) 017/2011, which addresses the safety of products from the light industry (Saidakhmet *et al*. 2022). Specimens 1, 3, and 4 did not contain any free formaldehyde, according to the testing findings that determined the residual formaldehyde content in cotton and linen textile materials. The toxicity of textiles may be affected by the modest amounts of formaldehyde found in the weaved fabrics made from evenly coloured cotton (specimen No.2) and linen treated with acidification (specimen No.5).

To measure acute immobility, two bioassays, one using bull spermatozoa to impair motility and another using *Daphnia magna*, were used to establish the toxicity of the fabrics. The results were compared to the spermatozoa sperm test, and we determined the acute daphnid test was far more sensitive. Acute immobility testing revealed that some fabrics deemed non-toxic by the sperm test had mild or low toxicity. It should be mentioned that the test may be conducted more simply with daphnids, as no special equipment is needed.

After trying out several doses, the daphnids test settled on 0.07 g/mL as the optimal ratio for determining the toxicity of textile extracts. In the case of very hazardous substances, all three test doses achieved complete immobilization of *Daphnia*. The dose of 0.07 g/mL was shown to be the most optimum for determining the toxicity gradation of medium- and low-toxic compounds. The kinetics of *Daphnia* immobilization were found to be smoother at this concentration. It was complicated to monitor the dynamics of organisms at concentrations of 0.09 g/mL, which inhibited mobility by over 50% of daphnids after 2 to 3 days of exposure, while at lower concentrations of 0.05 g/mL, no discernible impact was seen. The exposure durations showed that the sperm motility inhibition test was most closely correlated with a concentration of 0.07 g/mL.

The toxicity characteristics that were found by testing sperm and daphnids had an inverse association, according to the Pearson correlation coefficient. When comparing toxicity metrics, which take the opposite way into account as a positive or negative parameter, it was expected. In contrast, when the toxicity index was 100%, the immobility test with *Daphnia magna* revealed complete immobilization (only dead test organisms), and the motility inhibition test using bull spermatozoa revealed no inhibition (no toxicity) when the toxicity index was 70 to 120%. Acute immobility parameters are best defined within 1 or 2 days after the start of the test, as per the Pearson correlation analysis. During these times, the Pearson's correlation coefficients between the two bioassay findings were at their maximum. The sperm test-defined toxicity index was less strongly correlated with the outcomes of tests conducted with daphnids for longer durations (3 and 4 days). But even at low doses, the cumulative toxicity of toxicants may be assessed in a long-term experiment. Aqueous textile extracts that failed to exhibit toxicity during the first 48 h of testing were poisonous after further incubation times.

The use of sperm is a traditional method of biotesting. The main criterion for assessing the functional state of spermatozoa is the duration of their movement. Motility is assessed by microscopic examination of a drop of sperm from experimental solutions and comparing them with the state in the control sample every 10 minutes. The microscope stage must be constantly heated to a temperature of +40 °C. The motility time of spermatozoa is determined as the average between the last two measurements, of which the first determination registers the presence of at least one or two progressively motile spermatozoa, and the second - a complete cessation of progressive movement. The motility of bull spermatozoa depends on the disruption of cellular structures and functions affected by toxicants.

The sensitivity of *D. magna*, which is a natural filter feeder, depends on many factors. The variety of test functions of *D. magna*, including physiological, morphological and behavioural responses, makes it possible to obtain a response of the living system to various toxicants, which is actively used in studies of the state of environmental objects, primarily wastewater and industrial waste (Barata *et al.* 2008). The use of daphnia is also traditional and has the following advantages: convenience and relative simplicity of

cultivation, a sufficiently high level of organization of the living, and the assumption of similar impacts on other multicellular organisms. The presence of the circulatory and nervous systems is sufficient that it makes it possible to visually assess the responses, which in turn leads to the absence of the need for specialized measuring instruments, due to the sensitivity of daphnia to most pollutants and the comparative simplicity of performing experiments (Olkova et al. 2018). The methods are based on the use of various biosystems, but allow researchers to obtain a comparable response of living organisms to a toxic agent.

Along with comparing the outcomes of the two tests, the elements that affect the toxicity of various fabrics were determined. Through the comparison of two cotton samples, one dyed black and the other bleached, the presence of a dark dye had a direct impact on the motility of spermatozoa and the survival of daphnids, as shown by the organisms' 100% death rate after 24 h. From as little as 0.09 g/mL of the bleached cotton aqueous extract, daphnids were progressively immobilized. Problems with dye fixing and easy diffusion into an aqueous extract were observed in material No. 2. Chemicals such as trichloroethane (TCE) and nonylphenol ethoxylates (NPEs) are detergent-like substances used to prepare the fabric for dyeing. They are considered highly toxic to the human body. Disperse dyes, acid dyes, and azo dyes used in textile dyeing are potentially hazardous (Neamtu *et al.* 2004; Chung 2016).

The toxic effect of compounds depends on many factors: the content of organic substances in the environment of the biotest, pH, hardness, and other physical factors. The mechanism of action may be associated with the effect of individual molecules of the toxicant on cell organelles; complexes incompatible with the further vital activity of the biotest may be formed (Ricco *et al.* 2004). Inorganic substances that have isomers and isomers of low-molecular organic substances usually have a general toxic, non-specific effect, so their isomers do not differ in toxicity. Isomers of high-molecular toxicants often act specifically, that is, they have a high chemical affinity for a certain type of biomolecule in the body, which is spatially strictly organized. If the mechanism of the toxic process lies in the interaction of a radical or an atom responsible for isomerism, the toxicity of different isomers will differ significantly, and *vice versa* – a reactive isomeric part of the molecule has little effect on toxicity. In addition, there are mechanisms for protecting a living organism from the penetration and spread of toxicants in them. The more toxic substance in the test object's environment, the more dangerous it is for the organism, as it increases the rate of spread and, accordingly, the effect of the toxicant. Chemically active substances usually act as strong oxidizers or reducers. Chemically active substances are most dangerous for unicellular organisms. Acids and alkalis are dangerous due to their chemical activity as they change the pH of living organisms and lead to denaturation of macromolecules. The toxic process can be caused by substances that destroy hydrogen bonds in biological macromolecules, disrupting their spatial organization (Salnikow *et al.* 2008).

An abundance of research has shown that even trace amounts of dye in industrial effluents are very harmful to aquatic life (Kaur *et al*. 2018; Verma 2008). Extracts from coloured cotton and linen, when leached, were far more hazardous than those from non-dyed cotton and linen, according to research by Dave G. *et al*. (Dave and Aspegren 2010) Free formaldehyde in the water-based extract is another possible explanation for sample No.2's extreme toxicity. Underwear for children, such as diapers, bonnets, romper suits, and vests, should adhere to certain cleanliness standards, and test sample No. 3 ("Flannel") is a good example of this. Bull spermatozoa and daphnids showed very mild toxicity to the

water-based extract of this material (Garg *et al*. 2021), which is surprising given the printed finish. Based on the results of the sperm test, the sample had a toxicity index of 100.5%. The acute immobility parameter, measured after 2 days of exposure, was 8%, and after 4 days of exposure, it was 20%.

A sperm test showed that two linen samples (No. 4 and No. 5) were non-toxic. Dye is the deciding factor in textile toxicity; sample No.5, which consisted of acidified linen, did not contain any free formaldehyde and did not influence spermatozoa motility. These specimens demonstrated a minimal degree of toxicity when tested on the more delicate daphnids. Both textile extracts showed a steady death rate for the organisms tested. Since the immobilization of crustaceans took place after a period of 24 h, it has been determined that a concentration of 0.09 grams of textile per millilitre is too high. The dynamics of daphnid mortality were shown to be the most revealing when the content was 0.07 g/mL. Samples No. 4 (bleached linen) and No. 5 (linen treated with acid) were hazardous, which may be related to the residual levels of harmful compounds employed in textile finishing. An alkaline peroxide treatment, which includes alkaline boiling as well as peroxide bleaching, or a one-stage oxidative boiling with alkaline agents as well as peroxide are two examples of methods that are used in the manufacturing of bleached linen. There are other alternatives. Linen is acidified using organic acids, most often acetic or oxalic acid, to make it stronger and to make the dyed fabric more consistent in shade. Bioassay organisms may be poisoned by residual amounts of organic acids, peroxide, or alkaline substances (Beiras *et al*. 2021; Kanjal *et al*. 2023).

The level of toxicity was found to be low to medium in three synthetic material samples (test samples No.6-8) that were composed of polyester or polyamide fibres. The three samples' extracts showed comparable trends of daphnid mortality. The bleached polyamide sample was determined to be more hazardous than the bleached polyester sample (No. 8). Increasing the concentration of the raw monomers could make these materials toxic. For polyesters, this would be dimethyl terephthalate, which has a maximum evaluable content of 1.5 mg/L in an aqueous medium, and for polyamide, it would be caprolactam, with a maximum evaluable content of 1.0 mg/L in an aqueous medium-6. Additionally, dimethyl terephthalate is in a higher risk category than caprolactam.

Sample No. 9, which consisted of blue-dyed cotton and polyester, was determined to be hazardous by both bioassay procedures. A combination of the raw materials' makeup plus the presence of a dark, poisonous dye might cause the toxicity. Heavy metals like iron and copper are responsible for the extreme toxicity of dark colours (de Oliveira *et al*. 2018; Siti Aisyah *et al*. 2014). Dye and finishing chemical toxicity in textile effluent may be reduced using these newly discovered procedures (Mahmoodi and Arami 2009; Meriç *et al*. 2005)

Two synthetic knitted textiles (No. 10 and 11) were studied, and after 24 h, the daphnids were completely immobile. The two examples were both made using spandex, a kind of polyurethane fibre that provides the fabric its remarkable flexibility. Possible causes of the harmful effects include traces of polyurethane's ethylene glycol (1.0 mg/L is the maximum allowable concentration of a toxic substance in an aqueous medium) and polyester's dimethyl terephthalate (1.5 mg/L is the maximum allowable content of a toxic substance in a water medium) (Lusinyan *et al*. 2018).

Synthetic microfibres and the subsequent loss of monomers to water are two potential sources of toxicity in synthetic fibre clothing. Daphnids are endangered because they may ingest the synthetic fibres that are still in the textile extracts (Jemec *et al*. 2016; De Sá *et al*. 2018; Araujo *et al*. 2020).

A preprint has previously been published in Research Square; it has not been peer reviewed by any journal (Pekhtasheva *et al*. 2023).

CONCLUSIONS

1. *Daphnia magna* acute immobility test results were found to be in excellent agreement with the results of the motility inhibition test using bull spermatozoa, according to the findings of the research that used eleven different textile fabrics with varying compositions and finishings. In addition, the test that was performed using daphnids and spermatozoa showed a higher level of sensitivity than the usual test. In comparison to toxicity inhibition tests, the express visual *Daphnia magna* test is assumed to be less complicated. This is because it does not call for the use of additional analyzers and is accessible to a large number of researchers. Daphnids, on the other hand, are exceedingly sensitive to even low quantities of the toxicant it is exposed to. A preliminary experiment must be carried out under the influence of several different concentrations of the test substance to ascertain the range of concentrations that are considered to be significant.
2. For the acute immobility test, the fundamental standard was ISO 6341, which was concerned with the measurement of water quality. To evaluate the harmful effects of textiles on the human body, the following test criteria are suggested: The test solution should consist of 0.07 g of textile sample in 1 mL of distilled water; each daphnid should be given 2 mL of this solution. The Pearson correlation analysis suggests waiting at least 24 or 48 h following the start of the test to find an acute immobility parameter (*Daphnia magna* test). The toxicity characteristics based on the results of the sperm test and the daphnids test showed the strongest association during these testing periods when compared with other bioassays.
3. The study resulted in the identification of the critical elements that adversely affect textile toxicity. The presence of pure formaldehyde had no significant effect on sperm motility, ruling out its potential role as a toxicity determinant in textiles. Compared to undyed fabrics, dyed fabrics exhibited a higher degree of toxicity to the organisms used in the bioassay. Regardless of the material's composition, dark and black-colored fabrics exhibited the greatest levels of toxicity. Furthermore, residual monomers may have contributed to the high level of toxicity seen in mixed fabrics incorporating synthetic fibres.

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Data Availability Statement

Data are available on request from the authors.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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