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The novel measuring method for screening and assessing chromium content in clothes and shoes materials

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Abstract. The aim of this paper is to propose the bioindicative measuring method for screening and assessing the safety of textile and leather materials in relation to chemical threats. This method is based on toxicological assay in which Tetrahymena pyriformis, unicellular organism belonging to protozoans, is used as a test organism. For the realization of the research goal the sensitivity threshold of test organisms to chromium(VI) solutions was identified. The changes in cell development of test organisms in chromium solutions were registered by colorimetric measurements in the presence of alamarBlue® cell viability reagent. Empirical data enabled to fit logistic curves on the base of which the level of chromium toxicity was estimated. In the second step, harmfulness of aqueous extracts obtained from textile and leather samples containing chromium in relation to test organisms was evaluated. The performed research confirmed the high efficiency of the proposed method in screening and assessing chromium content in clothes and shoes materials and showed possibilities of using it in safety assessment of products with regard to chemical risks.

1. Introduction

Clothes and shoes materials, having a direct contact with a human body, may irritate a skin or cause other health problems, due to the presence of harmful substances. The level of a hazardous impact depends on the type and amount of chemical substances contained within textiles as well as their susceptibility to release from a product during its usage. Due to known toxicity of some chemicals, used in textile and leather industries, their use is limited or banned by law or other regulations. One of such substance is chromium(VI) that could be a compound of chemicals (potassium dichromate, potassium chromate, sodium dichromate, and sodium chromate) used in leather tanning or textile finishing processes. In accordance with ecological criteria (EU Ecolabel and Oekotex) this element should be no present in the final product. Chromium(VI) substances are classified as carcinogenic (cat. 1b), mutagenic (cat. 1b) and skin sensitizing (cat. 1) [1]. Dermal exposure to chromium compounds can cause contact allergic dermatitis in sensitive individuals. It could be very caustic and can cause burns that can facilitate the absorption of the compound and lead to systemic toxicity. Longer-term occupational exposure to them is especially dangerous [2]. It should be underlined however that hazardous effect of chromium compounds on humans is rather known in relation to pure chemicals. There is a lack of sufficient evidences on a causal link between allergic or other undesirable reactions and textile and leather products, which contain chromium(VI) or its chemical mixtures. This issue still needs further investigations.

To assess safety of textiles in relation to chemical risks many laboratory test, which enable to detect different harmful substances have to be performed. These methods are time-consuming and
expensive, moreover they do not inform about harmful effect of measured substance on a man. Such information could be given from bioassays that are common toxicological tests applied in the broad range of fields. What’s more, they are powerful tool to estimate a chemical content in various media [3, 4] and well known in dermatology and hygiene [5, 6]. It seems to be highly reasonable to ascertain a usefulness of a bioassay in assessing threats posed by textile and leather materials that contain harmful chemicals, which is the main aim of described studies. For the realization of the research goal the sensitivity threshold of test organisms to chromium(VI) solutions was identified. In the second step, harmfulness of aqueous extracts obtained from textile and leather samples containing known amount of chromium, in relation to test organisms, was evaluated.

2. Materials and methods

2.1. Tetrahymena pyriformis as a test organism

_Tetrahymena pyriformis_, an unicellular organism was chosen as a bioindicator based on facts that it meets most requirements for test organisms and is one of bioindicators commonly used in laboratory tests. _T. pyriformis_ shows similar sensitivity as human cells culture and allows tendentious statements about the toxicity of substances with regard to human organism [7]. It is an organism with cell membrane of quite different structure than those of bacteria, yeasts or algae that form a barrier to toxic compounds. In the case of _Tetrahymena sp._ the interior of the cell is separated from its environment with a thin cell membrane only, thus causing that ciliates are very sensitive even to the trace presence of toxic compounds in its environment. In addition, in the terms of vital functions, cellular structures and also gene functionality, _Tetrahymena_ cells are more close to human cells that other model microorganisms [8, 9].

For toxicometric purposes the strain _Tetrahymena pyriformis_ (Ehrenberg) Wolff 1947 of reference number CCAP 1630/1W coming from the Culture Collection of Algae and Protozoa, Ambleside UK was used. The culture was incubated for 48 hours and being in "log" phase, i.e. at intensive growth stage, was used.

2.2. Solutions and extracts preparation

To assess the toxicity of the textile and leather materials, test organisms were initially cultured in potassium dichromate within the range of 1 to 100 mg/L solutions, recomputed into pure chromium and then in water extracts of two materials with the content of 1.9 mg/kg of Cr\(^{6+}\) in sample No. 1 and 757 mg/kg of Cr\(^{6+}\) in sample No. 2. Due to extremely high content of chromium(VI) in extract of the sample No. 2, extract was diluted with water to 75 %, 50 % and 25 % of its original concentration. This enabled conducting more detailed observations. The use of water as the extraction agent was to simulate the contact of wet materials with the human skin. When using clothes or other textile products it does happen that textile is wetted more or less occasionally. The second reason was associated with the fact that natural water contain a low concentration of reducing materials, so chromium(VI) compounds are more stable, while in the presence of oxidizable organic matter chromium(VI) compounds may be reduced to chromium(III) [1].

The pure metal ions solutions and material extracts were placed in 1.5 mL microcuvettes, and then test microorganisms suspended in agar were added, thus obtaining a cell density of approx. 5 000 cells/mL. The samples prepared were incubated at 28 °C for 6 and 24 hours and the optical density of cell culture in solutions was directly measured. To observe the changes in proliferation of test cells the alamarBlue\textsuperscript{®} cell viability reagent was used, as well and the response of test organism to chromium solution and water extracts of the samples was registered by colorimetric measurements of absorbance at 570 nm, using 600 nm as the reference wavelength.

2.3. Estimation of the toxicity level based on the regression of logistic function

To assess the toxicity level for chromium(VI), expressed by the half inhibitory concentration (IC\(_{50}\)) in relation to _Tetrahymena pyriformis_, the data obtained with colorimetric measurements were
transformed to a form enabling the percentage absorbance increase suppression coefficient in relation to the control group to be determined for solutions of the substance under investigation [10]. To compute values of this coefficient the logistic curves (1) were fitted:

\[ f(c) = \frac{1}{1 + \exp\{b\left[\ln c - \ln IC_{50}\right]\}\} \]  

(1)

where:
c - test substance concentration,  
b - regression coefficient (informs about the rate at which the transition from zero to total suppression (100 %) of bioindicator growth occurs. The lower this parameter, the faster transition),  
IC_{50} - quantitative measure that indicates the concentration of a substance that is needed to inhibit a cell growth by half, in comparison to the control group.

Taking up the values of Tetrahymena pyriformis culture growth inhibition in the extracts, the chromium ion concentration (c), estimated on the base of transformed logistic function (1), an attempt of computing the chromium(VI) content within textile and leather samples (C) was made (2):

\[ C = \frac{c \times V}{m} \]  

(2)

where:  
V - extract capacity, ml,  
m - sample mass, g,  
c - approximated concentration of a substance in extract (mg/L), computed on the base of inhibition rate of bioindicator growth, resulted from approximation of logistic function (3):

\[ c = IC_{50} \left(\frac{1-f(c)}{f(c)}\right)^{\frac{1}{b}} \]  

(3)

The statistical environment R was used for data analysis.

3. Results and discussion

3.1. Evaluation of sensitivity threshold of bioindicator to chromium(VI) solutions

At the first step of the experiment, the sensitivity threshold of Tetrahymena pyriformis to chromium(VI) solutions was established. The results of alamarBlue® colour changes in solutions of test organisms incubated in potassium dichromate for 6 and 24 hours, are illustrated in figure 1.

![Figure 1](image)

**Figure 1.** Color changes of alamarBlue® in solutions of Tetrahymena pyriformis cultures incubated in chromium solutions at concentrations 1 – 100 mg/L: a/ after 6 hours of incubation, b/ after 24 hours of incubation.
The red color characteristic of the group control and potassium dichromate concentrations indicates large amount and high vitality of test cells that completely reduced alamarBlue® cell viability reagent. For the solution at higher concentration (depending on incubation time) the color violet can be observed, thus leading to the conclusion that the solution contains live cells much less numerous and of considerably lower vitality that those of the control. In solutions at concentrations 50 mg/L (after 6 hours of incubation) or 15 mg/L (after 24 hours of incubation) the color blue predominates. This color is characteristic for non-reduced form of alamarBlue® and indicates lack of live cells or loss of its metabolic abilities. Color changes visible to the naked eye are accompanied by proliferation changes of *T. pyriformis* (table 1) evaluated on the base of colorimetric measurements.

**Table 1.** Effect of chromium concentration in solutions on the proliferation of *Tetrahymena pyriformis* computed from colorimetric measurements in the presence of alamarBlue® cell viability reagent.

| Incubation time in alamarBlue® | Percent of alamarBlue® reduction in potassium dichromate solutions | Concentration of Cr(VI), mg/L |
|-------------------------------|---------------------------------------------------------------|-----------------------------|
| Control group                 |                                                              |                             |
| 6 h                           | 100                                           | 1                           |
|                               | 88.5                                          | 5                           |
|                               | 87.0                                          | 15                          |
|                               | 68.5                                          | 25                          |
|                               | 66.0                                          | 50                          |
|                               | 51.7                                          | 75                          |
| 24 h                          | 100                                           | 75                          |
|                               | 98.6                                          | 15                          |
|                               | 65.5                                          | 30                          |
|                               | 39.8                                          | 15.7                        |

The values of vitality index evaluated as percentage difference between treated and untreated cells presented in Table 1 indicate that chromium concentration of 1 mg/L seems to be safe for *T. pyriformis*, when 5 mg/L inhibits the proliferation of cells to the level 65.5 % of the group control in case of long-term impact of chromium on bioindicator. Significant negative influence on cells proliferation shows solution of 15 mg/L concentration. At this concentration the proliferation rate reached only 39.8 % of the group control (24 hours incubation). The colorimetric measurements for higher chromium concentration in solutions, above 50 mg/L, displayed very low absorbance values resulted in low values of vitality indexes at the level: 30 – 51 % of the group control. These findings may testify not only about inhibition of cells' proliferation but about high cells' mortality, as well.

The basic characteristics of approximated functions are listed in table 2.

**Table 2.** Logistic parameters for potassium dichromate.

| Incubation time, h | Parameters of logistic function | 95% confidence level for IC50 | R²  |
|--------------------|---------------------------------|-------------------------------|-----|
|                    | obtained on the base of optical density measurement |                                |     |
| 6                  | b = -2.056, p <0.01             | IC50 = 24.042, p <0.01        | 0.99|
| 24                 | b = -0.931, p <0.01             | IC50 = 5.754, p <0.01         | 0.96|
|                    | obtained on the base of colorimetric measurements (m2) |                              |     |
| 6                  | b = -0.963, p <0.01             | IC50 = 33.841, p <0.01        | 0.91|
| 24                 | b = -0.860, p <0.01             | IC50 = 12.414, p <0.01        | 0.96|

The inhibition of cell proliferation at 50 % of the control group (IC50) was recorded in the concentration range from 20.8 to 44.9 Cr mg/L using 6 hour incubation time, while after 24 hour of incubation the IC50 was reached at significant lower concentration, i.e. between 4.69 and 18.6 mg/L. These results show that the harmful effect of chromium(VI) on *Tetrahymena p.* highly depends on the
length of time exposure of this element on living organisms. The prolong exposure causes strong negative effect on bioindicator even in very low concentration of Cr(VI) solutions.

3.2. Assessment of the textile and leather materials toxicity

At the second step of the experiment, the inhibition rate of *Tetrahymena pyriformis* culture growth in water extracts of textile and leather materials was analyzed. The observations of colour changes of alamarBlue® cell viability reagent, performed by naked eye, showed consistent with the expectations results, i.e.:

— the occurrence of the red colour in the extract of the sample No. 1, which indicates large amount and high vitality of test organisms and lack of toxic effect of this sample on bioindicator;

— the occurrence of the blue colour resulted from not reduced form of alamarBlue® reagent in the extract of the sample No. 2 and its solutions (figure 2), testifying about strong harmful effect of this sample on living cells.

When analyzing the results presented in table 3, it can be assumed that estimated levels of chromium content in textile and leather materials by bioindicative measuring method are at similar levels to those determined by using analytical method. With regard to the sample No. 2 the chromium(VI) content was estimated at the level from 668 to 902 mg/kg by proposed method, taking

![Figure 2. Color changes of alamarBlue® reagent in the extract solutions of the sample No. 2 (after 6h of incubation with *T. pyriformis*).](image)

Table 3. The result of measurements related to water extracts of materials.

| Extract                  | Extract solution | Inhibition rate [%] | Chromium content estimated on the based of bioassay |
|--------------------------|------------------|---------------------|----------------------------------------------------|
|                          |                  | Incubation time     | In extracts [mg/L] | In materials [mg/kg] |                                   |
|                          |                  | 6 h 24 h            | 6 h 24 h | 6 h 24 h |                                   |
| 1                        | 100 % solution of extract of sample No. 1 | 0 0 | ≤3.45 | ≤1 | ≤34.5 | ≤10 |
| 2                        | 100 % solution of extract of sample No. 2 | 100 100 | ≥75 | ≥75 | ≥750 | ≥750 |
| 3                        | 75 % solution of extract of sample No. 2 | 100 100 | ≥75 | ≥75 | ≥1000 | ≥1000 |
| 4                        | 50 % solution of extract of sample No. 2 | 53.4 75.2 | 40.0 | 45.1 | 800 | 902 |
| 5                        | 25 % solution of extract of sample No. 2 | 36.6 56.3 | 19.1 | 16.7 | 764 | 668 |
into account the different methods of spectrophotometric measurement and time of bioindicator exposure in extracts, while using analytical method the amount of this element was established at 757 mg/kg.

4. Conclusions
The results of performed experiment have shown high suitability of the bioindicative measuring method based on alamarBlue® assay and *Tetrahymena pyriformis* for screening and assessing toxicity of chromium(VI) comprised in textile and leather materials.

The performed experiment showed the low cytotoxic effect of the extract obtained from the sample No. 1 (textile sample with 1.9 mg/kg Cr(VI) content) on test organisms. The strong harmful effect was observed in relation to the sample No. 2 and resulted from high content of chromium (757 mg/kg) within this sample. It is worth noting that the values of chromium content within the samples, estimated on the base of bioindicative method, was similar to those obtained using the analytical method (EN 71-3).

Research on toxicity of textile extracts by means of the proposed bioindicative method using *Tetrahymena pyriformis* as bioindicator and colorimetric measurements performed in a present of alamarBlue® cell viability assay confirmed the high efficiency of the newly invented method in the safety assessment of textile products in relation to chemical risks.

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