Safety Evaluation of Deinked Pulp Containing Offset Thermochromic Inks

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Evaluating the safety of thermochromic inks for offset lithography in deinked pulp samples is a major area of investigation. In this study, three offset inks were analyzed – one that dries by absorption and two that dry by oxypolymerization of vegetable oils. Inks were printed separately on strips of white uncoated paper, and the prints were recycled by chemical deinking flotation. Thermochromic inks, handsheets, filter pads, and process waters obtained from deinking were tested for the presence of heavy metals, while concentrations of bisphenol A (BPA), total organic compounds, and antimicrobial agents were examined in handsheets and filter pads. The concentration of heavy metals cations was determined from ashes of undeinked and deinked pulp handsheets as well as from ashes of blank paper, flotation froth, and process water filtrates. BPA originates from thermochromic inks, and a 50% reduction of BPA was noticed in the samples after flotation. Considering the results, deinked pulp is undesirable due to the presence of BPA. Despite the presence of BPA, there was no release of toxic components from deinked pulp.

Keywords: Recycled paper; Thermochromic inks; Heavy metals; Bisphenol A; TOC; Antimicrobial properties

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INTRODUCTION

Recycled paper is an important source of fibers for paper production. The quality of recycled pulp decreases as the collection rate of recovered paper increases and as more "marginal" paper fractions are collected (Bobu *et al*. 2010; Pivnenko *et al*. 2015). Most white recovered paper grades are recycled by deinking flotation. Using surface-active substances, hydrophobic printing ink particles are removed from pulp suspensions by adhesion to air bubbles, forming a froth that can be easily separated from the suspension (Blanco *et al.* 2013). The deinkability of printed products mostly depends on the printing process used, the ink characteristics, and the age of printed products (Faul 2010).

Studies on paper-based food packaging materials have identified a wide range of substances that may be present in recycled pulp (Boccacci Mariani *et al.* 1999; Summerfield and Cooper 2001; Jamnicki *et al.* 2012; Pivnenko *et al.* 2015; Vápenka *et al.* 2016), which mostly originate from printing and converting processes undertaken in the previous use of the paper. Those substances remain in the solid matrix during paper recycling and thereby end up in new products based on recycled fibers. Binderup *et al*. (2002) cited a large list of potentially harmful substances found in recycled paper,

including phthalates, solvents, azocolorants, diisopropyl naphthalenes, primary aromatic amines, polycyclic aromatic hydrocarbons, and benzophenone. Moreover, certain paper and board fractions in recovered paper derived from households contain relatively high concentrations of chemicals such as mineral oil hydrocarbons, phthalates, phenols, polychlorinated biphenyls, and toxic metals (Cd, Co, Cr, Cu, Ni, and Pb) (Pivnenko *et al*. 2016). In order to maintain the high paper recycling rates with satisfactory quality of recycled pulp, the presence of chemicals should be taken into account, and it is crucial that paper recycling processes are highly effective in ink and toxic component removal. Some harmful substances can be removed from recycled pulp with new deinking methods. Jamnicki *et al*. (2015) showed that adsorption deinking is more successful in the reduction of mineral oils than conventional flotation deinking.

If recycled paper is used in food packaging, it must comply with many specific requirements proposed by the European legislation (EC 2004, 2006). These requirements refer to the control and analysis of contaminants in recycled paper and board such as heavy metals, plasticizers, aromatic amines, poly-aromatic hydrocarbons, benzophenone, and BPA (Mohammadpour *et al*. 2016).

In the printing industry, many different types of inks and printing processes are used. Even though all printing inks are composed of four main ingredients (colorant, vehicle or binder, solvent, and additives), they differ in chemical composition. In order to satisfy the market innovations, there is a greater demand for traditional and functional printing inks, such as thermochromic printing inks.

Thermochromic printing inks are temperature-sensitive inks that exhibit color change at different temperatures. They come in two forms: liquid crystals or leuco dyes. In the cool state, leuco dyes exhibit color, and when heated, they become clear or translucent. The temperature of the color change transition is called the activation temperature (TA) (Homola 2008). Moreover, some leuco dye thermochromic inks change from one color to another, rather than changing from colored to colorless state. This is achieved with an ink that combines a leuco dye with a conventional ink colorant formulation. Commercially available thermochromic printing inks based on leuco dyes consist of microencapsulated leuco dye–developer–solvent systems and a resin binder (Tang and Stylios 2006; Seeboth and Lotzsch 2013). The thermochromic effect is caused by the formation of leuco dye–developer complexes in a reversible equilibrium redox reaction between leuco dye and developer (Seeboth *et al.* 2007). The most common dyes are phenylmethane and fluoran derivatives bearing a lactone ring moiety. In the lactone ring-closed state, these leuco dyes are either colorless or weakly yellow colored. Leuco dye–developer–solvent systems are colored in the solid state and become transformed on heating above the melting temperature of the solvent into a colorless liquid (Seeboth and Lotzsch 2008). Color developers are usually weak acids (bisphenol A, octyl phydroxybenzoate, methyl p-hydroxybenzoate, 1,2,3-triazoles, and 4-hydroxycoumarin derivatives) that assist opening the lactone ring of the leuco dye and stabilize the open form, while long-chain alkyl alcohols, esters, and acids are commonly used as solvents (White and LeBlanc 1999; MacLaren and White 2003). Vinković *et al.* (2017) found the presence of bisphenol A and benzophenone in commercially available thermochromic printing inks.

Deinkability of three different offset thermochromic inks printed on white uncoated paper was described in Vukoje *et al*. (2016), which was part of our previous research, and it was evaluated by calculating the flotation yield, pulp's brightness and whiteness increase and the color properties, determination of residual ink area, as well as ash content elimination. The obtained results indicate that thermochromic prints are poorly deinkable

because only 12.8% of the total dirt area of thermochromic ink was removed by deinking flotation and small increases in deinked handsheets brightness and whiteness (approx. 5% and 2%) were noticed. Although the classic offset prints are generally highly deinkable, poor deinkability of thermochromic offset prints was explained by high adhesion of inks onto the paper, which was similar to observations for toner formulations that are known to be very difficult to deink by conventional flotation process (Vukoje *et al.* 2016).

Due to poor deinkability of thermochromic offset prints and the presence of different harmful substances in their structure, the objective of this study was to evaluate the safety aspect of the deinked pulp made from paper printed with thermochromic inks, *i.e.*, its possible use in food contact applications. As paper material is considered safe to be brought in direct contact with foods only if it is free from any substances that have toxic potential, aim of this study was, thus, to determine the concentration of heavy metals and BPA, total organic compound (TOC), total carbon (TC) in recycled pulp containing thermochromic inks, as well as transfer of antimicrobial constituents from the recycled paper. Where it was possible, the concentrations of determined chemicals were compared with the proposed limits set out in Industry Guideline for the Compliance of Paper and Board Materials and Articles for Food Contact developed by the European paper and board food packaging chain (CEPI/CITPA/CEFIC/FPE 2012).

EXPERIMENTAL

Materials

Paper

A white uncoated paper was used as a substrate for printing. The characteristics of this substrate are presented in Table 1.

Table 1. Properties of Paper Substrate

White Uncoated Paper							
Basis weight (g/m^2)	Thickness (mm)	Bulk (cm^3/g)	Smoothness (sec)	CIE Whiteness (%)	ISO Brightness (%)	Opacity (%)	$CaCO_3$ (%)
140	0.159	1.14	12.3	142.51	95.59	96.95	25.07

Thermochromic inks

Three offset thermochromic inks, which differed in their activation temperature, color, and chemical composition were used for printing (Table 2).

Table 2. Offset Thermochromic Inks

Thermochromic Ink	Producer	Abbreviation	Activation Temperature	Drying Mechanism
Blue to Colorless	CHAMELEON®	BW	27 °C	Absorption
Green to Yellow	CTI®	GY	45 °C	Oxypolymerization
Burgundy to Blue	CTI®	BB	63 °C	Oxypolymerization

All thermochromic inks had the ability to change their color above the mentioned activation temperatures (TA). For example, thermochromic offset ink (GY) was green-colored below its activation temperature (63 °C) and changed to yellow above its activation point. The color change was reversible, *i.e.*, the original color was restored upon cooling.

Methods

Offset printing

The printing trials were carried out using a Prüfbau Multipurpose Printability Testing System (MZ II, Peissenberg, Germany) as described in Vukoje *et al*. (2016). For each print, the same printing parameters were used, *i.e*., the same amount of ink was applied on the rollers, and the printing force was 600 N. For printing on the Prüfbau Multipurpose Printability Tester, 45 mm wide paper strips were prepared. The papers were printed in full tone. About 74% of the total area of paper strip was covered with ink. Input material for deinking consisted of three sets of prints, which were mixed in equal proportions. To each printed strip one unprinted strip of paper was added in order to avoid too high grammage of applied ink in relation to the total mass of paper submitted to deinking. Thus the total area of print was 37% when compared to the total area of used paper.

Flotation deinking

Deinking flotation of thermochromic prints was conducted under laboratory conditions, according to the INGEDE method 11p as previously described (Vukoje *et al.* 2016). Handsheets and filter pads of blank (B), undeinked pulp (UP), and deinked pulp (DP) were prepared according to INGEDE method 1. Blank paper (B) samples were formed after the pulping of the original unprinted paper. Samples from undeinked pulp (UP) were formed after pulping of printed paper samples (without conducting flotation process), while deinked samples (DP) were formed after pulping and flotation of printed paper samples.

Determination of heavy metal content using atomic absorption spectroscopy (AAS)

The concentrations of heavy metals (Pb, Cd, Cu, Zn, Fe, Mn, Ni, Cr, Co, As) were determined in thermochromic printing inks, samples obtained from deinking process, and water filtrate by the AAS method. The concentrations of heavy metal cations were determined from ashes of undeinked (UP) and deinked pulp (DP) handsheets as well as from ashes of blank paper sample (B) and flotation froth. First, 5 g of sample was burned in a porcelain pot by slowly heating the electric hot plate and burner until it was completely carbonized. The "black" sample in porcelain pot was afterwards placed into the muffle furnace and heated to 540 °C until the ash achieved a light grey-white color. The ash content in the porcelain pot was dissolved in 2 mL of 6 M HCl and filtrated through double filter paper into a 50 mL volumetric flask. Before the measurements, three different known concentrations of tested metals were prepared to make calibration curves. Results were recorded by the Varian SpectrAA 220 atomic absorption spectrometer (Mulgrave, Victoria, Australia). The heavy metals concentration in process water filtrates were determined by the same procedure but without ignition pretreatment.

Determination of Bisphenol A concentrations

Bisphenol A was determined in filter pad samples made from undeinked (UP) and deinked pulp (DP) as well as in blank paper samples (B). Paper samples were cut in pieces, and 50 mL of solvent (methanol:water = 1:1) was added to 1 g of sample. The extraction

was performed in Soxtherm (O.I. Analytical, College Station, TX, USA) for 4 h.

Determination of total organic compound (TOC) and Total carbon (TC)

Total organic compound (TOC) and total carbon (TC) were determined in filter pad samples using TOC-VCSH analyzer with unit for solid samples SSM-5000A (Shimadzu, Kyoto, Japan).

Determination of the transfer of antimicrobial constituents

The handsheets obtained from the deinking process (B, UP, DP) were tested for their effect on the growth of bacteria *Bacillus subtilis* and fungus *Aspergillus niger*. The test determined whether the paper samples release antimicrobial constituents in quantities that inhibit the growth of the bacteria and fungus, which represents the toxicity of tested papers. Paper samples were cut to a shape of discs having 6 mm in diameter. Microbiological tests were performed according to HR EN 1104 (2002), in two ways:

1. *Bacillus subtilis* suspension (1 mL at 10^4 cell/mL) was transferred to three Petri dishes and flushed with nutrient agar (NA). The paper disc was placed in the middle of the dish. The same procedure was repeated for *Aspergillus niger* (10^5 cell/mL) with malt agar (MA) as the growing media.
2. In three Petri dishes containing nutrient agar (NA), 0.1 mL of *Bacillus subtilis* suspension (10^5 cell/mL) was added and homogenized with a Drigalski spatula. The paper disc was placed in the middle of the dish. The same procedure was repeated for *Aspergillus niger* (10^6 cell/mL) with malt agar (MA) as the growing media. Dishes were incubated for 3 to 5 days at 37 °C for bacteria and at 28 °C for fungus.

After incubation, an inhibition zone (in the growth of flora) indicated the release of antimicrobial constituents. A *Bacillus subtilis* penicillin sensitivity test was performed to confirm the results.

RESULTS AND DISCUSSION

Determination of Heavy Metals

Table 3 shows the obtained concentrations of heavy metals in the tested thermochromic inks. BW ink contained the highest concentration of Fe (20 mg/kg). In printing inks, iron is used in the form of iron oxide yellows (yellow pigment). Exceptional heat resistance makes them suitable for use in printed furniture surfaces and other high light specifications (Leach *et al.* 2007). The GY ink exhibited the highest concentrations of Co (500 mg/kg) and Mn (950 mg/kg). The GY ink contains vegetable oil as ink vehicles (Vukoje *et al*. 2017). Co and Mn salts are used as catalysts (siccatives) for oxypolymerization drying of vegetable oil. Moreover, in GY ink, notably lower concentrations of Fe (7.90 mg/kg) and Zn (3.80 mg/kg) were observed. In printing inks, Zn is used as white pigment and extender in the form of zinc white (ZnO) and zinc sulphide (ZnS). ZnO is a strong UV absorber and self-fungicidal. It discolors with copper compounds, and it becomes yellow when hot or white when cold. It is used as a white extender in many inks (Leach *et al.* 2007). GY ink had the highest concentration of Cr (0.26 mg/kg). Concentrations of other cations were less than 1 mg/kg of ink. In the BB ink, the highest concentrations of Co (700 mg/kg) and Mn (1100 mg/kg) were measured. Zn and Fe were present at concentrations of 1.80 and 10.0 mg/kg, while other cations were present at concentrations lower than 1 mg/kg. Thermochromic offset printing inks contain

remarkably lower amounts of heavy metals than digital offset printing inks (Barbaric-Mikocevic *et al*. 2004).

Table 3. Concentration of Heavy Metals in Used Thermochromic Offset Inks

Heavy Metals (mg/kg)	Thermochromic Printing Inks		
	BW	GY	BB
Pb	0.20	0.20	< 0.10
Cd	< 0.01	< 0.01	< 0.10
Cu	0.53	0.45	0.45
Zn	1.60	3.80	1.80
Fe	20.0	7.90	10.0
Mn	0.35	950	1100
Ni	1.30	0.34	0.44
Cr	0.12	0.26	0.14
Co	0.03	500	700
As	0.01	0.01	< 0.01

According to Rožić *et al.* (2005), certain cations present in paper can cause problems in paper usage or in paper recycling. Metal (Fe, Cu, and Mn) hydroxides/oxides in various additives catalyze paper acidification. During paper recycling, multivalent metal cations reduce swelling of fiber, due to reduction of electrostatic double layer at surfaces within the fiber microstructure. Reduced swelling inhibits bonding of fibers to each other and therefore paper sheets become weak. Taking into account that additives for paper production are not of high chemical purity, it is possible that some elements are introduced as impurities in calcium carbonate, which is used as filler (Rožić *et al.* 2005). Mertoglu-Elmas (2017) showed that presence of heavy metals in recycled corrugated board papers originates from chemical additives used in the process of pulp and paper manufacturing, as well as the finishing operations of paper, such as Pb, Cd, Zn, and Cu derived from colored pigments.

Table 4 shows the obtained concentrations of heavy metals in handsheet ashes of blank (B), before (UP) and after (DP) flotation, flotation froth, and process water filtrates that originated from blank (paper + deinking chemicals) and after flotation samples. The B sample contained the highest concentrations of Zn (144 mg/kg) and Fe (78.2 mg/kg), while the concentration was 7.10 mg/kg for Co and 12.2 mg/kg for Mn. Due to very high concentrations of Zn and Fe in B sample, it may be concluded that printing substrate is the main source of these metals. The high concentration of Zn can be explained by the Zn-based compounds used in paper production as fillers for the enchainment of some optical properties of paper such as opacity (Mertoglu-Elmas 2017). The UP sample contained slightly higher concentrations of Zn (165 mg/kg), Mn (16.2 mg/kg), Ni (0.87 mg/kg), and Co (11.7 mg/kg) than the DP sample. Larger concentrations of these cations are the result of thermochromic inks. The Pb, Cd, Cu, and Fe concentrations were lower in the UP and DP samples than in the B sample. Zn, Fe, Mn, and Co concentrations were reduced in the obtained handsheet after flotation (DP). However, in this sample (DP), the concentrations of Zn, Ni, Mn, and Co were greater than in the B sample due to print particles retention (*i.e.*, poor deinkability). Moreover, the determined quantities of Pb and Cd metals in the B, UP, and DP samples were all below maximum permitted limits in the CEPI/CITPA/CEFIC/FPE Industry Guideline. Even though current EU legislation on paper and board food contact materials, at the moment, does not provide specific migration limits for other determined heavy metals, the authors believe that the levels of determined

heavy metals (Cu, Zn, Fe, Mn, Ni, Cr, Co, As) could be of potential threat especially if recycled paper is to be brought in direct contact with moist and/or fatty food.

In our previous research, microscopic imaging revealed that flotation froth mostly contained ink particles originating from GY and BB prints, while the BW ink particles were observed in a smaller amount. Undeinked pulp (UP) handsheet contained mostly BB and GY ink particles while deinked pulp (DP) handsheet was cleaner but also contained mainly BB and GY ink particles. Since only small amounts of BW ink residues were present in all samples, their poor visibility was due to deformation and because of their mechanical damage during pulping. It was also assumed that BW ink particles (microcapsules) were retained in aqueous suspension (Vukoje *et al.* 2016). As deinking froth contained mainly short fibers, fillers, ink particles, and deinking additives, the highest concentrations of Zn, Mn, and Co that were found in GY and BB inks, were also present in filtration froth filter pad. A high concentration of Zn cations in filtration froth filter pad may originate from additives present in paper.

Determined concentrations of cations in process waters show that deinking does not affect heavy metals release from thermochromic inks. In process water filtrates, Zn and Fe were present in very low concentrations, while the concentration of other heavy metals was negligible (Table 4). Since thermochromic ink particles are difficult to separate from the pulp suspension, the release of heavy metals in process water is minimal.

Table 4. Concentration of Heavy Metals in Handsheets (B, UP, DP) and Process Water Filtrates Obtained from Blank and After Flotation

Heavy Metals (mg/kg)	Maximum Permitted Quantity* (mg/kg)	Samples Obtained from Deinking Process				Process Water Filtrate	
		Blank (B)	Undeinked pulp (UP)	Deinked pulp (DP)	Filter pad – flotation froth	Blank (chemicals and paper)	After flotation
Pb	3.0 **	2.60	1.70	1.60	4.20	0.01	<0.01
Cd	0.5 **	0.12	0.09	0.09	0.23	<0.01	<0.01
Cu	not established	2.60	2.40	2.50	4.90	0.01	0.01
Zn	not established	144	165	147	458	0.15	0.33
Fe	not established	78.2	75.9	71.2	142	0.08	0.17
Mn	not established	12.2	16.2	15.4	32.1	0.02	0.02
Ni	not established	0.67	0.87	0.79	1.75	<0.01	<0.01
Cr	not established	1.85	1.90	1.85	3.80	<0.01	<0.01
Co	not established	7.10	11.7	10.7	24.7	0.01	0.02
As	not established	<0.01	<0.01	0.01	0.01	<0.01	<0.01

* Purity requirements set out in the CEPI/CITPA/CEFIC/FPE Industry guideline.

** The concentration of these metals is usually determined in an aqueous extract according to EN 12498. Also, the materials are subjected to tests only if, in normal circumstances, the end use of the paper and board is known to be for contact with moist and/or fatty food.

Results imply that printing substrate is the main source of heavy metals concentrations (Fe, Zn) in the process waters produced by deinking flotation. In addition to this conclusion, it can also be assumed that Fe concentrations in process water may be result of BW thermochromic ink residues present in pulp suspension as described in previous research by Vukoje *et al.* (2016). Concerning the low concentrations of heavy metals in process water, it can be reused and returned into the recycling process.

Determination of BPA, TOC, and TC Content

Table 5 shows the concentrations of BPA, TOC, and TC in filter pad samples. Because no BPA was found in the blank sample, it was concluded that the BPA originated from the thermochromic printing inks. A relatively large reduction in BPA concentration was observed. Filter pads obtained after flotation contained 50% less BPA than the undeinked filter pads. Vinković *et al.* (2017) examined the BPA concentrations in commercially available printing inks where, among others, they also analyzed the same inks we used in this research. Their results showed that BPA was present only in BW thermochromic ink, while GY and BB inks did not contain any BPA.

A slight reduction of TOC and TC in filter pads after flotation compared with those obtained before flotation indicated a very slight decrease in the organic content. From the obtained results it can be concluded that TOC in samples originates from paper, *i.e.* cellulose, while only a small percentage is related to thermochromic printing inks. Small amount of inorganic carbon results from fillers (calcium carbonate) in paper. In our previous study, process water filtrates were examined in terms of TOC and chemical oxygen demand (COD). The obtained values for process water after flotation showed higher organic matter loads than the blank (Vukoje *et al*. 2016).

Table 5. Concentration of BPA, TOC, and TC in Obtained Filter Pads

Filter Pad Sample	BPA (mg/kg)	TOC (%)	TC (%)
Blank (B)	0	35.8	40.0
Undeinked pulp (UP)	15	37.2	40.8
Deinked pulp (DP)	7.5	36.1	40.2

Purity requirements set out in the CEPI/CITPA/CEFIC/FPE Industry guideline specify the limit value for BPA, which is shown in Table 6. However, the latest risk assessment by the European Food Safety Authority (EFSA) on the use of BPA (Bolognesi *et al.* 2015) stimulated the European Commission to strengthen the regulation on the use of BPA in food contact materials (FCMs). A draft regulation by the European Commission's (EC) Directorate-General for Health and Food Safety (DG SANTE) concerning the regulation of BPA in FCMs lowers the specific migration limit (SML) for food contact plastics from 0.6 mg/kg to 0.05 mg/kg (EC 2016). Because the existing SML value for BPA as stated in Industry guideline is derived from the current Regulation EC) 10/2011 on plastics FCMs, it is assumed that new lower SML values for BPA will also be adopted in the paper and board FCM regulations.

The conversion of BPA concentrations to the proposed limits presented in Table 6 was done by taking into account the actual grammage of analyzed filter pads (225 g/m^2) according to Eq. 1.

$$Qa = (Qm \times G)/100000 \quad (1)$$

where *Qa* is the concentration of substance in paper expressed as mg/dm², *Qm* is the concentration of substance in paper expressed as mg/kg, and *G* is the grammage of paper as expressed as g/m². *Qa* is the maximum quantity of the contaminant allowed in the packaging, if it is assumed that 100% of it will migrate into the food. The BPA content of the analyzed filter pads expressed as mg/dm^2 of material is presented in Table 6.

Table 6. Purity Requirements for BPA in Food Contact Paper and Board and Concentrations of BPA in Filter Pads Obtained from Deinking Process

Substance	Limit in Food	Tested in Paper and Board	Filter Pad Sample Measured concentrations of BPA (mg/dm^2)		
	SML (mg/kg food)	Limit (mg/dm^2)	Blank (B)	Undeinked pulp (UP)	Deinked pulp (DP)
BPA	0.6	0.1	0.0	0.034	0.017
New reduced limit for BPA (from draft regulation)	0.05	0.008			

The converted results indicated that the BPA in the analyzed filter pads obtained before and after deinking flotation were below the existing limits as set in Industry Guideline and current Regulation (EC) 10/2011 on plastics FCMs. However, under the stricter and likely new regulation limits, both analyzed filter pads failed, as the detected concentrations were above the permitted limits (Table 6). The results also showed that deinking flotation did not sufficiently eliminate the BPA content from the pulp to meet the draft regulation's new purity requirements. The presence of BPA in recycled pulp can be of major concern if the paper printed with thermochromic ink is used for production of paperboard where only pulping of paper is conducted, without performance of flotation process. Based on the obtained results for undeinked pulp (UP), it would mean even greater concentrations of BPA that would be left in recycled pulp. Based on the obtained results, in order to improve removal of thermochromic prints residues and toxic compounds, other recycling methods should be investigated.

Determination of Antimicrobial Properties

Components present in paper as fillers (Zn oxide, titanium oxide, heavy metals) or components of thermochromic inks (bisphenol A, heavy metals) may show antimicrobial activities (Munoz-Bonilla and Fernández-García 2012; Lemire *et al.* 2013). In order to examine release and possible transfer of the antimicrobial constituents from recycled paper pulp, microbiological tests were performed.

The total number of sporadic *Aspergillus niger* (Fig. 1a) was determined by counting in a Thomson Chamber and was 1.3×10^7 cell/mL. The total number of *Bacillus subtilis* cells in 1 mL was 1.4×10^9 cell/mL (Fig. 1b). The exposure of *Bacillus subtilis* to penicillin resulted in the formation of a 4-cm diameter inhibition zone (Fig. 1c).

Samples of B, UP, and DP were tested with *Bacillus subtilis* (Fig. 2) and *Aspergillus niger* (Fig. 3). There were no inhibition zones around the paper discs, indicating that the samples did not inhibit microbial growth and that there was no release of any toxic substances from recycled paper pulp. On malt agar, almost no discs were observed because of fungus overgrowth (Fig. 3).

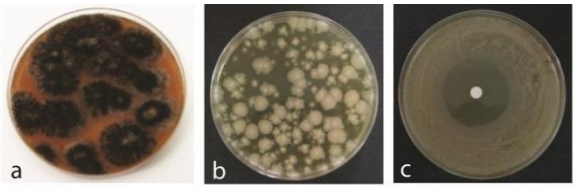

Fig. 1. Growth of fungus *Aspergillus niger* (a) and bacteria *Bacillus subtilis* (b); inhibition of *Bacillus subtilis* by penicillin (c)

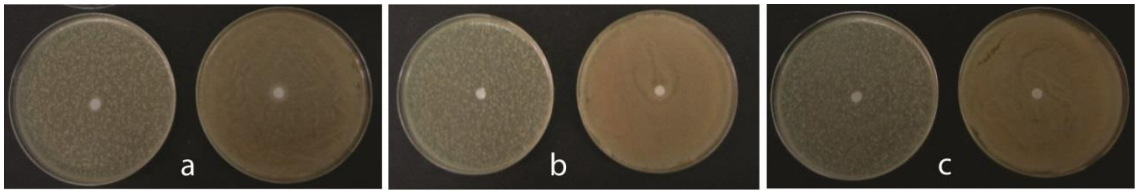

Fig. 2. Sensitivity test for bacteria *Bacillus subtilis* in samples B (a), UP (b), and DP (c). In each panel: (left) 1 mL of suspension flushed with s NA; (right): 0.1 mL of suspension homogenized with a Drigalski spatula

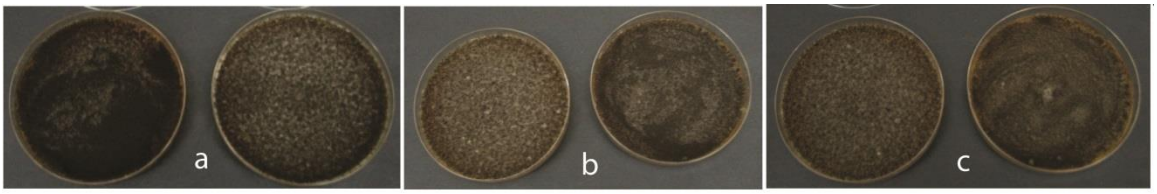

Fig. 3. Sensitivity test for the fungus *Aspergillus niger* in samples B (a), UP (b), and DP (c). In each panel: (left) 1 mL of suspension flushed with s NA; (right): 0.1 mL of suspension homogenized with a Drigalski spatula

CONCLUSIONS

1. Thermochromic printing ink dried by absorption (BW) contained only Fe in higher concentrations, while the other two oxypolymerization drying inks (GY and BB) contain notably higher concentrations of Co and Mn (that originate from their drying catalysts or siccatives).
2. The higher concentrations of Zn and Fe in all samples (B, UP, DP and process water filtrates) originate from printing substrate. The concentrations of Mn and Co in the DP handsheet and filtration froth originate from drying catalysts present in GY and BB inks.
3. Considering very low concentrations of heavy metals in process waters, they can be reused in recycling process.
4. BPA originated from BW thermochromic ink. The reduction of only 50% of BPA was noticed in the samples after flotation, which can be explained due to poor deinkability of thermochromic inks.
5. According to new stricter limits of maximum permitted quantity of BPA in FCMs, both analyzed samples failed the purity requirements, as both detected concentrations were

above maximum permitted limits.

6. The growth of *Bacillus subtilis* and *Aspergillus niger* was not inhibited in all tested samples (B, UP, DP), indicating that there was no release of toxic substances, despite the presence of BPA.

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