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Impacts of discarded coffee waste on human and environmental health

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ABSTRACT

Coffee is one of the most widely consumed beverages throughout the world. So far, many studies have shown the properties of coffee beverages, but little is known about its impacts on human and environmental health from its discard in the environment. So, the present work aims to investigate the mutagenic, genotoxic, cytotoxic and ecotoxic effects of leached (LE) and solubilized (SE) extracts from coffee waste, simulating the disposal of this residue in landfills and via sewage systems, respectively. Chemical analyses were also carried out. LE and SE induced mutagenicity in the TA98 Salmonella strain with and without exogenous metabolization (S9). In the T A100 only SE induced mutagenicity, what was observed without S9. An increase in the frequency of micronuclei was observed in HepG2 cell line after 3 and 24 h of exposure to both extracts. No cytotoxic effects were observed in HepG2 cells by WST-1 assay. The EC50 values for the LE and SE were 1.5% and 11.26% for Daphnia similis, 0.12% and 1.39% for Ceriodaphnia dubia and 6.0% and 5.5% for Vibrio fischeri, respectively. Caffeine and several transition metals were found in both extracts. Coffee waste discarded in the environment may pose a risk to human and environmental health, since this compound can cause DNA damage and present toxicity to aquatic organisms.

1. Introduction

Coffee has been consumed for over 1000 years and is currently one of the most widely consumed beverages around the world. According to the International Coffee Organization more than 8 million tons of this compound were consumed worldwide in 2014 (International Coffee Organization, 2015). Two species are of significant economic importance Coffea arabica (Arabica) providing 75% of the world production and Coffea canephora (Robusta), which provides 25% of the world production (Varnam, Sutherland, 1994; Belitz et al., 2009).

So far, many studies have shown the properties of coffee beverages, such as antioxidant (Gómez-Ruiz et al., 2007), antibacterial (Meckelburg et al., 2014), anti-inflammatory and anti-obesity properties (Jia et al., 2014), and effects on type 2 diabetes mellitus (Akash et al., 2014), amongst several others, but little is known about the impacts on human and environmental health from its disposal in the environment.

During the extraction of the beverage from coffee powder with hot water, a large amount of residue is produced, and considering the worldwide coffee consumption, it can be concluded that tons of coffee waste are generated from cafeterias and domestic production (Tokimoto et al., 2005). The exact values of the quantities discarded have not been established, but can be estimated based on the values generated by industry. This sector of the economy consumes about 50% of the world's coffee in the production of instant coffee, generating about 6 million tons of coffee waste annually (Tokimoto et al., 2005). Thus the amount of coffee waste generated from cafeterias and domestic production must be huge.

Some applications for the use of these residues have been speculated, but most remain unused and are discarded into the environment possibly, endangering human and environment health (Leifa et al., 2000).

Considering cafeterias and domestic production, the waste is mainly discarded in the trash and then sent to a landfill. Another way to discard it is down the sink, where the residue can reach water bodies through the sewage.

Thus in view of the large amount of coffee waste generated from coffee beverage making process and the lack of studies about the impact of this residue on human and environment health, this work aimed to investigate the mutagenic, genotoxic, cytotoxic and ecotoxic effects of

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leached and solubilized extracts from coffee waste, simulating the disposal of this residue in landfills and via the sewage, respectively, besides identifying the physicochemical properties and chemical constitution of these samples.

2. Material and methods

2.1. Sampling

The leached and solubilized extracts were prepared according to the Brazilian Association of Technical Standards 10005 and 10006, respectively (Brazilian Association of Technical Standards, 2004a, 2004b).

To prepare the leached extract, 50.0 g samples of traditional Pilão® coffee waste (about 70% Arabica and 30% Robusta, grain size < 9.5 mm) were transferred to polyethylene bottles and treated with 1.0 L of the extraction solution (5.7 mL of glacial acetic acid, 64.3 mL of 1.0 mol/L NaOH and 930 mL of Milli-Q water). The bottles were closed and shaken for about 18 h using a rotary shaker at 100 rpm (Nova Etica® model 430) at room temperature, and then filtered through a 0.45 μm membrane.

To prepare the solubilized extract, 25 g samples of Pilão® coffee waste were transferred to polyethylene bottles and treated with 100.0 mL of ultra-pure water. The bottles were closed, shaken for 5 min using a rotary shaker at room temperature and then left standing for 7 days before filtering through a 0.45 μm membrane.

2.2. Physicochemical analysis

The physicochemical parameters such as TDS (total dissolved solids), salinity and conductivity were determined in the leached and solubilized extracts using a multi-parameter analyzer (PCS Tester 35 – OAKTON).

2.3. Chemical analysis

2.3.1. Flame atomic absorption spectrometry – FAAS

For the metal analyses, 4 mL samples of leachate and solubilized extract were transferred to microwave vessels and treated with 1.0 mL of HNO_3 (P.A. Sigma) and 1 mL of H_2O_2 (30%, Vetec). The vessels were then placed inside the rotor of a microwave digestion system, sealed, tightened using a torque wrench and finally submitted to a microwave dissolution program for 4 min. After cooling, the digest contents were quantitatively transferred to a polyethylene bottle, diluted to 100 mL with Milli-Q water and stored at 4 ∘C until analyzed. The leached and solubilized extracts were analyzed for their metal contents by standard methods (American Public Health Association, 1998) using flame atomic absorption spectrometry – FAAS (SpectrAA-240, Varian, Australia).

2.3.2. Gas chromatography–mass spectrometry (GC–MS)

For the organic analysis, 100 mL of the leached and solubilized extracts were extracted three times with 30 mL n-hexane (HPLC degree, Tedia, USA). The organic phase was removed and 2 μL injected into the gas chromatograph–mass spectrometer GC–MS (456 GC – MS-TQ, Bruker Daltonics, Inc., Germany). This procedure was carried out in duplicate and with an analytical blank.

Chromatographic separation was achieved using a BR-5MS fused-silica capillary column (30 m × 0.25 mm with a 0.25 μm thick film), using helium as the carrier gas at 0.6 mL/min in the constant flow rate mode. The injections were made in the splitless mode. The MSD was operated by electronic impact (70 eV) in the scanning mode (40–400 *m/z*). The injector port was at 250 °C and the interface temperature was 280 °C. The oven temperature was first maintained at 60 °C, then increased to 240 °C at 3 °C/min, and held at this temperature for 2 min. The components were identified by comparison of their retention times and mass spectra with the corresponding data of reference compounds and by comparison of their mass spectra with those in the NIST libraries. This procedure was carried out in duplicate and with the analytical blank.

2.4. Salmonella/microsome mutagenicity assay

The Salmonella/microsome assay was carried out to evaluate mutagenic effects, using TA98 (hisD3052, rfa, Δbio, ΔuvrB, and pKM101) and TA100 (hisG46, rfa, Δbio, ΔuvrB, and pKM101) Salmonella typhimurium strains in the presence and absence of exogenous metabolization activation (S9), according to the International Organization for Standardization (ISO) protocol no. 16240 (International Organization for Standardization, 2005; Maron, Ames, 1983; Mortelmans, Zeiger, 2000). Briefly, 100 μL of overnight cultures of each strain, 500 μL of 0.1 M sodium phosphate buffer or S9 mix, 1 mL of different dilutions of the extracts in deionized water (100%, 50%, 25%, 12.5% and 6.25%) and 1 mL of molten soft agar were mixed and poured into a minimal agar plate. The plates were incubated at 37 °C for about 70 h and each experiment was carried out in triplicate. Deionized water was used as the negative control. For the TA98 and TA100 strains, the positive controls in the absence of S9 were 1.0 μg/plate of 4-nitroquinoline-1-oxide (4NQO; Sigma, St Louis, MO, USA) and 5.0 μg/plate of sodium azide (AS; Sigma, St Louis, MO, USA), respectively. In the presence of the S9 mix, 3.0 μg/plate of 2-aminoanthracene (2AA; Aldrich, Seelze, Germany) was added.

2.5. Micronucleus (MN) genotoxicity assay

The human hepatocellular carcinoma cell line HepG2 was kindly provided by Dr. Danielle Palma de Oliveira, Faculty of Pharmaceutical Sciences of Ribeirão Preto, São Paulo, Brazil. The cells were maintained in Eagle's Minimum Essential Medium (MEM) with 1.8 mM Ca^{++}, pH 7.4 (Gibco), and supplemented with 1.76 g/L $NaHCO_3$, 0.88 g/L pyruvate, 21.6 mg/L aspartic acid, 16.8 mg/L L-serine, 1% penicillin–streptomycin solution and 10% (v/v) heat-inactivated FBS. The cells were incubated at 37 °C with 5% CO_2 and a relative humidity (RH) of 95%. When the cells reached 80% confluence, the culture medium was removed the cells detached using 0.25% Trypsin-EDTA (Sigma Chemical Co.) for 5 min at 37 °C, centrifuged at 1500 rpm for 5 min, and the supernatant then removed. The cells were suspended in 1 mL medium supplemented with FBS (10%), and 1.0×10^5 cells/mL seeded into a 24well microplate containing a coverslip that had been pre-treated with 0.1 M nitric acid, and maintained at 37 °C with 5% CO_2 and 95% RH. After 24 h, the cells were treated with 100 μL each of the different dilutions of the leached or solubilized extracts in MEM supplemented with 10% FBS (6.25%, 12.5%, 25%, 50% and 100%), and the plates incubated for 3 and 24 h. After each incubation period, the cells were rinsed twice with MEM and cultured in fresh culture medium. After 24 h at 37°C, 5% CO_2 and 95% RH, the medium was discarded and the cells fixed with glacial acetic acid–methanol (1:3) fixative (Cornoy's fixative). The fixed cells were rinsed twice with McIlvaine's buffer (MI buffer: 21.01 g/L citric acid and 35.60 g/L Na_2HPO_4, pH 7.5), dried at room temperature and then stained for 40 min with 4′−6-diamidino-2-phenylindole (DAPI) (0.2 μg/mL) dissolved in MI buffer. The number of cells with micronuclei (2000 cells per dilution of the extracts) was determined in a fluorescence microscope (Reichert Univar) with an excitation wavelength of 350 nm to determine the induction of micronuclei. The negative control used was MEM supplemented with FBS (10%) and the positive control was N-methyl-N-nitro-N-nitrosoguanidine (MNNG) at a concentration of 0.5 mM. The MN test was carried out according to OECD guideline no. 487 (OECD, 2010) as described by Fenech (2000). All experiments were carried out in triplicate and repeated twice.

2.6. WST-1 cell viability assay

The viability of the HepG2 cell was measured using a colorimetric assay for 96-well plates containing 2-(4-iodophenyl) − 3-(4-nitrophenyl) − 5-(2,4-disulfophenyl) − 2H-tetrazolium monosodium salt (WST-1, Roche Applied Science). The assay was carried out according to the manufacturer's instructions. Briefly cells were seeded in the 96-well microplate at a concentration of 1.0×10^4 cells/mL in MEM supplemented with 10% FBS, and cultivated for 24 h at 37 °C with 5% CO_2 and 95% RH. The cells were then treated with100 µL of different dilutions of the leached or solubilized extracts in MEM supplemented with 10% FBS (6.25%, 12.5%, 25%, 50% and 100%) and the plates incubated for 3 and 24 h. After each incubation period, the dilutions were removed and replaced with fresh MEM supplemented with FBS (10%), 10 µL of WST-1 added, and the cells incubated for 4 h at 37 °C and 5% CO_2. The cell viability was measured at 450 nm in a microplate reader (µ-Quant, Bio-tek Instruments INC). The absorbance was proportional to the amount of dehydrogenase activity in the cell. Higher absorbance values are associated with an increased production of the formazan product (Weir, Xu, 2010). Cell viability was expressed as the percentage cell viability as compared to the controls. All incubations, including the controls and blanks, were carried out in triplicate and repeated twice.

2.7. Daphnia similis acute ecotoxicity assay

Acute toxicity tests with Daphnia similis were carried out according to the ABNT-NBR no. 12713 (Brazilian Association of Technical Standards, 2016). For each concentration, four replicates containing 5 neonates (6–24 h old) and 10 mL of sample diluted with soft water (hardness 44 mg/L as $CaCO_3$, pH 7.2) were prepared. The concentrations ranged from 6.25% to 100% for the solubilized extract, and from 0.19% to 100% for the leached extract. During the exposure time, the organisms were maintained at 20 ± 1 °C under a 12 h/12 h photoperiod and static conditions. After 48 h, the immobile daphnids were counted and recorded.

2.8. Ceriodaphnia dubia chronic ecotoxicity assay

Chronic toxicity tests with Ceriodaphnia dubia were carried out according to ABNT-NBR no. 13373 (Brazilian Association of Technical Standards, 2010). This method aims to measure the influence of the sample on fecundity of the organism. Ten neonates (6–24 h old) from 2 to 3 week-old mothers were placed one by one in 15 mL of test-solution in flasks, one for each concentration. The organisms were maintained in this static renewal test for 7 days at 24 ± 1 °C under a 12 h/12 h photoperiod. The number of neonates per replicate was counted and recorded daily and the organisms fed 3×10^5 cells/organism of Monoraphidiun dybowskii algae and 0.025 mL/organism/day of fish food Tetramin® suspension (10 g/L). The test-solutions were renewed on alternate days.

2.9. Microtox® acute assay

The assays were carried out according to ABNT-NBR no. 15411-3 (Brazilian Association of Technical Standards, 2012), using the analyzer Microtox®M500 with the Basic Test Protocol configuration (81.9%). The samples were first adjusted to 2% NaCl and pH 7.0 and a series of diluted samples prepared in 2% NaCl (2.8% to 90% for both extracts). These solutions were added to Vibrio fischeri bacterial suspensions (10^6 cells/replicate), purchased from BIOLUX® (batch103 Lyo 5), at 4 °C. Light emission was measured before and after 15 and 30 min of exposure. A 2% NaCl solution was used as the negative control.

2.10. Statistical analysis

For the Salmonella/microsome assay, ANOVA and a significant dose response were obtained using the Bernstein model (Bernstein et al., 1982), which uses both linear regression and ANOVA. For the MN and WST-1 assays, the results obtained were compared with the values obtained for the negative control using a one way variance analysis (ANOVA) followed by Tukey's HSD post hoc test. EC_{50} (median effect concentration) values for Daphnia similis were estimated using the Trimmed Spearman–Karber statistical method according to Hamilton et al. (1977). For the Ceriodaphnia dubia chronic ecotoxicity assay, the results were analyzed for significance from the negative control using a one way ANOVA and Dunnett's multiple comparison test to indicate the NOEC (no observed effect concentration) and LOEC (lowest observed effect concentration). For the Microtox® assay, the data were processed using the Microtox Omni Software, according to the Basic Test Protocol (90%), where toxicity was measured as the percent inhibition of light emission during exposure to the sample, corrected for loss of light in the control. The Effective Concentration (EC_{50}) was designated as the concentration of the sample (%) which produced a 50% decrease in light after exposure for 15 min.

3. Results

3.1. Physicochemical analysis

Table 1 shows the physicochemical properties of the leached and solubilized extracts. It can be seen that the leached extract showed higher conductivity, salinity and total dissolved solids (TDS) than the solubilized extract.

3.2. Chemical analysis

Table 2 shows the metals detected in the leached and solubilized coffee waste extracts.

According to the United States Environmental Protection Agency a material is considered as a hazardous waste if any metal detected is present at concentrations greater than 100 times those of the national primary drinking water standards (Arulrajah et al., 2014; Environmental Protection Agency, 1999). According to this criterion, the metals detected in both extracts were within the allowable limits.

Considering the organic compounds, both of the extracts analyzed (leached and solubilized) contained caffeine as the dominant compound (95%) besides of fatty acids (5%) such as, octanoic acid, hexadecanoic acid, oleic acid, and 9,12- octadecadienoic acid.

3.3. Salmonella/microsome mutagenicity assay

Fig. 1 shows the results obtained in the Salmonella assay. The leached extract obtained from the coffee waste exhibited mutagenic activity for the TA98 strain with and without the S9 mix (Fig. 1a). When tested with the TA100 strain, this extract did not induce a mutagenic response and metabolic activation generated metabolites that were toxic for this strain (Fig. 1b). The solubilized extract induced mutagenicity in the TA98 strain with and without the S9 mix (Fig. 1c), and in the TA100 strain in the absence of the S9 mix (Fig. 1d).

Table 1
Physicochemical properties of the leached and solubilized extracts.

Pilão® Waste	pH	Conductivity (µs/cm)	Salinity (ppt)	TDS (ppt)
Solubilized	5.7	2.60	1.34	1.89
Leached	5.0	5.10	2.63	3.62

TDS: Total dissolved solids.

Table 2
Metals detected in the leached and solubilized coffee waste extracts.

Pilão Waste	Metal Contents (ppm)							
	Cu	Zn	Fe	Mn	Ni	Co	Pb	Cd
Solubilized	N.D.[a]	N.D.[a]	0,73	0.75	0.18	0.10	0.25	0.03
Leached	0.18	0.15	0.73	0.77	0.15	N.D.[a]	0.25	0.03

[a] N.D: not detected; Cr e Al were not detected.

3.4. Micronucleus and WST-1 assay in HepG2 cells

The genotoxicity of the leached and solubilized extracts was assessed by the micronucleus assay in HepG2 cells and Fig. 2 shows the results obtained in the MN and WST-1 assays. The leached (Fig. 2a and b) and solubilized (Fig. 2c and d) extracts induced significant increases in the frequency of micronucleated cells in a dose-dependent manner either after 3 (Fig. 2a and c) or 24 h (Fig. 2b and d) of treatment. The leached extract induced micronuclei formation at higher concentrations after 3 (100%) and 24 h (50% and 100%) of treatment. These results suggest that the longer the exposure time the greater the genotoxic effects. Besides, a significantly elevated frequency of micronucleated cells was induced by the solubilized extract at a concentration of 6.25% after 3 h of treatment, a 10-fold increase as compared to the negative control. On the other hand, after 24 h, a significant increase in micronuclei only occurred in the HepG2 cells at the two highest concentrations tested (50% and 100%). At the same time, as shown in Fig. 2, using the WST-1 assay the cell viability was not affected after exposure for 3 and 24 h.

3.5. Ecotoxicity tests

Tests with three aquatic organisms were carried out to evaluate the ecotoxicity of the leached and solubilized extracts and the results are summarized in Table 3. The leached extract showed more toxic substances than the solubilized extract for the cladocerans D. similis and C.dubia, although both extracts showed similar results for the bacteria Vibrio fischeri. A decrease of at least 50% of dissolved oxygen was observed in a concentration dependent manner at above 6.25% of solubilized extract, after 48 h of exposure to D.similis. This low oxygen availability (< 2,00 mg/L) may contribute to the ecotoxicity of this sample. As expected, C.dubia was the most sensitive species for both samples.

4. Discussion

Coffee is one of the world´s most consumed beverages and consequently large amounts of residues are generated during the brewing process. These include the toxic solid residues obtained during the processing of steam to prepare instant coffee or coffee powder after extraction with hot water, which are hazardous to the environment (Campos-Vega et al., 2015; Mussato et al., 2011a, 2011b). Considering the above, the present authors investigated the mutagenic, genotoxic, cytotoxic and ecotoxic potential of the leached and solubilized extracts obtained from coffee waste.

Conflicting data on coffee mutagenicity have been reported in the literature. Some studies have shown that coffee produces mutagenic compounds (Ariza et al., 1988; Duarte et al., 1999; Glatt et al., 2012; Kosugi et al., 1983; Lee, Shin, 2010, Tang et al., 2010), whereas others have shown that it does not exhibit mutagenic activity (Heimbach et al., 2010; Monente et al., 2015; Shimizu, Yano, 1987).

In the present work, both the solubilized and leached extracts induced DNA damage, either by point mutations or chromosome breaks. Based on the Salmonella assay, both extracts induced frameshift mutation, and the solubilized one induced base pair substitution mutation. After metabolization, the solubilized extract was detoxified when tested with TA100 and the mutagenic potency of the leached

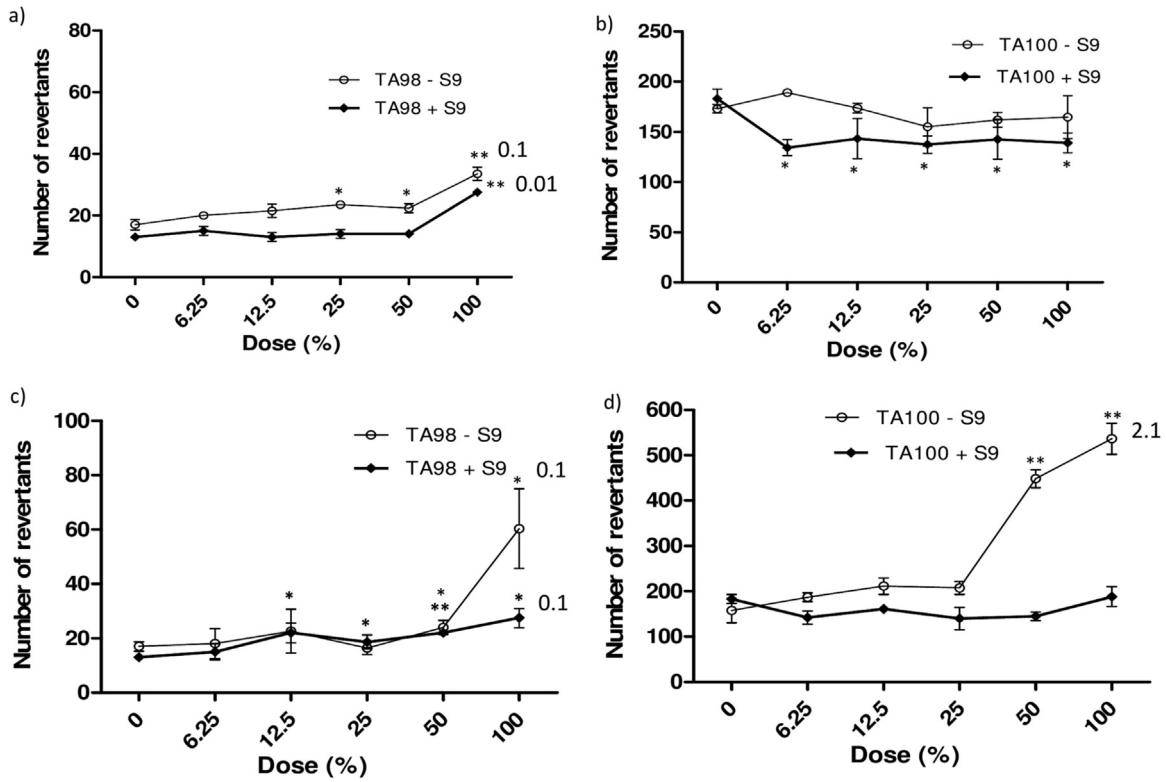

Fig. 1. Dose-response curve for the leached (a, b) and solubilized (c, d) extracts tested with Salmonella strains TA98 and TA100 in the presence and absence of exogenous metabolic activation (S9 mix). The numbers over each line were generated as the slope values by the model of Bernstein et al. (1982) and represent the potency of the compound expressed in revertants per microliter. * $p < 0.05$; ** $p < 0.1$. The results are presented as mean ± SD.

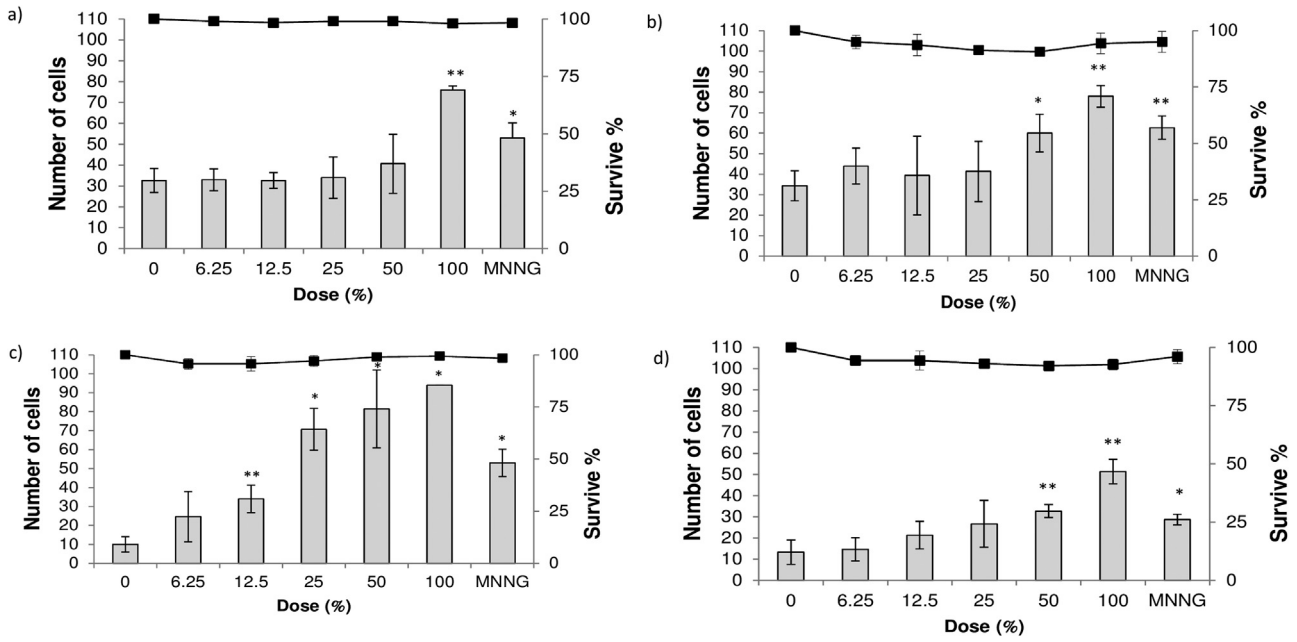

Fig. 2. Effects of the leached (a, b) and solubilized (c, d) extracts on frequency of micronucleated cells (gray bars) and viability (horizontal line over bars) of HepG2 cells after 3 (a, c) and 24 (b, d) hours of treatment. 0.5 μM MNNG (N-methyl-N-nitro-N-nitrosoguanidine) was the positive control. * $p < 0.05$; ** $p < 0.1$. The results are presented as mean ± SD.

Table 3
Aquatic toxicity to leached and solubilized extracts.

Pilão® Waste	Solubilized	Leached
Daphnia similis	EC50[1] 11,26% (7,83% to 16,21%)[5]	EC50[1] 1,50% (1,25% to 1,80%)[5]
Ceriodaphnia dubia	NOEC[2] 1,00%	NOEC[2] 0,06%
	LOEC[3] 2,00%	LOEC[3] 0125%
	IC50[4] 1,39% (1,03% to 1,54%)[5]	IC50[4] 0,12% (0,10% to 0,34%)[5]
Microtox®	15 min EC50[1] 5,90% (5,40% to 6,40%)[5]	15 min EC50[1] 6,24% (5,80% to 6,72%)[5]
	30 min EC50[1] 5,50 (5,05% to 6,00%)[5]	30 min EC50[1] 6,02% (5,62% to 6,45%)[5]

[1] EC_{50} (median effect concentration).
[2] NOEC (no observed effect concentration) $p = 0.05$.
[3] LOEC (lowest observed effect concentration) $p = 0.05$.
[4] IC_{50} (50% inhibitory concentration).
[5] Confidence range 95%.

extract decreased 10 times (from 0.1 to 0.01 rev/μL).

Several studies have shown the mutagenic properties of roasted coffee compounds (Ariza et al., 1988; Duarte et al., 1999; Kosugi et al., 1983) and the decrease of this effect after metabolization (Duarte et al., 1999), which corroborates with the present study. Similar to the results obtained in the bacterial system, it seems that coffee waste is detoxified in metabolically active HepG2 cells. The present results showed that the solubilized extract induced micronuclei formation in HepG2 cells during short term exposure, and after 24 h of treatment its genotoxic activity was reduced. Bravo et al. (2013) showed that aqueous extracts from coffee waste did not induce genotoxic effects (strand breaks or oxidized purines, formamidopyrimidine DNA glycosylase-sensitive sites) using the comet assay in HeLa cells after 2 and 24 h of treatment. However, the results for the leached extract showed an increased frequency of micronucleated cells, suggesting that the leaching process might have generated genotoxic compounds which were incapable of being detoxified by this system.

Several compounds may be associated with the mutagenic activity found in coffee, such as hydrogen peroxide and methylglyoxal, which present an important mutagenic synergistic effect (Ahmad et al., 2011; Al-Wasiti et al., 2016) polycyclic aromatic hydrocarbons produced during roasting (Shi et al., 2016) and metals (Wijewickreme, Kitts, 1998).

The GC-MS and FAAS analyses carried out during this study led to the identification of bioactive compounds in the leached and solubilized extracts of waste coffee, caffeine being the dominant compound as well as metals, in agreement with the data found in the literature (Campos-Vega et al., 2015; Canter et al., 2015; Mussato et al., 2011a).

Nehlig and Debry (1994) showed the mutagenic potential of caffeine in lower organisms, such as bacteria and fungi, and in mammalian cells in the absence of an exogenous metabolic activation system. These authors attributed the mutagenicity of caffeine mainly to chemically reactive components such as aliphatic dicarbonyls (Nehlig, Debry, 1994). On the other hand, other studies concerning the genotoxic and mutagenic potential of caffeine using animal models resulted in inconsistent and inconclusive data (Choundhury, Palo, 2004; Zarrelli et al., 2014). Apart from the mutagenic effects, it is important to point out that caffeine toxicity in humans has been associated with irritability, insomnia, nervousness, diuresis, muscle twitching, cardiovascular disorders and gastrointestinal disturbances (Stavric, 1988).

Although fatty acids were found in low proportions, it is important to highlight the possible contribution of these compounds to the genotoxicity found. The MAK-Collection for Occupational Health and Safety showed that oleic acid induces aneuploidy in pulmonary fibroblasts and yeasts and has tumor-promoting effect on the skin besides to stimulate cell proliferation in several organs and cell systems in vitro. Moreover, a mixture of linoleic acid (9,12-octadecadienoic acid) and oleic acid caused an increase in the incidence of gastrointestinal tumors in mice (MAK Value Documentation, 2002). Additionally, there is concern that long-term consumption of large amounts of linoleic acid might increase cancer risk (Zock, Katan, 1998).

Beeharry et al. (2003) showed that the hexadecanoic acid (palmitic acid) is a potent inducer of DNA damage in an insulin-secreting cell line and in primary human fibroblasts. Moreover the authors found that palmitic acid-induced DNA damage is prevented by linoleic acid, which act in this case as a protective agent against oxidative stress, instead of a source of mutagenic metabolites. This shows that the relationship of fatty acids to genotoxicity is very complex (Beeharry et al., 2003).

Considering the contribution of metals to the mutagenicity found, the mutagenic and carcinogenic properties of some metals identified in

this work have been demonstrated. Cadmium, for example, is carcinogenic to humans according to the International Agency for Research in Cancer (International Agency for Research in Cancer, 2012) and occupational and environmental exposures to lead have been associated with increases in chromosomal damage in humans (Maria et al., 2007). Wong (1988) showed that cadmium, cobalt, manganese and lead are mutagenic using the Salmonella assay in the absence of the S9-mix, and Pra et al. (2008) found that iron and copper are genotoxic and mutagenic to mice after sub chronic exposure. Moreover, the mutagenicity may be due to the formation of H_2O_2 through an auto-oxidation process, where polyphenolics, in the presence of transition metals, reduce the atmospheric oxygen, as demonstrated by Stadler et al. (1994).

Thus, even within the allowable limits, it is probable that the mutagenic effects found are due to the presence of metals or caffeine, or some interaction effect between them or even with other compounds not monitored in this study.

Comparing the mutagenic effect induced by both extracts, it can be deduced that the solubilized one presented a mutagenic effect on the T A100 Salmonella strain, unlike the leached extract, whereas in the micronucleus assay, the leached extract induced greater micronuclei frequency than the solubilized extract after 24 h of treatment. This behavior can be attributed to the composition of the extracts. Although the metal contents are similar in both extracts, Cu and Zn were not detected in the solubilized extract, while Co was not detected in the leached extract, the leached extract showing higher conductivity, salinity and total dissolved solids as compared to the solubilized extract. Moreover, the salinity can influence the speciation of metals. Anions form complexes with metals, thus reducing their bioavailability. In addition, high cation concentrations can compete with metal complexing agents, thus reducing the availability of the binding sites and altering the concentration of the free metal species.

The toxicological effects of caffeine showed that it does not seem to be a threat to the aquatic environment for short term exposure (Moore et al., 2008). This compound shows low acute and chronic toxicity to some organisms such as Brachionus calyciflorus (EC_{50} 24 h 1018 mg/L, EC_{50} 48 h 104 mg/L) (Zarrelli et al., 2014), the cladoceran Ceriodaphnia dubia (EC_{50} 7d 40 mg/L) (Moore et al., 2008), and the algae Pseudokirchneriella subcapitata (EC_{50} 72 h > 150 mg/L) (Zarrelli et al., 2014) and Scenedesmus subspicatus (EC_{50} 72 h > 100 mg/L) (Organization for Economic Co-operation and Development, 2002).

Notwithstanding, the long term continuous release of caffeine into the aquatic environment can lead to deleterious effects on living organisms (Zarrelli et al., 2014). Pires et al. (2016) showed that environmentally relevant caffeine concentrations induced sublethal effects such as oxidative stress and lipid peroxidation in the polychaetes Diopatra neapolitana and Arenicola marina. On the other hand, the ingestion of coffee beans by the common carp Cyprinus carpio L. was shown to be a negative modulator for zinc toxicity and bioaccumulation in the fish body (Abdel-Tawwab et al., 2015).

A coffee life cycle assessment made by Salamone, 2003 showed the consumption and disposal stages related to aquatic ecotoxicity due to the release of cadmium and chromium (III) into the environment. However the concentrations of leached and solubilized extracts which are toxic for cladocerans ($p < 0.05$) contain about 10–100 times less cadmium than the LC_{50} obtained by Rodgher et al. (2010) for D. magna. Chromium was not detected in either extract.

Other metals quantified, such as Cu, Zn, Fe, Mn and Pb showed acute toxicity for Daphnia sp. at concentrations of at least one or two orders of magnitude higher than the values found in both extracts (Cooper et al., 2009; Fjallbord et al., 2006). The same situation was observed when comparing the values of Cu, Zn and Pb for chronic toxicity for Ceriodaphnia dubia (Cooper et al., 2009).

The organic compounds present in the dissolved solids may be related to the toxicity found. The leached extract showed 3 times more total dissolved solids than the solubilized extract and was more toxic for cladocerans. The presence of fatty acids on samples may be act as a modulator of other compounds once they can activate or inhibited xenobiotic nuclear receptors involved in acclimation to toxicants as HR96 (Sengupta et al., 2015) and change rates of reproduction and survival after starvation period (Sengupta et al., 2016). Bertin et al. (2014) reported a pH-related toxicity of fatty acids to Rainbow trout gill cells and a raise of toxicity during a co-exposure of fatty acids and salts as $CaCl_2$, $MgSO_4$, and $FeSO_4$. The authors related the effect to an initial permeabilization of the gill cells by the fatty acids with a subsequent toxic response due to ionic dysregulation by the increased ion strength. Therefore the present results suggest that unregulated compounds can contribute to the toxicity of both extracts.

Considering the damages of coffee disposal to the environment, it is important to encourage the development of strategies for the removal of these compounds. Systems using ion-exchange resins, for example, can be improved to remove saturated and unsaturated fatty acids from environment (Maddikeri et al., 2012). Microbial degradation process using some bacterial and fungal strains such as Pseudomonas, Serratia and Aspergillus, Penicillium and Phanerochaete, respectively, has been shown to be efficient for caffeine degradation (Edwards et al., 2015; Dash et al., 2016) and the use of metal-resistant bacteria (cell and gene bioaugmentation), treatment amendments, clay minerals and chelating agents have been used to reduce bioavailable heavy metal concentrations (Olaniran et al., 2013).

5. Conclusion

Aside from the known effects of coffee itself, coffee waste may also induces mutagenicity, which remains in the leached extract after disposal in landfills, as well as in water supplies. Thus the coffee discarded in the environment may pose a risk to human and environmental health, since this compound can cause DNA damage and present toxicity to aquatic organisms. Follow with this study suggests the need of utilizing coffee wastes in other areas to reduce its impact on human and environmental health.

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Conflicts of interest

None declared.

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