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In vitro bioaccessibility of metals from tape tea – a low-cost emerging drug

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Abstract:	BACKGROUND: An in vitro physiologically relevant test based on the standard Unified Bioaccessibility Method (UBM) combined with inductively coupled plasma mass spectrometry was performed in this study to ascertain the elemental bioaccessibility pools of tape tea as emerging low-cost abuse drug under fasted conditions. METHODS: Elemental quantification in tape tea and body fluid extracts was performed by an inductively coupled plasma quadrupole mass spectrometer - ICP-MS, and for sample preparation of the bioaccessibility extracts prior to ICP-MS analysis, a microwave-assisted acid decomposition was applied by using a microwave oven. The Unified Bioaccessibility Method (UBM) was considered for investigation of elemental bioaccessibility in tape tea, required a full set of organic compounds, salts, and enzymes. RESULTS: Considering total element evaluation through ICP-MS, Co, Ni, Mn, and Zn are found at the highest concentrations in the sample, namely 415 ± 36, 202 ± 55, 1389 ± 225 and 2397 ± 197 μg L-1, respectively. Regarding the oral bioaccessibility test, after both gastric and gastrointestinal extractions Co, Ni, and Mn are fully bioaccessible while for Zn the bioaccessibility is ca. 66%. CONCLUSION: According to the first results in the literature proposed for these samples, the bioaccessibility results indicate a possible toxic effect caused by Co, Ni and Mn, which might be expected by regular consumption of tape tea, and can lead to diseases related to the high concentration of these metals in body fluids.
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May, 25th, 2020

Prof. Dirk Schaumlöffel– Editor
Journal of Trace Element in Medicine and Biology

Dear Prof. Schaumlöffel,

Enclosed, please find the revised version of our manuscript intitled *In vitro bioaccessibility of metals from tape tea – a low-cost emerging drug*, which are being submitted for possible publication in the JTEMB.

All the suggestions/corrections from the Reviewer were attained, and answered point-by-point in the Response to Review letter.

We believe that, mainly now, our contribution fits the journal standards.

Please, accept my best regards.

Yours sincerely,

Marco Aurélio Zezzi Arruda

Response to Reviewer

We would like to thank the reviewer #2 for the valuable comments regarding our manuscript in order to improve its quality. Taking into account the reviewer suggestions, we believe that our revised manuscript version has improved its quality and we believe that it now fits to the requirements for publication in JTEMB. Please, see below the responses from each comment.

Sentences highlighted in red color – removed from original version

Sentences highlighted in yellow color – changed from original version

Sentences highlighted in green color – inserted in the revised version

Reviewer #2: Major remarks:

(1) Introduction/Purpose of the study (General): The purpose of the study is not clearly revealed. Do metals which may migrate into the tape tea be known or suspected to produce hallucinogenic or similar effects? Or does the elucidation of the metal exposure may rather aim to the risk of side effects? Please clarify the purpose of the study.

R: We appreciate this suggestion. Prior to the Lehmann et al. [6] study, it was suspected the presence of some organic compounds in the tape tea, which could produce hallucinogenic effects. However, the authors did not find any organic compound in the infusion. In this context, the main purpose of our study was to evaluate the risk of side effects from tape tea consumption based on bioaccessibility results. Some additional information was inserted in the Introduction item for a better comprehension.

(2) Introduction (lines 48 - 56): The first paragraph of the introduction is not necessary for introducing to the aim of the study and should be deleted for a straight-forward preface.

R: Thank you for this suggestion. The first paragraph of the Introduction was removed in the revised version of our manuscript. A slight modification was made in the beginning of the new first paragraph.

(3) Introduction (line 62): I recommend to use the term "(audio and/or video) cassette tape" instead of "K7" and "VHS".

R: Thank you for this recommendation, the K7 and VHS terms have been replaced.

(4) Introduction (lines 64 – 65). In spite of that only few information is available on the constituents of the tapes, it is desirable to get the available information on the ingredients, which may justify the selection of the elements investigated. Particularly, I expected some indications for proofed or suspected psychoactive ingredients.

R: In fact, only few information regarding audio cassette tape ingredients are available. For this reason, prior to proceed with elemental determination and bioaccessibility assays, a qualitative experiment was carried out to investigate the most abundant elements in the tape tea. Afterwards, we selected the elements to be further evaluated in our study.

(5) Experimental / subchapter 2.3 (lines 134-135): Please describe clearly, how you dealt with samples which element concentration exceeded the dynamic range.

R: The solutions were properly diluted in deionized water and acidified accordingly in the linear working range for each element. This additional information was inserted in the revised manuscript.

(6) Experimental / subchapter 2.3 (lines 134-135): "determination was performed by ICP-MS" Please refer to subchapter 2.1.

R: This information was added in the revised manuscript.

(7) Experimental / subchapter 2.3 (line 136): Please revise the reference to "Table 2" instead to "Table 1".

R: This reference has been corrected.

(8) Experimental / subchapter 2.3 (lines 179-180): "determined by ICP-MS" Please refer to subchapter 2.1.

R: This information was added in the revised manuscript.

(9) Results and Discussion / subchapter 3.1 (lines 188 – 191): “elements that could lead to health issues”. Please specify whether you refer to hallucinogenic and related effects or to other health effects (side effects).

R: In fact, we decided to monitor some additional elements due to their possible side effects. A brief comment was inserted in the manuscript.

(10) Results and Discussion / subchapters 3.1 and 3.2 / issue "health risk assessment": In the discussion of possible health risks by the tape tea contaminants you refer several times to the "recommended daily intake (RDI)". Please consider that RDI indicates the required dose, but not the critical just tolerable dose. A toxicological risk assessment needs the comparison of the daily intake with the "tolerable daily intake (TDI)" or "acceptable daily intake (ADI)". Moreover, you may calculate the daily intake by tape tea consumption using an adequate exposure scenario, e.g. consumption of one liter of tape tea per day.

R: Thank you so much for this suggestion. In fact, the discussion based on tolerable daily intake (TDI) was missing in our original version, and now such information was inserted in the revised version in the Results and Discussion Section, as well as in the Conclusions. We have now calculated the daily intake by tape tea consumption under a proper scenario, and taking into account your suggestion of ingestion of one liter of tape tea per day.

(11) Results and Discussion / subchapter 3.2: I missed a comparison of the bioaccessibility rates found in the present study with bioaccessibility rates or oral absorption rates estimated in previous studies.

R: Thank you for this comment. In fact, we have not compared our results since there is no bioaccessibility study for tape tea infusion reported in the literature. In fact, we have found three works (Szymczycha-Madeja et al. 2020, Pereira Junior et al. 2018, Erdemir 2018) that studied element bioaccessibility in tea samples (but not from cassette tape), and a brief comparison is now present in the revised manuscript. In general, the authors reported bioaccessibility rates smaller than those found in our work. In fact, in our opinion, this result seems to be reasonable since the sample matrix content plays an important role in such matter. In this context, we believe that high bioaccessibility rates from tape tea could be explained to the lack of organic compounds in the infusion. Our hypothesis is also present in the revised version.

(12) Results and Discussion / subchapter 3.2 (lines 238-241): "The values of bioaccessible of Mn, Co and Ni indicate that they could provide a damage of the organism since these elements showed higher concentrations in tape tea than those of drinking water." I disagree with this conclusion. The higher element levels in tape tea

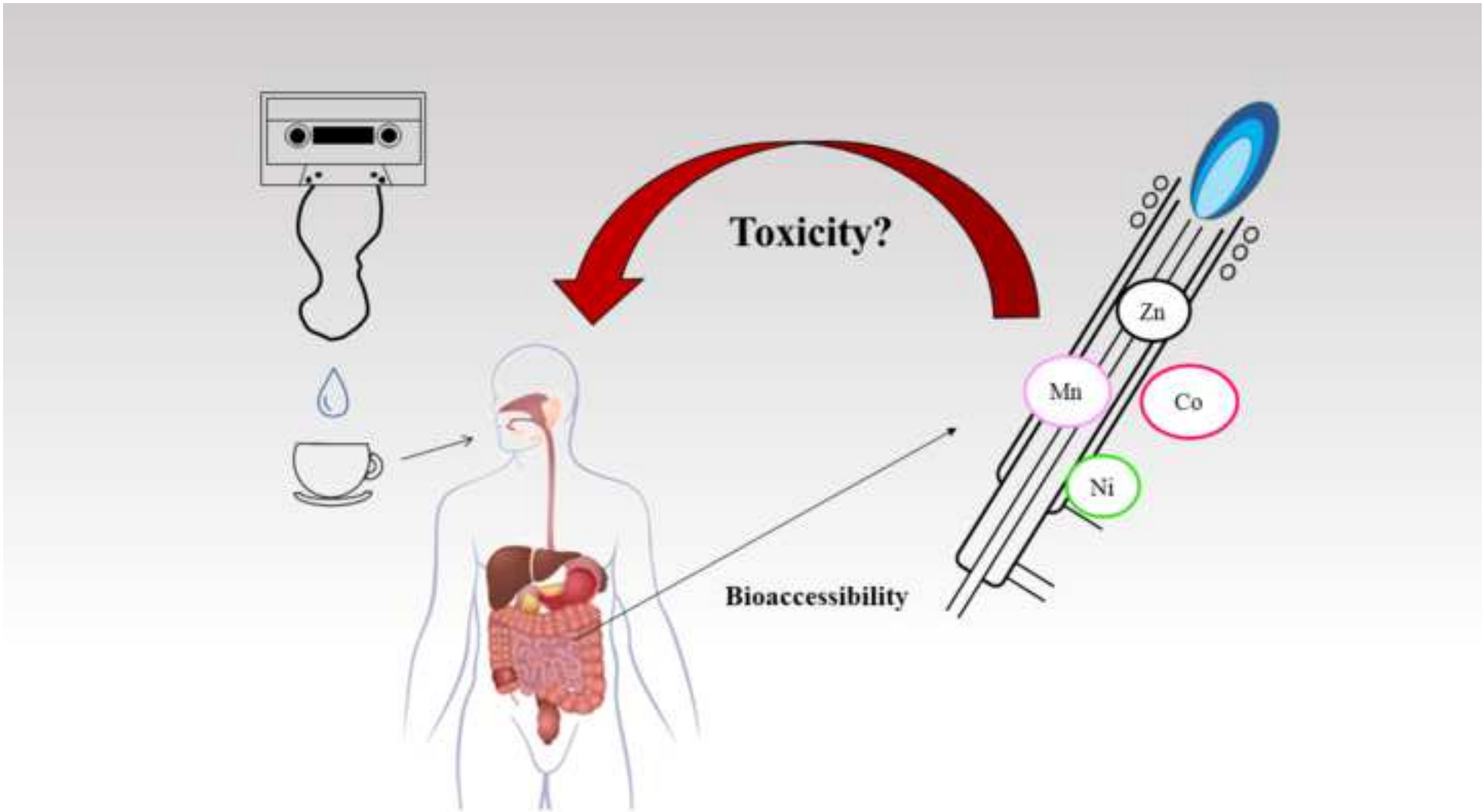
compared to drinking water can only indicate an additional exposure to these elements by tape tea consumption. This fact does not imply any health risk assessment. A health risk assessment need the comparison of the daily intake increase by tape tea consumption with TDI and ADI, respectively. Please consider this principle for the revision of your conclusions (also in the abstract) too.

R: Thank you so much for suggestion. We agree with your comments regarding our discussion in the subchapter 3.2, as well as our conclusions in the original version. In this way, we have considered the TDI values in the discussion of subchapter 3.2 and conclusions of our revised manuscript. In fact, there can be an associated risk related to contribution of tape tea ingestion, depending on the general diet of a person, as well as its weight and the level of the tape tea consumption. Afterwards, we believe it is now properly discussed.

HIGHLIGHTS

> Low-cost drugs have been used by teenagers for recreative purposes > total element determination of twelve isotopes in the tape tea > bioaccessibility study of those elements at concentration above recommended limit > complete bioaccessibility of toxic elements

Graphical Abstract



In vitro bioaccessibility of metals from tape tea – a low-cost emerging drug

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Abstract

BACKGROUND: An *in vitro* physiologically relevant test based on the standard Unified Bioaccessibility Method (UBM) combined with inductively coupled plasma mass spectrometry was performed in this study to ascertain the elemental bioaccessibility pools of tape tea as emerging low-cost abuse drug under fasted conditions.

METHODS: Elemental quantification in tape tea and body fluid extracts was performed by an inductively coupled plasma quadrupole mass spectrometer - ICP-MS, and for sample preparation of the bioaccessibility extracts prior to ICP-MS analysis, a microwave-assisted acid decomposition was applied by using a microwave oven. The Unified Bioaccessibility Method (UBM) was considered for investigation of elemental bioaccessibility in tape tea, required a full set of organic compounds, salts, and enzymes.

RESULTS: Considering total element evaluation through ICP-MS, Co, Ni, Mn, and Zn are found at the highest concentrations in the sample, namely 415 ± 36, 202 ± 55, 1389 ± 225 and 2397 ± 197 μg L^{-1}, respectively. Regarding the oral bioaccessibility test, after both gastric and gastrointestinal extractions Co, Ni, and Mn are fully bioaccessible while for Zn the bioaccessibility is *ca*. 66%.

CONCLUSION: According to the first results in the literature proposed for these samples, the bioaccessibility results indicate an increment in day-to-day total element concentration and depending on the concentration of each element that an individual consumes in its usual diet, the total concentration can exceed the TDI. There are several possible toxic effects caused by the excess of Co, Ni and Mn, which might be expected by their high total concentrations.

Keywords: tape tea, low-cost drugs, metal bioaccessibility, mass spectrometry, ICP-MS

1. Introduction

Illicit drugs, such as marijuana, cocaine, ecstasy, lysergic acid diethylamide (LSD), among others, have been extensively used with the appeal of making life more interesting/enjoyable [1,2]. However, for curiosity or for cheapening costs, low-cost abuse drugs, such as mushrooms [3], incense [4], salt baths [4], spice [5], and the quite exotic "tape tea", are already a reality. Although exotic, this infusion is prepared similarly to a tea infusion, being inexpensive, since either audio or video cassette tapes can be found in household or industry garbage.

The investigation of tape tea as abuse drug is imperative, once nothing or only few information is available in the literature about their constituents or hallucinogenic/side effects to the user's body. To this end, recently, Lehmann et al. [6] reported some chemical aspects of the tape tea, including concentrations of Mn, Co, Cr and Ni that are found 5 times superior to the maximum limit allowed for drinking water. In addition, no organic compound was found in tape tea and, according to the authors, a possible hallucinogenic effect could be a result of the high Mn concentration [6].

One of the current concerns across the forensic and toxicological fields lies on the actual effects of new types of low-cost abuse drugs, including the tape tea. In fact, there are several user reports on the web attesting hallucinogenic effects after the consumption of this low-cost drug. In order to obtain further biorelevant information regarding its toxicity, this work is aimed at evaluating the bioaccessible concentrations of metal species present in the prepared infusion to evaluate the risk of side effects from tape tea consumption. Bioaccessibility refers to the quantity of a nutrient/toxicant released from a given matrix (here video tape) and solubilized in the body fluids during

gastrointestinal digestion (bioaccessible fraction) thereby becoming potentially available for absorption in the small intestine [7,8]. The quality control of bioaccessibility results is herein done through mass balance validation on the basis of the total element content and the sum of bioaccessible and residual fractions for every individual target element using ICP-MS technology.

2. Experimental

2.1 Instrumentation

Elemental quantification in tape tea and body fluid extracts was performed by an inductively coupled plasma quadrupole mass spectrometer - ICP-MS (ICPMS 2030, Shimadzu Scientific Instruments, Maryland, USA), equipped with a mini torch, a concentric nebulizer (Meinhard®), and a cyclonic nebulization chamber cooled at a constant temperature of 5 ºC, and using an octapole collision cell with He as a collision gas. The experimental conditions used in all analysis are shown in Table 1. The daily ICP-MS instrumental calibration was carried out using a multielement standard solution containing the following species: Be at 10 µg L^{-1}, In, Bi and Ce at 2 µg L^{-1}, Co and Mn at 5 µg L^{-1}.

For sample preparation of the bioaccessibility extracts prior to ICP-MS detection, a microwave-assisted acid decomposition was applied by using a microwave oven (model DGT-100, Provecto Analitica, Jundiaí, Brazil) equipped with a temperature sensor, Teflon® vessels, and a magnetron of 2450 ± 13 MHz with a nominal power of 1200W.

Table 1 should be inserted here.

2.2 Reagents, solutions, standards and glassware

The solutions employed throughout were prepared with deionized water (≥ 18.2 MΩ cm) from a Milli-Q Direct-Q® 5UV water purification system (Millipore/Merck KGaA, Darmstadt, Germany). The standard stock solutions (1000 mg L^{-1}) of Li, K, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, Ba and Pb employed for ICP-MS measurements were purchased from Merck KGaA (Darmstadt, Germany).

To acidify or dilute samples/standards and decompose the extracts of the bioaccessibility tests, 65% (w/v) nitric acid (Merck KGaA), which was purified with a sub-boiling distillation system (Berghof, Eningen, Germany) was used. For the microwave-assisted sample decomposition, besides HNO_3, 30% (v/v) hydrogen peroxide was employed (Merck KGaA).

All glassware and other materials were cleaned in a 10% (v/v) HNO_3 bath to ensure the removal of residual metals. The cleaning procedure consisted of immersing the glassware in the acid bath for 24 h, and, then, washing each volumetric material three times with deionized water.

The Unified Bioaccessibility Method (UBM) developed by the Bioaccessibility Research Group of Europe [9], which was considered for investigation of elemental bioaccessibility in tape tea, required a full set of organic compounds, salts, and enzymes. All reagents employed are described in Table SM1 as constituents of the salivary, gastric, duodenal and biliary extractants (Table SM2), with the purpose of mimicking the distinct compartments of the human digestive tract. Additionally, the method was adapted to our samples (liquid tape tea), once it describes solid ones.

2.3 Sample preparation and total element determination

The tea tape infusion was prepared to mimic the user's condition according to Lehmann et al. (2016) [6]. However, in our study, instead of using the whole content one of a single tape to prepare the sample, a pool (N=20) was deemed more appropriate to offset variability between samples. In addition, the sample amount has been resized in order to ensure that all tests (total element and bioaccessibility) were carried out with the same pool.

The tape tea infusions (N=3) were prepared with 3.7 g of pooled sample and 80 mL of deionized water. In a hot plate, the samples were heated until boiling, and, then, maintained 5 more min under boiling with occasional stirring using a polymeric stick. The infusions were left to cold down to room temperature, then filtered in a Whatman quantitative filter paper and diluted with deionized water up to 100 mL. The sample solutions were properly diluted with deionized water and acidified accordingly in the linear working range for each element to be determined by ICP-MS (Section 2.1).

The accuracy of the ICP-MS for monitoring of the isotopes listed in Table 2 was evaluated using a Standard Reference Material® 1640a – "Trace Elements in Natural Water" by NIST.

2.4 Oral bioaccessibility test

To ascertain the actual amount of metals that are released from the sample matrix to body fluids, the tape infusions were subjected to the adapted UBM protocol [9] for in vitro bioaccessibility testing. Gastric (G) and gastrointestinal (GI) fractions as well as the blank containing only fluids, were obtained in triplicate.

In brief, twelve 50 mL-plastic flasks (Corning®, New York, USA) were used: six for the G fraction and six for GI the fraction including three blanks (15 mL of deionized

water) and three tape tea replicates (15 mL) for each fraction. In all flasks, 4.5 mL of salivary solution were added, followed by manually stirring for 10 s. Subsequently, 6.75 mL of gastric solution were added in all flasks. The pH of each solution was checked (1.20 ± 0.05) and, when necessary, adjusted with 1.0 mol L^{-1} HCl or NaOH. Then, all tubes were placed in a thermostatic bath (37 ± 2 ºC) with continuous orbital shaker agitation (150 rpm) (Quimis®, Brazil) for 60 min. After this time, the flasks named with G and their respective blanks were centrifuged for 15 min at 4500 *g*, and, then, the supernatant was acidified with concentrated HNO_3 (Figure 1). The supernatant (bioaccessible G fraction), as well as the residue (non-bioaccessible fraction), were collected and subjected to microwave-assisted acid decomposition (section 2.5).

In the six remaining flasks named GI, 13.5 and 4.5 mL of duodenal and biliary fluids were added, respectively, and the pH checked again (6.3 ± 0.5). Then, the tubes were placed in the thermostatic bath 37 ± 2 ºC and subjected to stirring at 150 rpm for 240 min. After that, the samples were centrifuged for 15 min at 4500 g, the GI supernatant separated, acidified, and, similar to the gastric fraction, subjected along with the GI residue to microwave decomposition. All decomposed samples (residue and bioaccessible fractions), from both fractions, were analyzed by ICP-MS. The bioaccessibility procedure is summarized in Figure 1.

Figure 1 should be inserted here.

2.5 Microwave-assisted decomposition

At the end of the bioaccessibility assay, all fractions were submitted to a microwave-assisted acid decomposition process (N=3). For residual fractions, the entire content was decomposed, while for bioaccessible fractions 5 and 2.5 mL-aliquots of GI and G samples were, respectively, decomposed. For both the residual and bioaccessible

GI fractions and the bioaccessible G fraction, 1.5 mL of sub-boiling nitric acid plus 1.0 mL of hydrogen peroxide 30% (v/v) (Merck KGaA) were employed. For the residual G fraction, 1 mL of sub-boiling nitric acid plus 667 µL of hydrogen peroxide 30% (v/v) were used. The samples were transferred to Teflon® vessels and subjected to the following microwave oven program: 8 min @ 330 W; 5 min @ 590 W and 40 min @ 720 W. After microwave digestion, the samples were made up to 25 mL (G and GI bioaccessible fractions) and 50 mL (G and GI residual fractions) with deionized water. Finally, the samples were properly diluted and the total element content was determined by ICP-MS (Section 2.1).

3. Results and discussion

3.1 Total element content in tape tea

Although the companies that manufactured the cassette tapes do not disclose their chemical composition, it is known that the magnetic cassette tapes require several metals for proper operation. Previous studies demonstrated the presence of metals like Co, Mn and Ni in infusions prepared from those tapes [6]. To expand the information regarding elemental content in such infusions, it was decided herein to monitor additional elements that could lead to health issues due to their possible side effects, such as Ba, Cd, Cu, Zn and Fe.

For further determination of the bioaccessibility fraction, one of the most important preliminary assays is to determine the total element content in the cassette of tape tea. This result is also required for mass balance validation based on the summation of the residual and bioaccessible fractions. To quantify the concentration of each element to be evaluated, the instrument conditions were previously validated by using a

Standard Reference Material® 1640a – "Trace Elements in Natural Water" by NIST. Satisfactory recoveries were obtained by all the tested elements as shown in the Table 2.

Table 2 should be inserted here.

The quantification of each element in the cassette tape tea was performed through standard instrumental data base conditions (ICPMS 2030, Shimadzu) (Section 2.1) and the infusions were prepared as described in Section 2.3. The results of the total element concentrations in the pooled sample are shown in Table 3.

Table 3 should be inserted here.

According to the Electronic Code of Federal Regulations for those specified metals, Mn and Ni showed concentrations above than those recommended for drinking water (Table 4) [10]. Although Co levels are not set in the legislation, such element was found at *ca.* 10 times higher level (Table 4) than the concentration observed in a sample of fresh water SRM 1640a (Table 2).

Potassium level is not specified for drinking water, and it was found below that of recommended daily intake (RDI) allowed, 4700 mg/day, for adults and children ≥ 4 years, according to FDA, similarly to Mo (45 μg/day) [11]. The other elements may not represent a potential risk, since their determined concentrations were lower than those established by FDA [11].

Table 4 should be inserted here.

In view of these results, the selected elements for which bioaccessibility assays need to be performed in risk assessment explorations are Mn, Co and Ni. In addition, Zn was monitored because the high total concentrations could lead to health problems.

3.2 Oral bioaccessibility assays

The aim of bioaccessibility is to determine the released quantity of an element from the sample matrix into the organism [12]. Its importance relies on the necessity of getting insight into the maximum pools that are potentially available for absorption through the small intestine, and through this information, pointing out if some elements pose potential risks to the human health. To the end of the UBM assay four fractions were obtained: bioaccessible gastric fraction (BGF), bioaccessible gastric-intestinal fraction (BGIF), residual gastric fraction (RGF), and residual gastric-intestinal fraction (RGIF). The last two fractions are important for mass balance assessment of all process.

The results in Tables 5 and 6 show that for the gastric fraction all of the elements are fully bioaccessible and thus potentially bioavailable. Looking to gastrointestinal values, the bioaccessible fraction is around 100%, except for Zn. GI bioaccessibility of Mn, Co and Ni is close to 100%, and, thus, they are readily bioavailable in the small intestine. The summation of residual and bioaccessible fractions for G and GI is not significantly different from the total concentrations and thus, the method accuracy is corroborated.

Tables 5 and 6 should be inserted here.

The bioaccessibility test is the first step to comprehend the behavior of the elements and with this information predict their absorption and toxicity [12]. In fact, the bioaccessible values of Mn, Co and Ni indicate that the consumption, e.g. 1 L, of the tape tea, besides the ingestion of food, 2 L of drinking water, among others, could result in a considerable increasing in the total amount of these elements in terms of daily diet.

Mn is a trace and essential element important in the organism [13] for enzymes and cells [14]. The toxicity through oral exposure of high concentrations of Mn is considered low, but studies have shown the possibility of neurotoxicity effects [13]. As

reported in the literature, the exposure to high concentrations of Mn might cause a syndrome named manganism. The symptoms resemble the Parkinson's disease accompanied with other effects such as limb rigidity, mild tremors, cock-like walk, excessive salivation and sweating and a disturbance of balance [14]. The RDI of Mn is 2.3 mg/day (adults and children ≥ 4 years) [11,15], then the total element content in tape tea, even fully bioaccessible, was lower than the total concentration recommended. On the other hand, the tolerable daily intake (TDI) is an estimative amount of a potentially harmful substance in food or drinking water that can be ingested daily over a lifetime without appreciable health risk [16]. For Mn the TDI value is 0.06 mg/kg [17], thus, for a 70 kg weight adult the TDI would be 4.2 mg/day. If such individual ingests 1 L of tape tea/day the consumption will be *ca.* 1.3 mg of Mn representing 31% of TDI. Taking into account that in a normal diet, people consume other foods and drinks that contain Mn, the total ingested quantity may exceed the TDI value.

Regarding Co, its continuous exposure can lead to a serious cardiac insufficiency [18,19]. A previous study [18] reported that rats whose diet had an additional amount of Co showed a reduction of 80% in the Mn-superoxide dismutase activity, and the activity of some respiratory chain enzymes, such as NADH-cytochrome was also decreased. As consequence, the mitochondrial ATP production was affected which in turn resulted in a respiratory insufficiency. In addition, the presence of Co in the organism may cause oxidative stress and lipid peroxidation as a result of the generation of reactive oxygen species generation and the increase of malondialdehyde (MDA) levels, respectively [20]. Once in the organism, the toxicity of bioaccessible Co can be explained taking into account its ionic form, since Co^{2+} can bind the serum albumin, thus reaching the red cells via the Ca^{2+} transport pathway [21]. Another reported situation related to high concentrations of Co is the ocular toxicity. For this element, there is no legislation of

minimum values for ingestion, however, the literature reports optic atrophy with values of 0.11 µg L^{-1} and 0.23 µg L^{-1} in blood [22]. Additionally, the retinal dysfunction was mentioned as a consequence of cobaltism as well [22].

In terms of Ni there is no recommended dosage established as RDI, however, according to European Food Safety Authority, the Ni TDI is 196 µg/day (adult, 70 kg) [23]. By comparing the TDI with the total concentration of Ni found in the tape tea, only the ingestion of one liter of tape tea would be enough to exceed Ni TDI. In fact, high concentrations of Ni in the organism can be related to some health disorders, such as allergies, carcinogenesis, renal and cardiovascular disorders [24-26]. The literature reports examples of studies where this situation resulted in oxidative stress, as well as lipid peroxidation, since the malondialdehyde (MDA) content was increased. In addition, some enzymes were affected by the surplus of Ni, such as superoxide dismutase and catalase, which had their activities reduced [24,25]. As previously reported, Ni^{2+} shows affinity for proteins and amino acids and this can lead to protein oxidation in cells and induce DNA damage in cell systems [25]. As additional effects, Ni can be related to sperm count and motility, as well as reduced number of pregnancies in rats, and toxicity of human placenta, and lipid peroxidation. A significant reduction in body and ovarian weights were found as a result of Ni exposure [27]. Nevertheless, as described in the drinking water guideline by WHO, the Ni TDI is 12 µg/kg [17], then, in the same situation described for Mn, a 70 kg weight adult could ingests a total amount of 840 µg/day. Taking into account our results, the consumption of a one liter of tape tea infusion would lead to an exposition of 202 µg of Ni, thus, representing 24% of the total concentration acceptable for this element in a day. Besides, WHO's recommendation, in terms of allocation up to 20% of the TDI for drinking water [17], the consumption of the tape tea, in addition to water, would reach almost half of the

total TDI. Moreover, this consumption together with other sources, e.g., foods, of Ni from a diet can exceed the total TDI.

Some bioaccesible rates reported in the literature, that also studied the same elements bioaccessibility in teas, were smaller than those found in our work, especially for Mn and Ni [15,28,29]. In general, despite of the differences in terms of bioaccessibility procedures considered in such studies, the results seem to be reasonable, since the sample composition plays an important role in elemental releasing from its matrix, thus, affecting the element bioaccessibility [30]. Then, it is hypothesized that the higher bioaccessibility rates found in our work could be justified by the lack of organic compounds in the tape tea infusion [6].

4. Conclusions

This is the first study on the oral bioaccessibility of trace metals in tape tea as a cost-effective abuse drug, and all the objectives of this work were attained. A modified UBM method was selected for the in-vitro investigation of bioaccessible fractions in the stomach and small intestine so as to simulate worst-case scenarios for prediction of human toxicity. Our findings indicate that Co, Mn and Ni are fully bioaccessible in the stomach and small intestine suggesting that an increment in total daily intake may occur by the consumption of the tape tea, and depending on the diet it can lead to an excess of tolerable daily intake of the evaluated elements.

Acknowledgements

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Tables

Table 1- ICP-MS operational conditions for elemental measurements.

Cyclonic nebulization chamber	Cyclonic
Nebulizer	Concentric
RF power (W)	1200
Plasma gas flow-rate (L min^{-1})	8.0
Auxiliary gas flow-rate – Ar (L min^{-1})	1.1
Nebulizer gas flow-rate – Ar (L min^{-1})	0.7
Cell voltage (V)	-21
Replicates	5
Collision cell gas flow (mL min^{-1})	6.0
Energy filter (V)	7.0
Correction equation	Not used
Monitored m/z	$^{7}Li^{+}$; $^{39}K^{+}$; $^{55}Mn^{+}$; $^{56}Fe^{+}$; $^{59}Co^{+}$; $^{60}Ni^{+}$; $^{63}Cu^{+}$; $^{64}Zn^{+}$; $^{98}Mo^{+}$; $^{114}Cd^{+}$; $^{137}Ba^{+}$ and $^{208}Pb^{+}$

Table 2- Total element concentrations and their respective recoveries of SRM 1640a® after elemental determination by ICP-MS.

Element*	Expected Values (µg L^{-1})	Obtained Values (µg L^{-1})	Recovery (%)
Li**	0.4066 ± 0.0094	0.44 ± 0.01	107 ± 3
K	579.90 ± 2.30	553 ± 14	95 ± 2
Mn	40.39 ± 0.36	38 ± 1	94± 2
Fe	36.80 ± 1.8	42 ± 6	111 ± 16
Co	20.24 ± 0.24	19.4 ± 0.3	96 ± 2
Ni	25.32 ± 0.14	24.4 ± 0.4	96 ± 1
Cu	85.75 ± 0.51	81.1 ± 0.4	94.6 ± 0.4
Zn	55.64 ± 0.35	59 ± 9	106 ± 17
Mo	45.60 ± 0.61	46 ± 1	101 ± 3
Cd	3.992 ± 0.074	3.73 ± 0.01	93.5 ± 0.2
Ba	151.80 ± 0.83	137 ± 1	90 ± 1
Pb	12.101 ± 0.050	12 ± 1	99 ±7

* The K, Fe, Mn, Zn, Cd, Ba and Ni determinations were performed employing a collision gas cell.

** Li determination was performed by the standards addition method.

Table 3- Total element concentrations in pooled cassette tape tea infusion as determined by ICP-MS.

Element	Dynamic range (µg L^{-1})	LOQ (µg L^{-1})	Concentration (µg L^{-1})
Li	0.1 – 10	0.008	11 ± 1
K	10 – 100	22.7	1264 ± 410
Mn	0.5 - 10	0.13	1389 ± 225
Fe	10 – 100	0.5	323 ± 43
Co	0.1 - 10	0.004	249 ± 28
Ni	0.1 - 10	0.08	202 ± 55
Cu	0.1 – 10	0.05	19 ± 3
Zn	10 – 100	0.8	2397 ± 197
Mo	0.1 – 10	0.005	< LOQ*
Cd	0.1 – 10	0.003	0.53 ± 0.08
Ba	0.1 – 10	0.03	8 ± 1
Pb	1 – 10	1.0	< LOD**

*LOQ = Limit of Quantification; **LOD = Limit of Detection

Table 4- Total element concentration in cassette tape tea infusion as determined by ICP-MS against e-CFR specified concentrations.

Element	Concentration (µg L^{-1})	Specified Concentration (e-CFR) (µg L^{-1})
Li	11 ± 1	Not specified
K	1264 ± 410	Not specified
Mn	1389 ± 225	50
Fe	323 ± 43	300
Co	249 ± 28	Not specified
Ni	202 ± 55	100
Cu	19 ± 3	1000
Zn	2397 ± 197	5000
Mo	< LOQ*	Not specified
Cd	0.53 ± 0.08	5
Ba	8 ± 1	2000
Pb	< LOD**	5

Table 5- Element concentrations in RGF and BGF using the UBM bioaccessibility test.

Element	Total ($\mu g\ L^{-1}$)	RGF ($\mu g\ L^{-1}$)	BGF ($\mu g\ L^{-1}$)	% Bioaccessible	% Recovery
Mn	1081 ± 160	10 ± 2	1269 ± 79	117 ± 7	118 ± 7
Co	249 ± 28	2.2 ± 0.5	248 ± 10	100 ± 4	101 ± 4
Ni	268 ± 41	1.4 ± 0.2	278 ± 22	104 ± 8	105 ± 9
Zn	2328 ± 280	< LD	2403 ± 149	103 ± 6	103 ± 6

Table 6- Element concentrations in RGIF and BGIF using the UBM bioaccessibility test.

Element	Total ($\mu g\ L^{-1}$)	RGIF ($\mu g\ L^{-1}$)	BGIF ($\mu g\ L^{-1}$)	% Bioaccessible	% Recovery
Mn	1081 ± 160	83 ± 4	1109 ± 7	102.5 ± 0.6	110.2 ± 0.6
Co	249 ± 28	8.4 ± 0.5	248 ± 21	100 ± 9	103 ± 9
Ni	268 ± 41	4.8 ± 0.9	252 ± 29	95 ± 11	96 ± 11
Zn	2328 ± 280	852 ± 114	1538 ± 332	66 ± 14	103 ± 14

Tables – Supplementary material

Table SM1- Reagents of the oral bioaccessibility assay.

Reagent	Brand	CAS
KCl	Mallinckrodt	7447-40-7
KSCN	Sigma Aldrich	333-20-0
$NaH_2PO_4 \cdot H_2O$	USB Corporation	10049-21-5
Na_2SO_4	Sigma Aldrich	7757-82-6
NaCl	Sigma Aldrich	7647-14-5
$NaHCO_3$	Sigma Aldrich	144-55-8
$CaCl_2 \cdot 2H_2O$	Vetec	10035-04-8
NH_4Cl	Merck	12125-02-9
KH_2PO_4	J.T. Baker	7778-77-0
$MgCl_2$	Sigma Aldrich	7786-30-3
HCl	Merck	7647-01-0
Urea	G.E. Healthcare	57-13-6
D-Glucuronic Acid	Sigma Aldrich	6556-12-3
Uric Acid	Sigma Aldrich	69-93-2
Glucosamine Hydrochloride	Sigma Aldrich	66-84-2
D-Glucose	Sigma Aldrich	50-99-7
Bile	Sigma Aldrich	8008-63-7
Pepsin	Sigma Aldrich	9001-75-6
Lipase	Sigma Aldrich	9001-62-1
Mucin	Sigma Aldrich	84082-64-4
Pancreatin	Sigma Aldrich	8049-47-6
α-Amylase	Sigma Aldrich	9001-19-8
Bovine Serum Albumin	Sigma Aldrich	9048-46-8

Table SM2- Chemical and enzymatic composition of the oral fluids.

	Composition (g L^{-1})			
	Gastrointestinal phase			
	Gastric phase			
	Oral phase			
	Salivary fluid (S)	**Gastric fluid (G)**	**Duodenal fluid (D)**	**Biliary fluid (B)**
Inorganic Compounds	KCl (1.8) KSCN (0.4) NaH_2PO_4 (1.8) $NaSO_4$ (1.1) NaCl (0.6) $NaHCO_3$ (3.4)	NaCl (5.5) NaH_2PO_4 (0.5) KCl (1.6) $CaCl_2$ (0.8) NH_4Cl (0.6) HCl (1.3% v/v)	NaCl (14.0) $NaHCO_3$ (6.7) KH_2PO_4 (0.16) KCl (1.13) $MgCl_2$ (0.1) HCl (0.04% v/v) $CaCl_2$ (0.4)	NaCl (10.5) $NaHCO_3$ (11.6) KCl (0.8) HCl (0.03% v/v) $CaCl_2$ (0.4)
Organic Compounds	Urea (0.4) Uric Acid (0.03)	Glucose (1.3) D-Glucuronic Acid (0.04) Urea (0.2) Glucosamine Hydrochloride (0.7)	Urea (0.2)	Urea (0.5)
Enzymes	α-Amylase (0.5) Mucin (0.05)	BSA (2.0) Pepsin (5.0) Mucin (6.0)	BSA (2.0) Pancreatin (18.0) Lipase (3.0)	BSA (3.6) Bile (6.0)
pH	6.5 ± 0.5	1.1 ± 0.1	7.4 ± 0.2	8.0 ± 0.2

Captions for figures

Figure 1- *In vitro* bioaccessibility UBM protocol by BARGE. (Adapted from reference 2).

Figures

Figure 1

In vitro bioaccessibility of metals from tape tea – a low-cost emerging drug

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Abstract

BACKGROUND: An *in vitro* physiologically relevant test based on the standard Unified Bioaccessibility Method (UBM) combined with inductively coupled plasma mass spectrometry was performed in this study to ascertain the elemental bioaccessibility pools of tape tea as emerging low-cost abuse drug under fasted conditions.

METHODS: Elemental quantification in tape tea and body fluid extracts was performed by an inductively coupled plasma quadrupole mass spectrometer - ICP-MS, and for sample preparation of the bioaccessibility extracts prior to ICP-MS analysis, a microwave-assisted acid decomposition was applied by using a microwave oven. The Unified Bioaccessibility Method (UBM) was considered for investigation of elemental bioaccessibility in tape tea, required a full set of organic compounds, salts, and enzymes.

RESULTS: Considering total element evaluation through ICP-MS, Co, Ni, Mn, and Zn are found at the highest concentrations in the sample, namely 415 ± 36, 202 ± 55, 1389 ± 225 and 2397 ± 197 µg L^{-1}, respectively. Regarding the oral bioaccessibility test, after both gastric and gastrointestinal extractions Co, Ni, and Mn are fully bioaccessible while for Zn the bioaccessibility is *ca*. 66%.

CONCLUSION: According to the first results in the literature proposed for these samples, the bioaccessibility results indicate a possible toxic effect caused by Co, Ni and Mn, which might be expected by regular consumption of tape tea, and can lead to diseases related to the high concentration of these metals in body fluids. the bioaccessibility results indicate an increment in day-to-day total element concentration, and depending on the concentration of each element that an individual consumes in its usual diet, the total concentration can exceed the TDI. There are several possible toxic

effects caused by the excess of Co, Ni and Mn, which might be expected by their high total concentrations.

Keywords: tape tea, low-cost drugs, metal bioaccessibility, mass spectrometry, ICP-MS

1. Introduction

Today we are living in a great dichotomy. If on the one hand the aim of the mankind is to enjoy life every second, well instilled by all types of social media, on the other, the pressure to live it day-by-day has become a great burden. In all cases, this appeal is even stronger by the younger population, which is vulnerable to the use of drugs, often illicit, and psychoactive substances for different recreational purposes, such as escaping from reality, facing daily problems or simply for seeking new hallucinogenic sensations. Regardless of the reason, socioeconomic, family, school, among others, the fact is that the addiction to drugs is vertiginous increasing in our society [1].

Illicit drugs, such as marijuana, cocaine, ecstasy, lysergic acid diethylamide (LSD), among others, have been extensively used with the appeal of making life more interesting/enjoyable [1,2]. However, for curiosity or for cheapening costs, low-cost abuse drugs, such as mushrooms [3], incense [4], salt baths [4], spice [5], and the quite exotic “tape tea”, are already a reality. Although exotic, this infusion is prepared similarly to a tea infusion, being inexpensive, since either audio or video cassette tapes can be found in household or industry garbage.

The investigation of tape tea as abuse drug is imperative, once nothing or only few information is available in the literature about their constituents or

hallucinogenic/side effects to the user's body. To this end, recently, Lehmann et al. [6] reported some chemical aspects of the tape tea, including concentrations of Mn, Co, Cr and Ni that are found 5 times superior to the maximum limit allowed for drinking water. In addition, no organic compound was found in tape tea, and, according to the authors, a possible hallucinogenic effect could be a result of the high Mn concentration [6].

One of the current concerns across the forensic and toxicological fields lies on the actual effects of new types of low-cost abuse drugs, including the tape tea. In fact, there are several user reports on the web attesting hallucinogenic effects after the consumption of this low-cost drug. In order to obtain further biorelevant information regarding its toxicity, this work is aimed at evaluating the bioaccessible concentrations of metal species present in the prepared infusion to evaluate the risk of side effects from tape tea consumption. Bioaccessibility refers to the quantity of a nutrient/toxicant released from a given matrix (here video tape) and solubilized in the body fluids during gastrointestinal digestion (bioaccessible fraction) thereby becoming potentially available for absorption in the small intestine [7,8]. The quality control of bioaccessibility results is herein done through mass balance validation on the basis of the total element content and the sum of bioaccessible and residual fractions for every individual target element using ICP-MS technology.

2. Experimental

2.1 Instrumentation

Elemental quantification in tape tea and body fluid extracts was performed by an inductively coupled plasma quadrupole mass spectrometer - ICP-MS (ICPMS 2030,

Shimadzu Scientific Instruments, Maryland, USA), equipped with a mini torch, a concentric nebulizer (Meinhard®), and a cyclonic nebulization chamber cooled at a constant temperature of 5 ºC, and using an octapole collision cell with He as a collision gas. The experimental conditions used in all analysis are shown in Table 1. The daily ICP-MS instrumental calibration was carried out using a multielement standard solution containing the following species: Be at 10 µg L^{-1}, In, Bi and Ce at 2 µg L^{-1}, Co and Mn at 5 µg L^{-1}.

For sample preparation of the bioaccessibility extracts prior to ICP-MS detection, a microwave-assisted acid decomposition was applied by using a microwave oven (model DGT-100, Provecto Analitica, Jundiaí, Brazil) equipped with a temperature sensor, Teflon® vessels, and a magnetron of 2450 ± 13 MHz with a nominal power of 1200W.

Table 1 should be inserted here.

2.2 Reagents, solutions, standards and glassware

The solutions employed throughout were prepared with deionized water (≥ 18.2 MΩ cm) from a Milli-Q Direct-Q® 5UV water purification system (Millipore/Merck KGaA, Darmstadt, Germany). The standard stock solutions (1000 mg L^{-1}) of Li, K, Mn, Fe, Co, Ni, Cu, Zn, Mo, Cd, Ba and Pb employed for ICP-MS measurements were purchased from Merck KGaA (Darmstadt, Germany).

To acidify or dilute samples/standards and decompose the extracts of the bioaccessibility tests, 65% (w/v) nitric acid (Merck KGaA), which was purified with a sub-boiling distillation system (Berghof, Eningen, Germany) was used. For the microwave-assisted sample decomposition, besides HNO_3, 30% (v/v) hydrogen peroxide was employed (Merck KGaA).

All glassware and other materials were cleaned in a 10% (v/v) HNO_3 bath to ensure the removal of residual metals. The cleaning procedure consisted of immersing the glassware in the acid bath for 24 h, and, then, washing each volumetric material three times with deionized water.

The Unified Bioaccessibility Method (UBM) developed by the Bioaccessibility Research Group of Europe [9], which was considered for investigation of elemental bioaccessibility in tape tea, required a full set of organic compounds, salts, and enzymes. All reagents employed are described in Table SM1 as constituents of the salivary, gastric, duodenal and biliary extractants (Table SM2), with the purpose of mimicking the distinct compartments of the human digestive tract. Additionally, the method was adapted to our samples (liquid tape tea), once it describes solid ones.

2.3 Sample preparation and total element determination

The tea tape infusion was prepared to mimic the user's condition according to Lehmann et al. (2016) [6]. However, in our study, instead of using the whole content one of a single tape to prepare the sample, a pool (N=20) was deemed more appropriate to offset variability between samples. In addition, the sample amount has been resized in order to ensure that all tests (total element and bioaccessibility) were carried out with the same pool.

The tape tea infusions (N=3) were prepared with 3.7 g of pooled sample and 80 mL of deionized water. In a hot plate, the samples were heated until boiling, and, then, maintained 5 more min under boiling with occasional stirring using a polymeric stick. The infusions were left to cold down to room temperature, then filtered in a Whatman quantitative filter paper and diluted with deionized water up to 100 mL. The sample

solutions were properly diluted with deionized water and acidified accordingly in the linear working range for each element to be determined by ICP-MS (Section 2.1).

The accuracy of the ICP-MS for monitoring of the isotopes listed in Table 2 was evaluated using a Standard Reference Material® 1640a – "Trace Elements in Natural Water" by NIST.

2.4 Oral bioaccessibility test

To ascertain the actual amount of metals that are released from the sample matrix to body fluids, the tape infusions were subjected to the adapted UBM protocol [9] for in vitro bioaccessibility testing. Gastric (G) and gastrointestinal (GI) fractions as well as the blank containing only fluids, were obtained in triplicate.

In brief, twelve 50 mL-plastic flasks (Corning®, New York, USA) were used: six for the G fraction and six for GI the fraction including three blanks (15 mL of deionized water) and three tape tea replicates (15 mL) for each fraction. In all flasks, 4.5 mL of salivary solution were added, followed by manually stirring for 10 s. Subsequently, 6.75 mL of gastric solution were added in all flasks. The pH of each solution was checked (1.20 ± 0.05) and, when necessary, adjusted with 1.0 mol L^{-1} HCl or NaOH. Then, all tubes were placed in a thermostatic bath (37 ± 2 ºC) with continuous orbital shaker agitation (150 rpm) (Quimis®, Brazil) for 60 min. After this time, the flasks named with G and their respective blanks were centrifuged for 15 min at 4500 *g*, and, then, the supernatant was acidified with concentrated HNO_3 (Figure 1). The supernatant (bioaccessible G fraction), as well as the residue (non-bioaccessible fraction), were collected and subjected to microwave-assisted acid decomposition (section 2.5).

In the six remaining flasks named GI, 13.5 and 4.5 mL of duodenal and biliary fluids were added, respectively, and the pH checked again (6.3 ± 0.5). Then, the tubes were placed in the thermostatic bath 37 ± 2 ºC and subjected to stirring at 150 rpm for 240 min. After that, the samples were centrifuged for 15 min at 4500 g, the GI supernatant separated, acidified, and, similar to the gastric fraction, subjected along with the GI residue to microwave decomposition. All decomposed samples (residue and bioaccessible fractions), from both fractions, were analyzed by ICP-MS. The bioaccessibility procedure is summarized in Figure 1.

Figure 1 should be inserted here.

2.5 Microwave-assisted decomposition

At the end of the bioaccessibility assay, all fractions were submitted to a microwave-assisted acid decomposition process (N=3). For residual fractions, the entire content was decomposed, while for bioaccessible fractions 5 and 2.5 mL-aliquots of GI and G samples were, respectively, decomposed. For both the residual and bioaccessible GI fractions and the bioaccessible G fraction, 1.5 mL of sub-boiling nitric acid plus 1.0 mL of hydrogen peroxide 30% (v/v) (Merck KGaA) were employed. For the residual G fraction, 1 mL of sub-boiling nitric acid plus 667 µL of hydrogen peroxide 30% (v/v) were used. The samples were transferred to Teflon® vessels and subjected to the following microwave oven program: 8 min @ 330 W; 5 min @ 590 W and 40 min @ 720 W. After microwave digestion, the samples were made up to 25 mL (G and GI bioaccessible fractions) and 50 mL (G and GI residual fractions) with deionized water. Finally, the samples were properly diluted and the total element content was determined by ICP-MS (Section 2.1).

3. Results and discussion

3.1 Total element content in tape tea

Although the companies that manufactured the cassette tapes do not disclose their chemical composition, it is known that the magnetic cassette tapes require several metals for proper operation. Previous studies demonstrated the presence of metals like Co, Mn and Ni in infusions prepared from those tapes [6]. To expand the information regarding elemental content in such infusions, it was decided herein to monitor additional elements that could lead to health issues due to their possible side effects, such as Ba, Cd, Cu, Zn and Fe.

For further determination of the bioaccessibility fraction, one of the most important preliminary assays is to determine the total element content in the cassette of tape tea. This result is also required for mass balance validation based on the summation of the residual and bioaccessible fractions. To quantify the concentration of each element to be evaluated, the instrument conditions were previously validated by using a Standard Reference Material® 1640a – "Trace Elements in Natural Water" by NIST. Satisfactory recoveries were obtained by all the tested elements as shown in the Table 2.

Table 2 should be inserted here.

The quantification of each element in the cassette tape tea was performed through standard instrumental data base conditions (ICPMS 2030, Shimadzu) (Section 2.1) and the infusions were prepared as described in Section 2.3. The results of the total element concentrations in the pooled sample are shown in Table 3.

Table 3 should be inserted here.

According to the Electronic Code of Federal Regulations for those specified metals, Mn and Ni showed concentrations above than those recommended for drinking

water (Table 4) [10]. Although Co levels are not set in the legislation, such element was found at *ca.* 10 times higher level (Table 4) than the concentration observed in a sample of fresh water SRM 1640a (Table 2).

Potassium level is not specified for drinking water, and it was found below that of recommended daily intake (RDI) allowed, 4700 mg/day, for adults and children ≥ 4 years, according to FDA, similarly to Mo (45 µg/day) [11]. The other elements may not represent a potential risk, since their determined concentrations were lower than those established by FDA [11].

Table 4 should be inserted here.

In view of these results, the selected elements for which bioaccessibility assays need to be performed in risk assessment explorations are Mn, Co and Ni. In addition, Zn was monitored because the high total concentrations could lead to health problems.

3.2 Oral bioaccessibility assays

The aim of bioaccessibility is to determine the released quantity of an element from the sample matrix into the organism [12]. Its importance relies on the necessity of getting insight into the maximum pools that are potentially available for absorption through the small intestine, and through this information, pointing out if some elements pose potential risks to the human health. To the end of the UBM assay four fractions were obtained: bioaccessible gastric fraction (BGF), bioaccessible gastric-intestinal fraction (BGIF), residual gastric fraction (RGF), and residual gastric-intestinal fraction (RGIF). The last two fractions are important for mass balance assessment of all process.

The results in Tables 5 and 6 show that for the gastric fraction all of the elements are fully bioaccessible and thus potentially bioavailable. Looking to gastrointestinal values, the bioaccessible fraction is around 100%, except for Zn. GI bioaccessibility of

Mn, Co and Ni is close to 100%, and, thus, they are readily bioavailable in the small intestine. The summation of residual and bioaccessible fractions for G and GI is not significantly different from the total concentrations and thus, the method accuracy is corroborated.

Tables 5 and 6 should be inserted here.

The bioaccessibility test is the first step to comprehend the behavior of the elements and with this information predict their absorption and toxicity [12]. The values of bioaccessible of Mn, Co and Ni indicate that they could provide a damage in the organism since these elements showed higher concentrations in tape tea than those of drinking water. In fact, the bioaccessible values of Mn, Co and Ni indicate that the consumption, *e.g*. 1 L of the tape tea, besides the ingestion of food, drinking water, among others, could result in a considerable increasing in the total amount of these elements in terms of daily diet.

Mn is a trace and essential element important in the organism [13] for enzymes and cells [14]. The toxicity through oral exposure of high concentrations of Mn is considered low, but studies have shown the possibility of neurotoxicity effects [13]. As reported in the literature, the exposure to high concentrations of Mn might cause a syndrome named manganism. The symptoms resemble the Parkinson's disease accompanied with other effects such as limb rigidity, mild tremors, cock-like walk, excessive salivation and sweating and a disturbance of balance [14]. The RDI of Mn is 2.3 mg/day (adults and children $\geq$ 4 years) [11,15], then the total element content in tape tea, even fully bioaccessible, was lower than the total concentration recommended. On the other hand, the tolerable daily intake (TDI) is an estimative amount of a potentially harmful substance in food or drinking water that can be ingested daily over a lifetime without appreciable health risk [16]. For Mn the TDI value is 0.06 mg/kg [17], thus, for

a 70 kg weight adult the TDI would be 4.2 mg/day. If such individual ingests 1 L of tape tea/day the consumption will be *ca.* 1.3 mg of Mn representing *ca.* 31% of TDI. Taking into account that in a normal diet, people consume other foods and drinks that contain Mn, the total ingested quantity may exceed the TDI value.

Regarding Co, its continuous exposure can lead to a serious cardiac insufficiency [18,19]. A previous study [18] reported that rats whose diet had an additional amount of Co showed a reduction of 80% in the Mn-superoxide dismutase activity, and the activity of some respiratory chain enzymes, such as NADH-cytochrome was also decreased. As consequence, the mitochondrial ATP production was affected which in turn resulted in a respiratory insufficiency. In addition, the presence of Co in the organism may cause oxidative stress and lipid peroxidation as a result of the generation of reactive oxygen species generation and the increase of malondialdehyde (MDA) levels, respectively [20]. Once in the organism, the toxicity of bioaccessible Co can be explained taking into account its ionic form, since Co^{2+} can bind the serum albumin, thus reaching the red cells via the Ca^{2+} transport pathway [21]. Another reported situation related to high concentrations of Co is the ocular toxicity. For this element, there is no legislation of minimum values for ingestion, however, the literature reports optic atrophy with values of 0.11 µg L^{-1} and 0.23 µg L^{-1} in blood [22]. Additionally, the retinal dysfunction was mentioned as a consequence of cobaltism as well [22].

Ni, similarly to Mn, is an essential element for the proper functioning of the body, and the recommended dosage is up to 196 µg/day according to European Food Safety Authority [22]. In terms of Ni, there is no recommended dosage established as RDI. However, according to the European Food Safety Authority, the Ni TDI is 196 µg/day (adult, 70 kg) [23]. By comparing the TDI with the total concentration of Ni found in the tape tea, only the ingestion of one liter of tape tea would be enough to exceed Ni

TDI. In fact, high concentrations of Ni in the organism can be related to some health disorders, such as allergies, carcinogenesis, renal and cardiovascular disorders [24-26]. The literature reports examples of studies where this situation resulted in oxidative stress, as well as lipid peroxidation, since the malondialdehyde (MDA) content was increased. In addition, some enzymes were affected by the surplus of Ni, such as superoxide dismutase and catalase, which had their activities reduced [24,25]. As previously reported, Ni^{2+} shows affinity for proteins and amino acids and this can lead to protein oxidation in cells and induce DNA damage in cell systems [25]. As additional effects, Ni can be related to sperm count and motility, as well as reduced number of pregnancies in rats, and toxicity of human placenta, and lipid peroxidation. A significant reduction in body and ovarian weights were found as a result of Ni exposure [27]. Nevertheless, as described in the drinking water guideline by WHO, the Ni TDI is 12 µg/kg [17], then, in the same situation described for Mn, a 70 kg weight adult could ingests a total amount of 840 µg/day. Taking into account our results, the consumption of a one liter of tape tea infusion would lead to an exposition of 202 µg of Ni, thus, representing 24% of the total concentration acceptable for this element in a day. Besides, WHO's recommendation, in terms of allocation up to 20% of the TDI for drinking water [17], the consumption of the tape tea, in addition to water, would reach almost half of the total TDI. Moreover, this consumption together with other sources, e.g., foods, of Ni from a diet can exceed the total TDI.

Some bioaccesible rates reported in the literature, that also studied the same elements bioaccessibility in teas, were smaller than those found in our work, especially for Mn and Ni [15,28,29]. In general, despite of the differences in terms of bioaccessibility procedures considered in such studies, the results seem to be reasonable, since the sample composition plays an important role in elemental releasing

from its matrix, thus, affecting the element bioaccessibility [30]. Then, it is hypothesized that the higher bioaccessibility rates found in our work could be justified by the lack of organic compounds in the tape tea infusion [6].

4. Conclusions

This is the first study on the oral bioaccessibility of trace metals in tape tea as a cost-effective abuse drug, and all the objectives of this work were attained. A modified UBM method was selected for the in-vitro investigation of bioaccessible fractions in the stomach and small intestine so as to simulate worst-case scenarios for prediction of human toxicity. Our findings indicate that Co, Mn and Ni are fully bioaccessible in the stomach and small intestine suggesting that an increment in total daily intake may occur by the consumption of the tape tea, and depending on the diet, it can lead to an excess of tolerable daily intake of the evaluated elements.

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Tables

Table 1- ICP-MS operational conditions for elemental measurements.

Cyclonic nebulization chamber	Cyclonic
Nebulizer	Concentric
RF power (W)	1200
Plasma gas flow-rate (L min^{-1})	8.0
Auxiliary gas flow-rate – Ar (L min^{-1})	1.1
Nebulizer gas flow-rate – Ar (L min^{-1})	0.7
Cell voltage (V)	-21
Replicates	5
Collision cell gas flow (mL min^{-1})	6.0
Energy filter (V)	7.0
Correction equation	Not used
Monitored m/z	$^{7}Li^{+}$; $^{39}K^{+}$; $^{55}Mn^{+}$; $^{56}Fe^{+}$; $^{59}Co^{+}$; $^{60}Ni^{+}$; $^{63}Cu^{+}$; $^{64}Zn^{+}$; $^{98}Mo^{+}$; $^{114}Cd^{+}$; $^{137}Ba^{+}$ and $^{208}Pb^{+}$

Table 2- Total element concentrations and their respective recoveries of SRM 1640a® after elemental determination by ICP-MS.

Element*	Expected Values (µg L^{-1})	Obtained Values (µg L^{-1})	Recovery (%)
Li**	0.4066 ± 0.0094	0.44 ± 0.01	107 ± 3
K	579.90 ± 2.30	553 ± 14	95 ± 2
Mn	40.39 ± 0.36	38 ± 1	94± 2
Fe	36.80 ± 1.8	42 ± 6	111 ± 16
Co	20.24 ± 0.24	19.4 ± 0.3	96 ± 2
Ni	25.32 ± 0.14	24.4 ± 0.4	96 ± 1
Cu	85.75 ± 0.51	81.1 ± 0.4	94.6 ± 0.4
Zn	55.64 ± 0.35	59 ± 9	106 ± 17
Mo	45.60 ± 0.61	46 ± 1	101 ± 3
Cd	3.992 ± 0.074	3.73 ± 0.01	93.5 ± 0.2
Ba	151.80 ± 0.83	137 ± 1	90 ± 1
Pb	12.101 ± 0.050	12 ± 1	99 ±7

* The K, Fe, Mn, Zn, Cd, Ba and Ni determinations were performed employing a collision gas cell.

** Li determination was performed by the standards addition method.

Table 3- Total element concentrations in pooled cassette tape tea infusion as determined by ICP-MS.

Element	Dynamic range (µg L^{-1})	LOQ (µg L^{-1})	Concentration (µg L^{-1})
Li	0.1 – 10	0.008	11 ± 1
K	10 – 100	22.7	1264 ± 410
Mn	0.5 - 10	0.13	1389 ± 225
Fe	10 – 100	0.5	323 ± 43
Co	0.1 - 10	0.004	249 ± 28
Ni	0.1 - 10	0.08	202 ± 55
Cu	0.1 – 10	0.05	19 ± 3
Zn	10 – 100	0.8	2397 ± 197
Mo	0.1 – 10	0.005	< LOQ*
Cd	0.1 – 10	0.003	0.53 ± 0.08
Ba	0.1 – 10	0.03	8 ± 1
Pb	1 – 10	1.0	< LOD**

*LOQ = Limit of Quantification; **LOD = Limit of Detection

Table 4- Total element concentration in cassette tape tea infusion as determined by ICP-MS against e-CFR specified concentrations.

Element	Concentration (µg L^{-1})	Specified Concentration (e-CFR) (µg L^{-1})
Li	11 ± 1	Not specified
K	1264 ± 410	Not specified
Mn	1389 ± 225	50
Fe	323 ± 43	300
Co	249 ± 28	Not specified
Ni	202 ± 55	100
Cu	19 ± 3	1000
Zn	2397 ± 197	5000
Mo	< LOQ*	Not specified
Cd	0.53 ± 0.08	5
Ba	8 ± 1	2000
Pb	< LOD**	5

Table 5- Element concentrations in RGF and BGF using the UBM bioaccessibility test.

Element	Total ($\mu g\ L^{-1}$)	RGF ($\mu g\ L^{-1}$)	BGF ($\mu g\ L^{-1}$)	% Bioaccessible	% Recovery
Mn	1081 ± 160	10 ± 2	1269 ± 79	117 ± 7	118 ± 7
Co	249 ± 28	2.2 ± 0.5	248 ± 10	100 ± 4	101 ± 4
Ni	268 ± 41	1.4 ± 0.2	278 ± 22	104 ± 8	105 ± 9
Zn	2328 ± 280	< LD	2403 ± 149	103 ± 6	103 ± 6

Table 6- Element concentrations in RGIF and BGIF using the UBM bioaccessibility test.

Element	Total ($\mu g\ L^{-1}$)	RGIF ($\mu g\ L^{-1}$)	BGIF ($\mu g\ L^{-1}$)	% Bioaccessible	% Recovery
Mn	1081 ± 160	83 ± 4	1109 ± 7	102.5 ± 0.6	110.2 ± 0.6
Co	249 ± 28	8.4 ± 0.5	248 ± 21	100 ± 9	103 ± 9
Ni	268 ± 41	4.8 ± 0.9	252 ± 29	95 ± 11	96 ± 11
Zn	2328 ± 280	852 ± 114	1538 ± 332	66 ± 14	103 ± 14

Tables – Supplementary material

Table SM1- Reagents of the oral bioaccessibility assay.

Reagent	Brand	CAS
KCl	Mallinckrodt	7447-40-7
KSCN	Sigma Aldrich	333-20-0
$NaH_2PO_4 \cdot H_2O$	USB Corporation	10049-21-5
Na_2SO_4	Sigma Aldrich	7757-82-6
NaCl	Sigma Aldrich	7647-14-5
$NaHCO_3$	Sigma Aldrich	144-55-8
$CaCl_2 \cdot 2H_2O$	Vetec	10035-04-8
NH_4Cl	Merck	12125-02-9
KH_2PO_4	J.T. Baker	7778-77-0
$MgCl_2$	Sigma Aldrich	7786-30-3
HCl	Merck	7647-01-0
Urea	G.E. Healthcare	57-13-6
D-Glucuronic Acid	Sigma Aldrich	6556-12-3
Uric Acid	Sigma Aldrich	69-93-2
Glucosamine Hydrochloride	Sigma Aldrich	66-84-2
D-Glucose	Sigma Aldrich	50-99-7
Bile	Sigma Aldrich	8008-63-7
Pepsin	Sigma Aldrich	9001-75-6
Lipase	Sigma Aldrich	9001-62-1
Mucin	Sigma Aldrich	84082-64-4
Pancreatin	Sigma Aldrich	8049-47-6
α-Amylase	Sigma Aldrich	9001-19-8
Bovine Serum Albumin	Sigma Aldrich	9048-46-8

Table SM2- Chemical and enzymatic composition of the oral fluids.

	Composition (g L^{-1})			
	Gastrointestinal phase			
	Gastric phase			
	Oral phase			
	Salivary fluid (S)	**Gastric fluid (G)**	**Duodenal fluid (D)**	**Biliary fluid (B)**
Inorganic Compounds	KCl (1.8) KSCN (0.4) NaH_2PO_4 (1.8) $NaSO_4$ (1.1) NaCl (0.6) $NaHCO_3$ (3.4)	NaCl (5.5) NaH_2PO_4 (0.5) KCl (1.6) $CaCl_2$ (0.8) NH_4Cl (0.6) HCl (1.3% v/v)	NaCl (14.0) $NaHCO_3$ (6.7) KH_2PO_4 (0.16) KCl (1.13) $MgCl_2$ (0.1) HCl (0.04% v/v) $CaCl_2$ (0.4)	NaCl (10.5) $NaHCO_3$ (11.6) KCl (0.8) HCl (0.03% v/v) $CaCl_2$ (0.4)
Organic Compounds	Urea (0.4) Uric Acid (0.03)	Glucose (1.3) D-Glucuronic Acid (0.04) Urea (0.2) Glucosamine Hydrochloride (0.7)	Urea (0.2)	Urea (0.5)
Enzymes	α-Amylase (0.5) Mucin (0.05)	BSA (2.0) Pepsin (5.0) Mucin (6.0)	BSA (2.0) Pancreatin (18.0) Lipase (3.0)	BSA (3.6) Bile (6.0)
pH	6.5 ± 0.5	1.1 ± 0.1	7.4 ± 0.2	8.0 ± 0.2

Captions for figures

Figure 1- *In vitro* bioaccessibility UBM protocol by BARGE. (Adapted from reference 2).

Figures

Figure 1

Figures

Figure 1

Table

Conflict of interest

We have to declare that there is no conflict of interest regarding this paper.

Contributions

Aline M. de Andrade was responsible for investigation, formal analysis, validation, and writing the original draft.

Rodrigo M. Galazzi was responsible for investigation and formal analysis.

Manuel M. Lladó was responsible for resources, as well as reviewing and editing the manuscript.

Marco A. Z. Arruda was responsible for conceptualization, reviewing and editing the manuscript, resources, supervision, and funding acquisition.