Environmental Research 166 (2018) 269–275

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Body burden of toxic metals and rare earth elements in non-smokers, cigarette smokers and electronic cigarette users

Mihaela Badea[a], Octavio P. Luzardo[b,*], Ana González-Antuña[b], Manuel Zumbado[b], Liliana Rogozea[a], Laura Floroian[c], Dana Alexandrescu[a], Marius Moga[a], Laura Gaman[d], Mariana Radoi[a], Luis D. Boada[b], Luis Alberto Henríquez-Hernández[b]

[a] Faculty of Medicine, Transilvania University of Brasov, Nicolae Balcescu 56, 500019 Brasov, Romania
[b] Toxicology Unit, Research Institute of Biomedical and Health Sciences (IUIBS), Instituto Canario de Investigación del Cáncer (ICIC), Universidad de Las Palmas de Gran Canaria, Paseo Blas Cabrera Felipe s/n, 35016 Las Palmas de Gran Canaria, Spain
[c] Faculty of Electrical Engineering and Computer Sciences, Transilvania University of Brasov, Bdul Eroilor 29, 500039 Brasov, Romania
[d] Faculty of Medicine, University of Medicine and Pharmacy Carol Davila, Bulevardul Eroii Sanitari, nr. 8, sector 5, Bucharest, Romania

ARTICLE INFO

Keywords:
Heavy metals
Electronic cigarette
Smoking
Exposure
Rare earth elements
Tobacco

ABSTRACT

Smoking is considered an important source for inorganic elements, most of them toxic for human health. During the last years, there has been a significant increase in the use of e-cigarettes, although the role of them as source of inorganic elements has not been well established. A cross-sectional study including a total of 150 subjects from Brasov (Romania), divided into three groups (non-smokers, cigarette smokers and electronic cigarettes smokers) were recruited to disclose the role of smoking on the human exposure to inorganic elements. Concentration of 42 elements, including trace elements, elements in the ATSDR's priority pollutant list and rare earth elements (REE) were measured by ICP-MS in the blood serum of participants. Cigarette smokers showed the highest levels of copper, molybdenum, zinc, antimony, and strontium. Electronic cigarette (e-cigarette) users presented the highest concentrations of selenium, silver, and vanadium. Beryllium, europium and lanthanides were detected more frequently among e-cigarette users (20.6%, 23.5%, and 14.7%) than in cigarette smokers (1.7%, 19.0%, and 12.1%, respectively); and the number of detected REE was also higher among e-cigarette users (11.8% of them showed more than 10 different elements). Serum levels of cerium and erbium increased as the duration of the use of e-cigarettes was longer. We have found that smoking is mainly a source of heavy metals while the use of e-cigarettes is a potential source of REE. However, these elements were detected at low concentrations.

1. Introduction

Contamination by heavy metals and, more recently, by rare earth elements (REE) and other minor elements (ME), has increased during the last decades due in part to their high use in technological and electronic devices (Hussain and Mumtaz, 2014). Although some heavy metals are necessary for life, most are considered non-essential and some have adverse health effects to humans—and other vertebrates—even at very low concentrations (Tchounwou et al., 2012). Moreover, some essential elements are included in the ATSDR's (Agency for Toxic Substances and Disease Registry) priority pollutant list for being toxic to living organisms at high concentrations (ATSDR, 2018; Tchounwou et al., 2012). Thus, a total of 23 elements are included in the ATSDR's priority pollutants list: silver (Ag), aluminum (Al), arsenic (As), barium (Ba), beryllium (Be), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), palladium (Pd), plutonium (Pu), antimony (Sb), selenium (Se), strontium (Sr), thallium (Tl), thorium (Th), uranium (U), vanadium (V), and zinc (Zn) (ATSDR, 2018).

REE and ME have been classified as evidently or potential occupational and environmental health risk factors by several international organizations (Pagano et al., 2015b). These elements have been increasingly and widely used in industry, agriculture, as well as in our daily life since they are very useful—or almost indispensable—for the manufacturing of all kinds of today's technological devices (Tansel, 2017). Thus, REE and ME are being mobilized from the few sites where they are abundant to be employed at an industrial scale, and therefore distributed all over the planet (Bozlaker et al., 2013). Thus, a number of

* Corresponding author.
E-mail addresses: mihaela.badea@unitbv.ro (M. Badea), octavio.perez@ulpgc.es (O.P. Luzardo), ana.gonzalezantuna@ulpgc.es (A. González-Antuña), manuel.zumbado@ulpgc.es (M. Zumbado), r_liliana@unitbv.ro (L. Rogozea), lauraf@unitbv.ro (L. Floroian), adanaso@unitbv.ro (D. Alexandrescu), mogas@unitbv.ro (M. Moga), laura.gaman@umfcd.ro (L. Gaman), mradoi_uniitbv@yahoo.com (M. Radoi), luis.boada@ulpgc.es (L.D. Boada), luis.henriquez@ulpgc.es (L.A. Henríquez-Hernández).

https://doi.org/10.1016/j.envres.2018.06.007
Received 16 April 2018; Received in revised form 24 May 2018; Accepted 4 June 2018
Available online 13 June 2018

"emerging pollutants" have appeared and are currently detected in living beings, including humans (Henriquez-Hernandez et al., 2017a, 2017b; Pagano et al., 2015a, 2015b).

Smoking is considered an important source for inorganic elements intake, mainly for some trace elements and other biochemically important elements (Chiba and Masironi, 1992). Thus, while cadmium or cupper are highly concentrated in cigarettes, for other elements—i.e. selenium—this association is inverted or even irrelevant (that is the case of mercury) (Bernhard et al., 2005). Anyhow, cigarette smoking interferes with the carefully controlled metal homeostasis of the human body and has to be considered as harmful to health. On the other hand, electronic cigarettes (e-cigarettes) are products that deliver a nicotine-containing aerosol—commonly called vapour—to users by heating a solution made up of propylene glycol or glycerol, nicotine and flavouring agents invented in their current form by Chinese pharmacist Hon Lik in the early 2000s (Grana et al., 2013). According to a report published by the Center for Tobacco Control Research and Education University of California, although young people are rapidly adopting e-cigarettes, there is a high level of dual use of e-cigarettes and conventional cigarettes among adults, mainly due to the belief that electronic cigarettes help to stop smoking. In that sense, all population-based studies of adult use show the highest rate of e-cigarette use among current smokers, followed by former smokers, with little use among non-smokers (Dockrell et al., 2013; King et al., 2013). However, e-cigarettes have not been proven to help people quit smoking (Grana et al., 2013). E-cigarettes pollute the air less than conventional cigarettes, but they do not emit "harmless water vapour" (Grana et al., 2013). Vapours' toxicant intake varies depending of which different e-liquids are used, the type of vaporizers, battery power settings and vaping regimes (Gillman et al., 2016; Sleiman et al., 2016). In that sense, formaldehyde, acetaldehyde, acrolein, diacetyl, acetol, glycidol, nicotine, nicotyrine, acenaphthene, isovaleraldehyde, formaldehyde, benzaldehyde and benzene have been detected in the vapour of e-cigarettes (Auer et al., 2017; Sleiman et al., 2016). However, other chemicals are not directly present in the e-liquids, but are either released from hardware components of the e-cigarette such as metal and silicate particles (Williams et al., 2013). It has been demonstrated that increasing battery outputs generates also increasing levels of some residues such as carbonyls (Kosmider et al., 2014). Moreover, the surface of the heating coil can reach temperatures as high as 110 °C using batteries > 10 watts, which conditions the level of volatile substances emitted by the e-cigarettes (Geiss et al., 2016). The effect that e-cigarettes may have in the uptake of inorganic elements—contained in e-liquids or as part of the electronic device—to the organism is unknown.

We have designed this study with the objective of measuring the blood concentrations of a total of 42 elements, including trace elements, elements in the ATSDR's priority pollutant list and REE—lanthanides and other ME—in a group of 150 subjects from Brasov (Romania). The series was divided into three groups (non-smokers, cigarette smokers and e-cigarettes users) and the results among groups were compared with the aim of disclosing the role of cigarette smoking and e-cigarette use as a source of inorganic pollutants.

2. Material and methods

2.1. Study design and participants

We conducted a cross-sectional study that included 150 Romanian subjects. All the subjects responded to a call made to participate in the present investigation. It was done in the context of the Faculty of Medicine of the Transilvania University of Brasov (Romania). Recruitment was done between December 2017 and February 2018. The series was formed by 58 non-smokers, 58 conventional cigarette smokers, and 34 e-cigarette users. All users of e-cigarette were ex-smokers. However, dual users—defined as persons who smoke cigarettes and use e-cigarette at the same time—were excluded from the study. The classification was based in self-reports of the participants. Demographical data were obtained and a face-to-face interview aimed to know details about the smoking status was also done. The questionnaire was designed exclusively for this purpose and data were recorded on paper and subsequently digitalized. Participation in the study was totally free and no one received any compensation.

All participants signed an informed consent before taking the sample. The study design was approved by the Ethical Committee of the Faculty of Medicine, Transilvania University of Brasov, Romania. Blood samples were obtained from all of the participants. All samples were obtained in the morning and the participants were informed not to smoke or use e-cigarette prior to the blood collection. Samples of blood were collected in 4 mL heparinized tubes (BD Vacutainer, LH 68 I.U. Lithium Heparin, BD-Plymouth, PL6 7BP, UK), maintained at 4 °C, and centrifuged at 1000 *g* for 15 min to separate the serum. The obtained serum was kept at − 20 °C until chemical analysis. Samples were sent to the University of Las Palmas de Gran Canaria for subsequent analysis.

2.2. Standards, samples and elements

We determined the serum concentration levels of 43 elements, including the trace elements and other REE and ME considered "emerging pollutants" (ATSDR, 2018; Tansel, 2017). Since, chromium was not considered for reasons of analytical confidence, the total number of elements finally included in this study was 42 (Additional file 1).

Samples consisted of 130 µL of serum, 1120 µL of ammonia solution (0.05% of EDTA, 0.05% of Triton X-100, and 1% of NH_4OH), and 50 µL of internal standards (ISTD) until a total final volume of 1.3 mL. ISTD solution was composed by Sc (scandium), Ge (germanium), Rh (rhodium), and Ir (iridium) at a stock concentration of 20 mg/mL each. Pure standards of elements in acid solution (5% HNO_3, 100 mg/L) were purchased from CPA Chem (Stara Zagora, Bulgary). Two standard curves (ten points, 0.005–20 ng/mL) were made to avoid interferences between elements: a) one using a commercial multi-element mixture (CPA Chem Catalog number E5B8·K1.5N.L1, 21 elements, 100 mg/L, 5% HNO_3) containing all the essential elements and main heavy metals; and b) other multi-element mixture tailor-made in our laboratory from individual elements (CPA Chem), which contained the REE and ME, as previously reported (Henriquez-Hernandez et al., 2017a).

2.3. Analytical methods

An Agilent 7900 ICP-MS (Agilent Technologies, Tokyo, Japan) with standard nickel cones, MicroMist glass concentric nebulizer, and Ultra High Matrix Introduction (UHMI) system was used for all measurements. The Integrated Sample Introduction System (ISIS) was configured for discrete sampling. The UHMI system was operated in robust mode. The 4th generation Octopole Reaction System (ORS4) was operated in helium (He) mode to reduce polyatomic interferences. A tuning solution consisting in a mix of Cs (cesium), Co (cobalt), Li (lithium), Mg (magnesium), Tl (thallium), and Y (yttrium) was used before the analysis for optimization of instrumentation. Quantification of the elements was made in the MassHunter v.4.2. ICP-MS Data Analysis software (Agilent Technologies).

The analytical method was optimized and validated, as previously reported (González-Antuña et al., 2017; Henriquez-Hernandez et al., 2017a). Recoveries obtained ranged from 89% to 128% for REE and ME, and from 87% to 118% for ATSDR's toxic heavy elements and essential elements. Linear calibration curves were found for all elements (regression coefficients > 0.998). The method limit of quantification (LOQ) was calculated by quantifying fifteen replicates of blanks, using 0.130 µL of alkaline solution. The LOQs were calculated as the concentration of the element that produced a signal that is three times higher than that of the averaged blanks. The accuracy and precision of this method was assessed using fortified alkaline solution (0.05, 0.5, and 5 ng/mL) in substitution of sample. In general, the calculated relative standard deviations (RSD) were lower than 8%, except for some few elements (Ti, Cr, Cu, Ni, Se, Fe, Ba, Zn, Sm), as the RSD raised to 15–16% at the lowest level of fortification. The precision improved at the highest level of concentration, as it was lower than 5% for all elements.

2.4. Statistical analysis

Descriptive analyses were conducted for all variables. Arithmetic means, standard deviation (SD), medians, ranges and percentiles 25th and 75th of the distribution were calculated for continuous variables. Proportions were calculated for categorical variables. Values below the LOQ but above LOD (Supplementary Table 1) were considered as ½ LOQ; and the values below the LOD were considered as null or non-detected for the calculation of frequencies of detection, and ½ LOD for the statistical analyses. Probability levels of less than 0.05 (two tailed) were considered statistically significant.

The normality of the data was tested using both, the Kolmogorov-Smirnov test (with Dallal-Wilkinson-Lillie for P value), and the D′Agostino-Pearson omnibus test. As expected, most of the data (i.e. concentrations of elements) did not follow a normal distribution. As a consequence we chose not assuming a normal distribution in any case, and comparisons between groups were performed using a non-parametric test (Kruskal-Wallis or Mann-Whitney *U* test). Differences in the categorical variables were tested by the chi-squared test. The correlation of inorganic elements with continuous variables was analysed by the Spearman's correlation test. We used PASW Statistics v 19.0 (SPSS Inc., Chicago, IL, USA) to manage the database of the study and to perform statistical analyses. Probability levels of < 0.05 (two tailed) were considered statistically significant.

3. Results and discussion

A total of 150 subjects were included in the present study, distributed in three different groups as follows: non-smokers (n = 58), cigarette smokers (n = 58), and e-cigarette users (n = 34). The series was mainly formed by females (76.7%), but gender distribution was not statistically different between groups (Table 1). We observed that mean age was statistically higher among e-cigarette users (35.2 ± 9.4 years old) compared with non-smokers and cigarette smokers ($P < 0.0001$; Table 1). This trend agrees with data published in the literature. In general terms, e-cigarette is used to quit smoking—although the success is quite limited—and therefore the use is initiated in older people who have been smoking previously, often during long periods of time (Adkison et al., 2013). This finding is supported by the fact that the mean of years smoking cigarettes was significantly higher among e-cigarette users (16.9 vs. 10.5 years in e-cigarette users and cigarette smokers, respectively, $P = 0.002$; Table 1). Moreover, population-based studies indicate that e-cigarettes are most commonly being used concurrently with conventional tobacco cigarettes (dual use) (Etter and Bullen, 2011). We observed that the median time of consumption of e-cigarettes was 12 months, a value higher than those reported in other studies which shows median values of 3 months in a group of 3587 e-cigarette users (Etter and Bullen, 2011). Shorter periods of use of e-cigarettes have also been reported (Goniewicz et al., 2013). No differences were observed regarding the age of onset to smoke. The highest proportion of e-cigarette users was shown among people from urban areas (Table 1), a finding which agrees with previous published data in other studies (Goniewicz et al., 2013).

3.1. Trace element status

The levels of 7 trace elements were measured in serum of the participants in the present study (Table 2). Since blood was collected in tubes containing anticoagulant, and anticoagulant suppose a bias for chromium determination (Mayo_Clinic), this element was deleted from the analysis. All trace elements were in the normal range in the group of non-smokers. As expected, the frequency of detection was 100% for all elements except for manganese. Copper (Cu), molybdenum (Mo), and zinc (Zn) were significantly higher among cigarette smokers ($P < 0.0001$ for all cases; Table 2). These results agree with the literature. Thus, higher serum levels of Cu have been reported among smokers (Chiba and Masironi, 1992) and it is known that Zn is present in cigarettes (about 70% is transferred to the smoke) (Chiba and Masironi, 1992). Although Zn serum concentrations in the average population have not been found to be affected by the smoking status (Galan et al., 2005), Zn concentration was higher in kidney cortex of smokers (Chiba and Masironi, 1992). Regarding to Mo, the available literature is brief and mainly report no differences of Mo between smokers and non-smokers (Kim et al., 2010). In the present study, while Mo concentration among non-smokers and e-cigarette users was quite similar (0.57 and 0.59 ng/mL, respectively), this concentration was higher among cigarette smokers (0.79 ng/mL). Mo requirement of plants varies with species, but its routine control is highly recommended for normal growth and development (Liu et al., 2000). Thus tobacco could be a source of Mo intake, whose origin could be in the supplements used for the best growth of the tobacco plant. To our knowledge, it is the first time reporting serum levels of Mo in a study like the present one.

We observed that e-cigarette users showed the highest level of selenium (Se), and this difference was significant even between cigarette smokers and e-cigarette users ($P = 0.022$; data not shown). Smoking has been shown to reduce blood Se levels through a not clear mechanism (Kafai and Ganji, 2003), although discrepancies has been reported in the literature (Lloyd et al., 1983). Selenium is a trace element also included in the ATSDR's priority pollutant list—together with Cu and Zn—, commonly employed in electronic devices (Tansel, 2017). Thus, a potential intake of this element through electronic cigarettes is plausible. However, serum level of selenium is affected by different factors including alcohol intake or diet (Lloyd et al., 1983), factors not

Table 1
Characteristics of the study population. Data presented as mean ± SD.

	Non smokers (n = 58)	Cigarette smokers (n = 58)	E-cigarette users (n = 34)	*P*
Gender (n, %)				0.307
Male	10 (17.2)	17 (29.3)	8 (23.5)	
Female	48 (82.8)	41 (70.7)	26 (76.5)	
Age (years)	24.5 ± 6.7	28.4 ± 10.8	35.2 ± 9.4	< 0.001[b]
BMI (n, %)[a]				0.078
< 18.5	8 (14.0)	3 (5.3)	1 (2.9)	
18.5–24.99	39 (68.4)	35 (61.4)	19 (55.9)	
25–29.99	8 (14.0)	14 (24.6)	8 (23.5)	
> 30	2 (3.5)	5 (8.8)	6 (17.6)	
Habitat (n, %)				0.012[c]
Rural	19 (32.8)	13 (22.4)	2 (5.9)	
Urban	39 (67.2)	45 (77.6)	32 (94.1)	
Age start smoking (years)	—	17.3 ± 3.9	18.4 ± 6.1	0.259
Time smoking cigarettes (years)	—	10.5 ± 8.7	16.9 ± 9.1	0.002[d]
Cigarettes/day	—	11.0 ± 5.9	—	n.a.
Time using e-cigarettes (months)	—	—	16.2 ± 15.5	n.a.

Abbreviations: SD, standard deviation; BMI, body mass index (kg/m^2); n.a., not applicable.
[a] 2 missed values.
[b] Kruskal-Wallis test.
[c] Chi square test.
[d] Mann-Whitney *U* test.

Table 2
Quantitative levels of trace elements in serum of non-smokers, cigarette smokers and e-cigarette users. The results were presented in ng/mL.

Element[a]	Non-smokers (n = 58) % of detection	Non-smokers (n = 58) Median (p25th–p75th)	Cigarette smokers (n = 58) % of detection	Cigarette smokers (n = 58) Median (p25th–p75th)	E-cigarette users (n = 34) % of detection	E-cigarette users (n = 34) Median (p25th–p75th)	P[b]
Cu (copper)[c]	100.0	611.35 (554.8–686.1)	100.0	961.28 (875.4–1089.9)	100.0	891.45 (798.6–958.2)	0.0001
Fe (iron)	100.0	1182.70 (771.8–1633.9)	100.0	970.74 (710.0–1221.0)	100.0	1150.62 (887.5–1514.8)	0.029
Mn (manganese)	86.2	0.67 (0.60–0.87)	91.4	0.79 (0.65–1.09)	94.1	0.78 (0.58–1.05)	0.145
Mo (molybdenum)	100.0	0.59 (0.47–0.75)	100.0	0.79 (0.65–0.95)	100.0	0.57 (0.40–0.93)	0.0001
Se (selenium)[c]	100.0	65.09 (58.3–76.9)	100.0	81.09 (70.2–92.4)	100.0	87.97 (79.6–95.0)	0.0001
Zn (zinc)[c]	100.0	645.67 (580.4–710.5)	100.0	1048.41 (934.5–1129.6)	100.0	870.75 (780.7–1008.3)	0.0001

Abbreviations: p25th–p75th, percentiles 25 and 75 of the distribution.
[a] Chromium was excluded from the analyses.
[b] Kruskal-Wallis test.
[c] Elements included in the ATSDR's priority pollutant list.

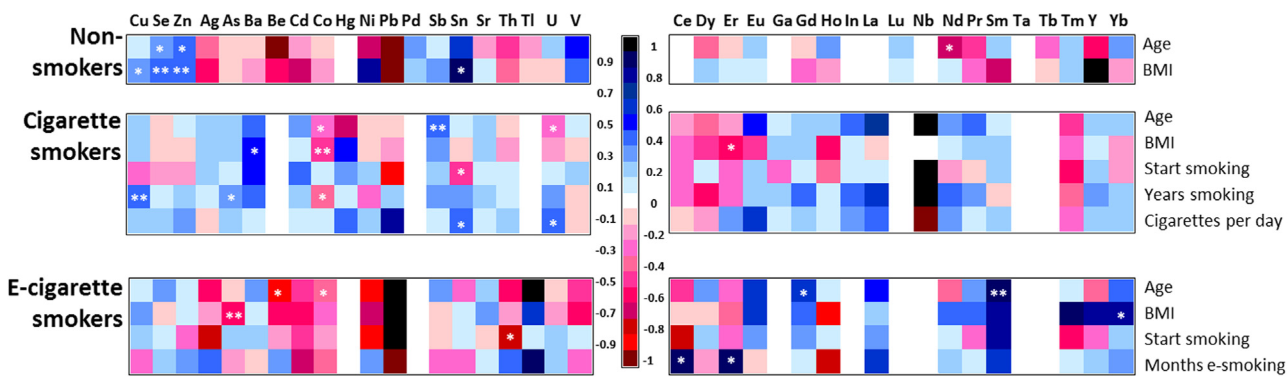

Fig. 1. Correlation between characteristics of the participants with trace elements included in the ATSDR's priority pollutants list—left panels— and with lanthanides and other rare earth elements—right panels—, in serum of non-smokers (upper panels), cigarette smokers (middle panels), and electronic cigarette smokers (lower panels). The correlations were analysed by the Spearman's rank correlation test. The higher the correlation coefficient, the deeper the coloring of the grating (blue for direct correlations and red for inverse correlations). *, P < 0.05; **, P < 0.01.

taken into account in the present study. It has been reported that BMI and age (Kimmons et al., 2006; Letsiou et al., 2014) influence the level of micronutrients including selenium. We observed a positive correlation of Se with age and BMI was observed, but only in the group of non-smokers (Fig. 1); thus, the presence of confounding factors—i.e. alcohol intake, diet or use of food supplements—has to be taken into account when interpreting this association.

3.2. Toxic elements in the ATSDR's priority contaminant list

A total of 20 toxic elements included in the ATSDR´s priority pollutant list were measured in the present study (Table 3). A number of 9 out of 20 elements were differentially distributed according to the smoking status.

We observed that antimony (Sb) and strontium (Sr) showed the highest concentrations among cigarette smokers (1.94 and 25.15 ng/mL, respectively). Both elements were detected in 100% of the participants. Those results absolutely agree with the literature (Bernhard et al., 2006; Huang et al., 2015). In the other hand, silver (Ag) and vanadium (V) were detected at the highest concentrations among e-cigarette users (Table 3), although the percentage was higher among smokers (48.3% vs. 20.6% for Ag and 93.1% vs. 35.3% for V, respectively). High concentrations of different elements—including Ag, Cr, Ni, Zn and V—were detected in the fluid and the aerosol of e-cigarettes (Aherrera et al., 2017; Saffari et al., 2014; Williams et al., 2017). According to a recent paper, the elements appeared to come from the filament (nickel, chromium), thick wire (copper coated with silver), brass clamp (copper, zinc), solder joints (tin, lead), and wick and sheath (silicon, oxygen, calcium, magnesium, aluminum) (Williams et al., 2017). With the exception of tin our results fit with the literature and support the theory that electronic cigarettes are a source of toxic elements intake. As stated, the device can reach temperatures as high as 110 °C, facilitating the incorporation of these elements through the vaping (Geiss et al., 2016). In relation to tin (Sn)—and also beryllium (Be)—, the result has to be taken with caution. Although both elements were detected at highest concentration among non-smokers, the frequency of detection was around 10%; while the highest frequencies of detection were observed among e-cigarette users and smokers (20.6% and 47.1% for Be and Sn, respectively; Table 3), and these differences were significant (X^2 test, $P = 0.003$ and $P < 0.0001$, respectively; data not shown).

As shown in Fig. 1, age of onset of smoking, years smoking and the number of cigarettes per day—in addiction to demographic variables—correlate with serum levels of some elements. It is of standing out the strong positive correlation between Cu and years of smoking as well as the strong positive association of cigarettes per day with Sn and uranium (which showed a frequency of detection ≈ 90% among cigarette smokers).

The number of detected elements was statistically different between groups (Fig. 2). Thus, 52 out of 58 (89.7%) cigarette smokers showed > 10 elements included in the ATSDR's priority pollutant list, compared with the 20.7% and 38.2% observed in non-smokers and e-cigarette users, respectively ($P < 0.0001$). These finding agree with the literature and places tobacco as an important source of toxic elements with potential adverse effects on people's health (Dai et al., 2015).

3.3. Rare earth elements and other minor elements

A total of 14 lanthanides and 5 minor elements (ME) were measured in the present study, and 5 of them (erbium, europium, gadolinium, holmium, and thulium) were differentially distributed between groups (Table 4). In any case, median values were very low. REE are present in

Table 3
Quantitative levels of elements included in the ATSDR's priority pollutant list in serum of non-smokers, cigarette smokers and e-cigarette users. The results were presented in ng/mL.

	Non-smokers (n = 58)		Cigarette smokers (n = 58)		E-cigarette users (n = 34)		
Element[a]	% of detection	Median (p25th–p75th)	% of detection	Median (p25th–p75th)	% of detection	Median (p25th–p75th)	P[b]
Ag (silver)	12.1	0.12 (0.1–0.2)	48.3	0.08 (0.1–0.1)	20.6	0.16 (0.1–0.5)	0.004
As (arsenic)	70.7	0.08 (0.0–0.2)	94.8	0.10 (0.1–0.2)	91.2	0.16 (0.1–0.3)	0.059
Ba (barium)	37.9	2.38 (1.7–3.7)	41.4	2.94 (2.2–5.6)	38.2	2.53 (1.9–3.1)	0.276
Be (beryllium)	5.2	0.75 (0.4–0.8)	1.7	0.26 (0.3–0.3)	20.6	0.30 (0.3–0.3)	0.026
Cd (cadmium)	13.8	0.04 (0.0–0.0)	74.1	0.04 (0.0–0.1)	23.5	0.03 (0.0–0.0)	0.579
Co (cobalt)	100.0	0.31 (0.3–0.4)	100.0	0.35 (0.3–0.5)	100.0	0.29 (0.2–0.4)	0.263
Hg (mercury)	1.7	0.47 (0.5–0.5)	13.8	0.55 (0.5–0.6)	2.9	0.49 (0.5–0.5)	0.564
Ni (nickel)	8.6	3.68 (3.5–4.6)	36.2	3.94 (3.5–4.6)	11.8	7.04 (3.9–10.0)	0.420
Pb (lead)	3.4	1.19 (0.9–1.5)	10.3	2.51 (1.1–4.6)	5.9	2.24 (1.0–3.5)	0.425
Pd (palladium)	15.5	0.01 (0.0–0.0)	0.0	—	2.9	0.01 (0.0–0.0)	0.375
Sb (antimony)	100.0	1.21 (1.1–1.4)	100.0	1.94 (1.6–2.2)	100.0	1.23 (1.1–1.6)	0.0001
Sn (tin)	10.3	8.95 (5.2–16.9)	48.3	3.35 (3.2–3.9)	47.1	5.38 (4.9–6.6)	0.0001
Sr (strontium)	100.0	20.52 (17.1–23.5)	100.0	25.15 (23.2–32.8)	100.0	23.18 (20.0–29.1)	0.0001
Th (thorium)	25.9	0.01 (0.0–0.0)	89.7	0.01 (0.0–0.0)	26.5	0.01 (0.0–0.1)	0.529
Tl (thallium)	65.5	0.03 (0.0–0.0)	0.0	—	8.8	0.03 (0.0–0.0)	0.482
U (uranium)	48.3	0.01 (0.0–0.0)	89.7	0.01 (0.0–0.0)	52.9	0.01 (0.0–0.0)	0.355
V (vanadium)	29.3	0.19 (0.2–0.2)	93.1	0.23 (0.2–0.3)	35.3	0.25 (0.2–0.3)	0.036

Abbreviations: p25th–p75th, percentiles 25 and 75 of the distribution.
[a] Results of elements included in ATSDR's priority list but also considered trace elements (Cu, Se and Zn) have been presented in Table 2.
[b] Kruskal-Wallis test.

computers and electronic devices (Tansel, 2017) and human exposure to elements such lanthanides (La), cerium (Ce), gadolinium (Gd) and lutetium (Lu) increases especially due to an occupational exposure (Pagano et al., 2015a). Little is known about the role of smoking as a source for REE, although some ME such as hafnium (Hf) and actinides like protactinium (Pa) and neptunium (Np) have been detected in tobacco, rolling paper and ash from different cigarette types (Nada et al., 1999). However, none of these elements was measured in the present study. It has been reported high concentrations of cerium (Ce) and lanthanum (La) in indoor air due to environmental tobacco smoke (Bohlandt et al., 2012), a findings supporting the fact that the frequencies of detection of erbium (Er) and gadolinium (Gd) were highest among cigarette smokers (39.7% vs. 17.6% and 48.3% vs. 35.3%; X^2 test, $P = 0.017$ and $P < 0.0001$, respectively; data not shown). These results are of interest since REE have been employed in the agricultural sector to improve tobacco growth (Boyko et al., 2011), a practice that can result in a higher level of exposure to these elements through smoked tobacco.

We observed a strong positive association between Ce and Er with the duration of the use of electronic cigarettes (Fig. 1), despite the fact that mean duration in the present study was 16.2 months. E-cigarettes have been suggested as a potential source for REE intake. This is the case of lanthanum, which has been detected in this type of devices (Williams et al., 2017). In the same line, we have observed an increased number of these elements encountered among e-cigarette users (Fig. 2). While 22 out of 58 cigarette smokers (37.9%) showed 5–10 different REE in the serum, 6 out of 58 (10.3%) of them showed > 10 different elements. In the other hand, these percentages were 11.8% and 11.8% among e-cigarette users (4 out of 34 in both cases), respectively (X^2 test, $P = 0.002$). A possible explanation to such scenarios is—as stated previously—the volatilization of this technology-related substances due to the high temperatures reached by the devices, a phenomenon previously reported for other chemicals (Sleiman et al., 2016).

To our knowledge, this is the first time that the level of such a large number of REE has been determined in a study like this, designed to understand the role of smoking cigarettes or using e-cigarettes as an additional source of exposure to this type of substance, especially taken into account that some of them (i.e. Ce or La) are associated with adverse health effects (Bohlandt et al., 2012).

3.4. Strengths and limitations of the study

One of the major limitations of this study is referred to the grouping of the participants. Due to the design of the study, based in self-reports

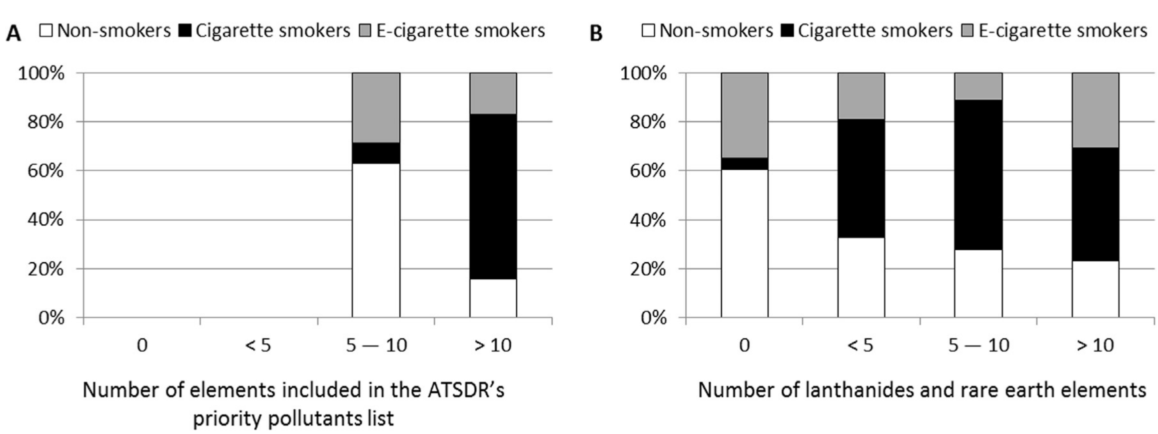

Fig. 2. Stacked bar graph showing the number of elements included in the ATSDR's priority pollutants list (A) and the number of lanthanides and rare earth elements (B)—expressed as absolute percentage—in the group of non-smokers, cigarette smokers and electronic cigarette smokers.

Table 4
Quantitative levels of lanthanides and other REE in serum of non-smokers, cigarette smokers and e-cigarette users. The results were presented in ng/mL.

	Non-smokers (n = 58)		Cigarette smokers (n = 58)		E-cigarette users (n = 34)		
Element	% of detection	Median (p5th–p95th)	% of detection	Median (p5th–p95th)	% of detection	Median (p5th–p95th)	P[c]
Ce (cerium)	0.0	—	41.4	0.14 (0.10–0.76)	17.6	0.21 (0.17–0.31)	0.058
Dy (dysprosium)	22.4	0.01 (0.01–0.05)	17.2	0.01 (0.01–0.02)	17.6	0.01 (0.01–0.02)	0.553
Er (erbium)	19.0	0.01 (0.01–0.04)	39.7	0.00 (0.00–0.01)	17.6	0.01 (0.00–0.01)	0.0001
Eu (europium)	22.4	0.01 (0.00–0.05)	19.0	0.00 (0.00–0.02)	23.5	0.00 (0.00–0.01)	0.042
Ga (gallium)[a]	1.7	0.12 (0.12–0.12)	37.9	0.09 (0.07–0.15)	0.0	—	0.171
Gd (gadolinium)	25.9	0.01 (0.00–0.05)	48.3	0.01 (0.00–0.03)	35.3	0.01 (0.01–0.21)	0.014
Ho (holmium)	22.4	0.01 (0.00–0.05)	17.2	0.00 (0.00–0.01)	11.8	0.00 (0.00–0.00)	0.001
In (indium)[a]	1.7	0.12 (0.12–0.12)	17.2	0.06 (0.04–0.09)	2.9	0.07 (0.07–0.07)	0.192
La (lanthanum)	0.0	—	12.1	0.14 (0.10–0.32)	14.7	0.12 (0.11–0.22)	0.871
Lu (lutetium)	20.7	0.01 (0.00–0.05)	1.7	0.01 (0.01–0.01)	2.9	0.00 (0.00–0.00)	0.235
Nb (niobium)[a]	0.0	—	3.4	0.12 (0.09–0.16)	2.9	0.21 (0.21–0.21)	0.221
Nd (neodymium)	20.7	0.02 (0.02–0.06)	36.2	0.03 (0.02–0.30)	26.5	0.05 (0.02–0.13)	0.183
Pr (praseodymium)	24.1	0.01 (0.00–0.06)	50.0	0.01 (0.00–0.05)	29.4	0.02 (0.01–0.05)	0.179
Sm (samarium)	8.6	0.01 (0.01–0.04)	46.6	0.01 (0.01–0.05)	14.7	0.02 (0.01–0.03)	0.633
Ta (tantalum)[a]	0.0	—	0.0	—	0.0	—	n.a.
Tb (terbium)	12.1	0.01 (0.01–0.05)	0.0	—	0.0	—	n.a.
Tm (thulium)	22.4	0.01 (0.00–0.05)	17.2	0.00 (0.00–0.00)	8.8	0.00 (0.00–0.00)	0.0001
Y (yttrium)[a]	5.2	0.02 (0.02–0.04)	69.0	0.03 (0.02–0.21)	26.5	0.04 (0.01–0.08)	0.497
Yb (ytterbium)	31.0	0.01 (0.00–0.04)	50.0	0.00 (0.00–0.01)	29.4	0.00 (0.00–0.01)	0.163
Σ lanthanides[b]	53.4	0.02 (0.00–0.36)	89.6	0.06 (0.00–0.81)	52.9	0.03 (0.00–0.70)	0.172

Abbreviations: REE, rare earth elements; p5th–p95th, percentiles 5 and 95 of the distribution; n.a., not applicable.

[a] Not in the lanthanides group.

[b] Promethium (Pm)—included in the lanthanides group—was not measured in the present study.

[c] Kruskal-Wallis test.

of the subjects about smoking, misclassification of smokers and e-cigarette users must be considered as a possible bias. In the sense, it is possible that e-cigarette users have not declared that they are actually smoking (even a small number of cigarettes per day or even occasionally) or viceversa. Although subjects who smoke and use e-cigarette were excluded, dual users suppose a bias that has to be taken into account, especially when the number of subjects is reduced. Potential bias due to cigarette smoking or e-cigarette users by self-declared non-smokers is also possible. Possible biases of this study have to be taken into account: i) difference in age distribution (that can influence the levels of certain elements) or ii) majority of women (whose serum levels of certain elements may be affected by gender). In addition, a potential selection bias can derive from the voluntary nature of the participants, especially if the wish to participate would be linked to the conditions and hypothesis under investigation, something that could condition the inclusion, for example, of heavy smokers or heavy e-cigarette users. Other variables associated to the intake of inorganic elements have to be taken into account, especially those related with diet, food supplements, alcohol intake or even the cigarette brand or the e-cigarette model (containing information about voltage and battery power). Finally, all the measurements were made in serum, and it is known that analysis in urine would be useful to differentiate acute vs. toxic exposure, especially for some elements such metals (Jain, 2018).

Notwithstanding these limitations, we consider that this work has important strengths. First, this population is rarely assessed at all, and most of these elements have been rarely, if at all, determined in a study like that. For many of these elements, the provenance from smoking has never been linked to concentrations of several of these metals in serum before. Although populations were limited, for many elements a statistical difference was found between non-smokers, smokers of cigarettes or users of e-cigarettes. Given the cross-sectional nature of the study design there is no possibility of establishing causality. Even so, these results, despite their limitations, are highly interesting and encourage further research.

4. Conclusions

In this research, a total of 42 inorganic elements (including trace elements, elements belonging to the ATSDR's priority pollutant list and REE) were measured in a series of Romanian people grouped according to the smoking status (non-smokers, cigarette smokers and e-cigarette users). We have found that tobacco smoke is a source for toxic elements such as copper, zinc, antimony, strontium or vanadium. On the other hand, e-cigarettes seem to be a new source for intake of silver, tin and REE as cerium, erbium or gadolinium. Although most of these elements were detected at lower concentrations, additional research is required aimed to explore the health effects produced by the continuous intake of the inorganic elements.

Acknowledgements

This work was possible with partial funding obtained by M. Badea from UEFISCDI – Grant for international mobility PN-III-P1-1.1.MC-2017-1797/2017 – ctr. 546/2017 and UTBV – Fellowship for international mobility 2017.

The authors thank Mrs. María de los Reyes Suárez Hanna for her technical assistance.

Competing financial interest declaration

There are no actual or potential conflicts of interest to declare for any author.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2018.06.007.

References

Adkison, S.E., et al., 2013. Electronic nicotine delivery systems: international tobacco control four-country survey. Am. J. Prev. Med. 44, 207–215.
Aherrera, A., et al., 2017. The association of e-cigarette use with exposure to nickel and chromium: a preliminary study of non-invasive biomarkers. Environ. Res. 159, 313–320.
ATSDR, 2018. Agency for Toxic Substances and Disease Registry.
Auer, R., et al., 2017. Heat-not-burn tobacco cigarettes: smoke by any other name. JAMA Intern. Med. 177, 1050–1052.

Bernhard, D., et al., 2006. Increased serum cadmium and strontium levels in young smokers: effects on arterial endothelial cell gene transcription. Arterioscler. Thromb. Vasc. Biol. 26, 833–838.
Bernhard, D., et al., 2005. Metals in cigarette smoke. IUBMB Life 57, 805–809.
Bohlandt, A., et al., 2012. High concentrations of cadmium, cerium and lanthanum in indoor air due to environmental tobacco smoke. Sci. Total Environ. 414, 738–741.
Boyko, A., et al., 2011. Potassium chloride and rare earth elements improve plant growth and increase the frequency of the Agrobacterium tumefaciens-mediated plant transformation. Plant Cell Rep. 30, 505–518.
Bozlaker, A., et al., 2013. Quantifying the contribution of long-range Saharan dust transport on particulate matter concentrations in Houston, Texas, using detailed elemental analysis. Environ. Sci. Technol. 47, 10179–10187.
Chiba, M., Masironi, R., 1992. Toxic and trace elements in tobacco and tobacco smoke. Bull. World Health Organ. 70, 269–275.
Dai, J.B., et al., 2015. The hazardous effects of tobacco smoking on male fertility. Asian J. Androl. 17, 954–960.
Dockrell, M., et al., 2013. E-cigarettes: prevalence and attitudes in Great Britain. Nicotine Tob. Res. 15, 1737–1744.
Etter, J.F., Bullen, C., 2011. Electronic cigarette: users profile, utilization, satisfaction and perceived efficacy. Addiction 106, 2017–2028.
Galan, P., et al., 2005. Serum concentrations of beta-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population. Eur. J. Clin. Nutr. 59, 1181–1190.
Geiss, O., et al., 2016. Correlation of volatile carbonyl yields emitted by e-cigarettes with the temperature of the heating coil and the perceived sensorial quality of the generated vapours. Int. J. Hyg. Environ. Health 219, 268–277.
Gillman, I.G., et al., 2016. Effect of variable power levels on the yield of total aerosol mass and formation of aldehydes in e-cigarette aerosols. Regul. Toxicol. Pharmacol. 75, 58–65.
Goniewicz, M.L., et al., 2013. Patterns of electronic cigarette use and user beliefs about their safety and benefits: an internet survey. Drug Alcohol Rev. 32, 133–140.
González-Antuña, A., et al., 2017. Simultaneous quantification of 49 elements associated to e-waste in human blood by ICP-MS for routine analysis. MethodsX 4, 328–334.
Grana, R., et al., 2013. Background paper on E-cigarettes (electronic nicotine delivery systems). In: U. o. C. Center for Tobacco Control Research and Education, San Francisco, A WHO Collaborating Center on Tobacco Control. Prepared for World Health Organization Tobacco Free Initiative. (Ed.).
Henriquez-Hernandez, L.A., et al., 2017a. Blood levels of toxic metals and rare earth elements commonly found in e-waste may exert subtle effects on hemoglobin concentration in sub-Saharan immigrants. Environ. Int. 109, 20–28.
Henriquez-Hernandez, L.A., et al., 2017b. Study of the influencing factors of the blood levels of toxic elements in Africans from 16 countries. Environ. Pollut. 230, 817–828.
Huang, Y., et al., 2015. Levels and risk factors of antimony contamination in human hair from an electronic waste recycling area, Guiyu, China. Environ. Sci. Pollut. Res. Int. 22, 7112–7119.
Hussain, M., Mumtaz, S., 2014. E-waste: impacts, issues and management strategies. Rev. Environ. Health 29, 53–58.
Jain, R.B., 2018. Concentrations of selected metals in blood, serum, and urine among US adult exclusive users of cigarettes, cigars, and electronic cigarettes. Toxicol. Environ. Chem. 100, 134–142.
Kafai, M.R., Ganji, V., 2003. Sex, age, geographical location, smoking, and alcohol consumption influence serum selenium concentrations in the USA: third National Health and Nutrition Examination Survey, 1988–1994. J. Trace Elem. Med. Biol. 17, 13–18.
Kim, Y.J., et al., 2010. Effects of smoking on trace metal levels in saliva. Oral Dis. 16, 823–830.
Kimmons, J.E., et al., 2006. Associations between body mass index and the prevalence of low micronutrient levels among US adults. MedGenMed 8, 59.
King, B.A., et al., 2013. Awareness and ever-use of electronic cigarettes among U.S. adults, 2010–2011. Nicotine Tob. Res. 15, 1623–1627.
Kosmider, L., et al., 2014. Carbonyl compounds in electronic cigarette vapors: effects of nicotine solvent and battery output voltage. Nicotine Tob. Res. 16, 1319–1326.
Letsiou, S., et al., 2014. Gender-specific distribution of selenium to serum selenoproteins: associations with total selenium levels, age, smoking, body mass index, and physical activity. Biofactors 40, 524–535.
Liu, S., et al., 2000. Spectrophotometric determination of trace amounts of molybdenum in vegetal and tobacco tissues with a new chromogenic reagent, dimethoxyhydroxyphenylflurone. J. Agric. Food Chem. 48, 5860–5863.
Lloyd, B., et al., 1983. Effect of smoking, alcohol, and other factors on the selenium status of a healthy population. J. Epidemiol. Community Health 37, 213–217.
Mayo_Clinic. Mayo Medical Laboratories. Test ID: CRS. Chromium, serum.
Nada, A., et al., 1999. Heavy metals and rare earth elements source-sink in some Egyptian cigarettes as determined by neutron activation analysis. Appl. Radiat. Isot. 51, 131–136.
Pagano, G., et al., 2015a. Rare earth elements in human and animal health: state of art and research priorities. Environ. Res. 142, 215–220.
Pagano, G., et al., 2015b. Health effects and toxicity mechanisms of rare earth elements-Knowledge gaps and research prospects. Ecotoxicol. Environ. Saf. 115, 40–48.
Saffari, A., et al., 2014. Particulate metals and organic compounds from electronic and tobacco-containing cigarettes: comparison of emission rates and secondhand exposure. Environ. Sci. Process Impacts 16, 2259–2267.
Sleiman, M., et al., 2016. Emissions from electronic cigarettes: key parameters affecting the release of harmful chemicals. Environ. Sci. Technol. 50, 9644–9651.
Tansel, B., 2017. From electronic consumer products to e-wastes: global outlook, waste quantities, recycling challenges. Environ. Int. 98, 35–45.
Tchounwou, P.B., et al., 2012. Heavy metal toxicity and the environment. EXS 101, 133–164.
Williams, M., et al., 2017. Elements including metals in the atomizer and aerosol of disposable electronic cigarettes and electronic hookahs. PLoS One 12, e0175430.
Williams, M., et al., 2013. Metal and silicate particles including nanoparticles are present in electronic cigarette cartomizer fluid and aerosol. PLoS One 8, e57987.