Evaluation of trace metal and polychlorinated biphenyl levels in tea brands of different origin commercialized in Italy

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ABSTRACT

The objectives of this study were to investigate the trace element (Hg, Cd, Pb, Cu, Zn, Ni, Fe, Cr and Se) and polychlorinated biphenyl (PCBs) content of several commercially available brands of green and black tea marketed in Italy. The concentrations these chemicals were found to be variable and largely dependent upon the type and brand of analysed tea. The most abundant element among the essential elements was Fe, followed by Zn, Cu, Se, Ni and Cr, whereas Pb was the predominant among the tested nonessential elements followed by Hg and Cd. Assessment based on several available guidelines showed that element content were low, except for Hg and Ni. The PCBs concentrations were generally low, with a homologue profile dominated by low-chlorinated congeners, namely three- and tetra-PCBs accounting for more than 60% of the total residue. Apart from trace elements, this is the first study documenting in detail the concentrations and congener distribution of PCBs in tea samples of different origin.

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1. Introduction

Tea, from the plant *Camellia sinensis*, is the second most consumed non-alcoholic beverage across the world next to water (Tea Association of the USA Inc., 2014), and consequently its commercial and social importance is obvious. According to Food and Agriculture Organization of the United Nations (FAO) world production is estimated to be 4.8 million tons in 2012 (FAO, 2012), with China and India being the main tea-producing countries. The consumption of tea beverages tea *per-capita* in the Italy is 0.10 kg/year (FAO, 2011). However, tea-drinking habit is considerably growing not only for its desirable aroma and refreshing taste, but also for its multiple health-promoting effects. Numerous epidemiological and pharmacological studies link, in fact, tea consumption to beneficial effects on human health, such as prevention from many types of cancer, diabetes by reducing the blood–glucose activity and Alzheimer's disease, besides to a enhancement of immune defences and a reduction of inflammations and blood cholesterol levels (Chacko et al., 2010). The therapeutic action of tea, especially of green tea, is mainly attributed to its polyphenol content, particularly flavanols and flavonols, which represent 30% of fresh leaf dry weight (McKay and Blumberg, 2002). However, even though the enormous benefit of tea it may contain substances undesirable, dangerous and toxic. Among them, elements such as mercury (Hg), cadmium (Cd) and lead (Pb), listed as the most toxic heavy metals as well as organic contaminants, including polychlorinated biphenyls (PCBs) are of special concern. As consequence of their environmental persistence and potential for bioaccumulation, these chemicals tend to bioconcentrate and/or biomagnify in the food chain representing a definite hazard for both wildlife and humans (Van den Berg et al., 1998). Exposure to these chemicals has been found to result in a variety of adverse human health effects. Hg, highly neurotoxic, explicates its negative effects at levels of 1×10^{-4} µg g^{-1}/day (US EPA, 2014), a long-term exposure to Pb levels of 4×10^{-3} µg g^{-1}/day (US EPA, 2010) may lead to memory deterioration and prolonged reaction times, while levels of Cd above 1×10^{-3} µg g^{-1}/day (US EPA, 2014) may determine kidney damage and also bone effects and fractures (Cao et al., 2010). Several studies have been made to assess tea quality by chemical analysis, usually with reference to trace elements. The results of these studies show a different accumulation according to the type of tea and geological source and suggest that tea leaves are especially prone to contamination by metals either through the roots from the soils or through the leaf blades from rain fall, atmospheric dusts, plant protection agents and fertilizers, as well as during the processing stage (Aksuner et al., 2012; Falahi and Hedaiati, 2013;

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Marbaniang et al., 2011). In contrast to great amount of literature on trace elements, data for PCBs are rare. Only small scale investigations producing initial information have been performed (Amakura et al., 2009; Nakata et al., 2002; Polder et al., 2010), but detailed reports on the content and congener distributions of PCBs are lacking. The knowledge and documentation of these chemical concentrations is, instead, essential, above all in food products from developing countries as China and India. In these latter countries a rapid and unorganized urban and industrial growth has contributed to elevated levels of pollutants into the environment (Sharma et al., 2009). Furthermore, the lack of regulation specifying the tolerance limits for many of these chemicals in tea imposes routine check. With the above background in mind, the present study was undertaken to quantify and to compare the concentration levels of non-essential (Hg, Cd, Pb) and essential elements (Cu, Ni, Zn, Fe, Cr, Se) as well as polychlorinated biphenyls (PCBs) in green and black tea imported in Italy from China and India. In addition, the trace element concentrations were compared with internationally approved legal limits.

2. Materials and methods

2.1. Sample collection

A set of 30 tea samples, including green and black tea from different geographical origins were purchased from various Italian supermarkets in boxes with 20 or 25 infusion bags each containing 1.0–2.0 g of the product material. For green tea, eleven samples were of Indian origin, four of Chinese production, and five were of unknown origin, but packed in Europe. For black tea the samples selected were half of Chinese production and half of Indian origin. To acquire a representative sample, the powder of 40 infusion bags from two boxes of each sample were uniformly mixed and oven dried at 100 °C to prepare the material for the chemical analysis.

2.2. Sample preparation and clean up

The extractive analytical procedure and the instrumental conditions for determine trace element concentrations have been described in detail elsewhere (Barone et al., 2013). Briefly, about 0.5 g of the samples were digested to a transparent solution with a mixture of HNO_3–$HClO_4$ (8:3) for cadmium (Cd), lead (Pb), nichel (Ni), zinc (Zn), copper (Cu) and iron (Fe) determination and with a mixture of H_2SO_4–HNO_3 (1:1) for mercury (Hg) and selenium (Se). The completely digested samples were allowed to cool temperature and diluted with deionized water according to the method recommended by Official Italian Agencies (G.U.R.I., 1994).

2.2.1. Instrumental analysis of trace elements

The content of elements was determined by atomic absorption spectrophotometry (Shimadzu AA 7000). Zn, Ni and Fe were analysed by flame, Cd, Pb, Cr, and Cu by using a graphite furnace (high-density tube) (GFA-7000), Hg and Se were measured by using a hydride vapour generator (HVG-1) after reduction by $NaBH_4$.

2.2.2. Instrumental analysis of PCBs

Concerning PCBs, the concentrations of 23 individual congeners (PCBs: 33, 53, 110, 142, 170; indicator PCBs: 28, 52, 101, 138, 153 and 180; "dioxin-like" PCBs: non-ortho PCBs 77, 81, 126, 169 and mono-ortho PCBs 105, 114, 118, 123, 156, 157, 167, 189) (Table 1) were determined using analytical procedures previously described and validated (Storelli, 2014). Briefly, about 40 g of powder were mixed with Na_2SO_4 and spiked with PCB 143 used as internal standard. The mixture was extracted with hexane:acetone (9:1) and the extracts were concentrated in order to determine the fat content by gravimetry. Next the extract was dissolved in hexane and cleaned by passing through 8 g of acid silica (H_2SO_4, 44% w. w.), using 50 mL of a mixture of hexane/dichloromethane (1/1, v/v) for elution of the analytes. The eluate was evaporated to dryness and redissolved in 100 μL of iso-octane. For the separation of non-ortho PCB congeners from other PCBs, the method reported by Tanabe et al. (1987), involving fractionation on 125 mg of activated carbon (434455 C. Erba, Milano, Italy), was used. For the analysis of PCBs, a Thermo Trace GC connected with a Thermo PolarisQ MS operated in electron impact ionization (EI) mode was equipped with a 30 m × 0.25 mm × 0.25 μm Rtx 200 capillary column (Thermo, Austin, Texas, USA). The MS was used in the SIM mode with two ions monitored for each PCBs homologue group in specific windows. One μl of the cleaned extract was injected in splitless mode (injector temperature 90 °C then to 300 °C with 70 °C/min), splitless time 1.50 min, pulse pressure time 1.50 min, pressure pulse 25 psi. Helium was used as carrier gas at constant flow (1.0 ml/min). The temperature of the Rtx 200 column was held at 90 °C for 1.50 min, then increased to 180 °C at a rate of 15 °C/min, further increased to 280 °C at a rate of 5 °C/min, further increased to 300 °C at a rate of 40 °C/min, held for 7 min.

2.3. Quality control and assurance

Reference tissue (Tomato leaves SRM1573a, NIST MD, USA) was treated and analysed in the same way as the samples for each batch of samples. The results (Hg: 0.039 ± 0.008; Cd: 1.45 ± 0.11; Cu: 4.92 ± 0.38; Ni: 1.54 ± 0.22; Zn: 29.20 ± 1.90; Fe: 353.00 ± 22.00; Cr: 2.01 ± 0.15; Se: 0.059 ± 0.008 μg g^{-1} dry weight) were in good agreement with the certified values (Hg: 0.034 ± 0.004; Cd: 1.52 ± 0.04; Cu: 4.70 ± 0.14; Ni: 1.59 ± 0.07; Zn: 30.90 ± 0.70; Fe: 368.00 ± 7.00; Cr: 1.99 ± 0.06; Se: 0.054 ± 0.003 μg g^{-1} dry weight). The results for standard reference material displayed recoveries of the elements ranging from 94% to 107% (n = 3). The limits of detection (LOD), defined as the concentration corresponding to three times the standard deviation of blanks are the following: Hg: 5.00; Cd: 0.10; Pb: 10.00; Cu: 25.00; Ni: 25.00; Zn: 25.00; Fe: 80.00; Cr: 5.00; Se: 0.50 ng g^{-1} wet weight. The limit of quantification (LOQs) defined as the concentration corresponding to ten times the standard deviation of blanks are the following: Hg: 13.00; Cd: 0.38; Pb: 40.00; Cu: 80.00; Ni: 80.00; Zn: 85.00; Fe: 250.00; Cr: 16.00; Se: 8.00 ng g^{-1} wet weight. Two blank samples were analysed together with each sample batch. Trace element concentrations in blanks were below the detection limits in all the analyses. Blanks and calibration standard solutions were similarly analysed as the digested sample solution, and calibration curves constructed. Analyses were triplicated and relative standard deviations among replicates were always less than 10%. Recovery tests were performed for the investigated trace elements in the selected samples by spiking analysed samples with aliquots of the standards and then carrying out digestion. The recovery percentages ranged from 96 to 99%. For PCBs quality control was performed through the analysis of procedural blanks, a duplicate sample and a standard reference material [CRM349 for PCBs (cod liver oils) (BCR, Brussels)] within each batch of samples. The recovery percentage of the standard reference material were in the range of 86–105%. For the samples and standard reference materials, the relative standard deviations (RSD) were <10% for all the detected compounds. The limit of detection (LOD) for PCBs ranged from 0.02 to 0.50 ng g^{-1} on a lipid weight basis, while the limit of quantification (LOQ) varied from 0.20 to 1.30 ng g^{-1} on a lipid weight basis. Appropriate standard solution was added to the samples and recovery values were between 82 and 104%. The trace element and PCB concentrations in the samples are expressed as μg g^{-1} and ng g^{-1} dry weight, respectively.