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Mercury content in beetroot and beetroot-based dietary supplements

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ABSTRACT

Total mercury (THg) concentrations in fifty-four beetroot-based DSs and seven lots of conventional and organic beetroots (divided into unpeeled, peeled, and skins) were determined by direct thermal decomposition-gold amalgamation cold vapour atomic absorption spectrometry. The analytical procedure was optimised and validated. The recovery (%) for Hg was 101.9 and 92.73 in BCR-463 and DOLT 4, respectively. The intermediate precision value (4.7%) exceeded the repeatability value, which was as expected. The estimated LOD and LOQ values of the analytical procedure used were 0.096 and 0.29 [ng], respectively, and were converted to corresponding MDL and MQL values, which were 0.96 and 2.9 [ng/g], respectively. The highest contents of THg were found in conventional (28.03 ng/100 g w.w.) and organic (56.2 ng/100 g w.w.) beetroot or powder supplements (0.65 ng/g). Statistical analysis confirmed the differentiation of the analysed group of products at the level of significance 0.05 and 0.001. There were found statistically significant relationships in terms of: dietary supplement-beetroot ($p < 0.001$), beetroot-part of vegetable ($p < 0.05$) and dietary supplement-pharmaceutical form ($p < 0.05$). In conclusion, the analysed DSs did not pose a significant risk for a consumer in terms of permissible contamination limit, Provisional Tolerable Weekly Intake realisation, and the Target Hazard Quotient.

1. Introduction

Mercury (Hg) is a silvery-white metal with an intense lustre and molecular weight of 200.59 g/mol (Clarkson and Magos, 2006). Classification of Hg and its compounds covers three main groups: metallic mercury (Hg^0), inorganic mercury (Hg^{2+}), and organic mercury (methyl mercury: CH_3Hg^+, etc.) (Sakamoto et al., 2018). Mercury compounds present in the earth's crust or fuels do not pose a threat to living organisms. Afterwards, it is released, it becomes very mobile as it effortlessly transforms into various chemical forms and remains permanently in the environment (Gworek and Rateńska, 2009). It is one of the elements with the highest accumulation factor (Gworek and Rateńska, 2009; Saletnik et al., 2016). The content of mobile Hg forms in the environment and their bioavailability to plants imply the real threat. In slightly acidic to alkaline soils, Hg can remain strongly bound to humic macromolecular substances and is not available to plants in this form (Clarkson and Magos, 2006; Peralta-Videa et al., 2009). Under these conditions, Hg can also be bound to low molecular weight humic acids that are readily soluble and facilitate its uptake by plant organisms. Mercury is taken up by plants from the soil as ions or by leaves from the atmosphere, then, accumulate Hg in the forms of Hg(0) and Hg(II) (Li et al., 2017). However, aquatic plants contain more organic Hg (methyl

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Table 1
Full characteristics of the analysed beetroot samples based on information in the place of purchase or label.

Form	Code	Water content (%)	Date of purchase	Certificate of organic cultivation	Place of purchase	Origin country
Conventional	1 Bp	85.9	11/05/2019	lack of certificate	large-area shop, Gdańsk (PL)	Poland (PL)
	1Bs	83.2				
	1Bu	85.1				
	3 Bp	85.2	11/14/2019	lack of certificate	retail shop, Kolbudy (PL)	Poland (PL)
	3Bs	84.2				
	3Bu	84.2				
	4 Bp	81.8	11/14/2019	lack of certificate	large-area shop, Gdańsk (PL)	Poland (PL)
	4Bs	78.4				
	4Bu	80.5				
	5 Bp	88.1	11/28/2019	lack of certificate	large-area shop, Gdańsk (PL)	Poland (PL)
	5Bs	86.8				
	5Bu	87.8				
Organic	2 Bp	83.2	11/05/2019	P 095 18, region: Greater Poland (PL)	large-area shop, Gdańsk (PL)	Poland (PL)
	2Bu	81.2				
	6 Bp	85.2	12/02/2019	PL-EKO-07–07904 Wilkowa Wieś, region: Pomeranian (PL)	grocery store (Internet), Gdańsk (PL)	Poland (PL)
	6Bs	80.9				
	6Bu	83.1				
	7 Bp	83.4	12/02/2019	PL-EKO-07–07904 Wilkowa Wieś, region: Pomeranian (PL)	grocery store (Internet), Gdańsk (PL)	Poland (PL)
	7Bs	80.0				
	7Bu	82.9				

Bp – peeled beetroot; Bs – beetroot skins, Bu – unpeeled beetroot.

mercury) than terrestrial plants (Li et al., 2017; Qiu et al., 2008). This element, like lead (Pb) and cadmium (Cd), does not participate in the vital functions of plants. After getting into the tissues, it is firmly bound by the sulfhydryl groups of proteins and may pose many threats to these organisms, i.e. disturb the processes of cellular respiration, mainly enzymatic transformations (Peralta-Videa et al., 2009). Moreover, Hg is toxic to higher organisms and accumulates in the subsequent links of the trophic chain (Konieczka et al., 2022; Peralta-Videa et al., 2009; Tchounwou et al., 2012).

Due to its high toxicity and bioaccumulation ability, Hg can be detrimental for humans at low concentrations in every form (Li et al., 2017; World Health Organisation, 2016). Organic Hg (such as methylmercury) is found to be the most toxic for humans because of its lipophilicity and ability to deposit in the central nervous system (Rani et al., 2019). The toxicity mechanisms are related to binding thiol groups in proteins by Hg ions, inhibiting enzymes, cofactors, and hormones by inactivating sulphur in their structures. As a consequence, the accumulation of Hg in the organism appears and leads to severe neurological disorders in children and adults (Mathieson, 1995). In 2008, the European Commission defined the permissible Hg contamination in dietary supplements at 0.10 mg/kg (The Commission of the European Communities, 2008). Moreover, the risk of human intoxication through diet intake can be assessed by estimating the realisation of Provisional Tolerable Weekly Intake (PTWI) which is 4 μg/kg body weight for Hg (Seventy-Second Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), 2012). Mercury might occur as a contaminant in food (Abernethy et al., 2010; Li et al., 2017; Qiu et al., 2008) as well as in DSs (Brodziak-Dopierała et al., 2018; Dolan et al., 2003; Kowalski and Frankowski, 2015; Puścion-Jakubik et al., 2021). The relatively easy and free procedure of introducing DS to the Polish market (Brzezińska and Grembecka, 2021) results in introducing thousands of new products appearing each year ("Register of products subject to the notification of the first marketing," n.d.). In Poland, the manufacturer is not obliged to submit documentation confirming the effectiveness of the introduced product or to carry out quality tests of the finished form, which carries the risk of the existence of products on the market of insufficient quality or bearing health risk for the consumer (Brzezińska and Grembecka, 2021). By definition, DS is a concentrated source of nutrients and bioactive ingredients (The Seym of the Republic of Poland, 2006). However, due to their concentrated form, they can also pose a higher risk to the consumer than conventional food. One of the most popular and readily available supplements are plant-based products, which are widely used in the non-pharmacy trade, especially online. Due to the lack of obligation to standardise the products used, they may contain contaminants, e.g., heavy metals, including Hg. Especially when they are distributed by small entities, often present on the market for one season.

Beetroot is a root vegetable that tends to accumulate toxic elements, particularly Cd, Hg, and Pb, which are considered harmful (Ćwieląg-Drabek et al., 2020; Saletnik et al., 2016). In the literature, some reports on the assessment of Hg content in different DSs can be found (Brodziak-Dopierała et al., 2018; Dolan et al., 2003; Fu et al., 2009; Kim, 2004; Kowalski and Frankowski, 2015; Puścion-Jakubik et al., 2021; Saper et al., 2008; Socha et al., 2013), however, there is a lack of data regarding those containing *Beta vulgaris* L. The study aimed to estimate Hg content in fifty-four beetroot-based DSs and seven lots of beetroots divided into three subgroups (peeled, unpeeled, skins), available on the Polish market. Furthermore, the health risk was assessed in view of the European Commission permissible Hg contamination in dietary supplements, realisation of PTWI of Hg, and the Target Hazard Quotient (THQ index) value. Statistical analyses were applied to verify the potential correlation between the concentration of Hg and beetroot parts. Moreover, the content of Hg in DSs was compared with the results obtained for vegetable samples.

2. Materials and methods

2.1. Sample preparation

Seven portions of raw beetroots were purchased in small-retail stores, large-retail stores (sales area >400 m^2), or grocery stores in Gdańsk (Poland, Europe) from November to December 2019. Four of them were cultivated conventionally and three were organic products. Every portion was washed and divided into 3 batches: peeled beetroot, skins, and unpeeled. Then, vegetables were chopped with ceramic tools (to avoid contamination with metal compounds). Three samples were prepared from every batch so a total of sixty vegetable samples were analysed. An alphanumeric code was used to mark the beetroot samples: the number represents the consecutive portions of vegetables, the letters Bp, Bs, Bu mean batches prepared from the same portion: peeled beetroot, beetroot skins, unpeeled beetroot, respectively. All samples were frozen (−30 °C) and then lyophilised (Alpha 1–4 LD plus freeze dryer; −42 °C, 0.1 mbar, 170 h and 20 min of drying off in −50 °C, 0.02 mbar,). Directly before analysis, the samples were homogenised in porcelain mortars. Full characteristics of the collected beetroot samples is shown in Table 1.

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Table 2
Full characteristics of the analysed beetroot-based dietary supplements based on information on the package.

Form	Code	Number of dosage units	Product net weight (g)	The content of beetroot extract or preserves/dosage unit	Declared weight of the dosage unit (g)	Recommendation (dosage units/day)	Origin country
capsules	C1A C1B	90	45	400 mg of root extract; 40 mg of nitrates	0.5	1 × 1 caps.	Poland (PL)
	C2A C2B	90	45	400 mg of root extract (15:1); gelatine	0.5	1 × 1 caps.	Poland (PL)
	C3A C3B C3C	60	35.76	dried juice concentrate; 38 mg of vitamin C; 2.8 mg of iron; capsule shell (gelatine of animal origin)	0.596	2 × 1 caps. during meal	Poland (PL)
	C4A C4B	30	11.3	268 mg of beetroot concentrate; 20 mg of vitamin C; 12 mg (1.4 mg iron) of iron gluconate; starch; anti-caking agent: magnesium salts of fatty acids; silicon dioxide	0.376	1 × 3 caps.	Poland (PL)
	C5A C5B C5C	60	41.4	550 mg of *Beta vulgaris* extract 4:1; pullulan capsule	0.69	1 × 2 caps.	Poland (PL)
	C6	100	ND	500 mg of beetroot; magnesium stearate; gelatine capsule	ND	3 × 2 caps	United States of America (USA)
	C7	60	ND	700 mg of organic prepared beetroot (beetroot extract, maltodextrin) corresponding to 4620 mg of dried beetroot); vegetable capsule shell (hydroxypropylmethylcellulose)	ND	1 × 2 caps. during meal	United Kingdom (UK)
	C8	100	ND	500 mg of beetroot; vegetable capsules (modified cellulose); cellulose; silica; magnesium stearate	ND	3 × 2 caps. during meal	United States of America (USA)
	C9	90	ND	500 mg of beetroot extract (*Beta vulgaris*) (standardised for 0.3% betanin); cellulosan; silicon dioxide; vegetable fatty; vegetable mineral salts	ND	3 × 1 caps. during meal	United States of America (USA)
	C10	60	ND	450 mg of beetroot extract; bulking agent: microcrystalline cellulose; shells: hydroxypropyl methylcellulose	ND	2 × 1 caps.	United States of America (USA)
	C11	90	ND	500 mg of beetroot extract (*Beta vulgaris*) (5:1); bulking agents: maltodextrin, microcrystalline cellulose; vegetable capsule shell: hydroxypropyl methylcellulose; anti-caking agents: silicon dioxide, vegetable magnesium stearate	ND	1 × 1 caps.	United States of America (USA)
	C12	60	ND	300 mg of freeze-dried juice from organic pickled beetroot; micronized apple fibre; cellulose capsule shell	0.3	2 × 1 caps. before meal	Poland (PL)
	C13	90	51.3	400.00 mg of beetroot extract standardised for 10% nitrates including nitrates 40 mg; cellulose capsule; hydroxypropyl methylcellulose; 70 mg of inulin Orafti GR	ND	1 × 1 caps.	Poland (PL)
	C14	60	36	200 mg of red beetroot extract (*Beta vulgaris* L. subsp. *vulgaris*); 200 mg of young barley extract (*Hordeum vulgare*); capsule (glazing agent: hydroxypropyl methylcellulose); 60 mg of niacin (nicotinamide); 18 mg of pantothenic acid (calcium D-pantothenate); bulking agent: microcrystalline cellulose; 4.2 mg of riboflavin; 4.2 mg of vitamin B_6 (pyridoxine hydrochloride); 3.3 mg of thiamine (thiamine hydrochloride); 600 μg of folic acid (pteroylmonoglutamic acid); 7.5 μg of vitamin B_{12} (cyanocobalamin)	ND	1 × 1 caps.	Poland (PL)
	C15	60	41.4	550 mg of beetroot extract (*Beta vulgaris*); glazing agent: pullulan	ND	2 × 1 caps.	Poland (PL)
	C16	100	39	300 mg of 10:1 extract of beetroot (*Beta vulgaris*); capsule: vegetable cellulose	0.39	2 × 1 caps.	Poland (PL)
	C17	30	ND	10 mg of iron (71.4% NRV); 40 mg of vitamin C (50% NRV); 400 mg of powdered red beetroot	ND	1 × 2 caps.	Poland (PL)
tablets	T1A T1B	60	33	500 mg of dried juice concentrate (refers to 2.75 g fresh beetroot); 1 mg of B_6; 1.25 μg of B_{12}; bulking agent: microcrystalline cellulose; anti-caking agents: fatty magnesium salts, silicon dioxide	0.55	1–2 × 3 caps.	Poland (PL)
	T2A T2B	60	39	488 mg of beetroot concentrate; 20 mg of vitamin C; 12 mg (1.4 mg iron) of iron gluconate; starch; anti-	0.65	1 × 3	Poland (PL)

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Table 2 (*continued*)

Form	Code	Number of dosage units	Product net weight (g)	The content of beetroot extract or preserves/dosage unit	Declared weight of the dosage unit (g)	Recommendation (dosage units/day)	Origin country
	T2C			caking agent: magnesium salts of fatty acids; silicon dioxide			
	T3A T3B	120	111	500 mg of dried juice (refers to 3.5 g of fresh beetroot); anti-caking agent: magnesium salts of fatty acids; silicon dioxide	0.925	1–2 × 3 tabl. during a meal or after a meal	Poland (PL)
	T4	100	35	132.375 mg of dicalcium phosphate; 132.375 mg of microcrystalline cellulose; 80 mg of beetroot extract (including 1% betaine); 2.25 mg vegetable magnesium stearate	0.35	2 tabl.	United Kingdom (UK)
	T5	60	37.8	500 mg of fresh beetroot; maltodextrin; 40 mg of L-ascorbic acid (vitamin C); 7 mg iron II fumarate (iron); bulking agent: sorbitols; anti-caking agents: magnesium salts of fatty acids, silicon dioxide	0.63	1–2 × 1 tabl.	Poland (PL)
	T6A T6B	60 60	39 39	beetroot concentrate 500 mg; vitamin C 20 mg; 12 mg iron (II) gluconate (1.4 mg iron); starch; anti-caking agent: magnesium salts of fatty acids, silicon dioxide	0.65	3 × 1 tabl.	Poland (PL)
	T7	60		500 mg of dried red beetroot concentrate; 1 mg vitamin B6; 1.25 mg vitamin B12; anti-caking agents: magnesium salts of fatty acids, silicon dioxide	0.5	3 × 1–2 tabl.	Poland (PL)
	T8A T8B	120	42	350 mg of beetroot extract 20:1 (80 mg of betanins); binder: dicalcium phosphate; emulsifier: microcrystalline cellulose; stabiliser: magnesium salts of fatty acids	0.35	1 × 1–2 tabl. during a meal	United Kingdom (UK)
	T9	100	146	1000 mg of beetroot extract; bulking agent: microcrystalline cellulose; anti-caking agent: stearic acid, magnesium stearate; stabiliser and solubiliser: sodium croscarmellose	1.46	1–3 × 1 tabl.	United States of America (USA)
	T10	90		300 mg of beetroot extract; bulking agents: dicalcium phosphate, microcrystalline cellulose; anti-caking agents: stearic acid, silicon dioxide, magnesium stearate; glazing agents: hydroxypropyl methylcellulose, glycerine, carnauba wax	ND	1 tabl.	United Kingdom (UK)
	T11	60	86	100 mg of beetroot root powder; 125 mg of L-arginine alpha-ketoglutarate; 125 mg of L-citrulline; 100 mg of Beta alanine; sweeteners: mannitol, xylitol and steviol glycosides; bulking agent: microcrystalline cellulose; stabiliser: sodium carboxymethylcellulose, cellulose gum; capsule shell: hydroxypropylmethyl cellulose; acidity regulator: citric acid; natural flavours (cherry and vanilla); emulsifier: hydroxypropyl cellulose; anti-caking agents: calcium salts of fatty acids and silicon dioxide	1.42	1 × 1–2 tabl. 20–30 min before training	United States (USA)
powders	P1	60 portions	43.32	beetroot root juice powder; vitamin C (L-ascorbic acid); iron fumarate. 1 serving (0.72 g) contains: iron fumarate 42 mg; including iron 14 mg; vitamin C 80 mg; powdered beetroot 600 mg.	ND	1 measure (0.72 g)	ND
	P2	ND	400	100% powdered red beetroot, whole ground, not peeled	ND	Mix 1 tablespoon of the product with water, juice or other drink. Ground beetroot is used as an addition to soups, dishes, cocktails, salads, yoghurts, cheese and other food products.	Poland (PL)
	P3	ND	200	100% powdered BIO red beetroot	ND	Mix 1 teaspoon (3 g) of the product with 200 mL of water, juice, yoghurt or add it as an ingredient in salads, cocktails, soups, desserts. The suggested daily dose for consumption during the day: 1–2 teaspoons.	Italy (IT)
	P4	ND	200	100% powdered red beetroot	ND	Add 1–2 teaspoons of beetroot powder (5–10 g) to shakes, smoothies or meals.	Poland (PL)
	P5	ND	340	beetroot powder	ND	1 × 1 spoon	United States of America (USA)
	P6	ND	100	beetroot tuber extract	ND	3 × 1 teaspoon	Czech Republic (CZ)
	P7	ND	110	powdered red beetroot	ND	1 × 1 teaspoon	Egypt (EG)
	P8	ND	100	powdered red beetroot	ND	1 teaspoon	Croatia (HR)
	P9	ND	200	powdered BIO red beetroot	ND	1 × 3 teaspoons	Germany (DE)

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Table 2 (*continued*)

Form	Code	Number of dosage units	Product net weight (g)	The content of beetroot extract or preserves/dosage unit	Declared weight of the dosage unit (g)	Recommendation (dosage units/day)	Origin country
	P10	ND	100	powdered beetroot 4:1	ND	1x half of teaspoon	Poland (PL)
	P11	ND	200	powdered organic beetroot	ND	1–2 teaspoons	China (CN)
	P12	ND	240	powdered red beetroot	ND	1 teaspoon	United States of America (USA)
	P13	ND	210	beetroot crystals made of concentrated beetroot juice, 4 g/100 g including nitrates	ND	2–3 teaspoons, 1–3 h before training or just after training	Ireland (IE)

ND – lack of data; NRV – Nutrient Reference Values (according to (Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011, 2011).

Fifty-four beetroot-based dietary supplements (from thirty-one different manufacturers), available on the Polish market, were obtained from various drugstores or online stores. Summary of the collected supplements samples characteristics is shown in Table 2. The following criteria were used to select products for analysis: availability in capsules, tablets, or powder; the main ingredient was beetroot preserve (i.e., dried juice, powdered root, dried extracts, lyophilisate); and availability for the Polish consumer via Internet sale or stationary in a drugstore. The products were purchased in three tranches over several months (in December 2019, November 2020, and October 2021) to get a representative group of products in terms of availability during this period. Some products were purchased with a different serial number and were analysed separately. Supplements were marked with an alphanumeric code: the first letter means the form in which the product was packaged (C –capsule, T – tablet, P – powder), the number means subsequent separate products, the letter (A, B, C) means different serial numbers of the same product. Products were homogenised directly before analyses using ceramic tools. In total, 74 samples of DSs and vegetables were analysed in triplicate.

2.2. Reagents and standards

Mercury standard-MSHG at a concentration of 100.10 ± 0.43 μg/mL in 10% HCl was purchased from Inorganic Ventures, INC (USA). N Acetyl – L Cysteine was obtained from Sigma Aldrich (Germany). Certified reference materials BCR-463 and DOLT - 4 were supplied by IRMM (Belgium) and NRC (Canada) respectively.

2.3. Determination procedure

Mercury/MA-3000 supplied by Nippon Instruments Corporation (NIC, Japan) was used to analyse mercury by cold vapour technique and oxygen was used as the carrier gas. The THg content in beetroot and food supplements samples was determined using the MA-3000 Mercury Analyzer in 3 repetitions.

2.4. Method validation

The Mercury/MA-3000 method was validated by linearity range, precision, accuracy, the limit of determination (LOD), and the limit of quantification (LOQ). The LOD and LOQ of the applied method were calculated using formulas (Eqs. (1) and (2)) proposed by Huber (Huber, 2003):

$$LOD = \frac{3.3 \bullet SD_a}{b} \quad (1)$$

SD_a – standard deviation of the intercept for the calibration curve;.
b – slope for the calibration curve.

When calculating the numerical limit of quantification (LOQ), the dependence described by Eq. (2) (Huber, 2003) was used:

Table 3
Validation parameters of the procedure for the determination of THg in beetroot and food supplements samples (assuming the weight = 100 mg).

Parameter		Value
		THg
Linearity	• 10 measurement points, • 3 repetitions, • concentration range: 10.25 [ng] ÷103 [ng] y = 1.0006x – 0.15	0.999
LOD [ng]		0.096
LOQ [ng]		0.29
MDL [ng/g]		0.96
MQL [ng/g]		2.9
Measuring range [ng/g]		2.9 ÷ 102.5
Repeatability CV [%]		2.8
Intermediate precision CV [%]		4.7
Recovery [%]		101.9 ± 1.2
BCR-463		92.73 ± 0.42
DOLT 4		

$$LOQ = 3 \bullet LOD \quad (2)$$

The validation parameters are presented in Table 3. The determination coefficient (R^2) was 0.999. Accuracy was determined based on CRMs analysis and was expressed as recovery which ranged from 92.73% to 101.9%. The precision was calculated as the coefficient of variation for all the results obtained in all the analysed samples. Values were obtained at an acceptable level and did not exceed 10%. The measuring range was from 2.9 to 102.5 ng/g of THg.

2.5. Calculations

The content of THg was determined in ng/g of dry weight (for beetroot samples) and ng/g of product for dietary supplements. Afterwards, the content of THg was recalculated to μg/100 g of wet weight (w.w.) of beetroot using the water content values (Table 1). Values in Table A.1 are expressed as an average content in a product ± expanded uncertainty (U) of measurement at 95% confidence level obtained for three replicates.

2.6. Health risk assessment

The estimated daily intake (EDI), the estimated weekly intake (EWI), and the estimated monthly intake (EMI) were calculated to evaluate the consumption of THg with the analysed products (Eq. (3)). The assumption was made that 100 g of beetroot was consumed daily (EDI). EDI for supplements was calculated based on the manufacturers' recommendations concerning daily intake (Table 2). The EWI was calculated by multiplying the EDI by 7 which equates to 7 days, while EMI by multiplying the EDI by 30.

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Table 4
The content of THg in the analysed beetroot samples and dietary supplements. The results are given for a group of products (based on n samples analysis).

Group of samples	n[1]	ng/100 g w.w. X	SD	Min	Median	Max	Q_1	Q_3
Conventional								
unpeeled beetroot	**4**	20	17	6	15	45	9.6	25.8
peeled beetroot	**4**	9.1	3.9	5.4	8.4	14.4	6.9	10.6
beetroot skins	**4**	28	22	12.9	19.6	60.1	13.9	33.7
Organic								
unpeeled beetroot	**3**	13.7	4.3	11.1	11.4	18.7	11.3	15.1
peeled beetroot	**3**	7.5	5.1	4.5	4.6	13.4	4.6	9.0
beetroot skins	**2**	56	23	40	56	72	48.2	64.2
	n[1]	**ng/g X**	**SD**	**Min**	**Median**	**Max**	**Q_1**	**Q_3**
Supplements in tablets form	**17**	2.6	3.9	0.3	0.7	12.2	0.53	1.72
Supplements in capsules form	**24**	0.64	0.39	0.32	0.53	2.31	0.46	0.66
Supplements in powder form	**13**	0.65	0.32	0.32	0.53	1.19	0.39	0.88

X–average, SD—standard deviation, Min—minimum, Max—maximum, n1 – number of samples with the determined content of the analysed element >LOQ.

$$EDI = C \times P \tag{3}$$

EDI – estimated daily intake (ng/day).
C – concentration of THg in the analysed product (ng/g).
P – portion of the analysed product consumed daily (g).

The determination of the Provisional Tolerable Weekly Intake realisation, expressed as %PTWI, was calculated, assuming that the average body weight of an adult in Poland is 70 kg, according to the following equation (Eq. (4)):

$$\%PTWI = \frac{EWI}{280} \times 100 \tag{4}$$

EWI – estimated weekly intake (μg of THg/week).
280 – the value of PTWI which is 4 μg/kg body weight for Hg (Seventy-second meeting of the Joint FAO/ WHO Expert Committee on Food Additives (JECFA), 2012) assuming that the average body weight of adult in Poland is 70 kg.

Furthermore, the Target Hazard Quotient (THQ index) was calculated according to the following equation (Eq. (5)):

$$THQ = \frac{EF \times ED \times P \times C}{RfD \times BW \times T} \times 10^{-3} \tag{5}$$

EF – frequency of exposure (365 days/year).
ED – the duration of exposure (70 years), equivalent of the average lifetime.
P – portion of the analysed product consumed daily (g).
C – concentration of THg in the analysed product (μg/g).
RfD – oral reference dose (0.3 μg/kg body weight/day).
BW – body weight (kg).
T – the average time of exposure for non-carcinogens (365 days/year × 30 years = 10,950 days).

To calculate THQ, the RfD value for mercury chloride (0.3 μg/kg body weight/day) corresponding to inorganic mercury compounds was used according to USEPA (United States Environmental Protection Agency (USEPA), 2002). THQ has been applied in researches to analyse the potential non-cancerogenic effect of the metals present in food and DSs. If the THQ > 1, it may indicate a potential risk related to the consumption of heavy metal with the analysed product. The THQ < 1 is associated with a low non-cancerogenic risk.

2.7. Statistical analysis

All data were analysed using the Shapiro-Wilk test to check the normality of the distribution of the random variable.

The non-parametric Kruskal-Wallis test was applied to check for relationships between the analysed variables in specific groups based on Hg content in the samples. The data were divided into the following groups: beetroot (vegetable)-dietary supplements containing beetroot, beetroot-type of cultivation (organic and conventional), beetroot-part of vegetable (unpeeled, peeled, and skin), dietary supplements-pharmaceutical form (capsule, tablet, and powder). Two groups, i.e., beetroot-part of vegetable (unpeeled, peeled, and skin) and dietary supplements-pharmaceutical form (capsule, tablet, and powder), were analysed by a post-hoc test (Dunn's test), which was performed to check the relationships between particular variables. All analyses were done using Statistica 13.3 (TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and discussion

3.1. Content of THg

The average results for two groups of beetroot samples (conventional and organic cultivations), divided into three subgroups (unpeeled, peeled, and skins), and for supplements in tablets, capsules, and powder forms are presented in Table 4. The number of the analysed samples with the determined content of Hg >LOQ, the mean content with the standard deviation for the group, the minimum, median, and maximum concentration of the determined element, first and third quartiles are given (Table 4). Results were expressed as ng/100 g fresh weight for beetroot samples and as ng/g for supplements.

The highest contents of THg in beetroot samples were observed for skins for both, conventional (28.03 ± 22 ng/100 g w.w.) and organic (56 ± 3 ng/100 g w.w.) cultivations. Simultaneously, organic beetroot skins contained more THg than conventional ones. Skins' batches (Bs) were characterised by the highest SD values. Such variability might be due to the fact that beetroot skins constitute the barrier for the majority of the contaminants and protect the inside of the vegetable. Vegetables absorb heavy metals from the ground as well as from deposits on the parts of vegetables exposed to air from the polluted environment. Moreover, these differences might be influenced by the contamination level of the cultivation place. The determined Hg contents in all beetroot samples were over ten times lower than those determined by Abbas et al. (2010) in sugar beetroots (0.005 ± 0.0003 μg/g which equals 500 ng/100 g w.w.).

The supplements in tablets (2.6 ± 3.9 ng/g) contained more THg than in capsules (0.64 ± 0.39 ng/g). The difference in THg content between capsule and tablet supplements may be related to the fact that more auxiliary substances are used in the formulation of the latter ones (such as fillers, binders, disintegrants, antiadherents, coating agents, and those affecting taste and aroma). These substances might be contaminated by trace amounts of this metal. Capsule supplements (0.64 ± 0.39 ng/g) contained a similar amount of THg to powder supplements (0.65 ± 0.32 ng/g). There is no literature data available on the THg content in beetroot-based DSs. Analyses of various groups of DSs, containing ingredients of plant origin, conducted by Puścion-Jakubik et al. (2021), showed that the mean Hg content was 3.37 ± 7.65 ng/g,

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Table 5
The content of THg in the analysed beetroot and dietary supplement samples expressed as a percentage of the maximum allowable level of its contamination (section A), as a percentage of the PTWI for 70 kg person (280 μg/70 kg/week) (section B) and as THQ (section C). The results are given for a group of products (based on n samples analysis).

		A			B: %PTWI		C: THQ	
Group of samples	n[1]	X (%)	SD (%)	Permissible contamination limit (mg/kg w.w.)	min	max	min	max
Conventional								
unpeeled beetroot	**4**	ND	ND	ND	0.015	0.112	0.0011	0.0078
peeled beetroot	**4**	ND	ND	ND	0.013	0.036	0.00093	0.00093
beetroot skins	**4**	ND	ND	ND	0.032	0.150	0.0022	0.0105
Organic								
unpeeled beetroot	**3**	ND	ND	ND	0.028	0.047	0.0019	0.0032
peeled beetroot	**3**	ND	ND	ND	0.011	0.034	0.00078	0.0023
beetroot skins	**2**	ND	ND	ND	0.100	0.181	0.0070	0.013
Supplements in tablets form	**17**	2.6	3.9	0.10	0.0012	0.0297	0.000082	0.0021
Supplements in capsules form	**24**	0.64	0.39	0.10	0.00053	0.00968	0.000037	0.00067
Supplements in powder form	**13**	0.65	0.32	0.10	0.00062	0.0596	0.000043	0.0041

X–average, SD—standard deviation, Min—minimum, Max—maximum, n1 – number of samples with the determined content of the analysed element >LOQ, ND – values cannot be calculated due to lack of the established permissible contamination limit of Hg in vegetables.

the median content was 1.69 ng/g, and the range of quartiles ranged from 1.10 to 2.86 ng/g. The above-mentioned data were higher as compared to those obtained in this study. Kowalski and Frankowski (2015) analysed 33 different DSs and the mean THg was 5.5 ng/g, while the median was 5.9 ng/g. These values were higher than the ones obtained for the analysed supplements in tablets (X = 2.78 ng/g, Me=0.80 ng/g), capsules (X = 0.63 ng/g, Me=0.53 ng/g), and powders (X = 0.65 ng/g, Me=0.53 ng/g). In another study, Brodziak-Dopierała et al. (2018) analysed herbal supplements (for example, containing violet, ginseng, artichoke, algae or bamboo) and found a THg content in the range of 0.02 and 4293.07 ng/g. The mean content was 193.77 ng/g, which was value almost 70 times higher than in our study for supplements in tablets (2.64 ng/g). These authors (Brodziak-Dopierała et al., 2018) reported the highest values for bamboo shoots (1806.12 ng/g) and alga *Chlorella pyrenoidosa* (1806.12 ng/g).

3.2. Health risk assessment

The maximum levels of contaminants in foodstuffs, including Cd, Pb, and Hg are regulated by European Commission Regulations No 1881/2006 and No 629/2008 (The Commission of the European Communities, 2008, 2006). The content of Hg in the analysed products was assessed in view of the above-mentioned regulations (Table 5). Mercury contamination is allowed at the level of 0.10 mg/kg of a commercially available form of dietary supplement (permissible contamination limit). No permissible limit of THg content for vegetables was established yet (The Commission of the European Communities, 2008). Detailed results for individual samples analyses are summarised in Table A.1. Furthermore, consumer exposure was assessed based on PTWI value for Hg (4 μg/kg/week) (Seventy-second meeting of the Joint FAO/ WHO Expert Committee on Food Additives (JECFA), 2012) and THQ index assuming that a consumer consumes 100 g of beetroot or the recommended daily intake of DSs by manufacturers (Table 5).

The lowest realisation of PTWI in the vegetable group was observed for organic peeled beetroots (0.011–0.034% PTWI for Hg) and conventional peeled beetroots (0.013–0.036% PTWI for Hg). The realisation of PTWI and THQ index was decreasing in order - beetroot skins, unpeeled beetroot, and peeled beetroot for conventional and organic samples.

The highest PTWI realisation was obtained for supplements in tablets (0.0012–0.0297% PTWI for Hg), while the THQ index ranged from 0.000082 to 0.0021. Percentages of PTWI realisation for supplements in capsules and powders were comparable, 0.00053–0.00968% and 0.00062–0.0596%, respectively. Similarly, THQ index for supplements in capsules amounted from 0.000037 to 0.00067 and for powders from 0.000043 to 0.0041. Moreover, Hg content in DSs was evaluated in view of the permissible contamination limit (0.10 mg/kg w.w.), which was not exceeded by any of the analysed products. The highest average contamination by THg was observed in the group of supplements in tablets (2.6 ± 3.9% of permissible contamination limit) (Table 5). It is worth noting that in the three capsule products (T4, T5, T8A), a relatively high THg contamination was determined (12.15, 9.42, 10.40% of permissible contamination limit, respectively) (Table A.1). However, it did not significantly influence the PTWI (0.021, 0.030, 0.018% PTWI, respectively) and THQ (0.0015, 0.0021, 0.0013, respectively) due to the small portion delivered (0.7–1.26 g/day). Average contaminations in the group of DSs in capsules and powders were comparable, 0.63 ± 0.39% and 0.65 ± 0.32% of the permissible contamination limit, respectively (Table 5). In conclusion, the analysed beetroot-based DSs in tablets, capsules, or powders did not pose a significant risk for consumers in view of permissible contamination limit, PTWI Hg realisation, and THQ index. Recommended portion of any analysed dietary contained less THg than the portion of any analysed beetroot samples. It may be related to a lower overall amount of the supplement (no more than 5 g for tablets and capsules, or 15 g for powders) delivered than beetroot (100 g w.w.).

There are no literature data available on the permissible contamination limit, Hg PTWI realisation, and THQ index in beetroot-based DSs. However, Puścion-Jakubik et al. (2021) calculated the percentage of Hg PTWI for DSs containing ingredients of plant origin available on the Polish market. The highest value (1.143% PTWI) was found in DS aimed at improving vitality and it was about 38 times higher than the highest results obtained for supplements in tablets investigated in this study (0.0297% PTWI). Brodziak-Dopierała et al. (2018) found that two from twenty-four analysed herbal DSs remarkably exceeded the PTWI (457.01% and 948.21%), while rest of the samples were characterised by values between 0.01% and 2.87% PTWI. In this study, all the analysed samples (DSs and vegetables) fulfilled PTWI at a noticeable lower level, 0.00053–0.0297% PTWI for DSs and 0.011–0.181% PTWI for beetroots.

Mercury is found as a contaminant emerging from the food chain (Tchounwou et al., 2012). Therefore, the presence of Hg in beetroot-based DSs is a consequence of the fact that plants are one of the most effective sorbents of Hg^{2+} from the soil and water (Peralta-Videa et al., 2009; Puścion-Jakubik et al., 2021). The concentration of this metal in beetroot material is affected by natural factors such as growing conditions, cultivation practices, and meteorological conditions. One of the most influential is the characteristics of the geological area of cultivation, i.e., its contamination with Hg-compounds (Saletnik et al., 2016). The use of such a raw material may result in the final product's contamination, even after processing (Brodziak-Dopierała et al., 2018; Puścion-Jakubik et al., 2021). Summing up, the content of THg in all the

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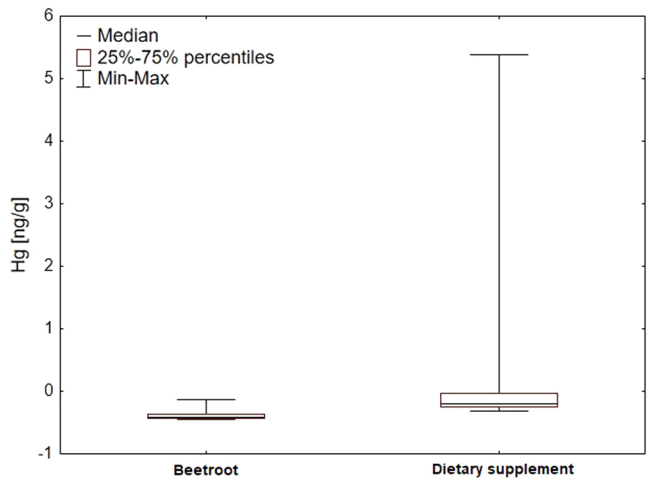

Fig. 1. Box-plot of Hg content [ng/g] in vegetable-beetroot and dietary supplement.

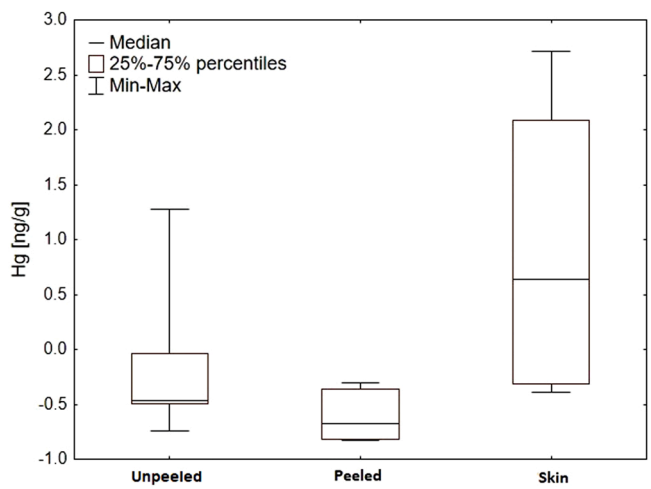

Fig. 2. Box-plot of Hg content [ng/g] in particular parts of vegetable-beetroot.

analysed samples was lower than reported in most of the literature for DSs containing ingredients of plant origin. In any analysed case, the PTWI value was not exceeded. Moreover, the calculated THQ index (THQ <1) does not indicate the increased non-carcinogenic risk as a result of the analysed products' consumption. Likewise, the content of THg in all DSs was lower than the permissible contamination limit. However, it is worth emphasising that Hg is a toxic element so in any amount it may pose a risk for the consumer. More stringent procedures for obtaining raw materials, THg contamination control, as well as decontamination process of plant material should be implemented into the production of DSs to eliminate the risk of contamination of the final product.

3.3. Statistical analysis

The applied non-parametric Kruskal-Wallis test proved the existence of statistically significant relationships between beetroot and dietary supplements containing beetroot at the significance level of $p < 0.001$ (H=30.67173) in terms of Hg content in the analysed samples (Fig. 1). Based on the results of the Kruskal-Wallis test for Hg content in the samples analysed, statistically significant relationships were found between beetroot samples and dietary supplements ($p < 0.001$) (Fig. 1), beetroot samples and the vegetable part, i.e., unpeeled vegetable, peeled and skins ($p < 0.05$) (Fig. 2), and dietary supplements and their

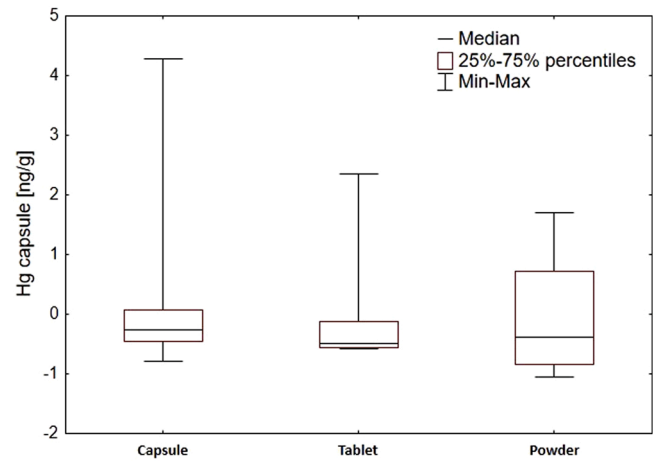

Fig. 3. Box-plot of Hg content [ng/g] in different forms of dietary supplements.

pharmaceutical forms, i.e., capsules, tablets and powders ($p < 0.05$) (Fig. 3). The test did not confirm the existence of statistically significant ($p > 0.05$) relationships in the vegetable-beetroot group vs. type of cultivation (organic and conventional). In addition, a post-hoc test (Dunn's test for multiple comparisons) was performed to determine which groups were significantly related. The results of the Dunn's test confirmed the existence of significant relationships between peeled beetroots and their skins at a significance level of 0.01. Dunn's test also revealed the presence of a significant relationship between capsules and tablets ($p < 0.05$) in the case of dietary supplements.

4. Conclusions

The study aimed to determine THg content in fifty-four beetroot-based DSs and seven lots of beetroots divided into three groups, i.e., peeled, unpeeled and skins using direct thermal decomposition-gold amalgamation cold vapour atomic absorption spectrometry. The results obtained for supplements were compared with beetroot samples. Moreover, the health risk assessment was conducted in view of Polish and European regulations on the allowed content of THg and Provisional Tolerable Weekly Intake (PTWI).

In this work, THg content in DSs and beetroot samples was successfully assessed and compared. None of the analysed beetroot samples or DSs did exceed the PTWI value, and the calculated THQ index did not indicate the increased non-carcinogenic risk resulting from these products' consumption. Statistical analyses confirmed differentiation of THg content in skins, unpeeled and peeled beetroots showing with the last ones characterised by the lowest amounts of this element. Peeling beetroots can reduce consumer's exposure to Hg compounds because skins were the most contaminated. It is worth emphasising that producers do not declare whether peeled beetroots were used to manufacture their products. The THg content varied significantly in the DSs group and the highest was found in tablets while comparable levels were determined in capsules and powders. In conclusion, a recommended portion of any of the analysed dietary supplements contained less THg than a portion of any of the analysed beetroot samples. It might be related to a lower overall amount of the supplement (no more than 5 g for tablets and capsules, or 15 g for powders) consumed than beetroot (100 g w.w.). Possible contamination with Hg can be associated with a direct threat to the consumers' health. Therefore, DSs should be examined before being released to the market and constantly monitored to ensure consumer safety.

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agencies in the public, commercial, or not-for-profit sectors.

CRediT authorship contribution statement

Joanna Brzezińska-Rojek: Conceptualization, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Visualization, Project administration. **Małgorzata Rutkowska:** Methodology, Software, Validation, Investigation, Resources, Data curation, Writing – original draft, Visualization. **Justyna Ośko:** Statistical analysis, Writing – original draft, Visualization. **Piotr Konieczka:** Methodology, Software, Resources, Writing – review & editing, Supervision. **Magdalena Prokopowicz:** Writing – review & editing, Supervision. **Małgorzata Grembecka:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Appendix A

See Table A1.

Table A1
Concentrations of THg (ng/g d.w.) in conventional, organic beetroot samples, and dietary supplement samples (xm ± U, (k = 2)).

Type	No. sample	THg (ng/g)	U (k = 2)	% Permissible contamination limit[1]	%PTWI[2]	THQ
Conventional beetroot samples	1 Bp	0.877	0.040	ND	0.036	0.0025
	1Bs	0.64	0.02	ND	0.032	0.0022
	1Bu	1.12	0.06	ND	0.049	0.0034
	3 Bp	0.537	0.021	ND	0.023	0.0016
	3Bs	1.32	0.06	ND	0.062	0.0043
	3Bu	0.58	0.05	ND	0.027	0.0019
	4 Bp	0.240	0.026	ND	0.013	0.00093
	4Bs	2.18	0.09	ND	0.150	0.010
	4Bu	1.85	0.04	ND	0.112	0.0078
	5Bo	0.5475	0.0087	ND	0.018	0.0013
	5Bs	0.94	0.05	ND	0.036	0.0025
	5Bu	0.44	0.04	ND	0.015	0.0011
Organic beetroot samples	2 Bp	0.662	0.099	ND	0.034	0.0023
	2Bu	0.81	0.02	ND	0.047	0.0032
	6 Bp	0.26	0.0071	ND	0.011	0.00078
	6Bs	3.05	0.29	ND	0.18	0.013
	6Bu	0.54	0.03	ND	0.028	0.0019
	7Bo	0.231	0.021	ND	0.011	0.00080
	7Bs	1.61	0.16	ND	0.100	0.0070
	7Bu	0.55	0.04	ND	0.029	0.0020
Dietary supplements - capsules	C1A	0.423	0.068	0.42	0.00053	0.000037
	C1B	0.529	0.03	0.53	0.00066	0.000046
	C2A	0.499	0.027	0.50	0.00062	0.000043
	C2B	0.463	0.042	0.46	0.00058	0.000040
	C3A	0.657	0.042	0.66	0.0020	0.00014
	C3B	0.615	0.043	0.62	0.0018	0.00013
Dietary supplements -capsules	C3C	0.68	0.074	0.68	0.0020	0.00014
	C4A	0.493	0.034	0.49	0.0014	0.000097
	C4B	0.45	0.059	0.45	0.0013	0.000088
	C5A	0.526	0.039	0.53	0.0018	0.00013
	C5B	0.345	0.033	0.35	0.0012	0.000083
	C5C	0.797	0.015	0.80	0.0027	0.00019
	C6	0.4938	0.0081	0.49	0.0044	0.00030
	C7	0.658	0.04	0.66	0.0027	0.00019
	C8	1.017	0.023	1.02	0.0097	0.00067
	C9	0.422	0.055	0.42	0.0024	0.00017
	C10	0.566	0.063	0.57	0.0021	0.00015
	C11	0.562	0.037	0.56	0.0011	0.000076
	C12	0.976	0.041	0.98	0.0019	0.00013
	C13	0.442	0.033	0.44	0.00057	0.000039
	C14	0.68	0.053	0.68	0.0010	0.000072
	C15	0.471	0.037	0.47	0.0016	0.00011
	C16	2.307	0.051	2.31	0.0045	0.00031
	C17	0.322	0.024	0.32	0.0010	0.000069
Dietary supplements -tablets	T1A	0.994	0.077	0.99	0.0082	0.00057
	T1B	0.552	0.038	0.55	0.0046	0.00032
	T2A	0.473	0.043	0.47	0.0023	0.00016
	T2B	0.487	0.021	0.49	0.0024	0.00017
	T2C	0.545	0.057	0.55	0.0027	0.00018
	T3A	0.582	0.045	0.58	0.0081	0.00056
	T3B	0.919	0.012	0.92	0.013	0.00089
	T4	12.15	0.64	12.15	0.021	0.0015
	T5	9.42	0.58	9.42	0.030	0.0021

(*continued on next page*)

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Table A1 (*continued*)

Type	No. sample	THg (ng/g)	U (k = 2)	% Permissible contamination limit[1]	%PTWI[2]	THQ
	T6A	0.5203	0.0035	0.52	0.0025	0.00018
	T6B	0.327	0.02	0.33	0.0016	0.00011
Dietary supplements -tablets	T7	1.716	0.08	1.72	0.013	0.00089
	T8A	10.4	0.42	10.40	0.018	0.0013
	T8B	0.671	0.028	0.53	0.0058	0.00040
	T9	0.529	0.041	1.64	0.010	0.00070
	T10	1.64	0.019	0.67	0.0012	0.000082
	T11	2.917	0.046	2.92	0.021	0.0014
Dietary supplements -powders	P1	0.34	0.058	0.34	0.00062	0.000043
	P2	0.68	0.039	0.68	0.025	0.0018
	P3	0.32	0.037	0.32	0.0024	0.00017
	P4	0.37	0.011	0.37	0.0091	0.00063
	P5	0.63	0.012	0.63	0.015	0.0010
	P6	0.47	0.052	0.47	0.014	0.00098
	P7	0.47	0.022	0.47	0.0035	0.00024
	P8	1.016	0.088	1.02	0.038	0.0026
	P9	0.53	0.0042	0.53	0.060	0.0041
	P10	0.88	0.042	0.88	0.0022	0.00015
	P11	1.19	0.063	1.19	0.018	0.0012
	P12	1.19	0.026	1.19	0.030	0.0021
	P13	0.39	0.031	0.39	0.014	0.0010

Bp – peeled beetroot; Bs – beetroot skins, Bu – unpeeled beetroot, ND – lack of data; 1 Permissible contamination limit (0.10 mg/kg w.w.), 2 280 – the value of PTWI which is 4 μg/kg body weight for Hg assuming that the average body weight of adult in Poland is 70 kg.

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