



Research Article

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# Determination of Fifty Trace Element Contents in Macro and Micro Follicular Colloid Nodular Goiter

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## Abstract

**Background:** Colloid nodular goiter (CNG) is the most common disease of the thyroid, even in non-endemic regions, but an etiology of CNG is unclear. It is known that not only iodine (I) but other trace elements (TE) are involved in goitrogenesis. The present study was performed to clarify the preferential accumulation of some TE either in the colloid or in cells of the thyroid gland.

**Methods:** Fifty TE (Ag, Al, As, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Fe, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Sc, Se, Sm, Sn, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr) in the thyroid tissues with diagnosed CNG were prospectively evaluated in 16 patients with macro-follicular CNG and 13 patients with micro-follicular CNG. Control group included thyroid tissue samples from 105 healthy individuals. Measurements were performed using a combination of non-destructive instrumental neutron activation analysis with inductively coupled plasma mass spectrometry.

**Results:** It was found that with a goitrous transformation the Ag, Al, B, Bi, Co, Er, Fe, Hg, Mo, Nd, Ni, Pb, Pr, Rb, Tl, U, Y, and Zn level in thyroid tissue can be significantly changed, and these changes depend on CNG histology. An association between B and Pb content and cell volume of CNG was observed.

**Conclusion:** There are considerable changes in TE contents in the goitrous transformed tissue of thyroid, which depend on the histology of goiter.

**Keywords:** Macro and micro follicular colloid nodular goiter of thyroid, Intact thyroid, Trace elements, Instrumental neutron activation analysis, Inductively coupled plasma mass spectrometry

**Abbreviations:** CNG: Colloid Nodular Goiter; TE: Trace Elements; INAA-LLR: Instrumental Neutron Activation Analysis with high resolution spectrometry of Long-Lived Radionuclides; ICP-MS: Inductively Coupled Plasma Mass Spectrometry; BSS: Biological Synthetic Standards; CRM: Certified Reference Material; IAEA: International Atomic Energy Agency; INCT: Institute of Nuclear Chemistry and Technology; DL: Detection Limit.

## Introduction

Colloid nodular goiter (CNG) is the most common disease of the thyroid, even in non-endemic regions [1]. CNG is clinically detected in about 4% of people older than 30 years [1]. CNG is benign lesion; however, during clinical examination, it can mimic malignant tumors. Furthermore, the origination of CNG can indicate the beginning of malignant transformation of the thyroid gland [2].

Up to now, an etiology of CNG is unclear and probably it is multifactorial [3]. There is opinion that CNG occurs when the thyroid is unable to meet the metabolic demands of the body with sufficient hormone production. The thyroid gland compensates by enlarging, which usually overcomes mild deficiencies of thyroid hormones. For over 20<sup>th</sup> century, there was the dominant hypothesis that CNG is the simple consequence of iodine (I) deficiency, because



I is an essential part of thyroid hormones. However, it was found that CNG is a frequent disease even in those countries and regions where the population is never exposed to I shortage [4]. Moreover, it was shown that I excess has severe consequences on human health and associated with the presence of thyroidal dysfunctions and autoimmunity, CNG and diffuse goiter, benign and malignant tumors of gland [5-8]. It was also demonstrated that besides I deficiency and excess many other dietary, environmental, and occupational factors are associated with the CNG incidence [9-11]. Among them a disturbance of evolutionary stable input of many trace elements (TE) in human body after industrial revolution plays a significant role in etiology of thyroidal disorders [12].

Besides I involved in thyroid function, other TE have also essential physiological functions such as maintenance and regulation of cell function, gene regulation, activation or inhibition of enzymatic reactions, and regulation of membrane function [13]. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of TE depend on tissue-specific need or tolerance, respectively [13]. Excessive accumulation or an imbalance of the TE may disturb the cell functions and may result in cellular degeneration, death, benign or malignant transformation [13-15].

In our previous studies the complex of in vivo and in vitro nuclear analytical and related methods was developed and used for the investigation of iodine and other TE contents in the normal and pathological thyroid [16-22]. Iodine level in the normal thyroid was investigated in relation to age, gender, and some non-thyroidal diseases [23,24]. After that, variations of TE content with age in the thyroid of males and females were studied and age- and gender-dependence of some TE was observed [25-41]. Furthermore, a significant difference between some TE contents in normal and cancerous thyroid was demonstrated [42-47].

Histologically, the CNG is cellular hyperplasia of the thyroid acini. There are two histological types of CNG: macro- and micro-follicular. It is obvious that these two types of CNG have different volume ratios "colloid to cells".

The present study was performed to clarify the preferential accumulation of some TE either in the colloid or in cells of the thyroid gland. Having this in mind, our aim was to assess the silver (Ag), aluminum (Al), arsenic (As), gold (Au), boron (B), beryllium (Be), bismuth (Bi), cadmium (Cd), cerium (Ce), cobalt (Co), chromium (Cr), cesium (Cs), dysprosium (Dy), iron (Fe), erbium (Er), europium (Eu), gallium (Ga), gadolinium (Gd), mercury (Hg), holmium (Ho), iridium (Ir), lanthanum (La), lithium (Li), lutecium (Lu), manganese (Mn), molybdenum (Mo), niobium (Nb), neodymium (Nd), nickel (Ni), lead (Pb), palladium (Pd), praseodymium (Pr), platinum (Pt), rubidium (Rb), antimony (Sb), scandium (Sc), selenium (Se), samarium (Sm), tin (Sn), terbium

(Tb), tellurium (Te), thorium (Th), titanium (Ti), thallium (Tl), thulium (Tm), uranium (U), yttrium (Y), ytterbium (Yb), zinc (Zn), and zirconium (Zr) contents in macro- and micro-follicular CNG tissue using consecutively non-destructive instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) and inductively coupled plasma mass spectrometry (ICP-MS). A further aim was to compare the levels of these TE in the macro- and micro-follicular CNG separately with those in intact (normal) gland of apparently healthy persons, as well as to find differences between the levels of these TE in the macro- and micro-follicular CNG.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre (MRRC), Obninsk. All the procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments, or with comparable ethical standards.

## Material and Methods

All patients suffered from CNG (n=29, mean age  $M \pm SD$  47 $\pm$ 14 years, range 30-64) were hospitalized in the Head and Neck Department of the Medical Radiological Research Centre. Thick-needle puncture biopsy of suspicious nodules of the thyroid was performed for every patient, to permit morphological study of thyroid tissue at these sites and to estimate their TE contents. For all patients the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. Histological conclusion for all thyroidal lesions was the macro-follicular CNG (n=16) and micro-follicular CNG (n=13).

Normal thyroids for the control group samples were removed at necropsy from 105 deceased (mean age 44 $\pm$ 21 years, range 2-87), who had died suddenly. Most deaths were due to trauma. A histological examination in the control group was used to control the age norm conformity, as well as to confirm the absence of micro-nodules and latent cancer.

All tissue samples were divided into two portions using a titanium scalpel [48]. One was used for morphological study while the other was intended for TE analysis. After the samples intended for TE analysis were weighed, they were freeze-dried and homogenized [49].

The pounded sample weighing about 10 mg (for biopsy) and 50 mg (for resected materials) was used for Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn measurement by INAA-LLR. Details of used nuclear reactions, radionuclides, gamma-energies, spectrometric unit, and sample preparation were presented in our earlier publications

concerning the INAA-LLR of TE contents in human thyroid, prostate and scalp hair [29,30,50,51].

After non-destructive INAA-LLR investigation the thyroid samples were decomposed in autoclaves and used for ICP-MS. The content of Ag, Al, As, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Se, Sm, Sn, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr was determined by ICP-MS using an ICP-MS Thermo-Fisher "X-7" Spectrometer (Thermo Electron, USA). The TE concentrations in aqueous solutions were determined by the quantitative method using multi elemental calibration solutions ICP-MS-68A and ICP-AM-6-A produced by High-Purity Standards (Charleston, SC 29423, USA). Indium was used as an internal standard in all measurements. Information detailing with the ICP-MS methods used and other details of the analysis was presented in our previous publication concerning TE contents in human thyroid, prostate, and scalp hair [35,52-55].

To determine contents of the TE by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [56]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten sub-samples of certified reference material (CRM) of the International Atomic Energy Agency (IAEA) IAEA H-4 (animal muscle) and IAEA HH-1 (human hair), as well as five sub-samples of CRM of the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2 Mixed Polish Herbs were treated and analyzed in the same conditions that thyroid samples to estimate the precision and accuracy of results.

A dedicated computer program for INAA-LLR mode optimization was used [57]. All thyroid samples were prepared in duplicate, and mean values of TE contents were used in final calculation. Using Microsoft Office Excel, a summary of the statistics, including, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels were calculated for TE contents. The difference in the results between normal thyroid and two groups of CNG (separately macro- and micro-follicular), as well as between two groups of CNG was evaluated by the parametric Student's t-test and non-parametric Wilcoxon-Mann-Whitney U-test.

## Results

The comparison of our results for the Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions (mg/kg, dry mass basis) in the normal human thyroid obtained by both INAA-LLR and ICP-MS methods is shown in Table 1.

**Table 1:** Comparison of the mean values ( $M \pm SEM$ ) of the chemical element mass fractions (mg/kg, on dry-mass basis) in the normal thyroid of males and females obtained by both NAA-LLR and ICP-MS methods.

Element	NAA-LLR M1	ICP-MS M2	$\Delta$ , %
Ag	0.0151 $\pm$ 0.0016	0.0122 $\pm$ 0.0014	19.2
Co	0.0399 $\pm$ 0.0030	0.0378 $\pm$ 0.0031	5.3
Cr	0.539 $\pm$ 0.032	0.451 $\pm$ 0.033	16.3
Fe	225 $\pm$ 11	221 $\pm$ 12	1.8
Hg	0.0421 $\pm$ 0.0041	0.0794 $\pm$ 0.0114	-88.5
Rb	7.37 $\pm$ 0.44	7.79 $\pm$ 0.46	-5.7
Sb	0.111 $\pm$ 0.008	0.079 $\pm$ 0.008	28.8
Se	2.32 $\pm$ 0.14	2.12 $\pm$ 0.14	8.6
Zn	97.8 $\pm$ 4.5	91.8 $\pm$ 4.3	6.1

**Note\*:** M - arithmetic mean, SEM - standard error of mean,  $\Delta = [(M_1 - M_2)/M_1] \cdot 100\%$ .

Tables 2-4 presents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Ag, Al, As, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Se, Sm, Sn, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr mass fraction in normal thyroid (n=105), macro-follicular CNG (n=16), and micro-follicular CNG (n=13), respectively. The As, Au, Eu, Ho, Ir, Lu, Pd, Pt, Te, Th, Tm, Yb, and Zr mass fractions in normal thyroid samples were determined in a few samples. The possible upper limit of the mean ( $\leq M$ ) for these TE was calculated as the average mass fraction, using the value of the detection limit (DL) instead of the individual value when the latter was found to be below the DL: where  $C_i$  is the individual value of the TE mass fraction in sample -i,  $n_i$  is number of samples with mass fraction higher than the DL,  $n_j$  is number of samples with mass fraction lower than the DL, and  $n = n_i + n_j$  is number of samples that were investigated. The As, Dy, Er, Gd, Ho, Ir, Lu, Nb, Pd, Pt, Tb, Te, Ti, and Tm contents in all samples of goitrous thyroid were under DL.

The comparison of Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tl, U, Y, and Zn mass fraction in normal thyroid with those in macro- and micro-follicular CNG is shown in Table 5, 6 respectively.

The ratios of means and the difference between mean values of Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Fe, Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tl, U, Y, and Zn mass fractions in macro- and micro-follicular CNG are presented in Table 7.

**Table 2:** Some statistical parameters of 51 trace element mass fraction (mg/kg, dry mass basis) in the normal thyroid.

Element	M	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Ag	0.0133	0.0114	0.0013	0.0016	0.0789	0.0102	0.00187	0.0333
Al	10.5	13.4	1.8	0.8	69.3	6.35	1.19	52.9
As	≤0.0049	-	-	<0.003	0.02	-	-	-
Au	≤0.0050	-	-	<0.002	0.0203	-	-	-
B	0.476	0.434	0.058	0.2	2.3	0.3	0.2	1.73
Be	0.00052	0.0006	0.00008	0.0001	0.0031	0.0003	0.0001	0.0022
Bi	0.0072	0.0161	0.0022	0.0003	0.1	0.0027	0.0005	0.0523
Cd	2.08	2.05	0.27	0.011	8.26	1.37	0.113	7.76
Ce	0.008	0.008	0.0011	0.001	0.0348	0.00475	0.00134	0.0293
Co	0.039	0.0276	0.0031	0.01	0.14	0.0285	0.013	0.124
Cr	0.495	0.261	0.031	0.13	1.3	0.43	0.158	1.08
Cs	0.0245	0.0166	0.0022	0.0022	0.0924	0.0198	0.00667	0.0723
Dy	0.00122	0.00183	0.00025	0.0003	0.0121	0.00063	0.0003	0.00519
Er	0.000377	0.000367	0.00005	0.0001	0.0022	0.000275	0.0001	0.0011
Eu	≤0.00039	-	-	<0.0002	0.0019	-	-	-
Fe	222.8	89.5	9.6	52	474	222	67.8	425
Ga	0.0316	0.0156	0.0021	0.01	0.081	0.0295	0.01	0.07
Gd	0.00105	0.00109	0.00015	0.0004	0.0065	0.0006	0.0004	0.00425
Hg	0.0543	0.0373	0.0043	0.007	0.151	0.046	0.00983	0.15
Ho	≤0.00040	-	-	<0.0001	0.0042	-	-	-
Ir	≤0.00028	-	-	<0.0002	0.001	-	-	-
La	0.00475	0.00461	0.00062	0.0004	0.0219	0.0027	0.0004	0.0171
Li	0.0208	0.0155	0.0022	0.0015	0.0977	0.0178	0.00412	0.0487
Lu	≤0.00020	-	-	<0.0001	0.001	-	-	-
Mn	1.28	0.56	0.07	0.47	4.04	1.15	0.537	2.23
Mo	0.0836	0.047	0.0062	0.0104	0.299	0.0776	0.0278	0.211
Nb	0.597	0.898	0.12	0.013	3.77	0.188	0.013	3.26
Nd	0.0041	0.0034	0.0004	0.0002	0.0165	0.003	0.00064	0.0137
Ni	0.449	0.344	0.046	0.074	1.8	0.33	0.12	1.39
Pb	0.233	0.246	0.033	0.023	1.6	0.18	0.0328	0.776
Pd	≤0.022	-	-	<0.014	0.07	-	-	-
Pr	0.00107	0.00086	0.00011	0.0001	0.0039	0.00073	0.0002	0.0035
Pt	≤0.00057	-	-	<0.00020	0.0138	-	-	-
Rb	7.54	3.65	0.39	1.21	22.6	6.84	3.54	17.4
Sb	0.0947	0.0692	0.0075	0.0047	0.308	0.0808	0.0117	0.279
Sc	0.0268	0.0329	0.006	0.0002	0.086	0.0064	0.000418	0.086
Se	2.22	1.24	0.14	0.32	5.8	1.84	0.776	5.58
Sm	0.000507	0.000469	0.000064	0.0001	0.0021	0.00035	0.0001	0.0015
Sn	0.0777	0.0677	0.0091	0.009	0.263	0.055	0.009	0.242
Tb	0.000198	0.000116	0.000016	0.00008	0.0006	0.00015	0.0001	0.00047
Te	≤0.0057	-	-	<0.003	0.0185	-	-	-
Th	≤0.0032	-	-	<0.002	0.01	-	-	-
Ti*	3.5	3.53	0.47	0.44	14.5	2.3	0.602	13
Tl	0.000932	0.000511	0.000068	0.0001	0.0029	0.0009	0.000294	0.00216
Tm	≤0.00014	-	-	<0.0001	0.0004	-	-	-

U	0.000443	0.000434	0.000059	0.0001	0.0026	0.0003	0.0001	0.00131
Y	0.0026	0.00234	0.00032	0.001	0.011	0.0017	0.001	0.00942
Yb	≤0.00059	-	-	<0.0003	0.0057	-	-	-
Zn	94.8	39.6	4.2	7.1	215	88.9	34.9	196
Zr	≤0.081	-	-	<0.03	0.48	-	-	-

**Note\*:** M - arithmetic mean, SD - standard deviation, SEM - standard error of mean, Min - minimum value, Max - maximum value, P 0.025 - percentile with 0.025 level, P 0.975 - percentile with 0.975 level.

**Table 3:** Some statistical parameters of 51 trace element mass fraction (mg/kg, dry mass basis) in macro-follicular colloid nodular goiter.

Element	M	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Ag	0.108	0.123	0.035	0.002	0.44	0.079	0.0022	0.371
Al	26.6	25	7.2	6.6	76.3	17.5	6.77	76.2
As	<0.004	-	-	-	-	-	-	-
Au	0.0115	0.0139	0.004	0.003	0.0517	0.008	0.003	0.0432
B	1.24	0.46	0.13	0.9	2	1	0.928	2
Be	0.00055	0.00032	0.00009	0.0002	0.001	0.0005	0.0002	0.001
Bi	0.056	0.066	0.019	0.0039	0.214	0.0289	0.0065	0.2
Cd	1.07	1.4	0.4	0.199	5.36	0.633	0.209	4.24
Ce	0.0189	0.0188	0.005	0.0046	0.0696	0.0111	0.005	0.0604
Co	0.0495	0.0254	0.0068	0.015	0.0914	0.0509	0.0155	0.0911
Cr	1.39	1.73	0.45	0.255	7.3	0.976	0.282	5.54
Cs	0.0159	0.0068	0.002	0.0083	0.0284	0.0161	0.0084	0.0276
Dy	<0.005	-	-	-	-	-	-	-
Er	0.00167	0.00089	0.00026	0.001	0.003	0.001	0.001	0.003
Eu	<0.001	-	-	-	-	-	-	-
Fe	431	390	98	65.1	1210	207	72.9	1151
Ga	0.0203	0.0092	0.003	0.01	0.034	0.02	0.01	0.0332
Gd	<0.001	-	-	-	-	-	-	-
Hg	1.43	1.34	0.35	0.1	5.18	1.34	0.109	4.42
Ho	<0.0002	-	-	-	-	-	-	-
Ir	<0.0003	-	-	-	-	-	-	-
La	0.0097	0.0096	0.003	0.0017	0.0356	0.00565	0.002	0.0308
Li	0.0268	0.0144	0.004	0.0073	0.0541	0.0252	0.00818	0.0535
Lu	<0.0002	-	-	-	-	-	-	-
Mn	1.35	0.67	0.18	0.56	2.7	1.2	0.57	2.63
Mo	0.165	0.066	0.019	0.049	0.259	0.173	0.0565	0.251
Nb	<0.013	-	-	-	-	-	-	-
Nd	0.0131	0.0093	0.003	0.0031	0.0331	0.0103	0.0032	0.0312
Ni	2.55	1.6	0.46	0.17	4.8	2	0.316	4.8
Pb	0.383	0.201	0.058	0.12	0.74	0.38	0.12	0.721
Pd	<0.012	-	-	-	-	-	-	-
Pr	0.00398	0.00334	0.001	0.00068	0.0119	0.0041	0.00071	0.0108
Pt	<0.0002	-	-	-	-	-	-	-
Rb	9.5	4.23	0.5	2.5	22.1	9.05	3.41	19.6
Sb	0.0704	0.0725	0.019	0.0102	0.267	0.041	0.0113	0.245
Sc	0.0229	0.0407	0.011	0.0002	0.113	0.0044	0.0002	0.112
Se	3.45	3.17	0.82	1.29	12.6	2.24	1.29	11
Sm	0.00149	0.00184	0.00053	0.0004	0.0069	0.00095	0.0004	0.0058

Sn	0.0467	0.0476	0.014	0.0143	0.172	0.0274	0.0149	0.155
Tb	<0.0001	-	-	-	-	-	-	-
Te	<0.007	-	-	-	-	-	-	-
Th	0.0067	0.0057	0.002	0.002	0.0209	0.005	0.002	0.0193
Ti*	<0.4	-	-	-	-	-	-	-
Tl	0.00151	0.00079	0.00023	0.00067	0.0035	0.00135	0.00069	0.00314
Tm	<0.0003	-	-	-	-	-	-	-
U	0.00165	0.00052	0.00026	0.0013	0.0024	0.00145	0.0013	0.00234
Y	0.0108	0.0114	0.004	0.0036	0.0346	0.0047	0.0036	0.0326
Yb	0.00024	0.00009	0.00003	0.0002	0.0004	0.0002	0.0002	0.0004
Zn	132	55	14	84	264	117	86.5	261
Zr	0.074	0.054	0.016	0.031	0.205	0.051	0.031	0.187

**Note\*:** M - arithmetic mean, SD - standard deviation, SEM - standard error of mean, Min - minimum value, Max - maximum value, P 0.025 - percentile with 0.025 level, P 0.975 - percentile with 0.975 level.

**Table 4:** Some statistical parameters of 51 trace element mass fraction (mg/kg, dry mass basis) in micro-follicular colloid nodular goiter.

Element	M	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Ag	0.263	0.285	0.09	0.002	0.842	0.157	0.014	0.811
Al	17.9	6.7	3	9.8	26	16.4	10.2	25.7
As	<0.004	-	-	-	-	-	-	-
Au	0.0097	0.0034	0.002	0.008	0.0148	0.008	0.008	0.0143
B	1.8	0.45	0.2	1	2	2	1.1	2
Be	0.0008	0.0004	0.0002	0.0002	0.001	0.001	0.00026	0.001
Bi	0.049	0.042	0.021	0.0115	0.0959	0.0445	0.0119	0.0942
Cd	1.44	0.99	0.44	0.26	3.01	1.31	0.362	2.84
Ce	0.018	0.023	0.01	0.0031	0.0582	0.011	0.00334	0.0538
Co	0.0667	0.0239	0.008	0.0387	0.123	0.0623	0.041	0.114
Cr	1	1.17	0.39	0.234	3.65	0.617	0.244	3.36
Cs	0.037	0.044	0.02	0.0095	0.114	0.0152	0.0099	0.106
Dy	<0.005	-	-	-	-	-	-	-
Er	0.0048	0.0051	0.002	0.001	0.0138	0.003	0.0012	0.0127
Eu	<0.001	-	-	-	-	-	-	-
Fe	434	744	224	96.5	2656	170	102	2088
Ga	<0.019	-	-	-	-	-	-	-
Gd	<0.001	-	-	-	-	-	-	-
Hg	0.86	0.71	0.23	0.139	2.22	0.544	0.182	2.11
Ho	<0.0002	-	-	-	-	-	-	-
Ir	<0.0003	-	-	-	-	-	-	-
La	0.0087	0.0086	0.004	0.0023	0.0237	0.0055	0.0025	0.0221
Li	0.0273	0.0056	0.003	0.023	0.0355	0.0254	0.0231	0.0348
Lu	<0.0002	-	-	-	-	-	-	-
Mn	2.32	1.73	0.71	0.45	5.5	1.86	0.578	5.16
Mo	0.305	0.184	0.082	0.157	0.627	0.239	0.165	0.591
Nb	<0.013	-	-	-	-	-	-	-
Nd	0.0183	0.0089	0.006	0.012	0.0246	0.0183	0.0123	0.0243
Ni	4.08	3.86	1.7	0.9	10.4	3	0.94	9.84
Pb	0.8	0.33	0.16	0.43	1.19	0.78	0.447	1.17
Pd	<0.012	-	-	-	-	-	-	-

Pr	0.0054	0.0057	0.003	0.00053	0.0131	0.0041	0.00063	0.0126
Pt	<0.0002	-	-	-	-	-	-	-
Rb	9.5	4.23	0.5	2.5	22.1	9.05	3.41	19.6
Sb	0.124	0.085	0.027	0.03	0.339	0.114	0.035	0.296
Sc	0.0088	0.0051	0.002	0.0016	0.0175	0.0071	0.0024	0.0172
Se	3.94	3.72	1.18	1.22	13.8	2.92	1.28	12
Sm	0.00142	0.0013	0.00058	0.0004	0.0037	0.001	0.00046	0.00343
Sn	0.05	0.026	0.011	0.0243	0.0775	0.0504	0.0243	0.0771
Tb	<0.0001	-	-	-	-	-	-	-
Te	<0.007	-	-	-	-	-	-	-
Th	0.006	0.0022	0.001	0.002	0.007	0.007	0.0025	0.007
Ti*	<0.4	-	-	-	-	-	-	-
Tl	0.00165	0.00065	0.00032	0.0011	0.0025	0.0015	0.00111	0.00245
Tm	<0.0003	-	-	-	-	-	-	-
U	<0.0003	-	-	-	-	-	-	-
Y	0.00815	0.00007	0.00005	0.0081	0.0082	0.00815	0.0081	0.0082
Yb	<0.0002	-	-	-	-	-	-	-
Zn	143	43	13	83	235	137	85.6	223
Zr	0.086	0.031	0.014	0.031	0.1	0.1	0.038	0.1

**Note\*:** M - arithmetic mean, SD - standard deviation, SEM - standard error of mean, Min - minimum value, Max - maximum value, P 0.025 - percentile with 0.025 level, P 0.975 - percentile with 0.975 level.

**Table 5:** Differences between mean values ( $M \pm SEM$ ) of trace element mass fractions (mg/kg, dry mass basis) in normal thyroid and macro-follicular colloid nodular goiter.

Element	Thyroid tissue				Ratio
	Normal thyroid n=105	Macro-follicular goiter n=16	Student's t-test p≤	U-test p	Goiter to Norm
Ag	0.0133±0.0013	0.108±0.035	0.022	≤0.01	8.12
Al	10.5±1.8	26.6±7.2	0.05	≤0.01	2.53
B	0.476±0.058	1.24±0.13	0.00008	≤0.01	2.61
Be	0.00052±0.00008	0.00055±0.00009	0.799	>0.05	1.06
Bi	0.0072±0.0022	0.056±0.019	0.027	≤0.01	7.78
Cd	2.08±0.27	1.07±0.40	0.05	≤0.01	0.51
Ce	0.0080±0.0011	0.0189±0.0050	0.073	>0.05	2.36
Co	0.0390±0.0031	0.0495±0.0068	0.176	>0.05	1.27
Cr	0.495±0.031	1.39±0.45	0.065	>0.05	2.81
Cs	0.0245±0.0022	0.0159±0.0020	0.0051	≤0.01	0.65
Er	0.000377±0.000050	0.00167±0.00026	0.00035	≤0.01	4.43
Fe	222.8±9.6	431±98	0.05	≤0.01	1.93
Ga	0.0316±0.0021	0.0203±0.0030	0.0078	≤0.01	0.64
Hg	0.0543±0.0043	1.43±0.35	0.0014	≤0.01	26.3
La	0.00475±0.00062	0.0097±0.0030	0.107	>0.05	2.04
Li	0.0208±0.0022	0.0268±0.0040	0.221	>0.05	1.29
Mn	1.28±0.07	1.35±0.18	0.73	>0.05	1.05
Mo	0.0836±0.0062	0.165±0.019	0.0012	≤0.01	1.97
Nd	0.0041±0.0004	0.0131±0.0030	0.009	≤0.01	3.2
Ni	0.449±0.046	2.55±0.46	0.00082	≤0.01	5.68
Pb	0.233±0.033	0.383±0.058	0.037	≤0.01	1.64

Pr	0.00107±0.00011	0.00398±0.00100	0.012	≤0.01	3.72
Rb	7.54±0.39	9.50±0.50	0.849	>0.05	1.26
Sb	0.0947±0.0075	0.0704±0.0190	0.241	>0.05	0.74
Sc	0.0268±0.0060	0.0229±0.0110	0.762	>0.05	0.85
Se	2.22±0.14	3.45±0.82	0.157	>0.05	1.55
Sm	0.000507±0.000064	0.00149±0.00053	0.093	>0.05	2.94
Sn	0.0777±0.0091	0.0467±0.0140	0.074	>0.05	0.6
Tl	0.000932±0.000068	0.00151±0.00023	0.029	≤0.01	1.62
U	0.000443±0.000059	0.00165±0.00026	0.016	≤0.01	3.72
Y	0.00260±0.00032	0.0108±0.0040	0.061	>0.05	4.15
Zn	94.8±4.2	132±14	0.017	≤0.01	1.39

**Note\*:** M - arithmetic mean, SEM - standard error of mean, statistically significant values are in bold.

**Table 6:** Differences between mean values (M±SEM) of trace element mass fractions (mg/kg, dry mass basis) in normal thyroid and micro-follicular colloid nodular goiter.

Element	Thyroid tissue				Ratio
	Normal thyroid n=105	Micro-follicular goiter n=13	Student's t-test p≤	U-test p	Goiter to Norm
Ag	0.0133±0.0013	0.263±0.090	0.021	≤0.01	19.8
Al	10.5±1.8	17.9±3.0	0.066	>0.05	1.7
B	0.476±0.058	1.80±0.20	0.0018	≤0.01	3.78
Be	0.00052±0.00008	0.00080±0.00020	0.257	>0.05	1.54
Bi	0.0072±0.0022	0.049±0.021	0.138	>0.05	6.81
Cd	2.08±0.27	1.44±0.44	0.257	>0.05	0.69
Ce	0.0080±0.0011	0.0180±0.0100	0.362	>0.05	2.25
Co	0.0390±0.0031	0.0667±0.0080	0.0083	≤0.01	1.71
Cr	0.495±0.031	1.00±0.39	0.232	>0.05	2.02
Cs	0.0245±0.0022	0.0370±0.0200	0.553	>0.05	1.51
Er	0.000377±0.000050	0.00480±0.00200	0.129	>0.05	12.7
Fe	222.8±9.6	434±224	0.369	>0.05	1.95
Ga	0.0316±0.0021	<0.019	-	-	-
Hg	0.0543±0.0043	0.86±0.23	<0.0061	≤0.01	15.8
La	0.00475±0.00062	0.00870±0.00400	0.368	>0.05	1.83
Li	0.0208±0.0022	0.0273±0.0030	0.107	>0.05	1.31
Mn	1.28±0.07	2.32±0.71	0.202	>0.05	1.81
Mo	0.0836±0.0062	0.305±0.082	0.054	≤0.05	3.65
Nd	0.0041±0.0004	0.0183±0.0060	0.264	>0.05	4.46
Ni	0.449±0.046	4.08±1.7	0.103	>0.05	9.09
Pb	0.233±0.033	0.800±0.160	0.038	≤0.01	3.43
Pr	0.00107±0.00011	0.00540±0.00300	0.22	>0.05	5.05
Rb	7.54±0.39	9.50±0.50	0.027	≤0.01	1.26
Sb	0.0947±0.0075	0.124±0.027	0.322	>0.05	1.31
Sc	0.0268±0.0060	0.0088±0.0020	0.0067	≤0.01	0.33
Se	2.22±0.14	3.94±1.18	0.179	>0.05	1.77
Sm	0.000507±0.000064	0.00142±0.00058	0.192	>0.05	2.8
Sn	0.0777±0.0091	0.0500±0.0110	0.088	>0.05	0.64
Tl	0.000932±0.000068	0.00165±0.00032	0.11	>0.05	1.77
U	0.000443±0.000059	<0.0003	-	-	-



Y	0.00260±0.00032	0.00815±0.00005	0.0001	≤0.01	3.13
Zn	94.8±4.2	143±13	0.0042	≤0.01	1.51

**Note\*:** M - arithmetic mean, SEM - standard error of mean, statistically significant values are in bold.

**Table 7:** Differences between mean values (M±SEM) of trace element mass fractions (mg/kg, dry mass basis) in macro-and micro-follicular colloid nodular goiter.

Element	Thyroid tissue				Ratio Macro- to Micro- follicular
	Macro-follicular goiter n=16	Micro-follicular goiter n=13	Student's t-test p≤	U-test p	
Ag	0.108±0.035	0.263±0.090	0.134	>0.05	0.41
Al	26.6±7.2	17.9±3.0	0.286	>0.05	1.49
B	1.24±0.13	1.80±0.20	0.049	≤0.01	0.69
Be	0.00055±0.00009	0.00080±0.00020	0.309	>0.05	0.69
Bi	0.056±0.019	0.049±0.021	0.822	>0.05	1.14
Cd	1.07±0.40	1.44±0.44	0.548	>0.05	0.74
Ce	0.0189±0.0050	0.018±0.010	0.973	>0.05	1.05
Co	0.0495±0.0068	0.0667±0.0080	0.117	>0.05	0.74
Cr	1.39±0.45	1.00±0.39	0.514	>0.05	1.39
Cs	0.0159±0.0020	0.037±0.020	0.337	>0.05	0.43
Er	0.00167±0.00026	0.0048±0.0020	0.249	>0.05	0.35
Fe	431±98	434±224	0.99	>0.05	0.99
Ga	0.0203±0.0030	<0.019	-	-	-
Hg	1.43±0.35	0.86±0.23	0.178	>0.05	1.66
La	0.0097±0.0030	0.0087±0.0040	0.841	>0.05	1.11
Li	0.0268±0.0040	0.0273±0.0030	0.919	>0.05	0.98
Mn	1.35±0.18	2.32±0.71	0.233	>0.05	0.58
Mo	0.165±0.019	0.305±0.082	0.165	>0.05	0.54
Nd	0.0131±0.0030	0.0183±0.0060	0.554	>0.05	0.72
Ni	2.55±0.46	4.08±1.7	0.433	>0.05	0.63
Pb	0.383±0.058	0.80±0.16	0.079	≤0.05	0.48
Pr	0.00398±0.00100	0.0054±0.0030	0.654	>0.05	0.74
Rb	9.50±0.50	9.50±0.50	0.954	>0.05	1
Sb	0.0704±0.0190	0.124±0.027	0.121	>0.05	0.57
Sc	0.0229±0.0110	0.0088±0.0020	0.239	>0.05	2.6
Se	3.45±0.82	3.94±1.18	0.739	>0.05	0.88
Sm	0.00149±0.00053	0.00142±0.00058	0.936	>0.05	1.05
Sn	0.0467±0.0140	0.050±0.011	0.852	>0.05	0.93
Tl	0.00151±0.00023	0.00165±0.00032	0.742	>0.05	0.92
U	0.00165±0.00026	<0.0003	-	-	-
Y	0.0108±0.0040	0.00815±0.00005	0.497	>0.05	1.33
Zn	132±14	143±13	0.591	>0.05	0.92

**Note\*:** M - arithmetic mean, SEM - standard error of mean, statistically significant values are in bold.

## Discussion

### Precision And Accuracy of Results

A good agreement of our results for the TE mass fractions with the certified values of CRM IAEA H-4, CRM IAEA HH-1, INCT-SBF-4, INCT-TL-1, and INCT-MPH-2 [29,30,35,50-55] as well as

the similarity of the means of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions in the normal human thyroid determined by both INAA-LLR and ICP-MS methods (Table 1) demonstrates an acceptable precision and accuracy of the results obtained in the study and presented in Tables 2-7.

## Effect of Goitrous Transformation on TE Contents

From Table 5, it is observed that in macro-follicular CNG the mass fraction of Ag, Al, B, Bi, Er, Fe, Hg, Mo, Nd, Ni, Pb, Pr, Tl, U, and Zn is 8.12, 2.53, 2.61, 7.78, 4.43, 1.93, 26.3, 1.97, 3.20, 5.68, 1.64, 3.72, 1.62, 3.72 and 1.39 times, respectively, higher, whereas the mass fraction of Cd, Cs, and Ga is 49%, 35%, and 36%, respectively, lower than in tissues of the normal thyroid. From Table 6, it is observed that in micro-follicular CNG the mass fraction of Ag, B, Co, Hg, Mo, Pb, Rb, Y, and Zn is 19.8, 3.78, 1.71, 15.9, 3.65, 3.43, 1.26, 3.13, and 1.51 times, respectively, higher, whereas the mass fraction of Sc is 67% lower than in tissues of the normal thyroid. Thus, if we accept the TE contents in thyroid glands in the control group as a norm, we must conclude that with a goitrous transformation the Ag, Al, B, Bi, Co, Er, Fe, Hg, Mo, Nd, Ni, Pb, Pr, Rb, Tl, U, Y, and Zn level in thyroid tissue can be significantly changed.

## Association Between TE Levels and Relative Volume of Colloid and Cells

Comparison mass fraction of Ag, Al, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Er, Fe, Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tl, U, Y, and Zn in macro- and micro-follicular CNG shown that level of B and Pb in macro-follicular goiter is 31% and 52%, respectively, lower than in micro-follicular goiter (Table 7). Because the relative volume of cells in the micro-follicular CNG is higher than in the macro-follicular CNG, it is possible to conclude that B and Pb increasingly associated with thyroid cells.

## Comparison With Published Data

The published data on TE contents in the CNG in comparison with normal levels are very scanty and contradictory. For example, information on B, Bi, Ce, Cs, Er, Ga, La, Li, Nd, Pr, Sb, Sc, Sm, Sn, Tl, and Y content in CNG was not found. Kovalev [58] found elevated levels of Ag in the CNG, but Gudzhedzhiani [59] did not. In study of Kamenev [60] the Al content in goitrous tissue was twice as much as the normal level, but Antonova, et al. [61] did not find any difference in Al content, whereas Ianchur, et al. [62], Elenevskaia, et al. [63], Bredikhin and Soroka [64], and Li [65] demonstrated a decrease of this metal mass fraction in goiter. A significant decrease of the Zn content during goitrous transformation was shown by Błazewicz et al. [66], but in the recent study this change was not confirmed [9]. Information on the TE contents in macro- or micro-follicular CNG, as well as about the association between TE level and relative volume of colloid and cells in goitrous tissue was not found.

## Limitations

This study has several limitations. Firstly, analytical techniques employed in this study measure only fifty TE mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of TE investigated in normal and goitrous

thyroid. Secondly, the sample size of macro- or micro-follicular CNG groups was relatively small and prevented investigations of TE contents in CNG group using differentials like gender, stage of disease, and dietary habits of healthy persons and patients with CNG. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on goiter-specific tissue of Ag, Al, B, Bi, Co, Er, Fe, Hg, Mo, Nd, Ni, Pb, Pr, Rb, Tl, U, Y, and Zn level alteration, demonstrates associations between B and Pb content and relative volume of cells in CNG, and shows the necessity to continue TE research of CNG of different histology.

## Conclusion

In this work, TE analysis was carried out in the tissue samples of normal and goitrous thyroid using a combination of non-destructive INAA-LLR and destructive ICP-MS. It was shown that this combination is an adequate analytical tool for the estimation of fifty TE (Ag, Al, As, Au, B, Be, Bi, Cd, Ce, Co, Cr, Cs, Dy, Er, Eu, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Mn, Mo, Nb, Nd, Ni, Pb, Pd, Pr, Pt, Rb, Sb, Se, Sm, Sn, Tb, Te, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr) content in the tissue samples of human thyroid in norm and pathology, including needle-biopsy cores. It was observed the considerable changes in TE contents in the goitrous transformed tissue of thyroid, which depend on the histology of goiter. It was found that B and Pb predominately accumulates in cells of CNG.

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## Conflicts of Interest

The authors declare that they have no financial conflicts of interest.

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