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Determination of Fifty Trace Element Contents in Normal and Goitrous Thyroid using a Combination of Instrumental Neutron Activation Analysis and Inductively Coupled Plasma Mass Spectrometry

Vladimir Zaichick

Department of Radionuclide Diagnostics, Medical Radiological Research Center, Obninsk, Russia

Abstract

Background: Nodular goiter (NG) is an internationally important health problem. The aim of this exploratory study was to evaluate whether significant changes in the thyroid tissue levels of fifty trace elements (TE) exist in the goitrous transformed thyroid.

Method: Thyroid tissue levels of fifty TE were prospectively evaluated in 46 patients with colloid NG and 105 healthy inhabitants. Measurements were performed using a combination of non-destructive and destructive methods: instrumental neutron activation analysis and inductively coupled plasma mass spectrometry, respectively. Tissue samples were divided into two portions. One was used for morphological study while the other was intended for TE analysis.

Results: It was found that contents of Ag, Al, Bi, Ce, Cr, Er, Fe, Hg, La, Li, Mn, Mo, Nd, Ni, Pr, Se, Sm, Tl, U, Y, and Zn in colloid NG tissue significantly increased whereas the levels of Cd, Ga, and Sn decrease in comparison with those in normal thyroid. Conclusion: There are considerable changes in TE contents in the goitrous tissue of thyroid.

Key words: Colloid nodular goiter, Intact thyroid, Trace elements, Biomarkers for goiter diagnosis, Instrumental neutron activation analysis, Inductively coupled plasma mass spectrometry

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*Correspondence:
Department of Radionuclide Diagnostics Medical Radiological Research Center
Korolyev St. 4, Obninsk, Kaluga Region, 249036, Russia
Tel: +7 48439 60289
E-mail: vzaichick@gmail.com

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Introduction

No less than 10 % of the world population is affected by goiter detected during the examination and palpation and most of these thyroidal lesions are nodular goiters (NG) [1]. However, using ultrasonography NG can be detected in almost 70% of the general population [2]. NG is also known as endemic nodular goitre, simple goitre, nodular hyperplasia, nontoxic uninodular goitre or multinodular goiter [3]. NG is benign lesions; however, during clinical examination, they can mimic malignant tumors. NG can be hyperfunctioning, hypofunctioning, and normal functioning. Euthyroid NG is defined as a local enlargement of

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thyroid without accompanying disturbance in thyroid function [3].

For over the 20th century, there was the dominant opinion that NG is the simple consequence of iodine (I) deficiency. However, it was found that NG 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 disfunctions and autoimmunity, NG and diffuse goiter, benign and malignant tumors of gland [5-8]. It was also demonstrated that besides the I deficiency and excess many other dietary, environmental, and occupational factors are associated with the NG 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 I and other TE contents in the normal and pathological thyroid [16-22]. Level of I 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].

To date, the pathogenesis of NG has to be considered as multifactorial. The present study was performed to clarify the role of fifty TE in the maintenance of thyroid growth and goitrogenesis. Having this in mind, our first aim is 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) mass fraction contents in NG tissue using a combination of non-destructive and destructive methods: instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR) and inductively coupled plasma mass spectrometry (ICP-MS), respectively. INAA-LLR and ICP-MS are the most powerful multi-element analytical tools for TE analysis. Using INAA-LLR it is possible to determine about 10 TE in thyroid samples [29,30] and using ICP-MS - about 50 [35,41]. However, as a non-destructive method INAA-LLR does not need in a special sample preparation (only freeze-drying and homogenization). Therefore, this method is an ideal method for some TE analysis of precious biopsy samples. By comparison TE results obtained both two methods allows to control possible losses of TE or contaminations by TE during acid digestion of thyroid samples that needs for ICP-MS.

A further aim is to compare the levels of these fifty TE in the goitrous thyroid with those in normal gland of apparently healthy persons.

Materials and Methods

Samples

All patients suffered from NG (n=46, mean age M±SD was 48±12 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 colloid NG.

Normal thyroids for the control group samples were removed at necropsy from 105 deceased (mean age 44±21 years, range 2-87), who had died suddenly. The majority of 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 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.

Sample preparation

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 [49-51].

The pounded sample weighing about 5-10 mg (for biopsy) and 50-100 mg (for resected materials) was used for TE measurement by INAA-LLR. The samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

After INAA-LLR investigation the thyroid samples were taken out from the aluminum foils and used for ICP-MS. The samples were decomposed in autoclaves; 1.5 mL of concentrated HNO_3 (nitric acid at 65 %, maximum (max) of 0.0000005 % Hg; GR, ISO, Merck, Darmstadt, Germany) and 0.3 mL of H_2O_2 (pure for analysis) were added to thyroid samples, placed in one-chamber autoclaves (Ancon-AT2, Ltd., Moscow, Russia) and then heated for 3 h at 160–200 °C. After autoclaving, they were cooled to room temperature and solutions from the decomposed samples were diluted with deionized water (up to 20 mL) and transferred to plastic measuring bottles. Simultaneously, the same procedure was performed in autoclaves without tissue samples (only $HNO_3^+H_2O_2^+$ deionized water), and the resultant solutions were used as control samples.

Certified Reference Materials

To determine contents of the TE by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [52]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. For quality control, ten subsamples of the certified reference materials (CRM) IAEA H-4 Animal Muscle from the International Atomic Energy Agency (IAEA), and also five sub-samples INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves and INCT-MPH-2 Mixed Polish Herbs from the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) were analyzed simultaneously with the investigated thyroid tissue samples. All samples of CRM were treated in the same way as the thyroid tissue samples. Detailed results of this quality assurance program were presented in earlier publications [53-59].

Instrumentation and methods

A vertical channel of nuclear reactor was applied to determine the content of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn by INAA-LLR. The quartz ampoule with thyroid samples, standards, and CRM was soldered, positioned in a transport aluminum container and exposed to a 24-hour irradiation in a vertical channel with a neutron flux of $1.3{\cdot}10^{13}$ $n{\cdot}cm^{-2}{\cdot}s^{-1}$. Ten days after irradiation samples were reweighed and repacked.

The samples were measured for period from 10 to 30 days after irradiation. The duration of measurements was from 20 min to 10 hours subject to pulse counting rate. Spectrometric measurements were performed using a coaxial 98-cm^3 Ge (Li) detector and a spectrometric unit (NUC 8100, Hungary), including a PC-coupled multichannel analyzer. Resolution of the spectrometric unit was 2.9-keV at the ^{60}Co 1,332-keV line.

Sample aliquots were used to determine 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 by ICP-MS using a Thermo X7 ICP-MS (Thermo Elemental, 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 INAA-LLR and ICP-MS methods used and other details of the analysis was presented in our previous publication concerning TE contents in human prostate and scalp hair [53-59].

Computer programs and statistic

A dedicated computer program for INAA-LLR mode optimization was used [60]. All thyroid samples were prepared in duplicate, and mean values of TE contents were used. Mean values of TE contents were used in final calculation for the Ag, Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn mass fractions measured by both two methods INAA-LLR and ICP-MS. 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 was calculated for TE mass fractions. The difference in the results between two groups (normal and goitrous thyroid) 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**.

Tables 2 and 3 present 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, 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 mass fractions in normal and goitrous thyroid tissue, 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 (≤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:

$$\leq M = \left(\sum_{i}^{n_i} C_i + DL \cdot n_j \right) / n$$

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 our results with published data for TE mass fraction in normal and goitrous thyroid [61-95] is shown in **Table 4 and 5,** 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 normal and goitrous thyroid are presented in **Table 6**.

Discussion

Precision and accuracy of results

Since there were no significant differences in TE concentration obtained by two methods, the losses and contaminations of the elements through acid digestion can be negligible for at least nine TE presented in **Table 1**. Moreover, a good agreement of our results for the TE mass fractions with the certified values of CRM IAEA H-4 and CRM IAEA HH-1 [53-59] 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 non-destructive INAA-LLR and destructive ICP-MS methods (**Table 1**) demonstrates an acceptable precision and accuracy of the results obtained in the study and presented in **Tables 2-6**.

Comparison with published data

Values obtained for Al, B, Cd, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, Lu, Mn, Nb, Nd, Ni, Pb, Pr, Pt, Rb, Sb, Sc, Se, Sm, Tb, Th, Ti, Tm, Yb, Zn, and Zr contents in the normal human thyroid (**Table 4**) agree well with median of mean values reported by other researches [61-83]. The obtained means for Ag, Au, Co, Mo, Sn, Y, and U were almost one-three orders of magnitude lower median of previously reported means but inside the range of means (**Table 4**). The mean obtained for As, Be, Bi, Ce, Eu, Ga, La, Li, and Tl were also one-three orders of magnitude lower than the median of previously reported data and outside the range of previously reported means (under a minimal value of published means). The mean obtained for Te was five orders of magnitude lower than the only reported result [83].

Data cited in **Table 4** also includes samples obtained from patients who died from different non-endocrine diseases. A number of values for TE mass fractions were not expressed on a dry mass basis by the authors of the cited references. However, we calculated these values using published data for water (75%) [75] and ash (4.16% on dry mass basis) [96] contents in thyroid of adults. No published data referring Ir and Pd contents of normal thyroid tissue were found.

In goitrous tissues (**Table 5**) our results were comparable with published data for Ag, Cd, Cr, Fe, Mn, Mo, Ni, Pb, Se, and Zn contents. The obtained means for Co and Ti were approximately one order of magnitude and for Rb and U two orders of magnitude lower median of previously reported means, herewith, means for Co and Ti were outside, while for Rb and U were inside the range of cited means (**Table 5**). Our result for As was some lower than the minimal published mean for this TE (**Table 5**). No published data referring Au, B, Be, Bi, Ce, Cs, Dy, Er, Eu, Ga, Gd, Hg, Ho, Ir, La, Li, Lu, Nb, Nd, Pd, Pr, Pt, Sc, Sm, Sn, Tb, Te, Tl, Tm, Y, Yb, and Zr contents of goitrous thyroid were found.

The ranges of means of TE content reported in the literature for normal and goitrous thyroid vary widely (**Tables 4 and 5**, respectively). This can be explained by a dependence of TE content on many factors, including the region of the thyroid, from which the sample was taken, age, gender, ethnicity, mass of the gland, and the goiter stage. Not all these factors were strictly controlled in cited studies. Another and, in our opinion, leading cause of inter-observer variability can be attributed to the accuracy of the analytical techniques, sample preparation methods, and inability of taking uniform samples from the affected tissues. It was insufficient quality control of results in these studies. In many reported papers tissue samples were ashed or dried at high temperature for many hours. In other cases, thyroid samples were treated with solvents (distilled water, ethanol, formalin etc). There is evidence that by use of these methods some quantities of certain TE are lost as a result of this treatment that concern not only such volatile halogen as Br, but also other TE investigated in the study [97,98].

Effect of goitrous transformation on trace element contents

From **Table 6**, it is observed that in goitrous tissue the mass fraction of Ag, Al, Bi, Ce, Cr, Er, Fe, Hg, La, Li, Mn, Mo, Nd, Ni, Pr, Se, Sm, Tl, U, Y, and Zn are higher than in normal tissues of the thyroid. The most increased (higher 3 times) group of TE was Ag (14.4), Bi (Bi), Er (7.9), Hg (21.7), Nd (3.4), Ni (5.9), Pr (3.7),Sm (3.3), U (3.3), and Y (4.4). In contrast, the mass fraction of Cd, Ga, and Sn are 39%, 34%, and 41%, respectively, lower. Thus, if we accept the TE contents in thyroid glands in the control group as a norm, we have to conclude that with a goitrous transformation the levels of Ag, Al, Bi, Ce, Cr, Er, Fe, Hg, La, Li, Mn, Mo, Nd, Ni, Pr, Se, Sm, Tl, U, Y, and Zn in affected thyroid tissue significantly increased whereas the levels of Cd, Ga, and Sn decrease.

Role of trace elements in goitrous transformation of the thyroid

Characteristically, elevated or reduced levels of TE observed in goitrous thyroid are discussed in terms of their potential role in the initiation and promotion of thyroid goiter. In other words, using the low or high levels of the TE in goitrous tissues researchers try to determine the goitrogenic role of the deficiency or excess of each TE in investigated organ. In our opinion, abnormal levels of many TE in tumor could be and cause, and also effect of malignant transformation. From the results of such kind studies, it is not always possible to decide whether the measured decrease or increase in TE level in pathologically altered tissue is the reason for alterations or vice versa.

Silver

Ag is a TE with no recognized trace metal value in the human body [99]. Ag in metal form and inorganic Ag compounds ionize in the presence of water, body fluids or tissue exudates. The silver ion Ag^+ is biologically active and readily interacts with proteins, amino acid residues, free anions and receptors on mammalian and eukaryotic cell membranes [100]. Besides such the adverse effects of chronic exposure to Ag as a permanent bluish-gray discoloration of the skin (argyria) or eyes (argyrosis), exposure to soluble Ag compounds may produce other toxic effects, including liver and kidney damage, irritation of the eyes, skin, respiratory, and intestinal tract, and changes in blood cells [101]. More detailed knowledge of the Ag toxicity can lead to a better understanding of the impact on human health, including thyroid function.

Aluminum

The Al is not described as essential, because no biochemical function has been directly connected to it. At this stage of our knowledge, there is no doubt that Al overload impacts negatively on human health, including the thyroid function [102]. Thus, the present study suggests that an excess of Al may be involved in the colloid NG etiology.

Bismuth

The Bi is the heaviest stable element. There is only limited information on Bi compounds effects and fate in the human body but Bi is seen as the least toxic trace metal for humans. It is widely used in medical applications for its good antibacterial properties [103]. Until now Bi is not considered a human goitro- or carcinogen. However, in recent publication Bi effects on thyroid function was shown [104]. Moreover, it was found that Bi replaces catalytic or structural metals such as iron, nickel and zinc in metalloproteins and the inorganic Bi derivatives can cause DNA single-strand breaks [105]. Thus, the present study suggests that an excess of Bi may be involved in the colloid NG etiology.

Cadmium

The Cd is well known as a category I carcinogen and mitochondria are considered to be the main intracellular targets for this trace metal. It was shown in many studies that Cd acts as a thyroid disrupter in both animals and humans [106]. Colloid cystic goiter, adenomatoid follicular hyperplasia with low-grade dysplasia and thyroglobulin hypo- and asecretion, and parafollicular cell diffuse and nodular hyperplasia and hypertrophy are often found in chronic Cd toxicity [107]. In the thyroid, Cd activates or stimulates the activity of various factors that increase cell proliferation and a reduction in normal apoptotic activity. In this connection our finding of lower Cd content in the goitrous thyroid is very astonishing.

Cerium, erbium, lanthanum, neodymium, praseodymium, samarium, and ittrium

The Ce, Er, La, Nd, Pr, Sm, and Y are rare earth elements (REEs). REEs are a series of 17 chemical elements. They include scandium (Sc), yttrium (Y), lanthanum (La) and the lanthanide series from Ce to lutetium (Lu), in the periodic table. Their adverse health effects, including toxicity affected embryogenesis, fertilization, cytogenetic and redox endpoints, are well known [108,109]. However, the available information is insufficient to ascertain the mutagenicity and carcinogenicity of lanthanides and their compounds. Thus, the present study suggests that an excess of lanthanides and their compounds may be involved in the colloid NG etiology.

Chromium

The Cr-compounds are cytotoxic, genotoxic, and carcinogenic in nature. Some Cr forms, including hexavalent chromium (Cr^{6+}), are toxicants known for their carcinogenic effect in humans. They have been classified as certain or probable carcinogens by the International Agency for Research on Cancer (IARC) [110]. Furthermore, it was found that an elevated intake of Cr may induce functional and cellular damage in animal and human thyroid [113,112]. Besides reactive oxygen species (ROS) generation, oxidative stress, and cytotoxic effects of Cr exposure, a variety of other changes like DNA damage, increased formation of DNA adducts and DNA-protein cross-links, DNA strand breaks, chromosomal aberrations and instability, disruption of mitotic cell division, chromosomal aberration, premature cell division, S or G2/M cell cycle phase arrest, and carcinogenesis also occur in humans or experimental test systems [113]. In this connection our finding of elevated Cr content in the goitrous thyroid confirms the role of this TE in the colloid NG etiology.

Iron

It is well known that Fe as TE is involved in many very important functions and biochemical reactions of human body. Fe metabolism is therefore very carefully regulated at both a systemic and cellular level [114,115]. Under the impact of age and multiple environmental factors the Fe metabolism may become dysregulated with attendant accumulation of this metal excess in tissues and organs, including thyroid [25,26,29-35]. Most experimental and epidemiological data support the hypothesis that Fe overload is a risk factor for benign and malignant tumors [116]. This goitrogenic and oncogenic effect could be explained by an overproduction of ROS and free radicals [117].

Gallium

Ga a group IIIa metal in the periodic system of elements, shares chemical properties with Fe. Ga is commonly used in industry and medicine. Data on the toxic potential of Ga are very limited [118]. Because there is a competition between Fe and Ga in biological systems [119], the lower level of Ga in the goitrous thyroid may be connected with the elevated Fe content in the affected gland.

Mercury

Hg is one of the most dangerous environmental pollutants [120]. The growing use of this metal in diverse areas of industry has resulted in a significant increase of environment contamination and episodes of human intoxication. Hg has been classified as certain or probable carcinogen by the IARC [121]. For example, in Hg polluted area thyroid cancer incidence was almost 2 times higher than in in adjacent control areas [122].

Negative effects of Hg are due to the interference of this metal in cellular signaling pathways and protein synthesis during the period of development. Since it bonds chemically with the sulfur hydride groups of proteins, it causes damage to the cell membrane and decreases the amount of RNA [123]. Moreover, it was shown that Hg may be involved in four main processes that lead to genotoxicity: generation of free radicals and oxidative stress, action on microtubules, influence on DNA repair mechanisms and direct interaction with DNA molecules [124]. Thus, the present study suggests that an elevated level of Hg in thyroid may act as a goitrogen.

Lithium

The results of lifelong Li-poor nutrition of animals show that Li is essential to the fauna, and thus, to humans as well [125]. Li-poor nutrition has a negative influence on some enzyme activity, mainly the enzymes of the citrate cycle, glycolysis, and of nitrogen metabolism [125]. On the other hand, Li is widely used in medicine as a mood-stabilizing drug. Because of the active transport of Na^+/I^- ions, Li is accumulated in the thyroid gland at a concentration 3 - 4 times higher than that in the plasma. It can inhibit the formation of colloid in thyrocytes, change the structure of thyroglobulin, weaken the iodination of tyrosines, and disrupt their coupling [126]. In addition, it reduces the clearance of free thyroxine in the serum, thereby indirectly reducing the activity of 5-deiodinase type 1 and 2 and reducing the deiodination of these hormones in the liver [126]. All these actions may cause the development of goiter.

Manganese

Trace element Mn is a cofactor for numerous enzymes, playing many functional roles in living organisms. The Mn-containing enzyme, manganese superoxide dismutase (Mn-SOD), is the principal antioxidant enzyme which neutralizes the toxic effects of reactive oxygen species. It was speculated that Mn interferes with thyroid hormone binding, transport, and activity at the tissue level [127]. However, an overall comprehension of Mn homeostasis and physiology, which is not yet acquired, is mandatory to establish Mn exact role in the thyroid goiter etiology and metabolism.

Molybdenum

The Mo is an essential TE and part of a complex called molybdenum co-factor, which is required for three mammalian enzymes—xanthine oxidase, aldehyde oxidase and sulphite oxidase [128]. Mo-dependent enzymes operate in the oxidative system of thyroid epithelial cells and also play role in the release of T_3 from the thyroid gland. However, there is data that even a slight increase Mo in the diet may accelerate and/or promote the process of thyroid cell transformation, thus acting as a tumor-promoting agent rather than a carcinogen [129]. Thus, the present study suggests that an elevated level of Mo in thyroid may act as a goitrogen.

Nickel

The peripheral connection between inorganic Ni and autoimmune thyroid diseases was mentioned in the literature [130]. Moreover, well known that human exposure to highly nickel-polluted environments, such as those associated with nickel refining, electroplating, and welding, has the potential to produce thyroid diseases. The exact mechanisms of nickel-induced thyroid diseases are not known. However, there is data that Ni-induced oxidative stress triggers cell proliferation, a process of great significance for thyroid goiter and cancer [131].

Selinium

The high level of Se content found just in the colloid NG cannot be regarded as pure chance. The seleno-protein characterized as Se-dependent glutathione peroxidase (Se-GSH-Px) is involved in protecting cells from peroxidative damage. This enzyme may reduce tissue concentration of free radicals and hydroperoxides. It is particular important for the thyroid gland, because thyroidal functions involve oxidation of iodide, which is incorporated into thyreoglobulin, the precursor of the thyroid hormones. For oxidation of iodide thyroidal cells produce a specific thyroid peroxidase using of physiologically generated hydrogen-peroxide (H_2O_2) as a cofactor [132]. It follows that the thyroid parenchyma must be continuously exposed to a physiological generation of H_2O_2 and in normal conditions must be a balance between levels of Se (as Se-GSH-Px) and H_2O_2. Thus, it might be assumed that the elevated level of Se in colloid NG tissue reflects an increase in concentration of free radicals and hydroperoxides during goitrous transformation.

Tin

For last four decades it was concluded that tin in an adequate level has beneficial effects on plants, animals and humans [133]. Sn and especially organotin compounds generates a wide variety of biological functions connected with the immune system, brain nervous system and endocrine glands, including thyroid. Among other several processes the biological functions of organotin compounds appear to be due to the inhibition of the membrane-mediated signal transduction system leading to DNA synthesis via phospholipid turnover and Ca^{2+} mobilization, as well as the involvement in cell proliferation, necrosis or apoptosis [134]. Thus, the possible goitrogenic effects of Sn deficiency on the thyroid gland cannot be ruled out.

Thallium

The Tl is a ubiquitous natural metal considered as the most toxic among TE. Moreover, Tl is a suspected human carcinogen [135]. We can't exclude the role of Tl elevated level in the NG etiology.

Uranium

The U accumulates in thyroid and its content is about an order of magnitude greater than the average soft tissue level [136]. It is known that U exposure may affect thyroid health [137].

Zinc

The Zn is active in more than 300 proteins and over 100 DNA-binding proteins, including the tumor suppressor protein p53, a Zn-binding transcription factor acting as a key regulator of cell growth and survival upon various forms of cellular stress. p53 is mutated in half of human tumors and its activity is tightly regulated by metals and redox mechanisms. On the other hand, excessive intracellular Zn concentrations may be harmful to normal metabolism of cells [138]. By now much data has been obtained related both to the direct and indirect action of intracellular Zn on the DNA polymeric organisation, replication and lesions, and to its vital role for cell division [139]. Other actions of Zn have been also described. They include its action as a potent anti-apoptotic agent [140]. All these facts allowed us to speculate that age-related overload Zn content in female thyroid, as was found in our previous study [25,29,31,33], is probably one of the factors in etiology of thyroid goiter and malignant tumors. Therefore, the elevated Zn level in colloid NG in comparison with normal level, detected in this study, supports our hypothesis.

Trace element levels as goiter markers

Our findings show that mass fraction of Ag, Al, Bi, Cd, Ce, Cr, Er, Fe, Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pr, Se, Sm, Sn, Tl, U, Y, and Zn are significantly different in colloid NG as compared to normal thyroid tissues (**Table 6**). Thus, it is plausible to assume that levels of these TE in affected thyroid tissue can be used as goiter markers. However, this subject needs in additional studies.

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 chemical elements investigated in normal and goitrous thyroid tissue. Secondly, the sample size of NG group was relatively small. It was not allow us to carry out the investigations of TE contents in NG group using differentials like gender, histological types of goiter, stage of disease, and dietary habits of healthy persons and patients with NG. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on goiter-specific tissue Ag, Al, Bi, Cd, Ce, Cr, Er, Fe, Ga, Hg, La, Li, Mn, Mo, Nd, Ni, Pr, Se, Sm, Sn, Tl, U, Y, and Zn level alteration and shows the necessity the need to continue TE research of goitrous thyroid.

Conclusion

In this work, TE measurements were carried out in the tissue samples of normal thyroid and colloid NG using two instrumental analytical methods: non-destructive neutron activation analysis with high resolution spectrometry of long-lived radionuclides and inductively coupled plasma mass spectrometry. It was shown that the combination of these methods is an adequate analytical tool for the estimation of fifty TE contents in the tissue samples of human thyroid, including needle-biopsy cores. It was observed that in goitrous tissues content of Ag, Al, Bi, Ce, Cr, Er, Fe, Hg, La, Li, Mn, Mo, Nd, Ni, Pr, Se, Sm, Tl, U, Y, and Zn significantly increased whereas the levels of Cd, Ga, and Sn decrease in a comparison with the normal thyroid tissues. In our opinion, the data of presented study strongly imply that TE play a significant role in thyroid health and the etiology of colloid

NG. It was supposed that the found differences in levels of TE in affected thyroid tissue can be used as colloid NG markers.

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Table 1. Comparison of the mean values (M±SEM) of the trace element mass fractions (mg/kg, on dry-mass basis) in the normal thyroid obtained by both NAA-LLR and ICP-MS methods

Element	NAA-LLR M_1	ICP-MS M_2	Δ, %
Ag	0.0151±0.0016	0.0122±0.0014	19.2
Co	0.0399±0.0030	0.0378±0.0031	5.3
Cr	0.539±0.032	0.451±0.033	16.3
Fe	225±11	221±12	1.8
Hg	0.0421±0.0041	0.0794±0.0114	-88.5
Rb	7.37±0.44	7.79±0.46	-5.7
Sb	0.111±0.008	0.079±0.008	28.8
Se	2.32±0.14	2.12±0.14	8.6
Zn	97.8±4.5	91.8±4.3	6.1

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

Table 2. Some statistical parameters of 50 trace element mass fraction (mg/kg, dry mass basis) in the normal thyroid (n=105)

Element	M	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Ag	0.0133	0.0114	0.0013	0.00160	0.0789	0.0102	0.00187	0.0333
Al	10.5	13.4	1.8	0.80	69.3	6.35	1.19	52.9
As	≤0.0049	-	-	<0.003	0.0200	-	-	-
Au	≤0.0050	-	-	<0.002	0.0203	-	-	-
B	0.476	0.434	0.058	0.200	2.30	0.300	0.200	1.73
Be	0.00052	0.00060	0.00008	0.0001	0.0031	0.00030	0.0001	0.0022
Bi	0.0072	0.0161	0.0022	0.000300	0.100	0.00270	0.000500	0.0523
Cd	2.08	2.05	0.27	0.0110	8.26	1.37	0.113	7.76
Ce	0.0080	0.0080	0.0011	0.00100	0.0348	0.00475	0.00134	0.0293
Co	0.0390	0.0276	0.0031	0.0100	0.140	0.0285	0.0130	0.124
Cr	0.495	0.261	0.031	0.130	1.30	0.430	0.158	1.08
Cs	0.0245	0.0166	0.0022	0.00220	0.0924	0.0198	0.00667	0.0723
Dy	0.00122	0.00183	0.00025	0.000300	0.0121	0.000630	0.000300	0.00519
Er	0.000377	0.000367	0.000050	0.000100	0.00220	0.000275	0.000100	0.00110
Eu	≤0.00039	-	-	<0.0002	0.00190	-	-	-
Fe	222.8	89.5	9.6	52.0	474	222	67.8	425
Ga	0.0316	0.0156	0.0021	0.0100	0.0810	0.0295	0.0100	0.0700
Gd	0.00105	0.00109	0.00015	0.000400	0.00650	0.000600	0.000400	0.00425
Hg	0.0543	0.0373	0.0043	0.00700	0.151	0.0460	0.00983	0.150
Ho	≤0.00040	-	-	<0.0001	0.00420	-	-	-
Ir	≤00.00028	-	-	<0.0002	0.0010	-	-	-
La	0.00475	0.00461	0.00062	0.000400	0.0219	0.00270	0.000400	0.0171
Li	0.0208	0.0155	0.0022	0.00150	0.0977	0.0178	0.00412	0.0487
Lu	≤0.00020	-	-	<0.0001	0.00100	-	-	-
Mn	1.28	0.56	0.07	0.470	4.04	1.15	0.537	2.23
Mo	0.0836	0.0470	0.0062	0.0104	0.299	0.0776	0.0278	0.211
Nb	0.597	0.898	0.120	0.0130	3.77	0.188	0.0130	3.26
Nd	0.0041	0.0034	0.0004	0.00020	0.0165	0.0030	0.00064	0.0137
Ni	0.449	0.344	0.046	0.0740	1.80	0.330	0.120	1.39
Pb	0.233	0.246	0.033	0.0230	1.60	0.180	0.0328	0.776
Pd	≤0.022	-	-	<0.014	0.0700	-	-	-
Pr	0.00107	0.00086	0.00011	0.00010	0.00390	0.00073	0.00020	0.00350
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	0.00470	0.308	0.0808	0.0117	0.279
Sc	0.0268	0.0329	0.0060	0.000200	0.0860	0.00640	0.000418	0.0860
Se	2.22	1.24	0.14	0.320	5.80	1.84	0.776	5.58
Sm	0.000507	0.000469	0.000064	0.000100	0.00210	0.000350	0.000100	0.00150
Sn	0.0777	0.0677	0.0091	0.00900	0.263	0.0550	0.00900	0.242
Tb	0.000198	0.000116	0.000016	0.0000800	0.000600	0.000150	0.000100	0.000470
Te	≤0.0057	-	-	<0.003	0.0185	-	-	-
Th	≤0.0032	-	-	<0.002	0.0100	-	-	-
Ti	3.50	3.53	0.47	0.440	14.5	2.30	0.602	13.0
Tl	0.000932	0.000511	0.000068	0.000100	0.00290	0.000900	0.000294	0.00216
Tm	≤0.00014	-	-	<0.0001	0.00040			
U	0.000443	0.000434	0.000059	0.000100	0.00260	0.00030	0.000100	0.00131
Y	0.00260	0.00234	0.00032	0.00100	0.0110	0.00170	0.00100	0.00942
Yb	≤0.00059	-	-	<0.0003	0.00570	-	-	-
Zn	94.8	39.6	4.2	7.10	215	88.9	34.9	196
Zr	≤0.081	-	-	<0.03	0.480	-	-	-

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 50 trace element mass fraction (mg/kg, dry mass basis) in the colloid nodular goiter (n=46)

Element	M	SD	SEM	Min	Max	Median	P 0.025	P 0.975
Ag	0.192	0.214	0.038	0.00200	0.842	0.102	0.00200	0.736
Al	27.1	24.7	5.3	6.60	95.1	20.5	6.92	85.2
As	<0.004	-	-	-	-	-	-	-
Au	0.0141	0.0152	0.0030	0.00300	0.0585	0.00800	0.00300	0.0551
B	5.50	17.8	3.8	0.900	85.2	1.00	0.953	43.1
Be	0.00072	0.00053	0.00011	0.000200	0.00200	0.000500	0.000200	0.00200
Bi	0.0585	0.0560	0.0130	0.00390	0.214	0.0433	0.00732	0.192
Cd	1.26	1.30	0.28	0.126	5.36	0.964	0.164	4.56
Ce	0.0186	0.0185	0.0040	0.00310	0.0696	0.0109	0.00340	0.0639
Co	0.0576	0.0282	0.0049	0.0150	0.147	0.0538	0.0163	0.128
Cr	1.18	1.38	0.24	0.144	7.30	0.659	0.200	4.47
Cs	0.0216	0.0232	0.0050	0.00760	0.114	0.0147	0.00793	0.0760
Dy	<0.005	-	-	-	-	-	-	-
Er	0.00299	0.00332	0.00100	0.00100	0.0138	0.00200	0.00100	0.0113
Eu	<0.001	-	-	-	-	-	-	-
Fe	449	597	92	62.0	2734	207	65.6	2623
Ga	0.0210	0.0080	0.0020	0.0100	0.0340	0.0200	0.0100	0.0328
Gd	<0.001	-	-	-	-	-	-	-
Hg	1.18	1.01	0.17	0.100	5.18	1.11	0.122	3.34
Ho	<0.0002	-	-	-	-	-	-	-
Ir	<0.0003	-	-	-	-	-	-	-
La	0.00990	0.00921	0.00200	0.00170	0.0356	0.00570	0.00199	0.0311
Li	0.0281	0.0117	0.0030	0.00730	0.0541	0.0259	0.00890	0.0530
Lu	<0.0002	-	-	-	-	-	-	-
Mn	1.77	1.13	0.23	0.450	5.50	1.60	0.516	4.12
Mo	0.183	0.121	0.026	0.0490	0.627	0.173	0.0511	0.437
Nb	<0.013	-	-	-	-	-	-	-
Nd	0.0139	0.0087	0.0020	0.00310	0.0331	0.0114	0.00326	0.0306
Ni	2.63	2.43	0.54	0.130	10.4	1.75	0.149	7.74
Pb	0.94	1.86	0.41	0.120	8.90	0.460	0.120	5.10
Pd	<0.012	-	-	-	-	-	-	-
Pr	0.00396	0.00359	0.00100	0.000530	0.0131	0.00360	0.000601	0.0125
Pt	<0.0002	-	-	-	-	-	-	-
Rb	9.50	4.23	0.50	2.50	22.1	9.05	3.41	19.6
Sb	0.127	0.113	0.019	0.00102	0.425	0.0865	0.0128	0.404
Sc	0.0196	0.0316	0.0060	0.000200	0.113	0.00655	0.000200	0.111
Se	3.54	3.31	0.56	0.860	13.8	2.37	1.02	12.8
Sm	0.00169	0.00156	0.00033	0.000400	0.00690	0.00100	0.000400	0.00522
Sn	0.0458	0.0384	0.0090	0.0143	0.172	0.0319	0.0154	0.142
Tb	<0.0001	-	-	-	-	-	-	-
Te	<0.007	-	-	-	-	-	-	-
Th	0.0074	0.0062	0.0010	0.00200	0.0210	0.00600	0.00200	0.0210
Ti	<0.4	-	-	-	-	-	-	-
Tl	0.00174	0.00093	0.00021	0.000520	0.00350	0.00155	0.000591	0.00345
Tm	<0.0003	-	-	-	-	-	-	-
U	0.00145	0.00053	0.00022	0.000820	0.00240	0.00130	0.000880	0.00230
Y	0.0113	0.0103	0.0030	0.00360	0.0346	0.00665	0.00360	0.0318
Yb	0.000246	0.000087	0.000024	0.000200	0.000400	0.000200	0.000200	0.000400
Zn	121	51	8	47.0	264	113	49.1	257
Zr	0.074	0.045	0.010	0.0310	0.205	0.0620	0.0310	0.174

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. Median, minimum and maximum value of means of trace element contents in the normal thyroid according to data from the literature in comparison with our results (mg/kg, dry mass basis)

Element	Published data [Reference]			This work
	Median of means (n)*	Min of means M or M±SD, (n)**	Max of means M or M±SD, (n)**	M±SD
Ag	0.25 (12)	0.000784 (16) [61]	1.20±1.24 (105) [62]	0.0133±0.0114
Al	33.6 (12)	0.33 (-) [63]	420 (25) [64]	10.5±13.4
As	0.068 (15)	0.0036 (131) [65]	500±48 (4) [66]	≤0.0049
Au	0.084 (3)	0.0014±0.0002 (10) [67]	<0.4 (-) [68]	≤0.0050
B	0.151 (2)	0.084 (1) [69]	0.46 (1) [69]	0.476±0.434
Be	0.042 (3)	0.000924(16) [61]	<0.12 (-) [68]	0.00052±0.00060
Bi	0.126 (4)	0.0339 (16) [61]	<0.4 (-) [68]	0.0072±0.0161
Cd	1.68 (20)	0.12 (131) [65]	47.6±8.0 (16) [70]	2.08±2.05
Ce	0.22 (1)	0.22 (59) [61]	0.22 (59) [61]	0.0080±0.0080
Co	0.306 (25)	0.016 (66) [71]	70.4±40.8 (14) [72]	0.039±0.028
Cr	0.69 (17)	0.088 (83) [73]	24.8±2.4 (4) [66]	0.49±0.25
Cs	0.066 (6)	0.0112±0.0109 (14) [74]	0.109±0.370 (48) [75]	0.025±0.017
Dy	0.00106 (1)	0.00106 (60) [61]	0.00106 (60) [61]	0.0012±0.0018
Er	0.00068 (1)	0.00068 (60) [61]	0.00068 (60) [61]	0.00038±0.00038
Eu	0.0036 (1)	0.0036 (60) [61]	0.0036 (60) [61]	≤0.00039
Fe	252 (21)	56 (120) [76]	3360 (25) [64]	223±90
Ga	0.273 (3)	<0.04 (-) [68]	1.7±0.8 (-) [77]	0.032±0.016
Gd	0.00256 (1)	0.00256 (59) [61]	0.00256 (59) [61]	0.00105±0.00015
Hg	0.08 (13)	0.0008±0.0002 (10) [67]	396±40 (4) [66]	0.054±0.037
Ho	0.00016 (1)	0.00016 (60) [61]	0.00016 (60) [61]	≤0.00040
Ir	-	-	-	≤0.00028
La	0.068 (3)	0.052 (59) [61]	<4.0 (-) [68]	0.0047±0.0046
Li	6.3 (2)	0.092 (-) [68]	12.6 (180) [78]	0.021±0.015
Lu	0.000224 (1)	0. 000224 (60) [61]	0. 000224 (60) [61]	≤0.00020
Mn	1.62 (40)	0.076 (83) [73]	69.2±7.2 (4) [66]	1.28±0.56
Mo	0.40 (11)	0.0288±0.0096 (39) [67]	516±292 (14) [72]	0.0836±0.047
Nb	<4.0 (1)	<4.0 (-) [68]	<4.0 (-) [68]	0.60±0.90
Nd	0.0108 (1)	0.0108 (60) [61]	0.0108 (60) [61]	0.0041±0.0034
Ni	0.44 (19)	0.0084 (83) [73]	33.6±3.6 (4) [66]	0.45±0.34
Pb	0.58 (25)	0.021 (83) [73]	68.8±6.8 (4) [66]	0.23±0.25
Pd	-	-	-	≤0.022
Pr	0.0034 (1)	0.0034 (59) [61]	0.0034 (59) [61]	0.00107±0.00086
Pt	0.00017 (1)	0.00017 (59) [61]	0.00017 (59) [61]	≤0.00057
Rb	7.8 (9)	≤0.85 (29) [67]	294±191 (14) [72]	7.5±3.7
Sb	0.15 (10)	0.040±0.003 (-) [79]	≤12.4 (-) [68]	0.095±0.069
Sc	0.009 (4)	0.0018±0.0003 (17) [80]	0.0135±0.0045 (10) [67]	0.0268±0.0329
Se	2.32 (21)	0.436 (40) [81]	756±680 (14) [72]	2.2±1.2
Sm	0.00216 (1)	0.00216 (60) [61]	0.00216 (60) [61]	0.00051±0.00047
Sn	0.67 (7)	0.0235 (16) [61]	-≤3.8 (17) [82]	0.078±0.068
Tb	0.000224 (1)	0.000224 (60) [61]	0.000224 (60) [61]	0.00020±0.00012
Te	109 (1)	109±82 (7) [83]	109±82 (7) [83]	≤0.0057
Th	0.00216 (42)	0.00044 (40) [81]	0.00528 (60) [61]	≤0.0032
Ti	1.42 (8)	0.084 (83) [73]	73.6±7.2 (4) [66]	3.5±3.5
Tl	<0.2 (2)	0.00138 (16) [61]	<0.4 (-) [68]	0.00093±0.00051
Tm	0.000124 (1)	0.000124 (60) [61]	0.000124 (60) [61]	≤0.00014
U	0.0060 (11)	0.00014 (66) [71]	0.428±0.143 (10) [67]	0.00044±0.00043
Y	<2.9 (2)	0.00225 (16) [61]	≤5.9 (17) [82]	0.0026±0.0023
Yb	0.00056 (1)	0.00056 (60) [61]	0.00056 (60) [61]	≤0.00059
Zn	110 (56)	2.1 (-) [63]	820±204 (14) [72]	95±40
Zr	<0.4 (3)	0.188 (60) [61]	<4.0 (-) [68]	≤0.082

M –arithmetic mean, SD – standard deviation, Min – minimum, Max – maximum, (n)* – number of all references, (n)** – number of samples.

Table 5. Median, minimum and maximum value of means of trace element contents in the thyroid nodular goiter according to data from the literature in comparison with our results (mg/kg, dry mass basis)

Element	Published data [Reference]			This work
	Median of means (n)*	Min of means M or M±SD, (n)**	Max of means M or M±SD, (n)**	M±SD
Ag	0.21 (4)	0.098±0.042 (19) [84]	2.56 (167) [85]	0.192±0.199
Al	3.84 (6)	2.45 (123) [86]	840 (25) [64]	27.3±23.6
As	0.0045 (3)	0.0044 (41) [81]	68±52 (11) [72]	<0.004
Au	-	-	-	0.0166±0.0194
B	-	-	-	4.65±15.0
Be	-	-	-	0.00090±0.00113
Bi	-	-	-	0.0706±0.0845
Cd	1.24 (4)	0.125±0.006 (64) [87]	1.72±0.13 (9) [88]	1.55±1.68
Ce	-	-	-	0.0181±0.0176
Co	0.67 (12)	0.110±0.003 (64) [87]	62.8±22.4 (11) [72]	0.0576±0.0324
Cr	3.66 (5)	0.72 (51) [89]	25.2 (25) [64]	1.17±1.19
Cs	-	-	-	0.0320±0.0471
Dy	-	-	-	<0.005
Er	-	-	-	0.00303±0.00328
Eu	-	-	-	<0.001
Fe	390 (5)	128±52 (13) [90]	4848±3056 (11) [72]	430±566
Ga	-	-	-	0.0211±0.0081
Gd	-	-	-	<0.001
Hg	-	-	-	1.15±1.04
Ho	-	-	-	<0.0002
Ir	-	-	-	<0.0003
La	-	-	-	0.00939±0.00882
Li	-	-	-	0.0295±0.0151
Lu	-	-	-	<0.0002
Mn	2.64 (21)	0.352 (130) [65]	34.9 (101) [91]	1.81±1.41
Mo	0.39 (4)	0.094-0.145 (77) [84]	512±16 (11) [72]	0.193±0.121
Nb	-	-	-	<0.013
Nd	-	-	-	0.0134±0.0075
Ni	1.00 (9)	0.404 (41) [81]	19.7±20.5 (11) [72]	2.89±2.52
Pb	0.76 (9)	0.156±0.156 (13) [88]	8.08±6.00 (514) [92]	1.31±2.27
Pd	-	-	-	<0.012
Pr	-	-	-	0.00389±0.00335
Pt	-	-	-	<0.0002
Rb	436 (2)	7,0 (10) [80]	864±148 (11) [72]	9.50±4.23
Sb	0.63 (1)	0.15 (1) [93]	1.10 (1) [93]	0.121±0.108
Sc	-	-	-	0.0239±0.0383
Se	2.60 (8)	0.248 (41 [81]	174±116 (11) [72]	3.20±2.92
Sm	-	-	-	0.00171±0.00181
Sn	-	-	-	0.0516±0.0399
Tb	-	-	-	<0.0001
Te	-	-	-	<0.007
Th	0.00026 (1)	0.00026 (41) [81]	0.00026 (41) [81]	0.0104±0.0155
Ti	4.12 (2)	2.69 (-) [94]	16.4±25.2 (514) [92]	<0.4
Tl	-	-	-	0.00190±0.00109
Tm	-	-	-	<0.0003
U	0.202 (4)	0.00052 130) [65]	0.280±0.256 (51) [92]	0.00116±0.00059
Y	-	-	-	0.0110±0.0108
Yb	-	-	-	0.000275±0.000133
Zn	146 (25)	22.4 (130) [65]	1236±560 (2) [95]	117.7±48.7
Zr	-	-	-	0.0733±0.0444

M –arithmetic mean, SD – standard deviation, Min – minimum, Max – maximum, (n)* – number of all references, (n)** – number of samples.

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

Element	Thyroid tissue				Ratio
	Norm n=105	Goiter n=46	Student's t-test *p*≤	U-test *p*	Goiter to Norm
Ag	0.0133±0.0013	0.192±0.038	**<0.000046**	**≤0.01**	14.4
Al	10.5±1.8	27.1±5.3	**0.0058**	**≤0.01**	2.58
B	0.476±0.058	5.50±3.8	0.200	>0.05	11.6
Be	0.00052±0.00008	0.00072±0.00011	0.165	>0.05	1.38
Bi	0.0072±0.0022	0.0585±0.0130	**0.00088**	**≤0.01**	8.13
Cd	2.08±0.27	1.26±0.28	**0.038**	**≤0.01**	0.61
Ce	0.0080±0.0011	0.0186±0.0040	**0.018**	**≤0.01**	2.33
Co	0.0390±0.0031	0.0576±0.0049	**0.0021**	**≤0.01**	1.48
Cr	0.495±0.031	1.18±0.24	**0.0088**	**≤0.01**	2.38
Cs	0.0245±0.0022	0.0216±0.0050	0.595	>0.05	0.88
Er	0.000377±0.000050	0.00299±0.00100	**0.0014**	**≤0.01**	7.93
Fe	222.8±9.6	449±92	**0.019**	**≤0.01**	2.02
Ga	0.0316±0.0021	0.0210±0.0020	**0.0014**	**≤0.01**	0.66
Hg	0.0543±0.0043	1.18±0.17	**<0.00000016**	**≤0.01**	21.7
La	0.00475±0.00062	0.00990±0.00200	**0.025**	**≤0.01**	2.08
Li	0.0208±0.0022	0.0281±0.0030	**0.037**	**≤0.01**	1.35
Mn	1.28±0.07	1.77±0.23	**0.048**	**≤0.01**	1.38
Mo	0.0836±0.0062	0.183±0.026	**0.0010**	**≤0.01**	2.19
Nd	0.0041±0.0004	0.0139±0.0020	**0.0010**	**≤0.01**	3.39
Ni	0.449±0.046	2.63±0.54	**0.00076**	**≤0.01**	5.85
Pb	0.233±0.033	0.94±0.41	0.098	>0.05	4.03
Pr	0.00107±0.00011	0.00396±0.00100	**0.0020**	**≤0.01**	3.70
Rb	7.54±0.39	9.50±0.50	0.108	>0.05	1.26
Sb	0.0947±0.0075	0.127±0.019	0.126	>0.05	1.34
Sc	0.0268±0.0060	0.0196±0.0060	0.387	>0.05	0.73
Se	2.22±0.14	3.54±0.56	**0.028**	**≤0.01**	1.59
Sm	0.000507±0.000064	0.00169±0.00033	**0.0037**	**≤0.01**	3.33
Sn	0.0777±0.0091	0.0458±0.0090	**0.013**	**≤0.01**	0.59
Tl	0.000932±.000068	0.00174±0.00021	**0.0012**	**≤0.01**	1.87
U	0.000443±0.000059	0.00145±0.00022	**0.0044**	**≤0.01**	3.27
Y	0.00260±0.00032	0.0113±0.0030	**0.014**	**≤0.01**	4.35
Zn	94.8±4.2	121±8	**0.0053**	**≤0.01**	1.28

M – arithmetic mean, SEM – standard error of mean, Statistically significant values are in **bold**.

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