International Journal of Multidisciplinary and Current Educational Research (IJMCER)
ISSN: 2581-7027 ||Volume|| 4 ||Issue|| 1 ||Pages 219-229 ||2022||

Comparison ofNineteen Chemical Elementsin Thyroid Tissue adjacent to Thyroid Malignant and Benign Nodules using Neutron Activation Analysis and Inductively Coupled Plasma Atomic Emission Spectrometry

Prof., Dr. V. Zaichick,
Medical Radiological Research Centre, Koroleyv St. 4, Obninsk 249036, RUSSIA

ABSTRACT

Background: Thyroid nodules (TN) are the most common endocrine disorder worldwide. Etiology and pathogenesis of thyroid benign and malignant nodules (TBN and TMN, respectively) are still not enough understood. The present study was performed to clarify the role of some chemical elements (Ches) in the origination and development of TN.

Methods: Contents of Ches such as aluminum (Al), boron (B), barium (Ba), calcium (Ca), chlorine (Cl), coper (Cu), iron (Fe), iodine (I), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), sulfur (S), silicon (Si), strontium (Sr), vanadium (V), and zinc (Zn) were prospectively evaluated in thyroid tissue adjacent to TBN (79 patients) and to TMN (41 patients). Measurements were performed using a combination of non-destructive and destructive methods: instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides and inductively coupled plasma atomic emission spectrometry. Results of the study were additionally compared with previously obtained data for the same ChEs in "normal" thyroid tissue.

Results: I was found that in thyroid tissue adjacent to TMN the mass fraction of I is 47% higher, while mass fractions Cl and Na 42% and 29%, respectively, lower those in thyroid tissue adjacent to TBN. The common characteristics of thyroid tissue adjacent to TBN and TMN were similar contents of B, Ba, Ca, Fe, K, Li, Mg, Mn, P, Sr, V, and Zn, as well as elevated levels of Al, Cl, Cu, I, Na, S, and Si, which overdrew those in "normal" thyroid approximately in 2.1, 2.2, 2.2, 1.4, 1.4, 1.2, and 2.1 times, respectively.

Conclusions: Role of Chesin etiology and pathogenesis TBN and TMN is similar and exessive accumulation of Al, Cl, Cu, I, Na, S, and Siin thyroid tissue may be involved in the TN origination and development.

Keywords: Thyroid; Thyroid malignant and benign nodules; Chemical elements; inductively coupled plasma atomic emission Spectrometry; Neutron activation analysis

I. INTRODUCTION

Thyroid benign and malignant nodules (TBN and TMN, respectively) are the most common endocrine disorder worldwide. Moreover, in some parts of the world, especially those of current or former iodine deficiency, thyroid nodules (TN) are still an endemic disease [1]. Incidence of TBN and TMN has been growing steadily over the past four decades, despite the use of iodine prophylaxis in many countries [2]. Some factors causing this higher incidence of TN were described in literature [3] and analysis of these data shown intriguing links between the etiologies of TBN and TMN [2,3]. In other words, the factors contributing to increases in the incidence of TBN are the same as those contributing to increases in TMN. However, the current state of knowledge regarding TN demonstrates that the etiology and pathogenesis of TBN and TMN are still not enough understood, because there are many not adequately explored chemicals, which induced thyroid hormone perturbations leading to these diseases.For over 20th century, there was the dominant opinion that TN is the simple consequence of iodinedeficiency [4]. However, it was found that TN is a frequent disease even in those countries and regions where the population is never exposed to iodine shortage. Moreover, it was shown that iodine excess has severe consequences on human health and associated with the presence of TN [5-8]. It was also demonstrated that besides the iodine deficiency and excess many other dietary, environmental, and occupational factors are associated with the TN incidence [3,9-11]. Among these factors a disturbance of evolutionary stable input of many chemical elements (ChEs) in human body after industrial revolution plays a significant role in etiology of TN [12].

Besides iodine, many other Ches have also essential physiological functions [13]. Essential or toxic (goitrogenic, mutagenic, carcinogenic) properties of Ches depend on tissue-specific need or tolerance, respectively [13].Excessive accumulation or an imbalance of the Ches may disturb the cell functions and may result in cellular proliferation, 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 Ches 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 many Ches content with age in the thyroid of males and females were studied and age- and gender-dependence of some Ches was observed [25-41]. Furthermore, a significant difference between some Ches contents in colloid goiter, thyroid it is, thyroid adenoma and cancer in comparison with normal thyroid was demonstrated [42-47].

The present study was performed to clarify the role of some Ches in the etiology of TBN and TMN. Having this in mind, the aim of this exploratory study was to examine differences in the content of aluminum (Al), boron (B), barium (Ba), calcium (Ca), chlorine (Cl), coper (Cu), iron (Fe), iodine (I), potassium (K), lithium (Li), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), sulfur (S), silicon (Si), strontium (Sr), vanadium (V), and zinc (Zn) inthyroidt issue adjacent to TN using a combination of non-destructive instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) and destructive method such as inductively coupled plasma atomic emission spectrometry (ICP-AES), and to compare the levels of these Ches in two groups of samples (tissue adjacent to TBN and TMN, respectively). Moreover, for understanding a possible role of Ches in etiology and pathogenesis of TN results of the study were compared with previously obtained data for the same Ches in “normal” thyroid tissue [43-47].

II. MATERIAL AND METHODS

All patients suffered from TBN (n=79, mean age M±SD was 44±11 years, range 22-64) and from TMN (n=41, mean age M±SD was 46±15 years, range 16-75) were hospitalized in the Head and Neck Department of the Medical Radiological Research Centre (MRRC), Obninsk. Thick-needle puncture biopsy of suspicious nodules of thethyroid was performed for every patient, to permit morphological study of thyroid tissue at these sites and to estimate their ChEs contents. In all cases the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials. Histological conclusions for benign nodules were: 46 colloid goiter, 19 thyroid adenoma, 8 Hashimoto's thyroiditis, and 6 Riedel’s Struma, whereas for thyroid malignant tumors were: 25 papillary adenocarcinomas, 8 follicular adenocarcinomas, 7 solid carcinomas, and 1 reticulosarcoma. Samples of visually intact thyroid tissue adjacent to TBN and TMN were taken from resected materials.

“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 MRRC. 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. Informed consent was obtained from all individual participants included in the study.All tissue samples obtained from tumors and visually intact tissue adjacent to tumors were divided into two portions using a titanium scalpel to prevent contamination by ChEs of stainless steel [48]. One was used for morphological study while the other was intended for Ches analysis. After the samples intended for Ches analysis were weighed, they were freeze-dried and homogenized [49].

The pounded samples weighing about 10 mg (for biopsy) and 100 mg (for resected materials) were used for ChE measurement by INAA-SLR. The content of Ca, Cl, I, K, Mg, Mn, and Na were determined by INAA-SLR using a horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor (Branch of Karpov Institute, Obninsk). After non-destructive INAA-SLR investigation the thyroid samples were used for ICP-AES. The samples were decomposed in autoclaves and aliquots of solutions were used to determine the Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractions by ICP-AES using the Spectrometer ICAP-61 (Thermo Jarrell Ash, USA). Information detailing with the NAA-SLR and ICP-AES methods used and other details of the analysis were presented in our earlier publications concerning Ches contents in human thyroid [33,34], prostate [50-54], and scalp hair [55]. To determine contents of the Che by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formal dehyde 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) IAEA H-4 (animal muscle) and 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 resultsA dedicated computer program for INAA-SLR mode optimization was used [57]. All thyroid samples for Ches analysis were prepared in duplicate and mean values of Ches contents were used in final calculation. Mean values of ChEs contents were used in final calculation for the Ca, K, Mg, Mn, and Na mass fractions measured by two methods.Using Microsoft Office Excel software, a summary of the statistics, including, arithmetic mean, standard deviationof mean, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for Ches contents in two groups of tissue adjacent to TBN and TMN.Data for “normal” thyroid were taken from our previous publications [43-47]. The difference in the results between two groups of samples “adjacent to TBN” and “adjacent to TMN”, as well as between “normal” and “adjacent to TBN and TMN combined” was evaluated by the parametric Student’s *t*-test and non-parametricWilcoxon-Mann-Whitney *U*-test.

III. RESULTS

Table 1 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 Al, B, Ba, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractionin thyroid intact tissue samples of two groups “adjacent to TBN” and “adjacent to TMN”.The ratios of means and the comparison of mean values of Al, B, Ba, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Znmass fractions in pair of sample groups such as “adjacent to TBN” and “adjacent to TMN”is presented in Table 2. Table 3 depicts 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 Al, B, Ba, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fractionin thyroid tissue adjacent “TTA” to TN (two groups “adjacent to TBN” and “adjacent to TMN” combined).The ratios of means and the comparison of mean values of Al, B, Ba, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Znmass fractions in pair of sample groups such as normal thyroid tissue “NT” and “TTA”is presented in Table 4.

IV. DISCUSSION

As was shown before [33,34,50-55] good agreement of the Al, B, Ba, Br, Ca, Cl, Cu, Fe, I, K, Mg, Mn, Na, P, S, Sr, V, and Zn mass fractions in CRM IAEA H-4, INCT-SBF-4, INCT-TL-1, and INCT-MPH-2 samples determined by both INAA-SLR and ICP-AES methods with the certified data of these CRMs indicates acceptable accuracy of the results obtained in the study of “adjacent to TBN”, “adjacent to TMN”,“NT”, and “TTA”groups of thyroid tissue samples presented in Tables 1–4.From Table 2, it is observed that in thyroid tissue adjacent to TMN the mass fraction of I is47%higher,while mass fractionsCl and Na 42% and 29%, respectively, lower those in thyroid tissue adjacent to TBN. In a general sense Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, P, S, Si, Sr, V, and Zn contents found in the “adjacent to TBN” and “adjacent to TMN” groups of thyroid tissue samples were similar (Table 2). It allowed combine data obtained for two groups for the purposes of finding a common ChEs composition of TTA to TN and improving statistical characteristics of results for this group of samples (Table 3). From obtained results it was found that the common characteristics of thyroid tissue adjacent toTBN and TMN were elevated levels of Al, Cl, Cu, I, Na, S, and Si, which overdrew those in “normal” thyroid approximately in 2.1, 2.2, 2.2, 1.4, 1.4, 1.2,and 2.1 times, respectively (Table 4). Thus, if we accept the Ches contents in “normal” thyroid glands as a norm, we have to conclude that with a nodular transformation the Al, Cl, Cu, I, Na, S, and Si contents in thyroid intact tissue adjacent to TN significantly changed.

Characteristically, elevated or reduced levels of Ches observed in thyroid nodules are discussed in terms of their potential role in the initiation and promotion of these thyroid lesions. In other words, using the low or high levels of the Ches in affected thyroid tissues researchers try to determine the role of the deficiency or excess of each Ches in the etiology and pathogenesis of thyroid diseases. In our opinion, abnormal levels of some Ches in TN could be and cause, and also effect of thyroid tissue transformation. From the results of such kind studies, it is not always possible to decide whether the measured decrease or increase in Ches level in pathologically altered tissue is the reason for alterations or vice versa. According to our opinion, investigation of Ches contents in thyroid tissue adjacent to TN and comparison obtained results with Ches levels typical of “normal” thyroid gland may give additional useful information on the topic because these data show conditions of tissue in which TN were originatedand developed.

Aluminum: Al is the most widely distributed metal in the environment. Environmental media may be contaminated by Al from anthropogenic sources and through the weathering of rocks and minerals [58]. The trace element Al is not described as essential, because no biochemical function has been directly connected to it. Toxic actions of Al induce oxidative stress, immunologic alterations, genotoxicity, and other disorders, including cell membrane perturbation, apoptosis, necrosis and dysplasia [58]. Furthermore, it was shown in experimental and epidemiological studies that Al can affect thyroid iodide uptake and hormones secretion [59,60]. Thus, it is possible to assume that an excessive accumulation of Al in thyroid tissue is involved in TBN and TMN etiology.

Chlorine and sodium: Cl and Naare ubiquitous, extracellular electrolytes essential to more than one metabolic pathway. In the body, Cl and Na mostly present as sodium chloride. Therefore, as usual, there is a correlation between Na and Cl contents in tissues and fluids of human body. Because Cl is halogen like I, in the thyroid gland the biological behavior of chloride has to be similar to the biological behavior of iodide. The main source of natural Cl for human body is salt in food and chlorinated drinking water. Environment (air, water and food) polluted by artificial on organic Cl-contained compounds, for example such as sodium chlorate ($NaClO_3$), and organic Cl-contained compounds, for example such as polychlorinated biphenyls (PCBs) and dioxin, is other source. There is a clear association between using chlorinated drinking water, levels $NaClO_3$, PCBs and dioxin in environment and thyroid disorders, including cancer [61-65}. Thus, on the one hand, the accumulated data suggest that Cl level in thyroid tissue might be responsible for TMNs development. However, on the other hand, it is well known that Cl and Namass fractions in human tissue samples depend mainly on the extracellular water volume [66]. TN and thyroid tissuesadjacent to nodules can be more vascular zed than normal thyroid. Because blood isextracellular liquid, it is possible to speculate that more intensive vascularization could be the reason for elevated levels of Cl and Na in thyroid tissue adjacent to TB and TMN. If that is the only case, the equilibrium between Cl and Na increases has to be, however, in comparison with "normal" thyroid the change of Cl level in adjacent tissue is significantly higher than change of Na level. Thus, it is possible to assume that an excessive accumulation of Cl in thyroid tissue is involved in TBN and TMN etiology.

Copper: Cu is a ubiquitous element in the human body which plays many roles at different levels. Various Cu-enzymes (such as amine oxidize, ceruloplasmin, cytochrome-c oxidize, dopamine-mono oxygenize, extracellular superoxide dismutase, lysyl oxidase, peptidyl glycineami datingmon oxygenize, Cu/Znsuper oxide dismutase, and tyrosine's) mediate the effects of Cu deficiency or excess. Cu excess can have severe negative impacts. Cu generates oxygen radicals and many investigators have hypothesized that excess copper might cause cellular injury via an oxidative pathway, giving rise to enhanced lipid peroxidation, thiol oxidation, and, ultimately, DNA damage [67-69]. Thus, Cu accumulation in thyroid parenchyma with age may be involved in oxidative stress, dwindling gland function, and increasing risk of TBN and TMN [25,26,31-34]. The significantly elevated level of Cu in thyroid tissue adjacent to TBN and TMN, observed in the present study, supports this speculation. However, anover all comprehension of Cu homeostasis and physiology, which is not yet acquired, is mandatory to establish Cu exact role in TBN and TMN etiology and metabolism

Iodine: To date, it was well established that iodine excess has severe consequences on human health and associated with the presence of TBN and TMN [4-8,70-73]. In present study elevated level of I in thyroid tissue adjacent to TBN and TMN was found in comparison with "normal" thyroid. Thus, on the one hand, it is likely that elevated level of I in thyroid tissue might be involved in the TN origination and development. On the other hand, however, elevated level of I in thyroid tissue adjacent to TN may explain by unusually intensive work of this tissue. Compared to other soft tissues, the human thyroid gland has higher levels of I, because this element plays an important role in its normal functions, through the production of thyroid hormones (thyroxin and triiodothyronine) which are essential for cellular oxidation, growth, reproduction, and the activity of the central and autonomic nervous system. As was shown in our previous study, TBN and, particularly, TMN transformation of thyroid gland is accompanied by a significant loss of tissue-specific functional features, which leads to a significant reduction in I content associated with functional characteristics of the human thyroid tissue [42-47]. Because the affected part of gland reduced productions of thyroid hormones, the rest "intact" part of thyroid tries to compensate thyroid hormones deficiency and work more intensive than usual.

Sulfur: Proteins contain between 3 and 6% of sulfur amino acids. Sulfur amino acids contribute substantially to the maintenance and integrity of the cellular systems by influencing the cellular redox state and the capacity to detoxify toxic compounds, free radicals and reactive oxygen species (ROS) [74]. ROS are generated during normal cellular activity and may exist in excess in some pathophysiological conditions, such as inflammation.

Therefore exploring fundamental aspects of sulfur metabolism such as the antioxidant effects of sulfur-containing amino acids [75] may help elucidate the mechanism by which the S content increases in thyroid tissue adjacent to TN. Thus, it might be assumed that the elevated S level in thyroid tissue adjacent to TNreflects an increase in concentration of ROS in this tissue.

Silicon: Si is the second most common Che in the Earth's crust. This Che is naturally present in foods as minerals: Si dioxide (SiO_2) and silicates. In potable water Si is mainly present as free orthosilicic acid (H_4SiO_4) [76]. Si is mainly absorbed from the diet. For European population average dietary intake of Si from uncontaminated food and water was estimated as 20–50 mg/day [77]. In such doze Si exerts beneficial effects on the human health, because as was shown in many *in vitro* and *in vivo* studies this element is involved in collagen synthesis, bone mineralization, structural integrity of nails, hair and skin [76]. However, over the last five decades Si compounds began widely use in medicine, for example, as oral supplements for improving osteoporosis, hair loss, nail, hair, and skin quality [78,79], as well as Si-containing breast implants [80]. Silicones are included in cosmetic formulations such as hair conditioners, shampoos, and facial creams [79]. Si compounds are also of high importance in food and consumer products [81]. Due to favorable properties like colloidal and chemical stability, the possibility for a covalent surface modification, and a high specific surface area, the application cases for Si- contained particles and Si-based materials have grown rapidly [81]. Thus, as a result of the above the Si intake constantly increases. There are many evidences for harmful effects of excessive Si accumulation in body tissues. For example, it was shown that reactive oxygen species (ROS) are generated on surface of SiO_2 particles distributed in the interstitium [82]. In addition, SiO_2 particles interact with macrophages, which secrete various inflammatory cytokines [83]. The elevated levels of ROS and inflammatory cytokines produce progressive inflammation [83]. Chronic inflammation induced by Si compounds can lead to allergic reaction, deficient humoral immune system, autoimmune diseases, fatal organ dysfunction, fibrosis, benign tumors, anaplastic large T cell lymphoma, and cancer [80-83]. Thus, it is possible to assume that an excessive accumulation of Si in thyroid tissue is involved in TBN and TMN etiology.

Limitations: This study has several limitations. Firstly, analytical techniques employed in this study measure only nineteen Ches (Al, B, Ba, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn) mass fractions. Future studies should be directed toward using other analytical methods which will extend the list of Ches investigated in thyroid tissue adjacent to TN. Secondly, the sample size of TBN and TMN group was relatively small and prevented investigations of Ches contents in this group using differentials like gender, functional activity of nodules, stage of disease, and dietary habits of patients with TN. Lastly, generalization of our results may be limited to Russian population. Despite these limitations, this study provides evidence on some Ches level alteration in thyroid tissue adjacent to TN and shows the necessity to continue Ches research of TN.

V. CONCLUSION

In this work, Ches analysis was carried out in the thyroid tissue adjacent to TBN and TMN using a combination of non-destructive INAA-SLR and destructive ICP-AES methods. It was shown that this combination is an adequate analytical tool for the determination of nineteen Ches (Al, B, Ba, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn) content in the tissue samples of human thyroid in norm and pathology. I was found that in thyroid tissue adjacent to TMN the mass fraction of I is 47% higher, while mass fractions Cl and Na 42% and 29%, respectively, lower those in thyroid tissue adjacent to TBN. The common characteristics of thyroid tissue adjacent to TBN and TMN were elevated levels of Al, Cl, Cu, I, Na, S, and Si, which overdrew those in "normal" thyroid approximately in 2.1, 2.2, 2.2, 1.4, 1.4, 1.2, and 2.1 times, respectively, and similar contents of B, Ba, Ca, Fe, K, Li, Mg, Mn, P, Sr, V, and Zn. Thus, from results obtained, it was possible to conclude that the role of ChEs in etiology and pathogenesis TBN and TMN is similar and exessive accumulation of Al, Cl, Cu, I, Na, S, and Si in thyroid tissue may be involved in the TN origination and development.

ACKNOWLEDGEMENTS

The author is extremely grateful to Profs. VtyurinBM and Medvedev VS, Medical Radiological Research Center, Obninsk, as well as to Dr. Choporov Yu, former Head of the Forensic Medicine Department of City Hospital, Obninsk, for supplying thyroid samples.The author is also grateful to Dr. Karandaschev V, Dr. Nosenko S, and Moskvina I, Institute of Microelectronics Technology and High Purity Materials, Chernogolovka, Russia, for their help in ICP-AES analysis.

Funding: There were no any sources of funding that have supported this work.

Conflict of Interest: The author has not declared any conflict of interests.

REFERENCES

1. Verburg FA, Reiners C. The association between multinodular goiter and thyroid cancer. Minerva Endocrinol 2010;35(3):187-192.
2. Simsir IY, Cetinkalp S, Kabalak T. Review of factors contributing to nodular goiter and thyroid carcinoma. Med PrincPract 2020; 29(1):1–5.
3. Foster JR, Tinwell H, Melching-Kollmuss S. A review of species differences in the control of, and response to, chemical-induced thyroid hormone perturbations leading to thyroid cancer. Arch Toxicol 2021;95(3):807-836.
4. Barrea L, Gallo M, Ruggeri RM, Di Giacinto P, Sesti F, Prinzi N, Adinolfi V, Barucca V, Renzelli V, Muscogiuri G, Colao A, Baldelli R. Nutritional status and follicular-derived thyroid cancer: An update. Crit Rev Food SciNutr 2021;61(1):25-59.
5. Zaichick V. Iodine excess and thyroid cancer. J Trace Elem Exp Med 1998;11(4):508-509.
6. Zaichick V, Iljina T. Dietary iodine supplementation effect on the rat thyroid ^{131}I blastomogenic action. In: Die Bedentung der Mengen- und Spurenelemente. 18. Arbeitstangung. Jena: Friedrich-Schiller-Universität; 1998:294-306.
7. Kim K, Cho SW, Park YJ, Lee KE, Lee D-W, Park SK. Association between iodine intake, thyroid function, and papillary thyroid cancer: A case-control study. EndocrinolMetab (Seoul) 2021;36(4):790-799.
8. Vargas-UricoecheaP, Pinzón-Fernández MV, Bastidas-Sánchez BE, Jojoa-Tobar E, Ramírez-Bejarano LE, Murillo-Palacios J. Iodine status in the Colombian population and the impact of universal salt iodization: a double-edged sword? J NutrMetab 2019;2019:6239243.
9. Stojsavljević A, Rovčanin B, Krstić D, Borković-Mitić S, Paunović I, Diklić A, Gavrović-Jankulović M, Manojlović D. Risk assessment of toxic and essential trace metals on the thyroid health at the tissue level: The significance of lead and selenium for colloid goiter disease. Expo Health 2019.
10. Fahim YA, Sharaf NE, Hasani IW, Ragab EA, Abdelhakim HK. Assessment of thyroid function and oxidative stress state in foundry workers exposed to lead. J Health Pollut 2020;10(27):200903.
11. Liu M, Song J, Jiang Y, Lin Y, Peng J, Liang H, Wang C, Jiang J, Liu X, Wei W, Peng J, Liu S, Li Y, Xu N, Zhou D, Zhang Q, Zhang J. A case-control study on the association of mineral elements exposure and thyroid tumor and goiter. Ecotoxicol Environ Saf 2021;208:111615.
12. Zaichick V. Medical elementology as a new scientific discipline. J RadioanalNucl Chem 2006;269:303-309.
13. Moncayo R, Moncayo H. A post-publication analysis of the idealized upper reference value of 2.5 mIU/L for TSH: Time to support the thyroid axis with magnesium and iron especially in the setting of reproduction medicine. BBA Clin 2017;7:115-119.
14. Beyersmann D, Hartwig A. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol 2008;82(8):493-512.
15. Martinez-Zamudio R, Ha HC. Environmental epigenetics in metal exposure. Epigenetics 2011;6(7):820-827.
16. Zaĭchik V, RaibukhinYuS, Melnik AD, Cherkashin VI. Neutron-activation analysis in the study of the behavior of iodine in the organism. Med Radiol (Mosk) 1970;15(1):33-36.
17. Zaĭchik V, Matveenko EG, Vtiurin BM, Medvedev VS. Intrathyroid iodine in the diagnosis of thyroid cancer.VoprOnkol 1982;28(3):18-24.
18. Zaichick V, Tsyb AF, Vtyurin BM. Trace elements and thyroid cancer.Analyst1995;120(3):817-821.
19. Zaichick V, ChoporovYuYa. Determination of the natural level of human intra-thyroid iodine by instrumental neutron activation analysis. J RadioanalNucl Chem 1996;207(1):153-161.
20. Zaichick V. *In vivo* and *in vitro* application of energy-dispersive XRF in clinical investigations: experience and the future. J Trace ElemExp Med 1998;11(4):509-510.
21. Zaichick V, Zaichick S. Energy-dispersive X-ray fluorescence of iodine in thyroid puncture biopsy specimens. J Trace Microprobe Tech 1999;17(2):219-232.
22. Zaichick V. Relevance of, and potentiality for in vivo intrathyroidal iodine determination. Ann N Y AcadSci 2000;904:630-632.
23. Zaichick V, Zaichick S. Normal human intrathyroidaliodine. Sci Total Environ 1997;206(1):39-56.

24. Zaichick V. Human intrathyroidal iodine in health and non-thyroidal disease. In: New aspects of trace element research (Eds: M.Abdulla, M.Bost, S.Gamon, P.Arnaud, G.Chazot). London: Smith-Gordon; and Tokyo: Nishimura; 1999:114-119.
25. Zaichick V, Zaichick S. Age-related changes of some trace element contents in intact thyroid of females investigated by energy dispersive X-ray fluorescent analysis. Trends Geriatr Health 2017;1(1):31-38.
26. Zaichick V, Zaichick S. Age-related changes of some trace element contents in intact thyroid of males investigated by energy dispersive X-ray fluorescent analysis. MOJ GerontolGer 2017;1(5):00028.
27. Zaichick V, Zaichick S. Age-related changes of Br, Ca, Cl, I, K, Mg, Mn, and Na contents in intact thyroid of females investigated by neutron activation analysis. Curr Updates Aging 2017;1:5.1.
28. Zaichick V, Zaichick S. Age-related changes of Br, Ca, Cl, I, K, Mg, Mn, and Na contents in intact thyroid of males investigated by neutron activation analysis. J Aging Age Relat Dis 2017;1(1):1002.
29. Zaichick V, Zaichick S. Age-related changes of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in intact thyroid of females investigated by neutron activation analysis. J GerontolGeriatr Med 2017;3:015.
30. Zaichick V, Zaichick S. Age-related changes of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn contents in intact thyroid of males investigated by neutron activation analysis. Curr Trends Biomedical EngBiosci 2017;4(4):555644.
31. Zaichick V, Zaichick S. Effect of age on chemical element contents in female thyroid investigated by some nuclear analytical methods. MicroMedicine 2018;6(1):47-61.
32. Zaichick V, Zaichick S. Neutronactivationand X-ray fluorescent analysis in study of association between age and chemical element contents in thyroid of males. Op Acc J Bio Eng Bio Sci 2018;2(4):202-212.
33. Zaichick V, Zaichick S. Variation with age of chemical element contents in females' thyroids investigated by neutron activation analysis and inductively coupled plasma atomic emission spectrometry. J Biochem Analyt Stud 2018;3(1):1-10.
34. Zaichick V, Zaichick S. Association between age and twenty chemical element contents in intact thyroid of males. SM GerontolGeriatr Res 2018;2(1):1014.
35. Zaichick V, Zaichick S. Associations between age and 50 trace element contents and relationships in intact thyroid of males. Aging Clin Exp Res 2018;30(9):1059-1070.
36. Zaichick V, Zaichick S. Possible role of inadequate quantities of intra-thyroidal bromine, rubidium and zinc in the etiology of female subclinical hypothyroidism. EC Gynaecology 2018;7(3):107-115.
37. Zaichick V, Zaichick S. Possible role of inadequate quantities of intra-thyroidal bromine, calcium and magnesium in the etiology of female subclinical hypothyroidism. IntGyn and Women's Health 2018;1(3):IGWHC.MS.ID.000113.
38. Zaichick V, Zaichick S. Possible role of inadequate quantities of intra-thyroidal cobalt, rubidium and zinc in the etiology of female subclinical hypothyroidism. Womens Health Sci J 2018;2(1):000108.
39. Zaichick V, Zaichick S. Association between female subclinical hypothyroidism and inadequate quantities of some intra-thyroidal chemical elements investigated by X-ray fluorescence and neutron activation analysis. Gynaecology and Perinatology 2018;2(4):340-355.
40. Zaichick V, Zaichick S. Investigation of association between the high risk of female subclinical hypothyroidism and inadequate quantities of twenty intra-thyroidal chemical elements. Clin Res: GynecolObstet 2018;1(1):1-18.
41. Zaichick V, Zaichick S. Investigation of association between the high risk of female subclinical hypothyroidism and inadequate quantities of intra-thyroidal trace elements using neutron activation and inductively coupled plasma mass spectrometry. Acta Scientific Medical Sciences 2018;2(9):23-37.
42. Zaichick V. Evaluation of twenty chemical elements in thyroid with Hashimoto's thyroiditis using X-ray fluorescent and neutron activation analysis. Journal of Medical Research and Health Sciences 2021;2 (10):1500-1510.
43. Zaichick V. Determination of twenty chemical element contents in macro- and micro-follicular colloid goiter using neutron activation analysis and inductively coupled plasma atomic emission spectrometry.International Journal of Medical and All Body Health Research 2021;2(2):5-18.
44. Zaichick V. Comparison of twenty chemical element contents innormal thyroid tissue and hypertrophic thyroid tissue. Universal Journal of Pharmaceutical Research 2021;6(4):32-42.
45. Zaichick V. Evaluationof twenty chemical element contents in thyroid adenomas using neutron activation analysis and inductively coupled plasma atomic emission spectrometry**.** World Journal of Advanced Research and Reviews 2021;11(03):242-257.

46. Zaichick V. Are there any differences between chemical element contents of goitrous and adenomatous thyroid? Journal of Medical Research and Health Sciences 2021;4(11):1576-1587.
47. Zaichick V, Zaichick S. Levels of chemical element contents in thyroid as potential biomarkers for cancer diagnosis (a preliminary study). J Cancer Metastasis Treat 2018;4:60.
48. Zaichick V, Zaichick S. Instrumental effect on the contamination of biomedical samples in the course of sampling. The Journal of Analytical Chemistry 1996;51(12):1200-1205.
49. Zaichick V, Zaichick S. A search for losses of chemical elements during freeze-drying of biological materials. J RadioanalNucl Chem 1997;218(2):249-253.
50. Zaichick S, Zaichick V. INAA application in the age dynamics assessment of Br, Ca, Cl, K, Mg, Mn, and Na content in the normal human prostate. J RadioanalNucl Chem 2011;288:197-202.
51. Zaichick V, Zaichick S. The effect of age on Br, Ca, Cl, K, Mg, Mn, and Na mass fraction in pediatric and young adult prostate glands investigated by neutron activation analysis. J Appl Radiat Isot 2013;82:145-151.
52. Zaichick V, Nosenko S, Moskvina I. The effect of age on 12 chemical element contents in intact prostate of adult men investigated byinductively coupled plasma atomic emission spectrometry. Biol Trace Elem Res 2012;147:49-58.
53. Zaichick V, Zaichick S. NAA-SLR and ICP-AES Application in the assessment of mass fraction of 19 chemical elements in pediatric and young adult prostate glands. Biol Trace Elem Res 2013;156:357-366.
54. Zaichick V, Zaichick S. Determination of trace elements in adults and geriatric prostate combining neutron activation with inductively coupled plasma atomic emission spectrometry. Open Journal of Biochemistry 2014;1(2):16-33.
55. Zaichick S, Zaichick V. The effect of age and gender on 37 chemical element contents in scalp hair of healthy humans. Biol Trace Elem Res 2010;134(1):41-54.
56. Zaichick V. Applications of synthetic reference materials in the medical Radiological Research Centre. Fresenius J Anal Chem 1995;352:219-223.
57. Korelo AM, Zaichick V. Software to optimize the multielement INAA of medical and environmental samples. In: Activation Analysis in Environment Protection. Dubna, Russia: Joint Institute for Nuclear Research, 1993:326-332.
58. Igbokwe IO, Igwenagu E, Igbokwe NA. Aluminiumtoxicosis: a review of toxic actions and effects.InterdiscipToxicol 2019;12(2):45-70.
59. Orihuela D. Aluminium effects on thyroid gland function: iodide uptake, hormone biosynthesis and secretion. JInorg Biochem 2011;105(11):1464-1468.
60. Benvenga S, Elia G, Ragusa F, Paparo SR, Sturniolo MM, Ferrari SM, Antonelli A, Fallahi P. Endocrine disruptors and thyroid autoimmunity. Best Pract Res Clin EndocrinolMetab 2020;34(1):101377.
61. Leko MB, Gunjača I, Pleić N, Zemunik T. Environmental factors affecting thyroid-stimulating hormone and thyroid hormone levels. Int J MolSci 2021;22(12):6521.
62. Schwartz GG, Klug MG. Thyroid Cancer Incidence Rates in North Dakota are Associated with Land and Water Use.Int J Environ Res Public Health 2019;16(20):3805.
63. National Toxicology Program. Toxicology and carcinogenesis studies of sodium chlorate (Cas No. 7775-09-9) in F344/N rats and B6C3F1 mice (drinking water studies). Natl Toxicol Program Tech Rep Ser 2005;517:1-255.
64. Parazzini F, Esposito G, Tozzi L, Tozzi S. Epidemiology of endometriosis and its comorbidities. Eur J ObstetGynecolReprod Biol 2017;209:3-7.
65. Sokal A, Jarmakiewicz-Czaja S, Tabarkiewicz J, Filip R. Dietary intake of endocrine disrupting substances presents in environment and their impact on thyroid function. Nutrients 2021;13(3):867.
66. Zaichick V. X-ray fluorescence analysis of bromine for the estimation of extracellular water. J ApplRadiatIsot 1998;49(12):1165-1169.
67. Li Y, Trush MA. DNA damage resulting from the oxidation of hydroquinone by copper: role for a Cu(II)/Cu(I) redox cycle and reactive oxygen generation. Carcinogenesis 1993;14(7):1303-1311.
68. Becker TW, Krieger G, Witte I. DNA single and double strand breaks induced by aliphatic and aromatic aldehydes in combination with copper (II). FreeRadic Res 1996;24(5):325-332.
69. Glass GA, Stark AA. Promotion of glutathione-gamma-glutamyltranspeptidase-dependent lipid peroxidation by copper and ceruloplasmin: the requirement for iron and the effects of antioxidants and antioxidant enzymes. EnvironMolMutagen 1997;29(1):73-80.

70. Kant R, Davis A, Verma V. Thyroid nodules: Advances in evaluation and management. Am Fam Physician 2020;102(5):298-304.
71. Leung AM, Braverman LE. Consequences of excess iodine. Nat Rev Endocrinol 2014;10(3):136-142.
72. Lee J-H, Hwang Y, Song R-Y, Yi JW, Yu HW, Kim S-J, Chai YJ, ChoiJY, Lee KE, Park SK. Relationship between iodine levels and papillary thyroid carcinoma: A systematic review and meta-analysis.HeadNeck 2017;39(8):1711-1718.
73. Aakre I, Evensen LT, Kjellevold M, Dahl L, Henjum S, Alexander J, Madsen L, Markhus MW. Iodine status and thyroid function in a group of seaweed consumers in Norway. Nutrients 2020;12(11):3483.
74. Townsend DM, Tew KD, Tapiero H. Sulfur containing amino acids and human disease. BiomedPharmacother2004;58:47-55.
75. Atmaca G. Antioxidant effects of sulfur-containing amino acids. Yonsei Med J 2004;45:776-788.
76. Boqué N, Valls RM, Pedret A, Arola L, Puiggrós F, Solà R. Relative absorption of silicon from different formulations of dietary supplements: a pilot randomized, double-blind, crossover post-prandial study. SciRep 2021;11:16479
77. Pennington JAT. Silicon in foods and diets. Food AdditContam1991;8:97-118.
78. Rondanelli M, Faliva MA, Peroni G, Gasparri C, Perna S, Riva A, Petrangolini G, Tartara A. Silicon: A neglected micronutrient essential for bone health.Exp Biol Med (Maywood) 2021;246(13):1500-1511.
79. Michalak M, Pierzak M, Kręcisz B, Suliga E. Bioactive compounds for skin health: a review. Nutrients 2021;13(1):203.
80. Colaris MJL, de Boer M, van der Hulst RR, Tervaert JWC. Two hundreds cases of ASIA syndrome following silicone implants: a comparative study of 30 years and a review of current literature. ImmunolRes 2017;65(1):120-128.
81. Kersting M, Olejnik M, Rosenkranz N, Loza K, Breisch M, Rostek A, Westphal G, Bünger J, Ziegler N, Ludwig A, Köller M, Sengstock C, Epple M. Subtoxic cell responses to silica particles with different size and shape. Sci Rep 2020;10(1):21591.
82. Fubini B, Hubbard A. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by silica in inflammation and fibrosis. Free Radic Biol Med 2003;34(12):1507-1516.
83. Kawasaki H. A mechanistic review of silica-induced inhalation toxicity. InhalToxicol 2015;27(8):363-377.

Table 1.Some statistical parameters of Al, B, Ba, Ca, Cl, Cu, Fe,I, K,Li, Mg, Mn, Na, P,S, Si,Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in thyroid tissue adjacent to thyroid benign (TATBN) and malignant (TATMN) nodules

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
TATBN	Al	25.7	14.3	7.1	16.1	46.7	19.9	16.2	44.8
	B	1.70	0.61	0.35	1.00	2.10	2.00	1.05	2.09
	Ba	1.53	1.28	0.64	0.420	3.30	1.20	0.449	3.17
	Ca	1537	1700	380	418	6466	994	442	6312
	Cl	9203	6033	1384	2881	23731	8161	3294	22429
	Cu	10.2	7.9	4.0	3.60	20.4	8.35	3.65	19.8
	Fe	217	142	24	41.5	620	171	58.2	557
	I	2158	1436	214	343	7912	1917	527	5441
	K	6764	4054	864	3406	18255	5392	3500	18077
	Li	0.035	0.022	0.011	0.0175	0.0666	0.0279	0.0180	0.0640
	Mg	316	275	59	15.0	987	292	15.0	890
	Mn	1.78	1.65	0.36	0.100	5.83	1.10	0.100	5.67
	Na	10850	5541	1209	4663	31343	9642	5548	23981
	P	4361	1095	548	3176	5824	4222	3248	5711
	S	9689	1251	625	8071	11122	9781	8196	11024
	Si	135	65	33	87.1	228	112	87.5	221
	Sr	6.28	5.17	2.59	1.30	13.5	5.15	1.54	12.9
	V	0.170	0.116	0.067	0.079	0.300	0.130	0.082	0.292
	Zn	105	68	12	34.2	344	86.4	44.1	304
TATMN	Al	19.4	11.1	5.6	7.10	32.9	18.9	7.66	32.2
	B	5.80	9.60	4.80	1.00	20.2	1.00	1.00	18.8
	Ba	0.50	0.32	0.16	0.230	0.920	0.415	0.232	0.894

Ca	917	524	131	81.0	1909	726	204	1822
Cl	5339	2252	581	2526	11767	4922	2595	10201
Cu	8.08	3.15	1.58	4.90	12.1	7.65	5.01	11.9
Fe	256	133	26	109	752	254	112	591
I	3183	1673	301	563	8240	2982	853	7766
K	5847	2558	661	2097	13092	5429	2559	11220
Li	0.0210	0.0203	0.0100	0.0096	0.0514	0.0115	0.0097	0.0485
Mg	359	402	104	15.0	1412	247	15.0	1287
Mn	1.77	1.60	0.40	0.410	6.78	1.25	0.429	5.54
Na	7726	2519	630	3865	14373	7434	4250	13009
P	9724	5592	2796	4023	15979	9446	4181	15738
S	9725	1999	999	6989	11323	10293	7176	11306
Si	77.3	56.2	28.1	28.8	135	72.7	28.8	134
Sr	1.16	0.29	0.14	0.830	1.40	1.20	0.843	1.40
V	0.082	0.026	0.013	0.0630	0.120	0.0725	0.0636	0.117
Zn	111	55	11	20.4	272	107	28.8	215

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 2. Differences between mean values (M±SEM) of Al, B, Ba, Ca, Cl, Cu, Fe, I, K,Li, Mg, Mn, Na, P,S, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in thyroid tissue adjacent to thyroid benign (TATBN) and malignant (TATMN) nodules

Element	Thyroid tissue adjacent to thyroid nodules				Ratio
	TATBN	TATMN	Student's t-test *p*≤	U-test *p*	TATMN/TATBN
Al	25.7±7.1	19.4±5.6	0.517	>0.05	0.75
B	1.70±0.35	5.80±4.80	0.456	>0.05	3.41
Ba	1.53±0.64	0.50±0.16	0.204	>0.05	0.33
Ca	1537±380	917±131	0.137	>0.05	0.60
Cl	9203±1384	5339±581	**0.017**	**≤0.01**	0.58
Cu	10.2±4.0	8.08±1.58	0.648	>0.05	0.79
Fe	217±24	256±26	0.283	>0.05	1.18
I	2158±214	3183±301	**0.0074**	**≤0.01**	1.47
K	6764±864	5847±661	0.405	>0.05	0.86
Li	0.035±0.011	0.021±0.010	0.384	>0.05	0.60
Mg	316±59	359±104	0.719	>0.05	1.14
Mn	1.78±0.36	1.77±0.40	0.976	>0.05	0.99
Na	10850±1209	7726±630	**0.029**	**≤0.01**	0.71
P	4361±548	9724±2796	0.150	>0.05	2.23
S	9689±625	9725±999	0.977	>0.05	1.00
Si	135±33	77.3±28.1	0.232	>0.05	0.57
Sr	6.28±2.59	1.16±0.14	0.142	>0.05	0.18
V	0.170±0.067	0.082±0.013	0.318	>0.05	0.48
Zn	105±12	111±11	0.728	>0.05	1.06

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

Table 3. Some statistical parameters of Al, B, Ba, Ca, Cl, Cu, Fe, I, K, Li, Mg, Mn, Na, P,S, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis)in in thyroid tissue adjacent (TTA) to thyroid benign and malignant nodules (combined)

Tissue	Element	Mean	SD	SEM	Min	Max	Median	P 0.025	P 0.975
TTA	Al	22.5	12.3	4.3	7.10	46.7	19.9	8.41	44.3
	B	4.04	7.14	2.70	1.00	20.2	1.00	1.00	17.5
	Ba	1.01	1.03	0.36	0.23	3.30	0.685	0.235	3.00
	Ca	1261	1336	223	81.0	6466	918	367	6182
	Cl	7498	5079	871	2526	23731	5456	2688	21344
	Cu	9.13	5.68	2.01	3.60	20.4	7.65	3.72	19.0

Fe	233	138	18	41.5	752	191	68.3	583
I	2577	1608	184	343	8240	2420	554	7646
K	6393	3513	577	2097	18255	5392	3275	17950
Li	0.0280	0.0209	0.0070	0.0096	0.0666	0.0207	0.00972	0.0639
Mg	333	328	54	15.0	1412	289	15.0	1092
Mn	1.77	1.61	0.26	0.100	6.78	1.15	0.100	5.93
Na	9499	4708	774	3865	31343	8283	4583	18091
P	7042	4704	1663	3176	15979	5068	3324	15416
S	9707	1544	546	6989	11323	9781	7178	11288
Si	106	64	23	28.8	228	104	28.9	212
Sr	3.72	4.36	1.54	0.830	13.5	1.40	0.860	12.2
V	0.120	0.084	0.032	0.0630	0.300	0.079	0.0642	0.275
Zn	108	62	8.1	20.4	344	101	34.0	284

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. Differences between mean values (M±SEM) of Al, B, Ba, Ca, Cl, Cu, Fe,I, K, Li, Mg, Mn, Na, P, S, Si, Sr, V, and Zn mass fraction (mg/kg, dry mass basis) in normal thyroid (NT) and thyroid tissue adjacent to thyroid benign and malignant nodules (TTA)

Element	Thyroid tissue				Ratio
	NT	TTA	Student's t-test *p*≤	U-test *p*	TTA/NT
Al	10.5±1.8	22.5±4.3	**0.029**	**≤0.01**	2.14
B	0.476±0.058	4.04±2.70	0.235	>0.05	8.49
Ba	1.12±0.15	1.01±0.36	0.785	>0.05	0.90
Ca	1682±106	1261±223	0.094	>0.05	0.75
Cl	3400±174	7498±871	**0.000049**	**≤0.01**	2.21
Cu	4.08±0.14	9.13±2.01	**0.040**	**≤0.01**	2.24
Fe	223±10	233±18	0.603	>0.05	1.04
I	1841±107	2577±184	**0.00076**	**≤0.01**	1.40
K	6418±290	6393±577	0.969	>0.05	1.00
Li	0.0208±0.0022	0.0280±0.0070	0.381	>0.05	1.35
Mg	296±16	333±54	0.514	>0.05	1.13
Mn	1.28±0.07	1.77±0.26	0.079	>0.05	1.38
Na	6928±175	9499±774	**0.0024**	**≤0.01**	1.37
P	4290±207	7042±1663	0.143	>0.05	1.64
S	8259±263	9707±546	**0.037**	**≤0.01**	1.18
Si	50.8±6.2	106±23	**0.047**	**≤0.01**	2.09
Sr	3.81±0.34	3.72±1.54	0.952	>0.05	0.98
V	0.102±0.005	0.120±0.032	0.604	>0.05	1.18
Zn	94.8±4.2	108±8.1	0.159	>0.05	1.14

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