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Soil heavy metals in Dagestan Republic and human health risk assessment

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Introduction. *The comprehensive studies of heavy metal content in soils around the world are extremely important for the assessment and prediction of environmental risks due to their impact on human health. To prevent and restore heavy metal pollution in soil, source identification and risk assessment of heavy metals are requiring.*

Aims and objectives. *The purpose of the present work is to find the correlations (direct links) between human's health (endocrine diseases) and a heavy metal to prevent the endocrine diseases of the population.*

Materials and methods. *A total of six hundred fifty seven samples of agricultural soil were collected and five heavy metals (Zn, Cu, Mn, Co, and Pb), were analyzed for their concentrations, pollution levels and human health impact.*

Results. *A total of 657 surface soil samples (0–20 cm) from agricultural areas of Dagestan Republic, five kinds of metals (Zn, Co, Cu, Mn, and Pb) were analyzed.*

Limitations. *To check of the reliability of the results obtained on the relationship between the content of Zn, Co, Cu, Mn, Pb in soils and blood of the population with the prevalence of endocrine diseases (diabetes mellitus, endemic goiter) in the population, repeated studies are needed to expand the list of heavy elements, diseases of the population, and the geography of coverage of the study area.*

Conclusions. *The relationship of endocrine diseases with the concentration of heavy metals in the soils of the flat zone of Dagestan Republic (DR) and patient's blood was found. The level of MPC (maximum permissible concentration) for Mn was is revealed to be within (0.45–1.29), while for Pb as 0.5–0.0). The results of the present study showed that the concentrations of Co and Cu in the soils of DR are low than MPC.*

Keywords: *agricultural soil; endocrinology; heavy metals; health risk assessment; ecological risk index; geo-accumulation index.*

Compliance with ethical standards. *The present study does not require the presentation of a biomedical ethics committee conclusion or other documents.*

For citation: Abdulagatov I.M., Yahyaev M.A., Salikhov Sh.K., Karaeva A.F. Soil heavy metals in Dagestan Republic and human health risk assessment. *Gigiena i Sanitariya (Hygiene and Sanitation, Russian journal*). 2023; 102(2): 113-120. https://doi.org/10.47470/0016-9900-2023-102-2-113-120 https://elibrary.ru/huxbgs

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Conflict of interest. The authors declare no conflict of interest.
Acknowledgement. The study had no sponsorship.

Received: November 16, 2022 / Accepted: December 8, 2022 / Published: March 25, 2023

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Тяжёлые металлы в почвах Республики Дагестан и оценка риска для здоровья человека

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Введение. *Комплексные исследования содержания тяжёлых металлов в почвах по всему миру чрезвычайно важны для оценки и прогнозирования экологических рисков и их влияния на здоровье человека. Для предотвращения загрязнения почвы тяжёлыми металлами и её восстановления требуется выявление источника и оценка риска загрязнения тяжёлыми металлами.*

Цель работы *– найти корреляционные связи (прямые связи) между здоровьем человека (эндокринными заболеваниями) и тяжёлыми металлами в почве для профилактики эндокринных заболеваний населения.*

Материалы и методы. *Всего было собрано 657 проб сельскохозяйственной почвы, и пять тяжёлых металлов (Zn, Cu, Mn, Co и Pb) были проанализированы на предмет их концентрации, уровней загрязнения и воздействия на здоровье человека.*

Результаты. *Поверхностные пробы почвы (0–20 см) из сельскохозяйственных районов Республики Дагестан были проанализированы на содержание пяти видов металлов (Zn, Co, Cu, Mn и Pb). Выявлено их влияние на здоровье населения в этом районе.*

Ограничения исследования. *Для проверки достоверности и надёжности полученных результатов о связи содержания Zn, Co, Cu, Mn, Pb в почвах и крови населения с распространённостью эндокринных заболеваний (сахарный диабет, эндемический зоб) населения необходимы повторные исследования с расширением списка тяжёлых элементов, заболеваний населения, географии охвата территории исследований.*

Заключение. *Исследования показали, что для всех исследованных регионов индекс потенциального экологического риска значительно ниже предела экологического риска, < 150. Однако относительно высокий экологический риск имеет Хасавюртовский район = 19,2, за ним следует Бабаюртовский район (18,9). Кроме того, Pb имеет относительно высокий экологический риск = 6,9, а Mn — самый низкий риск = 0,81. Фактор токсического отклика на все исследованные тяжёлые металлы относительно низкий. Индекс геоаккумуляции для наиболее изученных районов ⩽ 0 (в пределах от –0,25 до –0,89), кроме Zn = 0,02 и = 0,04 для районов Хасавюрта и Бабаюрта соответственно. Настоящее исследование показывает, что чем меньше Zn и Cu в почве и больше Pb, тем выше число больных сахарным диабетом.*

***Ключевые слова*:** *сельскохозяйственная почва; эндокринология; тяжёлые металлы; оценка риска для здоровья; индекс экологического риска; индекс геоаккумуляции*

Соблюдение этических стандартов. *Настоящее исследование не требует представления заключения комитета по биомедицинской этике или иных документов.*

Для цитирования: Абдулагатов И.М., Яхияев М.А., Салихов Ш.К., Караева А.Ф. Тяжёлые металлы в почвах Республики Дагестан и оценка риска для здоровья человека. *Гигиена и санитария*. 2023; 102(2): 113–120. https://doi.org/10.47470/0016-9900-2023-102-2-113-120 https://elibrary.ru/huxbgs (in English)

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Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов в связи с публикацией данной статьи.

Финансирование. Исследование не имело спонсорской поддержки.

Поступила: 16.11.2022 / Принята к печати: 08.12.2022 / Опубликована: 25.03.2023

Introduction

Heavy metals (Zn, Co, Cu, Mn, and Pb) at low concentrations play an essential role in the growth and development of plants and animals. However, at concentrations higher than certain threshold levels its cause toxicity to the plants [1]. The toxicity in plants (or shortage of heavy metals) depends on many factors such as concentration of heavy metals in the soil, pH of the soil, *etc*. Plants growing at contaminated sites take up heavy metals from affected soil-water, and keep on accumulating its. These heavy metals in plants are increasing by of food chains which may affect human health [2]. An exposure, greater than their permissible limits, leads to diseases. Shortage or excess of trace elements in soils leads to their migration in the human body occurs along the chain: soil-water-food (plants and animals)-human to the emergence of diseases of the population. When heavy metals enter the soil, they not only reduce environmental quality but also endanger the health of people and other organisms through the food chain. The soil continuously receives a significant number of pollutants from different sources including heavy metals due to rapid industrialization and urbanization. Accurate quantification of the risk related by heavy metals is essential for developing effective risk management strategies to protect the urban environment. According to numerous studies, the anthropogenic activity such as agriculture, urbanization, and industrialization is one of the key factors in increasing the heavy metal content in soils. Agricultural practices tend to increase heavy metals (Cu, Pb, Cd and Zn) contents. The comprehensive studies of heavy metal content in soils around the world are extremely important for the assessment and prediction of environmental risks. Heavy metals are very toxic, unlike some organic pollutants, may accumulate in soils over time because they are hardly removed by natural degradation process and resistant to biochemical degradation, which can cause potential threat to human health. Considering its direct connection with ecosystem and food safety, heavy metal contents in agricultural soil has drawn growing academic and public concern worldwide [3]. In order to the prevention and restoration of heavy metal pollution in soil source identification and risk assessment of heavy metals are requiring. Most researches have generalized agricultural impact as a factor in the analysis of heavy metal sources [4]. Therefore, it is of great importance to study the heavy metal contents (pollution characteristics of soil) and their sources in agricultural soil to protect the environment and human health. The heavy elements, such as Cu, Fe and Zn, could play important roles in plants growth and human health, while some metals such as Hg, Cd and Pb are toxic for biological activity. Many diseases of the population have a geochemical nature of distribution. The elemental status of the organism is largely determined by the ecological and biogeochemical characteristics of a particular area. In our previous series publications [5–8] we found a wide spread of some types of diseases among the local population due to geochemical factors, namely, the concentration of heavy metals Zn, Co, Cu, Mn, Pb in the soils of the flat zone of Dagestan Republic. This is initiated the present study. Many other researchers studied the relationship between noninfectious diseases of the local population in the different regions of Russia and the level of metals in human blood [9]. Other researchers [10] point to the geochemical nature of the spread of endocrine diseases (disruption) in the population. For a better assessment of soil pollution of Russia and its impact on the population health it is necessary to increase the area of surveys, to unify approaches to select the pollution indicators and to develop regulatory standards dependent on land use. This is one of the reasons which motivated the present work.

Dagestan Republic (South of Russia) is ecologically less studied region in the Russian Federation. Therefore, the main goal of the present work is to study the geochemical role of the concentration of five key heavy metals (Zn, Cu, Co, Mn, Pb) in soils and human blood related with the spread of endocrine diseases (disruption) among the population in the flat zone of Dagestan Republic. The concentration of selected heavy metals in soils and human blood was assessed in the Dagestan Republic. Another purpose of the present work is to find the correlations (direct links) between human's health (endocrine diseases or any disruption) and a particular chemical, namely, heavy metals (Zn, Co, Cu, Mn, and Pb). Considering the correlation between the composition of the environment and the spread of diseases of the population, an attempt is made to establish a connection of the concentration of Zn, Co, Cu, Mn, and Pb in the soils of the Dagestan Republic with the prevalence of endocrine diseases of the population. The results obtained in this study can provide reference for the prevention and control of heavy metal pollution from the perspective of source.

Materials and methods

Characteristics of study area. The study area is located in the Dagestan Republic (flat zone of Dagestan, western shore of the Caspian Sea, Southwestern Russia, 43°06'N – 46°53'E, coastal plain between the mountains and the Caspian Sea), six districts

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Table 1 / Таблица 1

Region characteristics

Характеристики региона

Characteristics Характеристики	Region / Регион					
	Kizilyurt Кизилюрт	Khasavyurt Хасавюрт	Babayurt Бабаюрт	Kizlyar Кизляр	Tarum Тарум	Nogay Ногай
Population / Население	70 996	128 096	42 033	59 466	29 198	22 094
Number of sites / Количество участков	16	57	22	85	22	17
Area, km² / Площадь, км²	524	1423	3109	3047	3109	8871

(Kizilyurt, Khasavyurt, Babayurt, Kizlyar, Tarum, and Nogay), which has a population of more than 350 000 (see Table 1). A total of 657 topsoil samples (0–20 cm depth) were collected from agricultural fields in the study area. The total study area was divided into six parts (regions) with different size (see Table 1), and the sites were selected at the different location of each regions. All samples sites were located in cultivated land in plains. At each sampling site, 6–10 subsamples of topsoil (0–20 cm) within 50–100 m radius were collected.

The area has an average annual temperature of 12.9 °C, and an annual average rainfall is about 250 mm (for Kizilyurt, Khasavyurt, Kizlyar, and Tarum), 138 mm for Babayurt and 99 mm Nogay regions. The prevailing wind comes from the Caspian Sea (East side), and the annual average wind speed is within (1.7 to 3.8) $m \cdot c^{-1}$. The relative air humidity is (72 to 74)%. Previous studies showed that the agricultural soil of Dagestan was greatly affected by atmospheric deposition [ссылка]. The samples collection was performed in the summer time (dry weather season). The main crops in this area are (rice, corn, wheat), vegetables (potatoes, cabbage, onion, beets, etc.), fruits (apricot, cherry, plum, apple, grape, pears, quince, etc.). All the samples were collected a few miles away from the main Federal Highway (Moscow–Baku) to eliminate the interference of traffic. The study area is the north part of the Dagestan Republic with a total area of about 17299 km² (see Table 1). The plain is covered by marine sediments, saline swamp soils are common, rolling sandy plain of the Nogay steppe, and mainly consist of sand, clay and sandy clay. The soil types in the study area are mainly paddy soil. It is predominantly flat region, with elevations approximately within of –15 m to 15 m. The area has a traditional mix of urban land uses, basically including residential and commercial areas. There are smaller-scale industrial activities in the regions. This region is commonly known for its rich natural surface geothermal springs (about 24 wells), Kizlyar (geothermal wells No. 6 and No. 17T).

The database of the Medical Information and Analytical Center of the Department of Health of the Dagestan Republic (MI & AC DH) has been used for the present study (Health Measures Survey [11]). Figure 1 shows the spread of endocrinology diseases among the population (per 1000 population) in the flat regions of Dagestan Republic according to the Health Measures Survey [11]).

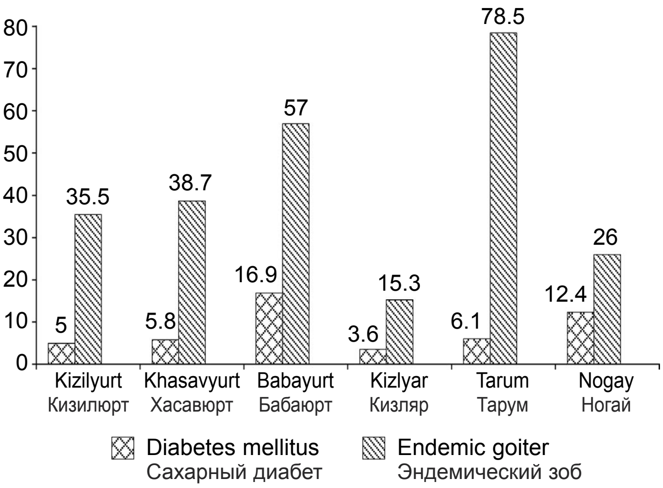
As one can see from figure, endemic goiter diseases are most common in the Tarum and in Babaurt regions, 78.5 and 57 patients per 1000 population, respectively. Relative low spreading of diabetes mellitus was observed in the Baburt (16.9 patients per 1000 population) and Nogay (12.4 patients per 1000 population) regions. All chemicals used in the present work and their purities were provided by Aldrich.

Soil sampling: samples collections, preparation and analysis. Soil samples were collected from different locations (657 sites), belonging to 6 distinct lands (Kizilyurt, Khasavyurt, Babayurt, Kizlyar, Tarum, and Nogay) of the Dagestan Republic using a wood spade by means of a completely randomized design, on the basis of sizes of agricultural area, waste discharging, irrigative water and the prevailing wind direction to assess the human activity on soil quality. Each main soil sample, consisted of 10–15 sub-samples, were randomly taken from the surroundings of each site, pooled and homogenized to form a representative sample and at least 500 m away from industry, traffic and residential areas.

The elemental composition of soils was determined at the biogeochemistry laboratory of the Pre-Caspian Institute of Biological Resources of the Dagestan Federal Research Centre of the Russian Academy of Sciences. All collected soil samples were air-dried at room temperature (20–23 °C), the roots, stones, plant materials and other debris were carefully removed before analysis according the requirements ISO-11464 [12], then passed through 2 mm polyethylene sieve. To analyze the soil samples with the mass of 0.2 g were selected. The unseeded soil lumps were rubbed and sifted again, then a sample was taken for analysis. The prepared soil samples were then stored in polyethylene bottles for analysis. These prepared samples were analyzed for pH and five main key metals Zn, Co, Cu, Mn, and Pb. Determination of mobile forms of the metals in soil was carried out by extraction of metals using 1 M hydrochloric acid. The extract was passed through a dry folded ash free (<0.01%) filter (ТУ 2642-001-68085491-2011) with 150 mm pore size (diameter) and average density and porosity. The obtained filtrates were used to metals determination. At the same time, we performed standard (control or reference) analysis. Soil pH was determined with the supernatants of water-soil ratio of 2.5:1 using a pH meter. The soil reaction ranged from (8.1 to 8.9) pH (an average value of pH is 8.5), see below Table 5.

Study population. The study population included the adults (age above 40 years old) living in the area under study (Dagestan Republic), which was diverse in term of the type of living environment, sex (50% males and 50% females), education and occupation (serving agriculture).

Blood sampling and analysis. Population-based biomonitoring is an efficient tool for characterizing the body burden of metals,



The spread of endocrine diseases among the population (per 1000 population) in the flat regions of the Dagestan Republic (Health Measures Survey [11]).

Распространение эндокринологических заболеваний среди населения на равнинных площадях Дагестана на 1000 населения.

https://doi.org/10.47470/0016-9900-2023-102-2-113-120

Table 2 / Таблица 2

Summary statistics of the heavy metals concentrations (mg/kg) in the agricultural soils of Dagestan Republic

Сводная статистика содержания тяжёлых металлов (мг/кг) в сельскохозяйственных почвах Республики Дагестан

Metals / Металл	Region / Регион	Parameters / Параметры						
		number of samples / число образцов / *n*	Min	среднее / Mean	Max	standard deviation / стандартное отклонение / SD	standard error / стандартная ошибка / SE	coefficient of variation / коэффициент вариации / CV
Zn	Kizilyurt / Кизилюрт	48	0.89	1.83	2.24	0.04	0.006	2.19
	Khasavyurt / Хасавюрт	171	0.67	1.72	2.38	0.13	0.001	7.56
	Babayurt / Бабаюрт	66	0.91	1.68	1.87	0.04	0.005	2.38
	Kizlyar / Кизляр	255	1.32	1.97	2.32	0.07	0.004	3.55
	Tarum / Тарум	66	0.93	1.48	1.84	0.11	0.014	7.43
	Nogay / Ногай	51	0.47	0.64	1.78	0.09	0.013	14.06
Cu	Kizilyurt / Кизилюрт	48	0.78	0.91	1.61	0.09	0.013	9.89
	Khasavyurt / Хасавюрт	171	0.52	0.71	1.14	0.04	0.003	5.63
	Babayurt / Бабаюрт	66	0.48	0.65	1.09	0.06	0.007	9.23
	Kizlyar / Кизляр	255	0.59	0.73	1.23	0.03	0.002	4.11
	Tarum / Тарум	66	0.1	0.45	0.63	0.04	0.005	8.89
	Nogay / Ногай	51	0.3	0.57	0.81	0.07	0.010	12.28
Mn	Kizilyurt / Кизилюрт	48	89	119	232	2.9	0.42	2.44
	Khasavyurt / Хасавюрт	171	149	167	247	3.3	0.25	1.98
	Babayurt / Бабаюрт	66	162	181	209	3.8	0.47	2.10
	Kizlyar / Кизляр	255	143	155	174	3.6	0.23	2.32
	Tarum / Тарум	66	131	156	170	4.2	0.52	2.69
	Nogay / Ногай	51	20	63	91	3.6	0.50	5.71
Co	Kizilyurt / Кизилюрт	48	0.64	0.83	1.08	0.09	0.013	10.84
	Khasavyurt / Хасавюрт	171	0.58	0.71	1.24	0.06	0.005	8.45
	Babayurt / Бабаюрт	66	0.49	0.67	0.85	0.09	0.011	13.43
	Kizlyar / Кизляр	255	0.32	0.48	0.71	0.03	0.002	6.25
	Tarum / Тарум	66	0.24	0.33	0.59	0.03	0.004	9.09
	Nogay / Ногай	51	0.18	0.23	0.64	0.04	0.006	17.39
Pb	Kizilyurt / Кизилюрт	48	2.5	3.1	4.3	0.9	0.130	29.03
	Khasavyurt / Хасавюрт	171	2.8	3.5	5.2	1.2	0.091	34.29
	Babayurt / Бабаюрт	66	3.5	4.2	5.0	0.9	0.111	21.43
	Kizlyar / Кизляр	255	2.5	3.7	4.9	1.8	0.113	48.65
	Tarum / Тарум	66	2.9	3.9	4.4	0.9	0.111	23.08
	Nogay / Ногай	51	2.8	3.6	4.7	1.2	0.168	33.33

toxics and nutrients as well. It is important for those metals with relevant health effects and that are inadequately controlled. Thus, data from a biomonitoring test can show where stronger environmental actions or medical decisions are required.

Blood was obtained in clinical laboratories of medical institutions from 10 patients in each studied area, prone to diabetes and endemic goiter. Whole blood samples were taken from the elbow vein (venous blood), in the morning, on an empty stomach (fasting). The samples were collected in vacuum containers Vacuette 456084 ("Greiner Bio-One GmbH", Austria) and stored in refrigerator until analysis in a frozen state. For determination of Zn, Co, Cu, Mn, and Pb concentrations in the blood, 0.2% aqueous solution of Triton X-100 and 0.2% solution of Antifoam-B were prepared for dilution whole blood. Equal amounts of whole blood and the prepared solution (5 cm^3) were combined, the resulting mixture was vigorously stirred for 30 seconds to obtain a complete dissolution of blood cell elements. The sample was centrifuged and a clean supernatant layer was analyzed. A solution of ammonium acid phosphate, $(NH_4)_3PO_4$, was prepared. For the purpose 2 g of this substance (supernatant) was dissolved in 100 cm^3 of distilled water. 1% solution of AMPC (ammonium – pyrrolidine – dithiocarbamate) was prepared in distilled water. This reagent was prepared daily. 5 cm^3 of AMPC solution was mixed with 100 cm^3 of ammonium acid phosphate solution. The solution was shaken in a separating funnel with 10 cm^3 MIBK (Methyl Isobutyl Ketone) for 2–3 minutes. The water layer was removed and the organic layer was separated. The extraction process was repeated a few times. The extracted compound is a modifier for the determination of trace elements in the blood, allowing to raise the temperature of ash and was stored in a polyethylene tube. Determination of the content of heavy metals in the blood was carried out according to the manual of the separating procedure, considering that 5 μl of the obtained modifier should be added to the analyzed sample. The metal content in the analyzed sample is calculated using a simple relation $x = kC$, where C is the metal content in the analyzed blood sample from the calibration curve or using calibration coefficients, mg/dm^3; and k is the coefficient considering dilution or concentration.

Table 3 / Таблица 3

Comparison of the median heavy metals' concentrations (mg/kg) for the present studied regions with the other regions of Russia

Сравнение медианных концентраций тяжёлых металлов (мг/кг) для исследуемых регионов с другими регионами России

Region / Регион	Metals / Металл					Reference / Источник
	Zn	Cu	Mn	Co	Pb	
Flat zone of the Dagestan Republic / Равнинная зона Республики Дагестан	1.31	0.91	0.92	1.14	1.18	This study / Данная работа
Volgograd Region / Волгоградская область	0.64	0.23	16.20	0.04	–	O.V. Sukhova et al. / О.В. Сухова и др. [13]
Krasnoyarsk Region / Красноярская область	0.53	0.24	6.10	0.08	–	A.P. Sergeev et al. / А.П. Сергеев [14]
Belgorod Region / Белгородская область	0.49	0.14	10.9	0.13	–	S.V. Lukin / С.В. Лукин [15]
Republic of Karelia / Республика Карелия	0.50	0.32	13.03	0.23	–	G.V. Akhmetova / Г.В. Ахметова [16]
Kemerovo Region / Кемеровская область	0.43	0.17	33.92	0.22	–	O.I. Prosyannikova and V.I. Prosyannikov [17] О.И. Просянникова и В.И. Просянников [17]
Sakhalin Lowland / Сахалинская низменность	3.40	0.26	4.26	–	0.70	E.A. Zharikova / Е.А. Жарикова [18]
Kabardino-Balcaria Republic / Кабардино-Балкарская Республика	7.50	1.00	23.0	–	2.7	C.M. Beslaneev et al. / С.М. Бесланеев и др. [19]

Heavy metals and whole blood concentrations measurements. The metal (Zn, Co, Cu, Mn, and Pb) concentrations in soils and whole blood were determined using atomic absorption spectroscopy with a graphite atomizer (Zeeman modulation polarization spectrometry, AAS-ETA Hitachi 170–70, Japan) in a high purity (99.9999 wt%) argon medium and a tubular graphite cuvette 170–5100 D. The concentration of metals was determined by comparing the analyzed solution with standard solutions Zn, Co, Cu, Mn, and Pb with a concentration of 1 mg/cm^3 using the calibration procedure. The uncertainty of a concentration measurements is less than 1% at the p=95% confidence level.

Statistical analyses. For all measured soil characteristic properties were assigned basic statistical parameters such as maximum and minimum, arithmetic mean, standard error (*SE*), standard deviation (SD), coefficient of variation (CV), and Pearson correlation coefficient. Statistical analyses of the results (type of sample distribution using the Shapiro–Wilk test, *t*-statistics, and Pearson linear correlation coefficient, Pearson and Altman and Gardner, were performed using the Statistic 6.0 application software package (StatSoft Inc., USA). The critical level of statistical significance was taken at $p \leqslant 0.05$.

Results

Summary statistics of heavy metals concentration (mg/kg) of the soils under study for each studied districts of the flat zone of Dagestan Republic are given in Table 2.

The highest content elements in the studied areas are Mn and Pb. Compared with the background value in Dagestan Republic, the average and median Cu and Co concentration in the agricultural soil were lower, while those of Mn, Pb, and Zn were much higher. However, in compared with the other industrial regions of Russia (see Table 3), the average concentrations of Co and Cu in Dagestan soil is higher. Also, the concentration of Zn was higher than in Volgograd, Krasnoyarsk, Belgorod, Karelia, and Kemerovo, although the Mn content is lower (Table 3) than in other industrial areas of Russia. In general, the agricultural soil heavy metal contents in this area did not exceed the risk MPC values. Table 3 shows the comparison of the heavy metals' distribution in the present studied area with other regions of the Russian Federation.

The concentration of Zn, Co, Cu, Mn, and Pb in the soils of the flat zone of Dagestan Republic is varying in the wide range (mg/kg): Zn – from (0,47 to 2,4); Cu – from (0,10 to 1,61); Mn – from (20 to 247); Co – from (0,18 to 1,24); and Pb – from (2,5 to 5,2) of soil (Table 4). The highest values of Zn, Cu, Mn, Co, and Pb concentration were found in Kizlyar (1,97), Kizilyurt (0,91), Babayurt (181), Kizilyurt (0,83), and Babayurt (4,2), respectively.

Table 4 / Таблица 4

The level of metal cations (mg/kg) in the soils of flat zone of Dagestan Republic

Уровень содержания катионов металлов (мг/кг) в почвах равнинной зоны Республики Дагестан

Region / Регион	Deptha* / Глубина *	n	Humus, % / Гумус, %	pH	Metals / Металл				
					Zn	Cu	Mn	Co	Pb
Kizilyurt / Кизилюрт	A	48	3.87	8.6	1.83	0.91	119	0.83	3.1
	B		1.79	8.5	1.48	0.68	158	0.79	2. 9
	C	10	0.95	8.7	1.31	0.96	172	0.70	2.7
Khasavyurt / Хасавюрт	A	171	3.39	8.1	1.72	0.71	167	0.71	3.5
	B		1.74	8.3	1.41	0.64	170	0.68	3.1
	C	10	0.81	8.4	1.03	0.76	187	0.64	2.4
Babayurt / Бабаюрт	A	66	3.42	8.4	1.68	0.65	181	0.67	4.2
	B		1.87	8.5	1.46	0.68	142	0.62	3.9
	C	10	0.76	8.8	1.02	0.79	183	0.54	3.3
Kizlyar / Кизляр	A	255	2.76	8.3	1.97	0.73	155	0.48	3.7
	B		1.49	8.5	1.73	0.67	167	0.44	3.5
	C	10	0.85	8.6	1.47	0.85	181	0.42	3.2
Tarum / Тарум	A	66	2.47	8.3	1.48	0.45	156	0.33	3.9
	B		1.53	8.5	1.21	0.51	162	0.30	3.6
	C	10	0.99	8.6	1.09	0.57	170	0.28	3.4
Nogay / Ногай	A	51	2.15	8.2	0.64	0.57	63	0.23	3.6
	B		1.34	8.4	0.55	0.52	57	0.28	3.5
	C	10	0.54	8.9	0.58	0.44	55	0.22	3.2

Note. * A – top layer, mostly humus, 0–25 cm; B – middle layer, clay and oxidized material, 76 cm; C – bottom layer, parent rock, loose rock and other mineral materials, 122 cm, are the genetic horizons.

Примечание. * A – верхний слой, в основном гумус, 0–25 см; B – средний слой, глина и окисленный материал, 76 см; C – нижний слой, материнская порода, рыхлая порода и другие минеральные материалы, 122 см, генетические горизонты.

https://doi.org/10.47470/0016-9900-2023-102-2-113-120

Table 5 / Таблица 5

Summary of heavy metal concentrations (mg/kg) together with standard deviations for the soils collected from flat regions of Dagestan Republic, $M \pm m$

Сводная концентрация тяжёлых металлов (мг/кг) вместе со стандартными отклонениями для почв, собранных в равнинных районах Республики Дагестан, $M \pm m$

Region Регион	Humus, % Гумус, %	pH	Metals / Металл				
			Zn	Cu	Mn	Co	Pb
Kizilyurt / Кизилюрт	3.87 ± 0.2	8.6 ± 0.2	1.83 ± 0.1	0.91 ± 0.03	119 ± 0.9	0.83 ± 0.03	3.1 ± 0.3
Khasavyurt / Хасавюрт	3.39 ± 0.3	8.1 ± 0.3	1.72 ± 0.3	0.71 ± 0.01	167 ± 1.0	0.71 ± 0.02	3.5 ± 0.4
Babayurt / Бабаюрт	3.42 ± 0.2	8.4 ± 0.2	1.68 ± 0.1	0.65 ± 0.02	181 ± 1.2	0.67 ± 0.03	4.2 ± 0.3
Kizlyar / Кизляр	2.76 ± 0.5	8.3 ± 0.4	1.97 ± 0.2	0.73 ± 0.01	155 ± 1.1	0.48 ± 0.01	3.7 ± 0.6
Tarum / Тарум	2.47 ± 0.2	8.3 ± 0.2	1.48 ± 0.3	0.45 ± 0.01	156 ± 1.3	0.33 ± 0.01	3.9 ± 0.3
Nogay / Ногай	2.15 ± 0.2	8.2 ± 0.3	0.64 ± 0.3	0.57 ± 0.02	63 ± 1.1	0.23 ± 0.02	3.6 ± 0.4
Ka [20] (Clark number, the relative abundance of a chemical element in Earth's crust / Число Кларка, относительная распространённость химического элемента в земной коре)			5	1.5	40	1	10
MPC / ПДК [21] (maximum permissible concentration / предельно допустимая концентрация)			23	5	140	5	6

Discussion

The concentration analysis of the studied heavy metals shows that their average values in the soils of the areas are lower than the Clark number (Ka) reported by Vinogradov [20]: Zn (2,5) – 8 times; Cu (1,6) – 3; and Co (1,2) in 4 times, while the concentration of Mn (1,5) in 4,5 times higher than Clark number Ka. Studies by Alexandrova et al. [21] show different levels and criteria for assessing the ecological status of soils, depending on the concentration of the elements under consideration. According to the data reported by Alexandrova et al. [21], the soils studied in the present work contain low concentration of Cu and Co, while Zn is at the level of the lower limit of the maximum permissible concentration of Zn. However, the concentrations of Mn and Pb are higher than MPC (the excepted values are 0,45–1,29 for Mn and 0,5–0,7 for Pb). The lower the Zn, Cu, Co and the more Mn contents in the soil, relative to the Clark coefficient (Ka) and MPC, the greater the population morbidity (sickness rate), i.e., in general, increase of the Mn and Pb content in the soil leads to the sickness rate increases, while the increasing of Zn, Cu, Co content leads to it decreases.

To assess the contamination levels of heavy metals, the pollution index (*PI*) was calculated $PI = C_i / C_{bi}$, where C_i is the concentration of the metal element *i* in topsoil (0–20 cm) samples, while C_{bi} is the background value of the target element. The derived values of *PI* for each individual heavy metals under study are presented in Table 6, where $PI<1$ is no contamination, $1<PI\leqslant 3$ is slight contamination. As one can see from Table 6, the *PI* values for Zn, Cu, Mn, Co, and Pb ranged from (1,02 to 1,54), (0,82 to 1,24), (0,81 to 1,09), (1,09 to 1,16), and (1,10 to 1,38), respectively. This indicates that, on average, heavy metal pollution for most studied regions is at a relatively low or slight level. However, the percentages of sampling locations exhibiting *PI* values larger than 1 were found to be: (2–54)% for Zn, 24% for Cu, 9% for Mn, (10–19)% for Co, (10–38)% for Pb, which indicate significant local accumulation of heavy metal pollutants within the study area.

Accordingly, the ecological risk index E_{ir} can be used to assess the risk of metals based on their toxicity response in the environment. The Hakanson potential ecological risk index E_{ir} was calculated using equation $E_{ir} = T_r^i C_i / C_{bi}$ and $RI = \sum_{i=1}^{m} E_{ir}$, where E_{ir} is the individual potential ecological risk index of heavy metal *i*, T_r^i is the toxic response factor of heavy metal *i*, C_i is the average content for heavy metal *I* (the present results, see above), and C_{bi} is the geochemical background reference value in soil, *RI* is the sum of the potential ecological risk of metals in the region, The toxic response factors of heavy metals studied in the present work are: Zn =Mn= 1 and Co =Cu = Pb = 5. The combined ecological risk (sum of the potential ecological risk, *RI*) of the studied metals are: Kizilyurt (17.8), Khasavyurt (19.2), Babayurt (18.9), Kizlyar (17.6), Tarum (17.37), and Nogay (19.4), As one can see, risk assessment showed that for all studied regions considerably lower than low ecological risk limit, $E_{ir}<150$. However, Khasavyurt area has the relatively high ecological risk $RI=19.2$, followed by Babayurt area (18.9). Also, Pb has the relative highest ecological risk $E_{ir}=6.9$ (Khasavyurt area), while Mn has the lowest risk $E_{ir}=0.81$ (Kizilyurt area).

The geoaccumulation index (I_{geo}) also evaluates the pollution level of metals in soils using the relation $I_{geo} = \log_2[C_i / (1.5 \cdot C_{bi})]$. The constant 1,5 is used due to potential variations in the baseline data. For $I_{geo}\leqslant 0$, the soil is practically uncontaminated, $0<I_{geo}<1$ – uncontaminated to moderately contaminated, $1<I_{geo}<2$ – moderately contaminated, and $2<I_{geo}<3$ – moderately to heavily contaminated. For the present results the most data is $I_{geo}\leqslant 0$ (within -0,25 to -0,89, soil is practically uncontaminated), except $I_{geo}=0{,}02$ and $I_{geo}=0{,}04$ for Zn, for regions of Khasavyurt and Babayurt, respectively.

Effects of metal content in the soil on human health. Under conditions of environmental distress, the immune, endocrine and central nervous systems react before other systems, causing a wide range of functional disorders. Then there are metabolic disorders and mechanisms of formation of environmentally dependent pathological process. In the present work, we have studied the

Table 6 / Таблица 6

The values of *PI* (Pollution Index) for heavy metals for studied

Значения индекса загрязнения (ИЗ) тяжёлыми металлами для изучаемых регионов

Region Регион	Metals / Металл				
	Zn	Cu	Mn	Co	Pb
Kizilyurt / Кизилюрт	1.26	0.83	0.81	1.16	1.15
Khasavyurt / Хасавюрт	1.52	0.89	0.90	1.09	1.38
Babayurt / Бабаюрт	1.54	0.84	0.88	1.19	1.23
Kizlyar / Кизляр	1.26	0.82	0.89	1.10	1.13
Tarum / Тарум	1.23	0.84	0.94	1.13	1.10
Nogay / Ногай	1.02	1.24	1.09	1.16	1.11

Table 7 / Таблица 7

Level of metal cations in blood (µg/L) of the studied population of the flat zone of Dagestan Republic

Уровень катионов металлов (мкг/л) в крови исследуемого населения равнинной зоны Республики Дагестан

Region / Регион	Metals / Металл: Zn	Cu	Mn	Co	Pb
	diabetes mellitus / endemic goiter (сахарный диабет / эндемический зоб)				
Kizilyurt / Кизилюрт	4110 / 4032	635 / 623	33.2 / 34.31	2.21 / 2.19	233 / 228
Khasavyurt / Хасавюрт	4078 / 4084	605 / 598	34.8 /35.28	1.74 / 1.79	249 / 239
Babayurt / Бабаюрт	3748 / 3752	557 / 534	37.3 / 36.41	1.98 / 1.85	272 / 251
Kizlyar / Кизляр	4184 / 4261	654 / 637	37.6 / 35.95	2.41 / 2.44	267 / 244
Tarum / Тарум	3945 / 4059	595 / 551	35.8 / 36.43	2.15 / 2.11	248 / 262
Nogay / Ногай	3814 / 3818	570 / 653	34.4 / 33.84	2.05 / 2.21	255 / 216
WHO [22] – The reference level in the blood Стандартный уровень в крови (ВОЗ) [22]	7000	1400	12	10	150

relationship of some endocrine (diabetes, endemic goiter) diseases of the local population with a concentration of Zn, Co, Cu, Mn, and Pb in the soils of the region and blood of patients. We compared the average concentration of trace elements in soils and the number of patients with pathologies. As a result, we have found a direct relationship of diseases of endocrinology with a concentration of Zn, Co, Cu, Mn, and Pb in the soils. Particularly, the following regularity was revealed: the lower Zn, Co, Cu and higher Mn, Pb relative to Clark number (Ka), according to Vinogradov [20], in soils, the more patients with the studied pathologies.

Using the Shapiro–Wilk criterion, it was determined that the sampling has a normal distribution. In this regard, the Pearson coefficient of linear correlation of metal content in soils and blood of patients with human endocrine pathology (disruption) was determined. Student's *t*-test (statistics) evaluation indicate the statistically significant differences (where $p<0,05$ was considered to be significant) in the compared samplings. Morbidity of the population with diabetes mellitus depended on the food content grown from biomass of cultivated plants on soils. For Pb the average strength of correlation coefficient is r=+0,48, while for Zn and Cu are r=-0,47 (average) and r=-0,31 (weak), respectively. Therefore, this implies the conclusion: the less of Zn and Cu in soils, and more Pb, the higher of the number of patients with diabetes. The concentration of Co and Mn did not have a significant effect on the pathology. The number of patients with endemic goiter depended on the concentration of Cu in soils, with the average strength of correlation coefficient (r =-0,57), while for Mn and Pb is (r=+0,43). The difference in the content of the studied metals in the whole blood of patients exposed to diabetes and endemic goiter was revealed (see Table 7).

As can be note, a statistically significant positive correlation with the studied pathologies was found for Mn and Pb and negative for Zn, Cu, Co (see Table 8).

Table 8 / Таблица 8

The coefficient of correlation between pathology and the concentration of the heavy elements

Коэффициент корреляции между патологией и концентрацией тяжёлых элементов

Parameter / Параметр	Correlation coefficient / Коэффициент корреляции: Soil / Почва	Blood / Кровь
Zn ↔ diabetes mellitus / Zn ↔ сахарный диабет	–0.468	–0.939
Zn ↔ endemic goiter / Zn ↔ эндемический зоб	+0.007	–0.288
Co ↔ diabetes mellitus / Co ↔ сахарный диабет	–0.130	–0.385
Co ↔ endemic goiter / Co ↔ эндемический зоб	–0.060	–0.500
Cu ↔ diabetes mellitus / Cu ↔ сахарный диабет	–0.314	–0.895
Cu ↔ endemic goiter / Cu ↔ эндемический зоб	–0.566	–0.875
Mn ↔ diabetes mellitus / Mn ↔ сахарный диабет	–0.060	+0.219
Mn ↔ endemic goiter / Mn ↔ эндемический зоб	+0.425	+0.566
Pb ↔ diabetes mellitus / Pb ↔ сахарный диабет	+0.613	+0.526
Pb ↔ endemic goiter / Pb ↔ эндемический зоб	+0.495	+0.713

Conclusions

In order to determine the concentrations and health risk of heavy metals in urban soils from the Dagestan Republic were collected and analyzed for Zn, Co, Cu, Mn, and Pb. A total of 657 surface soil samples (0–20 cm) from agricultural areas of Dagestan Republic, five kind of metals (Zn, Co, Cu, Mn, and Pb) were analyzed. $The concentration of metals in the soils of the flat zone of Dagestan has a wide range (mg/kg): Zn – from (0,47 to 2,4); Cu – from (0,10 to 1,61); Mn – from (20 to 247); Co – from (0,18 to 1,24); and Pb – from (2,5 to 5,2). The highest Zn, Cu, Mn, Co, and Pb concentrations were found in Kizlyar (1,97), Kizilyurt (0,91), Babayurt (181), Kizilyurt (0,83), and Babayurt (4,2), respectively. The relationship of some diseases of endocrinology with the concentration of Zn, Cu, Mn, Co, and Pb in the soils of the flat zone of Dagestan Republic and patient's blood was found. It is revealed that the level of MPC (maximum permissible concentration) for Mn was within (0,45–1,29), while for Pb is (0,5–0,7). According to the ecological risk assessment, a large proportion of the sampling sites presented no ecological risk (unpolluted). The results of the present study showed that the concentrations of Co and Cu in the soils of Dagestan republic are low than MPC. A risk assessment showed that for all studied regions considerably lower than low ecological risk limit, $E_{ir}<150$. However, Khasavyurt area has the relatively high ecological risk RI=19,2, followed by Babayurt area (18,9). Also, Pb has the relative highest ecological risk E_{ir}=6,9 (Khasavyurt area), while Mn has the lowest risk E_{ir}=0,81 (Kizilyurt area). Toxic response factor for all studied heavy metals relatively low, The geo-accumulation index (I_{geo}) for the most studied areas $I_{geo}\leqslant 0$ (within -0,25 to -0,89, soil is practically uncontaminated), except I_{geo}=0,02 and I_{geo}=0,04 for Zn, for regions of Khasavyurt and Babayurt, respectively. The conclusions suggested that Zn, Cu, Co, Mn and Pb were no pollution. The results of the risk analysis indicated that the heavy metal contents in the soils of flat zone of Dagestan Republic have a low ecological risk (unpolluted) in most of the study sites.

The present study showed that morbidity of the local population with diabetes mellitus depended on the food content grown from biomass of cultivated plants on soils. The less of Zn and Cu in soils, and more Pb, the higher of the number of patients with diabetes. The concentration of Co and Mn did not have a significant effect on the pathology. The difference in the content of the studied metals in the whole blood of patients exposed to endocrine diseases was revealed, for which a statistically significant correlation with pathologies was also found – positive for Mn and Pb and negative for Zn, Cu, Co. We showed the increase of the number of patients with pathologies in excess of Mn, Pb concentration and a decrease in Zn, Cu, Co. Increases of Pb content in the soil is a result of increasing sickness rate of population. The concentration of Co and Mn did not have a significant effect on the pathology. There was an increase in the number of patients with pathologies in excess of Mn, Pb concentration and decrease of Zn, Cu, Co in the soils. The number of patients with endemic goiter depended on the concentration of Cu in soils, with the average strength of correlation coefficient (r =-0,57), while for Mn and Pb is (r =+0,43). The difference in the content of the studied metals in the whole blood of patients exposed to diabetes and endemic goiter was revealed. To improve the health of the population, it is necessary to eliminate the influence of excess Mn and Pb and the shortage of Zn, Cu, Co in the humans, by improving the ecology of the environment. The present study may provide a scientific basis for strategies to protect human health in urban areas. The quality guidelines and standards, enforcing existed regional environmental regulations and laws, and conducting holistic environmental monitoring programs should be immediately acted. To improve the health of the population, it is necessary to eliminate the influence of the factors and restore the balance of trace elements in the human body. Optimize the ratio of trace elements is one of the factors for improving the ecology of the environment and its food.

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(п.п. 1–4, 11, 12, 22 см. References)

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