ORIGINAL ARTICLE

PHYSICO-CHEMICAL PARAMETERS USED FOR SOIL CHARACTERIZATION AND POLLUTION EVALUATION IN RACOS VOLCANO AREA, BRAȘOV, ROMANIA

LILIANA AVASILCAI [1#], OANA CIOANCA [1*], BRANCO-ADRIAN MORARIU [1], IONUT IULIAN LUNGU [1], MADALINA VIERIU [1#], CORNELIU TANASE [2], SILVIA ROBU [3#], MONICA HANCIANU [1], IONELA-DANIELA MORARIU [1]

[1]Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy Iaşi, Romania
[2]Faculty of Pharmacy, "George Emil Palade" University of Medicine, Pharmacy, Science and Technology of Târgu Mureş, Romania
[3]Faculty of Medicine and Pharmacy, "Dunărea de Jos" University of Galaţi, Romania

*corresponding author: oana.cioanca@umfiasi.ro
#Authors with equal contribution.

Abstract

The purpose of the study was the physico-chemical characterization of the soil from the extinguished volcano situated in Racoş area, from Braşov, Romania. The characterization of the soil was done in order to study the degree of soil pollution and to evaluate the regenerative power of nature in the volcano area. Soil health can be assessed through the analysed physico-chemical parameters, pH, density, humidity, ammoniacal nitrogen, organic nitrogen and albuminoid-telluric nitrogen, chlorides, organic substances, nitrates, nitrates and metal ions. The results of the determinations of the physico-chemical parameters showed that no pollutants were present in alarming levels in the analysed soil samples, which would explain the corresponding regeneration of the spontaneous flora in the volcanic area.

Rezumat

Scopul studiului a constat în caracterizarea fizico-chimică a solului din vulcanul stins situat în zona Racoş, din Braşov, România. Caracteristicile solului au fost analizate pentru a evalua gradul de poluare al acestuia, o dată cu urmărirea capacităţii de regenerare a vegetaţiei din zona vulcanică. Sănătatea solului poate fi apreciată prin parametrii fizico-chimici, pH, densitate, umiditate, azot amoniacal, azot organic şi azot albuminoid-teluric, cloruri, substanţe organice, nitraţi, nitraţi şi ioni metalici. Rezultatele determinărilor parametrilor fizico-chimici au arătat că nu au fost prezenţi poluanţi în niveluri alarmante în probele de sol analizate, ceea ce ar explica regenerarea corespunzătoare a florei spontane din zona vulcanică.

Keywords: volcano soil, organic nitrogen, albuminoid-telluric nitrogen, nitrates and metal ions, contamination evaluation

Introduction

"Racoşul de Jos" complex represents a geological area of national importance, with an area of 95.2 ha, integrated in the European Natura 2000 avifauna site since 2007.

The purpose of the study was the physical and chemical characterization of the soil from the extinguished volcano situated in Racoş area, Braşov, Romania, in order to study the degree of soil pollution in the area, due to a volcanic slag quarry that closed down in the 1990s and agriculture. Our study may be useful in asserting the regenerative power of nature in the volcano area. Soil health can be assessed through various physico-chemical parameters, plus the bio-chemical parameters and the pollution indicators [1, 6, 7, 15, 26].

One must take into account the fact that the vegetation has developed in an area seemingly poor in nutrients that was dominated by volcanic soils formed during the last eruption. Moreover, the soil contamination represents an important factor for the vegetal plant material that is intended for human and animal consumption, or for therapeutical use. Pollutants such as heavy metals, pesticides and drug residues that may contaminate the plants have usually high transfer rates in various extracts, and thus induce health risks for the consumer [9, 16, 18, 19]. Higher soil contamination is usually correlated with higher levels of contaminant both in edible and medicinal plants [5, 9, 18, 21].

Materials and Methods

In order to determine the physical and chemical parameters, soil samples have been collected from a depth of 0 - 25 cm of a 250 m^2 area that included the volcanic crater and natural pasture. To assess the appearance and density of the soil, the samples were dried at room temperature, after passing through a 2 mm

sieve. The organic substances, chlorides, ammonia, nitrites, nitrates, calcium, magnesium, iron and copper were subsequently determined using spectrophotometric and titrimetric methods; other parameters (humidity, pH, ammoniacal nitrogen, organic nitrogen and albuminoid-telluric nitrogen) were determined directly on soil samples [8].

In order to determine the density, the volume occupied by 50 g of soil collected from the pasture, and from the crater, respectively was measured and subsequently the ratio m/V as g/cm^3 was calculated.

Titrimetric methods were applied to determine the concentration of chlorides, organic substances, calcium and magnesium. The determination of chlorides in the aqueous soil extract was carried out using the Mohr method [2, 8]. Chloride concentration levels below 300 mg/L required the titration of the organic substances in acidic, hot environment, with 0.01 N potassium permanganate solution. The concentrations of calcium and magnesium ions were determined complexonometrically, by two successive titrations: the first for Ca^{2+} ions at pH = 12 - 13 and the second for the determination of the sum of Ca^{2+} and Mg^{2+} ions (at pH = 10) [6, 8, 10, 17].

Spectrophotometric methods have been applied to determine ammonia, nitrites, nitrates, iron and copper content. The ammonia present in the aqueous soil extract was treated with Nessler reagent in the presence of KOH and the yellow reaction product was analysed spectrophotometrically at λ = 430 nm [20, 22, 25]. The nitrites were treated with sulphanilic acid, in acidic medium, and the resulting diazonium salt was coupled with α-naphthylamine, forming a red azo compound with λ_{max} = 520 nm. Nitrates were analysed in anhydrous medium using phenoldisulfonic acid and the resulting yellow nitrophenoldisulfonic acid, was spectrophotometrically quantified at λ = 410 nm in the presence of ammonia. The spectrophotometric determination of iron was performed separately for Fe^{2+} and Fe^{3+}. Ortho-phenanthroline formed with Fe^{2+} ions a red-orange complex with λ_{max} = 510 nm. The assay of Fe^{3+} ions was carried out similarly, after reduction with hydroxylamine hydrochloride. The Cu^{2+} ions were treated with sodium diethyldithiocarbamate, at pH = 9 - 10. The resulting yellow complex was extracted in chloroform, and then it was spectro-photometrically quantified at λ = 436 nm [8].

The results were expressed as mg analyte/100 g soil or mg analyte/L aqueous soil extract, except for the concentration of oxidizable organic substances, which was expressed as mg [O]/100 g soil or mg [O]/L aqueous soil extract [20].

The soil moisture was determined gravimetrically, by drying the soil sample in the oven, at 105°C, until constant mass, and the results were expressed as percentages.

The soil pH was measured after the soil sample was dried at room temperature. It was then treated with 0.1 N potassium chloride solution, stirred and then left to rest for one hour. The pH of the supernatant was measures using a pH-meter.

The ammonia or ammoniacal nitrogen from the soil results from the self-purification processes that include the mineralization of organic substances that contain nitrogen. NH_3 was distilled in the presence of magnesium oxide and then it was retained in a standardized solution of sulfuric acid. The results were expressed as mg NH_3/100 g soil.

Various organic substances present in the soil (amines, amides, urea, fulvic substances, humic substances, etc.) might have contained nitrogen. The Kjeldahl method was used for their quantitative analysis. The soil sample was mineralized using concentrated H_2SO_4 and heat. After wet mineralization, the resulting gaseous ammonia was retained using 0.1 N H_2SO_4 solution. The excess sulfuric acid was back-titrated using 0.1 N NaOH solution. The organic nitrogen, expressed as mg NH_3 *per* 100 g soil, was calculated by subtracting the ammoniacal nitrogen determined previously [26].

Albuminoid-telluric nitrogen represents the nitrogen incorporated in the soil as its natural component and it was also determined through the Kjeldahl method. Substances containing protein nitrogen were precipitated using $Cu(OH)_2$, separated through filtration and mineralized with concentrated H_2SO_4. Organic nitrogen was transformed into ammonia, which was separated by distillation in alkaline medium and captured in 0.1 N H_2SO_4 solution. The excess sulfuric acid was back-titrated with 0.1 N NaOH solution. The result was expressed as mg NH_3/100 g soil [27].

The 10 soil samples collected from the volcanic crater and the 10 soil samples collected from the pasture were analysed in three replicates, and the mean results were calculated.

Results and Discussion

The soil collected from the crater was darker in colour, with dark brown hues to black, the particles were less adherent to each other and denser (ρ = 1.1904 g/cm^3), which caused faster sedimentation in the preparation step of the aqueous soil extract. The soil harvested from the pasture appeared in shades of ochre, reddish-brown to dark brown. The powder component was in a higher proportion, which required a longer time for sedimentation (ρ = 0.9259 g/cm^3) compared to the soil harvested from the crater.

The soil samples, whether harvested from the crater (pH = 6.02) or from the pasture (pH = 5.87), were weakly acidic and, according to the data of the National Institute of Research and Development for Paedology, Agronomy and Environmental Protection of Bucharest, Romania, they fell in the category of fertile soils, for which values between 5 and 7 pH units were characteristic [29].

The humidity of pasture soil samples (4.834%) was higher than that of volcanic crater soil samples (3.264%). That might have been a consequence of the fact that the particle size of pasture soil was smaller than that of crater soil, which allowed a more compact settlement, favourable for moisture retention. In addition, the vegetation, much more abundant in the pasture than in the crater, ensured a slower evaporation of water from the soil.

Ammoniacal nitrogen, organic nitrogen and albuminoid-telluric nitrogen (Table I) characterize the soil from the point of view of its fertility, while the ratio between albuminoid-telluric nitrogen and organic nitrogen is called the soil health number and provides information on the degree of soil impurity [29].

Table I

Determinations of various nitrogen forms

Parameter (mg NH_3/100 g soil)	Crater soil	Pasture soil
Ammoniacal N	20.725 ± 4.265	21.839 ± 0.312
Organic N	776.971 ± 27.033	478.231 ± 31.600
Albuminoid-telluric N	4.1955 ± 0.373	141.924 ± 0.842
Soil health number	0.0054 ± 0.000138	0.2967 ± 0.00645

It was noticed that the ammoniacal nitrogen concentrations had been almost the same in both soil samples, while the concentration of organic nitrogen was about 1.62 times higher in the crater soil compared to the concentration of the same parameter in the soil samples collected from the pasture. The concentration of albuminoid-telluric nitrogen in the pasture soil was approximately 34 times higher than in the crater soil. That explained the values of soil health number that were statistically distinct and significant for crater soil when compared to pasture soil. The data from the literature indicated that a value lower than 0.70 for the sanitary number of the soil, indicated a very contaminated soil. At the macroscopic level, those laboratory results were confirmed by the much more abundant pasture vegetation compared to the crater.

Chlorides, organic substances, ammonia, nitrates, nitrates and metal ions (calcium, magnesium, iron, copper) were determined from the aqueous soil extract (prepared in a 1:5 mass ratio of soil and water). Table II, Table III, Figure 4 and Figure 5, show comparatively the equations of the standard curves and the values of those parameters.

Table II

Characteristics of calibration curves for the determinations of soil chemical parameters

Parameter	Calibration curve equation	R^2
Ammonia	A = 0.0028C + 0.0006	0.9863
Nitrites	A = 0.0996C − 0.0070	0.9996
Nitrates	A = 0.0065C + 0.0053	0.9929
Iron	A = 0.0136C + 0.0048	0.9946
Cooper	A = 0.4277C − 0.0065	0.9913

Table III

Determinations on aqueous soil extracts

| Parameter (mg/100 g soil)	Crater soil	Pasture soil
Chlorides	8.04 ± 0.64	8.80 ± 0.17
Organic substances (as [O])	2.92 ± 0.09	9.02 ± 0.54
Ammonia	0.35 ± 0.115	0.47 ± 0.018
Nitrites	0.53 ± 0.063	0.25 ± 0.012
Nitrates	0.90 ± 0.058	2.05 ± 0.131

The presence of metal ions in soil may have beneficial effects on vegetation development, or may have toxic effects on soil biology by affecting the biochemical processes that occur in the soil, depending on their type [5, 16]. Interestingly, recent research on spices and medicinal plants from the South-East region of Romania indicated that wild-growing plants found near industrial areas can accumulate iron and copper in variable amounts, in certain cases the values exceeding the limits imposed within EU [9, 16]. Therefore, a proper assessment and monitoring of the soil would greatly increase the quality of the edible and medicinal plants.

The pollution of the environment with heavy metals, including copper, is a major problem worldwide [9, 16, 18, 21, 23]. Enzymatic reactions are inhibited in soil microbiota by metals through several mechanisms, such as: complexation of the substrate, combination with the active protein groups of the enzymes and reaction with the enzyme-substrate complex. Some ions may act as activators or co-factors, and at certain concentrations the activity of some enzymes may increase (*e.g.*, Mg, Ca, Ba, Co, Ni, Zn and Mn for
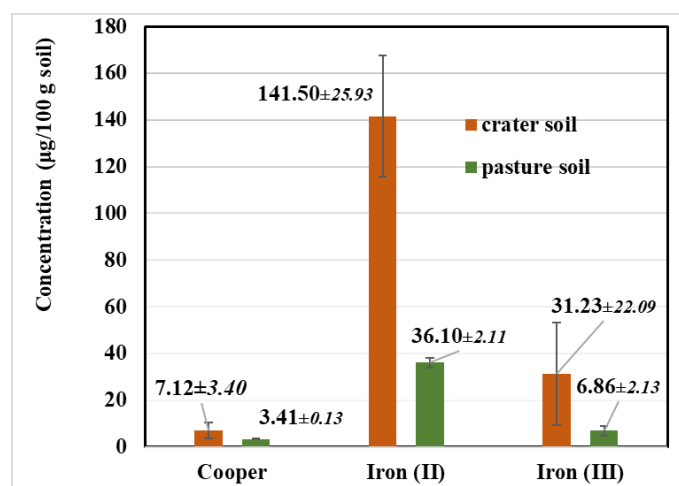
pyrophosphatase) [26, 32]. Metals can react with sulphide groups of enzymes causing inactivation or inhibition of enzyme activity. That process is influenced by the pH of the soil, in that the acidification of the soil reinforces the negative effect of heavy metal contamination [28].
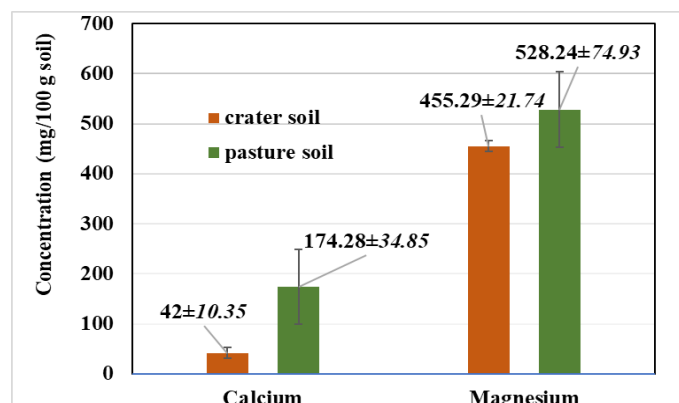
Figure 1.
Concentrations of calcium and magnesium ions in soil samples

Figure 2.
Concentrations of iron and copper ions in soil samples

Increasing levels of heavy metals such as Cr, Cu, Co, Cd and Pb in soil causes deterioration of soil health [13]. Although some metals from the soil are absorbed by plants as they are essential for their metabolism (*e.g.* Cu, Co, Cr), by accumulation in plants in concentrations exceeding safety levels in food and/or feed for animals, they represent a health hazard [29, 30]. At the same time, the content of heavy metals in soil and crops depends on the physico-chemical properties of the soil, cultivation technologies, the availability, type and solubility of metals in the soil, as well as the type of plant [4].

A recent study in China investigated the pollution, health risk and sources of heavy metals in volcanic agricultural soil samples and ranked copper as the main polluting element after nickel and chromium. Natural sources contributed the most to the accumulation of heavy metals in soil, followed by agricultural activities, emissions from everyday life and vehicles, and industrial activities. The high concentration of heavy metals in soil has been correlated with the composition of volcanic agricultural soils containing minerals rich in aluminium, Fe and Mn oxides, as well as organic carbon [31].

The results of the determinations of the physico-chemical parameters showed that no pollutants (organic substances, ammonia, nitrates, nitrates, chlorides, toxic metals - copper) were present in alarming levels in the soil samples analysed. On the contrary, the ratio between organic nitrogen and albuminoid nitrogen indicated that the soil was very strongly affected by the anthropic factor, probably due to the intense exploitation of the volcanic slag that took place until 2005.

The presence of calcium, magnesium, iron and copper ions revealed the mineral component of the soil. Higher calcium and magnesium concentrations in grassland soil compared to crater soil suggested the higher nutritive potential of the former, with favourable consequences for biotopes in the area of the extinguished

volcano from Racoș area, Romania. The presence of iron in higher concentrations in the soil of the volcano crater compared to the grassland soil could be correlated with the completely different colour of the two soil types: red-brick for the crater soil, and dark grey to black for the pasture soil. The low concentrations of copper and chlorides proved the absence of such pollutants in the natural reserve, declared as protected area by Government Decree no. 1581 from December 8th, 2005, published in The Official Journal of Romania no. 24 from January 11th, 2006 that established the regime of natural area for new areas.

From the obtained data we could notice quite a low content of the analytes determined from the aqueous soil extract, with the particularity that the level of organic substances, ammonia and nitrates were about 3 times, 1.34 times, and 2.27 times higher, respectively in pasture soil than in crater soil. In contrast, nitrites were found in crater soil, in double concentration than in pasture soil. That fact could be due to the mineralization process of the organic substances that was more intense in crater soil compared to pasture soil as the vegetation was better developed. Soil samples collected from pasture had a higher calcium and magnesium content than soil collected from the crater. Those concentrations were favourable for vegetation development because those ions enter the metabolism of plants, magnesium being essential in the synthesis of chlorophyll. The concentrations of iron and copper were much lower compared to those of calcium and magnesium, but were mainly found in the crater soil (in concentrations 3 times higher, in the case of Fe^{3+} ions, and 4 times higher for Fe^{2+} ions respectively, compared to the concentrations the same ions in pasture soil). That could explain the multitude of shades of crater soil (which was one of the tourist attractions) due to the specific colours of the ferrous and ferric compounds.

Geological studies proved that the Racoș volcano represents the oldest volcanic phase in the south-east of the Carpathian-Pannonic region, on the North-Western periphery of Brașov intramountain basin [1, 24]. The basaltic rocks and slag were investigated in terms of their mineral composition, but also in terms of their content of macro- and trace elements, when minerals from the silicate group were identified: olivine ($Mg_2Fe_2SiO_4$) and clinopyroxenes ($X_2Si_2O_6$, where X represents Na, Li, Mg, Ca, Fe, Mn, Ti, Zn or Al), as well as spinel ($MgAl_2O_4$, a mineral from the category of aluminium oxides). Although those minerals have a similar composition in which the macro- and trace elements are present in the form of oxides, they present different crystallization systems and have different hardness levels [11]. Those results were in accordance with those obtained in the quantitative determination of calcium, magnesium and iron from our study of the soil from the volcanic crater and from the neighbouring pasture, thus suggesting the inclusion

of basaltic rocks and slag in the soil formation process in the area.

A recent study traced the influence of climatic factors on pedogenesis, starting from the premise that soils formed from volcanic ash cover about 124 million hectares, about 1% of the Earth's surface, of which 60% in tropical areas [3]. Local environmental conditions control pedogenesis through a unique combination of factors and processes, especially in areas with rapid environmental change (such as the tropics), but the formation of volcanic ash soils follows similar patterns in different latitudinal zones. For that reason, we noticed a certain similarity between the mineralogical and implicitly chemical composition of the volcanic soil in the Racoș area and that of the tropical volcanic soil, characterized by the presence of olivine and pyroxenes alongside apatite and feldspars; consequently, large amounts of iron, but also Na, P, K, along with lower levels of Ca and Mg were found. An important aspect in volcanology is the chemical composition of underground waters. A study carried out in the Călimani-Gurghiu-Harghita volcanic chain area highlighted that the groundwater in the Racoș area, (latitude 46°02′24.78′′′′, longitude 25°24′45.96′′′′ and altitude 472 m), had pH = 6.52 [12]. The pH values determined both in the soil of the volcanic crater (pH = 6.02) and in the pasture soil (pH = 5.87) supported the statement that the waters and the soils they are in contact with have similar characteristics. The volcanic area is naturally characterized by high concentrations of heavy metals in environmental elements, which can degrade the quality of water, air, soil and food, endangering human health due to their persistence, high toxicity and bioavailability [26]. Following the research carried out by Yang *et al*. on the samples of volcanic soil turned into farmland (collected from depths within 1 - 20 cm), the concentrations of eight heavy metals and oxides were determined, including copper, whose concentration was 33.43 mg/kg soil. The level of copper concentrations determined in our study were approximately 980 times lower in pasture soil (3.41 μg/100 g soil), and 470 times lower in the soil collected from the volcanic crater (7.12 μg/100 g soil). Those differences can be explained on account of the different destinations of the volcanic soils: the high copper concentration levels from the volcanic soil in China could be the consequence of repeated agricultural treatments applied to crops, while the area of the inactive volcano Racoș has been a natural reserve since 2005, and slag mining ended more than 30 years ago.

Specialists in the field noticed similarities between the appearance of the Racoș volcano and that of the Etna volcano, where the same type of rocks and colours were identified. A recent study was initiated in the area of the Etna volcano, on its Southern flank, from where samples of "Ghiara" (a particular aggregate) were collected in different outcrops of the volcano,

whose distinctive feature is the exclusive presence under the solidified lava flows [14]. If in the past, the material was used in construction for its reddish hue and its ability to chemically react with calcium hydroxide, for the same reasons it has now aroused the interest of the scientific community involved in the formulation and production of ecological construction materials as an alternative to the traditional ones. From the chemical composition point of view the presence of calcium, magnesium and iron oxides (hematite - Fe_2O_3) responsible for the red, red-brown and grey shades, along with TiO_2, SiO_2, MnO, Al_2O_3, was found. If the architecture of Catania (Sicily, Italy) is characterized by the historical use of volcanic materials as blocks for masonry or aggregates for the production of mortars and plasters with shades tending to black and red, the first settlements of the village of Racoș were built of pumice stone and volcanic tuff; a testimony in that sense is the Sükösd-Bethlen Castle, Brașov, Romania, built in 1664, which represents one of the tourist attractions of the Racoș area.

Conclusions

The characterization of the soil in Racoș area – Brașov, Romania, through the assay of physical and chemical parameters was done in order to study the degree of soil pollution in the area and to evaluate the regenerative power of nature in the volcano area.

Soil quality depends largely on its natural composition, but the application of proper management through human interventions can produce its improvement. The fact that the Racoș area has become a protected area creates the premises for improving the health of the soil through its natural regeneration mechanisms.

Conflict of interest

The authors declare no conflict of interest.

References

1. Avasilcai L, Bireescu G, Vieriu M, Bibire N, Crivoi F, Cioanca O, Morariu I, Study on the Enzymatic Activity of the Soil in Racos Protected Area from Brasov. *Rev Chim.*, 2020; 71(3): 358-366.
2. Avasilcai L, Teliban G, Morariu DI, Stoleru V, Bibire N, Vieriu M, Panainte AD, Munteanu N, Parameters of Chemical Composition of *Phaseolus coccineus* L. Pods Grown in Protected Areas. *Rev Chim.*, 2017; 68(12): 2955-2958.
3. Barbosa AM, Francelino MR, Thomazini A, Schaefer CEGR, Anjos LHC, Pereira MG, Lyra GB, The thermal regime and mineralogical attributes of highland volcanic-ash soils from the Cotopaxi volcano, Ecuador: Absent permafrost and little pedogenesis. *Geoderma Reg.*, 2022; 29: e00496.
4. Bhatti SS, Kumar V, Singh N, Sambyal V, Singh J, Katnoria JK, Nagpal AK, Physico-chemical Properties and Heavy Metal Contents of Soils and Kharif Crops of Punjab, India. *Procedia Environ Sci.*, 2016; 35: 801-808.
5. Bigovic M, Roganovic M, Milasevic I, Djurovic D, Slavic V, Kosovic M, Pantovic S, Physico-chemical characterization of Igalo Bay peloid (Montenegro) and assessment of the pollution of potentially toxic elements in the sampling area. *Farmacia*, 2020; 68(3): 560-571.
6. Bireescu G, Munteanu N, Stoleru V, Avasilcai L, Bireescu L, Assessing the anthropogenic impact on environment by health soil card. *EQA Int J Environ Qual.*, 2014; 14: 1-9.
7. Bolan NS, Loganathan P, Saggar S, Calcium And Magnesium In Soils, In: Encyclopedia of Soils in the Environment, Elsevier Academic Press: Amsterdam, Holland, 2005; 149-154.
8. Cuciureanu R, Morariu I, Environmental and Food Chemistry. Methods of Analysis. Ed. Performantica: Iași, Romania, 2009, (available in Romanian).
9. Florea MA, Drumea V, Niță AR, Olariu L, Costea T, Gîrd CE, Investigation of pesticide residues and heavy metals content in various peppermint samples. *Farmacia*, 2020, 68(4): 704-709.
10. Garlitska N, Fira L, Lykhatskyi P, Boyko L, Biochemical mechanisms of oxidative stress in animals exposed to hexavalent chromium compounds in the case of isoniazid–rifampicin hepatitis, *Farmacia*, 2021; 69(2): 253-259.
11. Harangi S, Sági T, Seghedi I, Ntaflos T, Origin of basaltic magmas of Perșani volcanic field, Romania: A combined whole rock and mineral scale investigation. *Lithos*, 2013; 180-181: 43-57.
12. Italiano F, Kis BM, Baciu C, Ionescu A, Harangi S, Palcsu L, Geochemistry of dissolved gases from the Eastern Carpathians - Transylvanian Basin boundary. *Chem Geol.*, 2017; 469: 117-128.
13. Kaur M, Soodan RK, Katnoria JK, Bhardwaj R, Pakade YB, Nagpal AV, Analysis of physico-chemical parameters, genotoxicity and oxidative stress inducing potential of soils of some agricultural fields under rice cultivation. *Trop Plant Res.*, 2014; 1(3): 49-56.
14. Lanzafame G, Caggiani MG, Finocchiaro C, Barone G, Ferlito C, Gigli L, Mazzoleni P, Multidisciplinary characterization of the "Ghiara" volcanic paleosoil (Mt. Etna volcano, Italy): Petrologic characters and genetic model. *Lithos*, 2022; 418-419: 106679.
15. Lindsay WL, Schwab AP, The chemistry of iron in soils and its availability to plants. *J Plant Nutr.*, 1982; 5: 821-840.
16. Marinescu E, Elisei AM, Aprotosoaie AC, Cioanca O, Trifan A, Miron A, Robu S, Ifrim C, Hăncianu M, Assessment of heavy metals content in some medicinal plants and spices commonly used in Romania. *Farmacia*, 2020; 68(6): 1099-1105.
17. Mendez-Vilas A, Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, 2nd Edition. Formatex: Badajoz, Spain, 2010.
18. Mititelu M, Ghica M, Ionita AC, Morosan E, The influence of heavy metals contamination in soil on the composition of some wild edible mushrooms. *Farmacia*, 2019; 67(3): 398-404.
19. Morariu ID, Avasilcai L, Vieriu M, Cioanca O, Hancianu M, Immunochemical assay of chloramphenicol in honey. *Farmacia*, 2019; 67(2): 235-239.

20. Morariu ID, Avasilcai L, Vieriu M, Panainte AD, Bibire N, Novel Multiresidue Method for the Determination of Eight Trichothecene Mycotoxins in Pollen Samples Using QuEChERS-Based GC-MS/MS. *Rev Chim.*, 2017; 68(2): 304-306.

21. Official Journal of the European Union: Commission Regulation (EC) No. 420/2011 of April 29, 2011, amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs, 2011.

22. Oniga I, Toiu A, Benedec D, Vlase L, Comparative phytochemical profile of *Hypericum perforatum* and *Hypericum hirsutum (Hypericaceae), Farmacia,* 2022; 70(6): 1046-1049.

23. Ordak M, Wesolowski M, Radecka I, Muszynska E, Bujalska-Zazdrozny M, Seasonal variations of mercury levels in selected medicinal plants originating from Poland. *Biol Trace Elem Res.*, 2016; 173(2): 514-524.

24. Panaiotu CG, Jicha BR, Singer BS, Ţugui A, Seghedi I, Panaiotu AG, Necula C, 40Ar/39Ar chronology and paleomagnetism of Quaternary basaltic lavas from the Perşani Mountains (East Carpathians). *Phys. Earth Planet. Inter.*, 2013: 221: 1-14.

25. Singh B, Cowie A, Chan K, Soil Health and Climate Change: Volume 29 of Soil Biology. Springer: Berlin, Germany, 2011.

26. Sposito G, The Chemistry of Soils. 2nd Edition. Oxford University Press: New York, 2008.

27. Stadnic S, Pedology. "Alecu Russo" State University, Bălți, Republic of Moldova, 2010, (available in Romanian).

28. Utobo EB, Tewari L, Soil enzymes as bioindicators of soil ecosystem status. *Appl Ecol Environ Res.*, 2015; 13(1): 147–169.

29. Vlad V, Florea N, Toti M, Mocanu V, Method of correlation of the current Romanian soil classification system SRTS-2012 with the previous systems SRCS-1980 and SRTS-2003. The SRTS-2012+ system. *Res J Agric Sci.*, 2015; 47(3): 173-183.

30. Wang X, Chen T, Lei M, Song B, Wan X, Li Y, Selection of sugar cane varieties with a low heavy metal accumulation ability for the ecological remediation of contaminated farmland. *J Resour Ecol.*, 2012; 3(4): 373-378.

31. Yang J, Sun Y, Wang Z, Gong J, Gao J, Tang S, Ma S, Duan Z, Heavy metal pollution in agricultural soils of a typical volcanic area: Risk assessment and source appointment. *Chemosphere*, 2022; 304: 135340.

32. Yang ZX, Liu SQ, Zheng DW, Feng SD, Effects of cadmium, zinc and lead on soil enzyme activities. *J Environ Sci (China)*, 2006; 18(6): 1135-1141.