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Physical Properties and Ion Mobility Assays in Technosols Designed for Soil Restoration of Extractive Activities

M.M. Jordán[a]; E. García-Sánchez[a], M.B. Almendro-Candel[a], J. Bech[b]

[a]Department of Agrochemistry and Environment (GEA-UMH). University Miguel Hernández- Avda. de la Universidad s/n, Elche (Alicante), 03202, Spain

[b]Soil Science Laboratory, Faculty of Biology, University of Barcelona-Avda. 643, Diagonal,Barcelona, 08028, Spain

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A B S T R A C T

The rehabilitation technologies in areas degraded by extractive activities require the use of their own mine spoils. Reducing deficiencies in bulk density and aggregate stability, organic matter, and nutrients with a contribution of treated sewage sludge is proposed. This experiment was based on a controlled study using percolation columns. The assays was done using two mine spoils, both very rich in calcite. Two sewage sludge doses were undertaken (30,000 and 90,000 kg/ha of sewage sludge compost) in addition to a different mine spoils used as substrates. Irrigation water was provided by a device that simulated short duration rain. The leached water was collected 24 hours after the last application. Electrical conductivity, pH and the ions nitrate, ammonium, phosphate, sulfate, and chloride were determined. The experiment saw the bulk density decrease and the aggregate stability increase, thereby improving the structure. Significant nitrate concentrations appeared that may pose an environmental contamination risk. The resulting values for each irrigation application, the relationship between parameters, and the environmental risk are discussed.

Keywords: Sewage sludge, Irrigation, Ion mobility, Leachates, Bulk density

*Corresponding author: Jaume Bech; E-mail address: jaumebechborras@gmail.com

Introduction

From the ecological point of view, the restoration of extensive areas degraded by mining activities, the use of their own waste materials is required [1-4]. These materials do not possess the necessary fertility to ensure a successful process of restoration (implementation of adequate plant cover). Therefore, it requires the addition of organic amendments to achieve efficient substrate [5]. The obligation to restore abandoned mine and the correct application of biosolids is guaranteed by the legislation on waste management, biosolids and soil conservation [5]. Technosols are one of the latest additions to the World Reference Base for Soil Resources [6]. This new reference soil group contains a large range of artifacts and materials of natural and anthropic origin. They include a variety of refuse-based soil-like mine spoils, landfills, cinders, or sludge, whose properties and pedogenesis are dominated by their technical origin [7]. An adequate technosol selection can constitute a valuable and cost-effective solution for soil remediation and waste management [7]. Sewage sludge application in restoration has demonstrated its efficiency in previous studies [5, 8-10]. The use of treated sewage sludge can be a guarantee of success in the rehabilitation of areas affected by extractive activities, but it is important to preserve the environment with less risk of contamination of groundwater [10].

The experimentation was carried out on a controlled study using percolation columns. PVC pipe with a 10.5-cm interior diameter cut into 15-cm length pieces was used to make them. Two of these pieces were then stacked to construct each of the fifteen 30-cm tall columns (Fig. 1). Two treatments and a control were applied, which depended upon the quantity of sludge applied (Table 1 and Table 2). The sludge was applied on the surface and mixed with the soil, simulating a plowing or tilling action, producing a homogenous mixture within the uppermost 15 cm of soil.

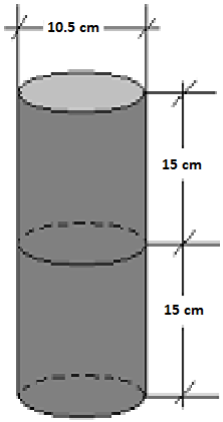

Fig. 1. Percolation columns used in the experiment

Table 1. *Quantity of sewage sludge applied in each treatment*

Sewage sludge quantity (kg/ha)	Designation
0	0
30,000	3
90,000	9

Table 2. *Experimental design and identifying symbols*

Symbol	Material contents
Z_0	30 cm column filled with aggregate (coarse).
D_0	30 cm column filled with degraded soil (fine).
$(Z+D)_0$	30 cm column filled from 0-15 cm with degraded soil and 15-30 cm with aggregate.
D_3	30 cm column filled with degraded soil. Sewage sludge dose (30,000 kg/ha) homogenous mixture first 15 centimeters.
D_9	30 cm column filled with degraded soil. Sewage sludge dose (90,000 kg/ha) homogenous mixture first 15 centimeters.
$(Z+D)_3$	30 cm column filled from 0-15 cm with degraded soil and 15-30 cm with aggregate. Sewage sludge dose (30,000 kg/ha) homogenous mixture first 15 centimeters.
$(Z+D)_9$	30 cm column filled from 0-15 cm with degraded soil and 15-30 cm with aggregate. Sewage sludge dose (90,000 kg/ha) homogenous mixture first 15 centimeters.

The experiment was carried out using two types of wastes rich in calcite (75-90%). The first, poor in quality was characterized by a predominance of the coarse element fraction (up to 75% by weight) and by containing high percentages of sand (Z). The second residual material tested came from the extraction of limestone. This waste was formed by levels of intercalated materials and residues of degraded soils (D). This usually has high heterometric stoniness (up to 60%), and is richer in clays (approx. 25%). These materials were amended with the biosolid according to quarry restoration methodology [11]. The characteristics of the mineral substrata employed appear in Table 3.

Table 3. *Characteristics of the substrata used in the experiment*

Texture	Clay (%)	Silt (%)	Sand (%)
Aggregate	15.40	16.00	68.60
Degraded	21.37	26.00	52.63

Parameter	Z	D
pH	8.25	8.92
EC S/cm (25 ºC)	257.20	56.32
OM (%)	0.53	0.27
P (mg/kg DM)	2.04	2.07
Ca (g/kg DM)	3.37	3.26
Mg (mg/kg DM)	134.13	337.57
Na (mg/kg DM)	222.15	63.27
K (mg/kg DM)	34.66	64.31
Fe (mg/kg DM)	2.25	1.48
Cu (mg/kg DM)	0.29	0.18
Mn (mg/kg DM)	2.08	1.07
Zn (mg/kg DM)	0.73	0.36
N (%)	0.03	0.02
$CaCO_3$ (%)	45-75%	55-70%
Act. Limestone (%)	18%	15%

The substratum used has a basic pH; therefore, it is a soil with an alkaline reaction. This means that most nutrients may have availability problems, making acidifying amendments necessary to lower the pH, facilitate element mobility, and improve the soil structure. The substratum has a relatively low nutrient content. Ca^{2+} is the element found in the greatest proportion within the soil solution. K^+ and Na^+ concentrations are moderate. This is reflected in the soil's electrical conductivity because these cations, especially Na^+, are very soluble and have considerable repercussions on this measurement when their concentrations are high. The equivalent calcium carbonate content is very high, as is typical for these types of tailings. The organic matter content is very low, just like that for assimilable phosphorous and Kjeldahl nitrogen compared with the desired normal content for a cultivated soil. With respect to the composition of assimilable nutrients (extract with ammonium acetate and DTPA), it is shown that, with the exception of Ca^{2+}, the remaining elements are found at low or very low levels, with iron, among others, standing out. Iron availability is normally low in limy soils whose cultivation practically makes any inorganic form of this metal inassimilable [12].

The biosolid used in this experiment comes from a wastewater treatment plant located near Aspe (Alicante). Prior to the composting process, the sludge needs to be mixed with a bulking agent, a supporting structure that favors aeration, absorbs humidity, and furthermore contributes carbon. Chopped hay and sawdust are used as the bulking agent, and silos exist for their storage. Hay favors aeration, sawdust absorbs humidity, and both materials constitute sources for carbon. The composition by volume of the sludge-bulking agent mixture is 50% sludge, while the remaining 50% is 1/4 hay and 3/4 sawdust. This sludge-bulking agent mixture progresses through the composting tunnel and is simultaneously homogenized by a tumbler, which in addition to permitting the progress and homogenization of the mixture, promotes its aeration. During the first weeks, the mixture is placed upon a porous base connected to an air injection system using fans or blowers, which maintains discontinuous forced aeration. Afterwards, the aeration is passive and natural.

For the sludge analysis, its total mineralization was carried out by electrothermal radiation (microwaves) in an acid medium. In the solution thus obtained, the solubilized elements except for nitrogen were assessed. This was determined by the Kjeldahl method, which quantifies the organic nitrogen and ammonium contents within the sample. The easily oxidizable organic carbon was calculated by sulfochromic digestion and subsequent assessment with Mohr's salt, an easily oxidizable organic matter by applying the 1.72 conversion factor, and the total by calcination in a muffle furnace at 500 ºC for 2-4 h.

As for macroelements, the sludge presents low contents of phosphorous and potassium, with medium contents for calcium and magnesium, all within the ranges cited by [13]. The total sodium content is of some importance, but cannot be considered dangerous for the soil. Analytically controlling the sludge at the time of its incorporation is important, especially with regards to sodium, as this element may cause soil salinity problems and alter its structure [14]. The C/N ratio is 12, indicating that the organic matter is partially mineralized and, therefore, the sludge can enhance soil fertility [15]. Many physical and chemical properties in soils amended with sludge, such as water adsorption, aggregate stability, contribution from N, P, and other nutrients for crop growth, depend, to some extent, upon the quantity of organic matter in the sludge that is added. Knowledge about the quantity of organic matter in the sludge can be used to estimate the quantities that must be applied to the soil [16]. Use sewage sludge and mine spoils as technosols constitute innovative strategies of waste management, whose application allowed the species growth and development [7]. This sewage sludge has an organic matter content that is very suitable for agricultural use (Table 4).

Table 4. *Sewage sludge composition (dry matter, DM) from the Aspe wastewater treatment plant*

Parameter	Value
Organic C (%)	44.4
Kjeldahl N (%)	3.7
C:N ratio	12
P (%)	0.29
K (%)	0.02
Ca (%)	0.09
Mg (%)	0.08
Fe (g/kg DM)	5.48
Cu (mg/kg DM)	289
Cd (mg/kg DM)	0.97
Ni (mg/kg DM)	18
Pb (mg/kg DM)	121
Zn (mg/kg DM)	768
Hg (mg/kg DM)	1.23
Cr (mg/kg DM)	21

2. Materials and methods

2.1. Irrigation

The soil contained in the columns was irrigated (8 applications) using tap water. The first five irrigations occurred every two weeks and the last three once per month. The irrigation applications lasted 6 months. Collection of the leached water was carried out 24 hours after the last application. The contribution of water was provided by a device that simulated short rainfall or a flood irrigation system that covered the surface and then percolated into the soil. It consists of a plastic recipient with holes punched in the bottom [10]. Water samples were taken from each one of them. Their saline characteristics were determined first, i.e., pH, electrical conductivity, the Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} cations, as well as the C^{l-}, $SO4^{2-}$, and HCO^{3-} anions [17].

2.2. Determination of some physical properties of the substratum

Bulk Density

Bulk density is defined as the ratio between the mass of the oven dry soil and the overall volume, which includes the volume of the particles and the porous space between them. It is dependent upon the soil particle densities (sand, silt, clay, and organic matter) and their type of packaging. Mineral particle densities are found within the range of 2.5 to 2.8 g/cm3, while organic particles are usually <1.0 g/cm3. The bulk density is a dynamic property that varies along with the structural conditions of the soil. It can serve as an indicator of the compaction and the restrictions to root growth (Table 5).

Table 5. *General relationship of soil bulk density to root growth based on soil texture*

Soil texture	Ideal bulk densities (g/cm³)	Bulk densities that may affect root growth (g/cm³)	Bulk densities that restrict root growth (g/cm³)
Sands, loamy sands	<1.60	1.69	>1.80
Sandy loams, loams	<1.40	1.63	>1.80
Sandy clay loams, loams, clay loams	<1.40	1.60	>1.75
Silts, silt loams	<1.30	1.60	>1.75
Silt loams, silty clay loams	<1.40	1.55	>1.65
Sandy clays, silty clays, some clay loams (35-45% clay)	<1.10	1.49	>1.58
Clays (>45% clay)	<1.10	1.39	>1.47

Source: USDA (1999). Soil Quality Test Kit Guide

Measuring the bulk density in every case is important due to its great variability. Between an organic horizon and a very compact Bt horizon, the values may vary from 0.1 to 1.80 or even more grams per cubic centimeter; under these conditions, the errors that may occur in the estimated parameters from it can be enormous. Determining the bulk density may be done by different methods, but preferably two are used. The best way to determine the bulk density is by taking a fixed volume of undisturbed soil and weighing it once dry, after heating it at 105º C until it reaches a constant weight. To do this, a metallic cylinder with a volume close to 100 mL is usually used. Once it is full and flush at both ends, the contained soil is extracted. Its volume corresponds to that of the cylinder and therefore known; it is then dried and weighed. The density is determined by the ratio between the weight obtained and the corresponding volume. The main drawback to this system is the presence of stones, so this can only be used in non-stony soil, which unfortunately, is less common. In this case, using another system is more convenient, one that is less precise but easier. It involves taking aggregate from the soil, as large as possible, drying it, and weighing it to learn its mass. A string is tied to it and it is submerged into molten paraffin to coat and waterproof its surface; once solidified, it can be weighed once again. The wax-coated aggregate is introduced into a graduated cylinder containing a known quantity of water. The volume gain of the water as a consequence of the introduction of the aggregate corresponds to its volume. This way, the two parameters necessary for the density calculation are learned. Although the paraffin layer is very thin and its volume negligible, it can be estimated based on its density and the weight increase undergone by the aggregate following the waterproofing process. The main drawback of this method is that it cannot specify the volume of the cracks and interned voids. However, by wetting the

soil, they all disappear; this serves to determine the behavior of moist soil.

Aggregate stability

Aggregate stability is a measure of the vulnerability of soil aggregates exposed to external disruptive forces. Soil aggregates consist of diverse particles that are bound to one another. Aggregates that resist the forces of water are called water-stable aggregates (WSA). In general, the higher the percentage of stable aggregate, the lower the soil erodibility. Soil aggregates are products of the soil microbial community, the organic and mineral components in the soil, the nature of the plant communities on the surfaces, and the ecosystem history. They are important in the movement and storage of soil water, erosion, root development, and microbial activity. The destruction of aggregates is the first step towards the development of crusting and surface sealing, which prevent water infiltration and increase erosion. Soil aggregation can vary over certain periods of time, such as a season or a year. Aggregates can form, disintegrate, and re-aggregate periodically. Aggregates improve soil quality by:

- Protecting the organic matter trapped in the aggregates from exposure to air and microbial decomposition.
- Decreasing soil erodibility.
- Increasing the movement of water and air (aggregates increase the amount of large pore space), thus improving the physical environment for root development and the habitat for soil organisms.

Determining the percentage of stable aggregates in the soil was performed by an artificial rain simulator according to the method described by [18].

2.3. Leachates

pH and electrical conductivity

Determining the electrical conductivity is performed by an electrical conductivity meter, which incorporates a conductivity cell, considering 25 ºC as the reference temperature, according to current analysis methods [19]. Measuring the electrical conductivity depends upon the type of ions present in the sample, their concentration, and the temperature at which the reading is taken.

Anions

The chloride content was determined by the Mohr method, based on the formation of silver chloride, an insoluble salt, detecting the turning point by the appearance of a red precipitate of Ag_2CrO_4, a compound used as an indicator [19]. Sulfates were determined following the nephelometric technique [20]. The nitrate content is determined by second-derivative ultraviolet spectroscopy [21].

The method for the determination of phosphorous is based on the formation of a phosphomolybdic complex in an acid medium, reduced by ascorbic acid, producing a blue coloration that is measured at 825 nm. The phosphorous is measured as a phosphate ion.

Cations

The method for determining ammonium is based on the development of indophenol blue by reaction of ammonium ions treated with a solution of sodium hypochlorite and phenol in the presence of nitroprusside acting as a catalyst. The Na^+, K^+, Mg^{2+}, and Ca^{2+} ions are measured directly in the sample or in appropriate dilutions by atomic emission spectrophotometry in the case of the first two ions, and by atomic absorption for the last two [10].

2.4. Linear regression analysis

Simple linear ***regression*** analysis was applied to the developed experimental data. It aims to predict and/or estimate the values of the dependent variable based on obtaining a linear function of the independent variable. Mathematically, the linear model or regression line is the following:

$$Y = a + b * X$$

Where *a*: cutoff point of the line with the response variable *Y*
Y: intercept
b: slope of the line, called the regression coefficient: the average rate of increase or decrease in Y caused by a unit increase in X.

Analyzing the degree of linear association between the dependent and independent variables is

necessary. To do this, among other statistics that permit assessing the goodness of fit of the data to the linear regression model, we have used:

Simple linear correlation, *r*, or what is also called the Pearson linear correlation coefficient, measures the degree of linear association between the variables, i.e., the joint variation of the two variables. This measure is unitless and independent of the scale at which the variables are measured. Its interpretation is very easy because it always takes values between 1 (strong positive linear association) and -1 (strong negative linear association). When the *r* values approach 0, no linear association exists between the considered variables, and therefore, determining the model and linear regression equation will be meaningless.

There may be an interesting and informative nonlinear relationship of one variable given the other. Consequently, basing the evaluation of the relationship exclusively on *r* is neither safe nor reasonable. In this sense, it is very convenient to accompany calculating *r* with the representation of the point cloud, since a visualization of the relationship between the variables will be obtained. A defined point cloud and a proximity between points close to the trend line that represents the correlation will indicate an acceptable relationship between the variables.

The squared correlation coefficient (R^2) represents the proportion of the variation of a variable that is explained by its linear association with another variable.

3. Results and discussion

3.1. Physical properties

The bulk density and percentage of stable aggregates have been determined as physical properties that can be modified following the application of organic amendments to the soil like is sewage sludge.

With respect to the substrata without amendment, aggregate (Z_o), degraded (D_o), and a mixture of aggregate and degraded $(Z+D)_o$, the results indicate that substratum Z_o has the lowest bulk density, logically due to its much thicker texture that is going to favor the availability of air voids in the column, decreasing the mass/volume ratio. As seen in Fig. 2, the sludge application decreases the bulk density in the distinct substrata tested with respect to the control, with the decrease being greater the greater the sludge dose. The organic content of the applied residue is going to improve the substratum structure by favoring the formation of pores that contribute to the bulk density decrease [22]. High doses of sludge (90,000 kg/ha) obtain a bulk density inferior to 1.4 g/cm^3, a value recommended by the USDA [23]. for sandy loams and loams (Table 5). Bulk density values greater than 1.6 g/cm^3 can affect root growth and even restrict it (Table 5).

Figure 3 shows that the control substrata used have a relatively low percentage of stable aggregates. The application of sludge significantly increases the percentage of stable aggregates, with this increase much larger for the greater application rate (90,000 kg/ha). The increase of organic matter

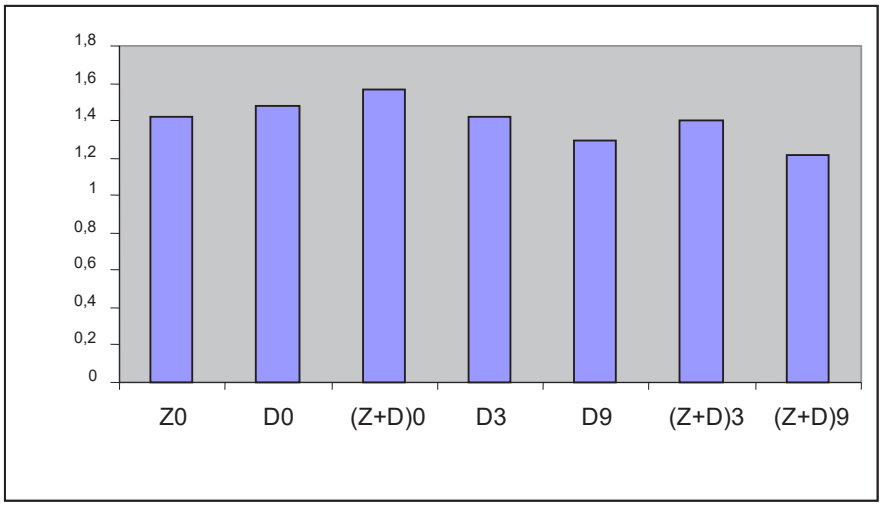

Fig. 2. *Bulk density (g/cm³) for the different substrata used*

in the soil will improve numerous properties thereof, among which all those related with the structure, like bulk density, aggregate stability, porosity, etc., can be highlighted. The improved soil structure decreases its vulnerability to degradation processes such as erosion and compaction. Nevertheless, no cases reached the values recommended by the USDA [23] for soils with a clay fraction percentage around 15%, which was situated between 65-70%. Considering the percentage of organic matter in the substrata, the aggregation percentage should have been 53% for the case of D_3 and 70% for the case of D_9 (USDA, 1999). During our experiment, these values were not reached (Fig. 3). It must be kept in mind that aggregate stability should increase over time.

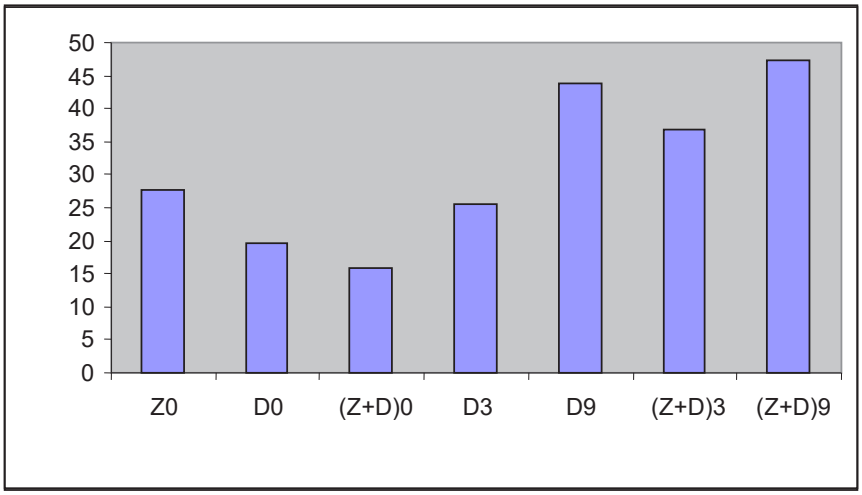

Fig. 3. *Aggregate stability (%) in the different substrata used*

3.2. Leachate analysis

The wash water of the mineral substratum can serve as a point of reference for possible contamination that may appear in groundwater when sewage sludge is applied as an amendment [5].

pH

No significant changes in the pH were produced between treatments; an acidifying trend was only seen in the first and third sampling in the treatments. Over time, it was observed that the pH values were more similar to that of the irrigating water before being added to the soil. The lowest pH values coincided with the beginning of the experiment (incorporation of residual matter and beginning of irrigation) and when the greatest degradation of the organic matter appears to have occurred, between the second and third irrigation.

Electrical conductivity

An increase in this property was observed in the water collected from the columns treated with sludge with respect to the control. This is due to the resulting wash of the soluble salts that the biosolid applied to the soil provided. The electrical conductivity values were closely related with the dose of biosolid applied, above all during the first 3 consecutive irrigation applications. However, the electrical conductivity values were only worrisome during the first three irrigation applications; beginning with the fourth and particularly the fifth ones, the electrical conductivity values stabilized as the preceding irrigations washed out the salts.

Inorganic nitrogen forms

Two of the three inorganic forms of nitrogen in the leachates are discussed: nitrates and ammonium. The nitrites analyzed in the wash water were very close to the detection limit of the technique used. Their results are not discussed because they were not significant.

An increase in nitrate concentration was observed in the water resulting from the soils treated with sludge with respect to the control soil. The treatments with high sludge doses (90,000 kg/ha) are those that contributed higher NO_3^- contents to

the water. The highest NO_3^- concentration in the leachates occurred in irrigations 1, 2, and 3 (2500-300 mg/L). From the fourth irrigation application onward, the wash of this anion was much scarcer. The nitrates exceeded the recommended values in the two treatments. In any case, these high nitrate concentrations would drop with the restoration and development of vegetative cover, which would assimilate a large portion of the nitrates, thereby reducing the possible risk of groundwater contamination.

The values obtained for ammonium were only significant for the first irrigation application. This cation increased with the biosolide dose, whose differences decreased over time. The ammonium quantities were far inferior to those obtained for NO_3^-, which is due to higher fixation of the NH_4^+ in the mineral substratum and its participation in the nitrification process to produce the more oxidized forms of the nitrogen.

Anions

A certain tendency was observed in the phosphorus to increase with the sludge dose treatment. The highest values were obtained in the columns filled with degraded soil (D) and with the applications equivalent to 90,000 kg/ha of composted and treated sludge. The recommended concentration limits were not exceeded in any treatments. High natural limestone in this soil impeded in part the displacement and loss of soil phosphorus (calcium phosphate precipitation).

Chlorides and sulfates are involved in mineral nutrition of plants. Furthermore, they are very relevant quality control parameters of water. Significant differences appeared in the chloride anions between the treatments with sludge and the control. Quite possibly, the most influential factor when determining the Cl^- in the leachates is the contribution from the sewage sludge, without forgetting that the substrata used (aggregate and degraded soil) contains abundant salts. In fact, in the first two irrigation applications, high chloride values resulted in the control columns with the presence of aggregate (Z or Z+D) with lower values in the control columns filled with degraded soil (D). This observation demonstrates the contribution of chlorides by the wash of the aggregate (Z) used as a mineral substratum. The chlorides were practically completely washed out in the first three irrigation applications. In the case of sulfates, an increase was noted with the treatment that decreased over time, whose highest values were reached in the second irrigation application. This circumstance corroborates the fact that the organic sulfur may have undergone organic matter mineralization processes and appeared in the leachates most significantly in the third sampling.

Cations

From the environmental point of view, the concentrations of Ca, Mg, Na and K in the leachates pose no risk. The contribution from soluble K^+ with the sludge does not appear to produce an increase of this element in the leachates. It is possible that the clayey nature of this degraded soil limits the displacement and loss of this nutrient that fixes relatively easily to the clay minerals. In the sodium, a clear increase was noticed in the first and second irrigation application with the treatment that was not significant for the remaining samplings. There was a tendency for its leaching to increase over time. The calcium seems to increase with the treatment and the sludge dose applied. Over time, its tendency is to decrease. The soil reaction with the sludge appears to have increased the presence of soluble Ca^{2+}, as it appeared in the leachates in considerable concentrations. The magnesium increased significantly with the treatment, and diminished with the passing of time.

3.3. Correlations

In this section, the linear regressions obtained by relating some parameters with others are analyzed, which gives us an idea about the linear relationship existing between the variables being studied. To do this, we used Pearson's linear correlation coefficient (r) that measures the degree of association between the variables, i.e., the joint variation that exists between the two variables. Its value is comprised between 1 (strong positive linear association) and -1 (strong negative linear association). When the *r* values approach 0, this means that no linear association exists between the considered variables. Since not all the obtained correlations produced significant results, we will only discuss those that did. Although there is no wide dispersion in the results, as can be seen in the different graphs, the linearity is more pronounced in the lower values, where a larger quantity of data exists. It would be desirable for

future research to complete these correlations using a wider range of values.

The electrical conductivity correlated well with the alkaline and alkaline earth elements analyzed in the wash water, with the exception of K^+ (Fig. 4, 5 and 6). In the case of Ca^{2+}, this correlation was excellent (Figure 4). It is obvious that the washed Ca^{2+} comes from both the substratum used as well as the sludge applied as organic amendment.

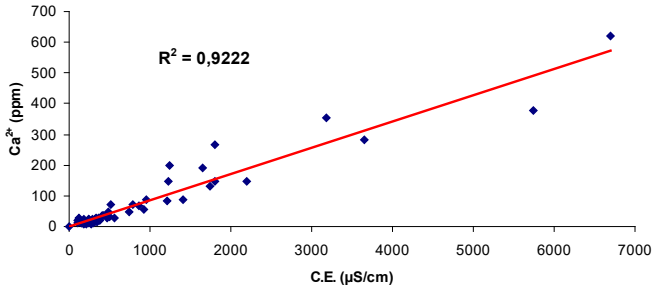

Fig. 4. *Correlation between the electrical conductivity and calcium*

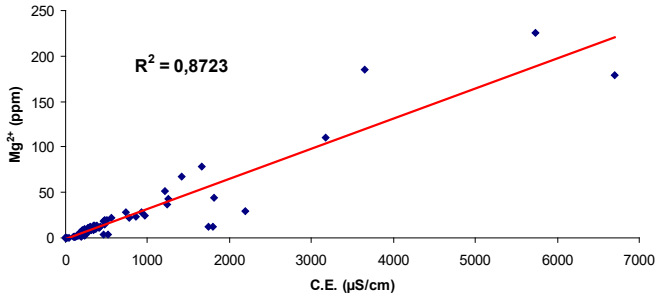

Fig. 5. *Correlation between the electrical conductivity and magnesium*

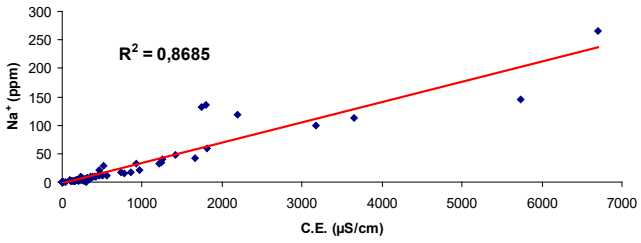

Fig. 6. *Correlation between the electrical conductivity and sodium*

Moreover, we can show that the electrical conductivity correlations with the anions presented a heterogeneous behavior. This correlation was excellent with NO_3^- (Fig. 7), but less so for either SO_4^{2-} or Cl^-, as could be expected (Fig. 8 and 9). This may be due to greater mobility and concentration of the NO_3^- anion. The resulting nitrate values in the first irrigation applications were very high and so they washed out quickly. In the case of the chlorides, the concentrations were lower and, possibly, the analytical method used (volumetric) was not the most appropriate because it brought a greater error than other analytical techniques.

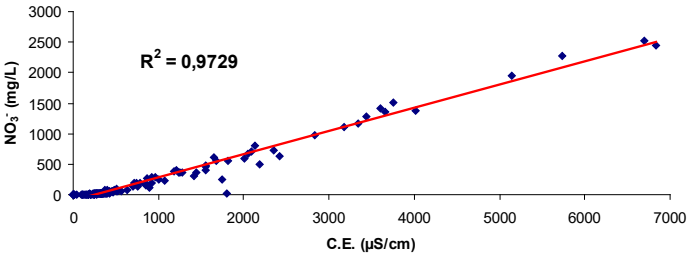

Fig. 7. *Correlation between the electrical conductivity and nitrates*

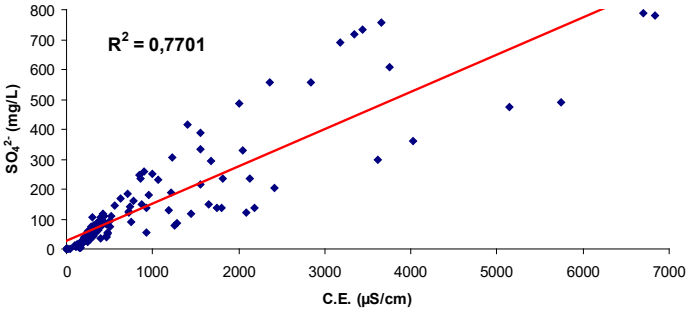

Fig. 8. *Correlation between the electrical conductivity and sulfates*

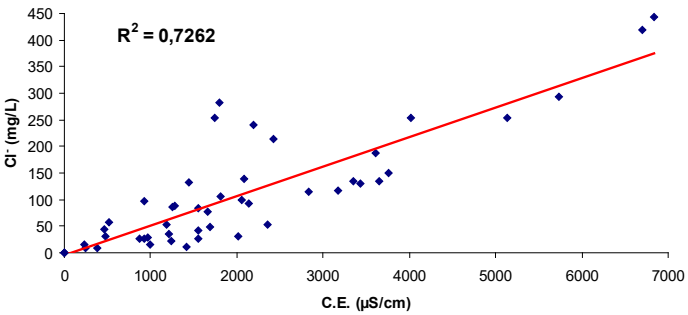

Fig. 9. *Correlation between the electrical conductivity and chlorides*

Sulfates are salts that have a lower solubility than halite. There were significant correlations between the SO_4^{2-} anion and the Mg^{2+}, Ca^{2+}, and K^+ cations (Fig. 10, 11 and 12). The highest correlation was obtained with Mg^{2+} that comes mainly from the substratum formed by magnesium limestone and dolomite subjected to a crushing process in the quarry plant (Figure 10). This may be due to the epsomite ($MgSO_4 \cdot 7H_2O$) having a higher solubility than the anhydrite and gypsum ($CaSO_4$ and $CaSO_4 \cdot 2H_2O$, respectively).

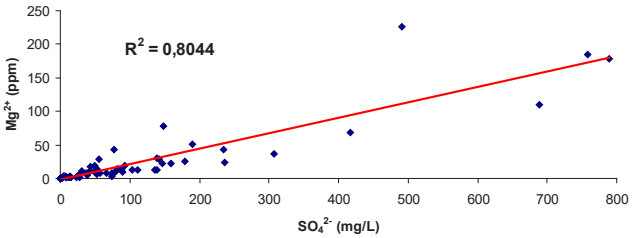

Fig. 10. *Correlation between the sulfates and magnesium*

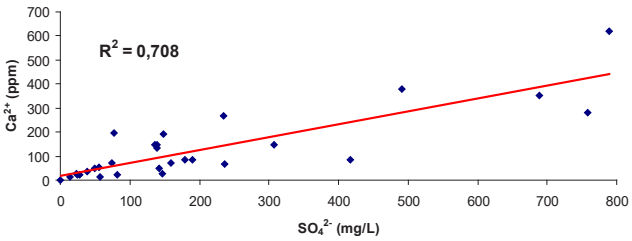

Fig. 11. *Correlation between the sulfates and calcium*

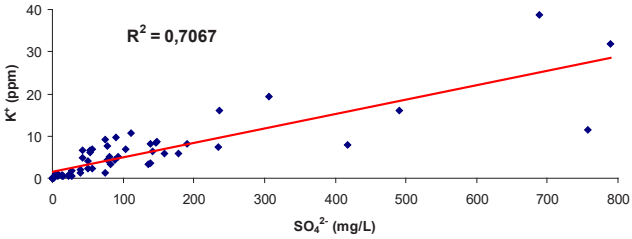

Fig. 12. *Correlation between the sulfates and potassium*

The good correlations obtained between the NO_3^- anion and the alkaline earth elements and the Na^+ were mainly due to their rapid percolation through the dissymmetrical columns, above all in the first irrigation applications (Fig. 13, 14 and 15).

Next, we will discuss the correlations obtained between the ions that formed very soluble salts (between 250 and 400 g/L). The only statistically significant correlation occurred with Na^+-Cl^- (Fig. 16). Predictably, the chlorides presented an excellent correlation with Na^+. NaCl (halite) is a very soluble salt and so geochemically it is very unstable and its mobility is very high.

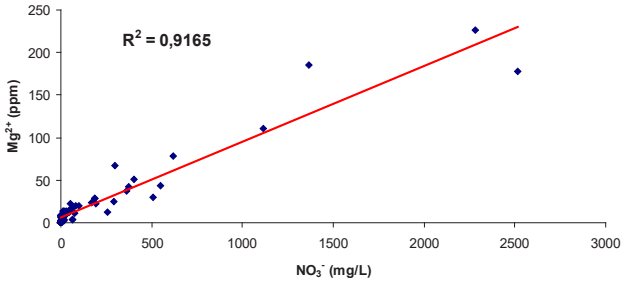

Fig. 13. *Correlation between the nitrates and magnesium*

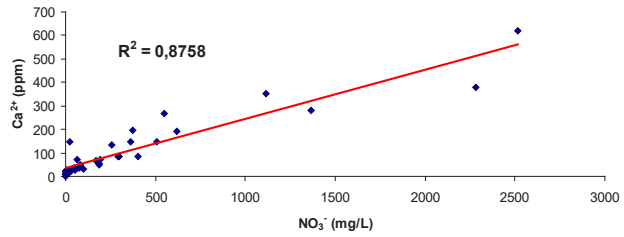

Fig. 14. *Correlation between the nitrates and calcium*

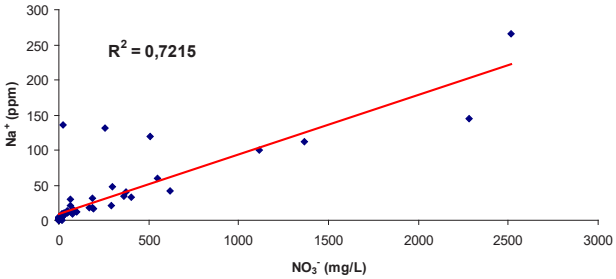

Fig. 15. *Correlation between the nitrates and sodium*

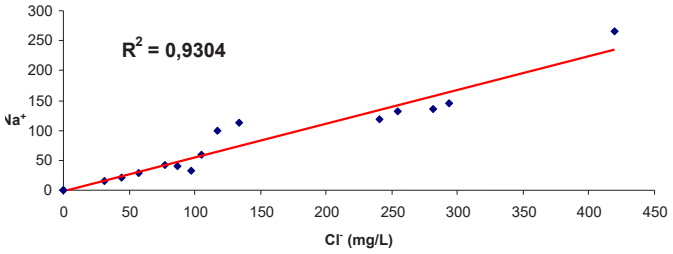

Fig. 16. *Correlation between the chlorides and sodium*

4.1. Physical properties of the substratum

The experiment saw the bulk density decrease and the aggregate stability increase, thereby improving the structure.

4.2. Environmental risk

As for the environmental risk with respect to the contamination of aquifers in the sierras de Callosa and Vega Baja del Segura from wash water, we performed a comparison between the concentrations of the contaminants obtained in the leachates from our experiment and the established limit values for water of the third quality group of the Segura Hydrographic Confederation.

- The pH values were found to be within the limit value range (5.5-9).
- The electrical conductivity limit value is <1000 µS/cm (Jordán et al. 2008). These values will be met from the fourth irrigation application onward, while the values up to that point were far superior. However, the quality of the aquifer's groundwater is quite poor, reaching conductivities of 5000 µS/cm, and so this parameter, in principle, would not represent any environmental risk to the aquifer [5, 10, 24].
- The limit values for the chlorides (<700 mg/L), phosphates (<27 mg/L), and sulfates (<800 mg/L) were very superior to those obtained in all the irrigation applications.
- Significant nitrate concentrations appeared that may pose an environmental contamination risk.

4.3. Correlations between leachate parameters

- The electrical conductivity correlated well with the cations, with the exception of the potassium.
- Regarding the electrical conductivity correlations with the anions, they were found to be excellent in the case of nitrates, which may be due to their high concentration and solubility; however, as expected, they were not as much with the sulfates and chlorides.
- For sulfates, significant correlations were obtained with the Mg^{2+}, Ca^{2+}, and K^{+} cations, with the magnesium correlation highest, which could be due to it being more soluble [10].
- The good correlations between nitrates and the Ca^{2+}, Mg^{2+}, and Na^{+} cations could mainly be due to their high solubility, as they percolated rapidly in the first irrigation applications [10].
- The chlorides showed excellent correlation with the sodium. NaCl (halite) is very soluble and so its mobility is high [5, 10, 24].

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Heritage wheats of Georgia

David Bedoshvili[a]* Marine Mosulishvili[bc], Gulnari Chkhutiashvili[d] , Mirian Chokheli[d], Nino Ustiashvili[b] & Ineza Maisaia[ef]

[a]Institute of Crop Science, Agricultural University of Georgia, 240, David Aghmashenebeli Ave., Tbilisi, 0159, Georgia

[b]Institute of Ecology, Ilia State University, 3/5, Cholokashvili Ave., Tbilisi, 0162, Georgia

[c]Georgian National Museum, 3, Purtseladze Str., Tbilisi, 0105, Georgia

[d]Scientific Research Center for Agriculture, 6, Marshal Gelovani Ave., Tbilisi, 0159, Georgia

[e]Institute of Botany, Ilia State University, 1, Botanikuri Str., Tbilisi, 0105, Georgia

[f]National Botanical Garden of Georgia, 1, Botanikuri Str., Tbilisi, 0105, Georgia

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A B S T R A C T

Georgia is characterized by remarkable diversity of the domesticated wheat. Five out of the fifteen wheat species found in Georgia, originate from Georgia and are local endemics. These wheat species are *Triticum carthlicum* Nevski, *Triticum macha* Dekapr. & Menabde, *Triticum palaeocolchicum* Menabde, *Triticum timopheevii* (Zhuk.) Zhuk., *Triticum zhukovskyi* Menabde et Eritczjan. *T. carthlicum* Nevski is free-threshing, while the other four wheats are hulled. The history of taxonomic identification, some specific morphological traits and the role they of these species in the ancient agriculture of Georgia are reviewed in the present paper. A traditional method of harvesting hulled wheats is also described and illustrated.

Keywords: *Triticum*, Hulled wheat, Free-threshing wheat, Dika, Zanduri, Makha.

*Corresponding author: David Bedoshvili; E-mail address: d.bedoshvili@agruni.edu.ge

Introduction

Georgian wheat species represent a special, living museum where the whole diversity of the wheat genus is presented. Hence, the Georgian wheat museum is unique, having an importance of a global scale, the analogues of which nowhere to be encountered. Fifteen species (*s.st.*) of wheat: eight free-threshing and seven domesticated ancient (hulled) wheat species are found in Georgia. Five out of 15 species are endemics to Georgia – one of them is free threshing, while four are hulled. None of the regions of the world has such diversity of wheat species and high level of endemism wheat. Free-threshing wheat is *Triticum carthlicum* (in Georgian Dika). Hulled endems are *T. palaeocolchicum* (Kolkhuri asli- in Georgian), *T. macha* (Makha), *T. timpoheevii* (Chelta zanduri), *T. zhukovskyi* (Zanduri). The purpose of the present paper is to review the endemic species of wheat of Georgia.

Triticum carthlicum Nevski (Karthlian wheat)

T. carthlicum Nevski (Karthlian wheat) was widely planted in Georgia. Its common name in Georgian is “Dika”. It is a free-threshing (AABB) tetraploid wheat, which is endemic to Georgia. This wheat has been cultivated for at least 8000 years in Georgia according to the data of the Neolithic archeological excavations [1]. Dika, as a highland crop, is well adapted to severe conditions. Supposedly, it was originated in highlands at 1000-2000 meters

above sea level, on the southern slopes of the Greater Caucasus, although its fields could have been encountered both above and below this range, even at heights that severely limit agriculture – 2200-2300 meters. Karthlian wheat is a result of the prolonged farming culture of Georgia. 'Dika' is mentioned in the 5th century Georgian historical documents [2-4].

Matsuoka in 2011 noted that *T. carthlicum* is strikingly similar to *T. aestivum* in morphology [5]. Karthlian wheat's spike morphology resembles more the morphology of common wheat (*T. aestivum*) rather than that of other subspecies of free-threshing tetraploid wheat [6]. Moreover, Kihara, *et al*. in 1950 had showed that the morphology of synthetic hexaploid wheat derived from crosses between *T. turgidum* subsp. *carthlicum* and *Aegilops tauschii* Coss., resembles that of common wheat and considered *T. turgidum* subsp. *carthlicum* as a candidate for the AB-genome donor of common wheat [7].

Karthlian wheat is of spring habit, early maturing, and somewhat resistant to fungus diseases (ergot). It was relatively resistant to frost, lodging and grain shattering. The growth habit of young plant is erect. It has strong yellow to light red stems. Spikes are flexible, tending to lean over. Glumes can be white or red. While several flowers are present in each spikelet only three usually develop kernels. Kernels are free-threshing, flinty, generally red. Dika can be distinguished from *T. aestivum* in the field by its stems which are usually solid below the spike instead of thin-walled and hollow. Also, its grain tends to be hairless on the top, which also differentiates dika from bread wheat. The landrace dika cultivated by farmers contained significant variability for glume and lemma color (white, red and black), glume hairness and grain color (red and white). Dika bread was highly valued by local population for its good taste and long "shelf live". [8].

Karthlian wheat was first identified by Flaksberger [9] as a variety of bread wheat *T. vulgare* var. *fuliginosum* Alef. (1866) p. p. Later N. Vavilov (1919) determined it as *T. persicum* Vavilov [nomen provisorium] [10]. In 1921 Zhukovskyi found it in Georgia and described as *T. persicum* Vavilov ex Zhuk. 1923 non Aitch & Hemsl. 1888. nom. illeg. [11]. This name [*T. persicum*] is a later homonym of *Triticum persicum* (Boiss.) Aitch. & Hemsl. =*Aegilops persica* Boiss. 1846 and therefore is illegitimate (ICN Art. 53.1). Seed of this wheat was sent to N. Vavilov by a German private company under the name of Persian wheat ("Persischer Weizen"). However, the German company itself had received the seed from Moscow, not from Iran [2, 12]. According to the "International Code of Nomenclature for algae, fungi, and plants" the earliest legitimate name of this species is *Triticum carthlicum* Nevski (ICN Art. 11.4) [13]. This endemic species was widely cultivated in Georgia and has never been associated with Iran [2].

Karthlian wheat is presented by 11 varieties in Georgia: *T. carthlicum* var. *fuliginosum* (Zhuk.) A. Filat.; *T. carthlicum* var. *nigrirubiginosum* (Flaksb.) A. Filat.; *T. carthlicum* var. *pseudorubiginosum* (Zhuk.) A. Filat.; *T. carthlicum* var. *pseudostramineum* (Flaksb.) A. Filat.; *T. carthlicum* var. *rubiginosum* (Zhuk.) A. Filat.; *T. carthlicum* var. *stramineum* (Zhuk.) A. Filat.; *T. carthlicum* var. *darginicum* (Berg & Muizhn.) A. Filat.; *T. carthlicum* var. *osseticum* (Greb.) A. Filat.; *T. carthlicum* var. *rarissimum* (Flaksb.) Mosul. & al.; *T.* x *cartlicum* var. *zhukovski* (Flaksb.) Mosul. & al.; *T. carthlicum* var. *dekaprelevichii* (Sicharulidze) Naskidashvili

All eleven varieties of *T. carthlicum*, were found only in Georgia. Eight out of the eleven varieties were found only in Georgia. *T. carthlicum* var. *fuliginosum* was recorded in the adjacent to Georgia mountainous regions of Dagestan, var. *stramineum* in Azerbaijan, while var. *rubiginosum* in Azerbaijan, Armenia and Turkey (on historical territories

Fig.1. *Triticum carthlicum Nevski (Karthlian wheat)*

of the Kartvelian [Georgian] people). It should be mentioned that Dika" doesn't have any name in other languages. Pure production fields of 'Dika' were registered only in Georgia. In all other countries, Dika is found as impurities in the fields of bread wheat (*T. aestivum*) [2].

Triticum macha Dekapr. & Menabde

T. macha is a hulled hexaploid (AABBDD) wheat, endemic to Georgia. It is called Makha wheat in Georgia. It was a major component of the Makha landrace, mainly cultivated in Racha-Lechkhumi, as well as in Lower Svaneti, Imereti and Samegrelo. The Makha landrace also included *T. palaeocolchicum*, a tetraploid wheat, which is described in the next section.

T. macha is a late-maturing winter wheat with tall, hollow stems. It is characterized by large above-ground phyto-mass and resistance smuts. The bush is semi-prostrate. Spikes vary in density from open to dense, with short awns. Kernels remain in the spikelets after threshing. They are elliptical, red, and intermediate in hardness.

In Makha fields, *T. macha* itself was presented in great variation for spike color (white and red) awnedness (awned, semi-awned and awnless) and hairness of glumes. The most widespread form was white spike with short awns and without hairs.

Fig. 2. *T. macha Dekapr. & Menabde (Makha wheat)*

T. macha was described by L. Dekaprelevich and V. Menabde from Lechkhumi in 1932. It is one of the oldest cultivated wheat and it was preserved only in Lechkhumi by the 1930-ies. It is characterized by traits of wild and domesticated wheat at the same time. It has brittle rachis and spikes fall down at late stages of maturity. Therefore, it used to be harvested in two steps: spikes were harvested with a local tool 'shnakvi' on the first place (see below its description) and straw was harvested after that [2, 14].

L. Dekaprelevich in 1954 proposed to split Makha into species: 1) Gvatsa Makha (*T. tubalicum* Dekapr.) – characterized by lax and fragile spikes and 2) Chelta Makha (*T. imereticum* Dekapr.) – characterized by stiffer and less fragile spike [3]. However, this proposal was not accepted.

There are 14 varieties of *T. macha* identified by Georgian wheat researchers: *T. macha* var. *colchicum* Dekapr. & Menabde; *T. macha* var. *georgicum* Menabde; *T. macha* var. *ibericum* Dekapr. & Menabde; *T. macha* var. *letshchumicum* Dekapr. & Menabde; *T. macha* var. *megrelicum* Menabde; *T. macha* var. *scharaschidzei* Menabde; *T. macha* var. *ericzjanae* Menabde; *T. macha* var. *rubiginosum* Menabde; *T. macha* var. *subcolchicum* Dekapr. & Menabde; *T. macha* var. *submegrelicum* Dekapr. & Menabde; *T. macha* var. *subletshchumicum* Dekapr. & Menabde; *T. macha* var. *palaeoimereticum* Dekapr. & Menabde; *T. macha* var. *palaeocolchicum* Dekapr. & Menabde; *T. macha* var. *planocompressum* Menabde

Dough mixed from Makha flour was stuck easily to walls of a bread-baking oven (*tonéé* in Georgian, or tondir in other languages of the region), so the bread would not fall off and burn. Makha's bread was considered as of high quality among the local population. It was white, tasty and flavorful, not to mention its ability to remain soft for several days. It was honor to treat guests to 'makha' bread at feasts.

Triticum palaeocolchicum Menabde

T. palaeocolchicum (Colchis emmer) is a hulled tetraploid (AABB) wheat, endemic to Georgia. It is very similar to wild forms of tetraploid wheat due to its morphological characteristics. Its spikes contain up to 40 fertile spikelets. Leaves are broad. The stems are strong and tall (up to 120 cm). It's important agricultural characteristics include resistance to fungal diseases. Grains of Colchis emmer are distinguished by high protein content, and

high lysine content in protein. Colchis emmer was widely spread as mixture of the Makha landrace in West Georgia. The local population did not differentiate it from Makha wheat and grain of both species were milled altogether

Fig. 3. *Triticum palaeocolchicum Menabde (Colchis emmer)*

Taxonomic identification of Colchis emmer seems to remain a stumbling stone and deserves a special consideration. It was first described as *T. dicoccum* var. *chvamlicum* Supat. [15], and shortly afterwards determined as *T. diccocum* grex (subsp.) *georgicum* Dekapr. and Menabde [16]. Later, V. Menabde considered Colchis emmer under the name of *T. palaeocolchicum* [17], while Dekaprelevich applied *T. georgicum* Dekapr [18]. Dorofeev first considered *T. dicoccum* subsp. *georgicum* Dekapr. & Menabde as the scientific name for Colchis emmer [19]. However later Dorofeev et al. identified Colchis emmer as *T. karamischevii* Nevski [20]. The confusion with Colchis emmer continues in the present days. Van Slageren considers Colchis emmer as *T. turgidum* subsp. *palaeocolchicum* (Menabde) A. Love & D. Love [21], while MacKey as *T. turgidum* ssp. *georgicum* (Dekapr. & Menabde) MacKey [22]. The authors of the present paper proposed to conserve the name *T. palaeocolchicum*.

Colchis emmer is represented by 3 varieties in Georgia: T. *palaeocolchicum* var. *chvamlicum* (Supat.) Menabde; *T. palaeocolchicum* var. *rubidium* Menabde and *T. x palaeocolchicum* var. *nigrescens* Menabde.

Zanduri

The following two species represent the Zanduri landrace. It consists of three species: *T. monococcum* var. *hornemanii* (Gvatsa [narrow] zanduri), *T. timopheevii* (Chelta [wide] zanduri) and *T. zhukovskyi* (Zanduri). Gvatsa zanduri (*T. monococcum* var. *hornemannii*) is not endemic to Georgia, as it was widely spread in other regions as well and it is not considered in the present paper. However, chelta zanduri and hexaploid zanduri are found only in Georgia. Zanduri landrace was widely distributed in Lechkhumi and Racha in1930-ies, when they were described by the wheat scientists.

Triticum timopheevii (Zhuk.) Zhuk.

T. timopheevii (Zhuk.) Zhuk (Timopheevi wheat) is called Chelta Zanduri by the population of Georgia. It is a tetraploid (AAGG) late-maturing hulled spring wheat with leaf blades that are pubescent on both sides. Spikes are very compact, rather short, somewhat pyramidal in shape with soft, thin, rather short awns. Spikelets usually contain two kernels. Kernels are medium long, slender and hard or flinty. *T. timopheevii* is known as drought and frost resistant plant. Chelta zanduri owing to its special immunity to fungal diseases deserved particular attention of wheat breeders. It was used as a source cytoplasmic male sterility in wheat breeding. It is known by adaptation to all kinds of soils (even to limestone). Among its negative features hulled grains and difficulty in threshing should be mentioned.

Zhukovski found a two-kernel wheat on the way to village Mokhisi of the Gori district, in the neighborhood of village Asarma in 1922. He described it as a new variation of wild emmer *Triticum dicoccoides* var. *timopheevii* Zhuk. [11]. Later E. Stoletova in 1923 came across with a field of this plant

Fig. 4. *Triticum timopheevii (Zhuk.) Zhuk (Timopheevi wheat, Chelta Zanduri)*

in Lechkhumi, West Georgia. She recorded that the local population called it Chelta Zanduri [23]. In 1928, P. Zhukovski promoted this variation to the rank of species *T. timopheevii* (Zhuk.) Zhuk. and ascribed it to domesticated wheats [2, 24].

Bread baked from 'chelta zanduri' (*T. timopheevii*) flour was rather widespread in West Georgia but it was not as tasty and flavorful as that of Makha or Dika. In some cases, Zanduri landrace was used to produce poultry feed. *Triticum timopheevii* is represented by three varieties: *T. timopheevii* var. *typicum* Zhuk., *T. timopheevii* var. *viticulosum* Zhuk. and *T. timopheevii* var. *nigrum* Ericzjan

Triticum zhukovskyi Menabde et Eritczjan

T. zhukovskyi Menabde et Eritczjan (Zanduri**)** is a hexaploid (AAGGAA), late-maturing hulled spring wheat, a member of the Zanduri landrace. It was found in a Zanduri population in 1959 by V. Menabde and A. Eritczjan. They proposed that it should have originated through allopolyploidization of diploid gvatsa zanduri (*T. monococcum*) and tetraploid chelta zanduri (*T. timopheevii*).

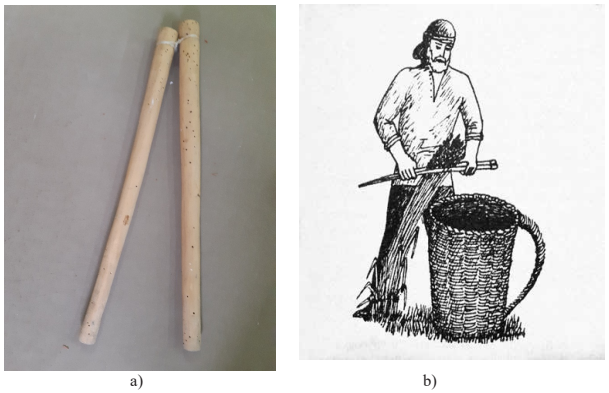
Fig. 5. *Triticum zhukovskyi Menabde et Eritczjan (Zanduri**)***

Farmers did not differentiate it from Chelta zanduri and the hexaploid plants did not have a special name. However, (results of karyological studies have shown) the authors found hexaploid plants (42 chromosomes), which were distinguishable from Chelta Zanduri through the ploidy level – *T. timopheevii* (28 chromosomes) and named it as *Triticum zhukovskiy* Menabde & Eritczjan – in honor of the outstanding researcher of *Triticum* and other cereals [25].

It is a very late maturing wheat. However, it is characterized by wide adaptation, frost and drought resistance. Bread baking quality is similar to that of Chelta zanduri.

Interestingly, the local population developed special tools top harvest hulled wheats Makha and Zanduri. First the wheat spikes were harvested by Shnakvi (Fig 6), a special tool consisting of two sticks tied together and gathered in baskets. After wheat stems were cut with sickles and bundled. The bundles were used to cover the roofs of houses and barns.

Georgian historians such as T. Kaukhchishvili and T. Mikeladze found evidence of wheat cultivation in ancient Georgia in the works of Ancient Greek historians Herodotus and Xenophon, respectively [26, 27]. Iv. Javakhishvili suggested in 1930 that the names of the ancient Georgian wheats: Dika, Zanduri, Makha and others were first mentioned in Georgian written sources as early as the V century AD [28]. Records about wheat production in Georgia are available in the works of Sulkhan-Saba Orbeliani (1658-1725; "Sitkvis Kona", Georgian Vocabulary), Vakhushti Batonishvili (1696–1757; "Description of Kingdom of Georgia, its habits and canons"), as well as in the travel notes naturalists of XVIII-XIX centuries such as Johann Anton von Güldenstädt [29], Johann Gottlieb Georgi [4, 30].



a) b)

Fig. 6. *a) Shnakvi, a special tool to harvest wheat heads in Georgia and b) A wheat farmer is harvesting wheat spikes using shnakvi [4]*

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Cluster Chelates on the Basis of Natural Raw Materials

Iamze Beshkenadze[a], Maia Gogaladze[a], Nazibrola Klarjeishvili[a], Nikoloz Zazashvili[b], Marina Chikaidze[b], Omar Lomtadze[a], Vakhtang Gabunia[a]

[a]Iv. Javakhishvili Tbilisi State University, P.Melikishvili Institute of Physical and Organic Chemistry, 31, A.Politkovskaia Str., Tbilisi, 0186, Georgia

[b]Biorational Technologies Research Center (BrTRC), 8, Iumashevi Str., Tbilisi, 0151, Georgia

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A B S T R A C T

The synthesis methods are shown and Mn, Zn and Cr cluster chelate compounds are synthesized on the basis of metal acetates and concentrate DAS (Ds) of vegetable origin. Different number of metals is bonded to each other in the synthesized compounds depending on synthesis conditions. The individuality of synthesized compounds is studied using trace element analysis and X-Ray radiography methods, as well as through melting temperature determination. Qualitative solubility of compounds in different solvents is defined. An experiment on earthworms was carried out in order to determine biological activity of synthesized cluster chelates. For this purpose three doses (maximum, minimum and normal) of mixtures of Mn, Zn and Cr compounds were prepared and their impact on earthworms' protein mass and degree of cocoon reproduction was studied. Effective and optimum doses of chelate compounds were established. Based on the results of conducted experiments it may be said that maximum mass change (115.66%) takes place in that test group, to which a minimum dose of chelate mixtures is added, while the degree of cocoon propagation reaches the maximum value (576.32%) in that test group, to which a normal dose of chelate mixtures is added. Thus, on the basis of carried out researches a conclusion is drawn that a balancing of earthworm substrate with different doses of Mn, Zn and Cr compound mixtures has a positive impact on both earthworm's protein mass gain and on the substantial increase of degree of cocoon reproduction, and 0.38g per 200 g of substrate is the optimum effective dose for cluster chelate mixtures. For the same chelate mixtures of Mn, Zn and Cr the preliminary check-up tests were conducted on broiler chickens. Based on analysis of obtained results (7.5% weight gain in the test group during upbringing period, and survival is 3.3% higher compared to control group) we deem reasonable to carry out experiments on a wider scale for determination of effective and optimum doses of chelate mixtures.

Keywords: Cluster, Chelate, Vermiculture, Worm, Cocoon, Concentrate.

*Corresponding author: Iamze Beshkenadze; E-mail address: iamzebeshkenadze@yahoo.com

Introduction

Population provision with high-quality, ecologically safe agricultural products is very topical as of today. Their provision with definite quantitative and qualitative composition and optimum ratio of microelements is one of the most important prerequisites of solution of this problem. It can be attained through creation and use of premixes containing chelate microelements, as far as the digestibility of chelate microelements by living organisms roughly equals to 60-70%, while in case of simple inorganic forms it is equal to 7-10%. Today the synthesized biologically active organic substances (amino-acids, oxy-acids etc.) are mainly used for microelements' chelation [1-15]. However there are only scarce data on chelates' obtaining on the basis of natural raw materials and on their use in the feed of poultry (agricultural birds) and farm animals [16-18]. Taking into account a number of positive factors (diversity and, respectively, high biochemical activity of natural raw materials, their cheapness and accessibility) we selected DAS – the concentrate of vegetable origin obtained on the base of

Biorational Technology Research Center as a research subject. It is distinguished by the diversity of organic substances of different classes entering its composition, and, respectively, by high biological and chemical activity [19-22]. In order to establish the biological activity of chelates obtained on their and metals' (Manganese, Zinc and Chromium) basis their impact on earthworm mass and degree of reproduction is studied. Experiment was conducted on a new species of earthworms, called "Georgian New", which is cultivated at the biofarm of the company Macro-Prim LLC by selectionist Guram Gejadze [23]. Preliminary check-up tests on broilers were conducted as well, taking into account the obtained results.

Methods used

- Trace element analysis – for determination of metal percentage in synthesized compounds;
- Melting temperature determination and X-Ray radiography study – for establishment the individuality of chelates;
- Solubility – for determination of qualitative solubility of chelate compounds in different solvents.

We have conducted the experiment on the earthworm according to the methodology, developed by us [24,25] and we have used for this purpose:

- Weighting method – for determination of earthworm mass; this method was used for determination of broiler live weight, as well;
- Count method – for identification of cocoons number.

Results and discussion

Within the frameworks of memorandum concluded between the Agrarian Chemistry laboratory of P. Melikishvili Institute of Physical and Organic Chemistry at Iv. Javakhishvili Tbilisi State University and Biorational Technology Research Center there were conducted studies aimed to determination of synthesis conditions for chelate compounds on the basis of Mn, Zn and Cr and concentrate DAS, as well as to synthesis and study of their biological activity. With observance of proper synthesis conditions and based on acetates and DAS so-called homonuclear, cluster-type chelate compounds of Manganese, Zinc and Chromium are obtained, in which the different number of metal atoms are bound to each other according to synthesis conditions (Table 1).

As is seen from the Table, in case of 0.02-0.03 mole Mn(CH3COO)2.4H2O+20ml.Ds ratio of reacting components the percentage of Manganese in compounds obtained in water bath through evaporation varies within the limits of 7.9-18.52%, while for that obtained through separation – within the limits of 23.10-43.43%. In case of 0.03-0.05 mole Zn(CH3COO)2.2H2O+15ml.Ds ratio the percentage of Zinc in compounds obtained in water bath through evaporation varies within the limits of 5.37-21.05%%, while for that obtained through separation – within the limits of 44.10-57.92%. For 0.03-0.05mole Cr(CH3COO)3+20ml.Ds ratio the percentage of Chromium in compounds obtained in water bath through evaporation varies within the limits of 10.63–18.22%, while for that obtained through separation – within the limits of 20.33-28.57%.

Table 1 . *Metal percentage in cluster chelates*

Ratio of reacting components	Synthesis conditions	Metal %
0.02–0.03 mole $Mn(CH_3COO)_2.4H_2O$ +20ml.Ds	Evapor. in water bath	7.9–18.52
	Separation	23.10–43.43
0.03–0.05 mole $Zn(CH_3COO)_2.2H_2O$ +15ml. Ds	Evapor. in water bath	5.37–21.05
	Separation	44.10–57.92
0.03–0.05 mole $Cr(CH_3COO)_3$+20ml. Ds	Evapor. in water bath	10.63–18.22
	Separation	20.33–28.57

In Table 2 some physical-chemical characteristics of cluster chelate compounds are given. As is seen from the Table, melting temperature of Mn and Zn compounds obtained via separation method is more than 300°C, while for Cr-chelate equals to 150°C. As for compounds obtained through evaporation, they are adhesive ones and melt at lower temperature, roughly within the limits of 55-65°C. Definite regularity is observed regarding solubility of compounds obtained through separation and evaporation in different solvents. In particular, compounds obtained via evaporation method are characterized by good water solubility, and compounds generated through separation are virtually insoluble Compounds obtained via both methods, are characterized by low or poor solubility in organic solvents.

Table 2. *Some physical-chemical properties of cluster chelates*

Chelates	Color	Melting ^{0}C	Solubility			
			Water	Alc.	Acet.	DMS*
Mn·Ds (via evapor.)	White-pinky	65	+	Low sol.	Low sol.	Low sol.
Mn·Ds (via selection)	Dark-pinky	>300	–	Virt. insol.	Virt. insol.	Virt. insol.
Zn·Ds (via evapor.)	White	60	+	Low sol.	Low sol.	Low sol.
Zn·Ds (via selection)	White	>300	_	Virt. insol.	Virt. insol.	Virt. insol.
Cr·Ds (via evapor.)	Blue-violet	55	+	Low sol.	+t	Low sol.
Cr·Ds (via selection)	Dark-violet	>300	+t	Low sol.	Low sol.	Low sol.

DMS*- Dimethylsulfoxyde

In addition to melting temperature determination, the individuality is established using diffractometric method, as well. X-ray diffractometric study is conducted using "ДРОН-4" at Cukα (λ=0.154184nm.) radiation. During exposition, the samples were rotated in their own plane by means of special device "ГП-13". For comparison purposes, the X-ray patterns of initial salts were taken, as well. As is seen from X-ray pictures that reflect the dependence between angle of reflection 2θ (in degrees) and relative intensity towards the greatest peak I/Io, chelate compounds containing chromium and Das are amorphous solid substances [pictures 1and 2]. X-ray pictures of Zinc-DAS, Manga-

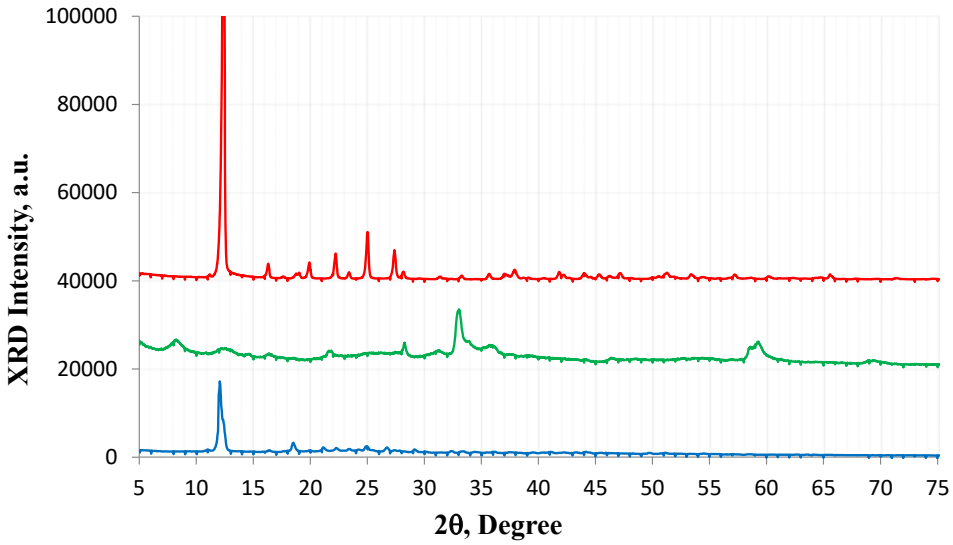

Fig. 1. *X-ray of Zinc-DAS chelate compounds*
Zn-acetate, ---- Zn·Ds (via evapor.), ---- Zn·Ds (via selection)

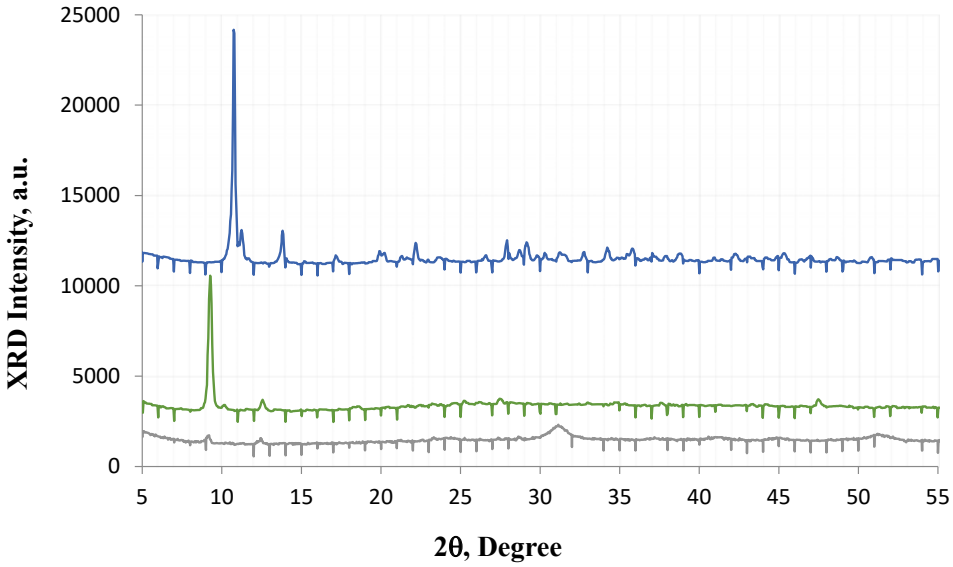

Fig. 2. *X-ray of Manganese -DAS chelate compounds*
---- Mn-acetate, ---- Mn·Ds (via evapor.), ---- Mn·Ds (via selection)

nese-DAS compounds, obtained via separation and evaporation, as well as of their initial salts differ markedly from each other.

X-ray pictures of new synthesized compounds don't contain diffraction maximums peculiar for initial compounds. So, based on X-ray patterns' analysis it may be said that Chromium-DAS, Zinc-DAS and Manganese-DAS clusters obtained via separation and evaporation are individual compounds.

In order to study biological activity, three doses of Mn, Zn and Cr-chelate mixtures (maximum, minimum and normal) were prepared based on concentrate DAS. When conducting tests on earthworms we set a goal to establish under the impact of chelate mixtures added to substrate: a) earthworm protein mass change; b) degree of cocoon reproduction and c) effective and optimal dose of chelate mixtures. In total, we tested four– one control and three test groups, each one with three repetitions: control group substrate of all three repetitions was dampened by ordinary water, I test group – by aqueous solution containing maximum (0.76g.) dose of chelate mixtures, II test group – by aqueous solution containing normal (0.38g.) dose of chelate mixtures, and III test group – by aqueous solution containing minimum (0.76g.) dose of chelate mixtures. 200g. of dry substrate and 5 earthworms of roughly equal mass were selected for all repetitions of both control and test groups. First weighing and cocoons calculation were made on the 21st day of experiment, while second and third – on 31st and 41st days, respectively. During all three weighing procedures, we calculated average mass and average number of cocoons for each group. After completion of the experiment an average mass change (in g. and percentage terms) and average number of cocoons (in pcs and percentage terms) overall for all stages was calculated for control and all three test groups (Table 3).

Table 3 . *Chelate mixture composition added to premix calculated for 50 kg of combined feed*

Mixture composition	Mass (g.)
Mn·Ds	18.2
Zn·Ds	11.54
Cr·Ds	2.11

As is seen from the Table, weight gain takes place at all three stages throughout the experiment. Compared to initial mass, the average mass change equals 1.98g. in control group and we conventionally took it as 100%. In parallel, the average mass change in I test group (Max.) is 99.49% (1.97g.), in II test group (Norm.) – 106.06% (2.1g.), while in III test group (Min.) – 115.66% (2.29g.). As the Table shows, the degree of cocoon reproduction is far more in all test groups, compared to control one. Particularly, if we conventionally take the average number (in pcs) of cocoon reproduction – 2.66 pieces as 100%, then it equals to 484.58% (12.89 pieces) in I test group, 576.32% (15.33 pieces) in II test group, while in III test group it is 559.65% (14.89 pieces).

Thus, based on the analysis of experimental results it may be said that maximum mass change takes place in III test group 115.66% (2.29 g.), when a minimal dose of chelate mixtures is added, while the degree of cocoon reproduction reaches its maximum value 576.32% (15.33 pieces) in II test group, to which a normal dose of chelate mixtures is added.

Preliminary check-up tests were conducted on broiler chickens for the same chelate mixtures. Two groups were composed for this purpose – test and control. 15 birds were included in each group, and experiment lasted for 35 days. First, the quantity of necessary combined feed was calculated (50kg.), and afterwards – the quantity of cluster chelate mixture added to premix intended for this quantity of feed (Table 4).

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Table 4 . *Main zootechnical indicators of broilers*

Indicators	Groups	
	Test	Control
Live weight (gr): 1-day	40	40
7-day	112	108
14-day	314	292
28-day		
Hen (female)	1026	965
Rooster (male)	1195	1039
Average	**1110.5**	**1039**
35-day		
Hen (female)	1715	1595
Rooster (male)	1845	1700
Average	**1780**	**1647.5**
Daily average weight gain in upbringing period, %	50.86	47.05
Bird survival in upbringing period, %	96.6	93.3

The basic experiment started on 1-day chickens, and combined feed was prepared and delivered in three stages: I stage – 1-7 days, II stage – 7-21 days, III stage – 21-35 days.

During the test there were studied:

- broiler growth and development – through individual weighing on 7, 14, 21, 28 and 35 days.
- average and daily gain;
- bird survival in upbringing period

Main zootechnical indicators of broilers are given in Table 5.

Table 5. *Main zootechnical indicators of broilers*

Groups (dose, gr.)	Container #	Test stages														Results			
		Initial		I weighing				II weighing				III weighing				Mass change		Aver. quant. of cocoons	
		mass (g)	mass (average)	mass (g)	mass (average)	quantity of cocoons	quantity of cocoons (ave.)	mass (g)	mass (average)	quantity of cocoons	quantity of cocoons (ave.)	mass (g)	mass (average)	quantity of cocoons	quantity of cocoons (ave.)	(g)	(%)	(piece)	(%)
Control 0.00	1	1.12	1.18	3.01	2.99	1	1.33	3.15	3.10	5	4.33	3.21	3.16	2	2.33	1.98	100	2.66	100
	2	1.23		2.94		1		3.01		4		3.11		2					
	3	1.20		3.01		2		3.15		4		3.16		3					
Max.(I) 0.76	4	1.34	1.21	3.80	3.43	4	3.67	3.14	3.21	20	19.33	3.12	3.18	17	15.67	1.97	99.49	12.89	484.58
	5	1.14		3.31		3		3.35		19		3.32		15					
	6	1.15		3.18		4		3.15		19		3.09		15					
Norm.(II) 0.38	7	1.24	1.19	3.31	3.22	7	7.67	3.47	3.31	19	19.67	3.49	3.29	18	18.67	2.1	106.06	15.33	576.32
	8	1.29		3.17		8		3.22		20		3.17		19					
	9	1.04		3.18		8		3.25		20		3.23		19					
Min.(III) 0.19	10	1.40	1.32	3.81	3.53	7	6	3.83	3.64	19	19.33	3.82	3.61	19	19.33	2.29	115.66	14.89	559.65
	11	1.41		3.46		5		3.71		19		3.66		19					
	12	1.16		3.32		6		3.37		20		3.35		20					

It is seen from the Table that in 14-day age the live weight of first group broilers prevails by 7.0% those of control group, by 6.4% at the age of 28 days. In the end of the upbringing period – in 35-day age the live weight of test group broiler is 1780g. in average that is 7.4% more than in control group. Daily weight gain in test group is 50.86g. throughout the upbringing period that is averagely 7.7% more compared to control group. Survival in test group was 96.6% in the upbringing period, and 93.3% - in control group.

On the basis of carried out experiments we deem necessary to conduct tests on a wider scale.

Conclusion

Based on carried-out researches the following conclusions can be drawn:

- So-called homonuclear cluster chelate compounds are obtained on the basis of microelements and concentrate DAS, where the atoms of the same metals, number of which depends on synthesis conditions, are bound to each other. Melting temperature and solubility in different solvents depend on number of atoms entering the composition of compounds, as well. Organic substances of different classes forming concentrate DAS are in coordinate bonds with metal atoms in chelate clusters. Under the proper synthesis conditions, in addition to homonuclear clusters, it is possible to obtain heteronuclear, similar- and different-ligand clusters. This fact makes it possible to obtain cluster chelate compounds with pre-planned desirable quantitative/qualitative composition and, respectively, with desirable biological activity.

Through study of biological activity of Mn, Zn and Cr-chelate mixtures prepared on the basis of concentrate DAS it was established that:

- Earthworm substrate balancing with different doses of mixtures has a positive impact on both earthworm protein mass increase and

on the substantial increase of degree of cocoon reproduction. 0.38g. per 200g. of substrate is considered as an optimum, effective dose of chelate mixtures. We suppose that the obtained effect is preconditioned by both Mn, Zn and Cr impact and by the diversity of biologically active compounds of different chemical classes entering into concentrate DAS composition. The obtained results are of great importance for that direction of vermiculture, in which a protein mass is used for balancing combined feed intended for poultry and farm animals as vitamin-protein, high-quality and concentrated additive.

• Based on the results of check-up tests (7.5% weight gain in the test group during upbringing period, and survival is 3.3% higher compared to control group) conducted on broilers for Mn, Zn and Cr-chelate mixtures we deem reasonable to carry out experiments on a wider scale for determination of effective and optimal dose of chelate mixtures.

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Drinking Water Treatment Technology for Microbial Contamination by Means of Cavitation Method

Nikoloz Enuqidze*, Tsitsino Kurtskhalia, Zaira Simonia, Nana Sakvarelidze, Nodar Ckeidze

G.Tsulukidze Mining Institute, 7, Mindeli Str., Tbilisi, 0186, Georgia

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A B S T R A C T

The aim of the work is to undertake systemic research of the treatment process of drinking water contaminated with microorganisms by means of cavitation method; development of technology and creation of experimental treatment system that will significantly reduce the cost and ensure high quality of treatment process. A jet micro-cavitator with the capacity of 5-10 l/h has been developed to conduct laboratory research. The selection of design of the cavitator was based on the following: low consumption of sample liquids, pressure range 1-5 bar, simple and flexible design. The novelty of the created activator is due to the fact that, as of today, activator with such a low capacity does not exist. On the basis of the research, cavitation and filtration were identified as two main nodes of the treatment system. Cavitation technology for treatment of the microbially contaminated drinking water has been developed and optimal technological parameters have been defined for the treatment. Target experimental technological system has been created. Water obtained in the system meets the requirements of drinking water technological regulation by its organoleptic, physical and chemical as well as microbiological parameters. The proposed system, in future may become a new technical installation for treatment of drinking water sharply reducing the costs of appropriate devices and simplifying their application and ensuring high level of safety.

Keywords: Water, Microorganisms, Cavitation, Filtration, Technology, Experimental System.

*Corresponding author: Nikoloz Enuqidze: E-mail address: enuqniko@gmail.com

Introduction

Quality of drinking water, its chemical and microbial safety, is among the main topics for ensuring human health. As of today, despite the existence of many treatment systems that apply various methods for removal of microbial contamination, the costliness and difficulty of maintenance of those systems restrict their widespread.

Ozonation, chlorination and ultraviolet (UV) radiation constitute the main conventional methods of water disinfection [1]. These three methods have strong disinfection effect on both bacteria and viruses. Application of chemical methods (ozonation, chlorination) result in byproducts that deteriorate water quality. In case of UV application required intensity of radiation should be defined (according to set regulations) and strongly followed to avoid photoreactivation (reparation), of microbes induced by incorrect dozes. Hence, application of the method requires high qualification of personnel.

Development of new technologies of water treatment and consequent technological systems ensuring reduced cost and simplified maintenance as well as required level of safety is very important.

Research Methods and Objects

The aim of the proposed work is to study treatment process of drinking water contaminated with microorganisms by application of cavitation method, develop technology by considering achieved results and create consequent technological system that would significantly reduce the costs and ensure high qulity of the treatment.

Hydrodynamic cavitation belongs to non-con-

ventional technologies of removal of microbial contaminants from water. Generally, cavitation is the breakdown of liquid continuity caused by sharp decrease of local pressure in comparison with the saturated vapor pressure of liquid. In the liquid- free zones bubbles containing mixture of liquid vapor and gases dissolved in liquid are formed. In the process of formation of bubbles minimal pressure varies in the range of 100-2500 kPa. Different types of inserts, such as microbubbles, solid microparticles, including microorganisms stimulate formation of bubbles. In the areas of low pressure bubbles grow, merge and form cavities. When pressure increases bubbles compress, deform, decompose or collapse. The process is accompanied with sharp increase of local temperature and pressure generation of cumulative microflows, synthesis of strong oxidizers (O, H_2O_2) that causes lysis of microorganisms in the liquid [2].

Cavitation can be generated by different mechanisms e.g. light photons of high intensity (laser), strong electric discharge, high frequency acoustic waves (ultrasound cavitation). Hydrodynamic cavitation takes place when rapid passage of water through pipes of various cross section is being applied. In case of pipes with small cross section the rate of flow increases, and the pressure decreases that induces breakdown of liquid phase and formation of bibles. When the liquid passes through the pipes with large cross section the flow rate of liquid decreases, pressure increases causing compression, deformation, fragmentation or collapse of bubbles. Cavitation actively progresses predominantly in vortex flows of liquids.

Influence of ultrasound cavitation of microorganisms is well studied [3,4], while data on effects of hydrodynamic cavitation on bacteria are rather limited [5].

When selecting hydrodynamic cavitators the following fact was considered: ranges of frequencies and intensities applied during production of emulsions coincide with that of ultrasounds being lethal for microorganisms.

Formation of emulsions in hydrodynamic emulgent-cavitator has been well studied. Since parameters (frequencies, ultrasound, power on 1cm^3, length of exposure) of influence of cavitation are close for microorganisms and emulsioning processes, for assessment of effects of cavitation on microorganisms characteristics of dispersion agents of various types can be applied (Table 1).

Table 1. *Characteristics of Various Types of Cavitation Dispersion Agents*

Types of dispersion agents	Capacity l/minute	Diameter of particles (μ)	Power kW	Specific power kW/m^3
Valvular	5000	0.8-2.5	37	7.4
Ultrasound	30	1.6	1.4	13.3
Jet	1000	1-1.25	4.4	4.4
Vortex on the basis of valvular A1-ОГ2М	5000	0.77-1.05	19	3.8
Hydrodynamic rotor-vortex Я5-ОЭА	300	1-2.5	0.15-0.175	0.5-0.6
Hydrodynamic rotor-vortex Я5-ОММ	3500-4000	1-2.5	2.1	0.5-0.6
Hydrodynamic rotor-vortex Я5-ОММ	6000-7000	-	2.5	0.4

As the table demonstrates rotor-vortex cavitators are more energy efficient in comparison to other types of cavitators [6]. Hydrodynamic cavitator consists of two main parts: cavitator itself and medium-pressure pump (0.4-1 MPa). Assemblage of hydrodynamic cavitator is simple and cheap. Main cost is related to pump. Cost of installations with the capacity of 30-40t/h is 4800- 100000 USD. Relative cost of the installation applied for cavitation treatment of 1tone liquid is within the range of 1600-2500 USD while the cost of the ultrasound installation with the capacity of 1m3 is in between 14000-22000 USD [7].

When comparing costs for treatment of water of certain volumes by various applicable methods cavitation method appearss to be the cheapest one. Expenditures are as follows: for cavitation 162 $, ultrasound treatment 261 $, chlorination 482 $ and ozonation 1600 $. [8]

The emphasis is made on hydrodynamic cavitation due to cheapness of its generation that results in cheapness of consequent device.

The study objects are drinking waters contaminated with microorganisms due to technogenic processes. Microorganisms - E.coli, St.faeculis, Ps. Aeruginosa and Typhimurium that according to ISO present indicators of contamination were used. Analysis were performed in line with the following ISO methods - ISO 9308–1:214; ISO 9899–2:00; ISO 16266–06 and ISO 19250–10.

Spectrophotometer DR-2800 LPV manufactured by „HACH LANGE" and Turbidimeter HI 93703 C and combined device HI 98204 – pH/ ORP/ EC/ T of "HANNA instruments" were used for determination of phisical and chemical parameters of water.

For laboratory research, the jet micro-cavitator (Fig. 1.) with capacity of 5-10 l/h was created, the selection of the design of which was based on the following considerations: Low consumption of sample liquids; Pressure range 1-5 bar; Simple and easily modified design.

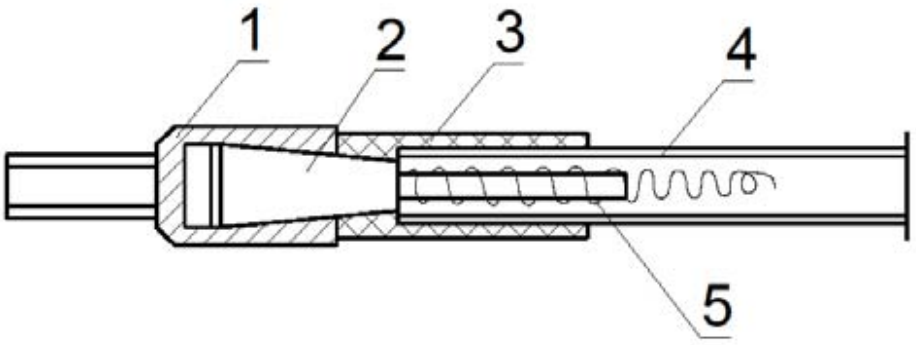

Fig. 1. *General scheme of jet micro-cavitator*

1. connector Luer-Lock; 2. injection needle (liquid accelerator); 3. tightener; 4. cavitation pipe; 5. hydraulic resistance (spiral).

It should be considered that production and operation of jet-cavitator is simpler and cheaper in comparison to other types of cavitators. In the given cavitator for acceleration of liquid stainless steel pipes (injection needles) with diameters of 0,1–0,55mm and length of 2-4 cm and for hydraulic resistance combination of spirals and nodes were used. The novelty of the proposed jet micro-cavitator derives from the fact that as of today low capacity micro-cavitator applicable for the continuous systemic laboratory research of cavitation processes does not exist [9–12]

Discussion and Analysis of the Results

To carry out experiments, cavitation test-bench (Fig. 2.) consisting of jet micro-cavitator produced by the authors was assembled.

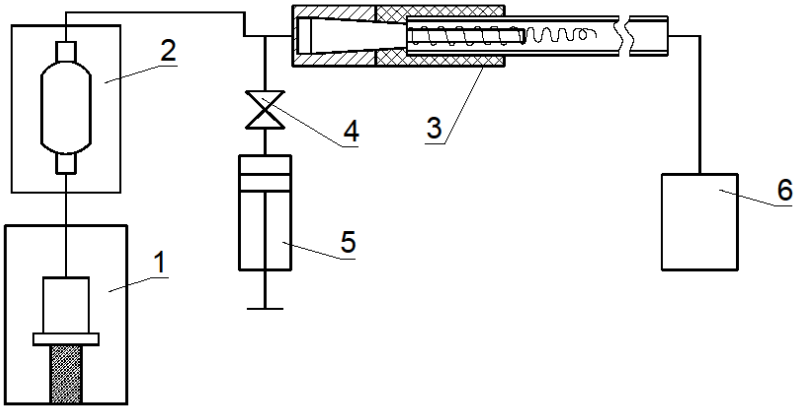

Fig. 2. *Hydrodinamic cheme of cavitation test-bench*

1. vessel for sample water; 2. pump-dozer with reversing valve and filter; 3. jet micro-cavitator; 4. Stopcock; 5. syringe for filling the hydraulic system; 6. vessel for cavitated water.

Experiments were conducted for the treatment of drinking water with microbial contamination. The data listed in the tables (Table 2.) are mean values of the parallel tests.

To avoid incomplete oxidation of microorganisms (due to insufficiency of oxygen – 1 l water contains 8 mg oxygen) in the process of cavitation some of the drinking water samples were pretreated with hydrogen peroxide (H_2O_2).

Table 2. *Microbiological Analysis of Cavitated Water*

#	Drinking water contaminated with microorganisms	Quantity of Microorganisms, CFU*/100 ml				
		Initial	Samples after cavitation			
			1	2	3	4
1	E.coli	900	Not present	Not present	Not present	Not present
2	Ps.aeruginosa	800	–,,–	–,,–	–,,–	–,,–
3	St.faecalis	7900	–,,–	–,,–	–,,–	–,,–
4	Typhimurium	850	–,,–	–,,–	–,,–	–,,–

* – Colony Forming Unit

For each microorganism four samples were prepared (table 2). First two samples (sample 1, sample 2) of each microorganism were not pretreated with hydrogen peroxide other two (sample , sample 4) were pretreated. For the first and third samples duration of cavitation was 5-10 minutes and for the second and fourth samples cavitation took place in the process of single passage. The same sequence was applied for all four samples. Cavitation process war conducted at pressure of 4-5 bar. According to results none of the cavitated samples contained live microorganisms. Hence, it can be concluded that in the conditions of given concentrations of microorganisms water can be purified without its pretreatment with hydrogen peroxide and the duration of cavitation does not exceed 5 minutes. The same results were obtained in the process of single passage of the solution.

The research was continued in the conditions of single passage of water through cavitator.

Subsequent experiments were carried out on samples treated with hydrogen peroxide to avoid incomplete oxidation in the conditions of different concentrations of contaminating microorganisms. To define optimal value of one of the main technological parameter-pressure, experiments on water samples contaminated with E. coli and Ps. Aeruginosa were carried out. Experiments were conducted on two different concentrations of each microorganism. Pressure was altered between 1-5 bar. Sample number indicates the pressure(bar) during the cavitation process (Table 3).

Table 3. *The Results of Cavitation of Water Contaminated with Microorganisms (in the conditions of single passage)*

Pressure during cavitation process	Quantity of microorganisms, CFU*/100 ml							
	E.coli				Ps. Aeruginosa			
	Initial	Cavitated	Initial	Cavitated	Initial	Cavitated	Initial	Cavitated
1	170	340	780	156	210	Not present	660	Not present
2	–,,–	185	–,,–	45	–,,–	–,,–	–,,–	–,,–
3	–,,–	Not present	–,,–	1	–,,–	–,,–	–,,–	–,,–
4	–,,–	–,,–	–,,–	Not present	–,,–	–,,–	–,,–	–,,–
5	–,,–	–,,–	–,,–	–,,–	–,,–	–,,–	–,,–	–,,–

* – Colony Forming Unit

At relatively higher pressure (4-5 bar) complete lysis of microorganisms takes place. When applied low pressure (1,2 bar) in case of E.coli increase of quantity of microorganisms is observed that is possibly associated with destruction of microbial organic components during cavitation and production of further deformation products representing food for the microorganisms and inducing their growth [1].

According to the obtained results it can be concluded that by means of proposed design of the jet cavitator high degree of lysis of microorganisms can be attained in the conditions of regulating pressure in the process of single passage of water.

By-products of cavitation - products of complete and partial oxidation of microorganisms and their lysis-destruction were analyzed. The results of the analysis enable selection of methods for removal of those substances.

Literature review shows [13-16] that main content of microbial cell is water (80-85%). Dry component presents 15-25% out of which 50-80% is proteins of various types. N, C, O, H are organogen of dry residue. In the presence of oxygen those elements transform into gaseous state and evaporate. Due to incomplete oxidation of amino acids that are building blocks of all proteins, cavitated drinking water may contain inorganic compounds containing nitrogen - NO_2^- , NO_3^- , NH_4^+. The turbidity of cavitated drinking water may also be ascribed to content of products of microorganisms' destruction (Table 4).

Table 4. *Characteristics of Cavitated Water*

Samples	Pressure, bar	Microorganisms														
		E.coli					Ps. Aeruginosa					St.faecalis				
		COD* mg O_2/l	Turbidity FTU**	NO_2^-,	NO_3^-,	NH_4^+,	COD*	Turbidity	NO_2^-,	NO_3^-,	NH_4^+,	COD* mg O_2/l	Turbidity FTU**	NO_2^-,	NO_3^-,	NH_4^+,
Water contaminated with microorganisms		13,0	0,6	0	0,1	0	14,0	1,03	0	0	0	12,0	1,0	0	0	0
Cavitated water contaminated with microorganisms	1	1,56	1,42	,,	0,2	,,	1,95	2,11	,,	0,20	,,	1,56	2,01	,,	,,	,,
	2	1,50	2,09	,,	0,2	,,	1,95	2,16	,,	0,20	,,	1,48	1,50	,,	,,	,,
	3	1,36	0,88	,,	0,2	,,	1,85	1,48	,,	0,20	,,	1,30	2,16	,,	,,	,,
	4	1,36	0,99	,,	0,2	,,	1,50	2,59	,,	0,25	,,	1,30	2,40	,,	,,	,,
	5	1,30	0,99	,,	0,2	,,	1,50	2,00	,,	0,25	,,	1,30	2,40	,,	,,	,,

* Chemical Oxygen Demand

** FTU Unit of Turbidity, defined according to Formazin

Experiments were carried out on the pressure between 1-5 bar. The obtained results reveal that in the conditions of cavitation the complete destruction of microorganisms takes

Experiments were carried out on the pressure between 1-5 bar. The obtained results reveal that in the conditions of cavitation the complete destruction of microorganisms takes place mainly due to their complete oxidation (sharp reduction of chemical oxygen demand). The tendency of increasing turbidity of cavitated water demonstrates that destruction of relatively small amount of microorganisms is linked with their fragmentation, splitting.

Low turbidity may be caused by small sizes of products of microorganisms' splitting. Applied method (turbidimetry) is based on the characteristics of particles to scatter transmitted light. The intensity of scattering effect depends on the size of particles.

Filtration method was applied for the removal of microorganisms' destruction splitting by-products. Tests were made on comparative filtration system (Fig.3).

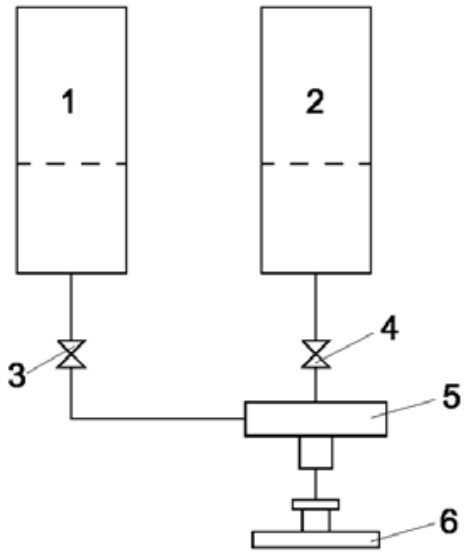

Fig. 3. *Hydrodynamic Shceme of Filtration Test-Bench*

1. blank reservoir; 2. sample reservoir; 3, 4. stopcock; 5. Trifurcate; 6. syringe filter

Syringe filters of 0,45 micron with 25 mm diameter were used as filters (6). Assessment of contamination level sensitive to filter is done on the test-bench: by means of connector filter (6) is fixed to the trifurcate (5), stopcock is opened (3) and the blank (saline) is injected into the filter. Height of hydraulic head is 65 cm. The flow rate of liquid in the filter is measured by timer(ml/sec.). Stopcock is closed (3) and opened (4)-sample is injected into the filter and the flow rate of the sample passing through the filter is measured (Table 5.).

Table 5. *Data of Filtration Processes*

Object of Study	Volume of filtrate, ml	Filtration time, sec	Flow rate of liquid, ml/sec.	Filtrate turbidity FTU		
				Microorganisms		
				E.coli	Ps. Aeruginosa	St.faecalis
Saline	10	70	0,14	0	0	0
	20	150	0,13	,,	,,	,,
Water contaminated with microorganisms	10	100	0,10	1,27	3,01	1,30
	20	260	0,07	,,	,,	,,
Cavitated water contaminated with microorganisms	10	120	0,08	0,70	0,72	0,68
	20	290	0,06	,,	,,	,,

* FTU Unit of Turbidity, defined according to Formazin

Waters with different levels of contamination (drinking water contaminated with microorganisms and its cavitate) and saline (pharmacological preparation) were filtered, the filtration rate of which was considered as relative standard. The flow rate (ml/sec) of cavitated (at various levels) water contaminated with microorganisms was measured in the conditions of obtaining equal volume of filtrate. The results (table 5.) confirm formation of small particles in the process of cavitation. The particles slightly affect increase of filter resistance.

As the result of performed experiments cavitation and filtration were identified as constituent nodes of the target system (experimental system of treatment of drinking water contaminated with microorganisms). A new experimental system was created (Fig. 4).

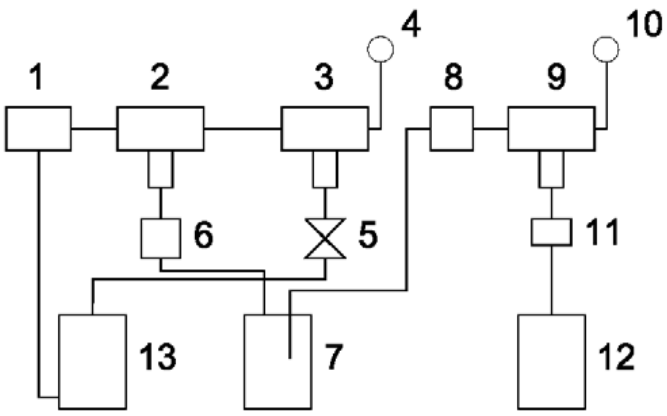

Fig. 4. *Hydrodynamic Shceme of ExperimentalSystem for Water Treatmentn*

1. pump-dozer BL-10; 2,3. trifurcate; 4. Manometer; 5. pressure regulator; 6. Cavitator; 7. reservoir for cavitated water; 8. pump-dozer BL-5; 9. trifurcate; 10. manometer; 11. 0,45 μm membrane filter; 12. reservoir for filtered water; 13. reservoir for raw water.

Sample water from reservoir (13) is pumped (1) to cavitator (6). In the system pressure is regulated with bypass stopcock (5) by controlling manometer (4). Liquid from the cavitator accumulates in the reservoir (7) from where it is pumped to the 0,45 μm membrane filter (11) by pump-dozer(8). Passing the membrane filter the liquid accumulates in the reservoir (12).

Synchronisation of the capacities of the cavitator and the filter is performed by regulating capacity of pump-dozer.

Table 6. *The Results of Treatment of Water with Microbial Contamination Conducted on the Experimental System*

#	Sample	Quantity of Microorganisms CUI/100მლ		Physical and Chemical Parameters					
				E.coli			Ps. Aeruginosa		
		E.coli	Ps. Aeruginosa	pH	COD* mg O_2/L	Turbidity FTU**	pH	COD* mg O_2/L	Turbidity FTU**
1	Water contaminated with microorganisms	700	800	8,12	13,00	1,07	8,13	14,00	1,70
2	Cavitated water contaminated with microorganisms	Not present	Not present	8,15	1,56	1,27	7,20	1,95	3,01
3	Filtrate of Cavitated water contaminated with microorganisms	Not present	Not present	8,17	1,50	0,70	8,50	1,90	0,72

* Chemical Oxygen Demand

** FTU Unit of Turbidity, defined according to Formazin

Water obtained in the experimental system, according to its chemical (pH, COD), organoleptic (turbidity) and microbiological parameters (table 6) meets the demands of Technical Regulation of Drinking Water.

Conclusion

Based on the obtained results of the systemic researches experimental system for treatment of microbially contaminated drinking water was created. The system consists of the two main nodes-cavitation and filtration. The capacity 5-10 l/h of the microcavitator created within the frameworks of the project has been applied. Water obtained in the system meets the requirements of drinking water technological regulation by its organoleptic, physical and chemical as well as microbiological parameters.

The proposed experimental system, in future may become a basis for a new technical installation for treatment of drinking water sharply reducing the costs of appropriate devices, simplifying their application and ensuring required level of safety.

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Evaluation of *Cerrena unicolor* BCC 300 and their Enzymes for Decolorization of Synthetic Dyes

Aza Kobakhidze*, Tamar Khardziani, Eva Kachlishvili, Vladimir Elisashvili

Institute of Microbial Biotechnology, Agricultural University of Georgia, Tbilisi, 0159, Georgia

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The aim of this study was to evaluate the capability of few WRB to produce lignin-modifying enzymes in the presence of dyes and to establish parameters providing maximum and fast decolorization of selected dyes by crude laccase. In the submerged cultivation in the glycerol-based medium supplemented with 0.3 mM synthetic dyes, *Cerrena unicolor* BCC300, Coriolopsis gallica BCC1184, and *Trametes versicolor* BCC13 produced 69.3-88.4 U/ml, 54.3-59.0 U/ml, and 1.7-14.3 U/ml laccase, respectively, and demonstrated a high decolorization potential of Amaranth, Remazol Brilliant Blue R, and Indigo carmine. Involvement of laccase in these dyes decolorization was assessed and proved using the crude laccase obtained from the *C. unicolor* BCC300 culture liquid. Against Amaranth, the *C. unicolor* BCC300 laccase was most effective at pH 6-7, toward Indigo carmine at pH 6, whereas the decolorization of RBBR occurred at a maximum rate at pH 5. The decolorization activity by the enzyme decreased with increasing dye concentration. Nevertheless, it completely decolorized 0.08% RBBR and Indigo carmine after 24 h incubation. Amaranth was more resistant to the laccase action and only 55% of dye was decolorized after 24 h incubation. Concentration of laccase of 1 U/ml was sufficient to completely decolorize RBBR and Indigo carmine. However, increasing the enzyme concentration to 3 and 10 U/ml enhanced the rate of decolorization. Overall, the isolated from *C. unicolor* BCC300 crude laccase showed catalytic properties required for their biotechnological applications.

Keywords: Basidiomycetes, Laccases, Synthetic Dyes, Decolorization, Enzyme, C.unicolor.

*Corresponding author: Aza Kobakhidze; e-mail address: a.kobakhidze@agruni.edu.ge

Introduction

In recent years, comprehensive and intensive studies have been carried out to detect easily available laccase sources with high catalytic efficiency, broad substrate specificity, and resistance to various environmental parameters for their biotechnological application, in particular, in bioremediation processes.

Due to low cost and color stability, synthetic dyes are used in many industrial process. However, during processing in textile industry, 10-15% of the used dyestuffs are lost in the industrial effluents leading to considerable environmental pollution [1]. Most of these dyes are health-risk factors with mutagenic and carcinogenic effect and cannot be completely removed by conventional wastewater treatment systems. Even low concentrations of dyes in aqueous ecosystems are highly visible and undesirable, reduce sunlight penetration, causing inhibitory effect on photosynthesis, and toxic aromatic amines can be generated, if dyes are broken anaerobically [2-5]. Application of different available physical or chemical methods for dye removal from wastewaters is limited due to expensive operational costs, formation of hazardous by-products, and intensive energy requirement [6]. As an alternative, biological processes have received increasing interest since they can offer a low-cost and environmentally friendly solution [6-8]. Various eukaryotic and prokaryotic microorganisms have been used for decolonization and degradation of synthetic dyes. Among them, most efficient in breaking down synthetic dyes are the white-rot basidiomycetes (WRB) which are capable of mineralizing a diverse range of persistent organic pollutants [6, 9]. This ability of

WRB to oxidize a wide variety of organic pollutants, including synthetic dyes, is due to an extracellular enzymatic system consisting of lignin peroxidases, manganese peroxidases, and laccases [1, 6, 10, 11] However, the cost of enzymes is still too high and there is need in new and potent enzyme sources and cheap production technologies. Moreover, fungal strain capable of growing in wide range of pH and temperature conditions and resisting the toxicity of the dyes at high concentrations should be chosen.

The objectives of this study were to evaluate the capability of few WRB to produce lignin-modifying enzymes (LME) in the presence of dyes and to establish parameters providing maximum and fast decolorization of selected dyes by crude laccase.

Materials and Methods

Organisms and inoculum preparation

The following WRB from the basidiomycete's culture collection of the Agricultural University of Georgia have been used in this study: *Cerrena unicolor* BCC300, *Coriolopsis gallica* BCC1184, *Trametes versicolor* BCC13. The fungal inocula were prepared by growing their mycelia taken from agar slants on a rotary shaker at 27 °C and 150 rpm in 250-ml flasks containing 100 ml of the medium (g/l): glucose, 10; NH_4NO_3, 2; KH_2PO_4, 1; $MgSO_4 \cdot 7H_2O$, 0.5; yeast extract, 2. After 7 days of cultivation the fungal biomass was homogenized in a Waring laboratory blender.

Cultivation conditions

The submerged cultivation of fungus was conducted in the Innova 44 shaker (New Brunswick Scientific, USA) at 27 °C and 150 rpm. The homogenized mycelium (5 ml) was used to inoculate the 250-ml flasks containing 50 ml of the medium (g/l): glycerol, 15; ammonium tartrate, 2; KH_2PO_4, 1; yeast extract, 3; $CuSO_4 \cdot 5H_2O$, 0.02; $MnSO_4$, 0.01; pH 5.8. The nutrient medium was supplemented with synthetic dyes (Amaranth, Indigo carmine, Remazol Brilliant Blue R) at the concentration of 0.3 mM. At predetermined time intervals, 1 mL of culture was sampled and solids were separated by centrifugation (Eppendorf 5417R, Hamburg, Germany) at 10,000 *g* for 5 min at 4 °C. The supernatants were analyzed for pH, dyes decolorization, and enzyme activities.

All experiments were performed twice using three replicates. All results were expressed as the mean ± SD with only $p \leq 0.05$ considered as statistically significant.

Analytical methods

The fungal biomasses were measured gravimetrically after recovering mycelium with centrifugation of whole cultures at 8000 *g* for 20 min and oven-drying at 60 °C for 24 h. Microbial decolorization of the synthetic dyes was evaluated in each taken samples by measuring the decrease in absorbance of culture liquids containing Amaranth (523 nm), RBBR (595 nm), and Indigo carmine (610 nm).

The laccase activity was determined spectrophotometrically (Camspec M501, UK) at 420 nm as the rate of 0.25 mM ABTS (2,2′-azino-bis-[3-ethyltiazoline-6-sulfonate]) oxidation in 50 mM Na-acetate buffer (pH 3.8) at room temperature [12]. MnP activity was measured at 610 nm by following by oxidation of Phenol Red [13] in the presence of 0.1 mM H2O2. One unit of laccase or MnP activity was defined as the amount of enzyme that oxidized 1 μmol of substrate per minute.

Enzymatic dyes decolorization

The decolorization experiments were performed using concentrated laccase preparation (400 U/ml) obtained by precipitation with ammonium sulfate from *C. unicolor* culture liquid. The enzymatic dyes decolorization was initiated by adding of 0.5 ml of the adequately diluted enzyme to 9.5 ml dye solution prepared by dissolving of each synthetic dye at a required concentration in 0.05 M citrate-phosphate buffer. The reaction mixture was incubated in 50 ml flasks at 27 °C and 150 rpm for 24 h. During incubation, 1 ml samples were taken after 0 h, 1 h, 2 h, 4 h, and 24 h and the solutions absorbance was measured at the λ_{max} of each dye. All experiments were performed twice using two replicates, the results were expressed as the mean and decolorization percent was calculated.

Results and discussion

Microbial decolorization of synthetic dyes

To elucidate the capability of the selected basidiomycetes strains to grow and produce lignin-modifying enzymes in the presence of three synthetic dyes their cultivation was performed in the submerged conditions in the glycerol containing medium. The fungi grew well in all media in the form of pellets. Slight delay in the fungal biomass accu-

mulation was visually observed during the first three days' cultivation in the presence of 0.3 mM dyes and the maximum values of dry biomass yields in the fungi cultivation in the presence of several dyes appeared to be rather lower as compared with those in the control media (Table 1). It is interesting that the *C. gallica* 1184 growth accompanied with increase of the media pH while in the cultivation of T. versicolor 13 the media pH decreased.

Table 1. *Effect of dyes on the basidiomycetes growth and enzyme activity in the submerged cultivation in the glycerol containing medium*

Dyes	Final pH	Biomass (mg/ml)	Laccase (U/ml)	MnP (U/ml)	Decolorization %
		C. unicolor BCC300			
Control	6.0 ± 0.2	6.2 ± 0.1	54.0 ± 4.8^{12}	0.26 ± 0.04^{10}	
Amaranth	5.5 ± 0.1	6.0 ± 0.2	69.3 ± 7.8^{10}	0.68 ± 0.09^{12}	100^{5}
RBBR	5.9 ± 0.2	7.0 ± 0.1	88.4 ± 14.2^{12}	1.18 ± 0.15^{7}	100^{5}
Indigo	5.9 ± 0.2	6.3 ± 0.1	74.2 ± 10.7^{7}	0.36 ± 0.05^{7}	100^{3}
		C. gallica BCC1184			
Control	6.5 ± 0.1	6.3 ± 0.3	40.2 ± 6.9^{10}	0.17 ± 0.02^{10}	
Amaranth	7.1 ± 0.1	5.6 ± 0.2	54.3 ± 8.7^{10}	0.24 ± 0.04^{7}	100^{5}
RBBR	7.4 ± 0.2	5.9 ± 0.2	59.0 ± 10.2^{10}	0.41 ± 0.07^{10}	100^{5}
Indigo	7.2 ± 0.2	5.6 ± 0.2	54.9 ± 7.7^{7}	0.33 ± 0.05^{7}	100^{3}
		T. versicolor BCC13			
Control	5.5 ± 0.1	5.1 ± 0.1	3.2 ± 0.4^{10}	0.07 ± 0.01^{10}	
Amaranth	5.5 ± 0.3	5.3 ± 0.2	9.0 ± 0.5^{15}	0.30 ± 0.01^{15}	100^{5}
RBBR	5.8 ± 0.1	4.7 ± 0.2	14.3 ± 0.3^{5}	0.39 ± 0.03^{15}	100^{5}
Indigo	5.7 ± 0.1	4.4 ± 0.1	1.7 ± 0.2^{5}	0.08 ± 0.01^{15}	100^{3}

The measurement of the laccase activity revealed that all dyes promoted this enzyme secretion by *C. unicolor* 300. Among them, RBBR followed by Indigo increased laccase activity by 64% and 34%, respectively, as compared with the control (Table 1). Analogically, 1.5-fold increase in laccase activity was detected when *C. gallica* 1184 was grown in the presence of RBBR and more than 30% increase of laccase activity was observed in the presence of other dyes. Another picture was revealed in the cultivation of *T. versicolor* 13. Specifically, RBBR caused almost 5-fold increase of laccase activity of this fungus while Amaranth promoted 3-fold increase of this enzyme activity as compared with the control medium. Taken into account that the specific laccase activity *T. versicolor* 13 in the control medium and media with RBBR and Amaranth are equal 0.63 U/mg biomass, 3.04 U/mg biomass, and 1.70 U/mg biomass, respectively, one may conclude that these dyes induced laccase production with the induction ratio of 4.8 and 2.7, respectively. By contrast, Indigo carmine not only delayed the biomass

accumulation of *T. versicolor* 13 but also decreased laccase activity of this strain.

RBBR and Amaranth are very appropriate elicitors of MnP synthesis. Especially, RBBR caused 4.5-fold and 5-fold increase of this enzyme activity of *C. unicolor* 300 and *T. versicolor* 13, respectively, as compared with their control media (Table 1). Other dyes, with the exclusion of Indigo in the cultivation of *T. versicolor* 13, also favored MnP secretion by the tested fungi. Undoubtedly, high enzyme activity accumulated during the submerged cultivation of the fungi provided efficient and complete decolorization of all dyes during maximum 5 days. It means that even at the dyes concentration as high as 0.3 mM, *C. unicolor* 300, *C. gallica* 1184, and *T. versicolor* 13 are capable to well grow and produce high activity of LME and they are promising strains for their application in bioremediation of textile industry effluents.

Effect of Initial Dye Concentration on *C. unicolor* BCC300 Growth and Enzyme Activity

Subsequently, the capability of *C. unicolor* BCC300 to withstand higher concentrations of two dyes and to produce the target enzyme activities was studied varying the Amaranth and RBBR concentrations from 0 to 1 mM. The data received evidence that in the submerged cultivation in the presence of Amaranth, the fungus formed the same biomass yields as in the control medium (Table 2). Lower concentration of RBBR even favored the fungal biomass accumulation. However, the highest concentration of RBBR significantly delayed the fungus growth and decreased the biomass yield by 31%.

Table 2. *Effect of dyes concentration on the C. unicolor BCC300 growth and enzyme activity*

Dyes	mM	Final pH	Biomass gain, (mg/ml)	Laccase, (U/ml)	MnP_{610}, (U/ml)	Decolorization, (%)
Control	0	6.0 ± 0.1	6.2 ± 0.1	54.0 ± 0.8^{12}	0.26 ± 0.05^{10}	
Amaranth	0.1	5.9 ± 0.1	6.0 ± 0.2	64.4 ± 0.7^{10}	0.52 ± 0.06^{12}	100^{5}
	0.3	5.5 ± 0.1	6.0 ± 0.2	69.3 ± 0.8^{10}	0.68 ± 0.12^{12}	100^{5}
	1.0	5.9 ± 0.2	6.1 ± 0.1	76.1 ± 1.1^{12}	1.24 ± 0.3^{12}	100^{8}
RBBR	0.1	5.7 ± 0.1	7.2 ± 0.1	85.9 ± 1.2^{12}	0.53 ± 0.06^{5}	100^{5}
	0.3	5.8 ± 0.2	7.0 ± 0.1	88.7 ± 1.1^{12}	1.18 ± 0.3^{7}	100^{5}
	1.0	5.6 ± 0.2	4.3 ± 0.1	94.5 ± 1.3^{12}	1.64 ± 0.2^{10}	100^{8}

C. unicolor BCC300 was found to produce both laccase and MnP activity in all the tested media (Table 2). However, the values of enzyme activity depended on the dyes concentration. In particular, the higher was dye concentration the higher was enzyme activity. However, laccase activity increase was not so remarkable as compared with that of MnP activity. Thus, the laccase activity in the presence of Amaranth increased by 19-41% while in the presence of RBBR by 59-75%. However, in the same cultivation conditions MnP activity increased 2-4.8-fold in the presence of Amaranth and 2-6.3-fold in the presence of RBBR.

The measurement of culture liquids absorbance during the submerged cultivation of *C. unicolor* BCC300 revealed an efficient decolorization of both synthetic dyes. It is worth noting that a complete decolorization of 1 mM Amaranth and RBBR needed a longer time and it was achieved after 8 days of the fungus cultivation in the synthetic glycerol-containing medium.

Enzymatic decolorization of synthetic dyes

The tested fungi, including the best enzyme producer *C. unicolo*r BCC300, demonstrated a high decolorization potential of three synthetic dyes. It was necessary to prove the involvement and role in this process of fungal extracellular enzymatic system. Therefore, the dyes decolorization was assessed using the crude laccase obtained from the *C. unicolor* BCC300 culture liquid.

Taking into account that laccase catalytic activity depends on the reaction mixture pH and on affinity towards the substrate, in the first set of experiments, the effect of pH on the selected dyes decolorization was studied using laccase at a final concentration of 1 U ml^{-1}. The reaction mixtures contained 0.04% of dyes (final concentrations). Data represented in Fig. 1 show that the dyes decolorization took place at all tested pH. However, the highest rate of Amaranth decolorization was observed at pH 6-7. Dye' treatment with laccase at this pH caused absorbance decrease by 57-63% after 24 h incubation of reaction mixture.

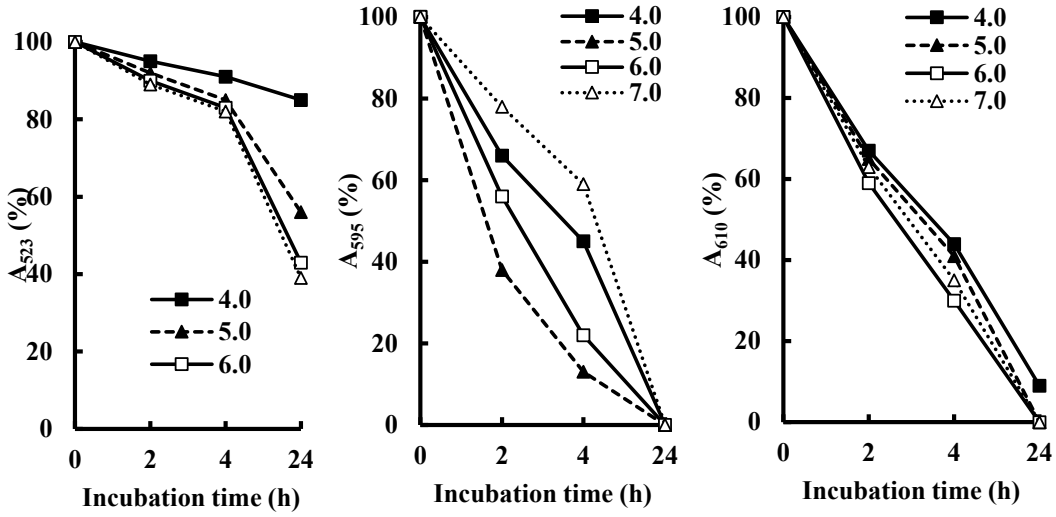

Fig. 1. *Amaranth (A), RBBR (B), and Indigo carmine (C) decolorization in dependence on the reaction mixture pH*

Unlike Amaranth, the highest rate of Remazol Brilliant Blue R decolorization was observed at pH 5.0-5.5. Dye' treatment with laccase at this pH caused 62% and 87% decolorization after 2 and 4 h incubation of reaction mixture, respectively (Fig. 1B). It is worth noting that a complete dye decolorization of RBBR was achieved at all pH after 24 h incubation. Finally, Fig. 4C show that the Indigo carmine decolorization efficiently occurred at wide range of pH with the highest rate observed at pH 6.0. Dye' treatment with laccase at this pH caused absorbance decrease by 41 and 70% after 2 and 4 h incubation of reaction mixture, respectively. Indigo carmine was completely decolorized by laccase during 24 h incubation at pH 5-7. It is worth noting that in all experiments the final laccase activity was measured after 24 h incubation of reaction mixtures. The results obtained showed that incubation at pH 4 caused a significant inactivation of enzyme and only 0.1-0.3 U/ml laccase activity was detected. At the same time, practically no enzyme activity loss was observed after 24 h incubation at pH 6-7. It means that the reduced dye discoloration at pH 4 is explained not only by the low catalytic activity of the enzyme, but also by its inactivation.

Subsequently, the ability of laccase from *C. unicolor* BCC300 to decolorize different concentrations of Amaranth, RBBR, and Indigo carmine was evaluated at the optimal for individual dyes pHs. The dyes concentration in solutions varied from 0.02 to 0.08% while the laccase concentration was 1 U/ml. Data represented in Fig. 2 show high decolorizing potential of laccase from *C. unicolor* BCC300. Enzyme is catalytically active even at dye concentration as high as 0.08% completely decolorizing RBBR and Indigo carmine after 24 h incubation. Amaranth was more resistant to the laccase action and only 55% of dye was decolorized after 24 h incubation at the initial dye concentration of 0.08%.

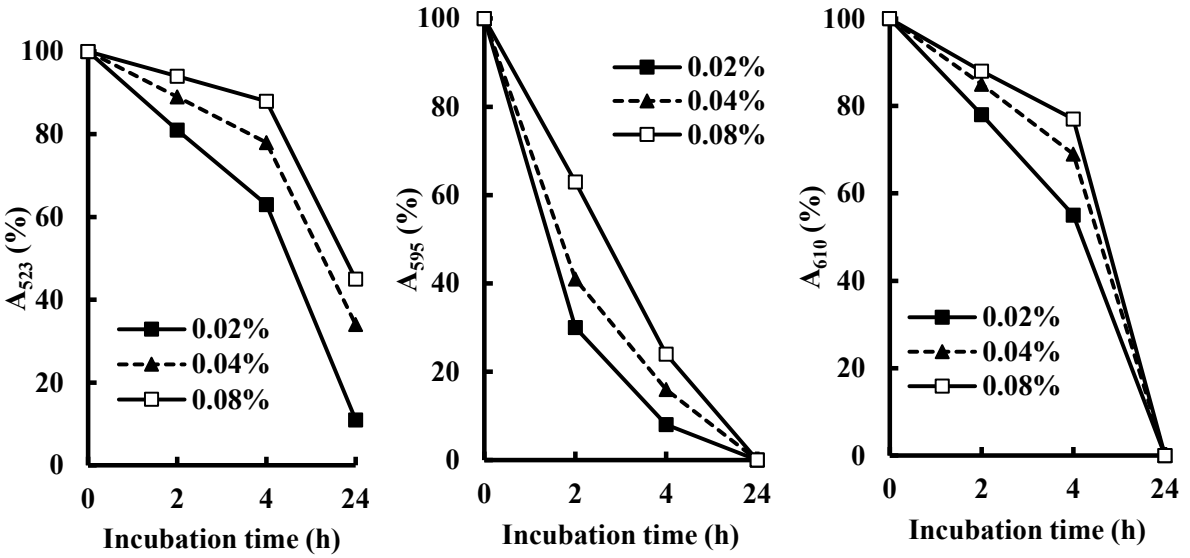

Fig. 2. *Amaranth (A), RBBR (B), and Indigo carmine (C) decolorization in dependence on the dye concentration*

Therefore, in the final experiment, the effect of the laccase concentration on Amaranth, RBBR, and Indigo carmine decolorization was studied at the dyes concentration of 0.08%. Enzyme loading is one of the reaction parameters required to achieve maximum rate of dye decolorization. Indeed, the higher was laccase concentration in the dyes solutions the higher was the rate of their decolorization. Among the dyes tested, RBBR was the most sensible to the catalytic action of *C. unicolor* BCC300 laccase (Fig. 3). Even at the laccase concentration of 1 U/ml the dye was completely decolorized after 24 h incubation. However, an increasing of laccase concentration highly accelerated the process of dye decolorization and it was practically completely decolorized after 4 h of incubation at the laccase concentration of 10 U/ml. Like in the previous experiments, Amaranth was the most resistant to the laccase catalytic activity. Nevertheless, complete decolorization of this dye was achieved when the concentration of laccase was increased to 10 U/ml.

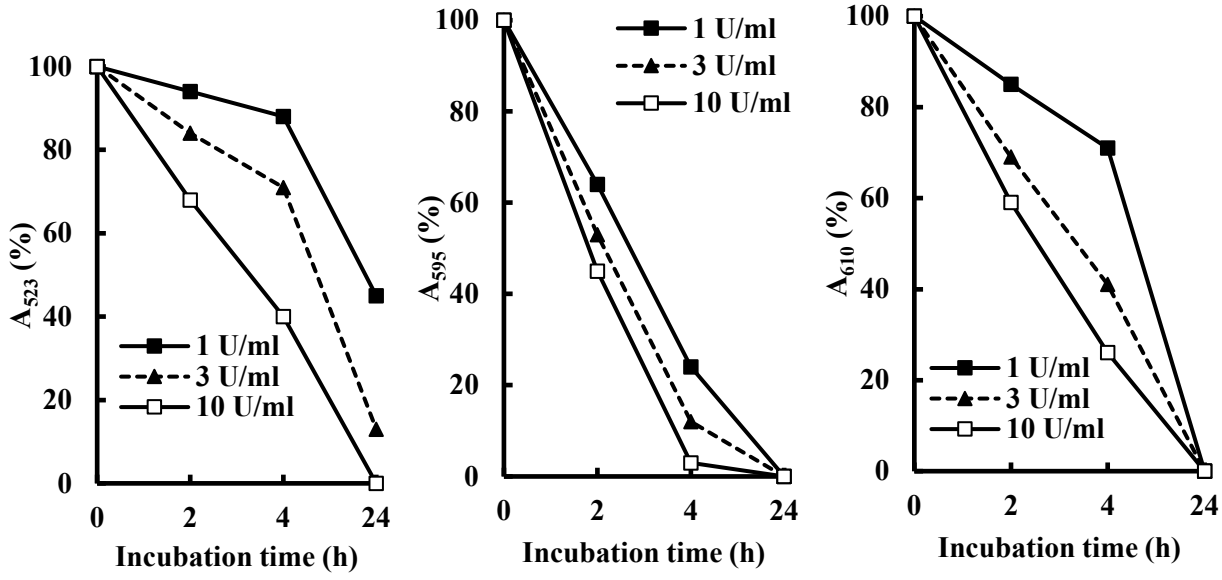

Fig. 3. *Amaranth (A), RBBR (B), and Indigo carmine (C) decolorization in dependence on the laccase concentration*

Discussion

This study showed that the selected white rot basidiomycetes had high decolorization capacity against azo dye Amaranth, anthraquinone dye RBBR, and indigoid dye Indigo carmine. *C. unicolor* BCC300, *C. gallica* BCC1184, and *T. versicolor* BCC13 were capable to withstand high concentration of dyes and to produce significant activities of laccase and MnP. Analogically, *B. adusta* CCBAS 232 was able to decolorize a number of chemically different synthetic dyes at concentrations of 2-4 g/l [14, 15]. *Polyporus* sp. S133 showed the fastest rate and efficiency (97% during 72 h) for decolorization of Amaranth (30 mg/l) under shaking conditions in glucose containing medium [16]. It is worth noting that as compared with the fungi tested in this study, *B. adusta* CCBAS 232 and *Polyporus* sp. S133 appeared to be poor enzyme producers.

Like in the study with *Trametes hirsuta* D7 enzyme [17], the obtained from liquid culture of *C. unicolor* BCC300 crude laccase was most effective at decolorizing anthraquinone dye RBBR. The crude enzyme showed good decolorization activity at acidic pH, but the optimal pH values for decolorization of the dyes were different. Against Amaranth, the *C. unicolor* BCC300 laccase was most effective at pH 6-7, toward Indigo carmine at pH 6, whereas the decolorization of RBBR occurred at a maximum rate at pH 5. At the same time, the most efficient decolorization of RBBR by crude enzyme from *Rigidoporus lignosus* W1 was obtained at pH 3.5 [18]. Enzyme extracts from *Coriolus versicolor* and *Pleurotus ostreatus* showed the maximum decolorization of RBBR at pH 4 [19].

The decolorization activity by the enzyme decreased with increasing dye concentration. In this study, the degree and rate of dyes decolorization decreased with an increase of dye concentration. Thus, 12, 22, and 37% of Amaranth decolorization was achieved during 2 h of incubation at dye concentration of 0.02, 0.04, and 0.08%, respectively. The highest rate of RBBR and Indigo decolorization was observed at the lowest dyes concentration. Similarly, maximum RBBR decolorization (95.6%) by laccase of *T. hirsuta* D7 was found at a lowest dye concentration (0.1%) [17].

In general, increasing the enzyme concentration enhanced the decolorization activity [17, 19]. In this study, concentration of laccase of 1 U/ml was sufficient to completely decolorize RBBR and Indigo carmine. Even lower concentration of laccase required to decolorize RBBR by *Trametes hirsuta* D7 [17]. By contrast, enzyme activity of 20 U/ml was optimum for decolorization of RBBR by *C. versicolor* and *P. ostreatus* [19]. However, an excessively high enzyme concentration would increase the cost of the treatment process.

Thus, three white rot basidiomycetes *C. unicolor* BCC300, *C. gallica* BCC1184, and *T. versicolor* BCC13 expressed an industrially relevant potential to decolorize structurally different synthetic dyes due to their capability to secrete extracellular laccase. Moreover, the isolated from *C. unicolor* BCC300 crude laccase showed catalytic properties required for their biotechnological applications.

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Small-headed milk-vetch tragacanthic shrubberies (Astracantheta microcephalae) in Tbilisi environs

N.J. Lachashvili[a*], L.D. Khetsuriani[b]

[a]Ilia State University, Institute of Botany; Georgian National Botanical Garden,
1, Botanikuri Str, Tbilisi, 0114, Georgia
[b]Ilia State University, Institute of Botany, 1,
Botanikuri Str., Tbilisi, 0114, Georgia

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A B S T R A C T

Small-headed milk-vetch tragacanthic shrubberies (Astracantheta microcephalae) of Tbilisi environs are studied for the first time. They are not appertaining to characteristic formations of Tbilisi environs. Its plant communities are fragmentary spread and with different plots area are inserted in the area of various vegetations. Their altitudinal range is from foothills to middle mountain belt (800 to 1400 m a.s.l.). In Tbilisi environs tragacanthic plant communities of A. microcephala mostly are secondary origin and derived as a result of digressive successions of oak forest (Querceta iberici). Primary plant communities are rare. Typological composition of formation is poor. 3 plant communities were identified in Tbilisi environs by us: (1) Astracanthetum graminoso-mixtoherbosum, (2) Astracanthetum festucosum valesiaci and (3) Astracantheto-Paliuroso-Rhamnosum. The first plant community is comparatively widespread and presented by different variants. The rest plant communities are rare and have local distribution area. Phytocoenological characteristics of plant communities are presented. Geo-botanical descriptions are represented in the form of consolidated table. General geo-botanical characteristics (general projective coverage, sodding degree, density, projective coverage, distribution and average height of each layer, floristic composition, coenetic role of each species – projective coverage, and etc.) and physical-geographical conditions (altitude, relief, exposure, inclination) are given. 200 species of vascular plants, which belong to 38 families and 133 genera, were recorded. In the floristic spectrum leading families are: 1. *Asteraceae* - 27 species (13,5%%), 2. *Fabaceae* – 19 (9,5%), 3. *Poaceae* – 18 (9%), 4. *Caryophyllaceae* – 17 (8,5%), 5-6. *Lamiaceae and Rosaceae* – 16-16 (8-8%), 7. *Brassicaceae* – 12 (6%), 8. *Rubiaceae* – 8 (4%), 9. *Apiaceae* – 7 (3,5%), 10. *Cistaceae* – 5 (2,5%). The life form spectrum is as follows: hemicryptophytes (including biennials) – 99 species (49,5%), therophytes – 55 (27,5%), phanerophytes – 16 (8/%), chamaephytes – 15 (7,5%), geophytes – 15 (7,5%).

Keywords: Astracantheta microcephalae, Plant community, Typology, Phytosociological characteristics, Floristic composition, Tragacanthic shrubbery.

*Corresponding author: Nikoloz Lachashvili: E-mail address: lachashvili@gmail.com

Introduction

Tragacanthic shrubberies are one of the characteristic for xerophilous vegetation of Caucasus. They have fragmentary distribution in Caucasus. Tragacanthic shrubberies are distributed both on Greater and Lesser Caucasus mountain ranges, in Zuvand, on Sheki and Iori plateaus [1-6]. Their altitudinal diapason is very large – from foothills to subalpine belt (800-2300 m a.s.l.). Tragacanthic shrubberies are developed on the thin skeletal and stony soils and rocks.

Species of genus *Astracantha*, *Astragalus*, *Acantholimon* and *Onobrychis* (*Astracantha denudata*, *A. aurea*, *A. microcephala*, *A. caucasica*, *Acantholimon hohenackeri*, *Onobrychis cornuta*, etc.) are dominants of Caucasus tragacanthic vegetation [1-6]. Florogenetically Caucasus tragacanthic vegetation is in the connection to relevant vegetation of Southwest Asia.

Astracantheta denudati, Astracantheta aurei and Astracantheta microcephalae are main tragacanthic formations in Georgia. Beside them Astracantheta caucasici and Astragaleta tanae are also distributed.

Tragacants of *Acantholimon* are rare (Acantholimonietum fominii, Acantolimonietum armenum and Acantolimonietum lepturoides). They are presented with small plots [5-9]. Floristic composition of Ass tragaleta denudati and Astragaleta aurei distributed on northern slopes of Greater Caucasus were studied by M. Ivanishvili [5] and Astcantheta microcephalae distributed in South Georgia (Meskheti) – by L. Khintibidze [6].

Tragacanthic shrubberies in Tbilisi environs do not belong to characteristic vegetation. But with its original phytocenological structure take distinguished position in the vegetation cover of Tbilisi surroundings. Their plant communities with different plots are fragmentary distributed and inserted in the area of various vegetations. Though, tragacanthic shrubberies in Tbilisi environs are represented by 3 formations. They are: (1) small-headed milk-vetch formation (Astracantheta microcephalae), (2) Caucasian milk-vetch formation (Astracantheta caucasici) and (3) Tana's milk-vetch formation (Astragaleta tanae) [8, 9]. From them the first formation is comparatively widespread. Caucasian milk-vetch formation is locally spread in the surroundings of vil. Martkopi on the foothills of Ialno ridge. Tana's milk-vetch formation is distributed on Mt. Didgori (Armazi ridge). Its small population also is on Mskhaldidi ridge near with vil. Mskhaldidi.

Literary data about tragacanthic shrubberies of Tbilisi environs is very scanty – only typological composition and distribution area of Astracantheta microcephalae and Astracantheta caucasici are given in Lachashvili et al. [8]. Phytocenological structure, floristic composition and distributed regularities were not studied. Their floristic and geo-botanical characteristics comparison to corresponding data of South Caucasus (Meskheti) and Greater Caucasus tragacanthic shrubberies would be interesting.

The aim of our research was to establish typological composition, distributed regularities and full floristic composition of Astracantheta microcephalae formation in Tbilisi environs; determine phytosociological structure for each distinguished plant communities.

Objectives and Methods

The object of research is small-headed milk-vetch tragacanthic shrubbery (Astracantheta microcephalae) of Tbilisi environs.

Phytosociological data was obtained by the route method in 2015-2019. 30 geo-botanical surveys (releve) were made. Geo-botanical surveys were carrying out on 25 m² plots. Number of species was quantifying on 1 m² plots also. During the geo-botanical surveys, studying the structure of phytocoenoses and identification of syntaxa, we were guided by the traditional geo-botanical methods [10-16]. Instead of the term "association" that is observed in soviet literature, we use the term "plant community" that is recognized through the Europe.

Life forms of the plants are separated on the basis of C. Raunkiaer [17] and I. Serebriakov classifications [18].

Soil types are founded on the modern classifications [19-21].

Results and Analysis

I. Areal and short physical-geographical characteristics

Small-headed milk-vetch formation (Astracantheta microcephalae) in Tbilisi surroundings is rare and is not appertaining to characteristic and widespread formations. Its plant communities are fragmentary distributed on Tabori, Mamadaviti, TeleTi, Sakaraulo and Mskhaldidi-Lisi ridges and with different plots area are inserted in the distribution area of various vegetation (steppe, shrubberies of different types, forest). Their altitudinal range is from foothills to middle mountain belt (800 to 1400 m a.s.l.).

Small-headed milk-vetch communities in Tbilisi environs mainly are spread in both climate zones: (1) moderately humid climate with moderately warm long summer and moderately cold snowy winter; average annual temperature is 7.4°-11°C, mean annual precipitation – 550-800 (900) mm, precipitation-evaporation ratio – within the range 1; (2) climate with insufficient humidity, dry hot summer and mild but well expressed winter; snow cover is unstable and brief; average annual temperature is 11,7°-12,5°C, mean annual precipitation – 380-550 mm, precipitation-evaporation ratio – within the range 0,4-0,6 [22-24].

Small-headed milk-vetch communities are formed on the cinnamonic skeletal soils and eroded slopes with scree and bare mother rocks.

Plant communities of *A. microcephala* in Tbilisi environs are either primary or secondary origin. Secondary origin communities are more frequent. They are derived as a result of digressive successions of oak forest (Querceta iberici) and relate to one of the last stage (IV stage) of post-forest vegetation succession [25, 26]. Primary plant communities are rare.

II. Typological composition and geo-botanical characteristic

Small-headed milk-vetch shrubberies in Tbilisi environs are not distinguished by typological variety. Within formation 3 plant communities were identified by us. They are: (1) Astracanthetum graminoso-mixtoherbosum (2) Astracanthetum festucosum valesiaci and (3) Astracantheto-Paliuroso-Rhamnosum. From them the first plant community is comparatively widespread and presented by different variants.

The distribution area in the environs of Tbilisi and consolidated geo-botanical tables are given below.

Abbreviations:

m – Meter cm – Centimeter s. – Specimen

E – East S – South
N – North W – West

Th – Therophyte G – Geophyte Ph – Phanerophyte
H – Hemicryptophyte Ch – Chamaephyte

1. Astracanthetum graminoso-mixtoherbosum

A. Surroundings of vil. Shindisi

Distribution in Tbilisi environs: Tabori ridge, surroundings of vil. Shindisi; *Altitude (m):* 835-845; *Topography:* slope; *Exposure (macro):* W, N-E; *Exposure (micro):* S-W, N-W, W, N-E; *Inclination:* 30°-35° (40°); *Soil:* cinnamonic, sceletal; *Species richness (1 m²):* 30,7; *Species richness (25 m²):* 52,4.

Table 1. *Astracanthetum graminoso-mixtoherbosum (surroundings of vil. Shindisi)*

Surveys	1	2	3	4	5	6	7
Exposure (macro)	W	W	W	W	W	W	N-E
Exposure (micro)	S-W	S-W	W-NW	W-NW	N-W	W	N-E
Inclination	30°	30°	40°	40°	35°-37°	35°-37°	32°-35°
General projective coverage (%)	70	70	80	75	85-86	75	70-73
Sodding degree (%)	0.5	-	-	-	-	-	-
I layer (Shrubs)							
Projective coverage (%)	40-45	40-45	50-55	32-35	43-45	50-55	38-40
Distribution	Uneven	Uneven	More or less evenly	Uneven	Uneven	Uneven	Uneven
Average height (cm)	40-50	40-50	45-50	50-60	50	50	45-50
Maximum height (cm)	70	70	100	70-80	80	85	75
II layer (Grass cover)							
Projective coverage (%)	58-60	62-65	73-75	65-68	70-73	60-63	58-60
Distribution	More or less evenly	Evenly	Evenly	Evenly	Evenly	Evenly	Evenly
Average height (cm)	20-25	20-25	25-30	20-25	20-25	22-25	25-27
Maximum height (cm)	60	60-70	45	37-40	60	70	70
III layer (Moss cover)							
Projective coverage (%)	-	-	-	+	-	-	+

Species richness							
Species richness - 1 m^2	31	27	28	33	36	31	29
Species richness - 25 m^2	53	53	50	54	51	53	54
Floristic composition	Projective coverage (%)						
Shrubs (Ph)							
Astracantha microcephala	40-45	40-45	51	32-35	43-45	50-53	45-50
Prunus incana	-	+	-	-	-	-	-
Paliurus spina-christi	-	+	1	+	-	+	-
Rhamnus pallasii	+	+	1	+	+	1	+
Spiraea hypericifolia	-	1	10	-	1	1	1-2
Semishrubs & dwarf semishrubs (Ch)							
Scutellaria orientalis	1	+	3-4	3	3-4	2-3	1-2
Teucrium nuchense	+	3-4	4-5	4-5	6-7	-	3
Teucrium orientale	-	-	-	-	-	-	+
Teucrium polium	+	3-4	4-5	-	+	3	2-3
Thymus coriifolius	15-17	16-18	-	11-12	14-15	-	9-10
Ziziphora clinopodioides	+	+	-	-	-	-	-
Perennial herbs (H)							
Achillea neilreichii	+	1	2-3	2-3	-	2	-
Alcea rugosa	-	-	-	+	-	-	-
Alyssum tortuosum	-	-	-	-	-	+	-
Artemisia absinthium	+	+	+	-	-	-	-
Astragalus brachycarpus	-	-	+	+	-	-	-
Astragalus bungeanus	3-4	4-5	5-6	-	2-3	5-6	1-2
Bothriochloa ischaemum	+	+	+	-	-	2-3	-
Campanula bononiensis	-	-	-	-	-	-	+ 1 s.
Carex liparocarpos subsp. *bordzilowskii*	+	+	-	0,5	+	-	-
Centaurea ovina	+	+	1-2	1-2	2-3	2-3	1
Cleistogenes serotina	-	+	3-4	-	-	-	0,5
Convolvulus cantabrica	-	-	-	+	-	-	-
Dactylis glomerata	-	-	-	+	-	-	-
Elymus repens	-	-	-	-	-	+	-
Eryngium campestre	+	+	+	0,5	1-2	-	1
Euphorbia boissieriana	2-3	1-2	1	+	-	0,5	-

Euphorbia seguieriana	+	+	1	+	-	-	+
Falcaria vulgaris	-	+	-	+	1	-	+
Festuca valesiaca	1-2	+	+	+	0,5	+	+
Galium verum	5-6	4-5	3-4	-	3-4	5-6	3-4
Inula oculus-christi	-	-	+	+	-	-	-
Jurinea blanda	-	-	-	-	+	+	-
Koeleria macrantha	2	2-3	3-4	3	4-5	3-4	2-3
Medicago caerulea	+	2-3	-	3-4	5-6	4-5	5-6
Melica transsilvanica	3-4	8-9	11-12	-	-	7-8	9-10
Nepeta racemosa	-	+	2-3	0,5	1-2	2-3	1
Onobrychis cyri	-	-	-	1	0,5	+	+
Petrorhagia saxifraga	+	1-2	1	-	0,5	1	+
Phleum phleoides	+	+	+	+	+	+	+
Plantago lanceolata	-	-	-	+	+	-	-
Poa angustifolia	-	-	-	-	-	-	+
Potentilla humifusa	1	-	-	0,5	+	-	+
Potentilla recta	+	-	+	+	0,5	0,5	+
Sanguisorba minor subsp. *balearica*	+	1-2	-	-	-	+	-
Salvia nemorosa	-	-	-	-	-	+	+
Salvia verbascifolia	-	-	-	0,5	-	+	-
Scabiosa columbaria	-	-	-	+	-	+	-
Scorzonera biebersteinii	+	0,5	-	-	-	+	-
Stachys atherocalyx	-	-	+	+	+	1	-
Stipa arabica	1	-	-	-	-	1	0,5
Stipa capillata	-	-	-	-	-	-	+
Taraxacum praticola	-	-	-	+	-	-	-
Thalictrum collinum	-	-	-	-	-	-	+
Trifolium ambiguum	-	-	-	+	-	-	-
Trifolium tumens	-	-	-	+	+	-	-
Leontodon asperrimus	-	-	+	-	-	-	-
Veronica multifida	-	-	-	-	+	-	0,5
Vinca herbacea	+	-	+	+	+	+	-
Perennial herbs (G)							
Allium pseudoflavum	+	+	-	-	+	-	-
Allium rotundum	-	-	-	-	-	+	+
Gagea tenuifolia	-	-	-	-	-	-	+
Gagea chlorantha	-	-	-	+	-	-	-
Iris caucasica	+	-	-	-	-	-	-

Poa bulbosa subsp. *vivipara*	-	+	-	-	-	-	-
Rumex tuberosus	-	-	-	-	+	-	-
Biennial plants (H)							
Carduus hamulosus	+	+	+	-	-	+	+
Chondrilla juncea	-	+	-	-	-	-	-
Lappula barbata	+	+	-	-	0,5	-	-
Silene latifolia	-	-	-	+	+	-	-
Picris strigosa	-	-	-	-	-	-	+
Silene cyri	-	-	-	-	-	+	-
Verbascum formosum	-	+	+	0,5	-	0,5	-
Annual plants (Th)							
Adonis flammea	+	-	-	-	-	-	-
Alyssum alyssoides	+	2-3	1-2	3-4	2-3	1-2	1
Alyssum desertorum	-	-	-	-	-	-	+
Alyssum linifolium	+	-	+	+	-	+	+
Arabidopsis thaliana	+	+	+	0,5	+	+	0,5
Arenaria serpyllifolia	-	-	-	+	+	-	+
Asperula arvensis	+	+	1-2	3-4	1	2-3	1-2
Astrodaucus orientalis	-	+	-	-	-	-	-
Bromus japonicus	-	-	-	-	+	0,5	-
Camelina microcarpa	+	+	+	-	-	+	-
Cerastium glutinosum	-	-	-	+	-	-	-
Clypeola jonthlaspi	+	+	0,5	+	0,5	-	+
Crepis sancta	+	+	1	2-3	1	1-2	1
Crupina vulgaris	-	-	+	-	-	-	-
Daucus carota	-	-	-	-	-	-	+
Draba nemorosa	-	+	+	-	0,5	+	-
Erodium cicutarium	+	+	3-4	-	2-3	-	-
Filago arvensis	+	-	-	-	-	+	-
Helianthemum salicifolium	+	+	1-2	4	2-3	2	1
Holosteum umbellatum	-	-	+	0,5	-	-	-
Kohlrauschia prolifera	-	-	-	-	-	-	+
Linaria simplex	-	-	+	+	+	-	-
Lolium rigidum	+	+	3-4	3	-	2	2-3
Medicago minima	+	-	-	-	0,5	+	-
Medicago rigidula	+	-	+	-	-	-	-
Papaver arenarium	+	+	+	+	+	+	+
Papaver dubium	-	-	-	-	+	-	-

Psilurus incurvus	+	-	+	-	+	+	-
Queria hispanica	-	-	-	-	+	-	-
Scabiosa micrantha	-	-	-	+	-	-	-
Sideritis montana	-	-	-	-	-	+	-
Silene conica	+	+	-	-	-	-	-
Thlaspi perfoliatum	+	3-4	5-7	4-5	5-6	4-5	3-4
Trifolium arvense	-	-	-	-	-	-	+
Trifolium campestre	-	-	-	-	-	-	+
Viola kitaibeliana	+	1	2-3	1-2	1	-	0,5
Xeranthemum squarrosum	+	+	3-4	4-5	2-3	2-3	0,5

B. Surroundings of vil. Mskhaldidi

Distribution in Tbilisi environs: Mskhaldidi ridge, surroundings of vil. Mskhaldidi; *Altitude (m):* 1220-1235; *Topography:* slope; *Exposure (macro):* S; *Exposure (micro):* S; *Inclination:* 20°-30°; *Soil:* cinnamonic, skeletal, surface small-stony; often fragments of bare mother rocks are on the surface; *Species richness (1 m²):* 17,2; *Species richness (25 m²):* 28,7.

Table 2. *Astracanthetum graminoso-mixtoherbosum (surroundings of vil. Mskhaldidi)*

Surveys	1	2	3	4	5	6	7	8
Exposure (macro)	S	S	S	S	S	S	S	S
Exposure (micro)	S	S	S	S	S	S	S	S
Inclination	22°-23°	30°	23°-25°	20°	30°-32°	20°	25°-26°	20°-22°
General projective coverage (%)	73-75	63-65	70-72	58-60	73-75	57-60	62-65	68-70
Sodding degree (%)	10	-	-	-	-	-	-	-
I layer (Shrubs)								
Projective coverage (%)	30	30	55	35	35	25-27	30-32	28-30
Distribution	Uneven	Uneven	Uneven	Uneven	More or less evenly	Uneven	More or less evenly	More or less evenly
Average height (cm)	25	30	25-27	20-25	20	25	28-30	25-28
Maximum height (cm)	35	40	40	30	30	35	40	37
II layer (Grass cover)								
Projective coverage (%)	70	50	40	40	60	40-45	50-55	55-60
Distribution	evenly	uneven	More or less evenly	More or less evenly	More or less evenly	uneven	More or less evenly	More or less evenly
Average height (cm)	5-7	5-7	8-10	6-8	5-7	5-7	7-8	6-8
Maximum height (cm)	50	50	55	45	55	50	52	57

III layer (Moss & lichen cover)								
Projective coverage (%)	-	-	-	-	-	-	-	-
Species richness								
Species richness - 1 m^2	22	16	15	11	22	16	17	19
Species richness - 25 m^2	30	24	30	27	32	29	30	28
Floristic composition	Projective coverage (%)							
Shrubs (Ph)								
Astracantha microcephala	30	30	55	35	35	25-27	30-32	28-30
Cotoneaster racemiflorus	+	-	-	+ 1 s.	+ 2 s.	-	-	-
Crataegus kyrtostyla	-	+	-	-	-	-	-	-
Rosa canina	-	-	-	-	+ 2 s.	-	-	-
Semishrubs & dwarf semishrubs (Ch)								
Artemisia caucasica	15-17	10	15	18-20	10	12-13	16-18	13-15
Dianthus orientalis	-	-	-	-	+	-	-	-
Fumana procumbens	-	-	-	1	12	+	-	1
Helianthemum georgicum	1	+	1	5-6	+	+	1	+
Hyssopus officinalis	-	-	1-2	-	-	-	-	-
Scutellaria orientalis	-	+	3	+	4	+	+	+
Teucrium nuchense	10	20	10	15	25	13-15	15-17	12-14
Teucrium polium	1	3	6-7	+	5	1-2	+	2-3
Thymus coriifolius	10	+	10	7-8	6-8	8-10	5-7	7-8
Ziziphora clinopodioides	-	+	1	-	-	-	+	-
Perennial herbs (H)								
Achillea neilreichii	+	+	+	+	-	+	+	-
Agrimonia eupatoria	-	-	+ 1 s.	-	-	-	-	-
Alchemilla sericata	-	-	-	-	+ 1 s.	-	+ 1 s.	-
Galium humifusum	-	-	+	-	-	-	-	-
Astragalus bungeanus	+	-	+	-	+	+	+	-
Bothriochloa ischaemum	+	+	-	+	-	-	+	-
Bromus biebersteinii	2	-	-	+	3	+	1	+
Carex liparocarpos subsp. *bordzilowskii*	15-16	18-20	-	17-18	8	10-12	10	13-15
Carex humilis	-	-	-	-	5	-	+	-

Centaurea bella	-	-	-	+	1	+	-	+
Centaurea ovina	-	-	-	+	-	-	+	-
Cleistogenes serotina	-	-	+ 1 s.	-	-	-	-	-
Clinopodium vulgare	-	-	+ 1 s.	-	-	-	-	-
Convolvulus cantabrica	-	-	+ 1 s.	-	-	+ 1 s.	+ 1 s.	-
Securigera varia	-	-	+	-	-	-	-	-
Dactylis glomerata	-	1-2	-	-	-	-	-	-
Euphorbia seguieriana	-	-	-	-	+	+	-	-
Festuca valesiaca	20-25	-	-	+	-	-	-	+
Fragaria viridis	-	1	12	-	-	-	-	-
Koeleria macrantha	+	-	-	+	+	+	-	+
Leontodon asperrimus	-	-	-	-	-	+	-	-
Lotus corniculatus	-	-	+	-	+	-	-	-
Medicago caerulea	+	-	-	-	-	-	-	-
Nepeta racemosa	2	12	15	-	+	3-5	8-10	5-6
Onobrychis cyri	-	-	-	-	-	+	-	+
Petrorhagia saxifraga	13-15	10	15	15	15-18	11-13	10-12	13-15
Phleum phleoides	-	-	-	+	-	-	-	-
Plantago lanceolata	1	+	-	+	+	+	+	+
Poa angustifolia	5	5	5	+	5	3	3-4	5
Potentilla humifusa	15-16	-	15	12-13	2-3	7-8	8-10	11-13
Potentilla argentea	-	-	+	-	-	-	-	-
Sanguisorba minor subsp. *balearica*	+	+	-	-	+	-	+	+
Pyrethrum sericeum	-	-	-	-	2	+	-	+
Scorzonera biebersteinii	+	-	-	-	-	-	-	-
Sempervivum transcaucasicum	-	-	-	+	-	-	-	+ 1 s.
Stachys atherocalyx	+	-	-	-	-	-	+	-
Stipa capillata	-	-	-	-	+	-	+	-
Thesium arvense	+	-	+	-	+	+	-	+
Trifolium tumens	+	+	-	-	-	-	-	-
Seseli grandivittatum	-	-	+	-	-	-	-	-
Biennial plants (H)								
Anthyllis lachnophora	-	-	-	-	+	+	-	-
Annual plants (Th)								
Clinopodium acinos	+	+	+	+	-	+	+	+
Alyssum desertorum	+	+	+	+	+	+	+	+

Asperula arvensis	+	-	-	-	-	-	+	-
Bromus squarrosus	+	+	+	+	-	-	+	+
Crepis sancta	-	+	-	-	-	-	-	+
Sideritis montana	-	-	+	+	+	+	+	+
Trifolium arvense	+	-	-	-	-	-	-	-

C. Surroundings of vil. Lisi, between vil. Lisi and Tsodoreti

Distribution in Tbilisi environs: foothills of Mskhaldidi-Lisi ridge, surroundings of vil. Lisi; *Altitude (m):* 800-850; *Topography:* slope; *Exposure (macro):* **N**; *Exposure (micro):* N-W, N; *Inclination:* 30°-33°; *Soil:* thin, cinnamonic, stony, with bare mother rocks; stones on the surface are results of process of break-up of mother rock; *Species richness (1 m²):* 10,2; *Species richness (25 m²):* 60;

Table 3. *Astracanthetum graminoso-mixtoherbosum (surroundings of vil. Lisi)*

Surveys	1	2	3	4
Exposure (macro)	N	N	N	N
Exposure (micro)	N-W	N-W	N-NW	N-W
Inclination (macro)	30°-32°	30°-32°	30°-32°	30°-32°
Inclination (micro)	25°-28°	20°	20°-22°	22°-24°
General projective coverage (%)	70	55	50	60
Sodding degree (%)	+	2-3	-	+
I layer (Shrubs)				
projective coverage (%)	28-30	26-28	17-20	27-28
Distribution	More or less evenly	Uneven	Uneven	More or less evenly
Average height (cm)	50	40-50	70-80	50-55
Maximum height (cm)	80	75	120	110
II layer (Grass cover)				
Projective coverage (%)	43-45	38-40	35-37	40-42
Distribution	More or less evenly	More or less evenly	Uneven	More or less evenly
Average height (cm)	20-30	20-30	20-40	25-27
Maximum height (cm)	80	120	120	115
III layer (Moss & lichen cover)				
Projective coverage (%)	20	30-32	15	20-22
Moss	20	30-32	15	20-22
Lichen	+	-	+	+
Species richness				
Species richness - 1 m^2	10.6	9.7	10,5	10.1
Species richness - 25 m^2	56	63	59	62

Floristic composition	Projective coverage (%)			
Shrubs (Ph)				
Astracantha microcephala	25	25-26	15-20	23-25
Carpinus orientalis	1-2	-	-	-
Cytisus caucasicus	+	-	-	-
Cotoneaster morulus	2-3	+	-	1-2
Cotoneaster racemiflorus	-	-	+	-
Juniperus oxycedrus	+	+	-	-
Lonicera iberica	+	-	-	-
Rosa canina	+	+	-	+
Spiraea hypericifolia	-	+	+	+
Semishrubs & dwarf semishrubs (Ch)				
Artemisia caucasica	+	10	5-6	4-5
Bupleurum exaltatum	-	+	-	+
Fumana procumbens	1-2	+	+	1
Helianthemum nummularium	5	7-8	5-6	5-6
Scutellaria orientalis	7	1-2	3-4	2-3
Teucrium nuchense	+	-	+	+
Teucrium polium	+	+	+	+
Thymus coriifolius	6	1-2	3-4	6-7
Perennial herbs (H)				
Achillea neilreichii	1-2	+	1	1-2
Alyssum murale	-	+	+	+
Anthyllis vulneraria subsp. *boissieri*	+	-	-	-
Asperula glomerata	-	+	-	-
Asperula prostrata	-	-	+	+
Astragalus bungeanus	+	+	+	+
Astragalus mollis	-	+	-	-
Bromus biebersteinii	2-3	+	1-2	+
Campanula alliariifolia	+	-	-	-
Carex humilis	1-2	2-3	1-2	2-3
Centaurea ovina	+	-	+	+
Securigera varia	1-2	-	-	-
Dactylis glomerata	+	+	-	+
Euphorbia boissieriana	+	+	+	+
Euphorbia seguieriana	+	+	+	+
Galium album	+	+	+	+

Galium verum	-	+	+	+
Hypericum perforatum	-	+	+	-
Jurinea blanda	3	+	1-2	1
Koeleria macrantha	+	+	+	+
Leontodon asperrimus	+	+	+	+
Linum tenuifolium	+	+	+	+
Medicago caerulea	13	+	4-5	6-7
Melica transsilvanica	-	7-8	2-3	2
Onobrychis cyri	1	+	1-2	2
Ononis pusilla	+	-	-	-
Petrorhagia saxifraga	+	+	+	+
Petrorhagia saxifraga	+	-	-	-
Poa angustifolia	-	-	+	+
Potentilla recta	+	+	+	+
Sanguisorba minor subsp. *balearica*	-	+	-	+
Psephellus carthalinicus	+	10	5-6	4-5
Pyrethrum sericeum	+	+	+	0,5
Salvia verbascifolia	+	-	-	-
Scabiosa columbaria	0.5	+	+	+
Scorzonera biebersteinii	+	+	+	+
Seseli grandivittatum	-	10	1-2	-
Silene italica	-	+	+	-
Stachys atherocalyx	+	-	+	1
Stachys iberica	+	-	-	-
Stipa capillata	-	+	+	+
Thesium arvense	+	+	-	+
Tragopogon graminifolius	-	-	+	+
Turritis glabra	-	+	-	+
Veronica multifida	-	-	+	+
Perennial herbs (G)				
Allium atroviolaceum	+	+	+	+
Allium pseudoflavum	-	+	+	-
Allium rotundum	+	+	+	+
Muscari szovitsianum	+	1	+	+
Rumex ruberosus	+	-	+	+
Tragopogon tuberosus	+	-	-	-
Biennial plants (H)				
Campanula sibirica subsp. *hohenackeri*	+	+	-	+

Annual plants (Th)				
Clinopodium acinos	+	+	+	+
Alyssum alyssoides	-	+	+	+
Arenaria serpyllifolia	-	+	+	+
Asperula arvensis	+	+	+	+
Bromus squarrosus	+	+	+	+
Crucianella angustifolia	-	+	+	-
Filago eriocephala	-	+	+	-
Kohlrauschia prolifera	-	-	+	+
Linaria simplex	-	-	+	+
Medicago minima	-	+	-	-
Melilotus neapolitanus	-	+	-	+
Crepis sancta	-	-	+	+
Queria hispanica	-	-	+	+
Scabiosa micrantha	+	-	-	-
Sedum pallidum	-	+	-	+
Sideritis montana	+	-	-	+
Silene conica	-	+	-	-
Thlaspi perfoliatum	+	-	+	-
Trifolium arvense	-	+	-	-
Trifolium campestre	-	+	-	-
Veronica arvensis	-	+	-	-

D. Surroundings of Kojori, Mt. Udzo

Distribution in Tbilisi environs: Mamadaviti ridge, Mt. Udzo; *Altitude (m):* 1390-1400; *Topography:* slope; *Exposure (macro):* S-W; *Exposure (micro):* S-W, rare S and S-E; *Inclination:* 30°-33°; *Soil:* thin, cinnamonic, sceletal, with small stones and bare mother rocks; *Species richness (25 m²):* 40; General projective coverage – 70-75%; Sodding degree – -;

Shrubs: projective coverage – 30-35%; Distribution – More or less evenly; Average height (cm) – 35-50.

Grass cover: projective coverage – 55-60%; Distribution – more or less evenly; Average height (cm) – from 15-25 to 40-60 (80).

Floristic composition

Shrubs (Ph)

1. *Astracantha microcephala* – 30-35%
2. *Crataegus meyeri* – +

Semishrubs & dwarf semishrubs (Ch)

1. *Cerastium argenteum* – +
2. *Teucrium nuchense* – 8-10%
3. *Teucrium orientale* – +

Perennial herbs (H)

1. *Achillea biebersteinii* – 2-3%
2. *Alyssum murale* – +
3. *Bilacunaria microcarpa* – 5%
4. *Bromus biebersteinii* – 25-28%
5. *Clinopodium vulgare* – +
6. *Convolvulus cantabrica* – +
7. *Dactylis glomerata* – +
8. *Eryngium campestre* – +
9. *Euphorbia seguieriana* – +
10. *Festuca valesiaca* – +
11. *Fragaria vesca* – +

Perennial herbs (G)

1. *Allium gramineum* – +
2. *Allium rotundum* – +

Biennial plants (H)

1. *Reseda lutea* – +

Annual plants (Th)

1. *Alyssum desertorum* – +

2. *Arenaria serpyllifolia* – +
3. *Bromus japonicus* – +
4. *Verbascum orientale* – +
5. *Filago eriocephala* – +

3. *Rhamnus pallasii* – + (2 s.)
4. *Rosa spinosissima* – + (1 s.)

4. *Teucrium polium* – 8-10%
5. *Scutellaria orientalis* – 3-5%
6. *Thymus coriifolius* – 2-3%

12. *Galium verum* – +
13. *Hypericum perforatum* – +
14. *Medicago caerulea* – +
15. *Melica transsilvanica* – +
16. *Onobrychis cyri* – +
17. *Petrorhagia saxifraga* – +
18. *Phleum phleoides* – 18-20%
19. *Potentilla recta* – +
20. *Scabiosa georgica* – +
21. *Stachys atherocalyx* – +
22. *Stachys iberica* – +

3. *Bellevalia speciosa* – +
4. *Sedum maximum* subsp. *ruprechtii* – +

6. *Alyssum linifolium* – +
7. *Sedum hispanicum* – +
8. *Trifolium arvevse* – +
9. *Trifolium campestre* – +
10. *Xeranthemum squarrosum* – +

E. Teleti-Sakaraulo ridge

Distribution in Tbilisi environs: Teleti-Sakaraulo ridge, surroundings of vil. Ertisi; *Altitude (m):* 1100-1160; *Topography:* slope; *Exposure (macro):* S-W; *Exposure (micro):* S-W; *Inclination:* 20°-25°; *Soil (Substrate):* bare sandstone mother rocks and its scree; soil weakly and fragmentary is formed between of bare mother rocks, thin, skeletal, surface is stony; *Species Species richness (25 m²):* 20; *General projective coverage* – 35%; *Sodding degree*: –;

Layer structure is not expressed. Plant community is inserted in the derivate of forest, post-forest shrubberies and steppe.

Shrubs: projective coverage – 18-20%; *distribution* – More or less evenly; average *height (cm)* – 20-40.

Grass cover: *projective coverage* – 17-18%; *distribution* – More or less evenly; *height (cm)* – from 10-15 to 50-70.

Floristic composition

Shrubs (Ph)

1. *Astracantha microcephala* – 30-35%
2. *Carpinus orientalis* – + (1 s.)
3. *Cotoneaster morulus* – +
4. *Rhamnus pallasii* – 2-3%

Semishrubs & dwarf semishrubs (Ch)

1. *Scutellaria orientalis* – 2%
2. *Teucrim polium* – 4%
3. *Thymus coriifolius* – +
4. *Fumana procumbens* – +
5. *Artemisia incana* – 1%

Perennial herbs (H)

1. *Alyssum tortuosum* – 4%
2. *Artemisia absinthium* – +
3. *Artemisia vulgaris* – +
4. *Centaurea ovina* – +
5. *Euphorbia seguieriana* – 2-3%
6. *Hypericum perforatum* – +
7. *Onosma tenuiflora* – +
8. *Sanguisorba minor* subsp. *balearica* – 1%
9. *Psephellus carthalinicus* – +
10. *Scabiosa columbaria* – +
11. *Stachys atheroccalyx* – +

Perennial herbs (G)

1. *Allium rotundum* – +
2. *Rumex tuberosus* – +

Annual plants (Th)

1. *Carduus pycnocephalus* subsp. *albidus* – +
2. *Gypsophila elegans* – +
3. *Trifolium arvense* – +

2. Astracanthetum festucosum valesiaci

Distribution in Tbilisi environs: Tabori ridge, surroundings of vil. Shindisi; *Altitude (m):* approximately, 850; *Topography:* slope; *Exposure (macro):* E; *Exposure (micro):* N-E, E; *Inclination:* 22°-28°, rarely 15°-18°; *Soil*: cinnamonic, sceletal; *Species richness (1 m²):* 28,4; Species richness (25 m2): 47,2.

Table 4. *Astracanthetum festucosum valesiaci*

Surveys	1	2	3	4	5	6	7	8
Exposure (macro)	E	E	E	E	E	E	E	E
Exposure (micro)	N-E	N-E	N-E	E	E	N-E	N	E
Inclination	25°-28°	15°-18°	23°-25°	22°-25°	22°-25°	25°-27°	25°-26°	20°-22°
General projective coverage (%)	95-96	93-95	95-97	93-95	95-96	95-98	90-93	92-95
Sodding degree (%)	14-15	17-20	10	22-24	15-17	12-13	8-9	13-14
I layer (Shrubs)								
Projective coverage (%)	26-30	45-50	45-50	23-25	45-50	32-35	22-23	25-28
Distribution	Uneven	Uneven	More or less evenly	Uneven	More or less evenly	Uneven	More or less evenly	More or less evenly
Average height (cm)	35-40	45-50	35-40	40-45	35-40	40	35-40	40
Maximum height (cm)	60-65	70-80	50	60	50-55	50-55	55-60	55-58
II layer (Grass cover)								
Projective coverage (%)	90-95	92-93	93-95	85-90	90-92	92-93	85-87	85-90
Distribution	evenly	evenly	evenly	evenly	evenly	evenly	evenly	evenly
Average height (cm)	12-15	15-20	15-20	15-20	15-20	15-20	15-20	15-17
Maximum height (cm)								
III layer (Moss & lichen cover)								
Projective coverage (%)	7-8	4-6	+	+	1-2	+	10-11	1-2
Moss	+	+	-	-	-	+	10	+
Lichen	7-8	4-6	+	+	1-2	+	1	1-2
Species richness								
Species richness - 1 m^2	29	27	27	29	25	31	31	28
Species richness - 25 m^2	53	46	47	43	42	47	53	47
Floristic composition	Projective coverage (%)							
Shrubs (Ph)								
Astracantha microcephala	25-30	45-50	45-50	23-25	45-50	32-35	22-23	25-28

Crataegus meyeri	+ 1 s.	-	-	-	-	-	-	-
Paliurus spina-christi	-	-	+ 1 s.	-	+ 2 s.	-	-	-
Rhamnus pallasii	-	-	-	-	-	+ 1 s.	+ 1 s.	-
Rosa spinosissima	+1c.	-	-	-	-	-	-	-
Semishrubs & dwarf semishrubs (Ch)								
Scutellaria orientalis	7-8	4-5	10	2-3	+	3-4	4-5	1-2
Teucrium nuchense	13-15	15-17	10-12	5-6	7-8	6-7	8-10	5-6
Teucrium polium	3-4	4-5	8-9	3-4	3-4	5-6	4	6-7
Thymus coriifolius	5-6	6-7	+	2-3	2-3	2-3	4-5	3-5
Teucrium orientale	-	-	-	+	-	-	-	-
Perennial herbs (H)								
Achillea neilreichii	1	+	+	3-4	4-5	1-2	2	+
Artemisia absinthium	-	-	-	-	-	-	+	-
Galium humifusum	-	+ 1 s.	-	-	-	-	-	-
Astragalus bungeanus	-	-	+	7-8	10	6-7	-	3-4
Bothriochloa ischaemum	-	-	+ 1 s.	-	-	-	-	-
Carex liparocarpos subsp. *bordzilowskii*	-	-	-	-	-	-	+	-
Centaurea ovina	+	1-2	+	+	+	+	+	+
Securigera orientalis	-	-	-	+	-	-	-	-
Securigera varia	-	-	-	-	-	+	+	-
Dactylis glomerata	+	-	+	-	-	-	-	-
Dianthus crinitus	-	+	-	-	-	-	-	-
Echium vulgare	-	-	-	-	-	+	-	-
Eryngium campestre	+	+	+	+	+	+	+	+
Euphorbia boissieriana	6-7	+	3-4	-	+	+	+	-
Euphorbia seguieriana	1	+	+	1	+	+	+	+
Falcaria vulgaris	8-9	+	8-10	2-3	10-12	4-5	3-4	5-6
Festuca valesiaca	25-30	35-37	15	42-45	26-27	23-25	20-22	24-26
Galium verum	+	3-4	-	-	-	6-7	+	1
Herniaria incana	+	-	-	+	-	+	-	-
Inula aspera	-	-	-		-	+	-	-
Jurinea blanda	+	1-2	-	-	-	+ 1 s.	-	-
Koeleria macrantha	+	+	6-8	1-2	6-8	4-5	3	3-4
Leontodon asperrimus	+	+	-	-	-	-	-	-
Lotus corniculatus	-	-	-	-	-	-	-	+
Medicago caerulea	3-4	+	6-7	3-4	7-8	4-5	5-6	4-5

Melica transsilvanica	-	-	-	-	-	-	-	+
Nepeta racemosa	-	+ 1 s.	-	-	+	-	-	-
Onobrychis cyri	+	+	+	+	-	-	+	+
Petrorhagia saxifraga	-	-	-	-	-	-	-	+
Phleum phleoides	-	-	-	-	-	+	+	-
Plantago lanceolata	+	+	+	2-3	10	-	+	1-2
Poa angustifolia	18-20	35-40	30	11-12	+	15-16	-	17-18
Potentilla humifusa	-	-	-	-	-	+	25-27	2-3
Potentilla recta	1-2	+	+	+	+	+	+	1
Sanguisorba minor subsp. *balearica*	1	+	+	+	+	-	-	+
Salvia nemorosa	15-16	3-4	+	1-2	7-8	-	3-4	4-5
Salvia verticillata	-	-	-	-	-	-	-	+
Scabiosa columbaria	-	-	+	5-7	4-5	-	-	-
Scabiosa georgica	-	-	-	-	-	+	-	-
Scorzonera biebersteinii	-	-	-	-	-	-	+	-
Seseli grandivittatum	-	-	-	-	-	-	+	-
Stachys atherocalyx	+	+	+	+	1	+	+	+
Stipa capillata	-	-	-	-	+	-	-	-
Taraxacum praticola	+	+	-	+	+	-	+	-
Thalictrum collinum	-	+	-	-	-	-	+	-
Thesium arvense	+	-	+	+	+	+	-	-
Tragopogon graminifolius	-	-	-	-	-	+	-	-
Trifolium ambiguum	+	-	+	-	-	-	-	-
Trifolium tumens	1-2	+	+	+	+	+	+	+
Turritis glabra	-	-	+	-	-	-	-	+
Veronica multifida	3-4	1-2	+	2-3	5	4-5	2	2-3
Vinca herbacea	1	-	-	-	-	-	-	-
Perennial herbs (G)								
Allium pseudoflavum	-	+	-	-	-	-	-	-
Allium rotundum	-	-	-	-	-	-	+	+
Bellevalia speciosa	-	-	+ 1 s.	-	+	-	-	-
Muscari szovitsianum	-	-	-	-	-	-	+	-
Poa bulbosa subsp. *vivipara*	+	+	+	-	-	+	+	+
Ranunculus illyricus	+	+	+	+	+	+	+	+
Rumex tuberosus	3-4	2-3	3-4	-	-	+	+	1
Biennial plants (H)								

Campanula sibirica subsp. *hohenackeri*	-	-	-	-	-	-	-	+
Carduus hamulosus	+	-	+	+	+	-	+	-
Lappula barbata	-	-	-	-	-	-	+	-
Annual plants (Th)								
Adonis flammea	-	+	+	+	+	+	-	-
Alyssum alyssoides	2-3	+	+	+	+	-	+	+
Arabidopsis thaliana	1	-	-	+	+	+	+	-
Arenaria serpyllifolia	+	-	+	+	-	+	+	+
Asperula arvensis	+	+	+	-	-	+	+	-
Filago arvensis	+	+	-	-	-	+	-	-
Carduus pycnocephalus subsp. albidus	-	-	-	-	-	+ 1 s.	-	-
Cerastium glutinosum	1-2	1	+	+	+	+	+	+
Clypeola jonthlaspi	-	-	-	+	+	+	+	-
Cruciata pedemontana	-	-	-	-	-	-	+	-
Crupina vulgaris	-	-	-	-	-	-	-	+
Draba nemorosa	+	-	-	-	-	-	+	-
Erodium ciconium	+	+	-	-	-	-	-	-
Erodium cicutarium	+	-	-	-	-	-	+	-
Helianthemum ledifolium	-	-	-	-	-	-	-	+
Helianthemum salicifolium	+	+	+	+	+	+	+	+
Holosteum marginatum	-	-	+	+	-	-	-	-
Kohlrauschia prolifera	-	-	-	-	-	-	-	+
Lolium rigidum	-	-	-	-	+	-	-	-
Lycopsis orientalis	+	-	-	-	-	-	+	-
Medicago minima	-	-	+	-	-	-	-	+
Medicago rigidula	-	+	-	-	-	-	-	-
Minuartia montana subsp. *wiesneri*	-	-	-	-	-	-	+	-
Crepis sancta	1	+	+	2-3	-	+	+	-
Queria hispanica	-	-	-	-	-	-	-	+
Thlaspi arvense	-	-	-	+	-	-	-	-
Thlaspi perfoliatum	5-6	2	3-4	3-4	2	1-2	4-5	1-2
Trifolium arvense	-	-	-	-	-	-	-	+
Trifolium campestre	-	-	-	-	+	-	-	-

Veronica arvensis	+	-	-	-	-	-	-	-
Viola kitaibeliana	+	+	+	+	+	+	+	+

3. Astracantheto-Paliuroso-Rhmanosum

Distribution in Tbilisi environs: Teleti ridge, between vil. Tabakhmela and vil. Kumisi; *Altitude (m):* 800-850; *Topography*: slope; *Exposure (macro)*: S-W; *Exposure (micro)*: S; *Inclination*: 25°-28°; *Soil (Substrate):* slope is very eroded; soil almost is not formed; substrate is presented with bare sandstone mother rocks and its scree; *Species richness (25 m2)*: 17; *General projective coverage*: 35-40%; *Sodding degree*: –;

I layer (Shrubs): *projective coverage* – 25-27%; *distribution* – uneven; *average height (cm)* – 35-40(45).

II layer (grass cover): *projective coverage – 10-12%; distribution* – uneven*; average height (c*m) – from 10-15 to 25-30.

Floristic composition

Shrubs (Ph)

1. *Astracantha microcephala* – 15-18%
2. *Atraphaxis caucasica* – +
3. *Cotoneaster morulus* – +
4. *Paliurus spina-christi* – 3-5%
5. *Rhamnus pallasii* – 5%
6. *Spiraea hypericifolia* – 1%

Semishrubs & dwarf semishrubs (Ch)

1. *Artemisia incana* – 1-2%
2. *Fumana procumbens* – 1%
3. *Scutellaria orientalis* – 0,5%
4. *Teucrium nuchense* – +
5. *Teucrium polium* – 1%

Perennial herbs (H)

1. *Agropyron cristatum* – 2-3%
2. *Alyssum tortuosum* – 1%
3. *Dactylis glomerata* – +
4. *Euphorbia seguieriana* – 1%
5. *Festuca valesiaca* – +
6. *Stipa arabica* – 1-2%

Biennial plants (H)

1. *Campanula sibirica* subsp. *hohenackeri* – +

Annual plants (Th)

1. *Bromus squarrosus* – +
2. *Carduus pycnocephalus* subsp. *albidus* – 1-2%
3. *Erodium cicutarium* – +
4. *Papaver arenarium* – +

III. Floristic composition

200 species of vascular plants, which belong to 38 families and 133 genera, were recorded. In the floristic spectrum leading families are: 1. *Asteraceae* - 27 species (13,5%%), 2. *Fabaceae* – 19 (9,5%), 3. *Poaceae* – 18 (9%), 4. *Caryophyllaceae* – 17 (8,5%), 5-6. *Lamiaceae* and *Rosaceae* – 16-16 (8-8%), 7. *Brassicaceae* – 12 (6%), 8. *Rubiaceae* – 8 (4%), 9. *Apiaceae* – 7 (3,5%), 10. *Cistaceae* – 5 (2,5%).

Floristic composition mainly consists of hemixerophytic species. Herbs are basic of floristic composition (170 species – 85%) and woody and semi-woody plants are few (accordingly, 16 species – 8% and 14 – 7%).

Composition of woody plants is poor. Beside *Astracantha microcephala* components of shibliak mostly are participated (*Paliurus spina-christi*, *Rhamnus pallasii*, *Cotoneaster morulus*, *C. racemiflorus*, *Crataegus kyrtostyla*, *C. meyeri* and etc.). But in most cases, they are presented with 1-2 specimens and their frequency of occurrence is insignificant.

Core of grass cover composition in the same way is created by steppe and shibliak components. Role of hemixerophytic and xerophytic chamephytes (semishrubs & dwarf semishrubs) is significant. They belong to characteristic plants of skeletal soils, stony and partially rocky ecotypes and frequency of occurrence of most of them is high. Composition of herbs is enriched with characteristic plants of different types of shrubberies and, partially, of dry meadows and steppe-meadows, but they are not appertained to constant species.

The life form spectrum is as follows: hemicryptophytes (including biennials) – 99 species (49,5%), therophytes – 55 (27,5%), phanerophytes – 16 (8/%), chamaephytes – 15 (7,5%), geophytes – 15 (7,5%).

Full list of recorded plants is given bellow. For each species life forms [18, 17] are indicated.

Abbreviations:
An. – Annual plant
Bien. – Biennual plant
P. – Perennial herb
S. – Shrub
Sh. – Semishrub & dwarf semishrub

Th – Therophyte
H – Hemicryptophyte
G – Geophyte
Ch – Chamaephyte
Ph – Phanerophyte

GYMNOSPERMAE
Cupressaceae
Juniperus oxycedrus L. / S. / Ph

ANGIOSPERMAE
DYCOTYLEDONEAE
Apiaceae (*Umbelliferae*)
Astrodaucus orientalis (L.) Drude / An. / Th
Bilacunaria microcarpa (M.Bieb.) Pimenov & V.N.Tikhom. / P. / H
Bupleurum exaltatum M.Bieb. / Sh. / Ch
Daucus carota L. /An. / Th
Eryngium campestre L. / P. / H
Falcaria vulgaris Berhn. / P. / H
Seseli grandivittatum Schischk. / P. / H

Apocynaceae
Vinca herbacea Waldst. & Kit. / P. / H

Asteraceae (*Compositae*)
Achillea biebersteinii Afan. / P. / H
Achillea neilreichii A.Kern. [*Achillea nobilis* subsp. *neilreichii* (A.Kern.) Velen.] / P. / H
Artemisia absinthium L. / P. / H
Artemisia caucasica Willd. / Sh. / Ch
Artemisia incana (L.) Druce / Sh. / Ch
Artemisia vulgaris L. / P. / H
Carduus hamulosus Ehrh. / Bien. / H
Carduus pycnocephalus subsp. *albidus* (M.Bieb.) Kazmi (*Carduus albidus* M.Bieb.) / An. / Th
Centaurea bella Trautv. [*Psephellus bellus* (Trautv.) Wagenitz] / P. / H
Centaurea ovina Pall. ex Willd. / P. / H
Chondrilla juncea L. / P. / H
Crepis sancta (L.) Bornm. / An. / Th
Crupina vulgaris Pers. ex Cass. / An. / Th
Filago arvensis L. /An. / Th
Filago eriocephala Guss. / An. / Th
Inula aspera Poir. / P. / H
Inula oculus-christi L. / P. / H
Jurinea blanda (M.Bieb.) C.A.Mey. / P. / H
Leontodon asperrimus (Willd.) Endl. / P. / H

Picris strigosa M.Bieb. / Bien. / H
Psephellus carthalinicus Sosn. / P. / H
Pyrethrum sericeum (Adams) M.Bieb. [*Tanacetum sericeum* (Adams) Sch.Bip.] / P. / H
Scorzonera biebersteinii Lipsch. / P. / H
Taraxacum praticola Dahlst. / P. / H
Tragopogon graminifolius DC. / P. / H
Tragopogon tuberosus K.Koch / P. / G
Xeranthemum squarrosum Boiss. / An. / Th

Boraginaceae
Anchusa arvensis subsp. *orientalis* (L.) Nordh. (*Lycopsis orientalis* L.) / An. / Th
Echium vulgare L. / P. / H
Lappula barbata (M.Bieb.) Gürke / Bien. / H
Onosma tenuiflora Willd. / P. / H

Brassicaceae (*Cruciferae*)
Alyssum alyssoides (L.) L. / An. / Th
Alyssum desertorum Stapf / An. / Th
Alyssum linifolium Stephan ex Willd. [*Meniocus linifolius* (Stephan ex Willd.) DC.] / An. / Th
Alyssum murale Waldst. & Kit. / P. / H
Alyssum tortuosum Willd. / P. / H
Arabidopsis thaliana (L.) Heynh. /An. / Th
Camelina microcarpa Andrz. ex DC. /An. / Th
Clypeola jonthlaspi L. / An. / Th
Draba nemorosa L. / An. / Th
Thlaspi arvense L. / An. / Th
Thlaspi perfoliatum L. / An. / Th
Turritis glabra L. / P. / H

Campanulaceae
Campanula alliariifolia Willd. / P. / H
Campanula bononiensis L. / P. / H
Campanula sibirica subsp. *hohenackeri* (Fisch. & C.A.Mey.) Damboldt / Bien. / H

Caprifoliaceae
Lonicera iberica M.Bieb. / S. / Ph

Caryophyllaceae
Arenaria serpyllifolia L. / An. / Th
Cerastium argenteum M.Bieb. / Sh. / Ch
Cerastium glutinosum Fr. [*Cerastium pumilum* var. *glutinosum* (Čelak.) E.Rico]
Dianthus crinitus Sm. / P. / H
Dianthus orientalis Adams / Sh. / Ch
Gypsophila elegans M.Bieb. / An. / Th
Herniaria incana Lam. / P. / H
Holosteum marginatum C.A.Mey. / An. / Th
Holosteum umbellatum L. / An. / Th
Kohlrauschia prolifera (L.) Kunth / An. / Th
Minuartia montana subsp. *wiesneri* (Stapf) McNeill / An. / Th
Petrorhagia saxifraga (L.) Link / P. / H
Queria hispanica L. [*Minuartia hamata* (Hausskn.) Mattf.] / An. / Th
Silene conica L. / An. / Th
Silene cyri Schischk. / Bien. / H
Silene italica (L.) Pers. / P. / H
Silene latifolia Poir. (*Melandrium boissieri* Schischk.) / Bien. / H

Cistaceae
Fumana procumbens (Dunal.) Gren. & Godr. / Sh. / Ch
Helianthemum georgicum Jus. & Pozdeeva / Sh. / Ch
Helianthemum ledifolium (L.) Mill. / Sh. / Ch
Helianthemum nummularium (L.) Mill. / Sh. / Ch
Helianthemum salicifolium (L.) Mill. / Sh. / Ch

Convolvulaceae
Convolvulus cantabrica L. / P. / H

Corylaceae
Carpinus orientalis Mill.

Crassulaceae
Sedum hispanicum L. / An. / Th
Sedum maximum subsp. *ruprechtii* (Jalas) Soó [*Sedum caucasicum* (Grossh.) Boriss.] / P. / G
Sedum pallidum M.Bieb. / An. / Th
Sempervivum transcaucasicum Muirhead / P. / H

Dipsacaceae
Scabiosa columbaria L. / P. / H
Scabiosa georgica Sulak. / P. / H
Scabiosa micrantha Desf. / An. / Th

Euphorbiaceae
Euphorbia boissieriana (Woronow) Prokh. / P. / H
Euphorbia seguieriana Neck. / P. / H

Fabaceae (*Leguminosae*)
Anthyllis vulneraria subsp. *boissieri* (Sagorski) Bornm. (*Anthyllis lachnophora* Juz.) / P. / H
Astracantha microcephala (Willd.) Podlech / S./ Ph
Astragalus brachycarpus M.Bieb. / P. / H
Astragalus bungeanus Boiss. / P. / H
Astragalus mollis M.Bieb. / P. / H
Cytisus caucasicus Grossh. [*Chamaecytisus caucasicus* (Grossh.) Holub.; *Cytisus ruthenicus* Wol.] / S. / Ph
Lotus corniculatus L. / P. / H
Medicago caerulea Less. ex Ledeb. / P. / H
Medicago minima (L.) L. / An. / Th
Medicago rigidula (L.) All. / An. / Th
Melilotus neapolitanus Ten. / An. / Th
Onobrychis cyri Grossh. / P. / H
Ononis pusilla L. / P. / H
Securigera orientalis (Mill.) Lassen (*Coronilla orientalis* Mill.) / P. / H
Securigera varia (L.) Lassen (*Coronilla varia* L.) / P. / H
Trifolium ambiguum M.Bieb. / P. / H
Trifolium arvense L. / An. / Th
Trifolium campestre Schreb. / An. / Th
Trifolium tumens M.Bieb. / P. / H

Geraniaceae
Erodium ciconium (L.) L'Hér. / An. / Th
Erodium cicutarium (L.) L'Hér. / An. / Th

Hypericaceae
Hypericum perforatum L. / P. / H

Lamiaceae (*Labiatae*)
Clinopodium acinos (L.) Kuntze [*Thymus acinos* L.; *Acinos arvensis* (Lam.) Dandy; *Acinos thymoides* Moench] / An. / Th
Clinopodium vulgare L. / P. / H
Hyssopus officinalis L. (*Hyssopus angustifolius* M.Bieb.) / Sh. / Ch
Nepeta racemosa Lam. (*Nepeta mussinii* Spreng. ex Henckel) / P. / H
Salvia nemorosa L. / P. / H
Salvia verbascifolia M.Bieb. / P. / H
Salvia verticillata L. / P. / H
Scutellaria orientalis L. / Sh. / Ch
Sideritis montana L. / An. / Th
Stachys atherocalyx K.Koch / P. / H
Stachys iberica M.Bieb. / P. / H
Teucrium nuchense [*T. chamaedris* subsp. *nuchense* (K.Koch) Rech.f.] / Sh. / Ch
Teucrium orientale L. / Sh. / Ch
Teucrium polium L. / Sh. / Ch
Thymus coriifolius Ronniger / Sh. / Ch
Ziziphora clinopodioides Lam. (*Ziziphora serpyllacea* M.Bieb.) / Sh. / Ch

Linaceae
Linum tenuifolium L. / P. / H

Malvaceae
Alcea rugosa Alef. / P. / H

Papaveraceae
Papaver arenarium M.Bieb. / An. / Th
Papaver dubium L. / An. / Th

Plantaginaceae
Linaria simplex Desf. / An. / Th
Plantago lanceolata L. / P. / H
Veronica arvensis L. / An. / Th
Veronica multifida L. / P. / H

Polygonaceae
Atraphaxis caucasica (Hoffm.) Pavlov / S./ Ph
Rumex tuberosus L. / P. / G

Ranunculaceae
Adonis flammea Jacq. / An. / Th
Ranunculus illyricus L. / P. / G
Thalictrum collinum Wallr. / P. / H

Resedaceae
Reseda lutea L.

Rhamnaceae
Paliurus spina-christi Mill. / S. / Ph
Rhamnus pallasii Fisch. & C.A. Mey / S. / Ph

Rosaceae
Agrimonia eupatoria L. / P. / H
Alchemilla sericata Rchb. ex Buser / P. / H
Cotoneaster morulus Pojark. / S. / Ph
Cotoneaster racemiflorus (Desf.) K.Koch / S. / Ph
Crataegus kyrtostyla Fingerh. / S./ Ph

Crataegus meyeri Pojark / S. / Ph
Fragaria vesca L. / P. / H
Fragaria viridis Weston / P. / H
Potentilla argentea L. / P. / H
Potentilla humifusa Willd. ex Schltdl. (*Potentilla adenophylla* Boiss.) / P. / H
Potentilla recta L. / P. / H
Prunus incana (Pall.) Batsch [*Cerasus incana* (Pall.) Spach] / S. / Ph
Rosa canina L. / S. / Ph
Rosa spinosissima L. / S. / Ph
Sanguisorba minor subsp. *balearica* (Bourg. ex Nyman) Mu¤oz Garm. & C.Navarro (*Poterium polygamum* Waldst. & Kit.) / P. / H
Spiraea hypericifolia L. / S. / Ph

Rubiaceae
Asperula arvensis L. / An. / Th
Asperula glomerata (M.Bieb.) Griseb. / P. / H
Asperula prostrata (Adams.) K.Koch / P. / H
Crucianella angustifolia L. / An. / Th
Cruciata pedemontana (Bellardi) Ehrend. / An. / Th
Galium album Mill. / P. / H
Galium humifusum M.Bieb. [*Asperula humifusa* (M.Bieb.) Besser] / P. / H
Galium verum L. / P. / H

Santalaceae
Thesium arvense Horv. / P. / H

Scrophulariaceae
Verbascum formosum Fisch. ex Schrank / Bien. / H
Verbascum orientale (L.) All. (*Celsia orientalis* L.) / An. / Th

Violaceae
Viola kitaibeliana Schult. / An. / Th

MONOCOTYLEDONEAE
Amaryllidaceae (*Alliaceae*)
Allium atroviolaceum Boiss. / P. / G
Allium gramineum K.Koch / P. / G
Allium pseudoflavum Vved. / P. / G
Allium rotundum L. / P. / G

Asparagaceae (*Hyacinthaceae*)
Bellevalia speciosa Woronow ex Grossh. / P. / G
Muscari szovitsianum Baker / P. / G

Cyperaceae
Carex humilis Leyss. / P. / H
Carex liparocarpos subsp. *bordzilowskii* (V.I.Krecz.) T.V.Egorova / P. / H

Iridaceae
Iris caucasica Steven (*Iris caucasica* Hoffm.) / P. / G

Liliaceae
Gagea chlorantha (M.BIeb.) Schult. & Shult.f. / P. / G
Gagea tenuifolia (Boiss.) Fomin / P. / G

Poaceae (*Graminae*)
Agropyron cristatum (L.) Gaertn. / P. / H
Bothriochloa ischaemum (L.) Keng / P. / H

Bromus biebersteinii Roem. & Schult. / P. / H
Bromus japonicus Thunb. / An. / Th
Bromus squarrosus L. / An. / Th
Cleistogenes serotina (L.) Keng / P. / H
Dactylis glomerata L. / P. / H
Elymus repens (L.) Gould / P. / G
Festuca valesiaca Schleich. ex Gaudin / P. / H
Koeleria macrantha (Ledeb.) Schult. [*Aira macrantha* Ledeb; *Aira cristata* L.; *Koeleria cristata* (L.) Pers.] / P. / H
Lolium rigidum Gaudin / An. / Th
Melica transsilvanica Schur / P. / H
Phleum phleoides (L.) H.Karst. / P. / H
Poa angustifolia L. / P. / H
Poa bulbosa subsp. *vivipara* (Koeler) Arcang. / P. / G
Psilurus incurvus (Gouan) Schinz & Thell. / An. / Th
Stipa arabica Trin. & Rupr. / P. / H
Stipa capillata L. / P. / H

Conclusion

Plant communities of small-headed milk-vetch formation (Astracantheta microcephalae) in Tbilisi environs are fragmentary distributed. Their altitudinal range is from foothills to middle mountain belt (800 to 1400 m a.s.l.). They are developed on slopes with various exposure and inclination, on the cinnamonic skeletal soils. Not rare surface of soils are covered with stones. Sometimes soil is not formed and substrate is presented with bare mother rocks and its scree.

In Tbilisi environs tragacanthic plant communities of Astracantha microcephala mostly are secondary origin and derived as a result of digressive successions of oak forest (Querceta iberici). Primaı ry plant communities are rare.

Typological composition of formation is poor. 3 plant communities were identified in Tbilisi environs by us: (1) Astragaletum graminoso-mixtoheroı bosum, (2) Astragaletum festucosum valesiaci and (3) Astracantheto-Paliuroso-Rhamnosum. The first plant community is comparatively widespread and presented by different variants. The rest plant communities are rare and have local distribution area.

In the phytocenological structure of plant communities 2 layer are expressed: I layer - shrubbery stratum, II layer – grass cover. Moss and lichen cover is not always developed. Average height of shrubbery stratum vary in (20)30-80(120) cm (mostly in 40-50 cm) and density of canopy from (20)30-35% to 45-50%. Layer structure in several cases is not expressed.

Floristic composition is rich – 200 species of vascular plants, which belong to 38 families and 133 genera, were recorded. In the floristic spectrum leading families are: 1. *Asteraceae* - 27 species (13,5%%), 2. *Fabaceae* – 19 (9,5%), 3. *Poaceae* – 18 (9%), 4. *Caryophyllaceae* – 17 (8,5%), 5-6. *Lamiaceae* and *Rosaceae* – 16-16 (8-8%), 7. *Brassicaceae* – 12 (6%), 8. *Rubiaceae* – 8 (4%), 9. *Apiaceae* – 7 (3,5%), 10. *Cistaceae* – 5 (2,5%).

Herbs are basic of floristic composition (169 species – 84,5%) and woody and semi-woody plants are few (accordingly, 16 species – 8% and 14 – 7,5%). The life form spectrum is as follows: hemicryptophytes (including biennials) – 99 species (49,5%), therophytes – 55 (27,5%), phanerophytes – 16 (8/%), chamaephytes – 15 (7,5%), geophytes – 15 (7,5%).

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Analysis of Heavy Metals Phases-carriers in Soils

Yu.N. Vodyanitskii[*a], A.T. Savichev[b,c]

[a]Lomonosov Moscow State University, Faculty of Soil Science, 1, Leninskiye gory, Moscow, 119234, Russia
[b]Geological Institute, Russian Academy of Sciences, 7, Pyzhevskiy per., Moscow, 119017, Russia
[c]Dokuchaev Soil Science Institute,7a, Pyzhevskiy per., Moscow, 119017, Russia

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A B S T R A C T

A method of extractograms analysis reflecting the dissolution of heavy metals (HM) in soils by dynamic sequential extraction has been developed. The technique is based on the deconvolution of complex extractograms on a series of symmetrical or asymmetrical Gaussians, corresponding to a separate HM phases-carriers. It is possible to determine after deconvolution the number of HM phases-carriers dissolved by the reagent (n), the share of HM in each phase-carrier (S) as a percentage dissolution of the HM by this reagent to the total HM-content, relative degree of each HM phase-carrier stable to the reagent (xmax), domination of fast- or slow-soluble particles in this asymmetrical phase by its asymmetry (As), dispersion of the HM phase-carrier by its dispersion (D). It was studied the action of three reagents: acetic acid, hydroxylamine and ammonium oxalate on Cu, Pb and Zn in contaminated soils. Soils contain two groups of HM carriers-carbonates with different acetic acid solubility. Soils and sediments contain three HM phases-carriers with different rates of dissolution by hydroxylamine. The number of HM-phases dissolved by ammonium oxalate depends on metal: it ranges from two for Zn to three for Cu. In total, contaminated soils contain 7-8 HM-phases, consistently soluble by three reagents. The abundance of the phases suggests that it is desirable to perform smaller fractionation of HM, taking into account the type of pollutants.

Keywords: Heavy metals, Chemical extraction, Extractograms, Deconvolution of spectra, Pollutants, Number of HM.

*Corresponding author: Yuri Vodyanitskii; E-mail address: yu.vodyan@mail.ru

Introduction

Methods of chemical dissolution of HM phases-carriers are used in the study of heavy metals (HM) in soils and sediments. Successive schemes of HM-extraction from soils are used now. The extraction of HM is used in two modifications: static and dynamic [1-5]. Static, technically simpler system of HM fractionation is known for decades [4]. Often for the sequential HM-extraction from of soil, the liquid phase is separated from the sediment by centrifuge. But the static system of HM-fractionation has many drawbacks, among them: the duration of analysis, the impossibility of automation, the risk of incomplete extraction due to precipitation, etc. [5].

In recent years, the system of dynamic sequential fractionation of HM, based on fundamentally different, more complex equipment has been developed. In particular, dynamic sequential HM-compounds analysis is performed in a rotating spiral column, which was originally developed for counter-current chromatography [5-8].

The extracted solution is analyzed for the content of the metal, and monitoring is possible in two modes. The accumulated portions of the effluent are analyzed in off-line mode, while differential HM-extractograms are built on points [9]. On-line analysis of the effluent is almost continuous, and the kinetic extractogram is formed a continuous curve.

The system of dynamic sequential HM-fractionation allows obtaining extractograms that characterize the dynamics of metal extraction by the reagents [9]. Already simple visual viewing reveals a wide variety of extractogram forms. In some experiments, as pyrophosphate extraction of lanthanides from soils, kinetic extractograms are the only symmetrical Gaussians [10]. This simple extractogram version has not interest and it is not considered in the paper.

But often the extractogram form is a more complex, because of fact of neighboring Gaussians

superposition, that is, the effect of "overlapping fractions" [5]. Meanwhile, there are no attempts to decipher the complex forms of kinetic HM-extractograms in soils.

The aim of the work is to decipher and analyze complex kinetic extractograms of HM phase-carrier in the soil in dynamic sequential fractionation.

Objects

The HM-extractograms obtained from contaminated soil through dynamic consistent chemical treatment were studied [9].

Montana Soil No. 1 SRM 2710 was near Butte City, Montana, USA. The soil is heavily contaminated with HM, contents are: Pb = 5530, Zn = 6950, Cu = 2950 mg/kg [9]. In this soil, the world's soil Clarks [11] is exceeded 369 times for Pb, 100 times for Zn, 54 times for Cu. The Fe-content in the soil is low: 33,800 mg/kg, twice below the Fe-Clark in the Earth's crust. The Mn-content is very high: 10,100 mg/kg: 10 times higher than the Mn-Clark in the Earth's crust. The Fe/Mn-ratio is 3.3, it is much lower for Clark-ratio in the Earth's crust: 62,000/1,060 = 58 [12]. Thus, the effect of Mn-oxides in soils on HM-fixing may be comparable with the influence of Fe-oxides.

Methods

The continuous HM-fractionation was performed according the well-known Kersten-Foerstner leaching scheme [10]. Applied reactants are the next. Step 1: 1 mol/l ammonium acetate, pH 7. Step 2: 1 mol/l acetic acid + NaOH to adjust to pH 5. Step 3: 0.01 mol/l $NH_2OH{\cdot}HCl$ + 0.01 mol/l HNO_3, pH 2. Step 4: 0.1 mol/l ammonium oxalate + HNO_3, pH 3. Step 5: 30% H_2O_2 %, pH 2.4 and 1 M NH_4OAc в 6% HNO_3 (v/v = 1/3) [9]. Reagents, soluble compounds and HM- phases are presented in the Table. 1.

The continuous fractionation of HM was performed on a planetary centrifuge with a vertical one-layer coil column drum fabricated in Dortmund, Germany. The rotation speed was 550-600 rpm. The mobile phase pumping rate was 1 ml/min. Before commencing the leaching procedure, the spiral column was filled by water. Then the solid sample (~0.5 g) was introduced into the column as a suspension in 10 ml of 1 ml/l ammonium acetate in schemes of Kersten-Foerstner [10]. Then, while the column was rotated, aqueous solutions of different reagents, used as the mobile phase, were continuously fed to the column inlet. The solid sample was retained inside the rotating column as the stationary phase under the action of the centrifugal force field throughout the experiment. In most cases 40 ml of each eluent were pumped through the column. Fractions (10 ml each) of the mobile phase (effluent) were collected. The contents of the elements in the separated fractions (10 ml each) were determined by inductively coupled plasma atomic emission spectrometry [10]. The resulting HM extractograms were then processed by the deconvolution procedure.

Table 1. *Leaching scheme of Kersten-Foerstner: applied reagents and HM phases-carriers [9].*

Step	Reagent	Soluble HM-phases	HM phases-carriers
I	1 M NH_4OAc, pH 7	Exchangeable, water soluble	Sorbates
II	1 M HOAc, pH 5	Acid soluble	Carbonates
III	0.01 M $NH_2OH{\cdot}HCl$ + 0.01 M HNO_3, pH 2	Easily reducible	Mn-oxides
IV	0.1 M oxalate buffer, pH 3	Moderately reducible	Amorphous Fe-oxides
V	30% H_2O_2, pH 2 T=85°C; 1 M NH_4OAc in 6% HNO_3	Oxidizable	Organic matter and sulfides

Method of kinetic extractograms deconvolution

Considering the extractogram as kinetics of metal extraction by reagent over time, it is natural to analyze it as a complex spectrum by the conventional chemical analysis. The main drawback of analytical spectrums is the effect of overlapping signals, in this case the "phase overlap effect", which is distorted the characteristics of the signal (the content of a separate phase). The problem of spectrum "cleaning up" has received a great deal of attention, and significant progress has been made in this direction. One of the common methods of eliminating the overlapping signals in the analytical spectrum is the procedure of its deconvolution, splitting into the original components. Deconvolulation is used to decipher IR-spectrums, nuclear magnetic resonance spectrums, synchrotron X-ray analysis, X-ray fluorescent analysis, laser diffraction and other spectra [13 – 18]. For the deconvolution of spectra, the shape of individual contours is given by the functions of Gauss or Lorenz. In the paper, the function of Gauss was used for deconvolution.

Let's describe the sequence of operations for extractograms deconvolution. In the experience of kinetic dissolution of HM-phases by one reagent in off-line mode were received six test points [9]. On the original extractogram, the projection of figurative dot on the *x*-axis is reflected the volume of the effluent in the interval from 0 to 50 ml. In other words, figurative points are reflected the kinetics of the loss of HM-mass (*y*-axis) when the volume of the leaked solution on *x*-axis. When processing extractograms for each effluent, the position of the dots on the *x*-axis was rationed to the maximum, and then their position is presented in shares of 1. The ordinates of each point are given as a percentage of the HM extracted, relative to its total amount in the soil. After such initial treatment, the extractogram was approximated with a 5-th degree of polynomial. At the same time, the determination factor reached R^2 = 0.995-1.00, which indicates a fairly accurate approximation of the extractogram with a smooth polynomial function.

Then a kinetic extractogram, recorded as a polynomial, was deconvoluted on a series of symmetrical or asymmetrical Gaussians according to the relevant computer program [18].

The symmetrical Gaussian for extractograms is described by the equation:

$$Y = A \cdot \exp\{-[(x - x_{max})/D]^2\}.$$

Asymmetrical Gaussian with a linearly changing dispersion along the contour is described by the equation:

$$Y = A \cdot \exp\{-[(x - x_{max})/(D \cdot (1 + grad \cdot (x - x_{max})))]^2\}$$

where: Y – the content of extracted metal (%), A – amplitude (%); x_{max} is the position of the top of the Gaussian, grad is a gradient of dispersion. For asymmetrical Gaussians with variable dispersion: D is dispersion in the center of the contour. After deconvolution of the extractogram with the help of asymmetrical Gaussian we get its additional properties: asymmetry As, and excess Ex. Asymmetry is positive with the stretched right branch of the Gaussian and is negative in the stretched left branch. The excess has a positive value with the acute top of the Gaussian and negative with the flat top.

Deconvolution based on symmetrical Gaussian is revealed three contours in each extractogram. Is this result reliable? To answer this question, let us compare the real distance between the projections of the two adjacent contours tops on the *x*-axis with the ideal projections of the three contours, when a distance is 0.33. The greater the distance of the x_{max}, the more reason to assume that the assumption of Gaussians independence is true. In almost all studied extractograms the distance between the third and second symmetrical Gaussians is $\Delta x_{max} = x_{max-3} - x_{max-2} > 0.33$, that is, there is a high probability that these Gaussians are independent of each other.

At the same time, the distance between the centers of the first and second Gaussians is short: $\Delta x_{max} = x_{max-2} - x_{max-1} < 0.33$, which raises doubts about the independence of these Gaussians. There is a possibility that the first and second symmetrical Gaussians were formed by splitting one asymmetrical Gaussian.

Therefore, deconvolution of extractograms on the second model, with a linearly changing dispersion on the line contour was performed additionally. This more complex program was revealed the two asymmetrical Gaussians in extractograms. There are two additional characteristics: the values of asymmetry and the excesses.

The choice between the two deconvolution models is based in favor of an option with a minimum deviation from 100%. For some processing options, this minimum is obtained by modeling the extractograms by three symmetrical Gaussians. For other options, the minimum is obtained when using a model with two asymmetrical Gaussians. For example, for Cu extracted with acetic acid from the soil, the de-

viation from 100% decreased from 0.16% for three symmetrical Gaussians to 0.09 % for two asymmetrical Gaussians. This speaks in favor of splitting this extractogram into two asymmetrical Gaussians. On the contrary, for Pb extracted with hydroxylyphine from the soil deviation from 100% increased from 0.32 % for three symmetrical Gaussians to 1.55 % for two asymmetrical Gaussians. This means that the extractogram is more adequately split into three symmetrical Gaussians.

Gaussian interpretation

The deconvolulation of kinetic extractograms provides new information about the number and properties of the HM-phase in the soil. As a result of deconvolution extractograms we get the following indicators.

1) The number of Gaussian (n) as HM-phases, soluble by this reagent.

2) The share of HM-phase (S) from the total metal dissolution by the reagent.

3) The relative degree of stability of each HM-phase in relation to the reagent. It is estimated by the projection of the top of the Gaussian on the *x*-axis (x_{max}). In the weakly resistant phase: x_{max} = min, in the highly resistant to dissolution phase: x_{max} = max, in the medium resistant to dissolution phase: min < x_{max} < max. Often the main phase is the most unstable phase, but sometimes the main phase is medium-resistant.

In addition, deconvolution reveals the heterogeneity degree of particle in each phase. Different phases show different indicators.

The heterogeneity of the symmetrical phase particles can be calculated assessed through the dispersion (D), the heterogeneity of the particles increases at high D values. The heterogeneity of asymmetric phase particles can be judged by its asymmetry (As). Slow-soluble particles dominate with As > 0, and fast-soluble particles dominate with As < 0.

Results and discussion

The results of 8 complex extractograms deconvolution obtained by reagents in the II, III and IV stages of soil processing are given in Figs. 1-3 and in the Table 2. The deconvolution revealed three symmetrical or two asymmetrical HM-phases in different variants of soil chemical treatment (Figs. 1-3).

Table 2. *Properties of HM-phases, obtained by extactograms deconvolution. Source primary data from [9].*

№ phase	x_{max}	D	A, %	As	Ex	S_{phase}, %
№ 8 Acetic acid, Cu; $S_{C\text{-}decon}$ = 99.84%; **$S_{AC\text{-}decon}$ = 100.09%**						
1-C	0.14	0.10	6.19			18.4
2-C	0.20	0.22	1.47			62.6
3-C	0.67	0.18	2.22			18.8
1-AC	**0.15**	**0.18**	**7.20**	**1.08**	**1.66**	**82.5**
2-AC	**0.70**	**0.18**	**1.40**	**-0.64**	**0.65**	**17.6**
№ 9 Acetic acid, Pb, $S_{C\text{-}decon}$ = 100.67%; **$S_{AC\text{-}декон}$ = 100.26%**						
1-C	0.12	0.12	5.72			30.6
2-C	0.26	0.22	5.04			51.8
3-C	0.69	0.18	2.08			18.3
1-AC	**0.15**	**0.18**	**7.20**	**1.08**	**1.66**	**82.5**
2-AC	**0.70**	**0.18**	**1.46**	**-0.64**	**0.65**	**17.6**
№ 7 Acetic acid, Zn, $S_{C\text{-}decon}$ = 100.43%: **$S_{AC\text{-}decon}$ = 100.06%**						
1-C	0.14	0.14	1.73			40.4
2-C	0.32	0.22	0.94			37.3
3-C	0.69	0.18	0.69			22.7
1-AC	**0.15**	**0.18**	**2.15**	**1.19**	**1.89**	**72.9**
2-AC	**0.70**	**0.20**	**0.68**	**-0.81**	**0.53**	**27.2**
№4 Hydroxylamine, Cu, **$S_{C\text{-}decon}$ = 100.58%**; $S_{AC\text{-}decon}$ = **100.66%**						
1-C	**0.15**	**0.14**	**4.17**			**25.9**
2-C	**0.32**	**0.24**	**4.70**			**52.6**
3-C	**0.76**	**0.26**	**1.82**			**22.1**
1-AC	0.19	0.23	7.24	0.91	0.38	90.8
2-AC	0.78	0.22	0.92	-0.81	0.31	9.9

№5 Hydroxylamine, Pb, **$S_{C\text{-}decon}$ = 99.68%;** $S_{AC\text{-}decon}$ = 101.55%						
1-C	**0.23**	**0.14**	**1.07**			**12.0**
2-C	**0.32**	**0.52**	**2.51**			**81.5**
3-C	**0.80**	**0.14**	**0.55**			**6.2**
1-AC	0.24	0.30	3.43	0.50	-0.66	96.3
2-AC	0.81	0.14	0.35	-1.20	1.02	5.2
№6 Hydroxylamine, Zn, **$S_{C\text{-}decon}$ = 100.32%;** $S_{AC\text{-}decon}$ = 101.51%						
1-C	**0.16**	**0.16**	**3.36**			**50.0**
2-C	**0.39**	**0.16**	**1.17**			**27.3**
3-C	**0.67**	**0.20**	**0.80**			**23.0**
1-AC	0.17	0.20	2.42	1.06	0.80	80.3
2-AC	0.68	0.24	0.50	0.57	-0.43	20.2
№ 10 ammonium oxalate, Cu, **$S_{C\text{-}decon}$ = 100.22%;** $S_{AC\text{-}decon}$ = 100.28%						
1-C	**0.14**	**0.12**	**5.30**			**26.3**
2-C	**0.28**	**0.22**	**6.14**			**57.1**
3-C	**0.94**	**0.22**	**1.82**			**16.8**
1-AC	0.18	0.20	9.53	1.16	2.00	85.8
2-AC	0.96	0.18	1.84	-0.43	0.15	14.5
№ 11 ammonium oxalate, Zn, $S_{C\text{-}decon}$ = 100.39%; **$S_{AC\text{-}decon}$ = 99.78%**						
1-C	0.14	0.12	2.86			28.8
2-C	0.30	0.22	2.93			55.9
3-C	0.92	0.29	0.95			15.7
1-AC	**0.18**	**0.20**	**4.70**	**1.11**	**1.73**	**85.8**
2-AC	**0.94**	**0.16**	**0.97**	**-0.73**	**0.81**	**14.0**

Note. Highlighted are bold and stressed options of deconvolution as close as possible on the *y*-axis to 100%. Amplitude A is expressed as a percentage of the total metal content in the soil. The share of the HM-phase S_{phase} is expressed as a percentage of the metal content soluble by the reagent.

TM-phases soluble with acetic acid

The deconvolution revealed three symmetrical or two asymmetrical HM-phases (Fig. 1). Acetic acid is considered as a reagent selective to HM, fixed by carbonates (Table 1). At the same time, it is known that different metals have different degrees of affinity for carbonates. According to [19], carbonate ornstein's in the desert zone of Kazakhstan contain an average of 45 mg cu/kg, 80 mg Pb/kg, 50 mg of Zn/kg. Considering the Clarks of these metals [11], we get the magnitude of their relative affinity: Cu = 45/14 = 3.2, Pb = 80/25 = 3.2 mg, Zn = 50/62 = 0.8. Thus, it is obvious that Zn affinity to the carbonates is much lower than the affinity of Cu and Pb.

Our data are quite consistent with this difference in the extraction of metals with acetic acid. Total amplitudes of Cu- and Pb-phases are reach: 8.60 and 8.66%, but Zn-amplitude is three times less (as for carbonate ortsteins): 2.83%. Thus, the three metals studied fall into two groups: high affinity with carbonates (Cu and Pb) and low affinity with carbonates (Zn). Let's see how this difference in affinity is reflected in the properties of HM-phases.

Presence of two symmetrical Gaussians are reflected two type of carbonates as HM-phases, they differ in resistance to the action of acetic acid. We can talk about the presence of two phases of HM-carbonate with different resistance to the action of acetic acid. It is possible that it is not only Ca-carbonates, but also Mg-carbonates.

The share of rapidly soluble HM-carbonates is very high and reaches 82% for Cu and Pb, but is noticeably lower for Zn - 73%. In addition, the Zn-carbonates are differ from the Cu- and Pb-carbonates in asymmetry. For the rapidly soluble Zn-phase the positive As-values are higher than As-values for the rapidly soluble of Cu- and Pb-phases. That is, the Zn-containing phase has a higher proportion of slow-soluble carbonates. Thus, in the contaminated soil metals are fixed with carbonates with different resistance to acetic acid.

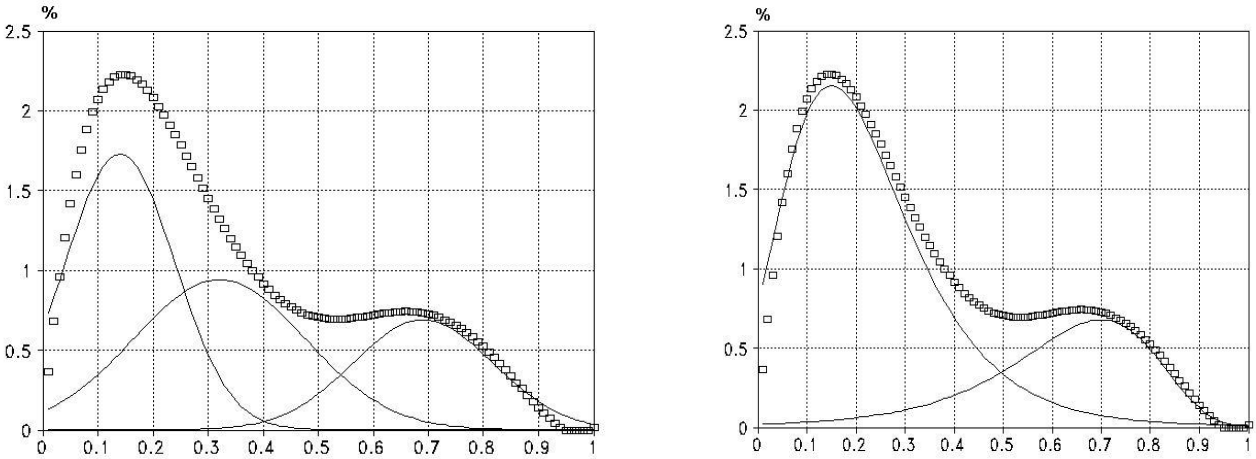

Fig 1. *Deconvolution of Zn-extractograms extracted from the soil with acetic acid. At the top: deconvolution using symmetrical Gaussians (S_{decon} = 100.43%); at the bottom: deconvolution using asymmetrical Gaussians (S_{decon} = 100.06%). The square dots reflect the shape of the original extractogram, the solid lines reflect the shape of the Gaussians..*

HM-phases soluble by hydroxylamine

The deconvolution revealed three symmetrical or two asymmetrical HM-phases (Fig. 2). Three symmetrical HM-phases, soluble during the third stage of treatment ($NH_2OH{\cdot}HCl + HNO_3$), have been identified in the soil. At the same time, there is a difference in the distribution of some metals infractions.

In the soil, the maximum Cu and Pb content falls on the second, medium soluble phase, and the maximum of the Zn falls on the first rapidly soluble phase. This indicates a difference in the solubility of HM-phases.

We will discuss the reasons for the variation of HM-phases stability to hydroxylamine. According to the Kersten-Foerstner scheme, hydroxylamine is selective in relation to HM, fixed by a single phase: Mn-oxides [9]. But obviously this phase is not the only soluble by hydroxylamine.

Here is an experience with the processing of soils of Ural, which showed that not only Mn, but also Fe is extracted by hydroxylamine solution [20]. All studied soils of Ural can be divided into three groups: soils of light and heavy texture and gley in soils of heavy texture. The Fe/Mn-ratio = (M ± m) was calculated, where M and m are average and its error of metals in hydroxylamine extracts from the soils of each group. This ratio was different in each group of soils. In extracts from the soils of the light texture, the Fe/Mn-ratio is 1.55 ± 0.22; in extracts from the soils of the heavy texture the Fe/Mn-ratio is 0.50 ± 0.04; in extracts from the gley in the heavy texture soils, the Fe/Mn-ratio is 0.25 ± 0.03. Thus manganese was extracted much more than iron only from the samples of gley by hydroxylamine. But from the rest of the soil, hydroxylamine together with Mn-oxides dissolves and a significant amount of Fe-hydroxides, which are HM-carriers. In fact, the hydroxylamine dissolves not two, but even three HM-phases.

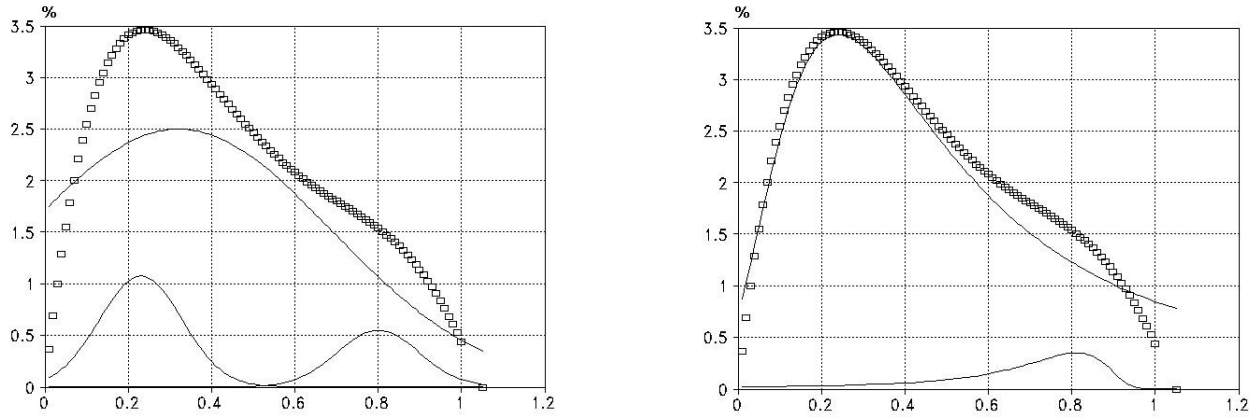

Fig. 2. *Deconvolution of Pb-extractograms extracted from the soil with hydroxylamine. At the top: deconvolution using symmetrical Gaussians (S_{decon} = 99.68%); at the bottom: deconvolution using asymmetrical Gaussians (S_{decon} = 101.55%). The square dots reflect the shape of the original extractogram, the solid lines reflect the shape of the Gaussians.*

HM-phases soluble by ammonium oxalate

The deconvolutions of HM-extractograms extracted by ammonium oxalate revealed two asymmetrical Gaussians for Zn and three symmetrical Gaussians for Cu. The deconvolution revealed three symmetrical or two asymmetrical HM-phases (Fig. 3).

The first of the asymmetrical phases accounts 86% of the Zn. The three Cu-phases are symmetrical, and the second phase accounts 57% of Cu. Thus, we can talk about the presence of two or three HM-phases with different resistance to the action of ammonium oxalate.

A large number of HM-phases are consistent to known data, that in addition to the expected X-ray aamorphous Fe-hydroxides as HM-carriers; oxalate also dissolves Fe(II)-minerals, including sulfides [21]. In addition, oxalate is able to dissolve loosely ordered silicates, such as allophanes [22].

Let us summarize. The determination of the number of TM carrier-phases makes it possible to evaluate the selectivity of each of the reagents used. There is an inverse relationship between the selectivity of the reagent and the number of phases soluble by it. This is illustrated by the action of two reagents contrasting in strength: water, with a minimum ability to dissolve solid phase particles, and royal vodka, in which this ability is expressed as much as possible. Their selectivity is very contrasting: in water, as a weak solvent, maximum selectivity, in royal vodka, which dissolves almost all minerals, there is no selectivity at all. In the schemes of sequential chemical extraction of HM, reagents are used in order of increasing their dissolving ability: from the weakest (often this is water) to the strongest (often hot nitric acid).

We calculate the average number of phases soluble by this reagent according to the formula:

$n_{cp} = (n_{Zn} + n_{Cu} + n_{Pb}) : 3.$

According to the Table 2, we obtain the average values of the number of phases soluble by acetic acid: $n_{aver(acetic)} = 2.0$, hydraxylamine: $n_{aver(hydroxylamine)} = 3.0$, ammonium oxalate $n_{aver(oxalate)} = 2.5$. As can be seen, the initial position of acetic acid in the Kersten-Foerstner scheme is quite consistent with its high selectivity (minimum $n_{aver\ (acetic)} = 2.0$). Compared with acetic acid, subsequent soil treatment with hydraxylamine showed a decrease in selectivity, since the value of $n_{aver(hydraxylamine)}$ increased to 3.0. But further soil treatment with oxalate showed an increase in selectivity: $n_{aver(oxalate)}$ decreased to 2.5. This was due to a decrease in the number of soluble Zn-phases. Thus, the selectivity of the reagent also depends on the type of HM-phases.

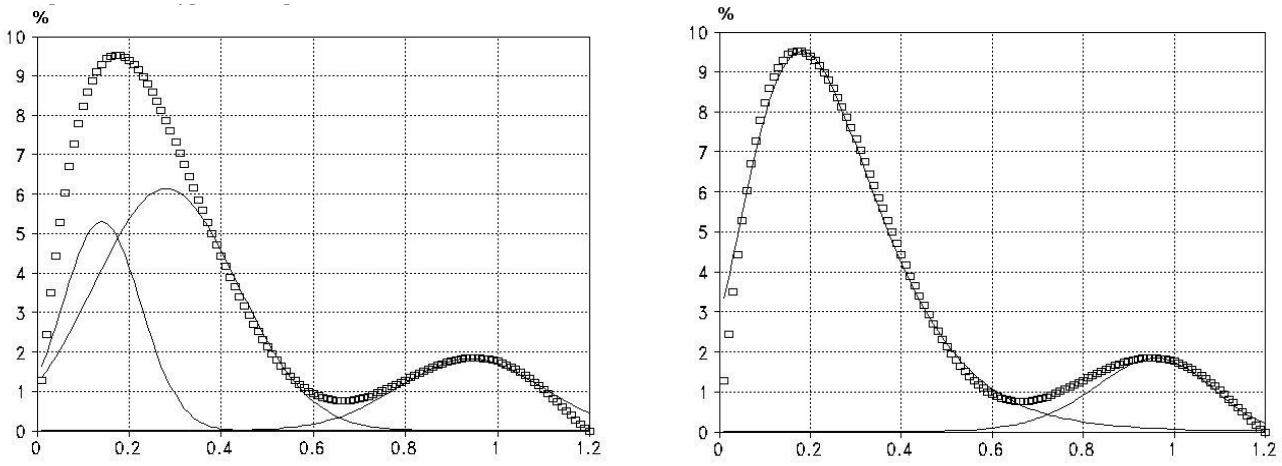

Fig. 3. *Deconvolution of Cu-extractograms extracted from the soil with oxalate. At the top: deconvolution using symmetrical Gaussians (S_{decon} = 100.39%); at the bottom: deconvolution using asymmetrical Gaussians (S_{decon} = 99.78%). The square dots reflect the shape of the original extractogram, the solid lines reflect the shape of the Gaussians.*

Conclusion

A method of extractograms analysis reflecting the dissolution of heavy metals in soils by dynamic sequential extraction has been developed. The technique is based on the deconvolution of complex extractograms on a series of symmetrical or asymmetrical Gaussian, corresponding to a separate HM phase-carrier. After deconvolition it is possible to determine the number of HM phase-carrier soluble by the reagent, the share of HM in each phase-carrier as a percentage dissolution of the HM by this reagent to the total HM-content, relative degree of each HM phase-hosted stability to the reagent (x_{max}), domination of fast- or slow-soluble particles in this asymmetric phase by its asymmetry (As), dispersion of the HM phase-carrier by its dispersion (D). It was studied the action of three reagents: acetic acid, hydroxylamine and ammonium oxalate on Cu, Pb and Zn in contaminated soil.

The soils consist of two groups of HM carbonate-carriers with different acetic acid solubility. Soils and sediments contain three HM phases-carriers with different rates of dissolution by hydroxylamine. The number of HM-phases soluble by ammonium oxalate depends on metal: it ranges from two for Zn to three for Cu. In total, the contaminated soil contains 7-8 HM-phases, consistently soluble by three reagents. The abundance of the phases suggests that it is desirable to perform smaller fractionation of HM, taking into account the type of pollutants.

The number of HM phases-carrier in soils and sediments depends on the deconvolution model of the extractograms: three symmetric and less often two asymmetric phases are detected. In any variant of deconvolution, the HM phases-carriers, fast soluble by this reagent, dominate soils and sediments. In contrast, the proportion of slowly soluble HM-phases is low, especially when exposed to ammonium oxalate: less than 18%. Judging by the presence of not one, but two or three phases, they differ in their resistance to dissolution by reagents.

So, in soils, three symmetrical Zn-phases were revealed; two of them: strongly and moderately soluble carbonates fix the main share of zinc. Two asymmetric carbonate Cu- and Pb-phases were found in soils. The presence of several phases of carbonates of HM-carriers with different resistance to acetic acid indicates the participation of Ca-, Mg-, and Fe-carbonates in the HM-fixation.

Hydroxylamine hydrochloride dissolves three symmetric HM phases-carriers in soils and sediments. Obviously, in addition to Mn-oxides, the reagent dissolves Fe-hydroxides, as well as a certain third phase. The oxalate buffer from the soil and sediment dissolves three symmetric Cu-phases and two asymmetric Zn-phases, and not the only one. It is possible that, in addition to the expected X-ray amorphous Fe-hydroxides, as HM carriers, oxalate also dissolves Fe(II)-minerals, including sulfides, as well as weakly ordered allophanes. Determination of the number and properties of HM phases-carriers allows the identification of the most suitable reagents for the extraction of different groups of TM-compounds from soils and sediments contaminated with various forms of pollutants.

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Electrification and Gasification in Georgian Agriculture Sector

Demur Chomakhidze

Georgian National Energy Regulatory Commission, 19, A. Mitskevich Str., Tbilisi, 0194, Georgia

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A B S T R A C T

The article provides a brief retrospective analysis of the level and dynamics of Georgia's electrification and gasification from the beginning of the process up to today. It is said that the production and consumption of electricity per inhabitant throughout the country reached its maximum in 1989. It is almost the same about gasification. As for these indicators in agriculture, it is far worse. However, it is noteworthy that in the last years of the country's independence the situation has been improved. The research has been provided throughout the country as well as in agriculture. The article provides suggestions for improvement of the situation. Namely to increase the level of electric power and gasification..

Keywords: Agriculture, Electrification, Gasification, Energy Balance, Energy Resources, Electricity Balance.

*Corresponding author: Demur Chomakhidze: E-mail address: demurchomakhidze@yahoo.com

Introduction

Power engineering is the most important basic for developing of any country whereas the level and dynamics of energy consumption and production per inhabitant is as objective and adequate parameter to characterize economic development as the Gross National Product.

Power engineering plays a leading role in economics because any process of manufacturing in all subfields of industry, agriculture, transport, all areas of population service and so on, is related to using more and more energy. Energy equipment is a fundamental material basis for the growth of productivity of social work. The development level of power engineering greatly influences the progress of dynamics and arrangement of industrial production across the country. It creates essential preconditions for raising living standards and improving labor conditions. Also, it is a ground for developing of all fields, including agriculture as well.

2. Electrification

It is well-known that electrification represents the most important indicator of energy security means; it implies the introduction of electricity in national farming and residential areas throughout the country. The integral indicator of electrification is electricity generation and consumption per capita. It is concentrated on the development of production forces in a particular country, the use of its ability and power. The level of production of electricity - consumption level, at the same time, is an indicator of the level of the people's life's quality, the socio-economic development and security of the whole country [1, 2].

The first power plant in Georgia was built in 1887 by Ilia Chavchavadze (an outstanding Georgian writer and public figure) in Tbilisi. The power generation was gradually rising, and in 1913 it reached 20 million kWh. This indicator reached its highest rate in the history of Georgia in 1989, while in the transition period of the market economy it was halved. There has been some growth since 2010 [3]. Consequently, the country's electrification indicator - electricity generation - consuming per person was changing (Table 1).

Table 1. *Electrification level and dynamics in Georgia in 1913-2016 [4]*

Years	Electricity production, mln kWh	Consumption of electricity per person kW
1913	20	10,0
1940	7417	205,3
1960	3916	948,4
1980	13984	2765,5
1989	15825	3330,0
2000	7446	1769,2
2010	9919	1902,9
2015	10593	2790,5
2016	11365	2966,5

Electricity consumption exceeded three thousands per person in 1989, and by the end of the century fell to 1769.2 kWh. By 2016, this figure made up 2966.5 kWh [4].

The image of rural electrification is the same throughout the country. It allowed us to utilize more fully the rural natural resources, the production forces, and to implement the achievements of scientific and technological progress, to provide effective socio-economic development and better living conditions for the population.

In the second half of the last century, the rural electrification was successful. In particular, consumption of electricity per person in rural population was as follows [5]:

Table 2. *Indicators of Electrification for agriculture 1960-1990*

Indicator	1960	1970	1980	1990
kWh	73,2	178,7	459,3	871,5

Therefore, in 1990 the inhabitants of rural areas used on average 871.5 kWh electricity, which was 11,9 times higher than the 1960 indicator. The same picture of electrification was in agricultural work and living. In the process of increasing the level of electrification made a significant contribution activation of scientific-research works. The reasoning and importance of electrification were given in them, and necessity of introduction and measures.

In particular, in the beginning of 1980, labor force in Georgia's Soviet farming calculation of one worker was 910 kWh, and in the farmers - 415 kWh. Electricity consumption in the household and service sphere per capita on average was 115.2 kWh.

Table 3. *Final use of total energy resources in Georgian agriculture, forestry and fishing in 2013-2016. Equivalent to 1000 tons of oil [6].*

Year	Final consumption in the country, total	agriculture, forestry and fishing	% for total consumption
2013	3726,3	13,7	0,367
2014	4022,8	12,1	0,301
2015	4174,6	18,7	0,448
2016	4330,5	29,6	0,683

Thus, the labor power equipment of Soviet industries was twice as high as in the farms. Both of them were characterized by growing tendency. The indicator of electricity consumption per capita in household and services were improving. However, all the above parameters were lower than in the industry and the city. For example, labor power generation in the industry was at 20242 kWh, or 22.2 times higher than in Soviet farms and 48.7 times more than in farms, while consumption of electricity in household and service sphere per capita was 636,2 kW. H, i.e. 5.5 times more than in the village [7]. It is noteworthy that the same indicators of the electrification of Georgia were even noticeably

lower,in comparison with average unionists [8, 9].

During the transition to the market economy, the indicators of electrification were significantly worsened throughout the country as well as rural areas. In particular, according to Geostat data, in 2015, only 42 kWh energy was be spent on village agriculture, forestry and fishery in Georgia, which is much (70.6) lower than the country's similar index [10].

According to the main indicator of electrification – by consumption of electricity per 1 person of population, Georgia still far behind the similar indicators of most of European countries and the world (Table 4):

Table 4. *Electricity consumption per 1 person in different countries of the world, 2014 [11*

Names of countries	kWh	% Compared to Georgia
The world on average, between them	3029,3	136,0
Asia	946,8	42,5
Africa	568,3	25,5
USA	12962,0	more than 5,8
Russia	6602,6	296,5
Germany	7035,7	316,0
Japan	7829,3	351,6
Georgia	2226,6	100,0
Azerbaijan	2201,0	98,8
Armenia	1897,0	85,2
France	6954,8	312,3
Latvia	3460,0	155,4
Lithuania	3820,0	171,5
The Ukraine	3410,0	153,1

3. Gasification

Gasification means the unity of maintenance and technical and design solutions implementation, construction and repair works and organizational activities that are aimed at moving the housing and communal facilities to the consumption of gas as the fuel and energy resource all over the country.

Gasification works in Georgia commenced in 1956. At the end of 1959, Tbilisi was supplied gas from the Republic of Azerbaijan. Annual capacity of the main gas pipeline was 1,8 billion m3 which, as a result of the reconstruction, achieved 4,6 m3. From the very beginning, gasification of the country developed very rapidly due to which the capacity of the existing gas pipeline as well as the gas resources became insufficient. It was necessary to find new sources. Vladikavkaz-Tbilisi gas pipeline was constructed and put into the operation from 1963. Over 1970-1978 Georgia was supplied gas from Iran too [10]. From November 1978, gas supply of Georgia from Iran was seized due to political developments in this country, and it was necessary to reconstruct Vladikavkaz-Tbilisi gas pipeline which commenced in 1985 and terminated in 1991. Annual capacity of the gas pipeline achieved 20 billion m3 due to which South Caucasus countries including Georgia moved to receiving the Turkmenistan gas. Within this period, Georgia was one of the leading countries according to the gasification level. 48 cities and 230 villages, up to 600 thousand flats, up to 800 industrial and agricultural plants, 1500 thermal power supply boilers, 2 thousand housing and communal facilities were gasified [3]. 10 thousand km gas pipeline including 2 thousand km main gas pipeline and 8 thousand km distribution network were constructed [11].

In 1989 gas consumption in Georgia exceeded 6,0 billion m3, and made 60% of the country's fuel balance. Natural gas was made available to almost every region of the country (except for the mountainous Svaneti and Adjara). In 1990, the consumption of natural gas in Georgia reached its maximum – 6046 million m^3 [12]. By this time, there were 576,5 thousand flats gasified in the country and the length of the gas pipelines was 4802,8 km. In 1990, the country's gas consumption gradually reduced and by the year 2000 it dropped down to 1094 million m^3, i.e. it reduced 5,5 times compared to the year 1990 [13] (please see Table 5 below).

In the process of increasing the level of gasification and electrification made a significant contribution activation of scientific-research works. The reasoning and importance of electrification and gasification were given in them, and necessity of introduction and measures [14-21].

Table 5. *Gasification Values of Georgia over 1990-2000 (by the year end)*

Year	Amount of gasified flats (thousand)	Gas pipeline length (km)	Consumption of natural gas (mln m^3)	Including agriculture
1990	576,5	4802,8	6046,0	180
1991	579,8	4937,8	4577,1	176
1992	587,2	4962,1	4633,7	49,6
1993	587,2	5158,4	3343,7	-
1994	587,4	5158,4	2595,2	-
1995	587,4	5158,4	910,5	-
1996	587,4	5151,9	947,0	12,4
1997	587,4	5151,9	830,0	13,6
1998	587,4	5151,9	846,0	14,9
1999	587,4	5151,9	1022,0	23,5
2000	587,4	5151,9	1094,0	26

The well-known developments of the past years had very negative impact on the gas plants. The country's gas supply stopped for a long time, gas was not supplied to Tbilisi as well as the entire Georgia (except Rustavi and Kazbegi region) during the entire 1995 and first half of 1996.

Within this period, Georgia's gasification level could not be improved due to the following issues:

1. Hard financial situation. No industrial enterprises were operating due to which the natural gas farms, in the environment of non-payment and low consumption, had very low revenues. There was not a single gas farm that had no debts (from several thousands to millions of GEL);
   Due to little volume of sold gas, gas cost was high as only small part of the network was functioning; however, depreciation and other taxes were fully charged;
2. Physical losses of gas were high and its percentage rate was high. Gas overconsumption by the residents (commercial losses) was added to the losses caused by the technical issues during the non-meter period, especially in winter;
3. Certain part of the residents had no money to install gas meters (approximately 100 USD);
4. Business plans could not be drafted and this made it difficult to attract investors.

Georgian gas supply was unstable over the following years too. It would even reduce during several years (Table 6).

Table 6. *Natural Gas Supply in Georgia over 2000-2016*

Year	Million m^3	Year	Million m^3	Year	Million m^3
2000	1094	2006	1860	2012	1933
2001	880	2007	1684	2013	1907
2002	700	2008	1450	2014	2197
2003	1011	2009	1200	2015	2416
2004	1231	2010	1094	2016	2261
2005	1440	2011	1750		

Until recently, the gasification of the villages in the country was conducted slowly. At present, this process is rather intensive. Major source of natural gas for Georgia now is Azerbaijan. So far the local production is still insignificant – only 0.3% of the total consumption. Both in villages and cities the consumption of the natural gas increases in the household sector. At the end of 2016, the amount of the consumers of this energy source exceeded a million (1055600) out of which 96.7% comes on the household [10]. Significant share in this number comes on villagers. In 2016, the average amount of natural gas consumed by one household consumer was 773 m^3. This value is 4.5% more than the value recorded in 2014. The increase is observed in Tbilisi too, and for the household consumers of the rest of Georgia, constant increase of the volume of the natural gas consumed by an individual subscriber indicates the increase of the role of the natural gas in the household sector. In the environment when it is more and more difficult to obtain wood in the country and its price increases respectively, natural gas is the most accessible (financially and in terms of the access to the energy) energy source in the environment of mass gasification of Georgian villages.

According to the aggregated energy balance of Georgia, natural gas consumption in agriculture, forestry and fishing amounted to 8,8 thousand tons of conventional fuels in 2016 which is 2,5 times higher than the same index in 2015. Now in this field the total cost of natural gas in the country is 0.656% (in 2015 – 0,256%) [20].

4. Conclusion

Energy development in Georgia is characterized by unsteady successes. Over the last 100 years (1913-2016) the manufacture of electricity has increased 568,3 times and the natural gas consumption has increased 5,0 times since the assimilation of it up to today (1960-2016). It is noteworthy that the maximum for both of them were achieved in 1989. Comparing the level of 2016, it was more than 39,2% in electricity manufacture and 2,7 times more in natural gas consumption. There is approximately the same trend in separate sectors in the use of these energy sources, including agriculture, which is traditionally characterized by low power equipment.

Analysis shows that in order to improve the situation in the future, it is necessary to take into account the characteristic specificity of both fields (energy and agriculture). For energy to work out successfully, the intensive and continuous funding is necessery in order to maintain the functioning capacity and at the same time to achieve progress in accordance with the Macroeconomic Environmental Requirements. It is necessary to attract a significant number of additional investments. This as a result of joint influence of other objective factors (ecological requirements, the need for more expensive energy resources, etc.), in the first place the capitalization of the sector and overall significance increase.

Also the peculiarities of Georgian agriculture electrification and gasification should be taken into consideration. First of all, here we assumed the country's mountainous relief, climate, seasonality, the existence of small settlements far away from the center, still high-handed labor, still remaining high share of manual labor and so on.

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Effect of the Using combined Environmentally Friendly Methods on Increasing the Yield of Agricultural Products

L.G.Eprikashvili[a*], M.A.Dzagania[a], G.V.Kekelia[b], N.B.Pirtskhalava[a], T.N.Kordzakhia[a], M.G.Zautashvili[a]

[a]Iv.Javakhishvili Tbilisi State University; P.Melikishvili Institute of Physical and Organic Chemistry, 3, Politkovskaya Str., Tbilisi, 0186, Georgia

[b]Georgian Technical University, 77, Kostava Str., Tbilisi, 0160, Georgia

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A B S T R A C T

The paper presents results of the combined use of environmentally friendly methods in order to increase the quantity and quality of a wheat harvest by intensifying the biological processes occurring in the seed with the use pre-seeding irradiation with a low-frequency acoustic range electromagnetic field (EMFAR) and with addition of natural zeolite - clinoptilolite to the soil. The experiment was carried out in eight variation, each in four replicates. The sowing qualities of wheat seeds were previously established. Laboratory germination of the test culture was 94%. Before sowing part of the seeds were soaked in water into the vegetation vessels, the other part – in a diluted solution NPK, and then exposed to the electromagnetic field of the acoustic range (EMFAR). The optimal parameters of EMFAR (frequency, voltage, exposure time) were established on the basis of previously testing. Prepared seeds were sown in vegetation vessels with composites containing soil and zeolite (clinoptilolite of the Handaki deposit, Georgia) on a 1: 1 ratio. The results of the carried out experiments showed that the life-sustaining activity parameters of test plants grown from seeds treated with EMFAR exceeded those of plants grown from untreated seeds. The proposed methods will increase the productive potential of wheat by improving the water- and energy-supply of plants (percent of germination, growth and yield parameters).

Keywords: Seeds, Stimulation, Electromagnetic field, Productivity, Natural zeolites, NPK.

*Corresponding author: Luba Eprikashvili; E-mail address: luba.eprikashvili@tsu.ge

Introduction

Today, the development of agriculture is in accordance with global trends in the transition from an extensive to an intensive way of managing.

Agriculture is one of the most important sector of the state's economy, not only from the point of view of ensuring food security, but also from the perspective of a common contribution to the development of the economy [1].

High productivity of crop species is one of the main indicators of the effectiveness of agricultural industry. In modern technologies, great importance is given to various methods of treating seeds and plants of grain crops with environmentally friendly methods that stimulate the growth and development of plants, increase their productivity and resistance to stress.

The solution of this problem is possible in two ways. The first way includes methods aimed at changing of environmental factors, creating favorable conditions for the growth and development of plants, such as fertilizing and feedings, the necessary agricultural activities, etc. In the process of evolution plants adapt to the environment, which determines their placement on land and in water. Within the area of distribution and biocoenosis, living conditions do not remain unchanged. In some years, changes in environmental factors are so significant that they make the plant need to mobilize all adaptive resources. The second way is associated with stimulating the growth and development of the

plant itself and includes the use of various agents, growth regulators, as well as presowing treatment that stimulate the development of plants. In countries where the most agricultural lands is at risk zone or unsustainable farming, the second way, i.e. the use of specimens and methods that increase plant resistance to uncomfortable growing conditions is the most relevant [2-3]. Development of new methods for increasing the viability of agricultural crops is the most important task of agrobiological sciences and agricultural industry. Over recent years in order to intensify plant growing, electrophysical methods of influencing plants and seeds of grain and vegetable have begun into the agriculture industry with the purpose of their stimulation (growth acceleration, yield enhancement and improvement of product quality). Stimulating effects such as electric and magnetic fields, sunlight, infrared and laser irradiation, high and ultrahigh frequency current have been tested in laboratory and field conditions. The influence of electrophysical factors on seeds is well reasoned and repeatedly tested in agriculture and has become widespread in many countries [4-11]. However, the response of the seeds to the same affecter may be different depending on the variety and quality of the seeds, duration of the treatment and radiation dose, waiting time from the moment of processing to sowing, on natural factors and other circumstances. For this reason, getting identical results of the efficiency of electrophysical processing is very difficult. Studies have established that under the influence of an electromagnetic field, forces are mobilized and the organism's energy reserves are released. Physiological and biochemical processes are activated in the early stages of seed germination; increasing in intra-exchange processes and a steady increase in germination energy, germination, initial growth strength, spring-summer survivorship rate takes place. All this favorably affects the entire subsequent period of plant development. A common drawback of all existing technologies using pre-sowing seed treatment by electrophysical methods is the low repeatability of the treatment results, and, as a consequence of this, the inability to determine the desired values of parameters of the acting electromagnetic field, which would provide a stable positive effect. Stimulation of plant growth is a complex problem. The difficulty of this study is insufficient knowledge of the metabolism of the plant organism, including such integral processes as growth and productivity. In many cases, the researchers' approach to the problem of seed stimulation remains empirical, because there are no sufficiently deep theoretical and experimental studies of the mechanism of action of various physical factors on seed.

Diseases and seed insects have a negative effects on the sowing quality of seeds. Chemical etching methods are currently used to prevent seed diseases. However, the use of toxic chemicals has a number of negative environmental consequences [12-14]. Therefore, it is necessary to stimulate seeds, improve their sowing properties and compensate for the inhibitory effect of etching.

In the framework of this study, the stimulation and intensification of the development of biological processes in the "seed + soil" system is assumed by the activation of elements at the nanolevel [15-17]. To implement research in this direction, it is necessary to harmonize processes that occur both at each stage of plant development and in the soil; i.e. the optimal set of field and soil parameters should be selected: field intensity and frequency, exposure time, ratio of the amount of zeolite and organic component. A significant improvement in the conditions of plant growth and development is achievable by stimulation and intensification of metabolic processes in plants via improving the supply of plants with water and nutrients from introducing organic-zeolite mixtures into the soil, as well as by pre-sowing seed treatment in an EMFAR (low frequency electromagnetic field of the acoustic range).

A number of researchers [14–16] have noted the high sensitivity of biological systems to the effects of low intensity electromagnetic fields of low-frequency range. According to some authors, the high sensitivity of seeds to a low-frequency electromagnetic field is explained by a change in the pH of the medium and by the release of proteins from the bound state into the aqueous medium. This, in turn, accelerates exiting of seeds from a dormant state and stimulates the development of restoration processes in them [17-19]. As a result, the impact of EMFAR contributes to an increase in crop productivity. At the same time, all types of electromagnetic radiation when exposed to seeds have stimulation and inhibition zones depending on the radiation dose. The use of electrophysical methods for pre-sowing seed treatment in agriculture is universal and the degree of their impact is mainly regulated by changing the power or exposure depending on the purpose of the treatment. Seeds initial quality and their viability is also affected on processing effect. We did not find any information on the combined use in agricul-

ture of natural zeolites and a low-frequency electric field of the acoustic range for the cultivation of organic crops. It should be noted that the ecological efficiency of using zeolite tuffs in the soil + plant system has been confirmed by researchers to improve the agrophysical and physical-chemical properties of soils, increase the yield and quality of plant products [20-27]. It has been experimentally established that introduction of unconventional mineral agricultural resources into the soil is economically viable and environmentally friendly agricultural method for increasing soil fertility and environmental resistance of both soils and plants to extreme conditions. In areas of environmental risk this agricultural method has a positive effect on the most important vital signs of grain, crop formation and grain quality parameters. Under certain environmental conditions natural zeolites, introduced into the soil are long-acting and able to change the ratio of cations in the soil absorption complex. Zeolite-containing species, in principle, are silicon-containing polymineral fertilizers because the main part of the chemical composition of various zeolites, just like the soil, is silicon. Silicon fertilizers and ameliorants on degraded soils of agricultural lands and soils with a low level of fertility can increase the provision of plants with silicon and effectiveness of the applied mineral and organic fertilizers, as well as plant protection products. The main function of silicon in plants is to ensure the organism's resistance to adverse environmental conditions, accelerate the growth and development of the root system, bind toxic compounds, and also ensure the synthesis of the necessary antioxidant protection enzymes and stress proteins. A positive reaction to the introduction of silicon is characteristically not only of silicophilous cereal plants, but also for other families.

Objects and Research Methods

Based on the foregoing, it seemed to us interesting to combine two methods: treatment of test seeds (wheat) with a low-frequency electromagnetic field of the acoustic range (EMFAR) and the introduction of natural zeolite into the soil. Due to this a laboratory mini-generator was designed. The optimal parameters of the electromagnetic field of the low-frequency acoustic range (frequency, voltage, exposure time) were established on the basis of earlier experiments. The sowing qualities of wheat seeds were established before the experiment. Seeds with good germination and high germination energy always give friendly and full-fledged seedlings. Seed germination energy – the ability of seeds of agricultural crops to grow together. This indicator is determined simultaneously with germination – the number of germinated seeds (as %) during a certain period for each crop. Determination of germination is one of the most important types of assessing the sowing qualities of seeds, since with poor germination the yield is reduced. Seed germination is understood as the number of normally germinated seeds in a sample taken for analysis (as %). Laboratory germination is the percentage of normally germinated seeds within 7-10 days in a sample taken for analysis. Germination should be close to 100% [6, 14]. In this case, the germination of the test culture (wheat) was 94%. Germination of seeds treated in EMFAR began one day earlier than in untreated seeds.

The experiment was carried out in eight variants, each in four replicates. Garden soil (gray-brown) and zeolite-clinoptilolite of Handaki deposit (Georgia) with a mineral content of 60-70% (ratio soil/zeolite was 1:1) were placed in vegetative vessels.

Before sowing the seeds (in vegetation vessels) were soaked in water or in a diluted solution of (0.5%) NPK. Some of them were treated with EMFAR, and then sown in prepared composites. Duration of the experiment was seven months (09.11.2017. -22.06.2018).

Results and its Discussion

First option – clean soil (absolute background) + wheat (test plant)+ water

Second option – soil + wheat + solution NPK.

Third option – soil + wheat + water, EMFAR.

Fourth option – soil + wheat + solution NPK +EMFAR.

Fifth option – soil + zeolite + wheat + water.

Sixth option – soil + zeolite + wheat + solution NPK.

Seventh option – soil + zeolite + wheat + water + EMFAR

Eighth option – soil + zeolite + wheat + solution NPK +EMFAR.

Table. *Biometric indicators of wheat development in various variants*

Variants	I	II	III	IV	V	VI	VII	VIII
Indicators of plant development	soil + water treated seeds (control)	soil + water treated seeds+ EMFAR	soil +seeds treated with solutions NPK	soil + seeds treated with solutions NPK + EMFAR	soil+zeolite+water treated seeds	soil+zeolite+water treated seeds+ EMFAR	soil +zeolite + seeds treated with solutions NPK	soil + zeolite + seeds treated with solutions NPK + EMFAR
Germination (%)	80	85	85	90	90	95	95	95
The average length of the stem (cm)	34,3	34,7	35,2	41,0	41,7	46,3	49,9	51,6
Average spike length (cm)	4,5	4,8	5,1	5,6	5,7	6,22	6.5	7,0
1000 seed weight (g)	19,7	20,0	25,0	25,00	26,3	26,32	29,00	31,5
Number of spike /m^2	255	349	382	400	410	440	450	502
The number of stems per 1 m^2	254	270	270	286	286	302	302	302
Green mass per 1 m^2 (g)	127,4	140,1	219,8	226,1	232,5	251,6	264,4	267,5

It is seen from the tabular data that the combination of the methods used (treating the seeds of the test culture with a low-frequency electromagnetic field of the acoustic range and introducing natural zeolite into the soil) positively affects the germination rate, green mass of plants and the increase in grain mass. It is likely that seed treatment (using EMFAR) before sowing activates the enzyme system in the initial period of seed development, which positively affects the final yield. The number of productive stems in plants from untreated seeds is less than in plants from treated seeds. Plant height and spike size in plants obtained from treated seeds is greater than from untreated seeds. Introduction of zeolite into the soil, as well as the preliminary soaking of seeds in an NPK solution, have a positive effect on the development of test plants. It is known that the number of grains in an spike is the main indicator of productivity. A mass of 1000 seeds shows what mass (in g) a thousand "pure seeds" have. As can be seen from the tabular data when combining all the options used in the experiment, the best results were obtained by sowing seeds soaked in an NPK solution and treated with EMFAR and sown in soil with zeolite.

Conclusion

Thus, the combination of the above methods contributes to reliable and stable activation of the physiological potential of plants, reduce environmental pollution and obtain environmentally friendly products. Vegetative plants grown from treated seeds are superior to untreated plants in terms of parameters.

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Reducing the Depth Migration of Radionuclides by Incorporation Organic and Inorganic Components into the Soil

Gogebashvili M.E.[ab*] , Gongadze A.D.[b], Tulashvili E.V.[c], Ivanishili N.I.[ab], Osidze I.G.[b], Kiparoidze S.A.[a]

[a]Laboratory of Radiation Safety Problems, I.Beritashvili Center of Experimental Biomedicine, 14, Levan Gulua Str., Tbilisi, 0166, Georgia

[b]I.Javakhishvili Tbilisi State University E.Andronikashvili Institute of Physics, 6, Mikheil Tamarashvili Str., Tbilisi, 0177, Georgia

[c]Material Research Institute, Faculty of Exact and Natural Sciences, I.Javakhishvili Tbilisi State University, 13, Chavchavadze Av., Tbilisi, 0179, Georgia

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A B S T R A C T

Purification of soils contaminated with radionuclides represents actual scientific-practical task. Depending on the volume, transportation-storage of such contaminated soils is associated with some difficulties. In this regard, phytoremediation methodology using is a relatively more appropriate. The main purpose of our research was to create a layer on remediation tank, which would facilitate the implementation of the phytoremediation method and at the same time prevented the migration of radionuclides into the lower layers of the soil. Modeling of radioisotope migration was performed by using the column method. The object of the study was soil contaminated by ^{137}Cs, while, various options containing organic and inorganic components were used as migration restricting factors. Maximum indicator of depth migration reduction was obtained by using astatine and manure mixture. The proposed method of using organic and inorganic components mixture to reduce depth migration of radionuclides into the soil is appropriate not only for our marker ^{137}Cs, also in the condition of other radioisotopes contamination..

Keywords: Radionuclides, Migration, Phytoremediation, Sorption, Radioisotopes, Nuclear Energy.

*Corresponding author: Micheil Gogebashvili; E-mail address: gogebashvili@gmail.com

Introduction

Georgia's orography and it's interaction with airThe rapid development of nuclear energy, the widespread use of radionuclides and ionizing radiation in various fields of human activity have determined the fact that radioactive substances have become one of the permanent constituents of the contaminated biosphere, ionizing radiation is discussed as ecological factor of environment. Global radioactive emission represents one of the way occurring radionuclides on the surface on the earth [1-3]. As a result of accident at Chernobyl and later, at Fukushima nuclear power stations has been accumulated the largest factual material about the intensity of Global occurrence of radioactive products from atmosphere to the soil, plant, food, human and animal body [4-7]. At the same time, problems of local type pollution emerged; Namely, in connection with the reorganization of scientific institutions, several specialized research laboratories have ceased to function in Georgia. Therefore, some problem is the radiological monitoring of the adjacent areas to the laboratories and the issue of radioactive materials utilization [8,9]. In this regard, it's particularly important to develop a methodology of the soils purification contaminated by radionuclides, because the mass of contaminated soils due to their volume does not allow for the storage of such soils [10]. One of the effective methods is

performing remedial work by using plant objects [11-13]. During planning phytoremediation methodology it's necessary to take into consideration the forms of radionuclides present in the study area. Specifically, analysis of the content of water soluble, exchangeable and non-exchangeable forms of radionuclides in the soil.

Water soluble forms of radionuclides, characterized by high rates of depth migration, they pose a significant ecological threat in terms of environmental pollution. Considering the fact that radionuclides can be introduced from the deeper layers of soil into the root system of perennials, or their spread through the groundwater into the environment, then it becomes clear how big are the environmental risks of water soluble forms of radionuclides existence in the soil [14,15]. Therefore, it's a certain scientific-practical task, apply such a methodology against depth migration of radionuclides which on the one hand will prevent the movement of radionuclides into the deeper layers of the soil and, on the other hand, allow the arrangement of tanks intended for phytoremediation.

Research Object and Methods

The object of the study was one of the most widespread soil types - brown soil in Georgia [16,17]. The soil was contaminated with the remains of closed radioisotope laboratory of the former Institute of Radiology and Ecology. Modeling of radioisotope migration was performed using the column method. Vertical columns of 50 cm were presented with combinations of different options: In the upper layers of the columns, contaminated soil was placed in equal amount. Intermediate fractions contained various types of organic and inorganic components. Ascanite was used as an inorganic component, mixing with the soil in different proportions, and the organic component was manure powder. The experiment also involved simultaneously the complex drug containing the both component-inorganic and organic. Universal humic-organic fertilizer "Agrovita", which contains: 1.5% humate, 3% nitrogen, 2.3% phosphorus and 1.6% potassium. The lower layer of the columns was provided with basic, clean soil. Fraction of acid-washed sand was used for the separation of boundaries between layers. The content of radionuclides in each fraction of column was determined by gamma spectrometry (Gamma-Beta Spectrometer "ATOMTEX MKC-AT-1315" and Gamma-Spectrometer "CANBERRA" with liquid nitrogen freeze germanium detector).

Results and discussion

The nature, velocity and diraction of migration of radionuclides distribution in the soil profile in different biogeocenosis are largely determined by their attachment or connecting strength to the soil [18]. Nowadays, studies on the attachment and connection of radionuclides to soil mainly represents laboratory researches for the study of radionuclide sorption (absorption) and desorption under static and dynamic conditions [19]. At the same time, in many cases, the study material is a mass, formed by the mixing of soils and various components over many years, the cleaning is a major problem due to its volume. Our study soil has been formed for 20 years as a result of complex creation processes of soil components and radionuclides. The characteristic radioactivity of the mentioned soil was 12230Bq/kg. Columns fractional compositions conventionally are reflected in the picture (Fig. 1). A-fraction contained contaminated soil which was washed periodically by the addition of water (for 3 months). Water was also added in equal amounts to each column. C-fraction contained a different ratio of basic soil and study organic and inorganic components. B-fraction in each column was also represented by the same amount of base soil. After the experiment, radioactivity of B-fraction was determined. Fig.1 reflects the results. In order to optimize the level of contamination, different amounts of contaminated soil to be included in the column was used - with the maximum rate 783.9 Bq/kg of radioactivity of column's B fraction of (Fig. 1-1), but minimum - 482.2 Bq/kg (Figure 1-2).

A- Contaminated soil fraction; B- Base (clean) soil fraction; Base soil and study components used in the C-model. Dotted lines indicate the radioactivity of B-fraction contaminated by the migration of 137Cs: 1-Double count of contaminated soil; 2 - Standard amount of contaminated soil; 3- Mixture of sand and ascanite; 4- Basic soil treated with "Agrovita" drug; 5- Manure powder; 6-Mixture of basic soil and manure powder; 7-Base soil mixture with ascanite and manure powder.

Since, experiment involved the introduction of the study components into the C-fraction, due to the volume of the column; minimal amount of contaminated soil was introduced in each column. – It's activity was 482.2 Bq/kg, so the second option

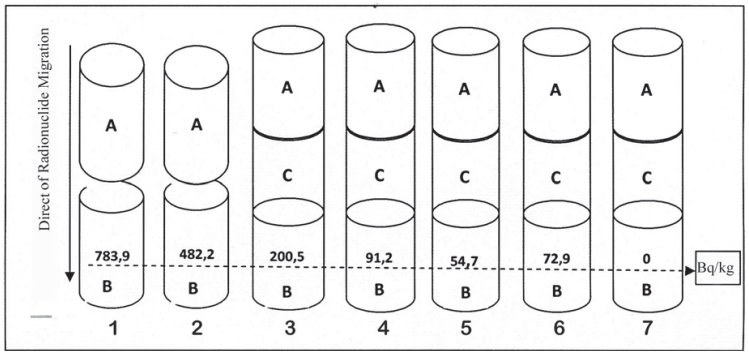

Fig. 1. *General Scheme of Radionuclide Migration Modeling*

(without C-fraction) was considered as a control option, where the standard amount of contaminated soil was included, as in other options used in the experiment. The C-fraction of 3th column contained a mixture of sand and ascanite (5: 1), whose radioactivity of the B-fraction amounted to 200.5 Bq/kg. The C-fraction of 4th column consisted a basic soil treated with "Agrovita" drug. The radioactivity of the B-fraction of the mentioned column was 91.2 Bq/kg. The C-fraction of 5th column was represented by a manure powder whose B-fraction radioactivity was 54.7 Bq /kg. In the C-fraction of 6th column, the base soil was introduced together with the manure powder (1: 1 ratio). The radioactivity of the B-fraction last one was 72.9 Bq/kg. As for 7th column, it's C-fraction contained a mixture of ascanite and manure powder, but no radioactivity was observed for the B-fraction of this column. It is clear that only water soluble forms of radionuclides are subject to depth migration from contaminated soil. Therefore, the presence of water-soluble forms of radionuclides is important during radiation contamination of soil-vegetation cover, on which depends the soil components connection strength of radionuclides to the soil components and there transport above ground parts of the plant. Radioactive decay products in form of anions, which are poorly connected with the soil, moving fairly fast in the soil profile. The quantitative characteristics of different forms of radionuclides (water soluble, exchangeable and non-exchangeable) are determined by their chemical properties — the heterogeneous nature of complex compounds formation with soil components. Figure 2 shows that the zones saturated with the test components used in our experiment are characterized by different radio capacity.

Figure 2 shows the influence of C-fraction's (Fig. 1) different content on the migration intensity (expressed in %) of radionuclides. The migration intensity of the control option radionuclides is assumed to be 100% (Fig. 2-1). In the second option (Figure 2-2) radioactivity of B-fraction amounted to 41.6% compared to the control; the same indicator was 18.9% in option treated by "Agrovita" (Fig. 2-3), and in the 4th and 5th option respectively 11.3 and 15.1% (Fig. 2-4.5). The maximum rate of sorption processes was obtained when using a mixture of ascanite and manure powder (Figure 2-6). Radioactivity feature of B-fraction was not observed in mentioned option.

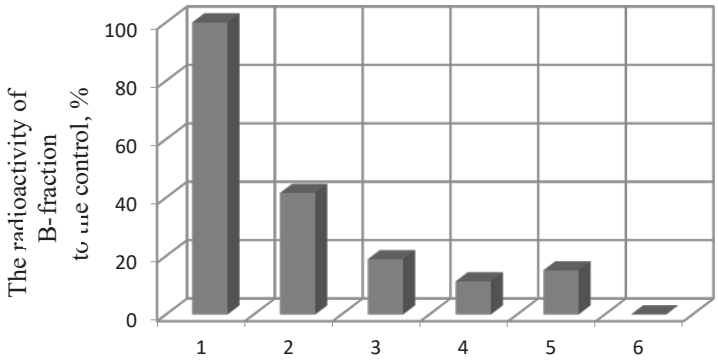

Fig. 2. *Influence of various organic and inorganic compounds On the intensity of radionuclide migration*

1 - Standard amount of contaminated soil; 2- Mixture of basic soil and ascanite; 3- Basic soil treated with "Agrovita" drug; 4- Manure powder; 5- Mixture of base soil and manure powder; 6- Base soil mixture with ascanite and manure powder.

Conclusion

The obtained results allow to establish the complex mechanism of radionuclides interaction with soil components. The results of the present study show the similarity of the final effects, despite the fact that experimental options are based on different mechanisms of the interaction of soil components with radionuclides. All options used in the experiment are characterized by relatively high rate of sorption, but maximum rate of deep migration reduction is obtained by using a mixture of ascanite and manure. In the last option, radioactivity of B -fraction reaches to the background level. Each component of the ascanite-manure option is characterized by quantitative constraints, namely, high concentrations of ascanite impede the flow of water into the column, and the use of pure manure is not conducive for plant cultivation intended for phytoremediation. Therefore, the proposed approach for the use of a mixture of organic and inorganic components for the depth migration reduction of radionuclides into the soil is appropriate not only for marker ^{137}Cs used by us, but also in condition of other radioisotope contamination.

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First report on diversity of probiotics in Dambalkhacho, traditional fermented milk product of Georgia

T.Japaridze*, V.Baramidze , M.Vakhania , L. Kutateladze, E. Elizbarashvili, K. Didebulidze

Agricultural University of Georgia, Kakha Bendukidze University Campus, 240, David Aghmashenebeli Alley, Tbilisi, 0159, Georgia

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A B S T R A C T

Dambalkhacho is a traditional Georgian fermented milk product, protected under the patented brand name N 1585/071, manufactured from cottage cheese, with unique starter cultures, in the mountainous regions of Georgia. This study was undertaken in order to gain insight into the microbiota, in particular probiotic strains that take part in the manufacture and ripening stages of Dambalkhacho. Traditional cheeses are an important reservoir of microbial diversity that can have important biotechnological applications, starter cultures consisting of autochthonous bifidobacterial strains are of particular interest. To determine the strain identity and evaluate their probiotic potential, we made use of both culturing and the culture-independent methods of PCR. The main species isolated were *B. bifidum, B. longum and B. longum subsp. Infantis.* Culturing methods enabled the determination of a number of viable microorganisms from different microbial groups and their isolation for potential future applications in specific starter cultures. Strains were tested for tolerance to low pH and high bile concentrations, simulating the human gastrointestinal conditions. The probiotic strain B (KB 1.4); C (TB 2); D (TB 6.2.1); B (TL 8.2); D (TL 2.4.1) met the suggested initial count of 106 CFU/ml with brand C recording the highest at 9.19 ± 0.14 log CFU/ml. The higher bile concentrations resulted in lower growth in all the strains. After pH 3.0 treatment, B (KB 1.4); D (TB 6.2.1); D (TL 2.4.1) have also met the requirement of survival at 2 % bile concentration. Based on the overall technological aptitude of the tested strains B (KB 1.4); D (TB 6.2.1); D (TL 2.4.1) met the initial count requirement, and exhibited good acid and bile tolerance therefore being a potentially good source of probiotic additives.

Keywords: Probiotics, Bifidobacteria, Fermented milk, Dambalkhacho, Important reservoir, Traditional cheeses.

*Corresponding author: Tamar Japaridze: E-mail address: t.japaridze@agruni.edu.ge

Introduction

New forms and range of food products favoring nutritional and therapeutic aspects are being increasingly developed. There has been a phenomenal growth in consumption of foods containing functional ingredients, particularly in Western Europe, USA and Japan [1]. Bacteria belonging to the genus Lactobacillus are members of the lactic acid bacteria family (LAB), a broadly defined group characterized by the formation of lactic acid as the sole or main end product of carbohydrate metabolism. The administration of probiotic Lactobacilli stimulated indigenous Lactobacilli and the production of short-chain fatty acids. This alteration of the intestinal environment should contribute to maintain the host's health. They can be found mostly in fermented foods (yogurt, cheese, olives, pickles, salami, etc.), as well as in the oral cavities, gastrointestinal tracts (GIT) and reproductive tract of animals [2-4]. One of the first microorganisms colonizing infants gut is Bifidobacterium species, but the number and variety of Bifidobacterium species decreases with age, from childhood to old age. Bifidobacterium longum, B. breve, and B. bifidum are generally dominant in infants, whereas B. catenulatum, B. adolescentis and, as well as B. longum are more prevalent in adults. Moreover, bifidobacteria have been associated with the production of a number of potentially health promoting metabolites including short chain fatty acids, conjugated linoleic acid and bacteriocins [5]. The identification and

enumeration of bifidobacteria has been reported by several research teams [6-9].

Dambalokhacho is a type of unique, traditional Georgian fermented milk products that is protected under the patented brand name N 1585/071. It has been produced for centuries in Georgia, predominantly in mountainous regions of the country. The production of the product is not industrialized thus, ancient fermentative microflora is maintained by isolated communities, and is harboring a wide pool for isolation of probiotic microorganisms. The search for new types of probiotics, from the microbiota of traditionally fermented foods especially exhibiting diverse composition of naturally occurring symbiosis of beneficial microorganisms, is the most promising pool providing novel strains [10] Increasingly, evidence is accumulating which shows beneficial effects of supplementation with bifidobacteria for the improvement of human health conditions ranging from protection against infection to different extra- and intra-intestinal positive effects [11-13]. Complex aproach is needed to identify probiotic properties of select strains. Several difierent methods have been reported but single system of tests has not been identified. Here we report the complex of experimental procedures, for identificar tin and evaluation of probiotic avtivity of bifidobacterial and lactobacillus strains isolated from spontaneously fermented, traditional milk products.

Recent studies have confirmed that bifidobacteria can protect from enteropathogenic infections through the production of acetate[14-15], which improves epithelial mediated intestinal defense, thus protecting the host against lethal infections. Furusawa et al. reported that a commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells, which have a critical role in suppression of inflammatory and allergic reactions [16]. These two studies are redefining the criteria for probiotic selection, providing us with a new means for evaluating the health beneficial effects of commensal bacteria.

Based on the above studies we intend to measure the acetate and butirate production of the selected strains to establish their probiotic activity. The acetate concentration will be evaluated by using EnzyChrom™ Acetate Assay Kit, which is designed for the quantitative determination of acetic acid or acetate and the evaluation of drug effects on acetate metabolism. Or with the same procedure as used for the butyrate, by gas chromatography, following the protocol developed and published by Richard E. Hiliman [17].

Materials and methods

Sample collection and bacterial strains

In total 30 samples were collected from specific ecological regions of Georgia, in small family farms or at local markets, from Pshavi, Ukana Pshavi Tianeti Pshavian villages. Samples were collected in sterile bags, labled, transported to the laboratory in cool boxes and stored at - 20 °C until further processing. All the samples were seeded onto specific selective media MRS, M17. BSM (sigma-aldrich), and reseeded up to 8 times, to obtain pure culture strains. 10 bifidobacterial strains were identified and selected for probiotic activity tests. The reference strains were purchased from DSMZ- Deutche Samml;ung von Mikrooganismen und Zellkulturen Gmbh. LactobacillusBulgaricus – ATCC 11842, Delbruck – ATCC 9649, Streptochochus tehrmophilus – ATCC 19258, Bifidobacterium Infantis– ATCC-15697, Bifodbacterium Longum-ATCC 15707, Bifidobacterium Bifidum- ATCC 2952. Gramm stained and visualized by light microscopy with Omax-wvr 1000 instrument [18].

DNA extraction and PCR

DNA was extracted using Milk DNA Extraction Kit (Norgen, Thorold, Ontario, Canada) bacterial DNA extraction kit, according to manufacturers instructions with slight modiffications as followed. Prior to DNA extraction 500 mg of dambalkhacho was homogenized in prelyzis buffer containing Tris 0.02 M, EDTA- 0.02 M. DNA quality and quantity was evaluated with nanodrop instrument ND-1000, NanoDrop Technologies, Inc.

Strain specific PCR was conducted for each pure strain using primers established by [19]. As well as designed by our team, using NCBI primer design toolConditions have been modified as follows; Initial heating for 2 min 95 °C, followed by 35 cycles consisting of denaturation 95 °C 15 s, annealing 57 °C 30 s, extension at 72 °C 40 s, and a final extension 72 °C 4 min. Amplicons were separated on 1 % agarose gel by electrophoresis and analyzed by ethidium bromide staining.

Probiotic activity tests

Strain survival in vitro conditions simulating the passage through the stomach and intestine was evaluated. All experiments have been carried out in triplicate.

Tolerance against Bile

MRS broth containing 0.3% and 2 % bile was inoculated with active overnight cultures. Culture with 0 % bile served as a control sample. The principle of assay was assessing the cell viability after exposure to bile salts (sigma) for 2, 4 and 6 h incubation with bile salts. Survival of strains were determined by pour plate counts of all the samples using 10-fold serial dilutions prepared in 0.1% peptone water. Viable cells were enumerated in 24 h anaerobic incubation and cell growth was monitored for growth every 3 h by measuring the absorbance at 620 nm [20].

Resistance to Low pH

Survival under low pH was evaluated based on methodology developed by Sahadeva and colleagues [21]. Active cultures were harvested by centrifugation and pelletswere washed once in phosphate-saline buffer (PBS, pH 7.2), re-suspended in PBS with pH adjusted to 1.5, 3.0 and 7.2 using 1M HCl (control with over 1.5 hour intervals) and incubated in MRS agar plates at 37˚C for 24 h.

Phosphoketolase assay

The ability of studied strains to transform fructose-6-phosphate into acethyl-phosphate and erythrose-phosphate by phosphoketolase, was studied according to the method developed by Zinedine et, all 2007.

Strains were grown in MRS broth for 24 h at 37°C, the culture was centrifuged and washed twice with a solution containing (PBS buffer 0.05% adjusted to pH 6.5 and added to 0.25% L-cysteine). Cells were then suspended in 2 ml of a lysozyme solution (10 mg/ml) and incubated a 37°C for 1.5 h followed by 1.5 h incubation at -20 °C. Afterwards 0.25 ml solutions containing 3 mg/ml of sodium fluoride and potassium iodoacetate 5 mg/ml was added, vortexed and incubated at 37°C for 30 min. Following incubation, 1.5 ml of a solution of hydroxylamine-HCl (13 g/100ml water, pH 6.5) was added and incubated at room temperature(RT) for 10 min. A reddish-violet color develops immediately with the addition of ferric chloride if the culture contains phosphoketolase activity. Accordingly, 3 ml of a solution of trichloroacetic acid (TCA at 15% w/v), 1.0 ml of 4N HCl and 1.0 ml of ferric chloride (FeCl, 6 H2O, 5% w/v in 0.1 N HCl) were added and incubated at RT for 10 min.

Acidification and Coagulation

Acidification and coagulation ability of LAB strains were assayed by inoculating 10% skim milk (RM1254, HiMedia, Mumbai, India) at 1% level and incubated at 37◦C for 28 h. Observation was made for commencement of clotting, followed by pH measurement [22-23].

Growth curve

The stock culture of each strain was separately prepared on MRS medium. Inoculated cultures were incubated at 37 ° C for 24 h and then stored in a refrigerator at 4 ° C. Batch submerged fermentation process was performed in separate flasks containing autoclaved milk as the substrate, incubations performed at 37 ° C. Cell number measured by specrophotometry at 620 nm wave length, measurements taken once a day for 3 days. Lactobacillus Bulgaricus – ATCC 11842 used as control.

Results and discussion

In total, 30 Dambalkhacho samples have been collected. Isolates have been characterized for cell morphology and Gram's character test. 18strains were selected for further experiments based on gram staining and microscopy. A number of bacterial isolates isolated from dambalkhacho in the present investigation were gram positive, branched and rod shaped, anaerobic, catalase negative, non-spore forming, which were identified as bifidobacteria (Fig. 1). Several different selective media for Bifidobacterial isolation and enumeration have been tested, the best results had been obtained with modified BSM media of Sigma (88517 Sigma-Aldrich BSM Agar). In the medium, we have increased the malt-dextrin concentration, which gives more carbon and energy for the selected strains.

After, initial tests the pure strains were subjected to further species identification procedures, by DNA extraction and 16s rDNA PCR analysis. In all the PCR amplification experiments the reference strains were used as positive controls. Identification of the isolated bifidobacterial strains to species was performed using PCR technique applying 16S rDNA-gene-targeted primers specific to *B. bifidum, B. longum* and *B. longum subsp. Infantis,* out of 18 strains with stringent molecular techniques only 5 were classified to *B. bifidum,* 4 *B. longum subsp. infantis* and 7 to *B. longum species* (Table 1).

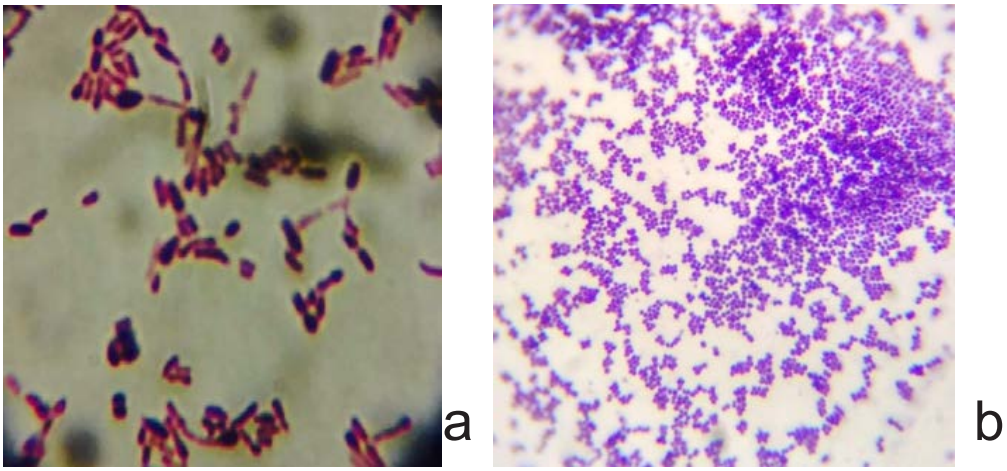

Fig. 1. *Representative pictures of select strains. Visualization by microscopy, x1000 magnification. Staining by Gramm stain. a) Sample seeded first time - mix culture; b) pure culture after 8 seedings*

all 3 species were isolated on MRS and BSM medium; In order to confirm the species specificity of isolated strains. Several PCR primer sets used (Table 2).

2 strains have not shown specificity of select primers and thus have not been assigned species. In this case, further studies are necessary. Strains belonging to

Table 1. *Names and the assigned species of the isolated strains*

Strains	Assigned species
A (KL10.2.1)	B. infantis
B (KL4.3.1)	B. infantis
C (TN5.7)	B. infantis
D (TN 5.3)	B. infantis
A (KB 12)	B.bifidum
B (KB 1.4)	B.bifidum
C (TB 2)	B.bifidum
D (TB 6.2.1)	B.bifidum
E (TUB 3.3.2.1)	B.bifidum
A (TL 1)	B. longum
B (TL 8.2)	B. longum
C (TL 5.3)	B. longum
D (TL 2.4.1)	B. longum
E (TL 3)	B. longum
F (6.5.5)	B. longum
G (2.1)	B. longum

Table 2. *Terget, Priner and Sequence*

Target	Primer	Sequence	(bp)
Bifidobacterium	g-Bifid-F	CTCCTGGAAACGGGTGG	549-563
	g-Bifid-R	GGTGTTCTTCCCGATATCTACA	
B. adolescentis	BiADOg-1a	CTCCAGTTGGATGCATGTC	279
	BiADOg-1b	TCCAGTTGACCGCATGGT	
B. bifidum	BiBIF-1	CCACATGATCGCATGTGATTG	278
	BiBIF-2	CCGAAGGCTTGCTCCCAAA	
B. breve	BiBRE-1	CCGGATGCTCCATCACAC	288
	BiBRE-2	ACAAAGTGCCTTGCTCCCT	
B. longum	BiLON-1	TTCCAGTTGATCGCATGGTC	831
	BiLON-2	GGGAAGCCGTATCTCTACGA	
B. infantis	BiINF-1	TTCCAGTTGATCGCATGGTC	828
	BiINF-2	GGAAACCCCATCTCTGGGAT	
B. lactis	Bflac2	GTGGAGACACGGTTTCCC	680
	Bflac5a	CACACCACACAATCCAATAC	

Some of the primers tested have shown crossreactivity to various other lactobacillus species (data not shown). Nonetheless, nonspecific PCR products were reduced, when annealing temperatures were increased to just below the maximum temperature at which no amplicons were produced. Primer sets selected during our investigations showed a considerable lack of specificity, even under our modified, more stringent PCR conditions. Experimental results of PCR are shown in Fig.2.

One of the most important criterion for probiotic selection is their tolerance to high acid levels, which is a determinant factor for the survival in the GI tract. The lowest pH in the GI tract according to Liong 2005 is 1.5 [21], [23], [28]. Whereas Leroy 2004, reports that pH 3.0 resistance is a threshold for best probiotic sources, and pH 7.2 is counted as control due to the constant probiotic counts [23]. Selected strains were incubated at different pH values of1.5, 3.0 and 7.2 (control) with over1.5 h intervals. Four strains, C (TB2); D (TB6.2.1); C(TL 5.3) and D(TL 2.4.1) have met the minimum initial count requirement asset by WHO/FAO2006.The acid tolerance for all isolates were the same where increasing level of acidity has negative impact on the viability of the probiotics (Fig. 3).

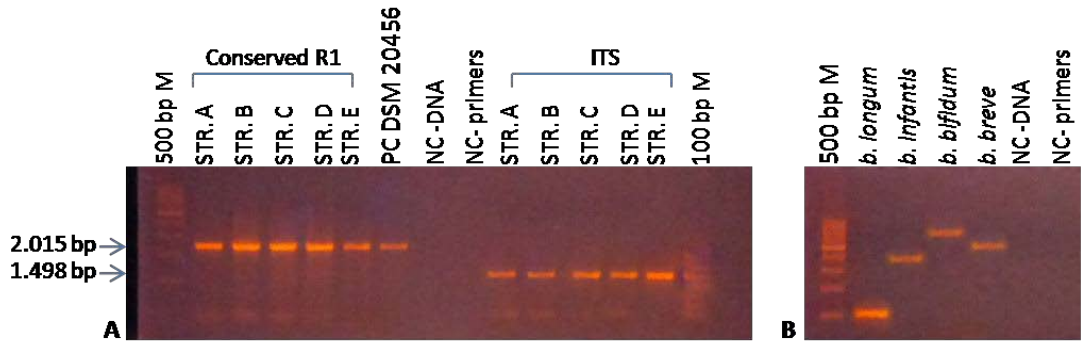

Fig. 2. *Representative agarose gel of amplification with PCR. A. Genus specific PCR Amplification using Bifidobacteria genus specific primers for ITS (g-Bifid-F; g-Bifid-R) and Conserver region 1 (LonU7; LonU8). Lane 1- molecular weight marker 500 bp. Lane 2-6 select strains, with the strain name indicated above the band. Positive control (PC+) reference strain. B. Multiplex PCR Lane 1-molecular weight marker 500 bp. Lane, 2 -5 control strains, with the strain species indicated above the band. Negative control (NC-) reaction without DNA or primers*

All strains have met the minimum requirement set by FAO/WHO (1 x 106 CFU/ml) except for strain E. The highest number of live probiotics was recorded by strain C with a count of 1.55 x 109 CFU/ mL and the lowest was recorded by strain E with a count of 2.40 x 105 CFU/ mL Judging by this initial count, strain A, B, C and D are considered good probiotic sources except for strain E (TUB 3.3.2.1). Amongst the reasons for the low count in strain E could be affected by the temperature during the fermentation process.. As a Brand E is strictly anaerobic, the oxygen that is dissolved in the product during manufacturing could stress the probiotics as too much oxygen will delay their growth [24]–[27]

Three selected strains showing the highest probiotic activity in previous tests have been subjected to cell viability and bile resistance tests for longer exposure times. Bile plays an important role in the physiology of intestinal bacteria. This is particularly important for probiotic bacteria, since their beneficial effects must be generated in their presence. It is known that the activities of intestinal bifidobacteria are deeply influenced by the presence of bile salts, and even some of them, such as cholesterol assimilation, have been directly correlated with bile salt metabolism in these bacteria.

To evaluate bile salt tolerance, viable cells were counted by measuring absorbance at 620 nm spectro-

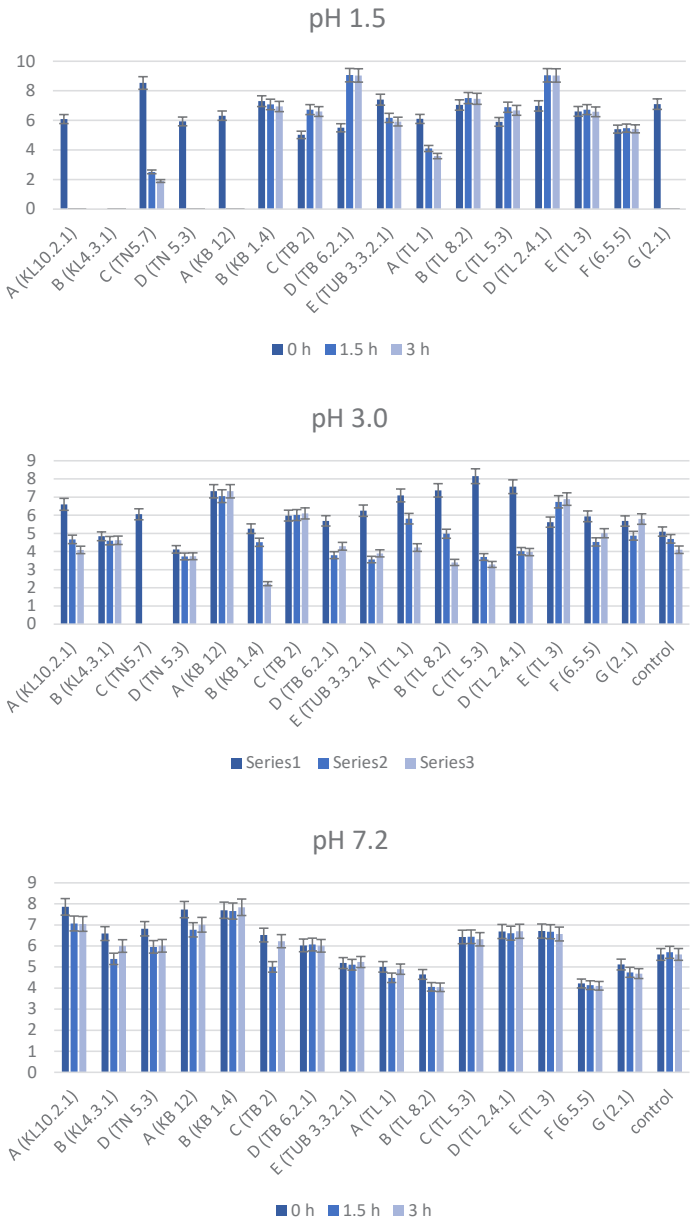

Fig. 3. *pH resistance of select strains. Total plate counts for five select strains on MRS agars at different pH values of 1.5, 3.0 and 7.2(control) over 1.5 hour interval*

photometrically using the method by [20]. The data shown in Fig 4 demonstrates, that all the select strains showed significantly higher resistance to bile salts compared to commercially available control strains.

increase in cell viability after 24 hours was 62% average among the select strains reaching its highest in strains A (KL10,2,1) whereas the viability increased by 91% after cultivating the cells for 72 hours, thus indicating the highest survival rate, *in vitro*, among the strains studied by the team. the mean values, derived from the experiment lead to the following observations:

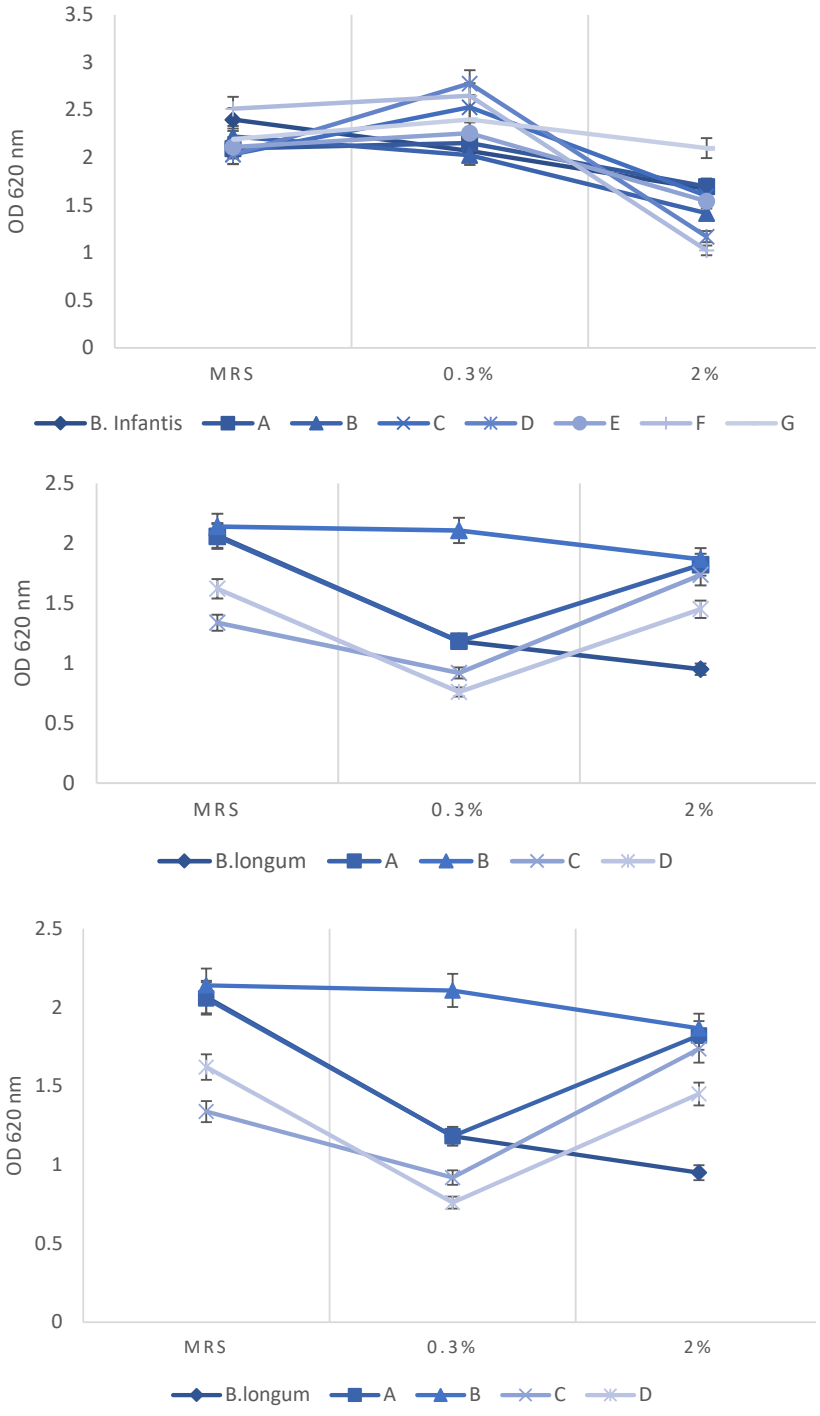

Fig. 4. *Bile salt resistance. Tolerance against bile salt (MRS, 0.15%, 0.3%) – OD620 nm values during 24 hours of incubation at 37°C. $p < 0.05$. results represent the mean, standard error pf three experiments (n¬=3)*

All bifidobacterial strains have shown a high acidification rate in the milk. During the fermentation we haven't seen the changes in shape and Gas production. The weakest congealing was detected in strains A (KB 12);D (TB 6.2.1)C (TL 5.3), results of growth and acidification Fig. 5. All other strains showed good structure formation and also very strong gas formation.

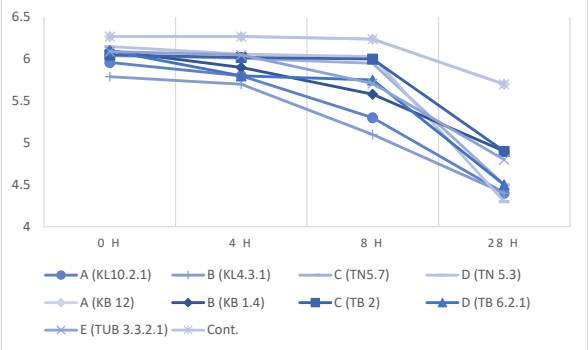

Fig. 5. *Milk acidification kinetics by select probiotic strains. All experiments were realised in the reconstituted milk at 37 °C. Commercial L. Bulgaricus – ATCC 11842 strain used as control.*

Tolerance to various pH and bile concentrations by simulating the human gastrointestinal pH and bile concentration (Fig. 4). The acid tolerance test was studied under pH 1.5 and 3.0 with 7.2 as control. The cell count for the acid tolerance test was obtained at an interval of 0, 1.5 and 3 hours respectively and was plated onto duplicate MRS agars to be incubated at 37C for 48 hours. All cells recovered after 3 hours of pH treatment were selected for bile tolerance test in MRS broth containing bile concentrations of 0% (control), 0.3% and 2.0% and cell counts were recorded after 24 hours of incubation. The probiotic strains in products A, B, C & D met the suggested initial count of 106 CFU/ml with brand C recording the highest at 9.19 ± 0.14 log CFU/ml. Strains in product A, B & C showed good tolerance to pH 3.0 and 7.2 recording a count of >106 CFU/ml after 3 hours with a range of 6.60 – 9.04 log CFU/ml. The higher bile concentrations resulted in lower growth of strains in all the brands. Upon pH 1.5 treatment, only brand C recorded growth in all bile concentrations. After pH 3.0 treatment, all brands except brand E met the requirement of survival at 0.3% bile concentration. Results showed probiotics in product A, B & C met the initial count requirement, and exhibited good acid and bile tolerance therefore being a potentially good source of probiotic.

Conclusion

Microflora of each Dambalkhacho, from difa ferent makers and different regions, varied considerably, based on preliminary exploration. Face tors such as milk, pH, and salt concentration may have contributed to the unique bacterial composition of each product, although no particular factor was determined to be responsible for differences in

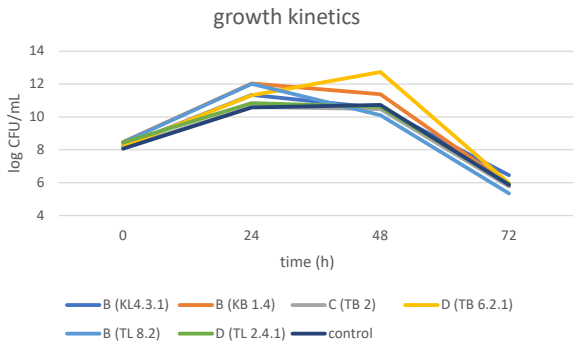

Fig. 6. *Growth kinetics of select probiotic strains during fermentation with six bifido strains. Each % culture by volume, with a final cell count of 8e9 log CFU/mL. Values are expressed as the mean AE standard batch was inoculated with 1deviation (n=3)*

abundance between the brands based on the limited available information. Pure strains of *B. bifidum, B. longum* and *B. longum subsp. Infantis* have been isolated from Dambalkhacho, collected from high mountainous regions of Georgia. All strains have been characterized microbiologically, and assigned species based on 16s variable region PCR aplification. Apart from 16 known strains the study resulted in 2 unassigned gramm positive, non-motile, catalase negative, rod shaped strains that require further investigation. Isolatedprobiotic strains in B (KB 1.4); C (TB 2); D (TB 6.2.1); B (TL 8.2); D (TL 2.4.1) met the suggested initial count of 106 CFU/ml with brand C recording the highest at 9.19 ± 0.14 log CFU/ml. The higher bile concentrations resulted in lower growth of strains in all the strains. After pH 3.0 treatment, B (KB 1.4); D (TB 6.2.1); D (TL 2.4.1) have also met the requirement of survival at 2 % bile concentration. Growth kinetic study also reviled, B (KB 1.4); D (TB 6.2.1) far exceeded con-

trol growth rates, of control strains. Based on the overall technological aptitude of the tested strains B (KB 1.4); D (TB 6.2.1); D (TL 2.4.1) met the initial count requirement, and exhibited good acid and bile tolerance therefore being a potentially good source of probiotic additive to the dambalkhacho.

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Innovative Technology of Making Tea "Mate"

Tamaz Megrelidze[a], Tamaz Isakadze, Givi Gugulashvili

Georgian Technical University, Department of Food Industry, 77, Kostava Str., Tbilisi, 0175, Georgia

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A B S T R A C T

It is known that „mate tea“ drink is made from leaves and reeds of South American plants. In South America, living without a „mate” is unthinkable. This is the national drink of Brazil, Paraguay and Argentina. Indians are confident that this drink can relieve mental and physical fatigue. Numerous studies have shown that mate tea contains almost all the vitamins and other nutrients necessary for normal human life. Raw materials for the production of ~mate tea~ are found in the tropical zone of South America. In Georgia, this raw material is not found. In addition, the price of these raw materials in the world market is quite high. Therefore, for the majority of our population, it is almost impossible to consume „mate tea“.

The article presents an alternative substitute for „mate tea“, which can be produced from the Caucasian rhododendron, in particular from its leaves, and also presents an innovative technology for processing the leaves of the Caucasian rhododendron. Caucasian rhododendron is found in the highlands of the Caucasus. The usefulness of the product obtained by processing the leaves of Caucasian rhododendron using innovative technology exceeds the usefulness of South American „mate tea“.

Keywords: Caucasian rhododendron, Raw materials, Vacuum, Biochemical composition, Food products, Tea.

*Corresponding author: Tamaz Megrelidze; E-mail address: tamazmegrelidze@gmail.com

Introduction

The purpose of the drying process of any product is to remove moisture. However, when it comes to food, in this case, in addition to removing moisture, another, important task arises - the preservation of the quality of raw materials in the drying process.

Any food product is manifested by its chemical and biochemical composition and ability to contribute to improving human health and longevity [1-3]. For this, food products must contain proteins, fats, carbohydrates, vitamins, enzymes, etc. [4-6]. Each product is distinguished precisely by its chemical and biochemical content and the more it is valued, the more it contains the indicated components useful to humans. Therefore, we can say that as the closing operation of almost all technological processes of processing the decisive majority of food products, the drying process in the food industry should ensure the maximum preservation of all positive quality indicators of processed rawmaterials.

The process of processing raw materials necessarily implies physical (mainly mechanical or thermal) impact, resulting in a change in certain of its properties. These changes are necessary to obtain the desired product and are quite acceptable. However, when processing raw materials, it is necessary to warn them against unacceptable changes that can lead to the loss of useful elements and, accordingly, to a deterioration in the quality of the final product.

Material and Methods

Such products that require special attention during processing include the Caucasian Rhododendron [7]. This plant can be called a pantry of healing components [8]. According to the results of numerous studies, Caucasian Rhododendron contains tannins, essential oils, tannins, arbutin, rutin, ursulic acid, gltcosides, vitamin C, flavonoids, etc. All of these components (with the exception of andromedotoxin glycoside) are extremely beneficial for human health [9,10]. These components give the plant such useful properties that it can be used to treat cardiovascular problems, viral infections,

rheumatism, obesity, colitis, to remove toxins and heavy elements from the body, to strengthen the human immune system.

To achieve such a wide range of useful properties, it is necessary to maximize the preservation of the chemical composition existing in the raw material during its processing [11]. Accordingly, the processing of raw materials should be carried out under special conditions that contribute to the maximum preservation of the initial chemical composition.

However, it cannot be said that at present, special attention is paid to preserving the chemical composition during the processing of raw materials. Harvesting of the Rhododendron of the Caucasus is carried out during flowering. Raw materials (leaves) are collected from two-three-year-old plants and dried indoors or in the air under a canopy to prevent the rays of the sun from falling, either in the attic or in the oven at a temperature of 50-600C; From time to time, the leaves are mixed by hand so that they dry faster and more uniformly. Finished leaves are stored in a glass container, which is tightly closed and put in a cool place away from sunlight.

This technology of drying and storage leads to the loss of healing and taste properties of the final product.

There are suggestions for processing the leaves of the Caucasian Rhododendron like processing tea leaves to produce green tea. In one case, raw materials (plant leaves) are subjected to fixing, frying, grinding, drying and sorting processes, and in the other case, fixing and drying, cutting, heat treatment (drying), repeated grinding and sorting.

In these proposed methods, the processing technology begins with fixation, which can, of course, ensure the preservation of the appearance of the sheet and its specific biochemical components. However, due to the use of high temperatures for fixing the sheet, some of its useful components will be lost. In addition, these methods involve two types of heat treatment - fixing and drying (or frying). But it is precisely the heat treatment that acts as negatively as possible on the preservation of the biochemical composition of the products.

Based on the foregoing, the authors propose a new technology for processing leaves of the Caucasian Rhododendron using freeze-drying.

Freeze drying is widely used in the food industry. This drying method is indispensable in the production of antibiotics, medications (blood plasma, blood substitutes, etc.), food. Sublimation dehydration technology ensures the maximum preservation of the beneficial properties of heat-sensitive products and is one of the modern progressive methods of preserving perishable agricultural products.

In addition, during freeze-drying, the initial mass of raw materials is significantly (4-10 times) reduced. This has a positive effect on the cost of transporting the finished product.

The industrial use of freeze-drying food is most widely developed in the USA, England, Germany, and France.

In freeze-drying, two well-known preservation methods are combined: freezing and vacuum drying. When freezing food products, changes in their intrinsic characteristics are minimized and vacuum drying, in turn, ensures the preservation of the composition, structure and taste of the product. The total effect of freeze-drying, in general, is the almost complete dehydration of the product with constant proteins, carbohydrates, fats, vitamins and enzymes.

When conducting freeze-drying, it is necessary to ensure two basic conditions: 1) most of the moisture (free moisture) in the product must be turned into ice, the proportion of which should not be less than 70%; 2) it is necessary to maintain a constant

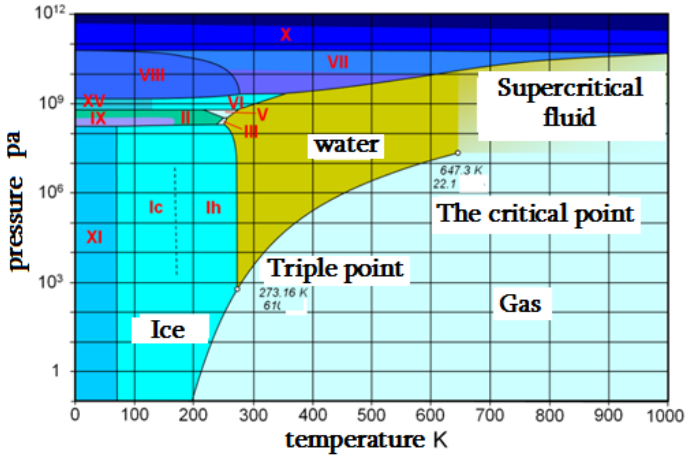

Fig. 1. *Triple point of water*

difference between the partial pressure of water vapor above the surface of the product and the steam in the drying chamber. As can be seen from fig. 1, the pressure at the triple point should be less than the pressure in the drying chamber. This condition ensures the transition of ice to a state of vapor, bypassing the liquid phase. Subsequently, the specified steam condenses on the evaporator of the refrigeration unit.

The quality of the final product largely depends on the speed of freezing. The higher the freezing rate, the higher the biological value of the frozen product. Therefore, in our case, the freezing of the leaves of the Caucasian Rhododendron is carried out directly in the sublimator by the method of shock freezing. Schematic diagram of the device for freeze-drying is shown in Fig. 2.

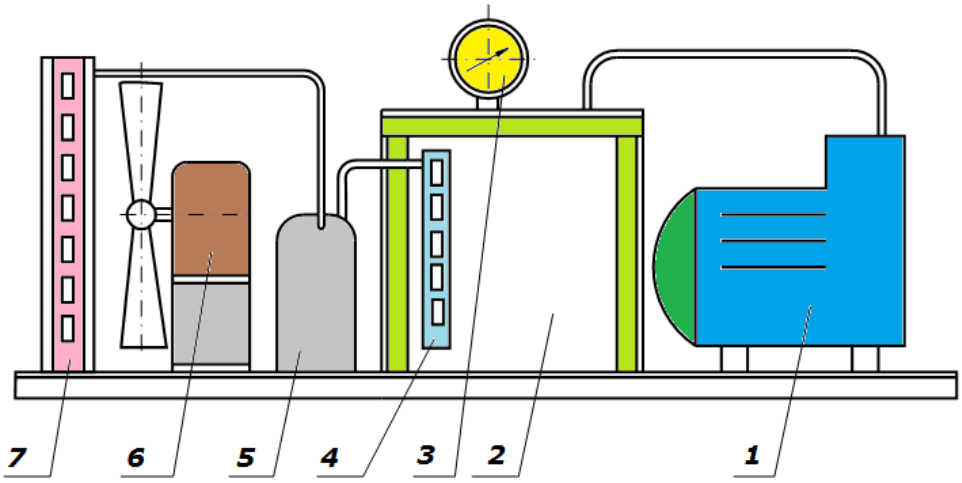

Fig. 2. *Schematic diagram of a device for freeze-drying*
1-vacuum pump, 2-drying chamber, 3-vacuum gauge, 4-evaporator, 5-rotation compressor, 6-axis fan, 7-condenser

After the drying process is completed, the obligatory operation is the packaging of the dried product, which should be carried out directly when the product is unloaded from the drying chamber. The purpose of the packaging is to prevent exposure of the product to ambient air, from which moisture, odor, microorganisms, etc. can get into the product.

A schematic diagram of the proposed technology for processing Caucasian Rhododendron leaves using freeze-drying is shown in Fig. 3.

Fig. 3. *The technological scheme of processing the leaves of the Caucasian Rhododendron using freeze-drying*

Conclusion

The presented new method of processing the leaves of the Caucasian Rhododendron ensures the preservation of not only the chemical composition and medicinal properties of the plant, but also its organoleptic characteristics (color, taste, aroma). Compared to the currently used processing method, the proposed method is, of course, more complex and expensive, but this complication is complemented by an increase in the number of stored healing components in the final product.

Acknowledgement.

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Validation of GPS derived integrated water vapor (IWV) of the atmosphere on the Georgia's territory

Giorgi Sokhadze[a*], Nato Kutaladze[b], George Mikuchadze[b], Gizo Gogichaishvili[b]

[a] Geosciences and Technology Development Institute,15a, Tamarashvili Str., Tbilisi, 0162, Georgia
[b]The Ministry of Environment Protection and Agriculture of Georgia, National Environmental Agency, 150, David Agmashenebeli, Ave., Tbilisi, 0012, Georgia

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A B S T R A C T

The delay in radio signals from the Global Navigation Satellite System (GNSS) is proportional to the integrated water vapor (IWV) in the atmosphere above the GNSS site. In this paper some peculiarities of atmospheric water vapor distribution derived from 7 GPS located on Georgia's territory are presented. We calculate ZPD data these GPS and converted into IWV using observed surface pressure and mean atmospheric column temperature obtained from the surface meteorological stations of Georgia's NHMS. The same variables have been obtained from the ECMWF ERA interim reanalyzes – for two years 2014-2015 period and compared with station observations. As radiosonde data for Georgia's stations are not available GPS derived IWV values were compared to the IWV from the ECMWF ERA interim reanalyses with a special focus on the monthly averaged difference (bias) and the standard deviation of daily differences. This comparison shows that the GPS derived IWV values are well suited for the validation of ERA interim reanalyzes of IWV. For most GPS stations, the IWV data agree quite well with the analyzed data indicating that they are both correct at these locations. Larger differences were mainly found in less humid areas during warm periods of year.

Keywords: GNSS delay, Integrated water vapor, ERA interim, Specific humidity, Time series, Satellite

*Corresponding author: Giorgi Sokhadze; E-mail address: sokhadze@gmail.com

Introduction

Global Navigation Satellite Systems (GNSS) not only revolutionized positioning, navigation and timing, but also provided an accurate sensor of the most common meteorological parameter, water vapor. A deeper understanding of the mechanisms distributing water vapor through the atmosphere and of water the vapor effects on atmospheric radiation and circulation is vital to estimate long-term changes in climate. This understanding is hampered by the fact that water vapor is the most variable of the major constituents of the atmosphere and our ability to measure time-varying global and regional water vapor distributions is still severely limited [1].

The NRT (near-real time) GNSS delay data contain information about the amount of water vapour above the GNSS sites. Surface based GPS-based measurements of zenith path delay (ZPD) can be used to derive vertically integrated water vapour (IWV) of the atmosphere. Surface based GPS-based measurements offer possibility to provide data at similar quality under all weather conditions. Regional networks, established all around the world providing temporally high resolved information of the integrated atmospheric water vapour with vertical profiling by satellite occultation techniques.

With the surface based technique, dual-frequency signals are collected at ground-based receivers and used to obtain the signal delay and thus the integrated water vapour along the path from the GPS satellites to the receiver [2-3]. It is interesting to note that this possibility occurred whilst exploring the cause of errors in geodetic measurements [4-5].

This "geodetic noise" is a valuable meteorological signal, as the so-called wet delay is nearly pro-

portional to the quantity of water vapor integrated along the signal path, which in turn can be transformed into an estimate of precipitable water. Given accurate delay measurements, precipitable water can be recovered with an accuracy of - 1 mm.

Radio signals from Global Navigation Satellite System (GNSS) slow down and bend, when passing from a GNSS satellite to a ground based receiver, causing a delay of the signals compared to a no atmosphere situation. The largest part of the delay is from the ionosphere, but this part is easily subtracted, due to its dispersive (frequency dependent) nature, and the fact that the GNSS satellites emit at two frequencies. The remainder of the delay is due to the neutral atmosphere, near the surface of earth [6-8].

A specially weighted average of the delays toward the individual satellites can be considered as zenith total delay (ZTD). The ZTD is typically given as a distance, corresponding to the actual delay multiplied by the speed of light, equal to the apparent extra distance the signals have been traveling.

It can be divided into two terms. The first term is the zenith hydrostatic or zenith dry delay (ZHD), which is proportional to the pressure at the GNSS receiver site. The second term is the zenith wet delay (ZWD), which is proportional to the integrated water vapor (IWV) above the GNSS site, and which depends weakly on the temperature and humidity profile above the site.

Water vapor plays a key role in formation of climate factors and some of the most important weather phenomena on Georgia's territory. This important meteorological parameter is not well studied yet, because of a big lack of humidity observations, mostly on upper atmosphere layers. Except of some valuable research devoted to atmospheric humidity temporal and spatial distribution on Georgia's territory [9].

Usage of ground based GNSS data is one means by which to improve this situation. We have 24 GNSS operating sites in Georgia with about 8 year period archived observations. For this study, we select 7 of them, located closely to automated weather stations (AWS), to avoid horizontal interpolation of surface pressure and temperature measured by AWS for calculation of vertically integrated water vapour with GAMIT/GLOBK software. GNSS and AWS locations, with corresponding elevations are presented on the map. Vertical interpolation of meteorological parameters from AWS elevation to GNSS ones was performed. This is a pilot investigation of GNSS derived meteorological parameters for country. Validity of the results was obtained by comparison of GNSS derived IWV against ECMWF ERA interim reanalyzes Total column water vapor (TCWV) parameter.

GPS observations and data processing

To obtain integrated water vapor (IWV) we selected 7 ground-based GPS station from the network operated by Georgian National Agency of Public registry (NAPR). All the available data at these 7 sites during the 2 year period of 2014–20215 has been analyzed along with the 12 far field IGS stations to reduce the influence of the strong correlation of tropospheric parameters. The NAPR stations distributed along territory of Georgia, which covers humid/dry and low/high elevation areas (Fig 1).

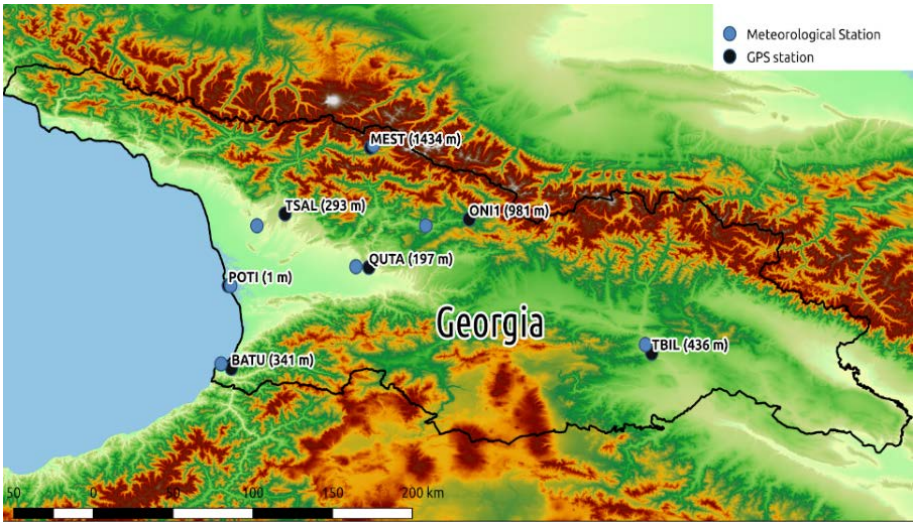

Fig. 1. *The map shows GPS and Meteorological stations location with the labeled name of the station and elevation in meters.*

We process GPS data using the GAMIT/ GLOBK software package [11] developed at the Massachusetts Institute of Technology, which uses double-difference phase observations to determine baseline distances between ground-based receivers and satellites. GPS satellite signal is delayed when it propagates through the atmosphere. Significant delay directly attributed to the passage of the signal to troposphere, which can be decomposed into a hydrostatic (dry) and a non-hydrostatic (wet) delay. Since these delays change with the elevation angle, the signal with low elevation angle has a longer delay through the troposphere than one with high elevation angle. The mapping functions VMF1 (Vienna mapping function) are applied to transform slant tropospheric delays into the zenith tropospheric delay (ZTD). The zenith hydrostatic delay (ZHD) accounts for nearly 90% of the total tropospheric delay and can be estimated more accurately if the surface pressure and temperature data is applied. In the absence of in situ meteorological sensor, we used vertically interpolated pressure data of the nearest station of the Georgian National Environmental Agency network with the average horizontal distance 10km. Zenith wet delay (ZWD) can be estimated by subtracting ZHD from the ZTD. Finally, ZWD can be converted to precipitate water vapor (PWV) using the following formula:

PWV=Π × ZWD

where can be calculated based on the for

$$\Pi = \frac{10^6}{\rho_\omega R_v (k_2 - \frac{k_3}{T_m}}$$

where ρ_ω is the density of liquid water, Rv is the specific gas constant for water vapor, k_2 and k_3 are the refractivity constants and T_m is the weighted mean temperature of the atmosphere given by

$$T_m = \frac{\int \left(\frac{e}{T}\right) dz}{\int \left(\frac{e}{T^2}\right) dz}$$

where e is the partial pressure of water vapor and Tis absolute temperature along the zenith path. The integral intervals are from the earth surface to the atmospheric top. Simply, T_m can be estimated [12] using the surface temperature measurement T_s and was interpolated from the nearest meteorological station:

$$T_m = 70.2 + 0.72T$$

ERA interim reanalysis

ERA-Interim is the latest global atmospheric reanalysis produced by the ECMWF [13]. ERA-Interim covers the period from 1 January 1989 onwards, and continues to be extended forward in near-real time. An extension from 1979 to 1989 is currently in preparation. Gridded data products include a large variety of 3-hourly surface parameters, describing weather as well as ocean-wave and land-surface conditions, and 6-hourly upper-air parameters covering the troposphere and stratosphere. Vertical integrals of atmospheric fluxes, monthly averages for many of the parameters, and other derived fields have also been produced [14-15].

The ERA-Interim atmospheric model and reanalysis system uses cycle 31r2 of ECMWF's Integrated Forecast System (IFS), which was introduced operationally in September 2006, configured for the following spatial resolution:

60 levels in the vertical, with the top level at 0.1 hPa;

T255 spherical-harmonic representation for the basic dynamical fields;

a reduced Gaussian grid with approximately uniform 79 km spacing for surface and other grid-point fields.

For comparison we extracted Total column water vapor (TCWV) from Era Interim archive at 6 hour time steps four times per day starting at 00 UT.

ZPD from GPS is usually measured instantaneously at 2 hour time steps starting at 01 UT. In order to obtain ZPD values at the 6 hourly times, the two ZPD measurements before and after the 6 hour time are averaged, i.e. the GPS results are mean values over four hourly intervals (e.g. a ZPD value at 12 UT is the average of the measurements at 11 UT and 13 UT). If only one of these two ZPD measurements is available, it alone represents the 6 hour time.

Validation strategy and obtained results

The GPS derived IWV cannot be compared directly with the IWV data from the reanalysis data. That information from different sources are averaged over different areas (GPS by some 100 km2 and ERA over an area of 10000 km2) and the GPS stations heights usually do not agree with the topography used in the ERA. Thus, it is necessary to interpolate ERA IWV not only horizontally but also vertically to the position of the GPS station. The horizontal interpolation of all OA values used, e.g. IWV or model height h, to a station coordinates is done by a weighted linear interpolation from the surrounding four grid boxes with the center coordi-

nates i. In order to compare the horizontally interpolated OA IWV values to the GPS derived IWV values, they have to be vertically interpolated from the model surface height to the GPS station height, more precisely the IWV difference between the model surface height and GPS station height has to be estimated. It is assumed that the mean relative humidity of the two lowest OA model levels (j = 1, 2) is representative for the atmospheric layer near the surface, especially at the station height *hS* and the model surface height *h(Xs)*. As only the specific humidity *q* is stored in the archive at model levels, the relative humidity r for both layers j with the pressure *pj* has to be computed [16-20].

The adjustment of the ERA interim IWV to the GNSS station height is obtained by the integration of specific humidity (q) over the height difference between the GPS station and the model surface.

$$IWV_{ERA} = IWV\chi_s + \sum_{i=1}^{n} \frac{q(h_{i-1})+q(h_i)}{2}(h_{i-1} - h_i)$$

This integration is numerically done in 30-m steps, which generally corresponds to a pressure difference smaller than 4 hPa and IWV 2 mm.

$$h_0=h(\chi_s),\ h_1=h(\chi_s)+30m,\ \ldots h_n=h_s$$

Corrected and averaged ERA interim and GNSS IWV 6 hourly data set was compared for 2014-15 period. Daily Biases were calculated and monthly statistics derived from daily ones have been analyzed. This comparison shows that the GNSS derived IWV values are well suited for the validation of ERA IWV and presented graphs are demonstrate such results (Fig.2 of individual GNSS and ERA interim IWV).

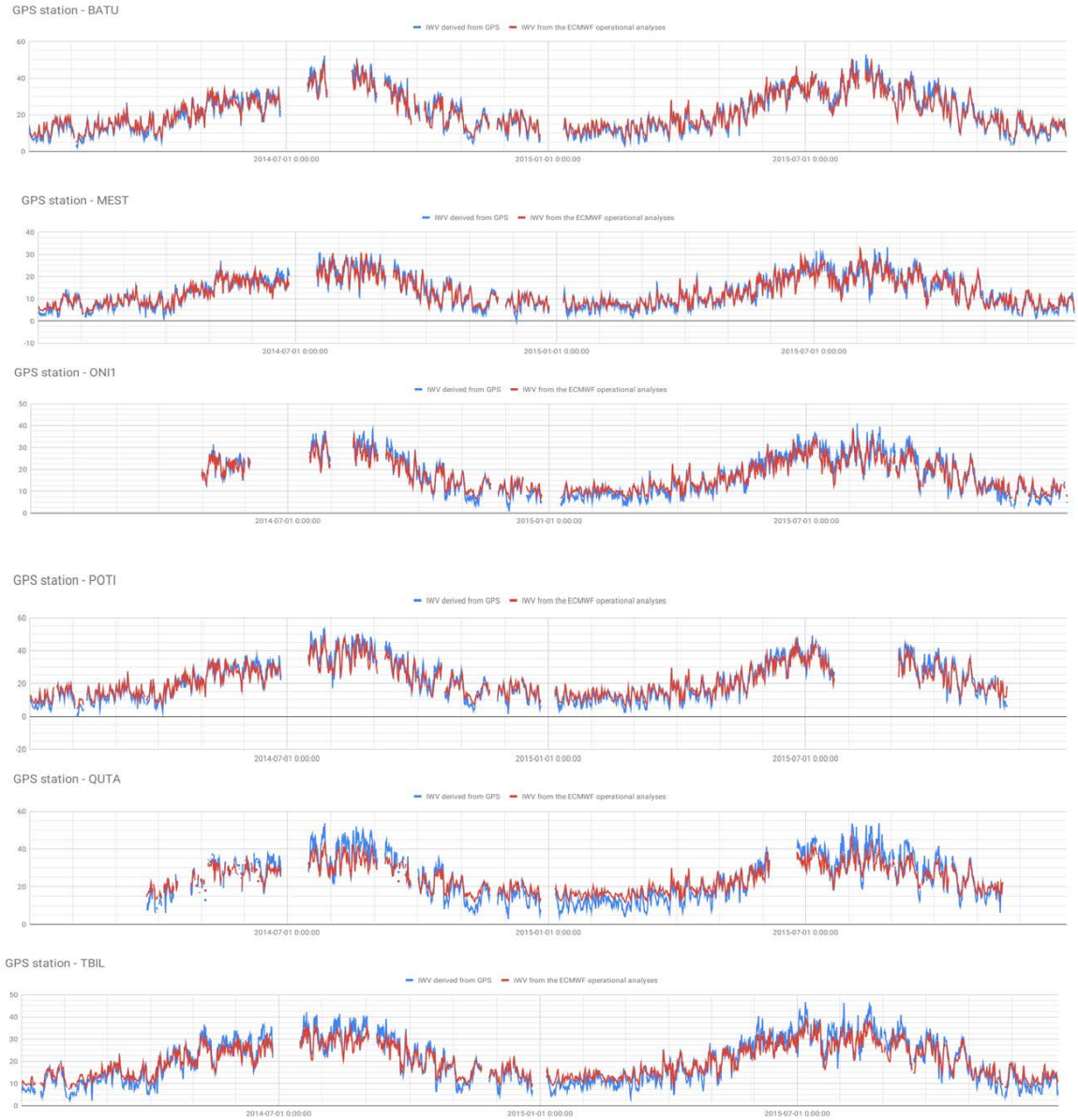

Fig. 2. *Time series of IWV derived from GPS and IWV from the ECMWF reanalysis*

For most GPS stations, the IWV data agree quite well with the analysed data indicating that they are both correct at these locations. From the graphs can be concluded, that in cold months GPS derived IWV have negative biases on all stations, as blue color is dominant in such a months, when in summer GPS overestimates water vapour and this dry bias is more evident on dryer stations (Qutaisi and Tbilisi). The Table 1 Shows the mean seasonal and annual biases and correlations, from where ones again we can see that in winter GPS derived IWV lower than IWV from Era across all stations and it is higher in summer, and this mean error is close to ± 2mm.

Table. 1. *Mean seasonal and annual biases and correlation between ERA and GPS mean daily IWV data*

Winter	spring	summer	autumn	year	statistics
-1.9	-0.7	2.15	0.4	0.0098	bias
0.8196	0.9202	0.8697	0.8834	0.906	correlation

On annual span mean error is almost 0 (0.0098) and Correlation also high. Fig. 3 also proves that all mean daily values over all sites are highly correlated and well fitted in regression model.

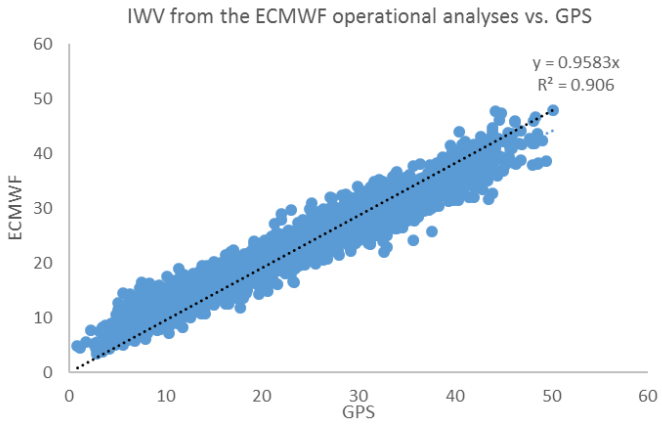

Fig. 3. *Scatterplot of GPS derived and Era interim mean daily integrated water vapor (IWV)*

Table 2 represents monthly RMSE of mean daily bias on the analyzed sites. It's evident that deference between GNSS stations and ERA interim IWV are higher on all stations for summer period. It should be mentioned that despite errors are small, results should be filtered taking into account uncertainty in the GPS derived IWV due to GPS measurements of ZPD (< 0.7 mm) and the use of surface pressure measurements from surrounding areas (0.5 mm).

Table. 2 *Monthly RMSE of mean daily bias for the 7 GNSS stations*

Stations	1	2	3	4	5	6	7	8	9	10	11	12
Tbilisi	4.2	3.1	2.7	2.6	3.1	3.8	3.7	5.1	4.2	2.4	2.5	2.5
Kutaisi	5.3	4.5	4.6	3.3	2.8	4.2	6	6.8	5.1	3.3	4.3	4.7
Poti	2.7	2.8	2.3	2.1	2.3	3.4	4.8	5	4.5	3.3	2.8	2.7
Mestia	1.5	1.2	1.3	1.4	1.5	2.4	2.7	2.1	2	1.7	1.1	1.6
Oni	2.5	2.1	2.1	1.9	2.2	3.1	3.3	4	3.1	2.1	2.1	2.4
Batumi	1.9	1.5	1.9	2.2	2.7	3.4	3.9	4	3.8	2.3	1.8	1.9
Tsalenjikha										3.2	2.4	2.1

Conclusion

This initial validation show that the GNSS derived IWV values are well suited for the validation of ERA IWV and presented graphs and tables are demonstrate such results. For most GPS stations, the IWV data agree quite well with the analyzed data indicating that they are both correct for the locations. This tendency is valid for all stations for all seasons and annually. It is noteworthy that all stations showed negative deviations during the winter season and dry biases during the summer and this tendency is more evident on dryer stations (Qutaisi and Tbilisi). From our results maximum RMSE values have been obtained on the stations where we had less difficulties with orography and horizontal pressure interpolations, which additionally gives errors in GPS derived IVW calculations, but on the stations from drier climate zones. It should be also mentioned, that this two stations are in urban areas and this possible effect worst to be investigated.

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Cesium-137 in the soils in the territory of Tbilisi City (Georgia)

Eremia Tulashvili*, Bezhan Tutberidze, Mariam Akhalkatsishvili, Lela Mtsariashvili, Manana Chkhaidze, Irina Ambokadze

Ivane Javakhishvili Tbilisi State University, 13, Chavchavadze Ave., ., Tbilisi, 0179, Georgia

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A B S T R A C T

In the present work there are given results of research of the content of technogenic radionuclide Cs-137 in various types of the soil in the territory of some districts of Tbilisi City – the capital of Georgia. 56 soil samples have been selected and analyzed in the studied territory. Activity concentration of radionuclide Cs-137 in the investigated samples varied from 0.19 to 118 Bq/kg (on the average - 18.7 Bq/kg). Some features of distribution of cesium-137, in particular, from the type of soil and an arrangement of sampling location points are noted.

Keywords: Radioactive background, Activity concentration, Ecological problems, Technogenic radionuclides, Biological circulation, Cs-137.

*Corresponding author: Eremia Tulashvili; E-mail address: eremia.tulashvili@tsu.ge

Introduction

One of the main components of the radioactive background of the Earth is made by the radioactivity of the soils which is caused by the presence of radionuclides in them. There are the natural and technogenic radioactivity.

The natural radioactivity of soils is caused by the natural radioactive isotopes which are always presented in soils and soil-forming rocks.

The technogenic radioactivity of soils is caused by ingress in the soils the radioactive isotopes formed as a result of nuclear and thermonuclear explosions, in the form of the waste of the nuclear industry or as a result of failures at the nuclear enterprises. Formation of isotopes in soils can occur owing to an induced radiation. The most often the technogenic radioactive pollution of soils is caused by isotopes U-235, U-238, Pu-239, I-129, I-131, Ba-140, Ru-106, Sr-90, Cs-137, etc. Regarding the ecological problems the greatest danger is represented by the radionuclides Sr-90 and Cs-137. It is caused by the long half-life period (29.1 years for Sr-90, and 30.0 years for Cs-137), high radiation energy and the easy ability to enter into biological circulation, in a feed circuit. By chemical properties strontium is close to calcium and is a part of bone tissue, and cesium is close to potassium and joins in many reactions of live organisms. Cesium-137 strongly fixed in soils and can extend over the food chain “plant-cattle-human being”. Therefore investigation of the content of technogenic radionuclides, first of all Cs-137, in this important environmental object represents an actual problem all over the world. Results of similar researches are given in many publications.

So, for example, in the work [1] there are given results of measurements of activity concentration of Cs-137 in the soil samples from the various areas of Punjab province, one of the most populated provinces of Pakistan. Activity concentration of Cs-137 was in the range from 1.1±1.0 to 5.3±2.5 Bq/kg. It is concluded that Cs-137 soil contamination does not pose radiation hazards to the population in the investigated areas.

The similar researches carried out spent for Gaziantep, an industrial and trade center in the southeastern part of Turkey [2], have shown, that the activity concentration for the fission product Cs-137 in the surface soil samples was determined as

8.02 Bq/kg. The measurement results obtained in this study indicate that the region has a background radiation level that is within the natural limits and shows no significant departure from the other parts of the country.

Results of a systematic study of soil radioactivity in the metallurgical center of the Republic of Macedonia, the city of Veles and its environs, were given in the work [3]. It has been shown, that Cs-137 originated from the atmospheric deposition and present in soil in the activity concentration range of 2–358 Bq/kg is irregularly distributed over the sampled territory owing to the complicated orography of the land.

In another work [4] there are given the results of a wider monitoring project of the agricultural soils in Lombardy, Italy. It has been established, that activity Cs-137 varied from 1.1 to 241 Bq/kg. The lowest activity of Cs-137 is in the plain, whereas the highest is in the North on soils kept as lawn or pasture. The Cs-137 activity of some samples suggests the presence of accumulation processes that lead to Cs-137 enriched soils.

Natural and artificial gamma-emitting radionuclides, including Cs-137, were measured in the soils of a small catchment in the Central Pyrenees, Spain [5]. By results of researches it was revealed, that concentration of activity Cs-137 changed in the range 4.4-64.7 Bq/kg (average value 30.9 Bq/kg).

In Georgia regular researches of natural (and also technogenic) radioactivity were not actually carried out. Rather detailed researches of radioactivity in various environmental objects have been carried out in 1986, after failure on the Chernobyl atomic power station and, basically, concerned technogenic radionuclides [6, 7, 8]. In these works it has been shown, that during this period in the territory of the Western Georgia, basically in the strip adjoining to the sea the big concentrations of technogenic radionuclides were observed (in particular, Cs-137 concentration made from several hundreds to some thousands of Bq/kg). It is possible to note also works [9, 10] in which results of research of radiation condition of coast of water area of Black sea during later period are given, in particular, presence of 7 natural (Ac-228, Ra-226, Bi-214, Pb-214, Pb-212, Pb-210, K-40) and 1 technogenic radionuclide (Cs-137) has been fixed in soil in some areas of Adzharia (Batumi, Gonio, Sarpi, Chakvi, Kvariati). Some results of the last period are given in a study by Kekelidze et al [11]. Urushadze and Manakhov studied content of technogenic radionuclides Cs-137 and Sr-90 in different types of soil in the territory of Georgia [12].

In the present work there are given the results of research of activity concentration of technogenic radionuclide Cs-137 in the soil for the territory of some districts of Tbilisi – the largest city and capital of Georgia.

Materials and method

Study area

The territory of Tbilisi city represents the crossed mountain area within average watercourse of the River Mtkvari. Tbilisi occupies deep kettle-shaped valley which width changes from 3000-4000 m to 35-40 m. River Mtkvari crossing a city practically in meridional direction, divides the city on the two appreciably divergent parts: more raised right-bank and considerably lowered left-bank. Various types of the soil are extended in the territory of Tbilisi.

Sampling was carried out in the whole territory of the city. 56 soil samples were selected from 56 sample locations (see Table 1), in particular, of the following types:

- cinnamonic – 52 samples (including cinnamonic calcareous (Cn-Cr) – 40 samples, cinnamonic (Cn) – 11 samples, and gray cinnamonic – 1 sample);
- alluvial – 4 samples (including alluvial calcareous (Al-Cr) – 2 samples, and alluvial (Al) – 2 samples).

Table 1. *List of locations (L), field numbers (FN) of investigated samples and their types (ST)*

#	L	Lt(N); Ln(E)	FN	ST	#	L	Lt(N); Ln(E)	FN	ST
1	Tx-1	41.65475; 44.74512	253	Cn	29	Md-2	41.69876; 44.79234	286	Cn-Cr
2	Sx-2	41.67025; 44.76433	255	Cn-Cr	30	Bg-2	41.68781; 44.80784	277	-"-
3	Kx-3	41.66384; 44.80876	223	-"-	31	Bg-4	41.68812; 44.80587	279	-"-

4	Kx-11	41.67016; 44.81483	220	-“-	32	Bg-6	41.68726; 44.80043	281	-“-
5	Kx-12	41.67191; 44.80206	229	-“-	33	Nr-2	41.68791; 44.80956	272	-“-
6	Kx-14	41.67262; 44.80532	231	-“-	34	Ty-2	41.68606; 44.81288	268	-“-
7	Ot-2	41.67087; 44.83550	217	Al	35	Ty-4	41.68580; 44.81202	270	-“-
8	Ot-3	41.66283; 44.87747	215	-“-	36	Un-4	41.71729; 44.71539	310	-“-
9	Zp-1	41.64281; 44.89925	204	Gr-Cn	37	Un-7	41.71841; 44.70726	313	-“-
10	Pl-1	41.63803; 44.93040	203	Al-Cr	38	Dt-3	41.72591; 44.70825	318	-“-
11	Pl-2	41.64055; 44.93427	202	-“-	39	Nc-2	41.73422; 44.71744	322	-“-
12	Ky-2	41.64574; 44.64459	235	Cn	40	Nc-5	41.72997; 44.73037	345	-“-
13	Ky-4	41.66170; 44.65142	237	-“-	41	Tq-2	41.74955; 44.68550	324	-“-
14	Dr-2	41.67016; 44.64579	240	-“-	42	Ls-2	41.73931; 44.73998	327	-“-
15	Dr-3	41.66658; 44.65155	238	-“-	43	Ls-4	41.74110; 44.74720	329	-“-
16	Dr-5	41.66899; 44.66050	243	-“-	44	On-2	41.73565; 44.73711	331	-“-
17	Kj-7	41.65925; 44.69699	250	-“-	45	Vs-2	41.74922; 44.76422	337	-“-
18	Kj-10	41.65904; 44.70620	247	-“-	46	Vs-4	41.75508; 44.75447	348	-“-
19	Kj-12	41.65857; 44.73178	252	-“-	47	Gs-2	41.73913; 44.77042	340	-“-
20	Tj-2	41.70367; 44.70393	295	Cn-Cr	48	Vd-2	41.76638; 44.75136	352	-“-
21	Tj-5	41.67601; 44.67910	298	Cn	49	Vd-4	41.76638; 44.75185	355	-“-
22	Tj-6	41.67651; 44.67930	299	-“-	50	Vd-6	41.77999; 44.71153	357	-“-
23	Bb-2	41.71312; 44.71512	291	Cn-Cr	51	Tr-8	41.76956; 44.81288	387	-“-
24	Bb-4	41.71396; 44.69906	293	-“-	52	Dg-5	41.79669; 44.74310	365	-“-
25	Bb-6	41.70601; 44.74229	301	-“-	53	Dg-7	41.80437; 44.74184	367	-“-
26	Bb-8	41.70564; 44.73715	303	-“-	54	Dg-8	41.81081; 44.70615	368	-“-
27	Vk-2	41.70603; 44.75459	289	Cn-Cr	55	Ll-2	41.69071; 45.01003	371	-“-
28	Sl-7	41.69055; 44.79087	264	-“-	56	Ll-5	41.69164; 44.99147	374	-“-

Sampling and analysis

Sampling

Samples were selected used the special hand auger directly in plastic containers (volume up to 2.0 L). After drying in laboratory conditions samples were grinded and sieved for their homogenization. Then samples were dried at the temperature 105 - 110°C to constant weight and their bulk density and weight were determined. These values were used at the description of sample geometry. The samples were sealed in Marinelli beaker.

Measurement of gamma radiation activity

Measurements were carried out using a Canberra GC2020 gamma spectrometer with a semi-conductor germanium detector with a relative efficiency of 24%. The gamma spectra acquisition time was 72 h. For the analysis, the software Genie-2000 S500 was used. Cs-137 activity concentration was determined by the 661.65 keV line.

Results

Results of measurements of Cs-137 activity concentration in the investigated soil samples are given in Table 2. Generalized data of measurement results – average (*av*), minimal (*mn*) and maximal (*mx*) values – depending on the soil type are given in Table 3.

Table 2. *Activity concentration (A, Bq/kg) of technogenic radionuclide Cs-137 in the investigated soil samples*

#	L	FN	ST	A, Bq/kg	#	L	FN	ST	A, Bq/kg
1	Tx-1	253	Cn	19.5	29	Md-2	286	Cn-Cr	1.52
2	Sx-2	255	Cn-Cr	18.5	30	Bg-2	277	-“-	90.9
3	Kx-3	223	-“-	19.7	31	Bg-4	279	-“-	103
4	Kx-11	220	-“-	4.85	32	Bg-6	281	-“-	118
5	Kx-12	229	-“-	15.4	33	Nr-2	272	-“-	62.1
6	Kx-14	231	-“-	2.8	34	Ty-2	268	-“-	36.9
7	Ot-2	217	Al	21.0	35	Ty-4	270	-“-	0.19
8	Ot-3	215	-“-	18.2	36	Un-4	310	-“-	3.11
9	Zp-1	204	Gr-Cn	1.53	37	Un-7	313	-“-	3.08
10	Pl-1	203	Al-Cr	8.70	38	Dt-3	318	-“-	17.7
11	Pl-2	202	-“-	5.94	39	Nc-2	322	-“-	1.83
12	Ky-2	235	Cn	6.54	40	Nc-5	345	-“-	0.51
13	Ky-4	237	-“-	14.1	41	Tq-2	324	-“-	4.39
14	Dr-2	240	-“-	33.1	42	Ls-2	327	-“-	37.8
15	Dr-3	238	-“-	2.61	43	Ls-4	329	-“-	38.1
16	Dr-5	243	-“-	19.8	44	On-2	331	-“-	16.3
17	Kj-7	250	-“-	3.31	45	Vs-2	337	-“-	10.1
18	Kj-10	247	-“-	24.2	46	Vs-4	348	-“-	33.2
19	Kj-12	252	-“-	15.4	47	Gs-2	340	-“-	2.94
20	Tj-2	295	Cn-Cr	2.79	48	Vd-2	352	-“-	2.79
21	Tj-5	298	Cn	4.82	49	Vd-4	355	-“-	3.92
22	Tj-6	299	-“-	34.7	50	Vd-6	357	-“-	17.6
23	Bb-2	291	Cn-Cr	3.74	51	Tr-8	387	-“-	11.9
24	Bb-4	293	-“-	7.00	52	Dg-5	365	-“-	0.24
25	Bb-6	301	-“-	7.77	53	Dg-7	367	-“-	36.7
26	Bb-8	303	-“-	28.4	54	Dg-8	368	-“-	31.1
27	Vk-2	289	-“-	9.13	55	Ll-2	371	-“-	1.02
28	Sl-7	264	-“-	5.12	56	Ll-5	374	-“-	1.80
								av	18.7
								mn	0.19
								mx	118

Table 3. *Cs-137 activity concentration depending on the type of soil*

#	ST	A, Bq/kg		
		av	*mn*	*mx*
1	Cn	16.2	2.6	34.7
2	Cn-Cr	20.3	0.19	118
3	Al	19.6	18.2	21.0
4	Al-Cr	7.3	5.9	8.7
5	Gr-Cn	1.5	-	-

Apparently from measurement results, Cs-137 activity in the studied samples changes in sufficiently wide limits – from 0.19 to 118 Bq/kg (average value – 18.7 Bq/kg). Thus, the highest values of activity were observed for soil of the type Cn-Cr – the average value was 20.3 Bq/kg, and a little bit more low – for soils of the types Al and Cn – 19.6 and 16.2 Bq/kg, accordingly. Activity concentration in the soil samples of the type Al-Cr (7.3 Bq/kg) is much less, and the least value – 1.5 Bq/kg – was measured for the soil sample of Gr-Cn.

Discussion

As may be seen from the received results, technogenic radionuclide Cs-137 was observed in all samples in sufficiently appreciable amounts. Usually its presence in soil is connected with deposition of an atmospheric precipitation in which cesium was as a result of nuclear tests in atmosphere, and also as a result of failure of the Chernobyl atomic power station in 1986. By a number of data, in particular, according to systematic observations for the flat areas of East Georgia [13], values of Cs-137 activity are now, basically, in the range 1-10 Bq/kg. With certain degree of convention it is possible to consider this level as background value for the whole territory of Georgia. Average value (18.7 Bq/kg) is greater this quantity that can be due to non-uniform precipitations following the accident. However it is impossible to exclude completely that the pollution fact could have rather recent history, considering presence of nuclear objects in surrounding geographical region. Results received for locations Bg-2, Bg-4, Bg-6, and also Nr-2 (see Table 2) located in the southern part of Sololaki ridge are of the special interest. Values of Cs-137 activity in these locations several times exceed background values as well as average value of activity.

As a whole, cesium-137 distribution in the soils of territories with a complex relief and slopes of a various layouts similar to the investigated territory in many respects depends on the many factors, such as soil moisture and a type of soil mode, a slope steepness, soil type, humus content and granulometric structure of soil fractions. Additional researches are necessary for specification of the processes influencing on the radionuclides migration in a various territories.

Conclusion

As a result of the carried out researches it was established, that activity concentration of technogenic radionuclide Cs-137 in the soil samples selected in 56 control points in the territory of some districts of Tbilisi City, changes in the range 0.19-118 Bq/kg (average value – 18.7 Bq/kg). Locations with the raised concentration of cesium are noted.

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Theoretical research on vibratory cutting of the plants stems in the dense environment: cutting with vibration

A.P. Tarverdyan, A.V. Altunyan, G.M. Yeghiazaryan

National Agrarian University of Armenia, 74, Teryan Str., Yerevan, 0009, Republic of Armenia

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A B S T R A C T

The second article of the current series deals with the issue related to the vibratory stems cutting of the plants in the dense environment, and in the water medium, in particular. A model and design schedule have been recommended, which enable to reveal the cutting effect with vibro-blade and the reasons promoting the significant decrease in the energy consumption of the cutting apparatus. As a result of theoretical investigations it has been found out that the vibro-cutting of the water plants (cane) stems enable to reduce the environmental resistance force factors in 10÷35 times and, therefore the energy efficiency grows up.

Keywords: Reservoirs and channels, Water plants, Dense medium, Vibro-cutting, Resistance forces, Energy efficiency

*Corresponding author: Arshaluis Tarverdyan; e-mail address: arshaluystar@gmail.com

Introduction

In the previous series of the current article it was already mentioned and justified that cleaning the reservoirs and channels from the water plants is an urgent issue worldwide [1].

The analyses of the operational indices and structures in the machines and particularly in their cutting apparatus designed for the solution of the mentioned problem indicate that they do not provide the needed efficiency and do not completely comply with the requirements of the technical and technological design.

The application of segmented cutting apparatus for the above mentioned purposes causes a number of difficulties both from the structural and technological perspectives. First, the imperative distance between the tractor and the cutting apparatus implies the use of a complex transmission mechanism with great marginal sizes, then such apparatus have got a high rate of rigidity in the knife blades, which doesn't allow to gage the segmented profile of the channels.

In this regard rotary cutting apparatus are preferable for cutting the plants stems and roots in the dense media (water, soil); anyhow, the experiments aimed at their application also doomed to failure [2], [3, 4]. The matter is, that in order to implement cutting without any pillars in the rotary cutting apparatus, the marginal cutting points should have 30÷50m/s linear velocity [2, 5]. Such velocities in the blades lead to an abrupt growth of resistance forces in the water and soil medium, which results in a rapid decrease of the rotation numbers in the rotor and hence, in the decrease of cutting velocities [1, 3]. In order to ensure the needed cutting velocity a rapid growth in the torque moment applied to the rotor's shaft or its equivalent horsepower should be provided. In the result of our theoretical and scientific-experimental investigations it has been proved that the double increase in the rotation numbers of the rotary cutting apparatus in the water environment leads to the growth of the torque moment or the horsepower in about five times [1,3].

All attempts to improve and update the existing cutting apparatus aimed at their efficiency increase in the dense environment were useless regarding the expected results [4]. Therefore, the only way to solve this problem is to design a fundamentally new cutting apparatus.

Throughout the long-term search for the problem solution and upon the laboratory and field research experiments it has been found out that the vibratory cutting is the most rational method for implementing efficient cutting with low energy consumption in such dense environments, like water (cutting of cane and cane-like plants) and soil (cutting of essential oil plants). In case of applying this method, the blade carries out moving (supplying) motion with low velocity (1÷5m/s) and vibratory motion in the cutting plane with low amplitude (2÷8mm) and relatively high frequency (50÷500s^{-1}) [2, 3, 6].

In the result of theoretical researches carried out on the vibratory cutting, the apparent advantages of the abovementioned cutting methods have been justified and some expressions have been derived, which enable to determine the parameters of the cutting apparatus [2, 3]. Nevertheless, it is worth mentioning that these results are true for air medium, the density of which doesn't have any effect on the resistance forces of the blade. The only factor is the resistance force of the very stem cutting.

While studying field-related scientific works we haven't found any investigations related to the theoretical research on vibratory cutting in the dense environment.

Thus, for the complete solution of the problem it is necessary to disclose the effect of vibratory cutting in the dense environment through the theoretical researches, since it is the only way to specify the real optimal parameters of the cutting apparatus.

Materials and methods

In the previous series of the current article it was already mentioned that the issue is going to be discussed from two perspectives [1]. In the first case the cutting process in the water environment has been examined without the blade vibration. When solving the problem from this perspective the factors of resistance force (without the force of the very stem cutting) affecting the blade throughout the environment have been derived, which are the following:

– resistance friction force along the blade sheet:

$$\boldsymbol{T_x = \frac{8}{15}\rho\omega^3 \cdot \sqrt{\frac{vb}{\omega}} \cdot \ell^2 \cdot \sqrt{\ell}}, \quad (1)$$

– resistance moments of the rotor shaft:

$$\boldsymbol{M_1 = \frac{c\lambda\omega^2\rho\ell^4}{8} + 4b\sqrt{\mu\rho\omega^3} \cdot \ell^3}, \quad (2)$$

$$\boldsymbol{M_2 = \frac{4}{9}\omega^3\rho\sqrt{\frac{vb}{\omega}} \cdot \ell^4 \cdot \sqrt{\ell}}, \quad (3)$$

where $\boldsymbol{\rho}$ is the density of the medium ($1000 kg/m^3$, the values are related to water medium), $\boldsymbol{\mu}$ is the coefficient of viscosity (0.1kg/m·s), $\boldsymbol{v}$ is the coefficient of kinematic viscosity, ($\boldsymbol{1 \cdot 10^{-6}}m^2/s$), **c** is the constant coefficient and depends on the form and sizes of the blade (in our case c=1.45), $\boldsymbol{\omega}$ is the rotation frequency of the rotor's shaft ($0 \div 100\mathbf{s^{-1}}$), **b** is the width of the blade sheet (0.03m), $\boldsymbol{\ell}$ is the length of the blade sheet (cutting edge) (0.3 m), **λ** is its thickness (0.001m).

In case of the mentioned numerical values of the units, when $\boldsymbol{\omega}$=100s^{-1}, we have received the following expression for the resistance force factors:

$$\boldsymbol{T_x = 450N/s}\ ,\ \boldsymbol{M_1 = 47.1N \cdot m}\ ,\ \boldsymbol{M_2 = 34.0}N \cdot m/s,$$

besides, as it has been already mentioned when the value of $\boldsymbol{\omega}$ is doubled from the $50s^{-1}$ up to $100s^{-1}$, the resistance force factors increase in up to 5 times.

Before passing to the studies of vibratory cutting, let's determine the resistance forces in case of 5 m/s velocity in the cutting edge points of the blade. It is the maximum value upon which the vibro-blade carries out its moving (supplying) motion in the water medium, so when $\boldsymbol{v = 5m/s}$, $\boldsymbol{\omega = 25s^{-1}}$. In case of the mentioned value of $\boldsymbol{\omega}$ we have the following expression:

$$\boldsymbol{T_x = 14.0N/s}\ ,\ \boldsymbol{M_1 = 5.0N \cdot m}\ ,\ \boldsymbol{M_2 = 1.06N \cdot m/s.}$$

Even upon the most approximate estimations when decreasing $\boldsymbol{\omega}$ from $50s^{-1}$ (the maximum threshold limit where the stem cutting occurs) to $25s^{-1}$ (twice) the resistance force factors are reduced in about 5 times. So, the theoretical and experimental investigations evidence that in case of low moving velocities (1÷5m/s) of the blade the resistance of the dense environment rapidly declines.

Similarly, it is indisputable that in case of the mentioned velocities (1÷5m/s) it is impossible to cut the plants stems without pillars. Anyhow, when the blade receives vibratory movement in case of the mentioned velocities, it becomes possible to carry out clean cutting of the stems with low energy consumption [2,3].

In the result of our theoretical researches we have derived the following expression for the critical forces of the stems vibratory cutting [3].

$$\boldsymbol{P_{cr} = \frac{D}{2b}\delta \cdot \Delta \cdot \sigma_p + \frac{2D^2{\sigma_p}^3 \cos\gamma}{2\pi E^2 \cos\varepsilon}(ctg\,\gamma \cdot \cos\gamma + \mu \sin\gamma) \cdot}$$
$$\boldsymbol{\cdot \Big\{[\sin\varepsilon - \cos\gamma\, tg\,\varphi\,(1-\cos\varepsilon)]\sin\gamma\, tg\,\varphi\cos\omega t +}$$
$$\boldsymbol{+\left[\sin\varepsilon\cos\gamma + \frac{2}{\pi}tg\,\varphi\,(\sin^2\gamma + \cos\varepsilon\cos^2\gamma)\cos\omega t\right] +} \quad (4)$$
$$\boldsymbol{+\,[\,tg\,\varphi\cos\omega t\,(\cos\varepsilon - \sin\varepsilon\cos\gamma\, tg\,\varphi\,)]\Big\},}$$

where $\boldsymbol{D}$ is the diameter of the cutting stem (D=20mm, the mentioned and further numerical data are related to our experiments conducted on the stem cutting of the cane), b is the amplitude of the blade vibration-b=8mm, $\boldsymbol{\delta}$ is the thickness of the blade - $\boldsymbol{\delta = 0.1}mm$, Δ is the width throughout the blade teeth - $\Delta = \boldsymbol{3.0}mm$, $\boldsymbol{\sigma_p}$- is the decomposition strain in the matter of the cane stem (mainly sclerenchyma and epidermis) - $\boldsymbol{\sigma_p = 20}N/mm^2$, E is the elasticity module of the cane stem - $\boldsymbol{E = 200}N/mm^2$, $\boldsymbol{\varphi}$ is the friction angle of the cane with the steel - $\boldsymbol{\varphi = 45^\circ}$, $\boldsymbol{\gamma}$ is the angle formed by the forward movement of the blade cutting edge and the cutting apparatus - $\boldsymbol{\gamma = 0 \div 90^\circ}$, $\boldsymbol{\varepsilon}$ is the blade fixation angle - $\boldsymbol{\varepsilon = 30^\circ}$, $\boldsymbol{\omega}$ is the frequency of the blade vibration - $\boldsymbol{\omega = 0 \div 100}s^{-1}$.

The diagram of the changing value in critical force $\boldsymbol{P_{cr}}$ during a stem cutting of the cane depending on the vibration frequency of the blade in terms of the mentioned numerical values in the air medium is introduced in fig. 1. The experiments have been conducted on two blade types: flat blade and that of with toothed cutting edges.

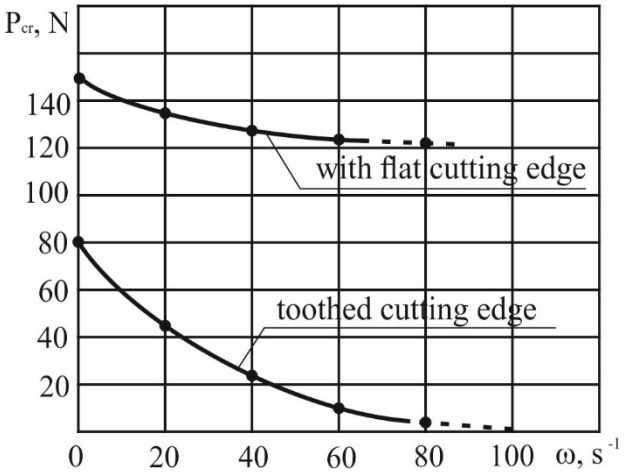

Fig. 1. *The dependence of the critical force P_{cr} of a stem cutting in the cane on the blade vibration frequency ω, (ε=30°, γ=45°) (composed by the author).*

The diagram (Fig. 1) shows that though in both cases the changing regularity of the critical force depending on the vibration frequency is the same, the critical force declines parallel to the frequency increase and their absolute values are quite different.

In case of the toothed cutting edge the value of the critical force is much lower, moreover, together with the increase of the value in $\boldsymbol{\omega}$ the discrepancy grows up. For example, when $\boldsymbol{\omega = 20 s^{-1}}$, the critical force for the toothed cutting edge is lower in about 2.8 times, while when $\boldsymbol{\omega = 80 s^{-1}}$, the declining discrepancy makes about 13 times [3].

The research experiments have shown that in case of vibratory cutting the blade should have toothed cutting edge which is of utmost practical significance.

In water environment the ratio of the cutting critical forces in the very stem is not subjected to considerable changes. The needed power (torque moment) transmitted to the rotor of the cutting apparatus rapidly grows up due to the environmental resistance. Besides, the latter exceeds the real cutting forces in 4÷5 times [1, 3, 6].

By numerous experiments [2, 3] it has been stated that in the air medium the blade vibration can be accompanied with high velocities (30÷80m/s), while in the dense environment it doesn't work due to the high rate of environmental resistance forces and unjustified energy consumption.

As it has been mentioned above, according to the expressions (1), (2) and (3) the decrease of the rotation frequency in the rotor already leads to the abrupt reduction of the environmental resistance forces. Anyhow, it is worth mentioning that the main reason for the decrease in the environmental resistance force caused by the blade vibration is not possible to explain through the mentioned expressions.

Results and discussions

For the solution of the current problem let's make use of a design schedule. Since we have already proved that it is relevant to conduct the plant stem cutting in the mutually vertical directions of the blade cutting edge in conditions of balanced oscillations [3], it is necessary to select the blade vibration through the elliptic law in order to disclose the vibro-cutting effect [2, 3, 7, 8].

Upon the mentioned law the vibration movement is transmitted to the blade by means of electromagnetic vibro-generator installed in the waterproof case of the rotor in the laboratory unit used for the study of plants stems vibro-cutting in the water medium [1].

The cutting edge points of the vibro-blade carry out complex movements which consist of the following moving modes:

- rotational or supplying movement of the blade with $\boldsymbol{\omega}$ angular velocity,
- vibratory complex movement along the blade cutting edge with $\boldsymbol{a_{(x)}}$ amplitude and $\boldsymbol{\omega_{(x)}}$ frequency,
- blade movement in vertical direction against the cutting edge with $\boldsymbol{a_{(z)}}$amplitude and $\boldsymbol{\omega_{(z)}}$ frequency.

The velocity of the arbitrary C point of the cutting edge in the vibro-blade will be determined in the following way:

$$\bar{\mathbf{v}}_{\mathbf{c}} = \bar{\mathbf{v}}_{\mathbf{c}}^{\mathrm{rot}} + \bar{\mathbf{v}}_{\mathbf{c}}^{\mathrm{vibr}}, \tag{5}$$

where $\bar{\mathbf{v}}_{\mathbf{c}}^{\mathrm{rot}} = \boldsymbol{\omega}\mathbf{r}$ (r is the rotation radius of the C point), $\bar{v}_c^{\mathrm{vibr}} = \bar{v}_{a(x)} + \bar{v}_{a(z)}$.

The vibration velocities in the direction of x and z axes will be:

$$\begin{aligned} \bar{\mathbf{v}}_{\mathbf{a(x)}} &= \mathbf{a}_{\mathbf{(x)}} \cdot \bar{\boldsymbol{\omega}}_{\mathbf{x}} \sin \boldsymbol{\omega}_{\mathbf{x}}\mathbf{t}, \\ \bar{\mathbf{v}}_{\mathbf{a(z)}} &= \mathbf{a}_{\mathbf{(z)}} \cdot \bar{\boldsymbol{\omega}}_{\mathbf{z}} \cos \boldsymbol{\omega}_{\mathbf{z}}\,\mathbf{t}\,. \end{aligned} \tag{6}$$

The diagram on the determination of the vibro-blade kinematic parameters is introduced in figure 2.

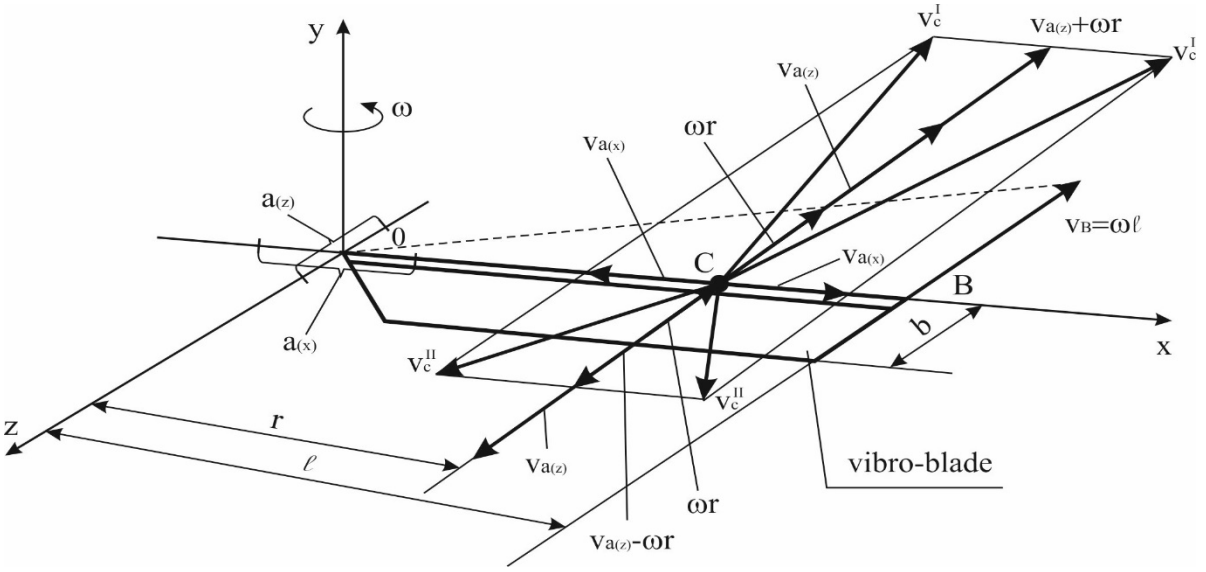

Fig. 2. *The diagram on the determination of vibro-blade kinematic parameters (composed by the authors)*

Upon the previously conducted theoretical and experimental investigations it has been proved that efficient vibro-cutting occurs, when $a_{(z)} = 0.1a_{(x)}$, $\boldsymbol{\omega_{(x)}} = \omega_{(z)} = \omega_1$ [3, 6].

Taking into account the aforementioned, for the C point of the vibro-blade cutting edge we'll get the following expression:

$$\bar{\mathbf{v}}_{\mathbf{c}} = \bar{\boldsymbol{\omega}}\boldsymbol{r} + \mathbf{a}_{(\mathbf{x})}\bar{\boldsymbol{\omega}}_{\mathbf{1}} \sin \boldsymbol{\omega_1 t} + \mathbf{0.1a_x}\bar{\boldsymbol{\omega}}_{\mathbf{1}} \cos \boldsymbol{\omega_1 t}. \quad (7)$$

The last expression testifies that depending on the vibration's phase-frequency ratio in the mutually vertical directions the velocity of the C point will have different directions and values. The 4 extreme values and directions of the mentioned velocities are depicted in figure 2. Thus, the values of the velocities will be:

$$\boldsymbol{v_c^I} = \sqrt{(\boldsymbol{\omega r} + v_{\boldsymbol{a(z)}})^2 + v_{\boldsymbol{a(x)}}{}^2}, \boldsymbol{v_c^{II}} = \sqrt{(v_{\boldsymbol{a(z)}} - \boldsymbol{\omega r})^2 + v_{\boldsymbol{a(x)}}{}^2},$$

or by inserting the abovementioned values we'll have:

$$\begin{aligned} \boldsymbol{v_c^I} &= \sqrt{(\boldsymbol{\omega r} + \mathbf{0.1}\boldsymbol{a_x\omega_1} \cos \boldsymbol{\omega_1 t})^2 + (\boldsymbol{a_x\omega_1} \sin \boldsymbol{\omega_1 t})^2}, \\ \boldsymbol{v_c^{II}} &= \sqrt{(\mathbf{0.1}\boldsymbol{a_x\omega_1} \cos \boldsymbol{\omega_1 t} - \boldsymbol{\omega r})^2 + (\boldsymbol{a_x\omega_1} \sin \boldsymbol{\omega_1 t})^2}. \end{aligned} \quad (8)$$

The received expressions show that the changing picture in the velocities of the cutting edge points of the blade is complicated, i.e. the velocity vector varies not only in the given point, but also along the cutting edge due to $\boldsymbol{\omega r}$ component ($0 \leq \boldsymbol{\omega r} \leq \boldsymbol{\omega l}$).

Since our discussions are related particularly to the vibration effect on the movement and resistance of the water environment, it is relevant to consider the moving components separately to simplify the solution of the problem. The effect of the velocity reduction in the blade's rotational movement on the rapid decrease of the resistance forces in the water environment has been already considered above. Let's turn to the design schedule used in the previous series of the related article to disclose the impact of the vibration movement on the resistance forces in the water environment [1, Figure 4].

When discussing the first part of the problem it has been found out that the resistance forces of the environment are mainly related to the liquid mass put into motion as a result of the blade movement [9, 10]. The liquid layer passing close to the blade surface gets the blade velocity and due to the internal friction of the fluid particles in the vertical direction to the blade surface the velocity gradually declines getting equal to zero in the distance of $\boldsymbol{\delta} = \sqrt{\frac{\boldsymbol{\nu b}}{\boldsymbol{k\omega r}}}$ [1].

The design schedule of the case study will look as follows (Fig. 3) (composed by the authors).

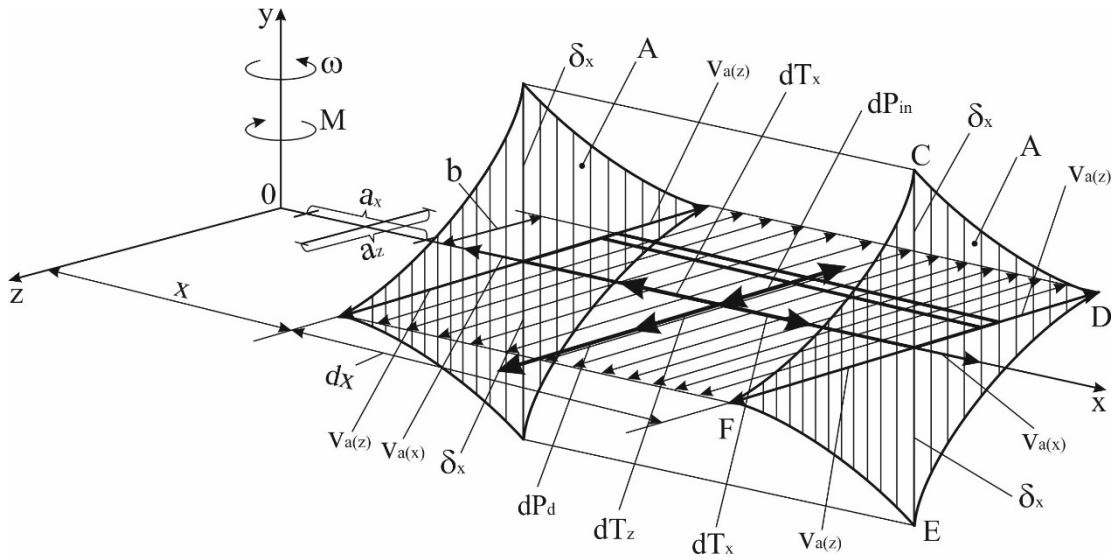

Fig. 3. *The diagram on determination of the resistance forces of the vibro-blade movement in the rotary cutting apparatus in water medium (composed by the authors).*

The water mass flow with basic volume in motion will be [1]:

$$\boldsymbol{dm = \rho\, dQ.} \tag{9}$$

In case of the blade vibration the moving water mass is surrounded by the surface with A base (unlike the first part of the discussed problem, in this case A is constant $along\ \ the\ \boldsymbol{l}$ length of the blade) and is within the volume of elementary prism $\boldsymbol{dQ}$ with dx height. The prism base is a combination of four parabolic triangles with δ_x and $v_{a(z)}$ sides, besides, the water mass within the prism volume changes the moving direction during one oscillation phase $\boldsymbol{t = \frac{1}{\omega_1}}$ caused by the vibratory movement and hence, the epure of the liquid motion velocities towards the vertical directions of the blade sheet will look as introduced in Fig. 4.

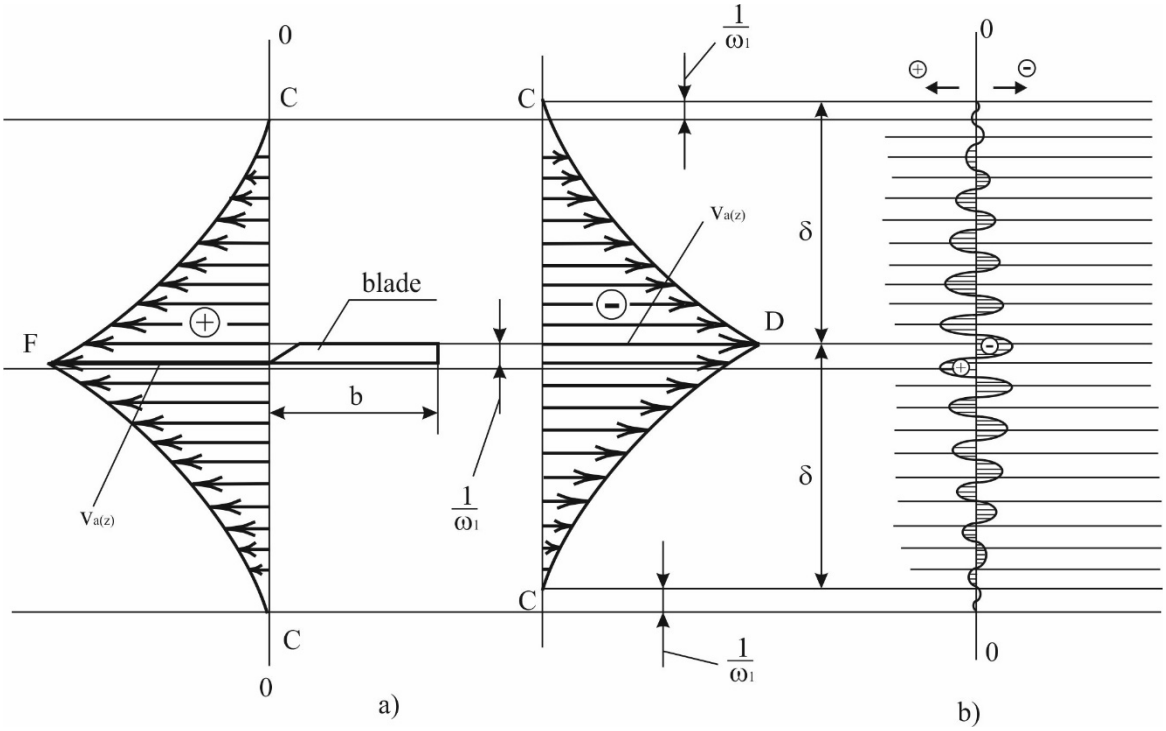

Fig. 4. *The epure for the velocities of the liquid motion in the vicinity of the vibro-blade moving in the liquid (composed by the authors).*

The particles of the liquid, getting in touch with the surfaces of the upper and lower b·dx (for the entire blade $\boldsymbol{b} \cdot \boldsymbol{l}$) blade areas. receive a motion in one direction $\frac{1}{2\omega_1}$, and after a second the new layers getting in touch with the mentioned surfaces get a motion in the opposte direction, while the particles of the former layers lose the velocity due to the lack of impulse signal in the previous direction, and the velocity gradually equals to zero. To get the summary epure of the velocities for the liquid particles in the vibro-blade vicinity it is necessary to reset the successive epures of the velocities considering their phasal deviation. As a result we'll get the summary epure for the velocities (Fig. 4 b).

It is easy to notice that the total area of the summary epure can be practically accepted as equal to zero, besides, the higher the vibration's frequency ω_1 is, the more reliable the abovementioned assumptions are. So, if the volume of the vibration-induced moving liquid is practically equal to zero (summary area of the elementary prism base: A→0), then the fluid mass in motion is $\boldsymbol{dm = 0}$ and all force factors, which are related to the fluid mass flow and generate resistance forces in the environment, practically become equal to zero. The afore mentioned is similarly applicable to the vibration towards the blade longitudinal direction.

In the previous series it was mentioned that the blade moving in the liquid medium is affected by the following resistance forces [1,9,10]:

1. friction forces in the longitudinal direction of the blade sheet:

   $\boldsymbol{T_x = \frac{8}{15}\rho\omega^3 \cdot \sqrt{\frac{\nu b}{\omega}}\ell^2 \cdot \sqrt{\ell} = 0}$, because $\boldsymbol{T_x}$ is related to the mass flow in motion which is equal to zero.

2. inertia forces $\boldsymbol{P_{in} = -\frac{2\omega^3 x^3 \rho}{b} \cdot \sqrt{\frac{\nu b}{\omega x}}\, dx}$, upon the same reason those forces are also equal to 0: $\boldsymbol{P_{in} = 0}$.

3. friction force in the latitudinal directions of the blade sheet: $\boldsymbol{T_z = 6b\sqrt{\mu\rho\omega^3 l^3} \neq 0}$, which is related to the rotation velocity of the blade.

4. Hydrodynamic resistance force:

   $\boldsymbol{P_d = c\lambda\omega^2\rho\frac{l^3}{2} \neq 0}$, this is also related to the blade rotary movement.

Thus, from the resistance force factors of the blade motion only $\boldsymbol{M_1}$ is available in case of vibration, the value of which is related to $\boldsymbol{T_z}$ and $\boldsymbol{P_d}$ forces [1].

$$\boldsymbol{M_1 = \frac{c\lambda\omega^2\rho\ell^4}{8} + 4b\sqrt{\mu\rho\omega^3} \cdot \ell^3}. \quad (10)$$

As it has been already mentioned, in case of vibratory cutting the blade rotation velocity (1.0-5.0m/s) is much lower than that of the stem cutting velocity (30-50m/s) without any pillars, as a result of which the resistance forces of the water environment rapidly decrease. Assuming that the rotary movement velocity in the vibro-blade is 3.0m/s ($\omega = 10\text{s}^{-1}$) and inserting the numerical values of the units - $\boldsymbol{\rho} = 1000\text{kg/m}^3$, c = 1.45, $\boldsymbol{\mu} = 0.1\text{kg/m} \cdot \text{s}$, b = 0.03m, $l = 0.3\text{m}$, $\lambda = 0.001\text{m}$ [1] in the expression (10), we'll have $M_1 = 1.315\text{N} \cdot \text{m}$.

In case of cutting without vibration the values of the resistance moment will be as follows:
when $\omega = 50\text{s}^{-1}$, $M_1 = 15.13\text{N} \cdot \text{m}$, when $\omega = 100\text{s}^{-1}$, $M_1 = 47.1\text{N} \cdot \text{m}$,
that is, in case of vibratory cutting not only a number of resistance forces are neutralized, but also the current force factor is also reduced in 10-35 times depending on the critical velocity needed for cutting.

Thus, the model and design schedule recommended for the solution of the problem enable to disclose the reasons for abrupt decrease of resistance forces in the water environment during the vibratory cutting.

The obtained results and particularly the summary epure for the velocities of the fluid motion in the vibro-blade vicinity serve as a background for conducting further discussions and finding new solutions for the current problem. Considering the fluid movement in the vertical directions against the upper and lower areas of the vibro-blade sheet as a shock (dying) oscillations, the problem can be theoretically interpreted otherwise, which can result in a more precise interpretation on the vibro-cutting effect in the dense medium and in the new opportunities for disclosing its consequences. The chapter related to the mentioned research aspects will be presented in the upcoming series.

Conclusion

1. A model and design schedule has been recommended, which enable to theoretically disclose and describe the stem's vibro-cutting effect in the dense environment.
2. Theoretical expressions have been derived, which enable to determine the factors of the resistance forces in the dense medium and their decline in case of stem cutting with vibratory blade. Particularly, it has been stated, that in case of implementing vibratory cutting in the water environment some part of environmental resistance force factors are missing at all, while the others decrease in 10÷35 times related to the cutting velocity.
3. The results obtained through the theoretical researches match up with those enhanced through scientific-experimental way with only 5 % deviation.

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