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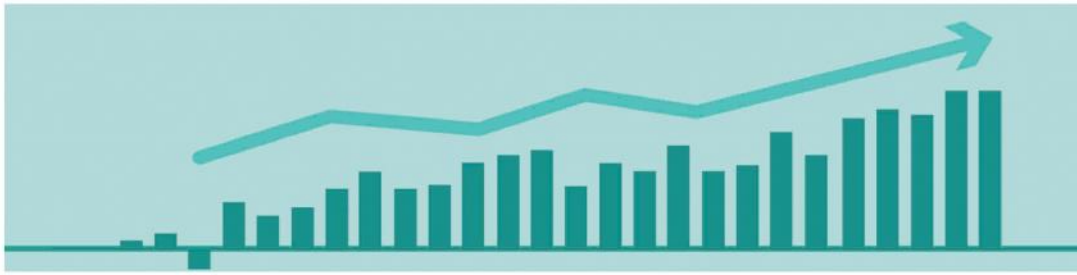

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"*Challenges in Biotechnological and Environmental Approaches*",

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(April 23 - 25, 2019)

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RESEARCH ARTICLE **(Open Access)**

Determining the best fit equations to represent the cumulative particle – size distribution curves in soils of Albania

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Abstract

There is no doubt about the need to replace the textural triangle by the cumulative particle – size distribution curves in the research context of Albanian soils. Beside all, it creates the necessary space to establish the relationships between the cumulative particle – size distribution curves and the most important hydraulic properties of soils. Therefore, having the cumulative particle – size distribution curve belonging to a specific soil, it becomes possible the recognition of hydraulic properties of this very soil. There are many efforts to determine the best fit equation reflecting the cumulative particle – size distribution curves. However, the best fit in our research seems to be exponential law function in its closed form. This conclusion became possible to be drawn based on a considerable large textural data base collected throughout the soils of Albania. Whether this conclusion is true for the soils where the clay predominates, or silt and sand predominate; or whether it has the same significance as for fitting to the process of data transfer from one scheme to another, it requires further research.

Keywords: cumulative particle – size distribution curve; exponential law function; textural triangle.

1. Introduction

Since the first theoretical proposal done by D. Hillel, there are plenty of efforts to establish equations of a relatively universal nature to describe the mineral particle size distributions in a particular typical soil. In his famous books [1, 2, 3, 4]. Hillel stated that the method to determine the texture of a given soil by the textural triangle is rather "arbitrary", which is a call for replacing it by the particle-size distribution curves. The efforts done later in this area of research did face difficulties, because of the existing of various models of mineral particles classifications, as it is shown in the following table:

Table.1 The various methods to determine the cumulative distribution of particles (clay, silt, sand) within the ranges called respectively as VF – very fine; F – fine; M – medium; C – coarse; VC – very coarse.

Particle size mm	The classification it belongs to	The particles Involved
0,001	Katschinski	clay fine
0,002	USDA&ISSS	clay
0,01	Katschinski	clay +fine silt
0,05	USDA&Katschinski	clay + fine silt + coarse silt
0,1	USDA	clay + fine silt + coarse silt + VF sand
0,25	USDA&Katschinski	clay + fine silt + coarse silt + (VF + F) sand
0,5	USDA&Katschinski	clay + fine silt + coarse silt + (VF + F+ M) sand
1	USDA&Katschinski	clay + fine silt + coarse silt + (VF + F + M + C) sand
2	USDA&ISSS	clay + fine silt + coarse silt + (VF + F + M + C+ VC) sand

2. Material and Methods

The method to be followed in this research is going to be in substance a quantitative analysis establishing relationships between the size (diameter) of mineral soil particles and their respective cumulative percentage in various textural soils. The quantitative analysis to be applied is the regression analysis. Meanwhile, the data base of the relative participation in percentage of mineral soil particles of various size ranges and in various typical textural soils, is going to be provided from the source referred as, [9].

In the following figure, the three typical particle – size cumulative curves are presented schematically

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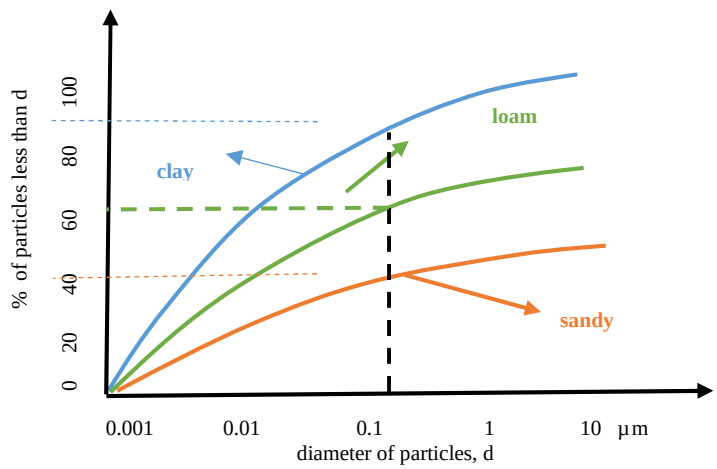

Figure 1. Particle-size distribution curves belonging to different textures schematically presented

The above seen curves will be quantified in a concrete way by applying the regression analysis and the constants that are going to get appeared in these equations, will be analyzing for their physical meaning. After, the found equations will be tested for their fitness towards the specific and various soils. Therefore, their significance will be applied to determine their suitability.

3. Results and Discussion

This study will use the final equations determined by Rousseva ,[7]. Both types of the equations are going to be considered: exponential and power equations in their closed forms, as it is appeared in the following:

$$F_1(D) = a + (100 - a)\frac{D^n}{1 + b^{mD}} \qquad (1)$$

$$F_2(D) = a + (100 - a)\frac{D^n}{1 + bD^m} \qquad (2)$$

; where

F1(D) is the function of "% of particles less than d" from the size range of a given mineral particle type in the case of having a closed exponential equation type.

F2(D) is the function of "% of particles less than d" from the size range of a given mineral particle type in the case of having a closed power equation type.

a is the percentage of particles less than 0,001 mm

b, m, n are the coefficients to be released by the regression analysis. However, they have an well determined physical meaning. So, after Rousseva, the coefficient b represents the ratio between the percentages of particles coarser than 0,01 mm with the particles between 0,001 mm and 0,01 mm. As the

coefficients m and n determine the slope of the curves represented by the above mentioned equations.

The both equations are going to be build up by using the data collected from the source referred as,[9].

The data found in this source are absolutely sufficient and with a trustable status to be used for meeting the aim of this study.

4. Conclusions

1. The data collected from various soils in Albania about their textural nature are sufficient and reliable to be applied for meeting the purpose of this research.
2. Both forms of closed equations, exponential and power, are going to be tested in the conditions of soils in Albania.
3. There are sufficient data to believe that the soils in Albania need to be grouped based on their texture nature in order to build more realistic and reliable equations for fitting them.

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RESEARCH ARTICLE **(Open Access)**

Evaluation of genetic diversity of cowslip populations (Primula veris L.) in Kosovo, based on some phenotypic traits and indicators.

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Abstract

Kosovo's territory is very rich in medicinal plants, and one of these plants is cowslip (*Primula veris* L.),which grows in almost all areas of the country. The study is focused in the western and eastern parts of Kosovo, in which are collected 20 cowslip samples, that represent 10 typical populations of this plant, distributed throughout the country. During three years (2016-2018), the populations included in this study, were analyzed for a number of bio-morphological indicators, as well as chemical analysis of the phenol content in flowers and roots are carried out.

For the analyzed populations, the plant phenological stages have been carefully observed, and a number of indicators and features have been measured. These phenotypic features and indicators have to do with: the number of flowering stalks, plant height, the number of leaves per plant, the size of the leaf, the number of flowers per plant, color of the flowers and leaves, the number of seeds, the type of the root system, etc. From the data obtained for each sample, the average parameters for each population are calculated , and then a statistical data processing is done. Based on the data processing, it is very evident that cowslip populations are characterized by a great diversity, between populations, as well as within the populations themselves. Diversity is present in the plant height, the height of the flowering stalks, the number of flowers per plants, the time of flowering and fructification etc. Some of these populations, such as those collected in Leqenat (P1M1) and Gollak (P8M1), have a great interest to be cultivated by the farmers, in the territory of Kosovo.

Keywords: plant diversity, plant population; phenotypic traits; chemical analysis

Introduction

Because of a very favorable geographic position of Kosovo, as well as the climatic and soil factors, the plant species diversity in this region is very large. There are about 1800 plant species in Kosovo, of which about 300 species are classified as medicinal and wild fruit tree species (Millaku 2010).

The aromatic and medicinal plants in Kosovo represent a great economic potential, for a sustainable rural development , as well as for the increase of income of farmer families, living in these rural areas.

Among the medicinal plants, that are grown in Kosovo, cowslip (*Primula veris* L.) is a very important plant, since it is collected, processed and marketed, for use in the pharmaceutical industry, due to the high content of phenols in flowers and roots. In spite of the great interest, cowslip plant is not studied in details as a medicinal plant, both in terms of its diversity and for the ability to use some wild populations of this plant, for extensive production in Kosovo.

The objective of this study, is to identify and evaluate some cowslip populations in the eastern and western parts of Kosovo, as well as, to assess the genetic diversity of these populations, based on some phenotypic traits and indicators.

The purpose of the study is that, based on the diversity among the cowslip populations, to select some of these populations that have better traits, in terms of morphological and chemical indicators of

roots and flowers. These populations can be used, for cultivation on open field, by farmers in Kosovo.

Materials and methods

The studied and evaluated plant material, is collected in 10 sites, distributed in the eastern and western parts of Kosovo. A total of 20 samples have been collected, representing 10 typical cowslip populations. The territory where the samples are collected, has a large variation in height above sea level. The collection of the plants, has started at the altitude of about 770 m over the sea level in Radishevë, and has ended at a height of approximately 1730 m over sea level in Rashkodol.

Field sampling is based on the Marshall & Browen method, which guarantees the collection of the maximum diversity of populations, included in the study.

For each population, two samples have been taken, which are approximately 10 km away from each other. For each sample, are taken 20 plants, at a distance of 50-100 m, from each other. For the evaluation of the bio-morphological variation, a total of 15 indicators have been described, of which, 10 are for quantitative features, and 5 for qualitative features. Quantitative features taken in the study are: the height of the plant; the diameter of the stalk; number of leaves in rosettes; the size of the leaf, the number of flower stalks per plant; the length of the flower stalk; number of flowers per plant; the number of seeds per flower; number of seeds per plant.

While the qualitative features taken in the study are: hair of the stalk; distribution of the leaves on the stalk; leaves shape; color of the flower ; the color of the calyx etc.

After collecting of the samples, measurements were made for each plant, and an average was calculated for each sample, as well as the average indicators for each population. For the main biomorphologic indicators, the correlation analysis of the features (Plascak 1993) was performed, based on the level of correlation: 0-0.1 no correlation; 0.1-0.25 very weak correlation; 0.25-0.4 weak correlation; 0.4-0.5 moderate correlation; 0.5-0.75 strong correlation; 0.75-0.9 very strong correlation; 0.9-1fully correlation.

Results and discussion

For each plant, included in the sample, measurements were made for some phenotypic indicators, and then the average for each sample was calculated. After this, the average was calculated for each of 10 populations, included in the study. The data for each population are presented in the table below:

For all indicators related to biomorphological features, correlation coefficients were calculated in order to analyze the level of correlation between the studied features.

Based on the data obtained from the processing, it results that there are very strong correlations between the height of the plant and the length of the flowering stalk ($r = 0.92$); there is a moderate strong correlation between the height of the plant and the number of seeds per flower ($r = 0.59$); as well as between the height of the plant and the number of seeds for plants ($r = 0.58$).

Between the height of the plant and the number of leaves per plant, there is a negative correlation ($r = -0.08$).

The correlation between the flowering stalk length and the number of stalks per plant, results to be poor ($r = 0.26$); also between the length of the flowering stalk and the number of seeds for flowers ($r = 0.32$). A moderate correlation exists between the flowering stalk length and the number of flowers per plant ($r = 0.47$); while strong correlation exists between the length of the flowering stalk and the number of seeds for plants. ($r = 0.57$).

Referring to the data on the table, for the phenotypic indicators, there is noted a high diversity among the populations, regarding the height of the plant, the diameter of the stalk, the number of leaves in rosettes, the number of seeds per plant, etc.

Table 1. Main phenotypic indicators

No	Populations	Phenotypic indicators										
		Altitude (m)	Plant height (cm)	Diameter of the stalk (mm)	No. of leaves per plant	Leaf length (cm)	Width of the leaf (cm)	No. of flowering stalks per plant	Length of the flowering stalk (cm)	No. of flowers per plant	No. of seeds per flower	No. of seeds per plant
1	Rroshk.	1672	23.2	1.7	5. 3	6.2	3.7	1.35	19.5	10.1	34.25	352
2	Leqenat	1513	26.1	2.25	11.5	6.7	3.75	2.6	23.2	22.5	31.5	550
3	Stankaj	1525	27.2	2.20	5.8	7.5	4.2	1.35	22.7	11.5	28.6	335
4	Istog 1	1500	25.3	1.85	7.2	4.78	2.6	1.75	22.1	9.2	37.5	550
5	Istog 2	1594	25.1	2.3	7.6	4.7	2.5	1.6	21.2	11.5	37.8	450
6	Kaçanik	950	21.2	2	7.5	4.45	2.2	1.55	18.2	14	28	410
7	Arte	900	25.7	2	7.7	4.6	2.56	1.6	22	14.1	33.6	480
8	Gollak	940	22.2	1.63	5.6	3.5	1.7	1.45	19.3	11.5	29.2	320
9	Sh. Baj.	992	23.3	1.8	6.5	4.2	2.5	1.5	20.6	12.3	32	390
10	Radishev	770	21	1.6	6.7	4.6	2.7	1.5	17.1	9	30.6	285

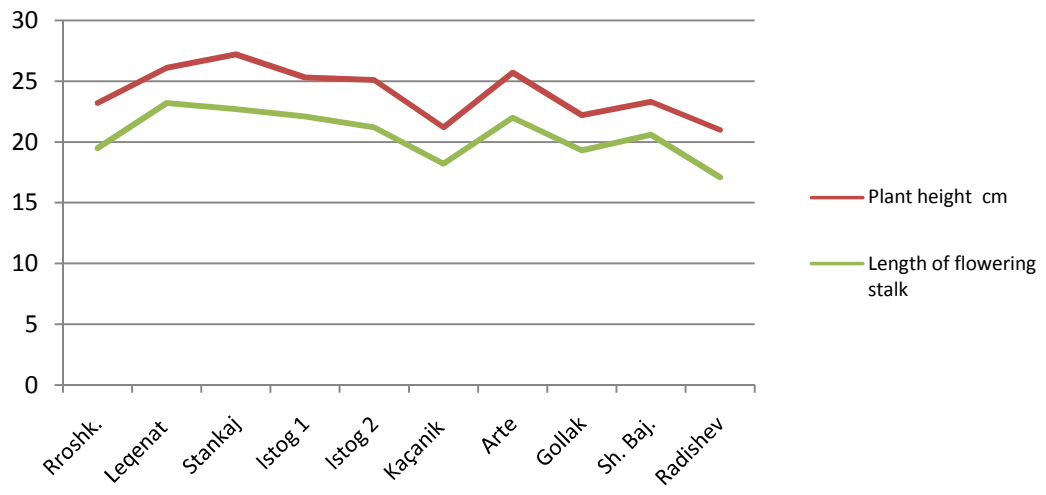

Figure 1. Variation for plant height and length of flowering stalks

The data show that the highest plants are those of the population taken in Stankaj (27.2 cm), while the plants with the lowest height are those of the population taken in Radisheve (21 cm).

Related to the flowering stalk, plants with the geatest length are those of the population collected in the Leqenat (23.2 cm) and with the smallest length, are those of the population collected in Radisheve (17.1 cm).

Referring to the data obtained from the measurements, it is shown that there is a great diversity for indicators: the diameter of the stalk, the number of leaves per plant and the size of the leaves. The largest number of leaves per plant, has the population taken in Leqenat (11.5) and the smallest number, has the population taken in Rroshkodol (3.5).

Also for the number of flowers per plant there is a great variation. The largest number of flowers for plants has the population taken in Leqenat (22.5) and the smallest number, the population collected in Radishevë (9).

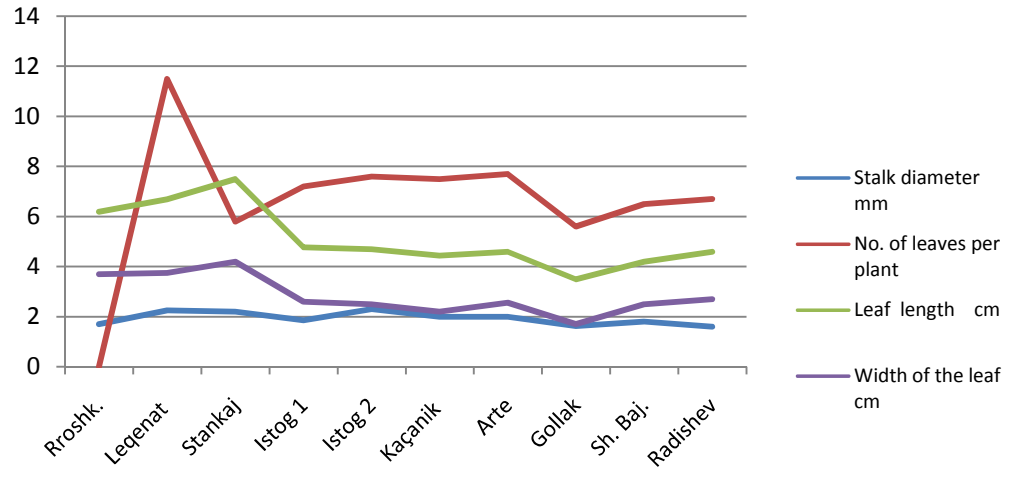

Figure 2. Variation for the stalk diameter, number of leaves and leaf dimensions

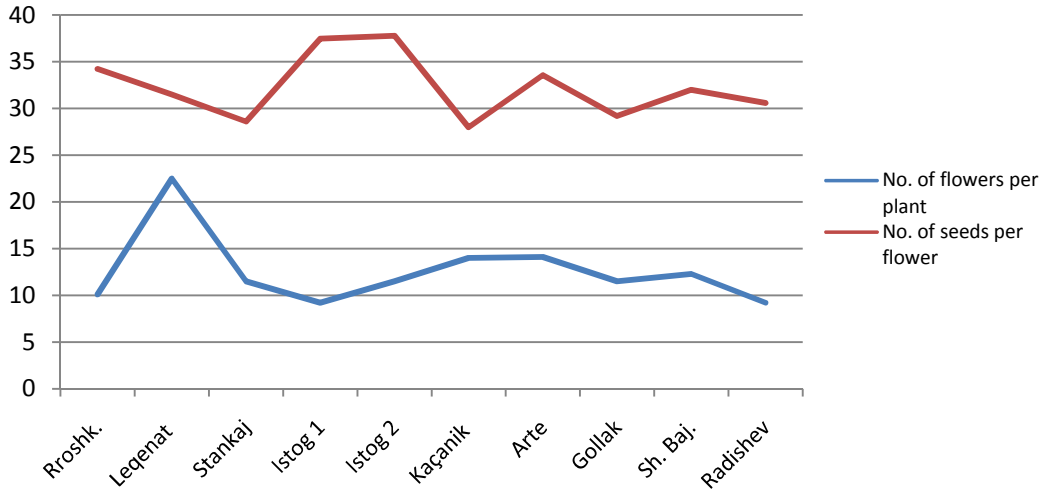

Figure 3. Variation for number of flowers per plant and number of seeds per flower

Conclusions

Cowslip plant is very common and widespread in the territory of Kosovo, and has a very large biomorphological diversity, both between the populations and within the populations themselves.

There is a significant variation in terms of the height of the plant, the number of flowering stalks per plant, the number of flowers for plant, the number of seeds for plant and so on.

Diversity for biomorphologic indicators is heavily influenced by climatic conditions, especially by the altitude over the sea level.

Some cowslip populations, such as those collected in Leqenat, Gollak and Kaçanik regions, are of a high interest to be selected and cultivated for extensive production by farmers in Kosovo.

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RESEARCH ARTICLE **(Open Access)**

Drought tolerance and thiols molarity in seedlings of two *Aegilops* accessions

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Abstract

Different species of the genus *Aegilops*, as wild relatives of wheat, are valuable sources of genes for wheat improvement, especially for the tolerance to abiotic stresses as drought and salinity. Thiols are actively involved in the maintenance of cellular redox homeostasis and in plant responses to almost all stresses. The most studied of thiols, glutathione can be found as reduced glutathione (GSH), which is oxidized to a disulphide form (GSSG), which in turn is recycled to GSH by a glutathione reductase. The redox potential of GSH/GSSG, which is responsible for the maintenance of cellular and extracellular homeostasis, is affected not only by the ratio GSH/GSSG, but also on the changes in synthesis and/or degradation of GSH. The recent study compared the seedlings drought tolerance for two *Aegilop*s accessions. Seedlings height, dry weight, first leaf length and leaf dry weight, have been evaluated prior to exposing to drought conditions. The seedlings were grown without irrigation for two weeks, and after that the number of survived seedlings, their fresh and dry matter were evaluated. The GSH content was also measured under controlled conditions, during the first three weeks after germination. For these, seeds were planted in pots with soil in laboratory conditions; GSH content was determined on extracts from stems, roots and leaves based on Ellman's test. From the results of the data processing via Software (*Sigma Plot 13.0)*, can be concluded that among the two *Aegilops* accessions do exist significant differences related to seedlings thiol molarity, drought tolerance, biometric parameters, and that the conical ear *Aegilops* accessions show advantages toward the cylindrical ones.

Key words: *Aegilops*, conical/ cylindrical ear, thiols, drought tolerance.

Introduction

Efficient strategy to solve loss of plant diversity consists of exploiting wild germplasm genomes of wheat species, which preserve a good part of their adaptive factor and diseases tolerance (1).

The genus *Aegilops* contains more than twenty species comprising both diploids and polyploids (2, 3, 4). The study of genetic diversity of *Aegilops* accessions may provide significant information regarding their potential for wheat breeding and genetic engineering in order to inclusion ones as many desirable features (5). *Aegilops* as relative to the cultivated wheat have many genes of agronomic interest and they can be important sources for resistance to diseases, pests and extreme environmental factors like drought, heating, salinity etc.

Independent of the strategy used to improve crop tolerance either by marker-assisted selection, recruitment of new potential crop species or gene transformation approaches, an in depth understanding of the mechanisms of stress tolerance is required (6).

Some recent research found that chromosome additions affected more the Glu/Gli ratio and the β-glucan content under drought than under control conditions. These results will contribute to the efficient transfer of wild alleles in introgression breeding programs to develop wheat varieties with improved health benefits and drought stress tolerance (7).

Drought stress, alone or in combination with other abiotic stresses, leads to ROS accumulation. It is accepted that ROS are signalling intermediates, required for normal plant growth and development and for inducing defensive stress responses. Enzymes, such as superoxide dismutase, catalase, ascorbate peroxidase, GSH peroxidase and Trx peroxidase (peroxiredoxin, PrxR), are important for ROS detoxification. Thiol and disulfide groups occur widely in natural products. They are found in small molecules such as lipoic acid,

glutathione, and thiamine, as well as in cysteine-containing peptides and proteins (hormones, enzymes, antibiotics). In all these substances, thiol and disulfide groups are clearly associated either with high chemical reactivity or with the consolidation of peptide and protein architecture (8). Plant thiols are apparently involved in plant response to almost all stress factors, and their accumulation, redox status and regulation are key to plant stress tolerance (6).

Evidence is emerging that a number of non-protein and protein thiols, together with a network of sulphur-containing molecules and related compounds, also fundamentally contribute to plant stress tolerance (9, 10).

GSTs involved in cellular detoxification seem to be produced in response to different stress factors, and furthermore are plant specific (11, 12, 13, 14,15).

2. Material and Methods

The plant material consists in seedlings of two *Aegilops* accessions, Ae.1 (Ardenica 5) and Ae.5 (Borsh5/4) from the collection of AUT Botanical Garden. The seedlings were established from seeds of each accession moistened with distilled water in Petri dishes (10 cm diameter). The experimental probe was arranged in a completely randomized design with three replicates established at room conditions, in Crop Physiology Laboratory, AUT. Seedlings height, first leaf length and the number of plants at third leaf stage have been evaluated prior to exposing to drought conditions. Plants were harvested on day 50 and the roots and shoots were collected separately, dried at 65°C for 2 days, weighed, and then used to calculate the total seedling dry weight (Sd DW), root dry weight (RDW), first leaf dry weight. Five-week-old well-watered plants were subjected to drought by withholding water for 14 days. After irrigation was resumed, the survival rate (per cent) and seedlings fresh weights in each sample were quantified one day later. The GSH content was also measured under controlled conditions, during the first three weeks after germination. For these, seeds were planted in pots with soil in laboratory conditions; Fresh leaves, roots and shoots from two week old plantlets were used to extract GSH (16) and GSH content was evaluated following Ellman's test (17).

One Way Analysis of Variance and the coefficients of correlations among different traits measured are evaluated using *Sigma Plot 13.0*.

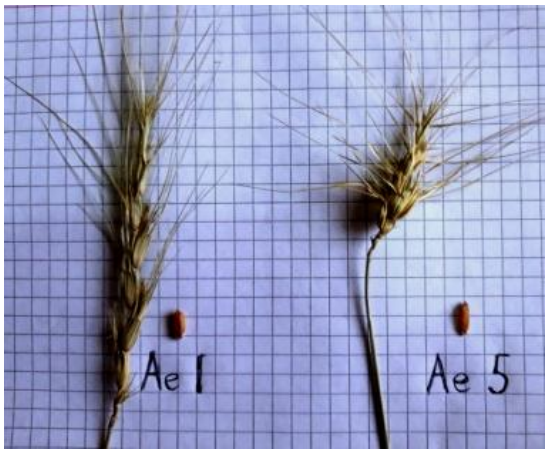

Figure 1. View of spikes of two *Aegilops* accessions, no.1 and no 5.

Figure 2. View from the experimental probe with *Aegilops* accessions at Laboratory of Crop Physiology.

Figure 3. Seedlings of *Aegilops* accessions no.1 and no.5.

3. Results and Discussion

Aegilops accessions biometric parameters

Several biometric parameters are very close related with plant vegetative and reproductive growth.

The following graphs and tables show data processing results related with some biometric parameters of 5-week seedlings of two *Aegilops* accessions included in the study.

These results, as is apparent from the graphs shown in Figures 4 and 5, are also in conformity with previous results (18), where it has been shown that the seedlings of conical shapes ears accessions exhibit significant differences in the biometric indicators from those with cylindrical ear shape. Besides first leaf length, that is greater at accessions Ae.5, the other parameters resulted greater at accession Ae.1 seedlings.

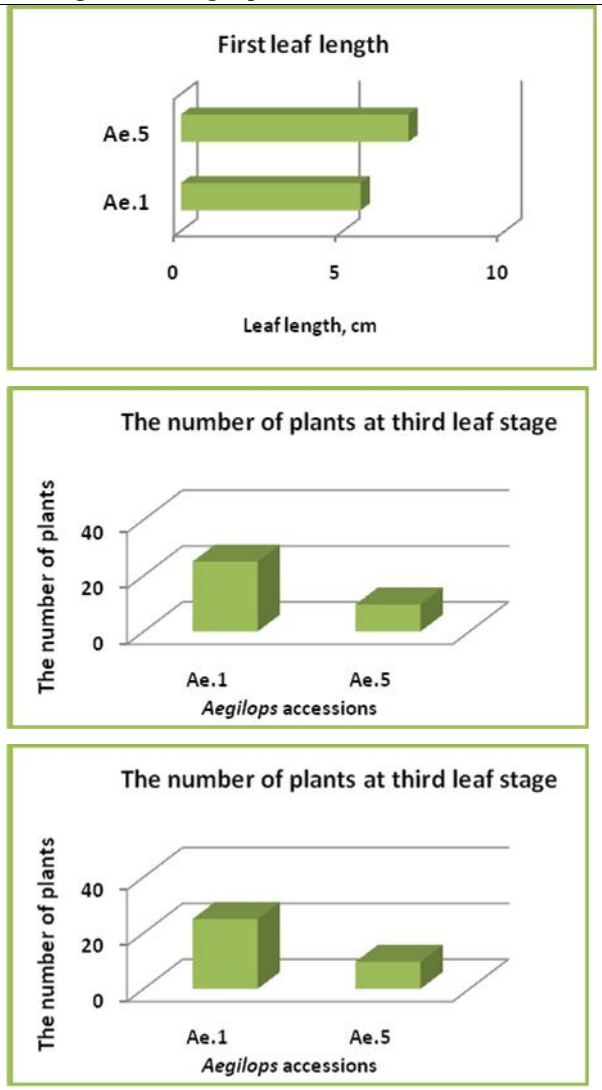

Figure 4. The biometric parameters of 5-weeks seedlings of two *Aegilops* accessions prior to drought tolerance test.

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Table 1. The results of One Way Analysis of Variance for two *Aegilops* accessions seedlings biometric parameters.

	F	P-value	F crit
First leaf length	24.72962	0.007636	7.708647
Seedlings height	8.71391	0.041889	7.708647
Third leaf stage plants	84.64	0.000775	7.708647

Drought tolerance

Biomass, biomass-related traits, and vegetative growth rates can be used as indicators of drought tolerance (19), and they are significantly correlated with grain yield under drought conditions (20).

Following earlier studies with different *Aegilops* accessions, this study was undertaken to investigate whether, besides the morphological and physiological differences previously reported, among the conical ear shape- accessions and cylindrical ones exist any difference in their tolerance to droughts. The graphs below show that the seedlings of conical ears accessions resisted better to drought than cylindrical ones. This result was found not only for the number of survived plants after drought stress, but even for drought survived plants fresh weights, one day after irrigation (Fig.6).

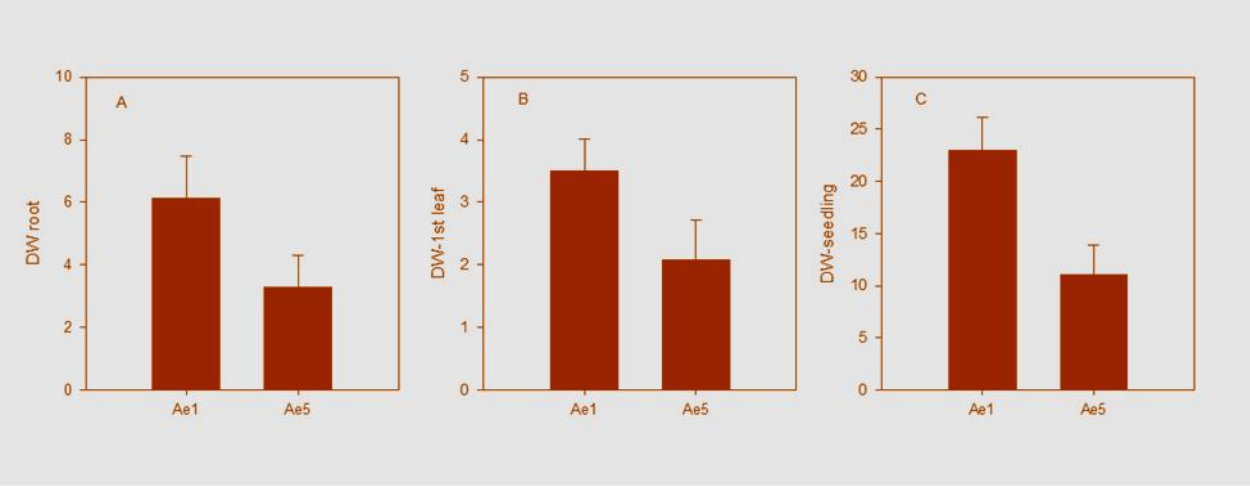

Figure 5. *Aegilops* accessions Ae.1 and Ae.5, root dry weight (A), first leaf dry weight (B) and seedling dry weight (C).

Table 2. The results of *Sigma Plot 13.0*, One Way Analysis of Variance for two *Aegilops* accessions (Ae.1 and Ae.5) seedlings biometric parameters.

Dependent Variable	***Normality Test (Shapiro-Wilk)***	***Equal Variance Test (Brown-Forsythe)***	*F*	*P*	*Comparisons for factor* ***Accession:***	*P*	***P<0.050***
DW-1st leaf	Passed (P = 0.130)	Passed (P = 0.347)	46.292	<0.001	t=6.804	<0.001	yes
DW-root	Passed (P = 0.088)	Passed (P = 0.416)	43.623	<0.001	t=6.605	<0.001	yes
DW-seedling	Passed (P = 0.057)	Passed (P = 0.631)	122.037	<0.001	t=11.047	<0.001	yes

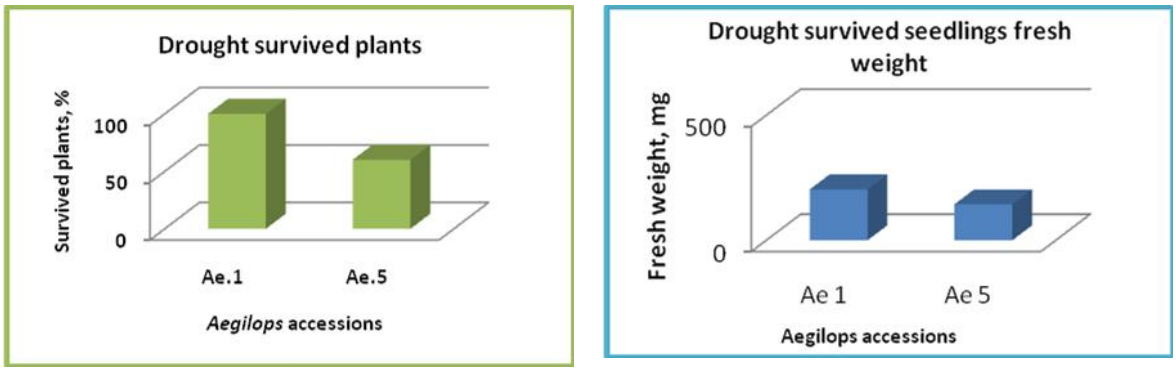

Figure 6. *Aegilops* accessions drought survived plants and their fresh weights 24 hours after irrigation.

The table below contains the results of the correlative analysis among *Aegilops* accessions seedlings different parameters; seedling dry weight, first leaf dry weight, root dry weight and seedling fresh weight (after two weeks drought stress). As it looks, their relations are positive and very strong. An important

result is that positive strong relations exist even among seedlings different parts (leaf, root) dry weights and seedling fresh weight after two weeks drought stress.

Table 3. The coefficients of correlations among *Aegilops* accessions seedling dry weight, first leaf dry weight, root dry weight and after drought stress seedling fresh weights.

	DW-1st leaf	***DW-root***	***FW-seedling***
DW - seedling			
	0.824 0.0000000220 30	**0.895** 2.622E-011 30	**0.976** 3.904E-020 30
DW-1st leaf			
		0.823 0.0000000246 30	**0.774** 0.000000526 30
DW-root			
			0.855 0.00000000174 30
FW-seedling			

Cell Contents:
Correlation Coefficient
P Value
Number of Sample

Different plant organs thiols molarity

GSTs activities have been reported to increase in a number of crops under drought and saline stresses (21, 22, 23). The results presented below (Fig.7 and Tab. 4) show that GSTF1 serves cells not only during the stress conditions but in a constant manner too (24, 25). Ae.1 and Ae.5 were selected through a drought tolerance test, as the most resistant *Aegilops* accessions among eight others taken in consideration (26).

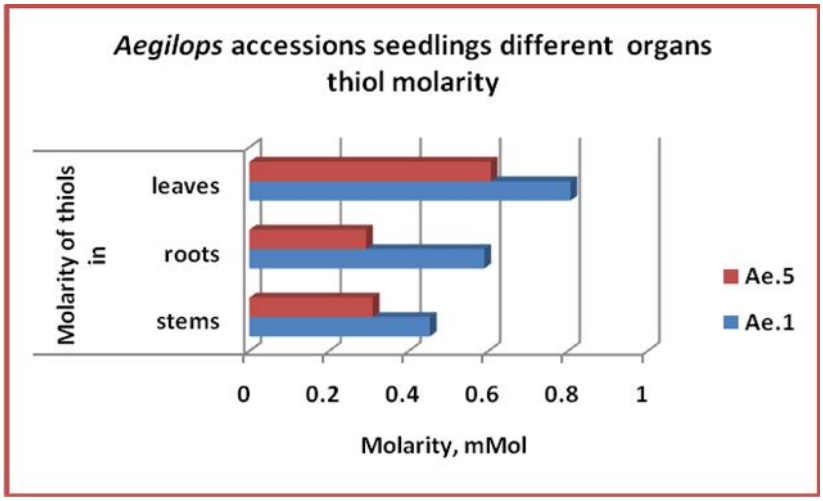

Figure 7. *Aegilops* accessions seedlings different organs thiol molarity.

Tabele 4. ANOVA main results of thiols molarity in leaves, roots and stems of two *Aegilops* accessions.

Anova results	*F*	*P-value*	*F crit*
Molarity of thiol in leaves	462.25	2.77E-05	7.708647
Molarity of thiol in root	1980.25	1.52E-06	7.708647
Molarity of thiol in stems	180	0.000179	7.708647

It was found that in both *Aegilops* accessions thiols exist in leaves, roots and shoots prior to drought stress. Their molarity is higher at Ae.1 then Ae.5 and among plant different organs, it is higher in leaves and roots than in stems.

4. Conclusions

Based on this study, there are significant differences of biometric parameters like seedling height, first leaf length and their dry weights, among the two *Aegilops* accessions seedlings Ae.1 and Ae.5. The conical ear *Aegilops* accessions show advantages toward the cylindrical ones for all mentioned parameters besides first leaf length. At the same time, conical ear *Aegilops* accession seedlings are more tolerant to drought stress. The level of tolerance toward drought, evaluated from the morphological parameters, correlates with the concentration of GSH in leaves, roots and stems at plants in the absence of stress conditions.

5. Acknowledgement

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RESEARCH ARTICLE (Open Access)

Statistical processing of the data for the presence of Q fever in human population in Western Macedonia

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Abstract

Q fever is an acute and rarely chronic, zoonotic disease. It is caused by *Coxiella burnetii*, an obligate intracellular, pleomorphic coccobacillus. It survives unfavourable conditions in the form of endospores and is extremely resistant to environmental effects. People are usually infected by inhaling the infected aerosol. The aim of study was to examine the frequence of Q fever in the human population in Western Macedonia and determining the statistical trend with descriptive and conclusive statistical methods. The serological test was conducted using the ELISA test kit. The serum isolated from the blood was kept at -30°C until testing. The sera were properly diluted based on therespective protocol using purified antigen of *C. Burnetii.* A total of 520 serums were checked of all ages from people with different epidemiological conditions, of which 114 resulted positive, with positivity rate of about 21.90%.The standard deviation of the infected population in general is 8.53, where in the female gender is higher than the masculine gender, while the age group of the two sexes the standard deviation ranges from 0.84 to 4.98.

The statistical analysis of the data results, prove that there is a connection and similarity among the samples from five regions in terms of the spread of the Q fever infection inhuman population, this is proven by the credibility boundaries with the Kloper-Pirson method. The frequency 78% negative per sample 520 is gained within the median limits from 74% to 86% of the sample.

Keywords: Zoonotic disease, human population, infection, serological test, regions.

1. Introduction

In August 1935, Dr. E. H. Derrick (MD, Melbourne University, 1922), the Director of the Laboratory of Microbiology and Pathology of the Queensland Health Department at Brisbane, Australia, was asked to investigate an outbreak of undiagnosed febrile illness among abattoir workers in Brisbane (10). This illness he named "Q" for "Query" fever. Derrick inoculated guinea pigs with blood or urine from the" fever patients. The guinea pigs became febrile. Derrick was unable to isolate the agent responsible for the fever so he sent a saline emulsion of infected guinea pig liver to Macfarlane Burnet in Melbourne. Burnet was able to isolate organisms which "appeared to be of rickettsial nature" (5). At about the same time Drs. Herald Rea Cox and Gordon Davis at Rocky Mountain Laboratory, Montana were working on the possible vectors of Rocky Mountain spotted fever and tularemia. Davis had ticks (the suspected vectors) feed on guinea pigs; the guinea pigs became ill. In May 1938, Dr. Rolla Dyer, the Director of the National Institute of Health, visited Cox in Montana to challenge Cox's report that he had cultivated rickettsiae in large numbers in embryonated eggs. Ten days later he became ill with retro-orbital pain, fever, chills and sweats. Five mL of his blood drawn on the sixth day of his illness resulted in fever when injected into guinea pigs. Subsequent studies showed that this agent was identical to the Nine Mile agent isolated from ticks. In April 1938, Burnet sent Dyer spleens from mice infected with the Q fever agent, Dyer showed that the Q fever agent was identical to Nine Mile agent (29). Cox named the Nine Mile agent Rickettsia diaporica (diaporica means having the property or ability to pass through) a reference to the filterable property of the agent (8). Meantime in Australia, Derrick proposed the name of

Rickettsia burnetii for the Q fever agent (11). In 1948 Cornelius B. Philip proposed that *R. burnetii* be considered as the single species of a distinct genus since it was now apparent that this organism was unique among the rickettsiae (30). He proposed the name *Coxiella*(30). The Q fever agent is now known as *Coxiella burnetii*.

The initial description of Q fever as an illness occurring among abattoir workers (10) was a strong portender of the epidemiology of this illness. The epidemiology of Q fever in man is linked to the epidemiology of fever in animals. Q fever is a zoonosis. Of all the animals infected by *C. burnetii*, man is the animal to usually develop illness as a result of infection (34). Man becomes infected following inhalation of *C. burnetii*. The desiccation resistant organism is shed from infected animals, usually cattle, sheep, goats or cats, especially at the time of parturition. Air samples are positive for weeks following parturition and viable organisms are present in the soil for periods of up to150 days (40).

Two characteristics of the organism are important in the epidemiology of the disease. These are its ability to withstand harsh environmental conditions, probably as a result of spore formation (28), and its extraordinary virulence for man. A single organism can cause disease in man (9).

Some studies have suggested that ingestion of raw (contaminated) milk is a risk factor for the acquisition of Q fever (16, 27, 22). Seroconversion, but not disease, did occur following ingestion of raw milk (3). It is likely that, in some populations, ingestion of infected material accounts for the high seropositivity rate. Ingestion of *C. burnetii* is probably important in the maintenance of Q fever in the animal population. Cats experimentally infected via the oral route did not become ill, whereas cats infected via the subcutaneous route became febrile and lethargic (14).

Percutaneous infection can occur (12, 13), but accounts for very few cases of Q fever worldwide.

While *C. burnetii* has been isolated from human placentas (35), there is little to suggest that vertical transmission occurs in man.

Person-to-person transmission has been documented but it is very unusual.

Sexual transmission of *C. burnetti* has been demonstrated in mice in the laboratory (38), but whether fever can be transmitted sexually under natural conditions and in other animal species is unknown.

Coxiella burnetii is a highly pleomorphic coccobacillus with a gram-negative cell wall. It measures 0.3 x 1 itm (2); unlike true rickettsiae it enters the cell by a passive mechanism. Within the cell it survives within the phagolysosome - the low pH of this environment is necessary for the metabolic functioning of *C. burnetii*.

Coxiella burnetii undergoes phase variation (21). In nature and laboratory animals it exists in the phase I state. Repeated passage of phase I virulent organisms in embryonated chicken eggs leads to gradual conversion to phase II avirulent forms (2). These two antigenic phases differ in the sugar composition of their lipopolysaccharides (33, 15), in their buoyant density in cesium chloride, and in their affinity for hematoxylin and basic fuchsin dyes. Plasmids have been found in both phase I and phase II cells. There are three different plasmid types varying in length from36 to 45 kilobases (32).

Man is the only animal known to almost always develop illness following infection with *C. burnetii* (1). Several clinical syndromes result from this infection - these are: a self-limited febrile illness, pneumonia, hepatitis, endocarditis, osteomyelitis, Q fever in infancy, neurological manifestations end complications of acute Q fever (25, 24).

While some *C. burnetii* infections are totally asymptomatic (7), the majority are mild self-limited febrile illnesses. It is difficult to know just what proportion of Q fever is truly asymptomatic.

Acute Q fever, which may be manifest as pyrexia of unknown origin, pneumonia or hepatitis, is almost always of abrupt onset. The manifestations of acute Q fever vary from country to country (15). Severe headache is a characteristic feature, so much so that it prompts a lumbar puncture to rule out

meningitis in some patients. Headache is more common with Q fever pneumonia than it is in pneumonia due to other agents (23).

Physical examination may be normal apart from an elevated temperature. Hepatomegaly and splenomegaly may be present (6). Uncommon features of acute Q fever include a variety of neurological manifestations: encephalitis, dementia, toxic confusional states, extrapyramidal disease (36), meningoencephalitis (26, 20, 4).

The strains of *C. burnetii* that cause chronic Q fever in man are probably different from those that cause acute disease (31, 39). The major manifestation of chronic Q fever is endocarditis. Another important predisposing factor is paralysis of specific cell-mediated immunity. Lymphocytes from patients with Q fever endocarditis are unresponsive to *C. burnetii* antigens (19) however this may be a secondary rather than a primary phenomenon. Phase I antibodies and antibodies in the IgA fraction predominate in Q fever endocarditis this is not so in acute Q fever.

Acute Q fever is readily treated with tetracycline (37). The treatment of chronic Q fever is difficult. It is probably best to use two antibiotics for at least two years. Some authorities recommend treating chronic fever for life. Strain differences may be very important in the management of chronic Q fever. The Priscilla isolate is less sensitive than the Nine Mile isolate to quinolones and it is resistant to rifampin (41).

Having in consideration that Q fever is a zoonotic disease, the aim of this study was to obtain a clearer picture over the epidemiological situation of the causes of Q fever in Western Macedonia, in relation to the serological presence of the Q fever in people.

2. Material and Methods

The stated study was conducted in the Laboratory of Virology of the Faculty of Veterinary Medicine – Tirana, Albania, using the ELISA Test in humans. The ELISA Kit was imported from the German Firm, SERION. The result‘s aim was to identify the IgG. The blood was taken from people of different pathologies, without any special preferences. It was collected from Gostivar, Tetova, Kercova, Struga and Dibra areas. The number of people tested is 520. The samples were provided in cooperation with the human service. The blood serum is separated by centrifugation at 6000 rpms in 20 minutes. The serum placed in plastic ampoules was kept frozen at -30^0C, until its testing. Positivity was based on the cut-off value, which in this case is over 0.5 OD. The sera were diluted before the test at a ratio of 1:400, in two steps. The first dilution was done at a ratio of 1:100, then the stated dilution, at a ratio of 1:4. The test was conducted based on the protocol of Serion Firm. The study‘s aim was to identify the presence of the infection in humans, and not interpret the diagnosis‘ decision. We have also tried to draw a link between human and animal infection by area, as people's blood is picked up at the areas where the blood of the animals was taken, which has resulted in specific seropositivities in the area (17, 18).

Additionally, since the number of serums is based on the minimum required number of serums for statistical processing, the results were processed from a statistical point of view to evaluate the reliability of the outcome that will result from the serological control of the serums in question. Results were processed by using statistical methods, such as the line equation of linear regression and the correlation coefficient.

3. Results and Discussion

In our study, as mentioned above, 520 people are involved. The initial male and female sample data have been separated from region to region (Tetovo, Gostivar, Debar, Kicevo and Struga). To facilitate the study and to assess the level of infection we have made a rough grouping, in terms of age groups: 0-20, 20-40 and over 40 years.

Hence, 520 sera of human were tested, of which 114 of them tested positive and results were

processed with statistical methods from which emerged the following results:

The work sample data of farm animals are shown in Table 1.

Descriptive statistics

Table 1. Data of the work sample divided in five regions with men and women of different age groups

i	Xmi(year)		Ni	Yoi(num)	Yoi(%)
Regio	Middle range of age groups of the region		Total number of tested persons	Numerical frecuency of persons positive with Q fever	Relative frequency of persons positive with Q fever
TETOVA	0-20	0,05 – 20,05	46	0	0,00%
	20-40	20,05 – 40,05	34	8	23,52%
	> 40	>40,5	75	21	28,00%
	Total:		**155**	**29**	**18,7**
GOSTIVAR	0-20	0,05 – 20,05	36	1	2,77%
	20-40	20,05 – 40,05	34	2	5,88%
	> 40	>40,5	70	7	10,00%
	Total:		**140**	**10**	**7,14**
DIBRA	0-20	0,05 – 20,05	6	0	0,00%
	20-40	20,05 – 40,05	8	2	25,00%
	> 40	>40,5	50	20	40,00%
	Total:		**64**	**22**	**34,37**
KERÇOVA	0-20	0,05 – 20,05	7	1	14,20%
	20-40	20,05 – 40,05	14	8	57,10%
	> 40	>40,5	53	23	43,30%
	Total:		**74**	**32**	**43,2**
STRUGA	0-20	0,05 – 20,05	47	10	21,20%
	20-40	20,05 – 40,05	14	5	35,70%
	> 40	>40,5	26	6	23,00%
	Total:		**87**	**21**	**24,13%**
	Total amount:		**520**	**114**	**21,90%**

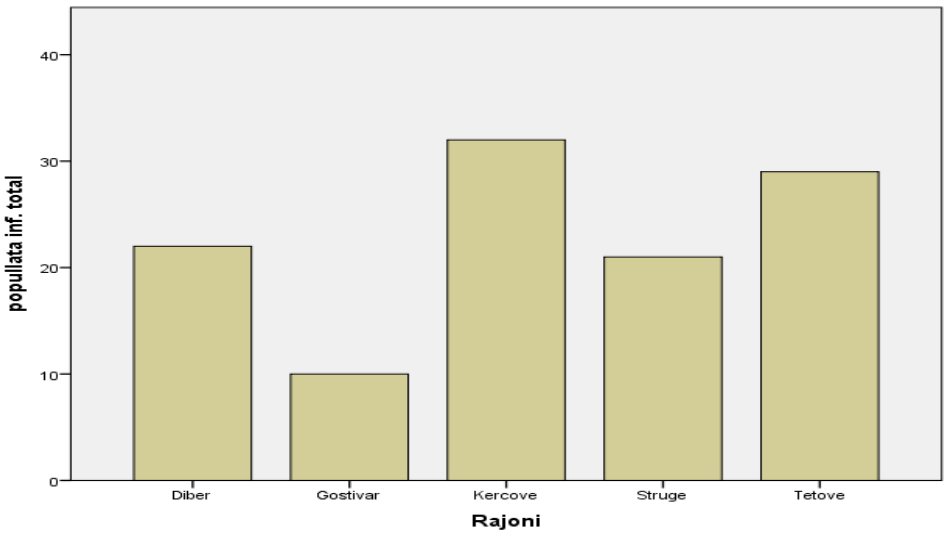


Figure 1. Dissemination of the frequency of infection to the human population

According to the data from Table 1 and Chart 1, where the numerical and relative frequencies are presented for the distribution of Q-affected women and men in the five regions of Western Macedonia (Tetovo, Gostivar, Debar, Kicevo and Struga), we conclude that the Kicevo region has a relatively higher frequency than the other regions, 43.2% of the 74 controlled entities.Positively resulted 32 people and the age group 21-40 is most affected by 57.10% relative frequency and less 0-20 year old age group with 14.20% relative frequency. Then comes the Dibra region with 34.37% relative frequency, where in contrast to the Kicevo region, the most affected here is the age group over 41 with 40.00% relative frequency and no case at the age group 0-20.This follows the region of Struga with 24.13% relative frequency and with the 21-40 age group affected by 35.70%, then Tetovo region with 18.7% and the most affected age group 41 and the last region with less affected by fever Q is the Gostivar region with 7.14% of cases and with the age group over 41 aged most affected. In general, 520 subjects were searched, where 114 subjects or 21.90% of cases were positively reported.

In a more detailed view we can conclude that the age group 0-20 in general for all regions is the least affected group by the Q fever. With simple mathematical calculations, although the table does not present this data, we gain: from a total of 142 controlled heads, 12 resulted positive with Q fever, or relative frequency of 8.45% of cases for the age group 0-20 years. But with the following statistical analysis we will verify this finding.

From Table 2, it is noticed that the standard deviation of the infected population in general is 8.53. Below we have a higher standard deviation of the female infected population than that of the male infected population, which means that the standard error in the calculation will be greater in the infected female population, whereas in the age groups of both genders the standard deviation ranges from 0.84 to 4.98.

Table 2. Descriptive statistics of the spreading of Q-fever

	Mean	Std. Deviation
Total inf. populaiton	22,80	8,526
Infected M	11,00	3,674
Infected F	11,80	5,119
F020 –Infected	1,40	2,608
F2040- Infected	2,80	2,588
F40- Infected	7,60	3,578
M020- Infected	1,00	1,732
M2040- Infected	2,20	,837
M40 –Infected	7,80	4,970

Conclusive statistic on the frequency of Q-fever in the human population

The rate of the frequency of the Q fever in the research regions should be analyzed with the binomial confidence test for each region separately. With the binomial test we obtain very low probability, almost zero for the regions of Tetovo, Gostivar and Struga and 0.018 ($p < 0.05$) for the Dibra region. So the probability for the theoretical frequency 50-50% to occur is very small in these regions. These four regions have a connection between them, which is not the case for the Kicevo region where the probability of occurrence of the theoretical frequency is 0.295 ($p > 0.05$), indicating that in 29% of the cases theoretical frequencies can occur. So this region is distinguished from the other four regions, according to the nature of the spread of the Q fever.

Below are also featured the confidence limits for 95% of the cases in the Tetovo region. The average of 81% of negative cases was obtained between the limits of 74% and 87% of the sample, while for the Kicevo region the average of 43% of positive cases was obtained between the limit of 31% and 35% of the sample.

Table 3. Testing of the hypothesis with the binomial test

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The categories defined by EQTE = Jo and Po occur with probabilities 0,5 and 0,5.	One-Sample Binomial Test	,000	Reject the null hypothesis.
2	The categories defined by EQGV = Jo and Po occur with probabilities 0,5 and 0,5.	One-Sample Binomial Test	,000	Reject the null hypothesis.
3	The categories defined by EQDI = Jo and Po occur with probabilities 0,5 and 0,5.	One-Sample Binomial Test	,018	Reject the null hypothesis.
4	The categories defined by EQKE = Po and Jo occur with probabilities 0,5 and 0,5.	One-Sample Binomial Test	,295	Retain the null hypothesis.
5	The categories defined by EQST = Po and Jo occur with probabilities 0,5 and 0,5.	One-Sample Binomial Test	,000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is ,05.

Table 4. Clopper -Pearson confidence coefficients

Confidence Interval Summary

Confidence Interval Type	Parameter	Estimate	95% Confidence Interval	
			Lower	Upper
One-Sample Binomial Success Rate (Clopper-Pearson)	Probability (EQTE=Jo).	,813	,742	,871
One-Sample Binomial Success Rate (Clopper-Pearson)	Probability (EQGV=Jo).	,929	,873	,965
One-Sample Binomial Success Rate (Clopper-Pearson)	Probability (EQDI=Jo).	,656	,527	,771
One-Sample Binomial Success Rate (Clopper-Pearson)	Probability (EQKE=Po).	,432	,318	,553
One-Sample Binomial Success Rate (Clopper-Pearson)	Probability (EQST=Po).	,241	,156	,345

Table 5. Chi-square coefficients

Eqnum			
	Observation N	Expetation N	Residual
Negative	406	260.0	146.0
Positive	114	260.0	-146.0
Total	520		

Test statistics	
	Eqnum
Chi-Square	163.969[a]
Df	1
Asymp. Sig.	.000

As in the final statistical analysis of animals (17,18), also in the human population, we will present Ho that has no link between regions for the diffusion of Q fever in the population. H1 has links between the regions, but let's look at the Hi-Square coefficient.

From the first table, the empirical and the theoretical frequency is seen, as well as the residual difference where empirical is 406 negative with Q-fever and 114 positive. While the expectation 260 with 260 cases and the Hi-Square coefficient is greater than zero.The important statistical indicator is 0,000, so the probability is less than 0.05, which is accepted that H1 has links between the regions during the spread of the Q fever in the population, although in the case of region to region analysis there is a difference between the region of Kicevo and the four other regions in the Hi-Square. In all regions, this distinction fades in favor of the other four regions.

Table 6. Statistical indicator of the hypothesis

Hypothesis Test Summary

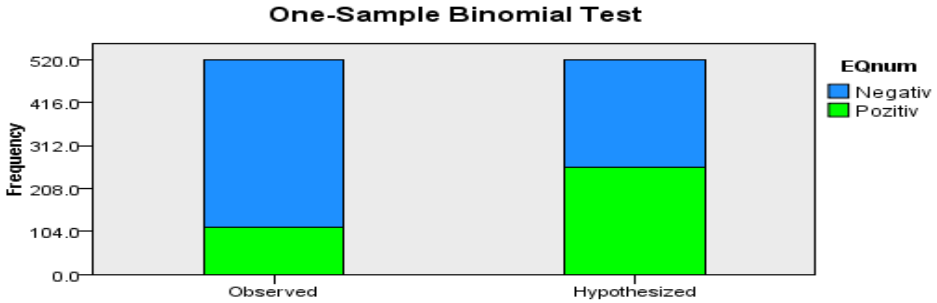
	Null Hypothesis	Test	Sig.	Decision
1	The categories defined by EQnum = Negativ and Pozitiv occur with probabilities 0.5 and 0.5.	One-Sample Binomial Test	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.

Table 7. The gained coefficients of the credibility of Clopper-Pearson

Confidence Interval Summary

Confidence Interval Type	Parameter	Estimate	95% Confidence Interval	
			Lower	Upper
One-Sample Binomial Success Rate (Clopper-Pearson)	Probability (EQnum=Negativ).	.781	.743	.816



Total N	520
Test Statistic	406.000
Standard Error	11.402
Standardized Test Statistic	12.761
Asymptotic Sig. (2-sided test)	.000

Figure 2. Comparison of our hypothesis with theoretical hypothesis

This finding is also verified by the binomial confidence test for 50-50% probability of the cases, where a 0,000 data is obtained, indicating that our empirical frequency distinguishes from the theoretical 50-50% with a statistically significant difference of 0,000. In the last column, the result of binomial analysis itself says H0 to be thrown and it can be automatically determined that there are links between the regions. Below we also gain the confidence limits with the Clopper-Pearson method. The 78% negative sample rate of 520 is gained within the median limits of 74% to 86% of the sample

From the above results, we conclude that there is a connection between the five regions (Tetovo, Gostivar, Debar, Kicevo and Struga) in the spread of Q fever in the human population, but with a special emphasis, the Kicevo region is distinguished from the other four regions in this connectivity and this phenomenon will be analyzed below with the difference of the differences of averages.

4. Conclusions

The statistical analysis of the data results in the conclusions:

-There is also a connection between the five regions when it comes to the distribution of the Q fever infection in people; however, the region of Kicevo is the most affected compared to the other four.

-Based on the analysis of mean difference, we can conclude that the regions can be divided into three sections depending on the prevalence of the Q fever infection: the Tetovo-Gostivar region is with low prevalence, the Debar-Struga region is with medium prevalence and the region of Kicevo is with high prevalence (based on the criteria obtained from the research results); however, this categorization can change with the increase in samples, due to the difference in the standard deviation for each region separately.

-The difference among mean values shows the distinction in the 40> age group as being the most affected by the Q fever infection, compared to the 20-40 and 0-20 age groups. If there is an increase in the number of examined patients, the difference between this age group and the latter two will also increase, because the standard deviation in calculations is lower in the 40> age group.

-There is a great similarity in terms of the distribution of the Q fever between sexes, which means that this kind of infection does not prefer one sex better than the other.

5. Recommendations

Based on the above-mentioned results, our recommendations are as follows:

Since Q fever is present in Western Macedonia and while the number of cases varies from year to year, veterinarians and physicians must be aware of the epidemiology of this disease. Probably the best approach to management of Q fever is to investigate outbreaks and apply appropriate control measures if necessary. Serological surveys of cattle, sheep and goats should be done periodically to monitor the endemic level of the presence of C. burnetii in a region as measured by seropositivity among the traditional reservoirs of this organism for man. Disease in humans is readily diagnosed as long as the manifestations of the disease and the provincial epidemiology of the disease are known to practicing physicians. Those at high (occupational)risk for this infection should also be aware of its signs and symptoms.

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RESEARCH ARTICLE (Open Access)

Sandy Dunes Vegetation in Narta

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Abstract

Extensive dunes occur on exposed coastline of Narta area from Vjosa mouth till Old Beach of Vlora. This region in particular has some of the largest dunes anywhere in Albania, with one of the largest expanses at " Zverneci hills ".

The sandy belt along the coastline is completely bare of vegetation to a length sometimes extending up to 30 m.

The Phanerogamic vegetation appears after this nudded belt, in a sandy belt already washed away by the considerable amounts of salt as a result of rain waters.

Pioneer species *Cakile maritima, Xanthium strumarium subsp. italicum, Salsola kali*, at the beginning isolated become more frequent when leaving the coastline. The vegetation of this sandy belt belongs to the pioneer association *Cakilo-Xanthietum italici.*

Gradually going away from the coastline and as the high of sandy dunes is increased, the physiognomy of vegetation is imparted by the species *Ammophila arenaria subs parundinaceae, Elymu sfarctus, Echinophora spinosa* etc. Ammophiletum association constitutes the last most evolved phase of the vegetation of sandy dunes or the borderline between dune vegetation and the Mediterranean pine forests. These forests are relatively going, cultivated (30-40y ago) recently in order to stabilize the sandy dunes and protect the agricultural lands.

The physiognomy of this formation is imparted by the species *Pinus maritima, P.pinea, P. pinaster* .The shrub layer is represented by typical Mediterranean species such as *Pistacia lentiscus, Erica manipuliflora, Myrtus communis*etc, characteristic species of the *Class Quercetea ilicis*. At the soda forest the shrub layer totally absents. The reason is a very high density of woody layer. These forests appear also very danger in many sectors (old beach of Vlora. Special interest in this formation present the endemic species such as *Orchis albanica Goelz&Reinhard* as well as a hybrid form *Orchis x paparisti*.

Keywords: Sandy dunes, Phanerogamy, Mediterranean pine forests, Endemic species.

1. Introduction

The studies mentioned above, even current data reflect, that the Narta area is a very important zone, especially by the presence of special flora and a diversity of habitats. The flora and vegetation of this area is very interesting. It is very rich in plant species. All these plant species make up a great national asset with economic and scientific values. Some of them are extremely rare, some others have scientific value and most of them make up widely used economic groups such as the medicinal, aromatic, industrial alimentary and decorative plants.

The aim of this study was to describe the basic types of vegetation in this area, and to show global importance of area of Narta and needs for their effective protection.

2. Material and Methods

The vegetation of the Narta area analyzed based on relevés follow the phytosociological method of Zurich-Montpellier school (Braun-Blanquet, 1932, 1964) This analysis conducted with the support of Flora of Albania (Paparisto et al.,1984-2000) and Flora Europaea (Tutin et al., 1964-1980). The nomenclature followed is Paparisto et al.,1984-2000. Number, size, distribution pattern of the stations and transects depend on the size and heterogeneity or diversity of habitats situated in the area of Narta, as well as on the bio-ecological

characteristics of the species or group of species. The GPS used to tell the exact location of stations or transects. The relevés were stored into the TURBOVEG database program (Hennekens&Schaminée 2001). The results of the classification were given in a vegetation table.

3. Results and Discussion

Our phytosociological research in the area Narta has recorded 10 associations. Sintaxonomical review is presented

1. CAKILETEA MARITIMAE Tüxen&Preising ex Br.-Bl. &Tüxen 1952

Euphorbietalia peplis Tx.ex Oberd. 1949

Euphorbion peplis Tx. ex Oberd. 1952

2. Cakilo - Xanthietum strumarii(Beg. 1941) Pign. 1958

Medicagini marinae-Ammophiletum australis Br.-Bl. 1921 corr. F. Prieto & T.E. Díaz 1991

3. ARTHROCNEMETEA Br.- Bl. et Tx. 1943 corr. Bol. 1957

Arthrocnemetalia fruticosi Br.-Bl. 1931 corr. Bol. 1957

Arthrocnemion fruticosi Br.-Bl. 1931 em. Riv. Mart. et al. 1980

4. Puccinellio festuciformis-Arthrocnemetum fruticosi (Br.-Bl. 1928) Géhu 1976(= Salicornietum fruticosaeBr.-Bl. 1928)

5. Limonietalia Br.-Bl. & O. Bolòs 1957

Limonion angustifolii Br.-Bl. (1933) 1934

Limonio-Artemisietum coerulescentis Horvatić (1933) 1934

6. JUNCETEA MARITIMI Br.-Bl. 1952 em. Beeft ink 1965

Juncetalia maritimi Br.-Bl. 1931 Juncion maritimi Br.-Bl. 1931

Juncetum maritimo-acuti Horvatic 1934

7. Populetalia albae Br.-Bl. ex Tchou 1948

Populiona lbae (Br. Bl. 1931) Tchou 1948

Populetum albae (Br. Bl. 1931) Tchou 1948

Table 1. Assoc *Cakilo-Xanthietum strumarii* Beguinot 1941, Pign. 1953

Numberofreveals	1	2	3	4	5	6	**Dominance**
Distance from the sea (m)	30	30	50	50	30	30	
Relevessurphace (m^2)	25	25	25	36	36	50	
Abundance (%)	25	15	20	20	25	25	
Abundance-Dominance	**A-D**	**A-D**	**A-D**	**A-D**	**A-D**	**A-D**	
Main sp of assoc.							
*Cakile maritima*Scop.	1.1	2.2	2.2	1.1	1.1	1.1	V
*Xanthium italicum*Mor.	2.2	2.2	1.1	1.1	1.1	1.1	V
Species of Cl. Cakiletea							
Salsola kali L.	1.1	1.1	1.1	-	1.1	1.1	V
*Euphorbia peplis*L.	1.1	-	2.2	1.1	-	1.1	IV
*Polygonum maritimum*L.	-	-	1.1	1.1	1.1	-	III
Species of Cl. Ammophiletea							
*Euphorbia paralias*L.	2.2	1.1	1.1	1.1	1.1	1.1	V
Elymus farctus(L.) P.Beauv.	-	-	1.1	2.2	1.1	1.1	IV
*Eryngium maritimum*L.	-	1.1	1.1	1.1	-	2.2	IV
*Echinophora spinosa*L.	1.1	1.1	1.1	-	1.1	2.2	IV
Convolvulus soldanella L.	1.1	1.1	-	-	1.1	+	IV
Sporoboluspungens(Schreber)Kunth	1.1	-	1.1	-	1.1	-	III
Others							
*Inulacrithmoides*L.	1.1	1.1	-	1.1	-	1.1	IV
*Lagurusovatus*L.	-	-	-	-	+	1.1	II
Cuscutasp	-	-	+	-	-	+	II

Table 2. Assoc*Cakilo-Xanthietumstrumarii* Beguinot 1941, Pign. 1953

Nr. of releves.	1	2	3	4	5	Prania
Releves surphace. (m2)	10	20	30	60	50	
Dominance. (%)	70	80	60	90	90	
Abundance-Dominance	A-D	A-D	A-D	A-D	A-D	
Main species.						
Arthrocnemum fruticosum (L.)Moq.	4.4	3.3	4.4	3.3	3.3	V
*Puccinellia festuciformis (*Host.) Parl	1.1	1.1	1.1	+	1.1	V
Sp. *Arthrocnemetea, Arthrocnemetalia and Arthrocnemion*						
Limonium vulgare Miller	1.1	1.1	1.1	+	1.1	V
Inula crithmoides L.	+	-	+	+	+	V
Halimione portulacoides (L.) Aellen	1.1	+	-	+	-	IV
Artemisia coerulescens L.	1.1	-	1.1	+	+	IV
Arthrocnemum glaucum (Delile) Ung.-Sterng.	+	+	1.1	-	+	IV
Sp. of Cl. Juncetea maritimi						
Juncus maritimus Lam.	+	+	-	+	1.1	IV
Juncus acutus L.	1.1	1.1	-	+	-	III
Suaeda maritima (L.) Dumort	-	+	+	+	-	III
Parapholis incurva L	-	+	+	-	-	II
Plantago crassifolia L.	+	-	-	+	-	II
Plantago coronopus L.	-	-	-	+	+	II
Salsola soda L	-	+	-	-	-	I
Sp. of Cl. Phragmito - Magnocaricetea						
Phragmites australis(Cav.) Trin &Stendel	-	+	+	-	+	III
Others						
Tamarix dalmatica Baum	+	+	+	+	1.1	V
Dittrichia viscosa L.	+	+	+	-	1.1	IV

Table 3. Assoc. *Limonio-Artemisietumcoerulescentis*Horvatic (1933) 1934

Nr. of releves.	1	2	3	4	5	6	Prania
Relevessurphace. (m2)	25	25	25	36	36	50	
Dominance (%)	25	15	20	20	25	25	
Abundance-Dominance	A-D	A-D	A-D	A-D	A-D	A-D	
Main species ofassosiacion							
Limonium vulgare Miller	4.4	4.4	5.5	3.3	5.5	5.5	V
*Artemisia coerulescens*L.	1.1	+	1.1	+	+	1.1	V
Sp. *Arthrocnemetea, Arthrocnemetalia*							
Limonium vulgare Miller	1.1	1.1	1.1	+	1.1	+	V
Inula crithmoides L.	+	-	+	+	+	1.1	V
Halimione portulacoides (L.) Aellen	1.1	+	-	+	-	1.1	IV
Artemisia coerulescens L.	1.1	-	1.1	+	+	+	IV
Arthrocnemum glaucum (Delile) Ung.-Sterng.	+	+	1.1	-	+	+	IV
Salicornia europaea L.	-	1.1	+	-	-	-	II
Suaeda maritima (L.)Dumort	+	-	-	+	-	-	II
Astertripolium L.	-	+	-	+	-	-	II
Salsola soda L	-	+	-	-	-		I
Sp.of Cl. Juncetea maritimi							
*Juncus maritimus*Lam.	+	+	-	+	1.1	-	III
Juncus acutus L.	1.1	1.1	-	+	-	+	III
Parapholis incurva L	-	+	+	-	-	+	III
Plantago crassifolia Forscal.	+	-	-	+	-	-	II
Plantago coronopus L.	-	-	-	+	+	-	II
Tamarix hampeana Boiss & Heldr	+	-	-	-	-	+	II
Sp. of Cl. Phragmito - Magnocaricetea							
Phragmites australis(Cav.) Trin. &Stendel	+	+	+	-	+	1.1	V
Scirpus maritimus L.	+	+	+	+	1.1	-	V
Tamarix dalmatica Baum	-	+	+	+	-	+	IV
Other							

Dittrichia viscosa L.	+	+	+	-	1.1		IV

Table 4. Associacion *Juncetummaritimo-acuti* Horvatič 1934

Nr. of releves.	1	2	3	4	5	6	7	**Dominance**
Releves surphace. (m2)	50	50	50	50	50	50	50	
Dominance. (%)	50	50	50	60	40	50	50	
Abundance-Dominance	A-D	A-D	A-D	A-D	A-D	A-D	A-D	
Main species ofasoc.								
Juncus acutus L.	2.2	2.2	1.1	3.3	1.1	2.2	2.2	V
Juncus maritimus Lam.	1.1	1.1	3.3	1.1	2.2	1.1	1.1	V
Sp. of Cl. Junceteamaritimi								
Scirpus holoschoenus L.	1.1	-	+	+	1.1	+	+	V
Tamarix dalmatica Baum	1.1	+	+	+	+	+	-	V
Plantago crassifolia Forscal	+	-	+	+	+	-	-	III
Plantago coronopus L.	-	+	+	-	+	-	+	III
Parapholis incurva(L.) C.E.Hubb.	+	+	-	+	+	-	-	III
Agropyron pungens (Pers.) R. & S.	-	+	+	-	-	+	+	III
Blackstonia perfoliata (L.) Huds	+	+		+	-	-	-	II
Limonium oleifolium Miller	-	+	+	-	-	-	+	II
Cynanchum acutum L.	+	-	+	-	+	-	-	II
Tamarix hampeana Boiss&Heldr	+	+	-	-	-	-	+	II
Juncus subulatus Forschal	-	+	-	+	-	+	-	II
Polypogon monspeliensis L.Desf	-	-	+	-	-	-	+	II
Aster tripolium L.	-	-	-	-	-	+	+	II
Aeluropus littoralis (Gouan)Parl.		+	-	-	-	+	-	II
Elymus pycnanthus Godron	+	-	-	-	+	-	-	II
Linum maritimum L.	-	-	-	+	-	-	-	I
Triglochin bulbosa L	-	-	-	-	-	-	+	I
Sp. of Cl. Arthrocnemetea								
Inula crithmoides L.	-	1.1	-	+	+	+	1.1	IV
Arthrocnemum fruticosum Moq	1.1	-	+	+	-	1.1	+	IV
Limonium vulgare Miller	1.1	-	+	+	+	-	+	IV
Artemisia coerulescens L.	+	-	+	+	+	-	-	III
Puccinellia festuciformis (Host.)Parl	+	+	+	-	-	+	+	III
Halimione portulacoides (L.) Aellen	-	-	+	+	-	+	+	III
Suaeda maritima (L.) Dumort	+	+	-	-	-	-	+	II
Arthrocnemum perenne Millex.Moss	-	-	+	-	-	-	+	II
Arthrocnemum glaucum Delile	-	-	+	-	-	-	+	II
Sp. of Order. Phragmitetalia								
*Phragmitesaustralis (*Cav.) Trin. &Stendel	1.1	1.1	1.1	+	-	1.1	+	V
Scirpus maritimus L.	1.1	-	+	+	-	1.1	+	IV
Equisetum ramosissimum L.	+	-	+	+	+	1.1	-	IV
Scirpus lacustris L.	1.1	-	+	+	+	-	+	IV
Chenopodium album L.	-	-	+	+	-	+	+	III
Foeniculum vulgare Miller	-	-	+	-	-	-	+	II
Piptatherum miliaceum(L.) Cosson	+	-	-	-	+	-	-	II
Sorghum halepense(L.) Pers	-	+	-	-	+	-	-	II
Phalaris arundinacea L.	-	-	-	+	-	+	-	II
Typha angustifolia L.	-	-	-	-	-	+	+	II
Others								
Dittrichia viscosa L.	1.1	-	-	+	-	1.1	+	III
Cynodon dactylon L.	+	+	+	-	-	-	+	III
Melilotus alba Medic.	-	+	-	+	+	+	-	III
*Vitexagnuscastus*L.	-	-	-	-	+	+	-	II
Daucus gutattus L.	+	-	-	-	-	-	+	II
Xanthium strumarium L.	-	-	+	+	-	-	-	II
Hordeum murinum L.	-	+	+	-	-	-	-	II

Potentilla reptans L.	-	-	-	+	+	-	-	II
Aster squamatus L.	+	-	-	-	-	+	-	II
Plantago lanceolata L.	-	-	-	-	-	+	+	II
Ononis variegata L.	-	-	+	+	-	-	-	II
Trifolium pratense L.	-	-	+	+	-	-	-	II
Calystegia sepium (L.) R.. Br	+	-	-	-	-	-	-	I
Arundo donax L.	-	-	+	-	-	-	-	I
Samolus valerandi L.	-	+	-	-	-	-	-	I
Lythrum salicaria L.	-	-	-	+	-	-	-	I
*Lotust enuis*W.&K.	+	-	-	-	-	-	-	I

8. FraxinionangustifoliaePedrotti 1970

Alnoglutinosae–Fraxinetumoxycarpae (Br. Bl. 1915) Tchou 1946.

9. QUERCETEA ILICIS Br. Bl. 1947

Pistacio lentisci – Rhamnetalia alaterni Rivas – Martinez 1975

Juniperion turbinatae Rivas – Martinez 1975 corr. 1987

Pistacio lentisci-Juniperetum macrocarpae Caneva, De Marco e Mossa (1981)

Quercionilicis (Br. Bl. 1936) Riv. Martinez 1975

10. Pinetumhalepensis-pineae prov.

Pinus maritima, P.pinea, P. pinaster

The presence of a dune system is result of factors, which determine the morphology of a sandy coast: abundant detritus depositing of fluvial or marine origin and presence of strong dominant winds. Moreover, the vegetation present in the area have to be considered as determining factor, since it has, due to its radical apparatus, a fundamental role in the consolidation and in the growth of the dune's height.

Perennial plants as a biological type dominate in floristic complexes, though in some cases (mainly on the beaches) the dominants are pioneer annual plants (*Cakile maritima, Salsola kali or Euphorbia peplis*). Analyzing the transversal profile of a dune (Uslu&Géhu 1990), starting from the shoreline, where the waves break, and continuing towards the inner part of the shore, one can observe a sequence of vegetation clusters which determine various habitats and various stages of growth of the dune's sandbar. In accordance with the specified definition of psammophytic vegetation succession dynamics and stages of dune formation, the following parts of the dune complexes have been evidenced: The higher parts of the beaches with pioneer vegetation Embryonic dunes Shifting dunes Pioneer vegetation on the higher parts of the beaches. The first vegetation clusters, found along the shore, find location at a distance from the sea, which safeguards them from the action of the wave-motion, and where sea storms may reach them only in rare cases. This association represents the first stages of development of littoral psammophytic vegetation in the higher beach places. Despite the poor floristic composition, the total abundance of the species is often very low. Pioneer plants are so called because they are the first plants capable of colonizing this type of hostile environment. The hostility is caused by strong thermal *Pinetum Pistacio lentisci – Juniperetum macrocarpae Medicagini marina – Ammophiletum australis, Euphorbio paraliae, Agropyretum Eryngio-Sporoboletum virginici, Cakilo – Xanthietum strumarii*.

Stabile dunes with, *Juniperusmacrocarpa* and *Pinuspinaster, P. halepensis, P. pinea*. White mediterranean dunes with *Amophylaarenaria* changes, poorness of water and finally, because of the variable saline content. The most representative plants among them are *Cakile maritima, Salsola kali, Inula crithmoides* and *Xanthium strumarium.*

Embryonic dunes.

Gradually going away from the coast line and as the height of sandy dunes is increased, the physiognomy of vegetation is imparted by the species *Eryngium maritimum, Euphorbia paralias, Echinophora spinosa, Elymus farctus, Cyperus capitatus, Sporobolu spungens*, that pertain to a more evolved phase of psammophytic

vegetation and from the beaches to the embryonic dunes. This type of vegetation represents a stable “potential” of the sandy banks. The discussed vegetation, in most of the cases, is under human impact determined by the developing tourism, intensive usage and intensive erosion. An obvious result of human impact is the expansive distribution of *Xanthium strumarium subsp. italicum* in the highest beach places and the embryonic dunes. The species was introduced from America and is perfectly adapted in these areas. Shifting dune vegetation. The increase of dune height is accompanied as well with the gradual change of the physiognomy of this vegetation. The highest dunes are colonized by the big tufts of *Ammophila arenaria* which grow especially on the crest of the dunes. This species is the real builder of the dunes. The presence of this species is an important factor in impeding the movement of sand quantities pushed away by the sea winds towards the continent. From this type of vegetation there are noticed two evaluative lines: Retro dune or degradation of sandy dunes and the formation of depressions. The end of depressions is closer to the level of salted ground waters. The ground becomes wetter and different vegetation grows from that of dunes, dominated by *Erianthus ravennae, Scirpus holoschoenus, Schoenus nigricans and Plantago coronopus.*

Mediterranean pine forest: These forests occupy a considerable part of the area distributed mainly on sandy dunes. In generally, they represent relatively young forests, cultivated recently in order to stabilize the sandy dunes and protect the agricultural lands. The physiognomy of this formation is imparted by the species *Pinus halepensis* and *Pinus pinea*. The shrubby layer is represented by typical Mediterranean species. The most spread shrubs in this formation are *Myrtus communis, Juniperus oxycedrus subsp. macrocarpa, Erica manipuliflora* and *Pistacia lentiscus*.. These forests constitute the last most evolved phase of the vegetation of sandy dunes.

On the Narta area, salt marshes are one of the most prevalent habitats around the coastline of Narta Lagoon. Plant species diversity is low, since the flora must be tolerant of salt and anoxic mud substrate. The most common salt marsh plants in Narta area is glasswort (*Salicornia europaea*), which have worldwide distribution. Glasswort is often the first plants to take hold in a mudflat and begin it's ecological succession into a salt marsh. Their shoots lift the main flow of the tide above the mud surface while their roots spread into the substrate and stabilize the sticky mud and carry oxygen into it so that other plants can establish themselves as well. Plants such as sea lavender (*Limonium vulgare*), Spiny rush (*Juncus acutus*) and Sea rush (*Juncus maritimus*) grow once the mud has been vegetated by the pioneer species. The *Salicornia europaea* (pioneer marsh communities) takes place in the space of just a few months between summer and early autumn. Following this layer is a wetland of sea-lavenders (*Limonium vulgare*), saltmarsh-grass (*Puccinellia festuciformis*), perennial glasswort (*Arthrocnemum fruticosum*) and *Halimione portulacoides*. These plants are tolerant of being covered by salt water for long periods. The development of the lower marsh communities is marked by the increasing diversity which follows the arrival of a range of new species. The next stage is the development of the plant communities dominated by Spiny rush (*Juncus acutus*) and Sea rush (*Juncus maritimus*) that cover a large surface in this area. In lagoon stretches enjoying similar conditions, a dense population of sea club-rush (*Bolboschoenus maritimus*) settles instead of the plant communities dominated by Spiny rush.

The development of the salt marshes in terms of plant species and communities is also accompanied by developments in the soil structure and micro-flora. These developments involve the establishment of populations of bacteria and fungi which are involved in biogeochemical processes controlling the breakdown of organic matter and the cycling of plant nutrients. Fresh waters vegetation.

The riparian forests, or alluvial forests, generally are those wooded areas suited to moist soils that cover both the river banks and the areas which are periodically submerged by flooding.

These forests occupy a considerable part of the Narta area. The dominant species of this wood are: the bay-oak (*Quercus robur*), common alder (*Alnus glutinosa*), ash (*Fraxinus angustifolia*), the white poplar (*Populus alba*), the elm (*Ulmus minor*), white willow (*Salix alba*) and the privet (*Ligustrum vulgare*). The area of riparian forests (typical for the region) is declining. The other riparian forests dominated by species such as *Alnu sglutinosa, Fraxinus angustifolia, Quercus ilex* and *Populus alba* can be found fragmentally. The relatively modest density of the vegetation is due to human pressure exerted on the area; building embankments, and poplar fields have greatly changed the original landscape.

This riparian forests includes several types:

1.*Riparian mixed forests.* - Mixed forests of *Quercus robur, Ulmus minor, Alnus glutinosa, Fraxinusangustifolia* are most distributed. The soil may be well drained between inundations or remain wet liable to flooding during regular rising of water level. The undergrowth is well developed.

2. *Riparian common alder forests*. – *Alnus glutinosa* type of riparian forest which require constant soil moisture throughout the year. Tree layer is dominated by *Alnusglutinosa* and rarely accompanied by *Populusnigra, Salix alba.*

3. *Riparian willow formations*. - The various species of willows, especially white willow (*Salix alba*).

- Another interesting association developed with high vitality in humidity and inundate environments in this belt is the one with White Poplar (*Populus alba*). Forest physiognomy is determined by White Poplar. In low humidity environment White Poplar are presented by weak development and very oft en cannot upper the shrubs level.

Formations of *Tamarix spp*., including *Tamarix dalmatica, Tamarix hampeana*, wet areas of fresh water and saline habitats of the Narta area. The relatively modest density of the vegetation is due to human pressure exerted on the area. All types occur on heavy soils (generally rich in alluvial deposits) periodically inundated by the annual rise of the river level, but otherwise well-drained and aerated during low-water.

4. Recomandations

The relations between these various types of habitats is of great ecological importance.

The importance of this area is illustrated by the fact that many of the threatened plant species in the Red Book of Albania occur in this area.

In general, the natural ecosystem of Narta can be seen as an essential part of Europe's natural heritage.

The most important environmental problems along the Narta area include:

- Development of tourism.

Today, the scale of this process is most extensive in Old beach of Vlora. Habitat loss is probably the most important effect. A number of coastal plant species are now believed to have become extinct due to total destruction of their habitats, especially sand beaches and dune areas

- Urban development.

Urbanization is a major space consuming process, still continuing in Soda forest.

- Intensification of traditional use.

Ecological values can be affected seriously by stronger forms of intensification on fishery and water culture, hunting, agriculture, production of salt, etc.

- Reclamation of land and cultivation.

In the past, reclamation was carried out on a large scale in this area.

As elsewhere in coastal region of Albania even in Narta area, forestation with maritime pines (*Pinus maritima, Pinu spinaster* and *Pinus pinea*), has had a major impact on much of the dune landscape throughout the area.

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RESEARCH ARTICLE (Open Access)

Evaluation of Phosphorus in Cola Drinks in Albanian Market

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Abstract

Phosphates are inorganic compounds, based on the element phosphorus (P), and combined with oxygen to form phosphates (PO_4), the form in which phosphorus is present in nature. Phosphate occurs naturally in the form of organic esters in many kinds of food, like meat, potatoes, bread, and milk. In the body, they are broken down to simple phosphate ions (PO_4), the basic building block of the many different biological molecules which include phosphorus. Inorganic phosphat is commonly used as an additive in industrial food production for a range of purposes including maintaining natural colors and flavors, acidity buffering, leavening, stabilization of texture, shelf-life quality.

High levels of phosphorus in the blood, has been associated with adverse health effects like organ damage, most notably of the kidneys, lower calcium levels which lead to brittle bone diseases,vascular calcification and cardiovascular diseases. Because of the potential damage to health from excessive phosphate consumption, a labeling requirement is advised for foods with added phosphate. The aim of our study was the evaluation of phosphate concentration in cola drinks in Albanian market and compliances with the current legal limits regarding this additive set by the Europian regulations. Phosphate concentration of selected drinks were evaluated using an UV spectrophotometric procedure. The results obtained indicated that phosphate concentration in cola drinks ranged from 887 mg/L to 1,800 mg/L. European regulations allow up to 700 mg/L of phosphate in cola drinks, a quantity that provide already 50% to 75% of the recommended daily intakeof phosphate for adults.

Keywords:inorganic phosphor, UV/Visible Spectrophotometer, Soft drinks, EU Regulations

Introduction

Phosphorus (as phosphate) is a natural constituent of human, animals and plants and is present in all biological materials. Phosphorus is widely present in food, largely as the phosphate ion. Phosphorus is an essential mineral and plays an important rolein the metabolism of carbohydrate, fat, protein and in bone remodeling.Even though they are naturally present in nearly all foods, protein-rich foods are especially high in phosphorus; these include dairy products (100–900 mg/100 g), meats (200 mg/100 g), fish (200 mg/100 g) and grain products (100–300 mg/100 g)[1].High levels of phosphorus in the blood, has been associated with adverse health effects like organ damage, most notably of the kidneys, lower calcium levels which lead to brittle bone diseases, vascular calcification and cardiovascular diseases[2]. In the light of the potential harmful effects associated with excessive phosphate consumption the average intake from foods in adults is estimated to be between 1,000 and 2,000 mg/day [3].The total phosphorus daily intake is depended also by contribution of added food phosphates. Phosphoric acid and phosphates (E 338-452) are food additives authorized[4]for a range of purposes[5] including maintaining natural colors and flavors, acidity buffering, leavening, stabilization of texture, shelf-life quality.The contribution of added food phosphates to total phosphorus intake is in low levels, usually 20-30% of dietary intake of phosphorus from natural foods, but latest evidence from studies showing a possible association between elevated serum phosphate concentration and an increased risk of cardiovascular disease (CVD)[6,7], call for a labelling requirement to be introduced for foods with added phosphates[8]

Food source is the main factor for phosphorus bioavailability. In plants, phosphorus is largely

present in the form of phytate, a form which is not bioavailable in humans because they lack the enzyme phytase, which is necessary to release phosphorus. Phosphorus in meat is typically found as intracellular organic compounds which can be easily hydrolyzedto release inorganic phosphate [9]. The phosphate as food additive is in form of inorganic salts. PTH (parathyroid hormone), and 1,25-dihydorxyvitamin D are the two hormones which in daily cycle regulate the body fluid phosphate concentration.

Must be evidenced that the dietary intake, absorption in intestines, exchanges with bone and intracellular compartments and renal excretion, play a vital role in the homeostasis of phosphorus. Skeleton and teeth bound, which contain phosphorus in solid form and counts for nearly 80% of total found in the body, and the other part is found in extracellular fluid and soft tissues. In general, the values ranging from 3.0 to 4.5 mg/dl (expressed as elemental phosphorus), are regarded to be common for serum phosphate concentration, and may change throughout the day succeeding a circadian rhythm, where the lowest values is recorded in the morning and the highest at night[9]. Although, scientists haven't found conclusive relationf regulatory hormones, cellular flux and renal excretion in the concentration of serum phosphorus due to complex interaction, it is known that the amount of dietary phosphorus intake doesn't influence it directly.

Nowadays, the dynamic way of living has increased the consumption of processed and ready-to-eat foods, increasing the concern that the phosphorous intake may be much higher and the use of phosphates as food additive may lead to an elevated phosphate serum concentration. Cola beverages are among the ready to drink products which contain phosphoric acid as food additive, and is one of the most favorable consumed drinks among teenagers and students[10–12].The aim of our study was the evaluation of phosphate concentration in cola drinks in Albanian market and compliances with the current legal limits regarding this additive set by the Europian regulations.

Material and Methods

Sample preparation

Cola drinks analyzed were taken in different Albanian market and were local and international brands. The samples were decarbonated prior of analyzing by vigorous stirring in room temperature for several hours in order to release all carbon dioxide present in drinks.

Instruments

Biochrom Libra S22 UV/Vis Spectrophotometer was used for the spectrophotometric measurement. The pH meter SI Analytic lab 845 was used for pH measurement of cola drinks.

Chemical estimation of phosphorus

The content of phosphorus was determined according to the method proposed by Lozano-Calero[13].The method consists of the formation of a complex of phosphate ion with a molybdate compound in acid solution. The colorless hexavalent molybdenum phosphate complex is reduced to a blue pentavalent form by ascorbic acid in acid medium. The intensity of the blue color is proportional to theamount of phosphate and is measured spectrophotometrically at 830 nm.

Results and Discussion

In Figure 1 is given the calibration curve of phosphor, which was prepared by using standard solutions of phosphorin the concentration range of 0-1.8 ppm and absorbance was measured on a UV/Vis spectrometer at wavelength 830 nm, as shown in Table1. Moreover, the corresponding equation and the correlation coefficient are given.

Figure 2 shows the absorption spectra of phosphor standard solution and all the samples. As it can be seen by the graph the wavelength of maximum absorption is at 830 nm. At this wavelength the system obeys Lambert–Beer's law in the concentration range of 0.5–5.0 μg/mL of

phosphate[14]. The phosphor content and pH of cola drinks are given in Tab.2.

Table 1 Phosphorous content of standard solution and the absorbance measurements

Phosphor P_2O_5 Content (ppm)	Absorbance at 830nm
0.0	0.000
0.6	0.034
1.0	0.056
1.4	0.081
1.8	0.107

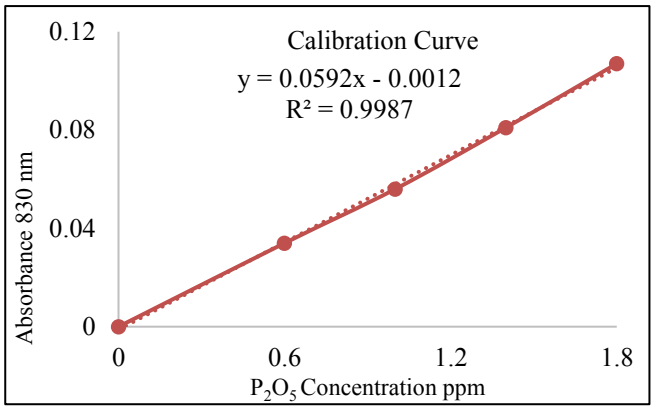

Figure 1 The calibration curve of phosphorous standard solution

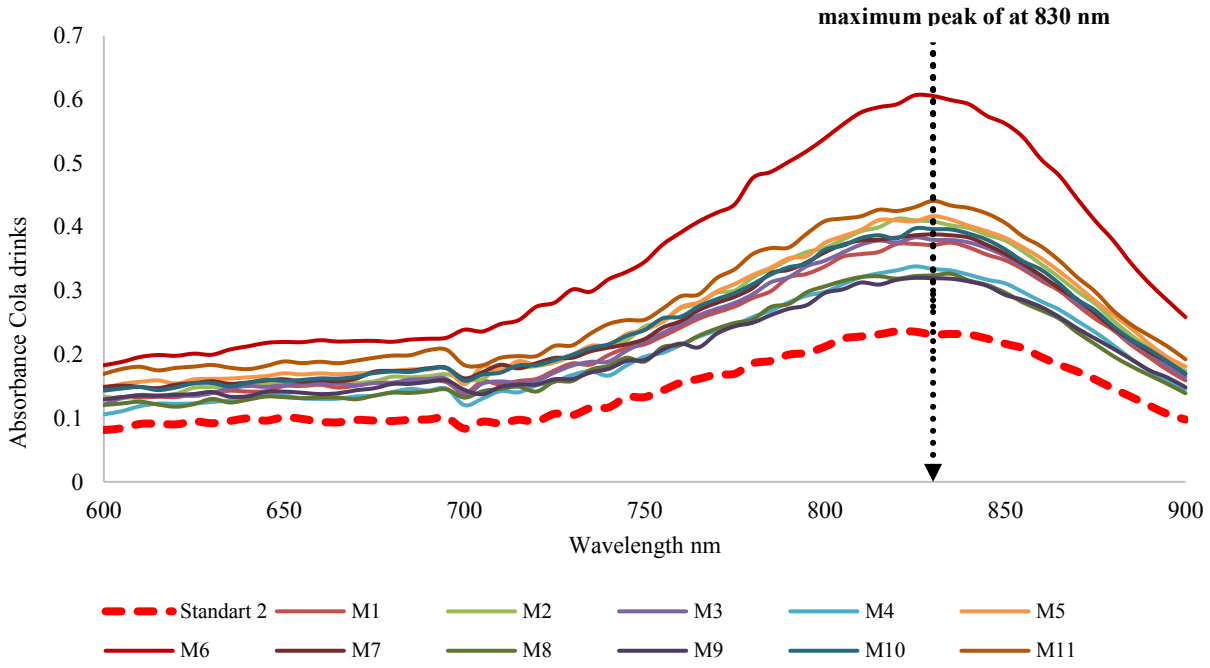

Figure 2 Absorption spectra of phosphorous standard solution and of all the samples.

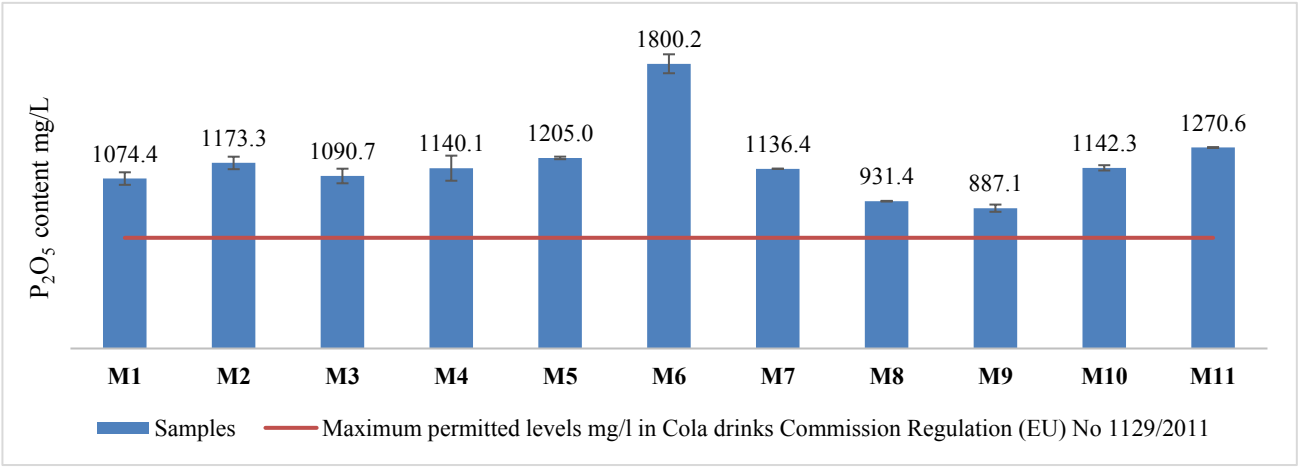

Figure 3 Phosphorus content in Cola drinks in Albanian market in comparison with MPL for this additive in EU

Table 2 Phosphorous content in Cola drinks

Sample	Absorbance830 nm	Phosphor content (P_2O_5) mg/L	pH
M1	0.363	1074.4±39.60	3.14
M2	0.397	1173.3±39.50	2.90
M3	0.369	1090.7±46.10	2.89
M4	0.385	1140.1±79.30	3.17
M5	0.407	1205.0±8.12	3.86
M6	0.609	1800.2±59.90	3.15
M7	0.384	1136.4±0.00	3.10
M8	0.315	931.4±2.08	3.25
M9	0.300	887.1±22.90	2.80
M10	0.386	1142.3±16.68	2.76
M11	0.430	1270.6±2.08	2.90

The higher phosphor content was found in sample M6, respectively 1800 mg/L and the lowest content was found in sample M9 (887mg/L). Phosphoric acid (E 338) is the only inorganic acid commonly used to give a specific taste profile to cola type drinks and has a strong effect on pH. The pH in our samples varied from 2.76 (M10) to 3.86 (M5) and no significant correlation was found between the low pH and high content of phosphor. A pH below 5.5 influence the health of our teeth as the acid begins to dissolve the hard enamel. Our bodies' ability to maintain a one-to-one balance between calcium and phosphorus in our systems it's the main factor for a good health. Soft drink consumption take away our bodies' calcium, leading to soft teeth. Excess amount of phosphoric acid in blood mean an extra work for kidneys, which are less able to excrete it. Soft drinks remove calcium from the body, and deposited in kidney, resulting in chronic kidney disease. An association between elevated serum phosphate levels and increase risk of cardiovascular disease in patients with chronic kidney disease has long been known[15,16].

Figure 3 shows the phosphor content in all samples compared to maximum permitted level (MPL) set by Commission Regulation (EU) No 1129/2011. The maximum permitted level for flavored drinks, a group which include cola drinks, is 700 mg/Lexpressed as P_2O_5[1],a quantity that provide already 50% to 75% of the recommended daily intake of phosphate for adults. Chart shows very clear that the samples are above the MPL set by EU for this additive in a range of 26.7% to 157% higher.

Conclusions

Data obtained from our study, shown that the content of phosphor in cola drinks in Albanian market is higher than the maximum limit authorized from the international authority of drug regulation. The exceed amount is higher at significant levels, respectively 26.7% to 157 %. Taking into consideration health problems arising from high phosphor daily intake, we suggest to the manufacturerto be transparent in the declaration of phosphor content in the label of the product in way to help consumer to makeinformed decisions about the purchase of products. The most important suggestion to the manufacturer should be: they always should produce in compliance withnational and international regulations and standards of food safety. Determination of phosphor was carried out by means of spectrophotometer UV-VIS as cheapest, fast, and excellent accuracy.

[1] **Food Additives Database in EU:**https://webgate.ec.europa.eu/foods_system/main/index.cfm?event=substance.view&identifier=128

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RESEARCH ARTICLE (Open Access)

Evaluation of Caffeine in Soft and Energy Drinks by Means of UV/Visible Spectrophotometer

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Abstract

The UV-VIS spectrometry is a method frequently used for routine caffeine determination in beverages. Caffeine absorb at UV-VIS region with maximum absorption band at 271 nm. In the present work we undertook various experiments to determine the pH and levels of caffeine concentration in ten soft drinks and five energy drinks available in local market in Albania. pH levels were measured by pH meter. Calibration solutions were prepared in the concentration range of 1-25 ppm from a 100 ppm stock solution. Concentration of caffeine in drinks was performed by a simple and fast standard UV spectrophotometric method at 271 nm. The minimum caffeine content of soft drinks was observed in *Brand-5* (24.55 mg/L), while the highest concentration of caffeine was observed in *Brand-9* (79.46 mg/L). Unlike the soft drinks, the concentration of caffeine in energy drinks were slightly higher with minimum amount at *Brand-4* (62 mg/L) and maximum at *Brand-5* (152 mg/L). The pH range of soft drinks were (2.51 to 3.34) and in energy drinks (2.33 to 3.49)

Keywords*:* Caffeine, UV/Visible Spectrophotometer, Soft and energy drinks

1. Introduction

1,3,7-trimethylxanthine widely known as Caffeine is a naturally alkaloid usually found in coffee beans and tea leaves or fruits [1]. Around sixty plant species contain caffeine [2]. Coffee plant is one of the common sources of caffeine, other sources of caffeine are found in the leaves of the tea, kola drinks, energy drinks, chocolates, cocoa beans, yaupon holly leaves, to quote only few [3]. For the first time, caffeine was isolated in laboratory as pure compound by German chemist Ferdinand Runge in 1819 [4]. It is a white odorless organic compound, powder crystalline and has bitter taste and react as stimulant drug in human body [5]. Mentioning some physical properties, the density of caffeine is 1.2 g/cm³, melting point of caffeine is 237 °C and the boiling point is 178 °C. Regarding solubility, is very low in water, moderate in ethyl acetate, pyrimidine, pyrrole, acetone, and very high in petroleum ether, ether, benzene and chloroform [6]. While coffee and tea beverages naturally contain caffeine and other derivatives, caffeine is found in varying quantities as an ingredient in many carbonated soft drinks including colas, Pepsi beverages, and in energy drinks its content varies from 10 to 50 mg of caffeine per serving. Caffeine is added as flavoring agent and is the most common additive found in soft and energy drinks in way to make them more addictive [7]. Caffeine has taken special attention in the past decades concerning its physiological and stimulatory effect. Caffeine as alkaloid directly affect at central nervous system as stimulant. It is worth to mention that the Food and Drug Administration (FDA) describe caffeine as a generally recognized as safe (GRAS) substance. Notwithstanding, FDA specifies that the maximum quantity in soft and energy drinks is limited to 0.02% (FDA 2006) [8]. As matter of fact, the highest legal amount of caffeine allowed in a 350 mL can of various drink is about 71 mg [8][a,b]. Regarding EFSA (European Food Safety Authority), Caffeine should not exceeded daily consumption for the adults up to 400 mg, 200 mg for pregnant women and less than 200 mg for children (however, is depended form their weight) [8][c]. Caffeine has drowned special interest of consumers and health professionals thanks to its wide consumption in the diet by a large percentage of the

population and its pharmacological effects in humans [9]. In this context, the physiological effects on human body systems have been stated by different researchers, including the central nervous, respiratory, gastrointestinal, cardiovascular, and renal systems, mentioning some of them [10]. Amongst several agencies, International Olympic Committee (IOC) has classified caffeine as a drug and when caffeine concentrations are found to be higher than 12 μg/mL in urine, is considered as abuse and punishments have been taken [11]. More in detail, considerable studies have proven caffeine to be a stimulant to human's central nervous system [12]. Also, it increases heart beat rate, dilate blood vessels and elevate levels of free fatty acids and glucose in plasma. In light of this, when amount of caffeine consumption is higher than amount allowed by FDA/EFSA, causes numerous body irregularity such as insomnia, nervousness, nausea, ear ringing, derillum and tremulosness. It is highly important avoiding overdose because in combination with alcohol, and some other drugs, these compounds generate a toxic effect and sometimes, even though in small occasion, lethal outcome [13,14]. In addition to, caffeine facilitates the conduction velocity in the heart which is responsible for the contractility of the heart and blood vessels. However, caffeine may significantly reduce cerebral blood flow by constricting of cerebral blood vessels. Although many negative consequences, when caffeine is consumed at acceptable level, provides a diuretic effect due to elevating the blood flow and glomerular filtration rate of the kidneys. Stomach problems like heart burn is an issue for some subjects' gastrointestinal system after consuming caffeine. Problems are reported for skeletal muscles and are predominantly increasing tremors incident [12,14]. Several methods exist for determining the caffeine content in tea, coffee, and beverages which are reported in literature. Including UV-Visible spectrophotometry, potentiometry, high performance liquid chromatography (HPLC), ion chromatography, high performance thin layer chromatography (HPTLC), capillary electrophoresis, micellar capillary electrophoresis, gas chromatography, and solid-phase microextraction gas chromatography [15 - 22]. The UV-VIS is a tool frequently used for routine caffeine determination in beverages for its effectiveness and is cheap compared with other techniques mentioned above. Caffeine absorb at UV-VIS region with maximum absorption band at 271 nm [23]. Accordingly, the aim of this work was determination of caffeine quantity in numerous soft and energy drinks available in Albanian market due to increasing consumption of above mentioned drinks in our daily life and then observing whether the concentration is at acceptable level allowed by FDA. In light of this we determined the amount of caffeine in ten different brands of soft drinks and five energy drinks by means of UV/VIS Spectrophotometer.

2. Material and methods

2.1 Chemicals and instrument

All glassware were washed with distilled water and then dried overnight in oven. Soft and energy drink were taken in different Albanian supermarket. The caffeine pure powder was provided from Sigma Aldrich. Biochrom Libra S22 UV/Vis Spectrophotometer was scanned from 10 – 400 nm. The pH meter SI Analytic lab 845 was used for pH measurement of drinks.

2.2 Standard solution preparation

The stock solution of Caffeine was prepared by dissolving 10 mg of pure caffeine in 1000 ml of distilled water to obtain 10 ppm caffeine solution. Standard solution was prepared by pipetting 0; 1; 1,5; 2 ml stock solution into 10 ml volumetric flask and then filled with distilled water up to the mark. The absorbance of each standard solution was measured at absorption maximum of 270 nm three time for each solution using 10 mm quartz cuvettes.

2.3 Sample preparation

All soft and energy drinks were decarbonated prior of using for absorbance measurement. More in detail, all sample were left under vigorous stirring in room temperature for several hours in order to release all carbon dioxide present in drinks. A beverage portion was drawn by pipette and filter off, then 1 ml of filtered sample was placed in 10 ml flask and was diluted with water. This procedure was repeated for all soft and energy drinks samples. The absorbance was measured three times for each sample at 270 nm in quartz cuvettes. Note of worth, the pH of each sample was measured after decarbonated.

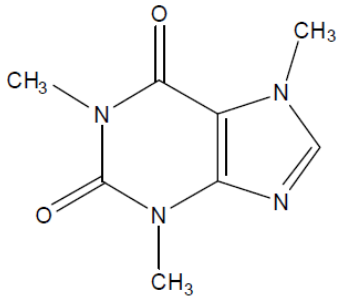

Figure 1. Chemical formula of caffeine

3. Results and Discussion

In fig 2 is presented absorption spectra of caffeine standard solution and the spectra of one sample from energy drinks and one from soft drink

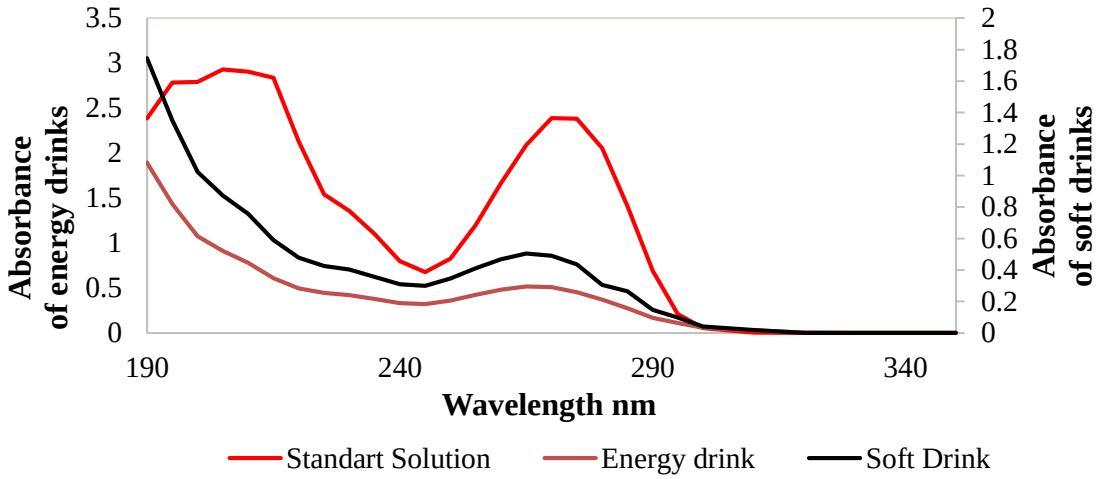

Figure 2. Absorption spectra of caffeine standard solution, energy drinks and soft drinks

The peak of caffeine standard solution is at 270 nm which is associated as n → π* electronic transition of caffeine [26]. This band at 270 nm is related with the C = O chromophore absorption. Concerning the absorption spectra of samples, the maximum absorption is at 265 nm. As it was expected, this shifted of maximum absorption is due to presence of other compound in sample and solvent that absorb in this region and strongly interfere [27].

In Figure 3 is plotted the calibration curve of caffeine, which was prepared by using standard solutions of caffeine in the concentration range of 0-2.5 ppm and absorbance was measured on a UV/Vis spectrometer at wavelength 270 nm, as shown in Table.1. Moreover, the corresponding equation and the correlation coefficient are given.

Table 1. Caffeine content of standard solution and the absorbance measurements

Caffeine Content ppm	Absorbance 271nm
0.0	0.0000
1.0	0.0218
1.5	0.0338
2.0	0.0450

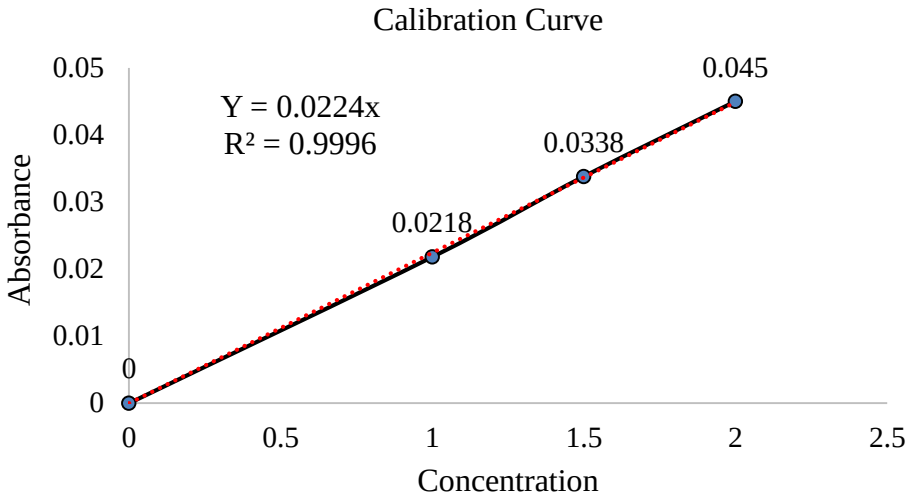

Figure 3. The calibration curve of caffeine standard solution

One of the main objective of this research was to observe the caffeine amount in soft and energy drinks collected from Albanian market and whether the quantity of caffeine is high or low of the FDA /EFSA recommended value[8] [a,b,c].

In the table below are given the corresponded concentration and the pH values of soft drinks. The maximum level of caffeine was found in Brand 5 which was 79.46 ppm and has a pH value 3.34 so it means Brand 5 has moderate acid pH and based on caffeine amount it is strongest central nervous system stimulant so it can be avoided from the consumer in market [8][a,b,c]. The minimum level of caffeine was found to be in Brand 6 which was 24.55 ppm and pH value is the amongst lowest compared to the other soft drinks so it means this brand is strong acidic. However, it can be sold in market and is a weak central nervous system stimulant regarding caffeine content. The US food and Drug Administration (FDA, 2006) limits maximum quantity of caffeine in soft drinks in range between 30-72 mg in 355 ml, and the EFSA limits maximum daily consumption about 3 mg/kg for adults, 5.7 mg/kg for habitual consumption and around 1.5 mg/kg for pregnant women and children [8][a]. In addition to, the most acidic drink is brand 3 with pH value at 2.51 and the less acidic is brand 5.

Table 2. Caffeine content of soft drinks

Sample	Absorbance 271 nm	Caffeine content mg/L	Con mg/L with dilution factor	pH
Brand 1	0.111	4.955	49.55	2.91
Brand 2	0.130	5.804	58.04	2.59
Brand 3	0.108	4.821	48.21	2.51
Brand 4	0.127	5.670	56.70	2.78
Brand 5	0.178	7.946	79.46	3.34
Brand 6	0.055	2.455	24.55	2.70
Brand 7	0.060	2.679	26.25	2.78
Brand 8	0.126	5.625	56.25	3.01
Brand 9	0.136	6.071	60.71	2.68
Brand 10	0.088	3.929	39.29	2.63

Taking into account the maximum limits of caffeine allowed from FDA and EFSA for the soft drinks, we found that the quantity is at the acceptable range and none of the drinks is in possession of higher amount of caffeine than FDA/EFSA regulation [8].

Presence of CO_2 in either soft or energy drinks is frequently considered as main contributor for the low pH values. It is worth to mention that other acids used as preservatives such as phosphoric acid, citric acid and tartaric acid, malic acid, ascorbic acid contribute to the acidic nature of these drinks. Due to presence of these acids in drinks, various microorganisms such as bacteria, fungi which may find a way to contaminate beverages, do not find an appropriate condition thanks to low pH level. Numerous studies have shown that consuming beverages daily can cause different dental problems [24,25].

Statistical analysis of soft drinks is shown in table below. The minimum of value Standard Deviation that subsequently correspond to the minimum of Variance is for brand 5. From the other side the maximum value of Standard Deviation and Variance is for brand 2.

Table 3. Statistical analysis of soft drinks

Sample	Mean	Standard Deviation	Variance
Brand 1	49.55	0.0818	0.0067
Brand 2	58.04	0.1081	0.0117
Brand 3	48.21	0.0888	0.0079
Brand 4	56.70	0.1509	0.0228
Brand 5	79.46	0.0458	0.0021
Brand 6	24.55	0.1248	0.0156
Brand 7	26.25	0.0953	0.0091
Brand 8	56.25	0.1276	0.0163
Brand 9	60.71	0.0655	0.0043
Brand 10	39.29	0.0754	0.0057

In the table below are given the corresponded concentration and the pH values of energy drinks.The highest concentration of caffeine was found in Brand 5 which was 152.68 ppm and has a pH value 3.38 so it means Brand 5 has moderate acid pH and based on caffeine amount it is strongest central nervous system stimulant so it can be avoided from the consumer in market. The lowest caffeine concentration was found to be in Brand 4 which is 62.05 ppm and pH value is the highest compared to the other energy drinks so it means this brand is less acidic, thus, it can be sold in market and is a weak central nervous system stimulant. Highlighting, the most acidic drink is brand 2 with pH value at 2.33 and the less is brand 4 as was mention above.

Table 4. Caffeine content of energy drinks

Sample	Absorbance 271 nm	Caffeine Content mg/L	Con mg/L with dilution factor	pH
Brand 1	0.141	6.2950	62.950	3.26
Brand 2	0.170	7.5890	75.890	2.33
Brand 3	0.170	7.5890	75.890	3.31
Brand 4	0.139	6.2050	62.050	3.49
Brand 5	0.342	15.268	152.68	3.38

As somehow it was expected the quantity of caffeine in energy drinks is higher compared with soft drinks. However, lower than maximum limited from FDA and EFSA (Range from about 80 – 150 mg per serving) [8]. Note of worth, when caffeine concentration exceeded 150 ppm should be mention in label.

Statistical analysis of energy drinks are given in table 5. Accordingly, the minimum of value Standard Deviation correspond minimum of Variance is for brand 1. In contrast, the maximum value of Standard Deviation and Variance is for brand 4.

Table 5. Statistical analysis of energy drinks

Sample	Mean	Standard deviation	Variance
Brand 1	62.95	0.036	0.0013
Brand 2	75.89	0.081	0.0067
Brand 3	75.89	0.105	0.0112
Brand 4	62.05	0.174	0.0304
Brand 5	152.68	0.153	0.0237

4. Conclusion

Analyzing the data gathered from our experiment approach, it is shown that the content of caffeine in soft drinks is not higher than the maximum limits authorized from the international authority of drug regulation. Even though the caffeine quantity in energy drinks are slightly higher than soft drinks, concentration is within maximum limits allowed form the international authority. Taking into consideration problems arising from the consumption of caffeine, even at permitted level, is suggested for the producer to mention the presence of caffeine quantity and health concerns in label, since we found out that those data are missing to the most selected drinks for this study. Determination of caffeine was carried out by means of spectrophotometer UV-VIS as cheapest, fast, and excellent accuracy.

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RESEARCH ARTICLE **(Open Access)**

Chemical profiling, antimicrobial and antioxidant activity of Albanian thyme essential oil

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Abstract

The present study shows the chemical composition, antifungal, antibacterial and radical scavenging effect of the essential oil from wild *Thymus vulgaris* L. Chemical analysis of essential oil was done by gas chromatography mass spectrometry (GC-MS). Antibacterial activity was tested using disc diffusion method on three food borne diseases while antifungal activity was tested on colony growth of three postharvest pathogens in volatile phase. The free radical scavenging activity of the oil was measured *in vitro* by 2,20- diphenyl-1-picrylhydrazyl (DPPH) assay. GC-MS analysis identifies 30 components with thymol and p-cymene as main component, 35.4 and 26.93% respectively. The oil of thyme (5 μL/disc) gave higher inhibition zones on the tested bacterial strains, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028 and *Staphylococcus aureus* ATCC 6538 compared with antibiotic cefazolin (30 μg/disc). In volatile phase, thyme oil (0.14 g/L) showed good antifungal activity on *Penicillium italicum*, *P. digitatum* and *P. expansum*, with percentages of mycelial inhibition 60, 71.4 and 73.5% respectively. The activity of the oil was fungistatic. Scavenging effect of the oil on DPPH radical varied from 36.4, 50.5 and 76.1% depending the concentrations used (1, 2 and 5 mg/mL). The *in vitro* biological activity of thyme oil can be attributed to aromatic monoterpenes thymol and p-cymene.

Keywords: *Thymus vulgaris*, thymol, p-cymene, antibacterial, antifungal, DPPH

1. Introduction

The use of synthetic antimicrobial and antioxidants in food industry is related with some problematics. Inapropriate use of these chemicals have been associated with the development of resistant spoilage microbes and food pathogens [1]. Some of these chemicals have been reported to be toxic and cancerogenic [2, 3]. In addition there is an increased consumer demand for products free of chemicals. As a result, there is a trend in alimentary industry to use natural compounds in order to replace (partially or totally) synthetic compounds. The most promising compounds are plant secondary metabolites. These plant secondary metabolites are bioactive components of essential oils and plant extracts of different plants, reported to have antimicrobial and antioxidant activity. In food industry the essential oils have been studied for their flavoring properties of foods, drinks and also for their antibacterial and antioxidant activity. *Thymus vulgaris* essential oil is a good candidate because wild and cultivated thyme apart from culinary and medicinal uses has been described to have different biological properties.

Thymus vulgaris L. is a native specie of Mediterranean Basin which belong the *Lamiaceae* family. In literature the extracts, essential oils and compounds extracted from this plant have been tested for their antimicrobial and antioxidant activities [4]. Main constituents found in thyme oil are thymol, p-cymene, γ-terpinene, carvacrol and linalool [5]. Chemical composition of wild thyme essential oil depend on factors such as chemotype, location were it

was collected (environmental condition) etc. The antimicrobial and antioxidant activity of the thyme oil have been attributed to monoterpenoid fenols such as thymol and carvacrol, and other monoterpenes (p-cymene, γ-terpinene). A few studies has been conducted on chemical and biological activity of Albanian *T. vulgaris* essential oil.

This study is aimed at assessing the essential oil composition, antimicrobial and antioxidant activities of Albanian *T. vulgaris*.

2. Material and Methods

Essential oil were provided from Albanian company "Mediterranean Spices & Imports" Tiranë. The wild thyme plant was harvested during July and the oil was steam distilled for four hours.

2.1. Chemical analysis of essential oils

The extracted thyme oil was analyzed by gas chromatography mass spectrometry (GC-MS) using an Shimadzu GC-2010 coupled to a Shimadzu GCMS-QP2010 Ultra mass detector (electron ionisation, 70eV) and equipped with a Teknokroma TRB-5(95%) Dimetil-(5%) diphenylpolisiloxane, 30m×0.25mm i.d. capillary column (0.25 µm film thickness). Working conditions were as follows: split ratio (20:1), injector temperature 300°C, temperature of the transfer line connected to the mass spectrometer 250°C, initial column temperature 70°C, then heated to 290°C at 6°C/min. Electron ionisation mass spectra and retention data were used to assess the identity of compounds by comparing them with those of standards or found in the Wiley 229 Mass Spectral Database.

2.2. Antibacterial bioassay

Antibacterial activity of the oil was investigated by disc diffusion method as already described by Sfeir et al. [6]. The bacterial suspension of *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028 and *Staphylococcus aureus* ATCC 6538 was adjusted to a density of bacterial cells of 1.0×10^8CFU/mL. A sterile swab immersed in this bacterial suspension was used to inoculate the entire surface of a nutrient agar. Five µL of thyme oil was applied on a sterile paper disc (8 mm) aseptically placed on the inoculated plates. Then, plates were incubated for 15 minutes at room temperature. Only one disc was tested per plate. After 24 h of incubation at 37°C in an incubator, the inhibition zones were measured in millimeters. Cefazolin (30 µg/disc) was used as a positive control for bacterial inhibition. All experiments were done in triplicate.

2.3. Antifungal bioassay

The antifungal activity of the oil was tested on colony growth using the methods given by Shao et al. [7] and Soylu et al. [8] with some modifications. Sterile plastic Petri dishes (90x15mm) with PDA medium were inoculated in the center with conidial suspension 10^5/mL prepared from 5-6 days cultures of *P. expansum P. digitatum* and *P. italicum*. Sterile filter paper discs (14 mm diameter) were attached to the inner surface of each Petri dish lid. The amount of 10 µL (0.14 g/L) of oil was added onto the filter paper, and the dishes were quickly covered. Petri dishes were wrapped with parafilm to inhibit the release of volatile components. The oil was allowed to volatilize inside the Petri dishes spontaneously at 24°C for three hours before the parafilm was removed. Controls were prepared similarly with the exception of the volatile treatment. Treatment was carried out with three replications. The efficacy of the oil was evaluated by measuring diameters of each colony after 7 days of incubation at 24°C.

Percentage mycelial inhibition (PMI) was calculated as follow:

PMI = $[(dc - dt)/dc] \times 100$, where *dc* is the mean colony diameter for the control sets and *dt* is the mean colony diameter for the treatment sets. All tests were repeated two times.

2.4. DPPH radical scavenging activity assay

The free radical scavenging activity of the oil was measured *in vitro* by 2,20- diphenyl-1-picrylhydrazyl (DPPH) assay according to Saeed et al. [9]. Concentrated solution was prepared by dissolving 24

mg DPPH in 100 mL methanol. The working solution was achieved by diluting DPPH stock solution with methanol to attain an absorbance of about 0.98±0.02 at 517nm using the spectrophotometer (UV-1202 SHIMADZU). Three mL aliquot of this solution was mixed with 100 µL of the sample at 1, 2 and 5 mg/mL concentration. The reaction mixture was shaken and incubated in the dark for 30 min at room temperature. Then the absorbance was measured at 517 nm. Control was prepared as above without essential oil. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as follow:

Scavenging effect % = {(control absorbance - sample absorbance) / (control absorbance)} x 100.

3. Results and Discussion

3.1. Chemical analysis of essential oils

GC-MS analysis identifies 30 components with thymol and *p*-cymene as main components, 35.4 and 26.93% respectively with lesser quantities of γ-terpinene (5.16%), β-caryophyllene (3.92%), linalool L (3.68%) and carvacrol (2.71%). In table 1 are presented the other components of thyme essential oil.

Table 1. Identified compounds of thyme oil

Peak	Base m/z	R.Time	Area %	Compounds
1	93.10	4.958	0.13	β-phellandrene
2	93.10	5.104	1.36	α-pinene
3	93.10	5.426	1.05	Camphene
4	93.10	6.058	0.13	β-pinene
5	93.10	6.353	0.93	β-myrcene
6	93.10	6.753	0.13	α-phellandrene
7	93.10	7.042	1.21	α-terpinene
8	119.15	7.042	26.93	*p*-cymene
9	68.05	7.377	0.83	Limonene
10	43.00	7.43	2.32	1,8-cineole
11	93.10	8.209	5.16	γ-terpinene
12	93.10	9.13	0.15	Terpinolene
13	71.05	9.433	3.68	Linalool L
14	95.10	10.867	1.10	Camphor
15	95.10	11.57	2.31	Borneol L
16	71.05	11.977	1.67	4-terpineol
17	59.05	12.451	0.38	α-terpineol
18	149.15	13.88	1.48	Carvacrol methyl ether
19	149.15	14.2	1.41	Thymyl methyl eter
20	93.10	15.685	0.61	Isobornyl acetate
21	135.15	15.865	35.14	Thymol
22	135.15	16.177	2.71	Carvacrol
23	105.10	18.696	0.40	α-ylangene
24	81.10	19.005	0.15	β-bourbonene
25	93.10	20.131	3.92	β-caryophyllene
26	161.15	21.968	0.48	α-amorphene
27	105.10	22.731	0.13	α-murolene
28	161.20	23.16	0.72	Germacrene D
29	159.15	23.438	1.25	δ-cadinene
30	79.05	25.283	2.11	Caryophyllene oxide

Thyme oil was the subject of numerous studies conducted earlier. Previous works reported that the oil of *T. vulgaris* contains as main constituens thymol, p-cymene, γ-terpinene and carvacrol [10, 11, 12]. Our results are in line with those repots. Thyme oil based on chemical composition can be clasified in different chemotypes. Thyme oil in this study belongs to thymol chemotype in agreement with those reported erlier for Albanian thyme oil [13].

3.2. Antimicrobial activity

Antimicrobial activity of thyme oil is shown in table 2. The antibacterial activity is expressed as inhibition zone diameter in mm.

The oil of thyme (5 μL/disc) gave higher inhibition zones on the tested bacterial strains, *E. coli* ATCC 25922, *S. typhimurium* ATCC 14028 and *S. aureus* ATCC 6538 compared with antibiotic cefazolin (30 μg/disc).

In volatile phase, thyme oil (0.14 g/L) showed good antifungal activity on *Penicillium italicum*, *P. digitatum* and *P. expansum*, with percentages of mycelial inhibition 60, 71.4 and 73.5% respectively. The activity of the oil was fungistatic.

Table 2. Inhibition zone diameters (mm) on three bacterial species and percentages of fungal mycelial inhibition of thyme essential oil.

Pathogens	*T. vulgaris*	Control	Cefalozin
Bacterial species			
E. coli	45.7±1.5	0.0	19.3±0.6
S. typhimurium	31.7±5.5	0.0	18.3±0.6
S. aureus	48.0±2.0	0.0	33.3±0.6
Fungal species			
P. digitatum	71.4±5.1		
P. expansum	73.5±1.3		
P. italicum	60±7.9		

Values in the table represent the mean value of three replicates (± standard deviation).

T. vulgaris essential oil, thymol chemotype, have shown a broad scale of antibacterial activity against food borne pathogens. In a study conducted by [14] thyme oil (64% thymol) inhibited the growth of different bacterial species including E. coli and S. typhimurium. In an earlier study the thymol chemotype (49% thymol and 19% p-cymene) of thyme oil showed good antibacterial activity against S. aureus and S. typhimurum [15]. Our results are in agreement with those reported in literature [14, 15].

Essential oil extracted from different *Thymus* species have been studied for their antifungal activity and in many studies the oils have shown to be fungicidal or fungistatic. The antifungal activity of *T. vulgaris* essential oil and other oils are with high interes in agricultural sector in particular for the control of postharvest pathogens of fruits and vegetables. Due to the volatilization of oil it can be useful as fumigant for the control of postharvest pathogens especially in some fruits such as strawberries that are difficult to be sanitized with aqueous solution.

In a study conducted by Klarić *et al.* [16] thymol and *T. vulgaris* essential oil with main costituents *p*-cymene (36.5%) and thymol (33.0%) showed strong fungistatic or fungicidal against diferent fungal species such as *Aspergillus*, *Penicillium*, *Cladosporium*, *Trichoderma*, *Mucor* and *Rhizopus*. In these experiments thymol reduced the fungal growth three time higher than the oil. In another study with *P. digitatum* wich cause green mould in citrus fruits the thyme oil showed good antimicrobial activity in contact and volatile phase [17]. Propably the

fungistatic activity in three *Penicillium* species tested is due to phenolic costituent thymol, these confirmed also by other studies [11, 16].

3.3. DPPH radical scavenging activity

Scavenging effect of thyme oil on DPPH radical varied from 36.4, 50.5 and 76.1% depending the concentrations used (1, 2 and 5 mg/mL). Previous studies have demonstrated that the antioxidants activity of thyme oil is related to phenolic compound thymol and the monoterpene p-cymene [18, 19]. Our data is in agreement with these reports given that the oil used in this study has as main compounds thymol (35.4 %) and p-cymene (26.93%).

4. Conclusions

Chemical analysis of the essential oil of *T. vulgaris* identified different compounds with thymol and p-cymene as main compounds. *In vitro* experiments the oil showed good antibacterial activity on the three food borne pathogens and inhibited the colony growth of the three *Penicillium* species. The oil also presents good scavenging activity on DPPH radical. The biological activities of the oil maybe attributed to major compounds (thymol and *p*-cymene).

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RESEARCH ARTICLE **(Open Access)**

Determination of the proper time and effectiveness of insecticide, for protection against coodling moth

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Abstract

In Albania, apple is one of the most important tree, especially in the coldest north and south - eastern areas, with a production of 80,000 tons per year. Main developed region for this fruit is Korca, it's provide about 65% of production with 70% of the total number of apples trees planted in Albania. The tendency to increase the apple production per unit of surface, also to introduce new varieties and rootstocks has opened up many problems for apple growers especially with the management of key pests and diseases as **coodling moth**. Important is the increase of reliability for the effectiveness of the chemical preparations used. It is necessary to conduct a series of studies to provide the proper answer and assistance to apple growers in this direction. My study part of the PhD thesis is based on several experiments aims on the determination of the proper time of application and the effectiveness of insecticides. For this purpose, three insecticides with the lowest negative impact on the consumer and the environment (**l.a.granulosevirus, cpgv, l.a indoxacarb, l.a. diflubenzuron**) were tested, with the dosage and predetermined amount of solution used. Insecticide treatments were carried out based on monitoring and by alternating preparations and for two basic cultivars Golden Delicious and Star King in both generations of the pest. The effectiveness of insecticides was determined by the rate of infection for each cultivar, compared to control trial.

This experiment could serve to the apple cultivars to increase the reliability on the chemical preparates for the proper time, dosage and the amount of solution used.

Keywords: *insecticide, effectiveness, coodling moth, generation.*

1. INTRODUCTION

Apples are part of our national wealth not only for the presence of many native cultivars but also for the presence of a tradition inherited from generation to generation. In Albania, apple is one of the most important tree, especially in the coldest north and south - eastern areas. Today it is providet a production of 80,000 tons per year.

Naturally, the main center of development of this culture is the Korça region, where about 65% of production is currently provided, in this context, this region has 70% of the total number of apples planted in Albania. *(Thomaj F, Domi H, Spahiu T, 2013)*

The tendency to increase apple-cultivated areas, to increase production per unit area, as well as the introduction of new varieties and rootstocks has opened up a lot of trouble for apple cultivators in Korca region to manage key pests and diseases where one of the most important is the Coodling moth (*Laspeyresia (Carpocapsa) pomonella L*) which is considered "key" pest. Another important fact is to increase the reliability of the effectiveness of the preparations used for the protection against this pest. It is therefore necessary to conduct a series of studies and experiments to provide the most accurate answer and assistance to apple growers in this direction. *(Çakalli D, Shahini SH, Varaku S 2005)*

One of them is my study part of the PhD thesis based on several experiments set up in a 5 ha apple orchard in the village of Dvoran in Korca region regarding the determination of the proper time for application and the effectiveness of insecticides. To this aim, were tried three insecticide part of the Yellow and Green list with the lowest negative impact on the consumer and the environment

(***granulosevirus, cpgv, indoxacarb, and diflubenzuron***) with a predetermined dose and amount of solution used. Insecticide treatments were carried out based on the prognosis - signaling system by alternating preparations and for the two basic cultivars in Star King and Golden Delicious variety and for the two generations of the pest (Coodling moth). The effectiveness of insecticides was determined by the rate of infection for each cultivar, compared to untreated control field. *(CABI, Skënderasi B. 2011)*

This experiment could serve some apple cultivars to increase the reliability of the chemical preparations, the time, the dose and the amount of solution used.

2. MATERIALS AND METHODS

2.1 Materials

The experiment was carried out during 2018 in one of the most well-known villages for fruit and apple cultivation in Dvoran village of Korça region in a parcel of 5 Ha where most dominant are Star King and Golden Delicious varieties divided into separate rows. To carry out the experiment will be taken an area of 100 m^2 with 240 apple trees from both varieties.

So in the 3 blocks will be studied only 2 main apple cultivars in the orchard;

1. Golden deliciosus, 2. Starking,

Cultivators represent 2 variations while the blocks represent 3 replicants. Each variation in each replication is represented by 10 apple trees. The trees of each variation were labeled in the first and the tenth tree, *e.g* P1 a1 b2.

The scheme is a twofactorial with randomized block. (Figure 1) The chemical treatments in this scheme were carried out on the basis of forecast - signalisation and the following insecticides were used:

Madex (*granulosevirus*) (*cpgv*), 2.Dimiline (*diflubenzuron*), 3.Avaunt (*indoxacarb*).

The block that serves as a control block was not treated with any kind of pesticide.

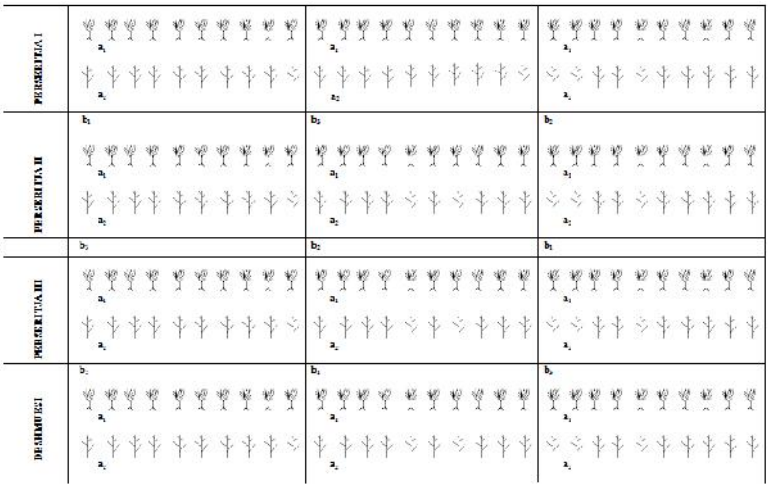

Figure 1. The randomized application scheme of pesticides

2.2 Methods

Will be studied insecticides part of the Green and Yellow list with the lowest negative impact on the consumer and the environment (grade III preparations), this insecticides are used also in integrated protection programs. Chemical treatments will be made based on forecast - signalisation programs by alternating them. Two basic apple cultivars will be treated: Golden deliciosus and Starking.

Variation a1, a2 cultivars:

1.Golden deliciosus 2. Starking.

Variation b1, b2, b3 Insecticides:

1. Madex (granulosevirus) (cpgv)

2. Avaunt (indoxacarb)

3. Dimiline (diflubenzuron)

These chimical preparations were used for both generations. The spraying of the trees were carried out with a spraying backpomps using an average of 1 - 1.5 liters of solution/tree.

The effectiveness of insecticides was determined by the infection rate for each cultivar, compared with untreated cintrol cultivars. At the beginning of July for the first generation and in the third week of August for the second generation, were analyzed 10 labeled apple trees for each variety by 10 fruits per tree = 100 fruits in total. Infection rate indicates the number of fruit affected, in percentage, in 100 analyzed fruits. *(Isufi E. 2000)* Infection on apple fruits was considered complete when the pest had deeply penetrated in the fruit. (until the seeds). (Figure 2) The time of chemical treatments was determined by the data collected from other experiments conducted for the monitoring of coodling moth by the traditional method as well as modern method using sexual pheromone.

Figure 2. Monitoring of the pest infection rate

3. RESULTS

After the application of the insecticides tested at the time and in the appropriate quantity, an infection (damage) analysis was performed on the fruit to see the effectiveness of these preparations. Based on the analysis it resulted that;

For the first generation of the pest at the first replication from the application of Madex *(granulosevirus)* at the Golden Delicious variety resulted 3 infected fruits while for the other preparations the number of infected fruit was 0; (*Table 1*)

For the first generation of the pest at the first replication from the application of Madex *(granulosevirus)* at Star King variety resulted 4 infected fruits while for the usage of Dimiline *(diflubenzuron)* resulted 2 infected fruit and during the usage of the third preparation Avaunt (*indoxacarb*) resulted 1 infested fruits; (*Table 1*)

From the analysis made at the control field it was found that for the first replication in the first generation of the pest the infection was slightly higher, in the Golden Delicious variety resulted 4 infected fruits while in the Star King varieties resulted 10 infected fruits; (*Table 1*)

For the first generation of the pest at the second replication from the application of Madex *(granulosevirus)* at the Golden Delicious variety resulted 1 infected fruit while for the other preparations the number of infected fruit was 0 for the Avaunt (*indoxacarb*) and 1 for Dimiline *(diflubenzuron);* (*Table 2*)

For the first generation of the pest at the second replication from the application of Madex *(granulosevirus)* at Star King variety resulted 2 infected fruits while for the usage of Dimiline *(diflubenzuron)* resulted 3 infected fruits and during the usage of the third preparation Avaunt (*indoxacarb*) resulted 1 infested fruit; (*Table 2*)

From the analysis made at the control field it was found that for the second replication in the first generation of the pest the infection was slightly higher, in the Golden Delicious variety resulted 9 infected fruits while in the Star King varieties resulted 13 infected fruits; (*Table 2*)

For the first generation of the pest at the third replication from the application of Madex

(granulosevirus) at the Golden Delicious variety resulted 1 infected fruit while for the other preparations the number of infected fruit was 0 for the Avaunt (*indoxacarb*) and 0 for Dimiline *(diflubenzuron)* ; (*Table 3*).

For the first generation of the pest at the third replication from the application of Madex *(granulosevirus)* at Star King variety resulted 2 infected fruits while for the usage of Dimiline *(diflubenzuron)* there are no infected fruits and during the usage of the third preparation Avaunt (*indoxacarb*) resulted 3 infested fruits; (*Table 3*)

From the analysis made at the control field it was found that for the third replication in the first generation of the pest the infection was slightly higher, in the Golden Delicious variety resulted 10 infected fruits while in the Star King varieties resulted 9 infected fruits; (*Table 3*).

To go further with the results of the analysis of the infection rate in fruit during the second generation of the pest:

Related to the second generation of the pest at the first replication from the application of Madex *(granulosevirus)* at the Golden Delicious variety resulted 10 infected fruits regarding the other preparations as Avaunt (*indoxacarb*) the number of infected fruit was 0 and for Dimiline *(diflubenzuron)* the number of infected fruit was 0; (*Table 4*)

For the second generation of the pest at the first replication from the application of Madex *(granulosevirus)* at Star King variety resulted 13 infected fruits while for the usage of Dimiline *(diflubenzuron)* resulted 1 infected fruit and during the usage of the third preparation Avaunt (*indoxacarb*) resulted 3 infested fruits; (*Table 4*)

From the analysis made at the control field it was found that for the first replication in the second generation of the pest the infection was high, in the Golden Delicious variety resulted 32 infected fruits while in the Star King varieties resulted 37 infected fruits; (*Table 4*)

For the second generation of the pest at the second replication from the application of Madex (granulosevirus) at the Golden Delicious variety resulted 5 infected fruit while for the other trails there was no infection; (*Table 5*)

For the second generation of the pest at the second replication from the application of Madex (granulosevirus) at Star King variety resulted 9 infected fruits while for the usage of Dimiline *(diflubenzuron)* resulted 1 infected fruit and during the usage of the third preparation Avaunt (*indoxacarb*) resulted 3 infested fruit; (*Table 5*)

From the analysis made at the control field it was found that for the second replication in the second generation of the pest the infection was high, in the Golden Delicious variety resulted 31 infected fruits while in the Star King varieties resulted 33 infected fruits; (*Table 5*)

In the third replication from the second generation in the application with Madex *(granulosevirus)* for the Golden Delicious variety resulted in 7 infected fruits while for the other preparations there was no infection; (*Table 6*)

For the second generation of the pest at the third replication from the application of Madex *(granulosevirus)* at Star King variety resulted 9 infected fruits while for the usage of Dimiline *(diflubenzuron)* resulted no fruit infected and during the usage of the third preparation Avaunt (*indoxacarb*) resulted 3 infested fruit; (*Table 6*)

The analysis shows that at the control field it was found that for the third replication in the second generation of the pest the infection was high, in the Golden Delicious variety resulted 38 infected fruits while in the Star King varieties resulted 33 infected fruits; (*Table 6*)

Table 1. Analysis of infected fruits for the first replicant of Star King and Golden Delicious varieties for 1st generation

Replicant I	Generation I		Factors							
			$P_1a_1b_1$	$P_1a_1b_3$	$P_1a_1b_2$	$P_1a_2b_1$	$P_1a_2b_3$	$P_1a_2b_2$	Cont a_1	Cont a_2
	Number of trees	Tree 1	0	0	0	1	0	0	0	1
		Tree 2	0	0	0	0	0	1	1	0
		Tree 3	1	0	0	0	0	0	0	2
		Tree 4	0	0	0	0	0	0	1	1
		Tree 5	0	0	0	1	1	0	0	1
		Tree 6	0	0	0	0	0	0	0	2
		Tree 7	1	0	0	1	0	1	1	2
		Tree 8	0	0	0	0	0	0	0	0
		Tree 9	1	0	0	0	0	0	1	0
		Tree 10	0	0	0	1	0	0	0	1
	Sum		**3**	**0**	**0**	**4**	**1**	**2**	**4**	**10**

Table 2. Analysis of infected fruits for the second replicant of Star King and Golden Delicious varieties for 1st generation

Replicant II	Generation I		Factors							
			$P_2a_1b_3$	$P_2a_1b_2$	$P_2a_1b_1$	$P_2a_2b_3$	$P_2a_2b_2$	$P_2a_2b_1$	Cont a_1	Cont a_2
	Number of trees	Tree 1	0	0	0	0	0	0	1	1
		Tree 2	0	0	0	1	0	0	1	1
		Tree 3	0	0	1	0	0	0	0	2
		Tree 4	0	0	0	1	0	0	1	2
		Tree 5	0	0	0	0	0	0	2	1
		Tree 6	1	0	0	0	0	1	0	2
		Tree 7	0	0	0	0	0	0	1	1
		Tree 8	0	0	0	0	1	0	2	1
		Tree 9	0	0	0	1	0	0	1	1
		Tree 10	0	0	0	0	0	1	0	1
	Sum		**1**	**0**	**1**	**3**	**1**	**2**	**9**	**13**

Table 3. Analysis of infected fruits for the third replicant of Star King and Golden Delicious varieties for 1st generation

Replicant III	Generation I		Factors							
			$P_3a_1b_2$	$P_3a_1b_1$	$P_3a_1b_3$	$P_3a_2b_2$	$P_3a_2b_1$	$P_3a_2b_3$	Cont a_1	Cont a_2
	Number of trees	Tree 1	0	0	0	1	0	0	1	0
		Tree 2	0	0	0	0	0	0	2	0
		Tree 3	0	0	0	0	1	0	0	2
		Tree 4	0	1	0	0	0	0	1	2
		Tree 5	0	0	0	1	0	0	2	1
		Tree 6	0	0	0	0	0	0	1	2
		Tree 7	0	0	0	0	0	0	0	1
		Tree 8	0	0	0	1	0	0	1	1
		Tree 9	0	0	0	0	1	0	0	0
		Tree 10	0	0	0	0	0	0	2	0
	Sum		**0**	**1**	**0**	**3**	**2**	**0**	**10**	**9**

Table 4. Analysis of infected fruits for the first replicant of Star King and Golden Delicious varieties for 2nd generation

Replicant I	Generation II		Factors							
			$P_1a_1b_1$	$P_1a_1b_3$	$P_1a_1b_2$	$P_1a_2b_1$	$P_1a_2b_3$	$P_1a_2b_2$	Cont a_1	Cont a_2
	Number of trees	Tree 1	1	0	0	1	0	0	4	4
		Tree 2	1	0	0	2	0	0	3	4
		Tree 3	1	0	0	0	0	0	4	3
		Tree 4	0	0	0	3	0	0	3	3
		Tree 5	2	0	0	1	0	0	3	5
		Tree 6	0	0	0	0	0	2	4	4
		Tree 7	1	0	0	1	0	0	2	3
		Tree 8	1	0	0	2	1	1	5	4
		Tree 9	1	0	0	2	0	0	3	3
		Tree 10	2	0	0	1	0	0	3	4
	Sum		**10**	**0**	**0**	**13**	**1**	**3**	**32**	**37**

Table 5. Analysis of infected fruits for the second replicant of Star King and Golden Delicious varieties for 2nd generation

Replicant II	Generation II		Factors							
			$P_2a_1b_3$	$P_2a_1b_2$	$P_2a_1b_1$	$P_2a_2b_3$	$P_2a_2b_2$	$P_2a_2b_1$	Cont a_1	Cont a_2
	Number of trees	Tree 1	0	0	0	0	0	1	3	3
		Tree 2	0	0	1	0	0	0	3	4
		Tree 3	0	0	1	0	1	2	3	2
		Tree 4	0	0	0	0	0	0	3	4
		Tree 5	0	0	1	0	0	1	2	3
		Tree 6	0	0	0	0	0	1	3	2
		Tree 7	0	0	0	1	0	0	3	3
		Tree 8	0	0	0	0	0	1	4	4
		Tree 9	0	0	2	0	1	2	3	5
		Tree 10	0	0	0	0	1	1	4	3
	Sum		**0**	**0**	**5**	**1**	**3**	**9**	**31**	**33**

Table 6. Analysis of infected fruits for the third replicant of Star King and Golden Delicious varieties for 2nd generation

Replicant III	Generation II		Factors							
			$P_3a_1b_2$	$P_3a_1b_1$	$P_3a_1b_3$	$P_3a_2b_2$	$P_3a_2b_1$	$P_3a_2b_3$	Cont a_1	Cont a_2
	Number of trees	Tree 1	0	1	0	0	0	0	4	2
		Tree 2	0	0	0	0	1	0	4	4
		Tree 3	0	0	0	2	1	0	3	3
		Tree 4	0	1	0	0	0	0	4	4
		Tree 5	0	0	0	0	2	0	5	4
		Tree 6	0	0	0	0	0	0	2	2
		Tree 7	0	2	0	0	3	0	4	4
		Tree 8	0	0	0	1	0	0	4	4
		Tree 9	0	3	0	0	1	0	5	3
		Tree 10	0	0	0	0	1	0	3	3
	Sum		**0**	**7**	**0**	**3**	**9**	**0**	**38**	**33**

4. DISCUSSION

The results obtained show that by comparing the three chemical preparations, the highest effectiveness in the protection from the coodling moth infection had two insecticides Dimiline *(diflubenzuron)* and Avaunt *(indoxacarb)* compared with the other Madex *(granulosevirus)* this visible for both generations of the pests. While comparing the effectiveness of this three preparations between the two generations, higher efficacy these preparations have given in the first generations visible this in the average of the infected fruits *e.g* for the first generation of Star King varieties in the first replicant treated with Madex (granulosevirus), the average infected fruit were 2.6 compared with the second generation average 10.3. (*Table 7*)

While when comparing the results between the data obtained from the tree rows were are applied chimical preparations with the data obtained from the fruit analysis in the control field, the difference is apparent with a very high level of infection of the witness:

The average infection rate for the first generation in both varieties with insecticides used for

protection was much lower compared to the control field: variety Golden Delicious 1.6, 0 and 0.3 compared with 7.6 as well as in the variety Star King 2.6, 2 and 1.3 compared with 10.6. (*Table 7*)

The average infection rate for the second generation in both varieties with insecticides used for protection was much lower compared to the control field: variety Golden Delicious 7.6, 0 and 0 compared with 33.6 as well as in the variety Star King 10.3, 3 and 0.6 compared with 34.3; (*Table 7*)

Table 7. Summary table for the rate of fruit infection by the pest

Year	Generation	Preparations	Factors	Golden					Starking				
				P1	P2	P3	∑	Avarage	P1	P2	P3	∑	Avarage
2017	I	Madex	b1	3	1	1	5	1.6	4	2	2	8	2.6
		Avaunt	b2	0	0	0	0	0	2	1	3	6	2
		Dimiline	b3	0	1	0	1	0.3	1	3	0	4	1.3
		Control		4	9	10	23	7.6	10	13	9	32	10.6
	II	Madex	b1	10	5	7	23	7.6	13	9	9	31	10.3
		Avaunt	b2	0	0	0	0	0	3	3	3	9	3
		Dimiline	b3	0	0	0	0	0	1	1	0	2	0.6
		Control		32	31	38	101	33.6	37	33	33	103	34.3

5. CONCLUSIONS

From the experiment data, were reached these conclusions:

- The highest effectiveness of protection against coodling moth results to have had two chemical preparations Avaunt (*indoxacarb*) and Dimilina *(diflubenzuron)* during both first and second generations;
- Insecticide Dimilina *(diflubenzuron)* compared to the Avaunt (*indoxacarb*) has had a better control of the pest infection especially during the second generation;
- It turns out that during the use of granulovirus Madex *(granulosevirus)* has had a slight infection from the pest during two generations;
- Infection from the pest appears to be higher and sometimes more difficult to control during the second generation, seeing the average of the infected fruits;
- The rate of infection from the pest result with no significant change betwen the two apple varieties *(Golden delicious and Star King)*, it was observed a slightly higher infection rate at the Star King variety;
- Compared the data obtained from the controle field the damage caused to the fruit by the pest is many times higher than when it is treated with chemical preparations;
- It's loks to be necessary the monitoring of coodling moth *(mainly with the modern method using sexual pheromone)* as well as applying chemical preparations to succeed and to have guaranteed protection from this pest.

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RESEARCH ARTICLE **(Open Access)**

The effect of "Natuphos" phytase on improving of performance parameters of weaned piglets in albanian farm conditions.

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Abstract

Today's studies demonstrate the nutritional and ecological importance of using microbial phytase in the feeding of monogastric animals, such as pigs and poultry. The use of phosphorus containing grain cereals, oats and their by-products is relatively low. Only 20-40% of phosphorus is used by the animal organism, the rest 60-80% is in the form of phytic acid**,** which can also be called anti nutritional factor.

The aim of this study was to test the effects of the microbial phytase (NATUPHOS) on the performance parameters of weaned piglets in albanian farm condition.

The microbial phytase preparation (*Aspergillus niger*, NATUPHOS) was supplemented to a basal ration 750 FTU/kg feed and the effects on growth performance of weaned piglets were studied. The supplementation of microbial phytase improved slightly daily weight gain, feed conversion ratio and reduces the amount of inorganic phosphorus needed to maximize growth and bone mineralization and markedly reduces fecal excretion of phosphorus. Overall a positive effect of the microbial phytase on performance parameters was observed.

Keywords: Weaned piglets, microbial phytase, performance parameters, P- reduction

1. Introduction

The majority of the phosphorus contained in foods of plant origin is in the form of phytate-phosphorus. Its content represents about 60-70% of phosphorus in cereals and 50-60% in oil plants and industrial by-products, used as "animal feed " [4]. Non ruminant animals, poultry and pigs, have limited ability to digest phosphorus. Much of it is eliminated with faeces without being affected by fluids and enzymes of the digestive tract. For this reason, monocalcium and bivalent minerals phosphates with the highest solubility are used in non ruminant animal food rations to ensure the necessary levels of phosphorus. In addition, the phyto-phosphate groups of fats contained in plant foods, being relatively saturated with negative loads, form unburnt complexes and mineral elements: Ca, Mg, Zn, Cu as well as protein fractions, preventing partial use of them. Eliminating such unstable phosphorous and mineral phosphate complexes creates problems, significantly affecting environmental pollution. This includes contamination of soil, groundwater and the disruption of nitrogen and phosphorus balance in the arable soil. Consequently, effective nutrition management is a key to sustainable livestock production, enabling the reduction of the amount of animal excreted phosphorus. "For this reason, in some countries there are legally binding restrictions on their use in animal rations" [3] Today's studies demonstrate the nutritional and ecological importance of using microbial phytase in the feeding of non ruminant animals, such as pigs and poultry. The use of phosphorus containing grain cereals, oats and their by-products is relatively low. Only 20-40% of phosphorus is used by the animal organism, the rest 60-80% is in the form of phytic acid**,** which can also be called anti nutrition factor. The use of enzymes in the feeding of pigs increases the utilization of phytate-phosphorus, calcium and other mineral substances. The enzyme phytase catalyses the stepwise hydrolysis of phytate to inorganic phosphates and inositol with

lower phosphorylated inositol phosphates as intermediates [1,5]. Hydrolysis of phytate by phytases occurs when phytin is in solution [6].

2. Material and Methods

Forty piglets (Large White x Landras) of four litters were transferred to flat-decks and allocated to 2 groups (A and B) with 20 animals (10 male and 10 female), respectively. Four piglets from different litters (1 male and 1 female), with the same body weight were housed in every box (experimental unit). The litter origin was taken into account, avoiding that piglets from the same litter were allocated in the same treatment. There were five replications per control group and five also per treated group. The control group (A) was feed with a balanced diet, containing mono calcium phosphate. The experimental group (B) was feed with low level of phosphorus, without inorganic phosphorus. All the phosphorus in this group originates from soybean meal. This group was supplemented with NATUPHOS phytase 750 FTU/kg feed. Ambient room temperature was maintained at 17-22^{0}C for three first weeks and lowered by 1^{0}C for each week thereafter. The ventilation also was provided to ensure good air quality. The basal diet mainly contained maize and soybean meal and the nutrient contents met or exceeded nutrient requirements recommended by NRC. The diets were offered ad-libitum and animals had free access to water.

Table 1. The calculated nutrient concentration of diet

Nutrient concentration	**Control group (A)**	**Experimental group (B)**
ME (MJ/kg)	9.62	9.87
Crude protein %	17.5	17.1
Crude fibre %	3.24	2.75
Calcium %	0.80	0.80
Phosphorus %	0.54	0.52
Lysine%	1.2	1.18
Metionine+Cystine%	0.83	0.68

During six weeks experimental period Body Weight (BW), Daily Weight Gain (DWG) and Feed Conversion Ratio (FCR, kg feed/kg Body Weight Gain were measured weekly. Data are presented as arithmetic means with standard deviation of the mean (Mean ± SD). One-way analysis of variance and Student's *t*-test ($P<0.05$) were performed to test the differences between two groups.

3. Results and Discussion

Feeding phytase NATUPHOS was slightly improved the production parameters respectively: Final Body Weight (FBW) by 6.32% and Daily Weight Gain (DWG) by 10.36%, compare with control group.

Table 2. The effect of phytase on production parameters.

Parameters		*Control group*	*Experimental group*
Production	n[1]	X ± SD	X ± SD
Initial BW, kg	20	6.40 ± 0.22	6.43 ± 0.24
BW 6th week [2]		15.64±0.68	16.63 ± 0.69
DWG, g [3]		220.0 ± 6.5	242.8 ± 6.7
FCR [4]		1.85 ± 0.04	1.75 ± 0.03

[1] Number of animals, (20 piglets/ every group, at the beginning of the experiment)
[2] BW at the end of the trial.
[3]DWG for whole experimental period.
[4]FCR for whole experimental period.

Feed Conversion Ratio (FCR) was reduced (-5.4%) to compare with control group, but the differences were not significant. The effect of microbial phytase use as a partial replacer of the bicalcium phosphate in the layers and weaned piglets, aiming a better utilization of the phytic phosphorus and decrease of the environment pollution was documented [7]. Utilization of microbial phytase (Natuphos) on the nutritive ration of weaned piglets (28 days old), was accompanied with improved performance parameters. The dosage of enzymes depends by the phytase activity and total phosphorus of cereals used in nutritive ration. [2] provides a first estimate of phytase activity, phytate and total phosphorus of some cereals grown in Albania and used in non-ruminant feed rations. It is only an estimate, because the magnitude of the “phytate problem” in terms of nutrient management in agricultural and livestock production is very important.

4. Conclusions

The utilization of phytase on the nutritive ration of piglets at the weaning moment, can improve the performance parameters of them. Enzymes utilization is very important, especially in the extensive and semi extensive pigs farm conditions. The utilization of phytase will be considered as a good possibility for increasing of meat production and decreasing of cost per production unit. The supplementation of diets with microbial phytase, besides the nutritional and environmental benefits, also has economic benefits. They relate to cost-effective ratios, especially in some countries of the world, which have high cost sanctions if organic fertilizers contain high levels of phosphorus. This may be due to soil contamination and the breakdown of the N and P balance in the ground and in groundwater. So pigs' diets should be prepared with low levels of nitrogen and phosphorus to reduce the excretion of nitrogen and phosphorus, while maintaining optimal growth and animal health.

5. Acknowledgements

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RESEARCH ARTICLE **(Open Access)**

Correlation of thyroid antibodies with thyroid hormones in autoimmunethyroid disease

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Abstract

Autoimmune diseases have a high prevalence in the population, and autoimmune thyroid disease (AITD) is the most common organ specific auto immune disorder resulting in the dysfunction of the thyroid gland (hypo or hyperfunction) .The clinical diagnosis of autoimmune thyroid diseases (AITD) is usually confirmed by detection of various antibodies in the patient's blood or by histopathological evaluation.In this study, I evaluated the prevalence of serum antibodies and assessed the correlation between antibodies and thyroid dysfunction. Included in this study were 410 patients, them underwent testing for thyroid function and thyroid antibodies. 304 were females and 106 were males, the age of them ranged from 20-65 years.Thyroid peroxidase antibodies (TPO Ab) tested positive in 83.4% of patients and negative in 16.5% . Antithyroglobulinantibody (ATG Ab) estimation was positive in 57.5% patients and negative in 42.5%. By thyroidfunction testing and serum antibody evaluation , of the 342 TPO- positive patients , 63.4% were hypothyroid, 9 % were hyperthyroid and 27.4 % euthyroid. In 236 ATG Ab - positive patients, 57.6% were hypothyroid, 6.7% were hyperthyroid and 33.1% were euthyroid. But in the 174 ATG Ab-negative patients, 58.6% were hypothyroid. However, 62.6% of patients who were negative for antithyroglobulin antibodies were hypothyroid. It should be noted that only one subject who was negative for thyroid peroxidase was hypothyroid.

This shows that thyroid peroxidase antibodies are more sensitive than antithyroglobulin antibodies in predicting hypothyroidism. Similarly, thyroid peroxidase antibodies were more sensitive than antithyroglobulin antibodies in autoimmune thyroiditis (99.5% vs 58.4%, $p< 0.05$). It is well recognized that autoimmune thyroid disease correlates with excess iodine intake.

Hypothyroidism was the most frequent thyroiddysfunction in patients with positive TPO and ATG antibodies.

Keywords: Antithyroid antibodies, antithyroglobulin antibodies, hyperthyroidism, hypothyroidism, thyroid peroxidase antibodies, autoimmune thyroid disease.

Introduction

Autoimmune diseases (AD) represent a spectrum of disorders caused by inflammation of organs due to production of antibodies against self-structures and cytotoxic action of T cells. The patients commonly present in outpatient endocrine clinics with goiter or thyroid dysfunction. The human AITDs broadly include Graves' disease (GD) and Hashimoto's thyroiditis (HT) which are the most common causes of thyroid gland dysfunctions and nonendemic goiter .These conditions arise due to complex interactions between environmental and genetic factors and are characterized by reactivity to self-thyroid antigens which are expressed as distinctive inflammatory or antireceptor autoimmune diseases. The etiology of AITD is multifactorial. Susceptibility to the disease is determined by a combination of immune mechanism, genetics, and environmental (iodine, infection, and stress) and constitutional factors.The clinical diagnosis of autoimmune thyroid disease is usually confirmed by the detection of various antibodies in the patient's blood sample. Three types of antibodies are most commonly assayed: antithyroglobulin, thyroid peroxidase (previously termed antimicrosomal), and TSH receptor antibodies. The clinically pertinent classification of autoimmune thyroid disease, listed in Table 1, includes Hashimoto's thyroiditis and its variants, autoimmune atrophic thyroiditis (myxoedema) and Grave's disease., this study has two aims: (1) to find the regional prevalence of thyroid peroxidase and anti-thyroglobulin antibodies, and (2) to determine the

correlation of the antibody to thyroid dysfunction in patients with histological diagnoses of autoimmune thyroiditis and Hashimoto's thyroiditis.Included in this study were 410 patients who attended the outpatient clinic and had a histological diagnosis of autoimmune thyroiditis

Table .1.Classification of autoimmune thyroid disease.

Etiology	*Clinical presantation*
Hashimoto's thyroiditis	Goitre, Hypothyroidism
Painless thyroiditis	Small goiter, transient hyper- or hypothyroidism
Atrophic/primary thyroiditis	Hypothyroidism/ thyroid atrophy
Grave's disease	Hyperthyroidism /goitre

Materials and methods

Included in this study were 410 patients, which have an history of thyroid disorders, each of them underwent the following investigations: blood tests for thyroid function (T_3, T_4 , TSH), thyroid peroxidase antibodies, and antithyroglobulin antibodies. Thyroid function tests and antibodies were estimated by the chemiluminiscent method. Following are the values of the reference ranges for thyroid function and antibody tests in our laboratory: T_3(0.8-2ng/ml > 21 years); T_4 (5.13-to-14.06 µg/dL); TSH (0.27-4.12 uUI/ml);; Anti peroxidase antibodies (< 34 IU/ml) and antithyroglobulin antibodies (< 115 IU/ml).Data entry was done in MS. Excel and analysis were done using SPSS20. Statistical analyses were performed using Chi- square and Pearson correlation test. P-value less than 0.05 were statistically significant.

Table.2.Age distribution of patients

Age	*Male*	*Female*	*Total*	*%*
20-30	14	91	105	25.6
31-40	42	118	160	39.2
41-50	23	61	84	20.4
51-65	27	34	61	14.8

Results

Of 400 patients, 304 were females and 106 were males, the age of the studied patients ranged from 20 to 65 years.

Thyroid peroxidase antibodies were estimated in all patients; 342 patients (83.4 %) tested positive and 68 (16.5%) tested negative. Antithyroglobulin antibody estimation was also done in all patients; 236 patients (57.5 %) tested positive and 174 patients (42.4 %) tested negative (Table III).Of the 342 patients who were positive for thyroid peroxidase, 217 (63.4 %) were hypothyroid; 31 (9 %) were hyperthyroid. and 94 (27.4%) were euthyroid. However, all patients who were negative for thyroid peroxidase antibodies (100%) were euthyroid. This enhances the predictive value of positivity for thyroid peroxidase antibodies and thyroid dysfunction. Of 236 patients who were positive for antithyroglobulin antibodies, 135 (57.6%) were hypothyroid; 16 (6.7%) were hyperthyroid, and 78 (33.1%) were euthyroid. Also, of the 174 patients who were negative for antithyroglobulin antibodies, 102 (58.6%) were hypothyroid, 9 (5.2%) were hyperthyroidism and 63

(36.2 %) were euthyroid. In predicting hypothyroidism (Table IV), thyroid peroxidase antibodies were much more sensitive than antithyroglobulin antibodies (99.5% vs 56.9%). About correlation of Anti-TPOand Anti-TG with TSH and other hormones, was a low correlate r=0.90 and r=0.87respectively.

Table.3.Assay results of antibody levels

Antibody	*Positive*	*Negative*
Anti-TG*	236	174
Anti- TPO**	342	68

** Antithyroglobulin Ab. ** Thyroid peroxidase Ab*

Table .4.Correlation between antibody levels and thyroid status

Antibody	*Status*	*Number of patients*	*Number and % of hypothyroid patients*	*Number and % of hyperthyroid patients*	*Number and % of euthyroid patients*
Anti –TPO	Positive	342	217 (63.4%)	31 (9%)	94 (27.4 %)
	Negative	68	1 (1.5%)	0	67 (98.5%)
Anti -TG	Positive	236	135 (57.6%)	16 (6.7%)	78 (33.1%)
	Negative	174	102 (58.6%)	9 (5.2%)	63 (36.2%)

Discussion

The prevalence of autoimmune thyroiditis is increasing. We observed every day that about a large number of patients who present to outpatient clinics in our geographical region with thyroid problems have autoimmune thyroiditis.. However, inflammation of the thyroid gland early in the disease may cause thyroid follicular destruction with thyroid hormonal release from the follicles. This results in transient hyperthyroidism. These antibodies can even lyse the thyroid cells. B cells present the thyroid antigen to T cells. T cells secrete cytokines that activate a variety of other immune cells, and has a role in antibody production (Th2 cells) and apoptotic destruction of thyroid cells by activating cytotoxic T cells (Th1 cells). Genetic and environmental factors such as toxins, bacterial and viral infections or iodine excess, appear to interact, leading to the appearance of auto antigens and accumulation of antigen-presenting cells in the thyroid. Consequently, due to loss of immune tolerance, auto-reactive immune cells (T lymphocytes) activated by antigen-presenting cells invade the thyroid gland, interacting with the thyroid cells, and the apoptotic pathways are activated by certain cytokines produced locally by the T lymphocytes. It is likely that the regulation of apoptosis during this interaction between the invading lymphocytes and the defending thyroid cells, may play an important role in the clinical expressio. Of the patients in this study, 74.1% were female, and 50.9 % of these were in the reproductive age group. Since thyroid dysfunction can lead to antenatal and neonatal complications, the diagnosis and correction of any thyroid disorder is very important in pregnant patients. Thyroid autoimmunity is a risk factor for pregnancy loss.

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RESEARCH ARTICLE **(Open Access)**

Inheritance of Spike Traits in F1 Generation in Wheat Depending on Parents' Genetic Diversity

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Abstract

The aim of research was to assess the inheritance of wheat spike length (SL), based on the parents diversity as genes donors, identification and determination the best cross combinations in the F1 generation, as well estimation of heterosis indicators as relation: parents/offspring (P1/F1 and P2/F1). Field trials were conducted during the years 2016/17 and 2017/18, at the Experimental Didactic Farm of the Faculty of Agriculture and Veterinary in Prishtina. Experimental design was a RCBD, with plot size $1m^2$ in three replications.The experimental formula was: Parents (20-P) +F1-genotypes (10-GF1) x Replications (3-R) x Plants (10P) =900 results. The mean genes value effect (μ) and genotype variation (gv) for (SL)were: for female parents set (P1) μ = 8.99cm $spike^{-1}$and gv = ±38.93 %, for the male parents set (P2) μ=8.40cm $spike^{-1}$ and gv= ±39.66 %, for mid-parent (MP), the μ value was 8.69cm $spike^{-1}$ and gv = ±30.67%; while for better parent (BP) μ=9.31cm $spike^{-1}$ and gv = ±37.61%, and for F1 generation μ=10.07cm $spike^{-1}$and gv = ±22.74%. Magnitude of variation for heterosis (Ht) was 3.47 to 35.30% and for Hb was -11.39 to 28.57%. Through, cross combination of parents, it was possible to identify the superior genotypes in F1 generation: G5; G13 and G24 for SL (11.0cm $spike^{-1}$). The obtained results were with wide range variability and highly significant differences between parents and genotypes of the F1 generation, on the level of LSDp=0.05 and LSDp=0.01.

Keywords: Wheat, inheritance, parents, spike, F1-generation, hetrosis.

1. Introduction

In principle wheat production is important in both terms: for grain and seed, due to the fact of its use for food and as reproduction material (Fetahu et al., 2017; 2019). Food production, in sufficient and high quality, healthy and economically reasonable, today is a challenge for mankind, but this challenge will be even greater in the future (Fetahuet al., 2013a; 2015a).

The precondition for developing of new genotypes with high production capacity, requires genetic distance among parents that participating in the breeding program (Martin et al. 1995; Morgan, 1998; Dedaniya et al., 2018). Parental impact in the development of new cultivars and the efficient identification of superior combinations is a fundamental issue in plant breeding programs (Morgan, 1998; Gowda et al., 2010). Therfore, it was suggested that the heterosis in relation to the better parent could be useful to optimize heterozygous combination (Kant et al.,2011; Fetahu et al., 2015a). The crop cultivation and realized yield per unit area depends on the influence of factors such as: genotype, environment and applied agrotechnique from the moment of sowing until harvest, including the economic cost (Fetahu et al., 2014b). Information on genotype variability to environmental changes helps to predict the yield of cereals (Yong et al 2004; Fetahu et al., 2014a). Cultivar selection for a specific location is one of the essential challenges for improving yield for grain or seed, without increasing the cost of wheat production (Fetahu et al., 2013b; 2014b; 2019). The goal of each breeding program is to obtain high yielding cultivars and excellent quality, which meets market requirements (Williams et al. 2008). Hybridization is the crossing of two cultivars differing from each other in one or more characters to generate offspring with new desirable characters, as a result of genetic recombination. The hybrid of F1 generation is

superior over either parent for one or more characters, and it is known as hybrid vigor or heterosis (Fetahu et. al., 2015a). From the perspective of the breeder, heterosis of superiority over BP, is more effective than heterosis of MP, particularly in the breeding of self-pollinating crops, when the objective is identification of superior genotypes in F1 generation (Fetahu et al., 2015a). The variability, heritability, and components of phenotypic variation for spike length are important for the cultivar creation, as it is recommended by Kraljevic-Balalic et al. (1995). Selection of parents with genetic diversity is the crucial stage from the standpoint of breeding programs in order to develop new genotypes having desirable characters. Therefore, the aim of the research was to assess the inheritance of wheat SL, based on the diversity of parents as genes donors, identification and determination of the best cross combinations in F1generation, as well estimation of heterosis indicators as relation parents/offspring (F1/MP and F1/BP).

2. Material and Methods

2.1. Plant material and experimental design

Twenty parents with genotype diversity and variability were included in the combination and crossing program in order to obtain the hybrid generation in F1. Diversity of crossing parents, both with genotypes of F1 generation were: Jagger x Brea/F1-G5; Lira x Brea/F1-G7; Lorena x Brea/ F1-G12; Gabi/Boema x Gabi/F1-G13; Marina/Dora x Marina/F1-G14; Soys248 x Apach/F1-G16; Victoria/Mambo x Victoria/F1- G18; Lorena x Euclid/ F1- G19; Anica x Srpanjka/F1- G22 and Boema x Lutescens/F1-G24.

Field trials were conducted in randomized complete block design with three replications (RCBD) at Experimental Didactic Farm (EDF), Faculty of Agriculture and Veterinary (FAV) in Prishtina, located in geographical position: 42°38'97"N and 21°08'45"E, altitude of 570 m.a.s.l.

In the autumn of 2015, the seed of the parents were sown in plastic pots, under greenhouse conditions, while in May 2016, emasculation of female parents was done, and then crossings were realized. Then, hybrid genotypes obtained in F1, both with parents, were sown in micro-plots with 500 seeds per $1m^2$in autumn of 2016. During the growth period, normal agronomic and plant protection care was applied, except irrigation. Plant harvest was done during the sumer of 2017 at full maturity stage, with maxiumum of 14% grain moisture.

2.2. Measurements

After harvesting, the spike length (SL) was determined in 10 plants, for parents P1, P2 and F1. After that, the spike length of the mid-parents (MP) and the better-parents (BP) is determined.

The experimental formula was: EF = 20-Parents + 10-GF1-Genotypes x 3-Replications x 10-Plants = 900 spikes. Heterosis indicators (Ht) and (Hb) are defined as percent increase or decrease of F1 genotype over MP as wellas BP. The possible heterotic effects, was estimated according to formula of Fonseca and Patterson (1968):

$$\boldsymbol{Ht}(\%) = \frac{\boldsymbol{F1 - MP}}{\boldsymbol{MP}}\boldsymbol{x1}$$

$\boldsymbol{Hb}(\%) = \frac{\boldsymbol{F1-BP}}{\boldsymbol{BP}}\boldsymbol{x100}$ (Fetahu et al., 2015b)$\boldsymbol{gv}(\%) = \frac{\boldsymbol{Xg-\mu}}{\mu}\boldsymbol{x100}$

Where,*gv-genotype variability; Xg-average genotype value, μ-mean value population;Ht-hetrosis and Hb-heterosis for beter parents.*

2.2. Statistical analysis

The research data were subject of ANOVA, according to the program MINITAB-18©, while the significance is determined for the level of probability P_{005} and P_{001}. The parent's variation P1; P2 and F1, as well as the effect of the parents and the interaction of P1/F1 and P2/F1 were calculated.

3. Results and Discussion

Spike length (SL), has been considered one of the most important contributing factors associated with increases of wheat yield that have occurred in the

modern plant breeding. Average results of our research and comparisons for general and genotype variation (gv) for P1; P2; F1; MP and BP are presented in (Table 1), while heterosis indicators for F1/Ht and F1/BP in Figure 1. The variance analysis for spike length shows differences among parental geneotypes P1; P2 and F1; Parents x F1 interaction was also highly significant (Table 2). The significant difference indicates the existence of genetic variability among the parents that is precondition for cross breeding, as objective to obtain F1 genotypes with high heterosis (Ht) over MP and BP.

The average spike length for the parent's set P1 was μP1= 8.99cm spike^{-1}. Among the parents with the maximal and minimal values, were cultivars: Lira with 10.5cm spike^{-1} and Victoria with 7.0cm spike^{-1}, the differences between them were 3.5cm spike^{-1}, and the interval of general variation was gv=±38.93%.

Cultivar Lira, compared with the μP1 value, had a longer spike for 1.51cm or gv = +16.78%, while Victoria cultivar, had shorter spike for -1.99cm or gv = -22.14%. The P2 parent set had average spike length of μP2 = 8.40cm spike^{-1}. While, the genotype Gabi / Brea had maximal length (10.33cm spike^{-1}), whereas cultivars: Victoria and Srpanjka, had minimal length or 7.0cm spike^{-1}, and the difference was 3.33cm or the general variability of gv = ±39.66%. The genotype Gabi/Brea compared to the μP2 value, had longer spikes +1.93cm or gv = 22.98%, while the cultivars Victoria and Srpanjka differed for -1.4cm spike^{-1} or gv = -16.67%.

Average spike length for mid parent (MP) was μMP = 8.69cm spike^{-1}. Genotypes with maximal and minimal values were: G-16 with 9.67cm spike^{-1}, respectively G-7 with 7.0cm spike^{-1}, with a difference 2.67cm spike^{-1} or with general variability gv = ±30.67%.

The combination, Soys248 x Apach/G-16, had longer spike for 0.97cm or gv = 11.22%, respectively the combination Victoria/Mambo x Victoria/G18, was shorter for -1.69cm spike^{-1} or gv = -19.48%.

The average spike length for the better parent (BP) was μBP = 9.31cm spike^{-1}. The genotypes G-7 and G-18 differs between them for 3.5cm spike^{-1}, with a general variation interval of ±37.61%. Genotype G-7, had longer spikes than μBP for 1.19cm or gv = 12.82% and genotype G-18, had shorter for -2.31cm or gv= -24.79%.

Table 1. Spike length (cm) in F1

Nr.	*P1*	*gv (%)*	*P2*	*gv(%)*	*MP*	*gv(%)*	*BP*	*gv(%)*	*F1*	*gv(%)*
G-5	8.60	-4.34	7.66	-8.81	8.13	-6.44	8.60	-7.63	11.00	9.24
G-7	10.50	16.80	7.66	-8.81	9.08	4.49	10.50	12.78	10.00	-0.70
G-12	7.83	-12.9	7.66	-8.81	7.75	-10.87	7.83	-15.90	10.00	-0.70
G-13	9.83	9.34	8.50	1.19	9.17	5.47	9.83	5.59	11.00	9.24
G-14	9.66	7.45	9.33	11.07	9.50	9.26	9.66	3.76	10.00	-0.70
G-16	9.00	0.11	10.33	22.98	9.67	11.22	10.33	10.96	10.00	-0.70
G-18	7.00	-22.14	7.00	-16.67	7.00	-19.45	7.00	-24.81	9.00	-10.63
G-19	7.83	-12.90	9.66	15.00	8.75	0.63	9.66	3.76	10.00	-0.70
G-22	9.83	9.34	7.00	-16.67	8.42	-3.16	9.83	5.59	8.71	-13.51
G-24	9.83	9.34	9.16	9.05	9.50	9.26	9.83	5.59	11.00	9.24
μ	8.99		8.40		8.69		9.31		10.07	

The spikes of F1 generation were of average length μF1 = 10.07cm spike^{-1}, and exceeded the spike length of parents set P1 and P2. F1 genotypes, in relation to the μP1 values, were longer for 1.08cm spike^{-1}or genotype variation was gv = 10.72%, whereas with μP2, the difference was 1.67cm spike^{-1} or gv = 16.58%. Three genotypes: G5/F1; G13/F1 and G24/F1, were 11.0cm long, while the G22/F1 genotype had a length of 8.71cm spike^{-1}, the difference between them was 2.29cm spike^{-1}, with a total variability of ±22.74%. Genotype variability for G5/F1; G13/F1 and G24/F1, compared with μF1, the

difference was +0.93cmspike^{-1} or gv = 9.24%, while with G22/F1 genotype, the difference was -1.36cm spike^{-1} and gv = -13.51%.

The results for spike length are in agreement with data of Ullah et al. (2014), who reported results for spike length for the parents from 9.57 to 12.87cm, but not matched the results of Rasul et al. (2002), who reported SL=15.50cm among parents. The similar results were obtained by Fetahu et al. (2008). In the studies of Bilgin et al. (2011) they obtained different results for SL at different wheat genotypes from 8.2 to 11.0cm. Among the F1 hybrids, the maximal spike length was 10.0cm, while the minimal spike length was 8.6cm. Our results are higher for genotypes of F1 generation. These results are also in accordance with Fetahu et al. (2014a; 2015b; 2016); Kant et al. (2011) and Mirza et al. (2017), but not with the results of Kondić et al. (2017) who reported shorter SL.

Heterosis (Ht and Hb): Results and effects of spike length inheritance for F1generation, for heterosis indicators: Ht/F1/MP and Hb/F1/BP are presented in Fig. 1. The magnitude of heterosis provides a basis for genetic diversity and guideline for the selection of desirable parents for the development of superior F1 hybrids in order to exploit the hybrid vigor and for building gene pool to be exploited in population improvement.

All genotypes of F1 generation exceeded MP values, and Ht had positive values with variation intervals of 3.47 to 35.30%, which is realized in genotypes G-16 and G-5. However, such an indicator did not identify all possible differences of MP and F1 values. The heterosis indicator Hb, as a BP/F1 ratio, identified two opposite directions of the inheritance for parents exceed for the spike length. The highest desirable heterosis of F1 generation, over BP, was recorded in cross breeding combination G-5 (Jagger x Brea) with Hb=35.30%, while with negative Hb was the genotype G-22 (Anica x Srpanjka), with Hb=-13.31%.

Table 2. The variance analysis for spike length in parentsgeneotypes P1; P2 and F1

	Parental (P1 and P2)					*F1 generation*			
S. variation	**d.f.**	**MS**	**F**	**LSD0.05**	**Lsd0.01**	**SS**	**F**	**$LSD_{0.05}$**	**$LSD_{0.01}$**
Repition	2	0.175	0.41	/	/	0.0121	0.028	/	/
Tretmans	9	3.915	9.419**	1.1061	1.5152	1.717	4.025**	1.1207	1.5352
Error	18	0.415				0.4026			
Total	29								

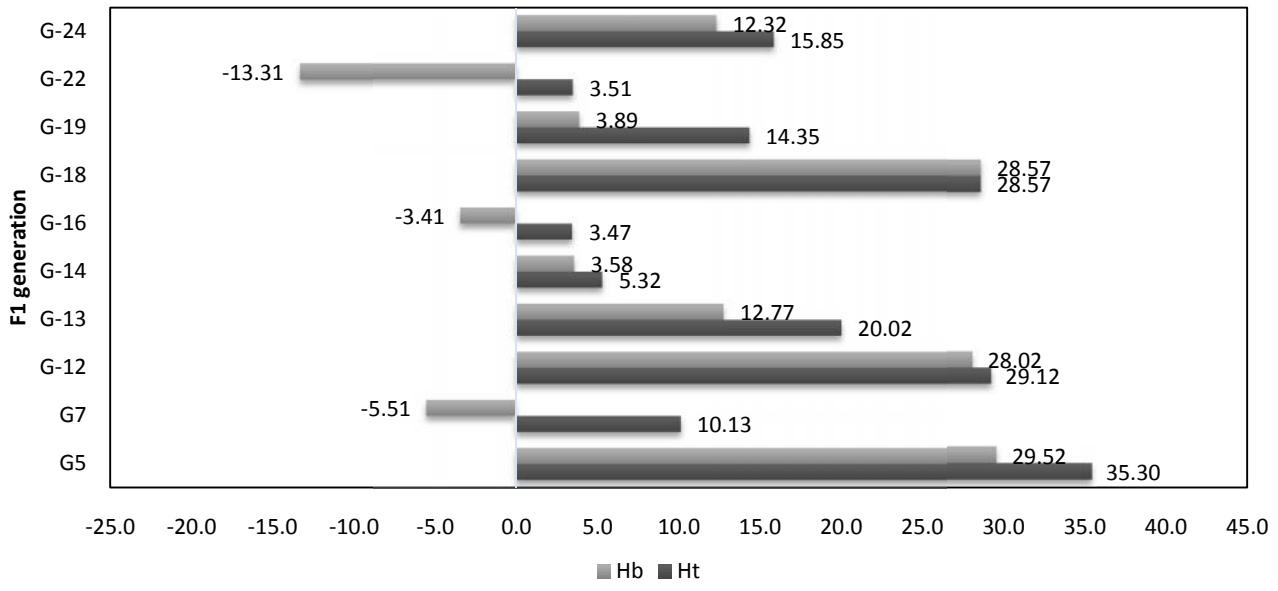

Figure 1. Heterosis inheritance

Table 3. The effect of inheritance from parents to the F1 generation

P1 /F1					
Sources of variation	**d. f.**	**SS**	**F**	**LSD0.05**	**Lsd0.01**
Blok	2	0.063	0.15	/	/
Parents (A)	9	3.869	9.54**	0.640	0.876
F1-generation (B)	1	12.97	31.98**	0.388	0.529
Interaction A x B	9	1.764	4.350 **	1.077	1.471
Total	59	0.405	/		
P2/ F1					
Blok	2	0.154	0.210	/	/
Parents (A)	9	4.075	5.54**	1.040	1.425
F1-generation (B)	1	40.671	157.85**	0.273	0.372
Interaction A x B	9	1.511	5.86**	1.205	1.648
Total	59	0.257	/		

Rasul et al. (2002), has reported positive results for heterosis over MP and over BP, with values of Ht = 7.42% and Hb = 6.24%, while our results are significantly higher. The positive heterosis for the spike length is a desirable trait, especially those that exceed BP. In their research Bilgin et al. (2011), reported magnitude of heterosis for SL over MP (8.77 to -18.18%) and BP (8.14 to -25.64%), similar to our research results.

Inamullah et al. (2006), for the Ht and Hb values, also reported different results (+19.11 and +16.2), while Zaazaa, et al. (2012), reported values of MP, Ht (15 to 20.59%) and Hb (13.02 to 19.60%)

4. Conclusions

The identification of the highly significant differences of genotype variation for the spike length and the hterosis indicators for the three sets (P1, P2 and F1), shows the presence of parental diversity, combination abilities through crossing (intraspecific hybridization), to create heterozygous organisms in generation F1, with hybrid vigour.

Research findings identified three superior hybrid genotypes in F1 generation: G-5; G-13 and G-24, obtained from the parents: Jagger x Brea; Gabi/Boema x Gabi and Boema x Lutescens.

Heterosis in relation to mid-parent (Ht/MP) was positive, while the Hb/BP ratio identified the heterosis with two-opposite directions. Therefore, Hb enable to identify parents who have better combinations ability during crossing and expresses maximal heterosis.

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RESEARCH ARTICLE **(Open Access)**

Variability of chamomile (*Matricaria chamomilla* L.) populations as a valuable medicinal plant in Albania evaluated by morphological traits

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Abstract

Study for the evaluation of variability of 30 chamomile (*Matricaria chamomilla* L.) samples, representing 10 chamomile populations collected in nine different natural growing areas of Albania (Berat, Fier, Skrapar, Tirana, Lezha, Lushnja, Korca, Kucova and Kruja), using morphological traits was carried out in the Experimental field of Agricultural University of Tirana, during two growing seasons.The 10 chamomile populations, grown in a randomized block with three replications, were assessed by seven quantitative morphological characters (plant height, number of principal branches, length of internodes, leaf length, number of cones produced per plant, fresh cones yield and dry cones yield). ANOVA, PCA on correlation and cluster analysis reveal presence of significant diversity, and the association among different morphological characters. Comparisons of means for each pair using Student test ($t = 2.08596$ and $\alpha = 0.05$) show the significant differences between chamomile genotypes at the $P_{0.05}$ and $P_{0.01}$ levels of the probability. Relationships between the morphological characters and chamomile genotypes using Principal Component analysis found the morphological traits: plant height, number of cones produced per plant, leaf length and internode length as the most important for the PC1 that account for 36.46% of the total variance, and internode length, fresh and dry yield of cones for the PC2 that account for 31.24% of the total variance. Cluster analysis (Ward's method) divides the whole chamomile populations into three cluster groups. Correlation relationships among morphological quantitative traits range from 0.27 (internode length and dry yield of cones) to 0.76 (number of cones produced per plant and leaf length). The study identifies number of cones produced per plant, leaf length and internode length as characters with agronomic interest which account for evaluation of genetic diversity and breeding programs of chamomile populations.

Keywords: Chamomile(*Matricaria chamomilla)*, morphological characters, cluster analysis.

1. Introduction

Chamomile (*Matricaria chamomilla* L.) is one of the important medicinal herb native to southern and eastern Europe. Chamomile is a widely recognized herb in Western culture. Its medicinal usage dates back to an[2]. Chamomile has been used in herbal remedies for thousands of years, known in ancient Egypt, Greece, and Rome[8].

Chamomile (*Matricaria chamomilla* L.) is a well-known medicinal plant species from the Asteraceae family often referred to as the "star among medicinal species." Nowadays it is a highly favored and much used medicinal plant in traditional medicine. It's multitherapeutic, cosmetic, and nutritional values have been established through years of traditional and scientific use and research. Chamomile is a rich source of natural products, and its chemical constituents of essential oils and plant parts have valuable pharmacological properties. Today chamomile is a common ingredient in herbal teas because of its calming, carminative, and spasmolytic properties. It is also a popular ingredient in topical health and beauty products for its soothing and anti-inflammatory effects on skin. Chamomile is used mainly as an anti-inflammatory and antiseptic, also antispasmodic and mildly sudorific [13]. The chamomile drug is included in the pharmacopoeia of 26 countries[14] because of several pharmacological actions as antibacterial, antifungal, anti-inflammatory, antispasmodic, anti-ulcer, antiviral, and sedative effects.The flowers of *M. chamomilla* contain the blue

essential oil from 0.2 to 1.9%, [4];[12] which finds a variety of uses.

There are two species of chamomile, generally, used in traditional herbalism, *Matricaria chamomilla or Chamomilla recutita*(German chamomile or Hungarian chamomile), and *Chamaemelumnobile*(Roman chamomile). In most formularies and reference books, *Matricaria chamomilla* L. is regarded as the correct species name. However, according to the International Rules of Botanical Nomenclature, *Chamomilla recutita*(L.) Rauschert is the legitimate name for this species [17]. Both are annual herbs belong to the Asteraceae/Compositae family and are similar in physical appearance, chemical properties, and general applications. German chamomile, however, is the more familiar and more commonly used of the two. The two species of Chamomile (Roman and German) have been used for medicinal purposes for more than a thousand years [11]. Both species are among the most widely used medicinal plants in the world.

Chamomile plant is considered indigenous to northern Europe and grows wild in central Europeancountries; it is especially abundant in Eastern Europe including Albania. Chamomile is also found in westernAsia, the Mediterranean region of northern Africa, and the United States ofAmerica. It is cultivated in many countries [1]; [21]; [20].

Albania with its geographical position in the Mediterranean region and in the Balkan Peninsula is very rich in medicinal and aromatic plants. Medicinal and aromatic plant collections of Albania, compounded by more than 300 species (in the ex situ, in situ and on farm status of conservation), and represent about 10% of the Albanian flora[3].Medicinal and aromatic plants are economically important plants grown over a wide range of ecological habitats in the country [15];[18];[7]. Chamomile plants *Matricaria chamomilla* (L.) are collected and used as raw materials in the pharmaceutical, cosmetic and food industry [15].

Domestic and foreign markets for medicinal plants are growing rapidly and provide important opportunities for the development and diversification of Albanian agriculture. Currently, sage (*Salvia officinalis*) dominates the medicinal crops of Albania. Smaller surfaces are also cultivated with other medicinal species including chamomile (*Matricaria chamomilla).*Despite its economic importance, however, chamomile is little known about the extent and nature of the variability of this species. Therefore, the information about extent of uses of various gene pools are extremely valuable for the rational planning of the use of germplasm in breeding programs [22];[7];[6].

The aim of the study was the analysis of variability between chamomile (*Matricaria chamomilla)* populations growing naturally in different areas of Albania, and identification of the most important morphological characters, to be used in breeding programs of chamomile germplasm.

2. Materials and methods

Plant material: The analysis was performed on ten 10 chamomile (Matricaria chamomilla L.) populations, originated from nine collecting areas of Albania (Berat, Fier, Skrapar, Tirana, Lezha, Lushnja, Korca, Kucova and Kruja).

Morphological characters: Plant height (PH) (cm), Number of branches per plant (No.br), length of internodes (L.Int), leaf length (LL), number of cones per plant (No.C), fresh yield of 1000 cones (YF) and dry yield of 1000 cones (YD).

The experimental site and field observations: The study for the assessment of chamomile variability was carried out at the experimental field of Agriculture University of Tirana (latitude: 402405N; longitude: 0194108E; elevation: 40m). Chamomile populations were grown in a randomized block design with three replication and field observations and measurements of morphological traits were realized on 20 plants of each plot.

Statistical Analysis: The differences between chamomile populations for the mean values of

quantitative traits evaluated were carried out using ANOVA analysis. Identification of chamomile populations of relatively similar or different characteristics and identification of the most important morphological characters that influence highly on the total variation, was realized using Principal Components Analysis (PCA) on correlation and cluster analysis methods. All statistics data were calculated employing the [19], and a dendrogram (ward method) and a relationship diagram (chamomile populations x morphological characters) were carried out.

3. Results and Discussions

Analysis of morphological quantitative traits: ANOVA analysis found the presence of differences between chamomile populations for the most number of morphological traits significant at the probability $P_{0.05}$ and $P_{0.01}$. Except leaf length (LL), all other traits were significant at the probability $P_{0.05}$ (Table 1). High degree of variation was observed for No.C, No.br, and YF traits.

Tab.1. Analysis of variance for all chamomile traits analysed

Source of Variance. Traits	Sum of Squares (population) (df=9)	Sum of Squares (Error) (df= 20)	C. Total (df = 29)	Mean Square (population)	Mean Square (Error)	F' Ratio	Prob>F
PH	103.57575	41.95357	145.52932	11.5084	2.0977	5.4863	0.0008*
No.br	1.0084300	0.059000	1.0674300	0.112048	0.00295	37.9823	<.0001**
L.Int	0.9542133	0.0519333	1.0061467	0.106024	0.002597	40.8307	<.0001**
LL	0.8187867	0.9584000	1.7771867	0.090976	0.047920	1.8985	0.1115
No.C	848.5787	538.5933	1387.1720	94.2865	26.9297	3.5012	0.0094*
YF	3631.4083	359.1667	3990.5750	403.490	17.958	22.4681	<.0001**
YD	124.35867	18.80000	143.15867	13.8176	0.9400	14.6996	<.0001**
F ratio values significant at the $P_{0.05}$ level of the probability (*) and $P_{0.01}$ level of the probability (**).							

Principal Components Analysis: Principal Components Analysis on Correlations identified the variances of the principal components and the proportion of the total variance each factor accounts for (Table 2). Based on the mineigen criterion [10] three principal components that account for 84.5% of the total variation are retained for further analysis. PCA results show that the major sources of variation in the measurements are given by the first two PCs (67.7%).

All quantitative variables contribute to 100% of total variation. The percentages of total variation accounted for by each of the first three PCs are 36.5%; 31.2% and 16.8% respectively (Table 2). The first three PCs explain 84.5% of the original variation, and the variation > 80.0% is acceptable for evaluation of plant populations in general and for characterization of plant collections in a genebank [5]; [9].

Tab. 2. Eigenvalues of principal components (chamomile populations x morphological traits)

Principal Components/factor analysis						
PC No.	Eigenvalue	Percent variance	Cumulative Percent	χ^2	df	Prob. > χ^2
1	2.5521	36.459	36.459	129.455	19.859	<.0001*
2	2.1868	31.241	67.699	103.760	17.182	<.0001*
3	1.1789	16.841	84.540	65.681	14.112	<.0001*
4	0.5532	7.902	92.443	36.730	9.643	<.0001*

χ^2 – Chi Square, df–degree of freedom; Prob.–probability; *significance equal to 0.05 / 0.01 of probability

The maximum information from morphological data was received using ordination methods in combination with cluster analyses [**9**]. Dimensional scaling of relationships (chamomile populations x morphological traits) that contributes for the larger proportion of the total variance in PC1, PC2 and PC3 revealed by PCA indicate that the contribution of each chamomile populations and of each quantitative morphological traits on the total of variation was found not equal.

PCA and cluster analysis found 10chamomile samples included in PC1 representing the characteristics of three chamomile populations (Karbunare, Verbas and Dukas), 8 chamomile samples included in PC2 representing especially the characteristics of two chamomile populations (Poshnje and Provnik), and twelve chamomile samples in PC3 representing the characteristics of three chamomile populations (Mat, F. Preze and Kozare). Two chamomile populations as Larushk and Sheqeras have presented mix of characteristics like chamomile populations of PC3 and PC1 (Table 3, Figure 1; Figure 2).

Tab. 3. Matrix of vectors of three PC for 10 chamomile populations x 7 morphological traits

No	Morphological quantitative traits		PC1	PC2	PC3
1	Plant height	PH	0.51859	0.10957	-0.27035
2	Number of branches per plant	No.br	-0.12810	0.14227	0.83706
3	Length of internodes	L.Int	0.31650	0.33258	0.37598
4	Leaf length	LL	0.54347	0.00746	0.17438
5	Number of cones per plant	No.C	0.55962	-0.14228	0.02963
6	Fresh yield of 1000 cones	YF	-0.06664	0.64893	-0.15723
7	Dry yield of 1000 cones	YD	-0.03883	0.64477	-0.16992

In bold all eigenvectors > 0.30

For PC1 (with 36.5% contribute on the total variation) characters as PH, L.Int, LL and No.Cwere the most important source for the variation of PC1.The characters as No.br, YF and YD showed not important negative influence on the PC1 variance (Table 3, Figure 1).

Variation in PC2 (= 31.2% of total variation) was mainly result of differences in L.Int, YF and YD morphological traits. Traits as YF and YD account for nearly the same amount of variance on PC2 (Table 3, Figure 1).

For PC3 (with only 16.8% contribute on the total variation) characters as No.br and L.Intwere the most important source for the variation on the PC3 variance. The characters as PH, YF and YD showed negative influence on the PC3 variance (Table 3, Figure 1).

Cluster analysis: Relationships between 30 chamomile samples that represent 10 chamomile populations assessed by quantitative morphological traits and genetic similarity/distances coefficients revealed by cluster analyses categorized all chamomile samples (populations) into three clusters (Figure 2).

There were five morphological quantitative traits of PC1 and PC2 that have influenced on the differentiation of three clusters. The first cluster (with ten samples of three chamomile populations) was differentiated by PH, L.Int, LL and No.C traits of PC1.Correlation analysis found moderate and strong positive correlation between these characters (r range from 0.36 to 0.76). Negative correlation was found between PH and No.br (r = - 0.33). The second cluster includes eight samples of three chamomile populations and was differentiated by L.Int, YF and YD traits of PC2.Moderate positive correlation between these traits was found (r range from 0.27 to 0.33).The third cluster includes twelve samples of three chamomile populations and was differentiated by No.br and L.Int traits of PC3 (Figure 2).

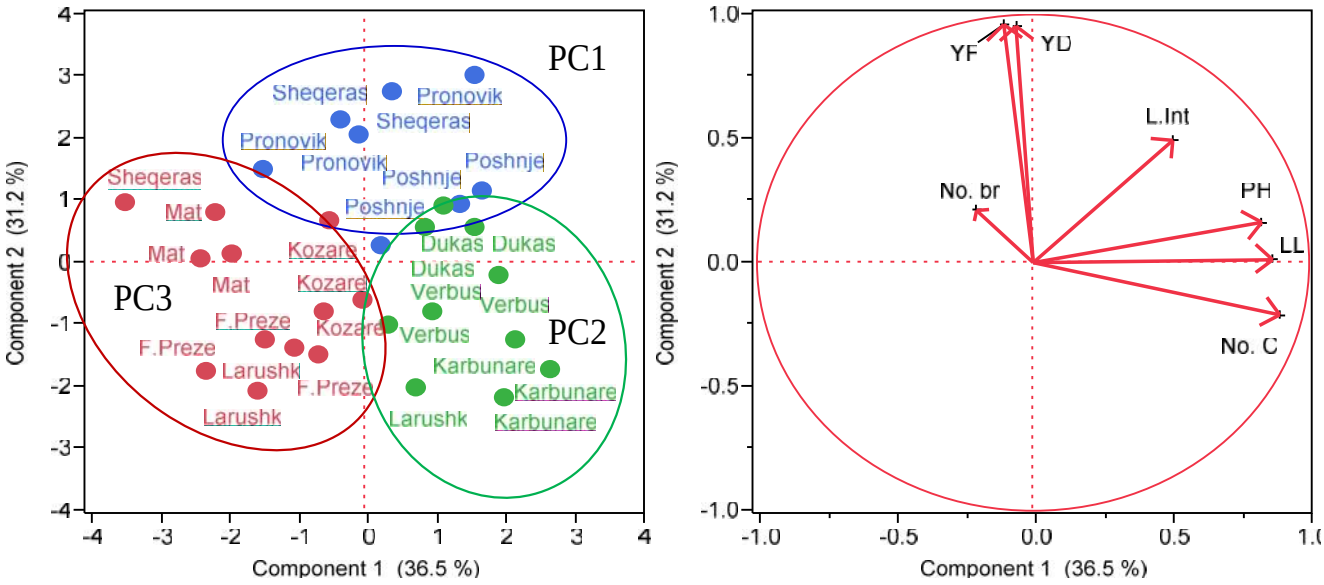

Fig. 1.Relationships among 10 chamomile populations on morphological traits revealed by PCA

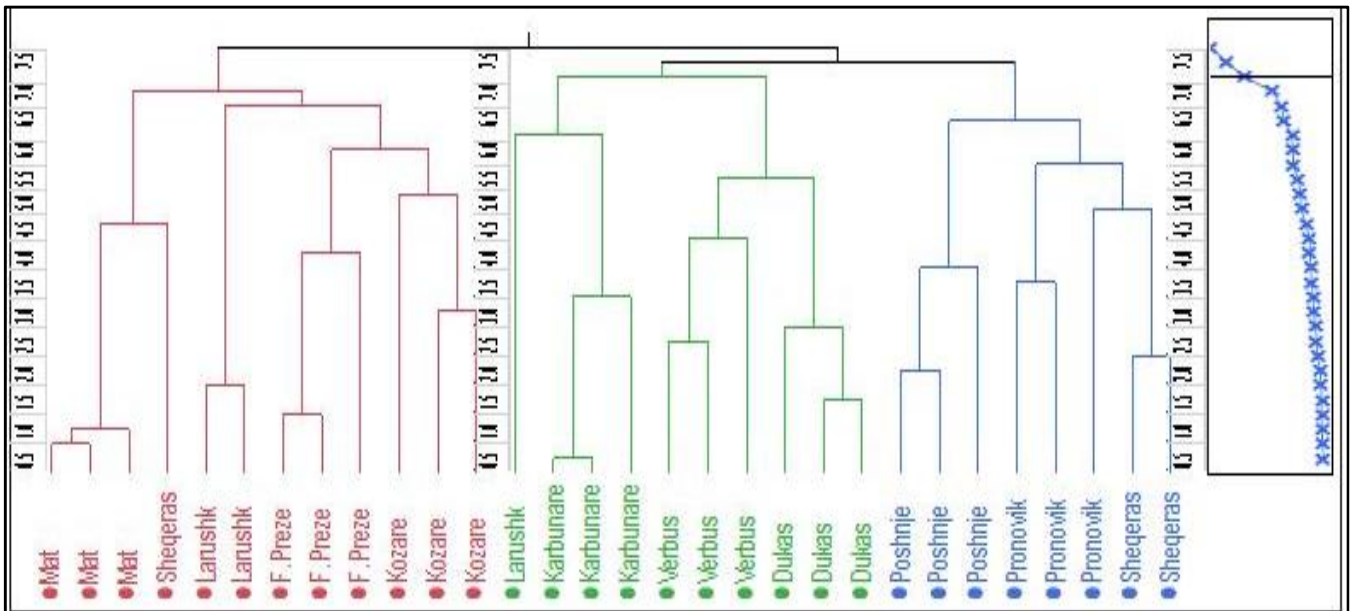

Fig. 2. Dendrogram of relationships among chamomile populations and morphological quantitative traits

PCA and cluster analysis found clear differences among following chamomile populations collected in Karbunare, Verbas, DukasPoshnje, Provnik, Mat, F. Preze and Kozare. Chamomile populations collected in Larushk and Sheqeras sites present a mix of characteristics. So, Larushk chamomile show similar morphological characteristics with chamomile collected in F. Preze and Karbunare. AndSheqeras chamomile show similarity with chamomile collected in Provonik and Mat (Figure 2).

Knowledge of genetic similarity or distances generates a better understanding of germplasm sampling. Separation of chamomile populations into three clusters could be useful with regard to chamomile cultivation and chamomile breeding purposes.

4. Conclusions

The field evaluation test permitted the first evaluation of chamomile populations collected in different areas of Albania and identified the most

important morphological traits with high medicinal value.

PCA and cluster analysis identified the morphological traits with more significant weighting on PC1 variance (PH, L.Int, LL, No.C), which can be used successfully as morphological markers for evaluation of the chamomile germplasm.

The amount of genetic variability found in this study is sufficient for selection of desirable traits for creation of new gene combinations to sustain chamomile breeding programs.

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RESEARCH ARTICLE **(Open Access)**

Analyse of life forms and floristic elements of some medicinal plants in Bredhik Reserve, Sharr Mountain in Kosovo

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Abstract

This study represents the results for inventory of medicinal plants on the territory of Bredhik Natural Strict Reserve (Sharr Mountain, Kosovo) during 2018. The inventory of medicinal flora, are made for the first time in this area. The study shows the presence of 92 taxa within 70 genera and 42 families. Their taxonomic structure, life forms and biological types are discussed. Detailed information on their phytogeographical structure by major floral types and conservation status of taxa is presented to. The species with any conservation status by the international legislation are 60 or 65.22% of the total taxa's number. The analysis of life forms has shown that medicinal flora in this area predominates by hemicryptophytes (43 species) and phanerophytes (32 species). The phytogeographical elements dominated by taxa of chorological types Euro Mediterranean-Sub Mediterranean, Boreal – Subboreal, Euro-Asian and Euro-Siberian. The relic species are 16 (17.4% of the total species number) and are represented mainly by tertiary relicts. The biological types are represented mainly by perennial herbaceous plants (53.26%), followed by trees (18.48%) and shrubs (15.22%). Presence of a high number of threatened species (listed in IUCN List and European Red List of Medicinal Plants), indicates the importance of this Reserve for biodiversity conservation.

Key words*:* Flora, conservation status, relict species, medicinal plants

Introduction

Kosovo has the greatest diversity of plants and it is the habitat of over 2500 plant species, because of its geographical location and climatic diversity. The Sharr Mountain is located in the region of Kosovo and Macedonia and comprises an overall area of 1,600 km^2 (Pulaj, 2013). The territory of Sharr National Park lies in the south and expanding towards southeast of Kosovo and far beyond into North Macedoniaand to some extent into Albania at southwest side. Sharr has significant natural and ecological value, scientific, cultural, educational and recreational tourism. Within their space a huge diversity of both species and habitats exists (Tillemann, 2012). Regarding the floristic aspect they fall into the range of richest mountains in Kosovo and the Balkan's Peninsula and represent a treasure of plant species with a large number of endemic, relict, rare and endangered species (Pulaj, 2013). On this territory there are about 1500 species of vascular floras (Veselaj & Mustafa, 2015; Mustafa et al., 2018), which all belong to life forms and number of ecologic types, from the Mediterranean to the Arctic (Mejzini, 2007). Also, the area is rich in more than 250 medical plants species (Pulaj, 2013). Due to high values of biodiversity, Sharr National Park has been identified as Important Plant Area (IPA) (Veselaj & Mustafa 2015). IPAs are natural or semi-natural sites with exceptional botanical richness and/or supporting an outstanding assemblage of rare, threatened or endemic plant species and/or vegetation of high botanic value. They are unique places where the jewels of Europe's wild plants can be found (Anderson et al., 2005).

The climate is diverse, from mild continental climate to the alpine climate above 2000 m. The cold continental climate dominates the areas with an altitude between 550 and 1,000 meters, whereas in areas with altitude higher than 1,000 (1,000 – 1,700) meters there is a harsh alpine climate. The continental climate is characterized by cold and wet winters and dry and hot summers. The alpine climate is characterized by long and cold winters with heavy snowfalls and short and cool summers (Abdii et al., 2017).

On the Shar Mountain, part of Kosovo's territory, are located 16 strict protected areas, one of which is Bredhik Nature Reserve (GRK, 2016). The total area of the Bredhik is 126.16 hectares. Vertical amplitude of the study area is about 600 m (between 1200 and 1800 m a.s.l.). There is no specified information about the climate in the research area. The Bredhiku Reserve distinguished for Hellenic beech forests with *Abies borisii-regis*, an endemic species of Balkan (in Kosovo is found only in the Sharr Mountains, in Restelica), in a highness of 1500 m – 1580 m and a forest with firs (habitat *Abietum alba koritniensis*) with highness of 1100 m – 1600 m, important habitats that are classified in Annex 1 of the European Directive on Habitats 92/43/EEC (MMPH/AMMK, 2013).

Medicinal plants play an important role in the development of human cultures and these plants are consider as valuable bio resources with social and economic importance. Identification of medicinal plants is necessary in management of their use. It gets an even greater significance when a significant part of it in certain areas remains unknown. Until now, no study has been performed of the medicinal plants of Bredhik Reserve. The aim of this study was to inventory medicinal plants and to conduct floristic analysis of the data obtained. Also, their national and international conservation status was considered.

Materials and methods

This study for inventory medicinal plants on the territory of the Strict Nature Reserve of Bredhiku was conducted during 2018. Identification of the collected plants is made according to

Atlas of Kosovo plants (Berisha et al., 2012), The red book of vascular flora of the Republic of Kosovo (Millaku et al., 2013), Flora of Albania (Paparisto et al. 1988, 2000; Qosja et al.. 1992, 1996), Excursion flora of Albania (Demiri, 1983). The names of the species are under the databases of the Plant List (2013). The abbreviations of the authors' names of the plants are according to the International Plant Names Index (IPNI). The medicinal plants are according to Allen et al. (2014) and Millaku (2010; 2015).

For the classification of taxa as particular life forms, the Raunkiaer system (1934) was followed. For their determination we used different literature sources (Millaku, 1999: Millaku et al., 2013; Tomović et al., 2014; Vuksanović et al., 2016). Taxa were classified as chamaephytes (Ch), geophytes (G), hemicryptophytes (H), d phanerophytes (Ph) and therophytes (Th).

For the floristic elements, the classifications of Assyov & Petrova (2012) were used. Biological types are defined by Paparisto et al. (1988, 2000), Qosja et al. (1992, 1996), Berisha et al. (2012) and Millaku et al. (2013). The relics are presented according to some botanical literature sources (Millaku et al. 2008; Zahariev, 2016; Hajredini et al., 2013).

The conservation statute is recognized using the following documents: The red book of vascular flora of the Republic of Kosovo (Millaku et al., 2013), the IUCN Red List of Threatened Species (IUCN, 2018), European Red List of Medicinal Plants (Allen et al., 2014), Annex II to EU Habitats Directive (Directive 92/43/EEC), Appendix I to Bern Convention (CE, 1979), and

EU Wildlife Trade Regulation (Council Regulation (EC) No 338/97 of 9 December 1996 on the protection of species of wild fauna and flora by regulating trade therein) (Council Regulation 338/97). The floristic list is arranged in alphabetical order of the taxa (Table 1).

Results and discussion

An inventory of medicinal plants on the territory of Bredhik Natural Strict Reserve on Sharr Mountain is made for the first time. The total number of the found medicinal plants on the study area of the Bredhiku Reserve comprised 92 medicinal species that have been classified into 42 families and 70 genera (Tab. 1). Asteraceae and Rosaceae were the most represented families in the study area (14 sp. each). These were followed by Lamiaceae and Primulaceae (5 sp.), Cupressaceae (4 sp.), Gentianaceae and Plantaginaceae (3 sp.). The remaining families were represented by one (25 family) or two (10 family) species each.

Most genera are found in the family Asteraceae (11) and Rosacea (7), others have 1–4 species. Only 4 genera (5.71%) have 3–5 species species. Most of them have 1-2 species. Most species-rich genus were *Primula* (Primulaceae) with 5 taxa, *Prunus* (Rosaceae) with 4, *Juniperus* (Cupressaceae) and *Rubus* (Rosaceae) with 3 species each genus. The majority of genus, 44 (62.85%) are presented with one genera, and 11 (15.71%) have by 2 genus.

The individual species in a community can be grouped into various life forms classes on the basis of their growth performance. The life forms spectrum of the medicinal flora of Bredhiku Reserve can be divided into five main types (Gaf. 1). The conditions of the site reflected in the life form of species. The biological spectrum shows a pronounced hemicryptophyte character of this area. Hemicryptophytes represent 46.74 % (43 sp.) of the total number of medicinal plants. Phanerophytes was the next dominant with an overall representation with 32 species (34.78%). Geophytes and therophytes comprised 6 species (6.52%) each, and chamaephytes represented by 5 species (5.44%).

Climate determines the type of plants that exist in each ecosystem. The life form spectrum is the indicator of micro and macroclimate (Asmus, 1990). Biological range of the investigated flora has shown a variety of forest and grassland communities in the mountain area with a mild continental climate to the alpine climate. Domination of hemicryptophytes and phanerophytes in the biological spectrum can be explained by the location of the study, which is in the temperate climatic zone and by the significant contribution of forest habitats on its territory. The phanerophytes are life forms that are at the lowest altitude of study area.

The specific physical and geographical conditions of Bredhiku Reserve determine considerable diversity of floristic elements. We have established 17 different floristic elements (Fig. 2). The phytogeographical elements structure of the flora shows that they are dominated by taxa of floristic elements Euro-Asiatic (17; 18.68%) and European (13; 14.28%). The studied flora have also high numbers of medicinal species from Euro-Siberian (10; 10.98%), Euro-Mediterranean (9; 9.89%), Sub-Boreal (9; 9.89%) and Sub-Mediterranean (8; 8.79%) chorological groups. Boreal and Cosmopolitan elements are represented by 6 taxa (6.59%) each. The percentage of adventive species is small, 3 species (3.29%). The remaining floristic elements (8 groups) are relatively underrepresented. They are presented with 1-2 species (1.10-2.19%).

An important group of medicinal plants present in the Bredhiku Reserve are the relict plant species. Relict species are often viewed as fascinating ‘living fossils’ or remnants of old times (Grandcolas et al., 2014). They provide a unique opportunity to understand past and recent biogeographical and evolutionary processes (Kozlowski et al., 2013). In total, 16 (17.4% of the total species number) relict species are recorded in this study. They are represented mainly by tertiary relicts. Of them, only 2 plants species are glacial relict (*Arctostaphylos uva-ursi* (L.) Spreng., *Gentiana asclepiadea* L.). The presence of an imposing number of relict taxa has a great significance from the aspect of conservation biology of the area, emphasizing the fact that the Bredhiku area represents an important center of the important plant species in Kosovo.

Table 1. List of the registered plants species in the study and some their floristic elements

Species	Family	Life forms	Floristic element	Biological type	Relict plants	Red Book Kosovo & EU Policy species*	European Red List	IUCN Red List
1	2	3	4	5	6	7	8	9
Achillea millefolium L.	Asteraceae	H	Eur-Sib	p			LC	LC
Aconitum napelus L.	Ranunculaceae	H	Eur	p		b	LC	
Agrimonia eupatoria L.	Rosaceae	H	Eur- Med	p			LC	
Allium ursinum L.	Alliaceae	G	Eur	p			LC	
Anthyllis vulneraria L. *subsp. polyphylla* (De gand) Nym.	Fabaceae	Ch	Eur-Med	p				
Arctostaphylos uva-ursi L. (Spreng)	Ericaceae	Ph	Boreal	sh	gl	c	LC	
Artemisia absinthium L.	Asteraceae	H	Pont-Med	p			LC	
Artemisia vulgaris L.	Asteraceae	H	subBoreal	p			LC	
Atropa bella-donna L.	Solanaceae	H	Eur	p				
Bellis perennis L.	Asteraceae	H	Eur-As	p				
Betula pendula Roth	Betulaceae	Ph	Eur-Sib	t	te		LC	LC
Carlina acaulis L.	Asteraceae	H	Eur	p				
Castanea sativa Mill.	Fagaceae	Ph	Eur-subMed	t	te		LC	LC
Centaurea jacea L.	Asteraceae	H	Eur-Sib	p				
Centaurium erythraea Rafn	Gentianaceae	Th	subMed	a-b				LC
Chenopodium album L.	Chenopodiaceae	Th	Cos	a				
Cichorium intybus L.	Asteraceae	H	Eur-Sib	p			LC	
Clematis vitalba L.	Ranunculaceae	Ch	Eur	p	te			
Colchicum autumnale L.	Liliaceae	G	Eur	p			LC	LC
Cornus mas L.	Cornaceae	Ph	subMed	sh				LC
Corylus avellana L.	Corylaceae	Ph	Med-CAs	sh-t	te			LC
Corylus colurna L.	Corylaceae	Ph	Pont-CAs	t	te			LC
Crataegus monogyna Jacq.	Rosaceae	Ph	subBoreal	sh-t			LC	
Digitalis lanata Ehrh.	Scrophulariaceae	H	subMed	b-p			LC	
Echium vulgare L.	Braginaceae	H	Eur-As	b-p				
Epilobium angustifolium L.	Onagraceae	H	subBoreal	p				LC
Equisetum arvense L.	Equisetaceae	H	Boreal	p			LC	LC
Euphorbia myrsinites L.	Euphorbiaceae	G	subMed	p				
Fragaria vesca L.	Rosaceae	H	subBoreal	p			LC	
Fraxinus ornus L.	Oleaceae	Ph	subMed	t	te			LC
Galium verum L.	Rubiaceae	H	Eur-As	p			LC	
Gentiana asclepiadea L.	Gentianaceae	H	Eur	p	gl			
Gentiana punctata L.	Gentianaceae	H	Alp-Carp	p		LC	LC	LC
Geranium macrorrhizum L.	Geraniaceae	G	Eur-Med	p				
Geranium robertianum L.	Geraniaceae	Th	subBoreal	a-b				
Humulus lupulus L.	Cannabaceae	H	Eur-Sib	p	te		LC	
Hypericum alpigenum Kit.	Hypericaceae	H	Eur -Sib	p				
Hypericum perforatum L.	Hypericaceae	H	Cos	p			LC	
Juglans regia L.	Juglandaceae	Ph	Eur-As	t	te			LC
Juniperus communis L.	Cupressaceae	Ph	subBoreal	sh	te		LC	LC
Juniperus nana Willd.	Cupressaceae	Ph	Eur- As	sh				
Juniperus oxycedrus L. *subsp. oxycedrus*	Cupressaceae	Ph	Med	sh	te		LC	LC
Leucanthemum vulgare (Lam.) DC.	Asteraceae	H	Eur-Sib	p				
Lilium martagon L.	Liliaceae	G	Eur-As	p			LC	
Malus domestica Borkh	Rosaceae	Ph	Eur-As	t				
Malus sylvestris (L.) Mill.	Rosaceae	Ph	Eur	t			DD	DD
Malva sylvestris L.	Malvaceae	Th	Cos	a			LC	
Matricaria chamomilla L.	Asteraceae	Th	Eur-As	a			LC	

Mentha longifolia (L.) Huds.	Lamiaceae	H	Eur-Sib	p				LC
Morus alba L.	Moraceae	Ph	Adv	t				
Morus nigra L.	Moraceae	Ph	Adv	t				
Orchis morio L.	Orchidaceae	G	Eur-subMed	p				
Origanum vulgare L.	Lamiaceae	H	Eur-As	p			LC	
Petasites albus (L.) Gaertn	Asteraceae	H	Eur-Pont	p			LC	
Petasites hybridus (L.) G. Gaertn., B.Mey. & Scherb.	Asteraceae	H	Eur	p			LC	
Phyllitis scolopendrium (L.) Newman	Aspleniaceae	H	subBoreal	p				
Plantago lanceolata L.	Plantaginaceae	H	Cos	p			LC	
Plantago major L.	Plantaginaceae	H	Boreal	p			LC	LC
Primula acaulis L.(Gruff)	Primulaceae	H	Eur	p				
Primula elatior **(**L.) Hill	Primulaceae	H	Eur	p				
Primula veris Huds. subsp. *columnae* (Ten.)	Primulaceae	H	Eur-Med	p				
Primula veris L.	Primulaceae	H	Eur-Med	p			LC	
Primula vulgaris Huds.	Primulaceae	H	Eur-As	p				
Prunus avium L.	Rosaceae	Ph	subMed	t				LC
Prunus cerasus L.	Rosaceae	Ph	Eur	t				
Prunus cocomilia Ten.	Rosaceae	Ph	Eur-Med	t				LC
Prunus spinosa L.	Rosaceae	Ph	sPont	sh			LC	
Pteridium aquilinum (L.) Kuhn	Dennstaedtiaceae	H	Cos	p				
Pyrus communis L.	Rosaceae	Ph	Eur-As	t				LC
Robinia pseudoacacia L.	Fabaceae	Ph	Adv	t				LC
Rosa canina L. subsp lutetiana (Lem.) Hay.	Rosaceae	Ph	subMed	sh			LC	
Rubus fruticosus L.	Rosaceae	Ph	Eur-Med	sh			LC	
Rubus idaeus L.	Rosaceae	Ph	subBoreal	sh			LC	
Rubus ulmifolius L.	Rosaceae	Ph	Eur-Pont	sh				
Salix alba L.	Salicaceae	Ph	Eur-As	t	te		LC	LC
Salix caprrea L.	Salicaceae	Ph	subBoreal	t	te			
Sambucus nigra L.	Caprifoliaceae	Ph	Eur-Med	sh		a; b	LC	
Solidago virgaurea L.	Asteraceae	H	Boreal	p				
Taraxacum officinale (L) Web.	Asteraceae	H	Cos	p			LC	
Teucrium chamaedrys L.	Lamiaceae	Ch	Eur-Med	p			LC	
Teucrium montanum L.	Lamiaceae	H	subMed	p			LC	
Thymus sp.	Lamiaceae	Ch		p				
Tilia cordata Mill.	Tiliaceae	Ph	Eur	t			LC	LC
Tussilago farfara L.	Asteraceae	H	Eur-As	p			LC	
Urtica dioica L.	Urticaceae	H	Boreal	p			LC	LC
Vaccinium myrtillus L.	Ericaceae	Ph	Boreal	sh	te		LC	
Veratrum album L.	Melanthiaceae	H	Eur-As	p			LC	
Verbascum thapsus L.	Scrophylariaceae	H	Eur-As	b			LC	
Veronica officinalis L.	Plantaginaceae	H	Eur-Sib	p				
Viburnum opulus L.	Adoxaceae	Ph	Eur-Sib	sh				
Viola tricolor L.	Violaceae	Th	Eur-As	a			LC	
Viscum album L.	Loranthaceae	Ch	Eur-As	sh	te		LC	

Legend

- ***Life forms****:* Ch - Chamaephytes; H - Hemicryptophytes; G - Geophytes; Ph - Phanerophytes, Th - Therophytes;
- ***Life cycle***: a – annual; a-b – annual to biennial; b – biennial; b-p – biennial to perennial; p – perennial; ssh - semi-shrub; sh – shrub; sh-t – shrub to tree; t – tree.
- ***Relict specie*****:** Tertiary relict: te; Quaternary glacial relicts: gl

Conservation Status:

[*] *EU Policy species* - The plants that are listed under European policy instruments:

a - EU Habitats Directive (Council Directive 92/43/ EEC of 21 May 1992)

b - Bern Convention - EU Convention on the Conservation of European Wildlife and Natural Habitats

c - EU Wildlife Trade Regulation (Council Regulation (EC) No 338/97 of 9 December 1996)

– *The Red Book of Vascular Flora of the Republic of Kosovo 1*: LC - Least Concern.

– *European Red List of Medicinal Plants*: LC-Least Concern, DD- Data deficient.

– *The IUCN Red List of Threatened Species*: LC-Least Concern, DD- Data deficient.

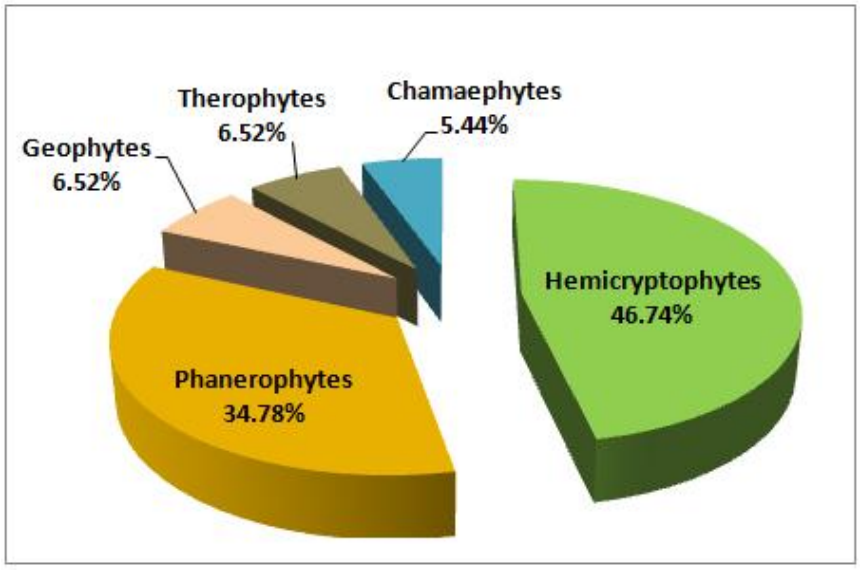

Fig. 1. Spectrum of life forms in the medicinal flora of Bredhiku Reserve.

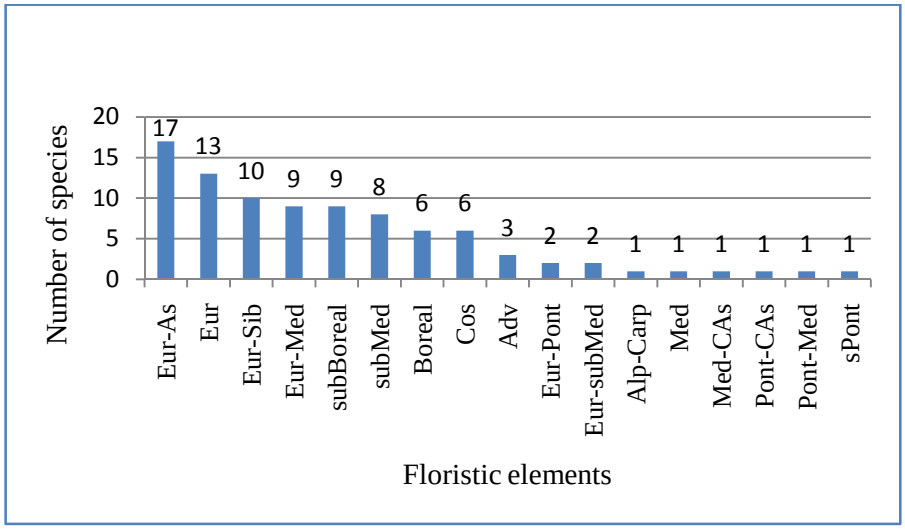

Fig. 2. Distribution of the medicinal plants by floristic elements.

Table 2. Distribution of the species by biological type

Biological type	Number of species	Percentage of all species
Annual (a)	4	4.35
Annual to biennial (a-b)	2	2.17
Biennial (b)	1	1.09
Biennial to perennial (b-p)	2	2.17
Perennial (p)	50	54.35
Shrub (sh)	14	15.22
Shrub to tree (sh-t)	2	2.17
Tree (t)	17	18.48

All biological types are represented among the medicinal plants of Bredhiku Reserve. Regarding the biological types (Table 2) the medicinal species dominated perennial herbaceous plants, 50 species (54.35%). Next are the groups of tree plants, 17 species (18.48%), and shrubs, 14 species (15.22%). With a lower number of species are represented annual herbaceous plants, biennial herbaceous plants and the transitional forms between the basic biological types, 1-2 species. The largest number of perennial

herbaceous plants can be explained by the wide variety of habitats and communities of subalpine plant species on study area. The relatively high number of shrubs and trees results from settlements and preserved forest habitats.

The species described in the studied area with conservation status by the international and national legislation are 60 or 65.22% of the total taxa's number. From them, 48 species (52.17 %) are protected under the European Red List of Medicinal Plants, and 25 are included in the Red List of Threatened Plants of the International Union for Conservation of Nature (IUCN, 2018). In the Red Book of Kosovo is included only one species, *Gentiana punctata*L. All species with conservation status are under the category of Least Concern (LC), except of *Malus sylvestris* (L.) Mill., which included in category Data deficient (DD).

There are 3 protected species listed in the *EU Policy species* (the plants that are listed under European policy instruments). One of them is *Sambucus nigra* L.. It is included in Annex II of Directive 92/43/EEC (Plant and animal species of Community interest whose conservation requires the designation of special areas of conservation) and in Appendix I In Appendix I of Convention on the Conservation of European Wildlife and Natural Habitats (Berne Convention). In the Bern Convention is included also *Aconitum napelus* L., whereas species *Arctostaphylos uva-ursi* L. (Spreng) is listed in Council Regulation (EC) No 338/97 (EU Wildlife Trade Regulation).

The number of the relicts species with conservation status identified in this study is significant (13 species), despite how small the territory of the study is.

Conclusion

The medicinal flora of Bredhiku Strict Nature Reserve (Sharr Mountain in Kosovo) includes 92 taxa. Inventory of medicinal showed significant taxonomic diversity. The phytogeographical elements dominated by taxa of chorological types Euro-Asiatic and European. Euro-Siberian, Euro-Mediterranean, Sub-Mediterranean and Sub-Boreal chorological groups have also a high percent of species. The results of the analyses of the biological spectrum have shown a pronounced hemicryptophyte character of this area. They represent 46.74 % of the total number of medicinal species. The list of medicinal plants includes 60 species with conservation importance. The medicinal relic species in Bredhik Mountain are describe for the first time. The obtained results can be used for comparison with the data on the flora of different Strict Nature Reserve in Sharr National Park, as well as in the whole country. This inventory is only the first step in a series of studies required for mapping of the distribution, conservation status, threats of the species, as well as identify conservation measures within the protected strict area.

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RESEARCH ARTICLE **(Open Access)**

The endemic and relic alpine–subalpine plant species in Shutman natural reserve of Kosovo and their conservation status

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Abstract

On the territory of the Sharri National Park of Kosovo there are 16 strict natural protected areas, one of which is Shutman Reserve. Until now the biodiversity of endemic and relic plants in Shutman has not been completely described. The present study is the first on this subject for the Shutman Reserve. The purpose of the study is to investigate the biodiversity of endemic and relic plant species also their conservation status. The plants were collected during the vegetative period, May – September 2018. In total, in this paper are presented 42 vascular plants. From which, 24 species are Balkan endemics, 2 species are sub-endemics, 2 species are steno-endemics and 1specie is Central and South-eastern Europe endemic. Nearly 12 of Shutman's investigated vascular plants are tertiary and glacial relics. A total of 24 taxa have a protection status. The biological type of species represented mainly by perennial herbaceous plants (95%). The largest percentage of floristic elements to describe appertain the Balkan type (40.48%) and Boral type (9.52%), whereas the hemi-cryptophytic dominate among the life forms, with 59.52%. The data of the paper showed that Reserve of Shutman has a very high level of endemic and relic species.

Keywords*:* Vascular plants, endangered species, floristic element, life form.

Introduction

Kosovo is one of the Balkan countries with the greatest biodiversity, including biodiversity of endemic and relic plants. Sharri Planina is one of the largest and highest mountain ranges of the Balkan Peninsula, stretching between the Prizren-Metohia valley and the Polog valley in the border areas of the Kosovo and Republic of North Macedonia. The Sharri National Park in Kosovo is located in south-eastern part of country. It is one the most important Balkan and European centers and one of the most qualitative endemism including relics, endemics and endemic- relics and types (Abdii & Xhulaj, 2016; Tomović et al., 2014). Nowadays, on the territory of the Sharri Mountain there are 16 strict natural protected areas, one of which is area of high mountains "Shutman", putting under natural strict protection on Decision of GRK No. 17/74, 10 February 2016 (GRK, 2016).

The Reserve of Shutman covers an area of 5057.39 hectares that consists of a wide mountain belt from 1600 m to 2536 m above sea level (Vraca e Madhe). The subalpine zone extends above the upper forest belt from 1600 - 2100 m (rare 2200 m.) above sea level (Abdii & Xhulaj, 2016). Higher up is found the alpine zone, where environmental conditions become more extreme due to decreasing temperatures, increasing solar radiation, strong winds, etc.

Shutman area is dominated by an alpine and continental climate, at times influenced by a median continental climate (MMPH/AMMK, 2013). The annual average temperature is 8.6° C. Summers are short and fresh with an average temperature of 18.1° C, with long and relatively cold winters (average temperature -0.4° C). The annual average precipitation reaches is 1370 mm, with snow cover for around 280

days per year. Approximately 50% of total annual rain falls is from April to September, whereas months with less rainfall are January, February, March and August (MMPH/AMMK, 2013; Bank et al., 2012; Voit et. al., 2013).

Shutman have a combination of underwater land systems, including mountain lakes, oligotrophic interior streams, marshes, extensive pasture, shrub and herbaceous vegetation and vegetation of rocky surfaces (MMPH/AMMK, 2013). The geographical location, climate and hydrological characteristics, different rock substrata and soil types, are factors contributing to a rich flora and vegetation and considerable number rare, relic and endemic plant species. The paleo-environmental conditions allowed the survival of relic taxa, including some of tertiary origin (Olšavská et al. 2016). But, the biodiversity of endemic and relic plants in Shutman is poorly explored and no detailed studies are available**.** The present study is the first on this subject in the Shutman Reserve. The aim of this study was to provide a list of the endemic and relic plant species together with their conservation status and an analysis of floristic elements, biological types, life form for each species. This information is important due to the high conservation value of strict natural protected area of Shutman.

Material and methods

Study area

Shutman Reserve is a part of Sharri National Park of Kosovo. This covers an area of 5057.39 hectares that consists of a wide mountain belt from 1600 m to 2536 m above sea level (Vraca e Madhe). The subalpine zone extends above the upper forest belt from 1600 - 2100 m (rare 2200 m) above sea level and upper them is found the alpine zone, where environmental conditions become more extreme due to decreasing temperatures, increasing solar radiation, strong winds, etc.

Data collection and species determination

Based on our study, this survey was conducted in the vegetation period of year 2018 on the territory of the Strict Nature Reserve of Shutman. Identification of plant material were determined according to the Flora of Albania (Anonymous, 1988-2000), Atlas of Kosovo Plants (Berisha et al., 2012), Red Book of Vascular Flora of the Republic of Kosovo (Millaku et al., 2013) and Excursion Flora of Albania (Vangjeli, 2015; Demiri, 1983). The nomenclature used was according to the databases of the Plant List (theplantlist.org).

The floristic elements are given after Assyov & Petrova (2012), supplemented with data from Asenov (2015) and Vuksanović et al., (2016). The life forms of the taxa are represented in the systems of Raunkiaer (1934). The following abbreviations apply: *Ph = phanerophyte, Ch = chamaephyte, H = hemicryptophyte, G = geophyte, T = therophyte*. Biological types are defined by Berisha et al. (2012), Millaku et al. (2013) and Anonymous (1988-2000).

Information on endemic and relic elements of vascular plants are presented under same botanical publications (Zahariev, 2016; Mustafa et al., 2015; Tomović et al., 2014; Vuksanović et al., 2016; Millaku et al. 2008; Millaku et al. 2017; Hajredini et al., 2013; Mahmutaj, 2015; Petrova & Vladimirov, 2010).

Threatened status of plant taxa is represented according to the Red Book of Vascular Flora of the Republic of Kosovo (Millaku et al., 2013), the IUCN Red List of Threatened Species (IUCN, 2018), Bern Convention (Council of Europe 1979), Annex V of Directive 92/43/EEC (Habitat Directive), European Red List of Vascular Plants and Appendix II of CITES Convention (CITES 2011). The floristic list (Table 1) is provided in alphabetical order by families and species.

Results and Discussion

The floristic list of endemic and relict species or important plant species within this paper contains 42 vascular plants belonging to 20 families and 28 genera, (Table 1). The most important contributors at

family rank with the highest number of the important plant species are Asteraceae (5 taxa), Caryophyllaceae (5), Rosaceae (5) and Campanulaceae with 4 species. Two families, especially Gentianaceae and Saxifragaceae, are represented by 3 species. As many as 14 families were represented by 1-2 species. *Achillea, Campanula, Dianthus* and *Gentiana are* the genera with the highest number of species (first genera presented 4 species and others genera presented with 3 species).

Table 1: The floristic list of the endemic and relic present in Shutman Natural Reserve

Taxa	Life forms	Floristic element	Biological type	Relic and endemic plants	Rareness & Conservation status
ASTERACEAE					
Achillea abrotanoides Vis.	Ch	Bal	p	Bal endemic	
Achillea chrysocoma Friv.	H	Bal	p	Bal endemic	EN
Achillea corabensis (Heimerl) Micevski	Ch	Bal	p	Bal endemic	EN
Achillea tanacetifolia Mill.	H	Eur-Sib	p		LC- IUCN
Centaurea nervosa Willd.	H	Alp-Carp-Bal	p	Bal endemic	
CAMPANULACEAE					
Asyneuma limonifolium (L.) Janch.	H	Ap-Bal	p	Tertiary relics	
Campanula albanica Witasek	H	Bal	p	Bal endemic	EN
Campanula foliosa Ten.	H	S-E-Eur	p	Bal endemic	LC
Campanula spatulata Sibth. et Sm.	G	Bal	p	Bal endemic	
CAPRIFOLIACEAE					
Sambucus racemosa L.	Ph	Boreal	sh	Tertiary relics	LC- IUCN
CARYOPHYLLACEAE					
Cerastium decalvans Schloss. & Vuk.	H	Bal	p	Bal endemic	
Dianthus integer Vis.	H	Bal	p	Bal endemic	
Dianthus scardicus Wettst.	H	Bal	p	Steno-endemic	NT
Dianthus superbus L.	H	Eur-As	p	Bal endemic	LC
Silene roemeri Friv.	H	Bal-Ap	p	Bal endemic	
COLCHICACEAE					
Colchicum autumnale L.	G	Eur	p		LC- IUCN
CRASSULACEAE					
Sempervivum heuffelii Schott	H	Bal	p	Sub-endemic	LC
Sempervivum kosanini Praeger	H	Bal	p	Bal endemic	NT
EMPETRACEAE					
Empetrum nigrum L.	H	Boreal	p	QG relics	Rare
FABACEAE					
Onobrychis scardica (Grseb.) Halacsy	H	Eur-Med	p	Bal endemic	
GENTIANACEAE					
Gentiana lutea L.	H	Eur	p	Tertiary relics	VU; HD V; LC – ERL
Gentiana punctata L.	H	Alp-Carp	p	C & SEE endemic	LC; LC- IUCN
Gentiana verna L.	H	Eur-As	p	QG relics	
GERANIACEAE					
Geranium subcaulescens L'Hér. ex DC.	G	Med	p	Bal endemic	LC
IRIDACEAE					

Crocus scardicus Kosanin	G	Bal	p	Steno-endemic	NT; LC- IUCN
Crocus veluchensis Herb.	G	Bal	p	Bal endemic	
LAMIACEAE					
Lamium garganicum L.	H	Med	p	Sub-endemic	NT
LILIACEAE					
Lilium albanicum Griseb.	G	Bal	p	Bal endemic Tertiary relics	LC
ORCHIDACEAE					
Nigritella nigra (L.) Rchb. F.	G	Eur	p	QG relics	CITES II
PRIMULACEAE					
Soldanella alpina L.	H	Bal	p	Bal endemic	
RANUNCULACEAE					
Anemone narcissiflora L	G	Boreal	p	QG relics	
ROSACEAE					
Dryas octopetala L.	Ch	Boreal	p	QG relics	Rare
Geum montanum L.	H	Alp-Carp-Bal	p	QG relics	
Potentilla calabra Ten.	H	S-Eur	p	Bal endemic	EN
Potentilla doerfleri Wettst.	Ch	S-Eur	p	Bal endemic	EN
Potentilla speciosa Willd.	Ch	S-Eur	p	Bal endemic	
SALICACEAE					
Salix reticulata L.	Ch	Arct -Alp	s-sh	QG relics	LC
SAXIFRAGACEAE					
Parnassia palustris L.	H	Sub-Boreal	p	QG relics	LC- IUCN
Saxifraga scardica Griseb.	Ch	Bal	p	Bal endemic	LC
Saxifraga sempervivum K. Koch	Ch	Bal-Anat	p	Bal endemic	LC
SCROPHYLARIACEAE					
Linaria peloponnesiaca Boiss. & Heldr.	H	Bal	p	Bal endemic	
Verbascum scardolicum Bornm.	H	Bal	p	Bal endemic	

Life cycle: p – perennial; ssh – semi-shrub; sh – shrub.

Life forms*:* Ch – Chamaephytes; H – Hemicryptophytes; G – Geophytes; Ph – Phanerophytes.
Conservation Status: The Red Book of Vascular Flora of the Republic of Kosovo 1: EN – Endangered, VU – Vulnerable, NT - Near Threatened, LC - Least Concern. The IUCN Red List of Threatened Species: IUCN - LC (Least Concern). Convention on International Trade in Endangered Species of Wild Fauna and Flora: CITES II - Annex II. Directive 92/43/EEC (Habitat Directive): HD5 – Annex V. European Red List of Vascular Plants: ERL- LC (Least Concern).
Relic and endemic plants: QG relic - Quaternary glacial relics; C & SEE endemic - Central & Southeastern Europe endemic; Bal endemic - Balkan endemic

The total number of endemic taxa described in this paper is 29 species (69% of all species). The endemic taxa are represented by 24 Balkan endemic species; 2 Balkan sub-endemic species; 2 steno-endemics species and 1 Central and Southeastern Europe endemic specie. The genera *Achillea, Campanula, Dianthus* and *Potentilla* are amongst those most rich in endemic species. The number of the endemics species identified in this study is significant. The high number of endemic taxa recorded in Shutman Reserve makes this massif important for the endemic flora in Balkan and Kosovo, a centre of the highest floristic richness of the Balkan endemic plants.

An area of endemism must have at least two endemic species (Linder, 2001; Morrone, 2008; Casagranda & de Grosso, 2013). Areas of endemism have a central role in biogeography as they are the analytical units in historical biogeography, and are also considered quite

relevant for biodiversity conservation (Casagranda & de Grosso, 2013). In the last years, endemism has acquired importance in conservation biology since it is considered an outstanding factor for delimitation of conservation areas (Casagranda & de Grosso, 2013). Endemism is an important concept in conservation biology and it is one of the criteria used to set priorities for species conservation efforts (Shevock, 1996). Shutman Reserve, as an important area of endemism with high biodiversity value, requires special attention for plant conservation.

In Shutman Reserve are investigated 12 vascular plants which are relic species. Eight of this species are quaternary glacial relics and the remaining four are tertiary relics. *Lilium albanicum* Griseb., is endemic and tertiary relict specie. Presence of relic species of olden epochs show how old and preserved is the investigated flora. Tertiary relics are ancient representatives of Arctic tertiary flora (Zahariev, 2017). Quaternary glacial relic species have migrated from the north of Europe to the south in the period of the last glaciations and the mountains of the Balkan Peninsula have become their refuge (Zahariev, 2017). A detailed list of relict plants is needed not only for the purposes of ecology and environmental protection; it will be particularly useful in floristic studies, in conservation biology and in biogeography (Zahariev, 2016).

The spectrum of life forms is an important indicator of the ecological conditions in the communities. Also, it is an indicator of a complex interdependence of plant life forms and the climatic and edaphic characteristics features and it reflects adaptation of plants through time and the current conditions, in agreement with the environmental conditions in which these plants survive (Tomović et al., 2014). The life forms spectrum of the endemic and relict flora of the Shutman consisting by four main types. Their number and percentage of them are presented in *table 2.* The biological spectrum of the endemic flora shows a pronounced hemi-cryptophytic character of this area. The hemi-cryptophytic dominate among the life forms, with 25 species or 59.52% of the total number of species, followed by chamaephytes and geophytes both with 8 species (19.05 %) and phanerophytes with only one species (2.38%). Domination of hemi-cryptophytic in the biological spectrum of the important plant species of the Shutman Reserve matches the influences of the cold mountain climate. The prevalence of hemicryptophytes is characteristic for the floras of cold mountain climates. The higher percentage of chamaephytes and geophytes can be explained with resistance of these plant species to stressful environment. Chamaephytes are especially important in stressful environments, for example in alpine ecosystems and on rock and nutrient-poor soils (Matevski etj. 2011), like Shutman conditions. Geophytes have their buds buried, and thus protected from cold under the ground, therefore these plant species are resistant to stressful environment and can adapt to harsh environment.

The specific physical and geographical conditions of Shutman Reserve determine considerable diversity of floristic elements. We have established 16 different floristic elements (table 2). The largest number of species belongs of the Balkan type (40.49%) and Boral type (9.53%), following by floristic elements European and South Eastern European both by 7.14%. The distribution of the floristic elements correlates with the geographic location of the study area, and confirms the diversity of conditions in the area.

The floristic analysis indicates that the biological types are represented mainly by perennial herbaceous plants (95.24%), and only one of the species is included in the biological types of is semi-shrub (2.38%) and shrub (2.38%). The dominance of perennial herbaceous plants can be explained by the influence of environmental factors on alpine–subalpine species.

Table 2: Floristic elements in the flora of Shutman Mountain

No.	Floristic elements		Number of species	% of the total number of species
1	Balkan	Bal	17	40.49
2	Boreal	Boreal	4	9.53
3	European	Eur	3	7.14
4	South Eastern European	S-E-Eur	3	7.14
5	Alpo-Carpathian-Balkan	Alp-Carp-Bal	2	4.76
6	Eurasian	Eur-As	2	4.76
7	Mediterranean	Med	2	4.76
8	Alpo-Carpathian	Alp-Carp	1	2.38
9	Apenninian-Balkan	Ap-Bal	1	2.38
10	Arctic-Alpine	Arct -Alp	1	2.38
11	Balkan-Anatolian	Bal-Anat	1	2.38
12	Balkan-Apenninian	Bal-Ap	1	2.38
13	Euro-Mediterranean	Eur-Med	1	2.38
14	Euro-Siberian	Eur-Sib	1	2.38
15	South European	S-Eur	1	2.38
16	Sub-Boreal	Sub-Boreal	1	2.38
	Total		**42**	**100.00**

In these paper we have describe 24 species with protection status. Five of them are included in the Red List of Threatened Plants of the International Union for Conservation of Nature (IUCN, 2018). All of them are under the category of “Least Concern” (LC). Nineteen species included in the Red Book of Vascular Flora of the Republic of Kosovo. Nine of them are under the category of “Least Concern” (LC), 5 are endangered (EN), 4 species are near threatened” (NT) and one species is vulnerable (VU). Of the registered plants one of them *Nigritella nigra* is included in the Appendix II of CITES. *Gentiana lutea* L. is included in the Annex V of Directive 92/43/EEC (Habitat Directive) and in the European Red List of Vascular Plants under the category of “Least Concern” (LC).

Conclusions

In the present study was found a high number of endemic taxa of subalpine–alpine plant species. This makes Shutman Reserve an important area of endemism in Kosovo and Balkan that requires special attention for plant conservation. The list of relics described has a significant number to. It will be useful in floristic studies, in conservation biogeography and in biology. The number of endemics, relics and endemic-relic species of conservation status is higher in Shutman area. A total of 24 plant species have a national or international conservation status. However, a significant number of the endemic and relic species has not been evaluated yet according to the IUCN criteria mainly due to insufficient information on their populations. The floristic studies targeted for species with conservation importance should be carried out in order to preserve the great floristic richness of the area. The high number of endemic, relict and protected species emphasizes not only the high conservation status of the region, but and the need of effective management for protection of biological diversity.

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RESEARCH ARTICLE **(Open Access)**

Advanced technology of energy use of biomass. The study case: the burning of the distilled waste of sage in Albania.

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Abstract

Biomass waste management systems with low environmental impact, which are able to protect the health and safety of inhabitants, are now gaining global attention. There is currently an increasing interest in new renewable energy generation methods, where its production from biomass is constantly growing. Burning or incineration is a known method for disposal of biomass residues. One of the main objectives of the National Energy Strategy for 2018-2030 for Albania is to provide incentives for implementing the necessary climate change policies, such as achieving renewable energy (RES) and efficiency energy targets and reduce negative environmental impacts. According to a study by the Ministry of Agriculture in the field of renewable energies and specifically in assessing the potential of biomass in our country, the conclusion is reached that biomass represents a considerable source which has not yet been fully exploited.

In this paper, there is going to be presented the most advanced technology that is used in our days, to burn the waste after the distillation of sage. Like we know, the practice methods for the burning of biomass and waste that are used for electric uses, for example to produce heat and electricity, they are

- Burning in grilling boilers

- Burning in boilers with food from below

- Fluidized base technology

This article treats arguments that technology with dusty base the most appropriate and is one efficient technology for the cremation of biomass, secondary fuels and coal, as well as for combustion of other combustible waste.

There are two essential factors that give impetus to the application of this technology in the energy industry:

First, the use of this technology is possible to burn with efficient fuel efficiency with low calorific power such as household waste or other combustible waste.

Second, an avand of this is that it gives the possibilty of a low chance of pollution from the cermation, without the need of the expensive machines of cleaning of the gaseous current.

Keywords: Biomass, electrical energy, renewable energy source.

1. Introduction

Problem Spreading

The Energetic Situation in Albania

In 2014, 2015, 2016 total primary energy production in Albania was respectively 2.014 ktoe, 2117 ktoe, 2013 ktoe. The domestic consumption of RES in 2014, 2015, 2016 was respectively 2,342 ktoe, 2219 ktoe, 2309 ktoe, while net imports were 1888 ktoe. The primary energy supply in Albania is dominated by oil, hydro power plants and imported electricity. Figure 1 shows which imports of petroleum products, electricity and a small amount of coal account for over 56% of all primary energy consumption. Figure 2 shows that the transport sector consumes the largest amount of energy, followed by the residential sector and the industry sector, and the three most important fuels are petroleum products, electricity and firewood.

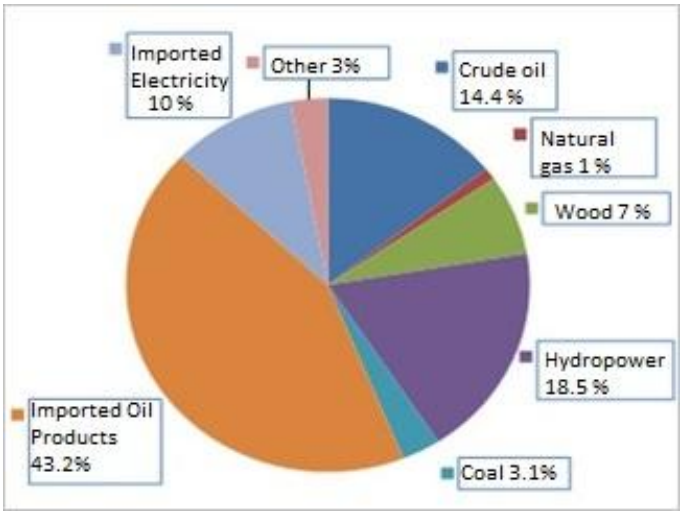

Figure 1: Type of energy used

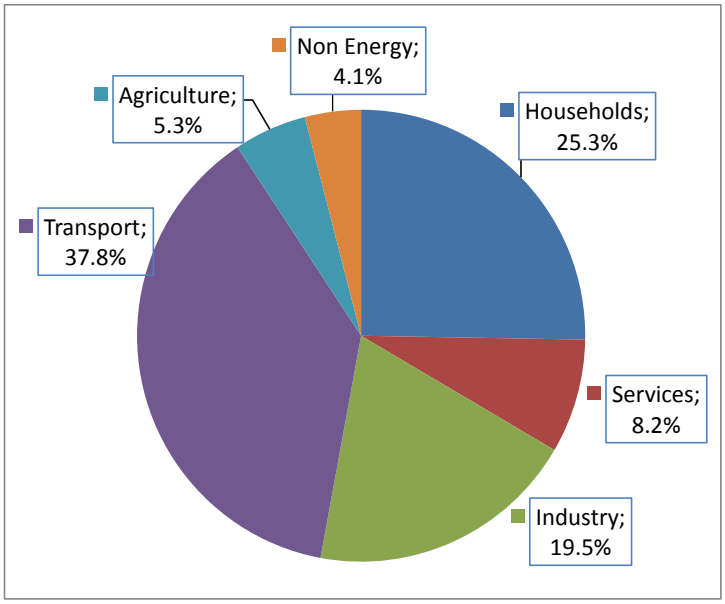

Figure 2: Energy consumers

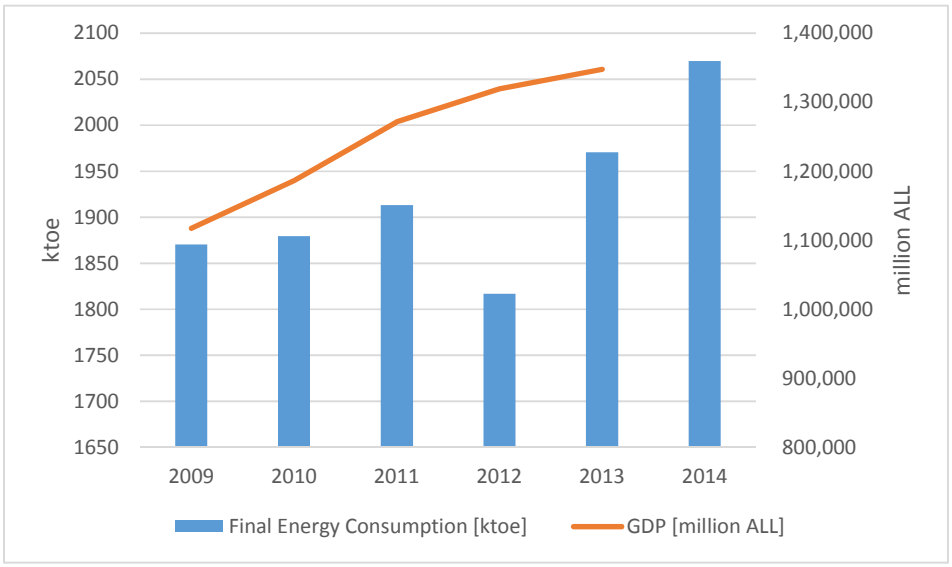

Figure 3: Final energy consumption data (Source: Albanian Energy Balance Sheet 2009-2014, AKBN; INSTAT)

From 2009-2016, final energy consumption in Albania increased from 1871 ktoe to 2060 ktoe, an increase of around 10,13%, but growth was not stable over the years. As shown in Figure 3, the final energy consumption in Albania grew gradually between 2009 and 2011, before falling in 2012, coinciding with a slowdown in the Albanian economy. This was mainly due to rising demand for housing. Power consumption rose sharply in 2014, driven mainly by the iron and steel industry sectors

2. Materials and Methods

2.1 Basic concepts in the field of renewable energy.

Renewable energies are energies that are derived from resources that do not have the basis of burning fossilized processed matter. This kind of energy aims to be as clean and less harmful to the environment as a whole. Alternative sources from which this type of energy can be generated are wind, solar radiation, water, etc.

In the technical context of the range of renewable energies are classified:

- Hydropower
- The Aeolian Parks
- Photovoltaic Parks
- Geothermal Resources
- Biomass

2.2 Challenges of the energy sector in Albania

Fulfilment of economic developments in different sectors and the increasing level of energy consumption per capita;

Improving the energy intensity trend;

Increasing the security of energy supply by improving energy efficiency, increasing the share of renewable energy sources and other conventional energy sources, and enhancing regional cooperation and integration.

In addition to the above challenges, three future challenges for the Albanian energy system are the achievement of the RES objective in 2020 and beyond, the EE target for reducing the final energy use and the target for reducing GHG emissions.

2.3 Energy Policy Scenarios related to the use of renewable energies

Renewable Energy Resources (RES): This scenario guarantees that Albania fulfils the commitments of the Energy Community Treaty by reaching the target of 45.03% of the renewable energy contribution versus the total in 2020 by implementing the National Renewable Energy Action Plan.

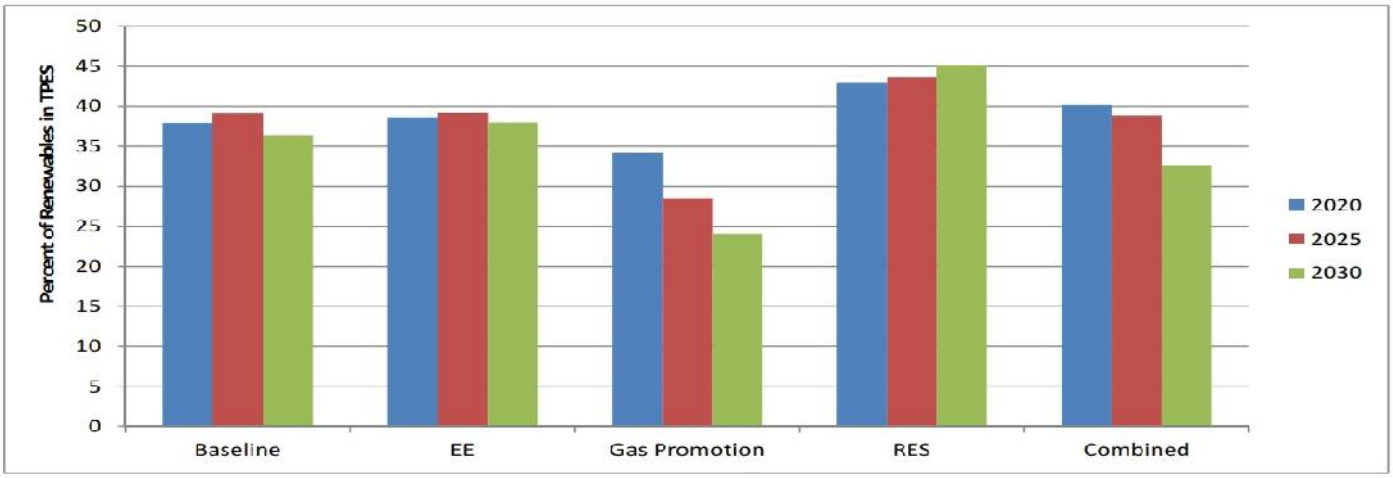

Figure 4: The volume of RES that is going to have until year 2030

Table 1:. The share of renewable energy sources in primary energy supply. (Source: National Energy Strategy 2017-2030)

Year/% Scenarios	Percentage		
	2020	2025	2030
Base	37.98	39.09	36.33
EE	38.65	39.16	38.00
Gas Promotion	34.20	28.54	24.04
RES	42.95	43.64	45.03
Combined	26.35	28.97	26.87

2.4 Renewable energy

Table 2: The energy provided by renewable energy sources that cover the thermal energy demand (ktoe)

Viti/kToe	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030
Fire wood	195	217	233	249	265	280	296	295	295	294	295	295	295	298	300	303	306
Solar Energy	12	17	20	24	28	33	38	40	43	46	49	53	56	60	64	69	74
Biodiesel	29	66	75	83	92	100	108	117	125	133	142	150	159	167	175	184	192
Biomass / pellet	-	2	5	8	11	15	19	22	26	30	34	38	42	46	50	54	59
Residues of agricultural crops	-	3	6	9	12	15	17	20	22	23	25	26	27	28	28	29	29

2.5 Biomass sources that can be used for energy purposes

Table 3: Biomass sources (Source: International Renewable Energy Agency 2017)

Rural Resources	Urban Resources
Forest waste, wood	Urban wastes, wood packaging etc.
Agricultural residues from corn crops, grain harvest residues	Solid waste from sewage purification
Barriers and trees	Gas landfill
Biogas from animal extracts	Solid urban waste

2.6 Some data related to the cost of biomass use plants for energy purposes

The total cost of biomass-generating energy technologies depends on the type of technology used and is different from one country to another.

- The total cost of boiler installations is between 1880 USD/kW ÷ 4260 USD/kW
- With fluidized base is 2170 USD/kW and 4500 USD/kW.

- The anaerobic cost systems are in the amounts of 2570 USD/kW and 6100 USD/kW.
- Gasification technologies, such as fixed and fluidized base rockers, cost 2140 USD/kW ÷ 5700 USD/kW.
- Gas production from the landfill cost 1920 USD/kW - 2440 USD/kW.
- The cost of CHP plants is much higher than that of plants built only for electricity.

(Source : International Renewable Energy Agency 2017)

3. Results and Discussion

3.1 The processes of transformation of the sage biomass

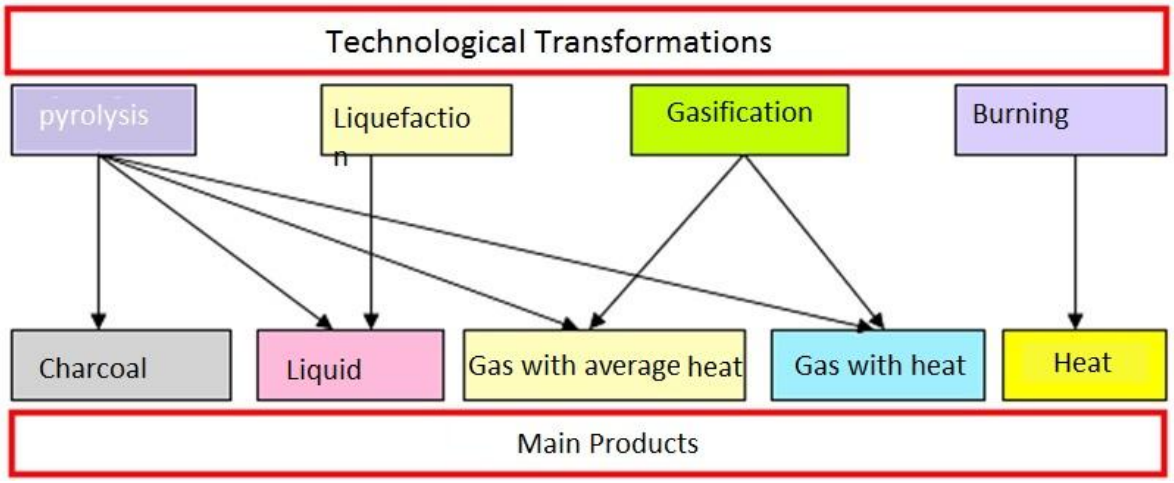

Figure 5: Thermochemical transformation processes and main products. (Bridgwater, 1993)

3.2 Advanced biomass energy utilization technologies

Direct biomass burning for energy production is a mature and available technology in the market and can be applied in a wide range of several MW to 100 MW or more and is the most common form of biomass energy production.

There are two main ways of using biomass for energy purposes: 1) Biomass burning to produce technological steam; and 2) Use of steam on a steam turbine, which is then used for generating electricity.

The two most common forms of boilers are 1) skis that can be fixed or movable and 2) with fluidized base. The fuel can be completely biomass or combine biomass with coal or other solid fuels (EPA, 2008).

3.3 Boilers with fixed conveyors

Fixed scales can be used in boliers with manual operation. They are suitable for small combustion systems (<1.0 MWh). Between staircases, flat planks are most usable. Plane planks, have larger surface area and air distribution more uniformly.

These types of boilers are suitable for combustion of high volatility fuels. The maximum temperature of the area is very close to the grid surface. Air combustion is partly utilized inside the base and partly in the combustion chamber to burn volatile matter. Burning in a griddle can lead to different dispersions of flying ash from fuel. (Nussbaumer, 1998).

3.4 Mobile Grill Boliers

Mobile grills, such as fixed grids, are suitable for high humidity biomass, ranging in size and high ash content. Wood mixtures can be used, while mixing of

wood with straw, cereals and herbs can not be due to various behaviors during combustion, low humidity and low melting point. (Obernberger, 1998)

Transport of the object on the grill should be as homogeneous and light as possible in order to keep the coat of grass as smooth and homogeneous as possible to avoid the formation of "cavities", the formation of unburned particles.

Table 4: Biochemical and thermochemical process of biomass

Thermo chemical processes	
Burning	**Burning is the complete oxidation of CO2 and H2O fuels.**
Gasification	**The gasification process can be defined as the thermal decomposition of the biomass substance (partial combustion in an environment with low oxygen content).**
Pyrolysis	**Pyrolysis is the thermal degradation of carbonate materials in the absence of an external oxidizing agent and occurs at temperatures of 400-800 ° C. Pyrolysis products include gases, juices and solid charcoal**
Bio Chemical Processes	
Anaerobic process	**It is a process of decomposition of biological materials and favored by the conditions of relatively high temperatures, moisture and lack of air. Products are mainly methane gas and carbon dioxide**

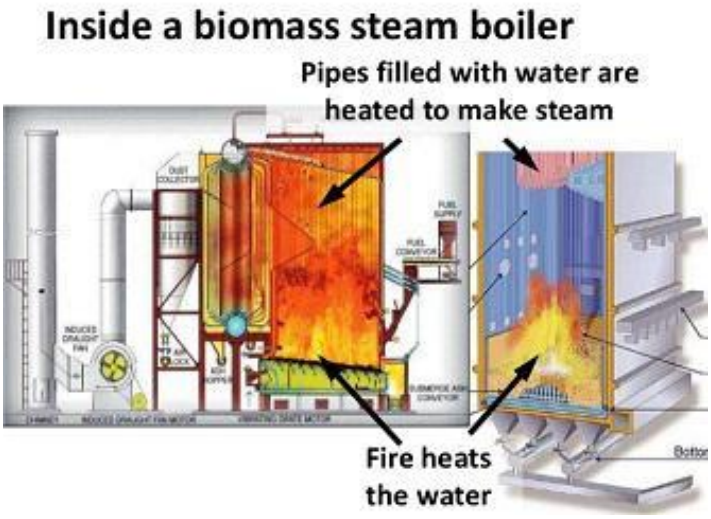

Figure 6: Boilers with fixed conveyors (Source: Holtham, 2013)

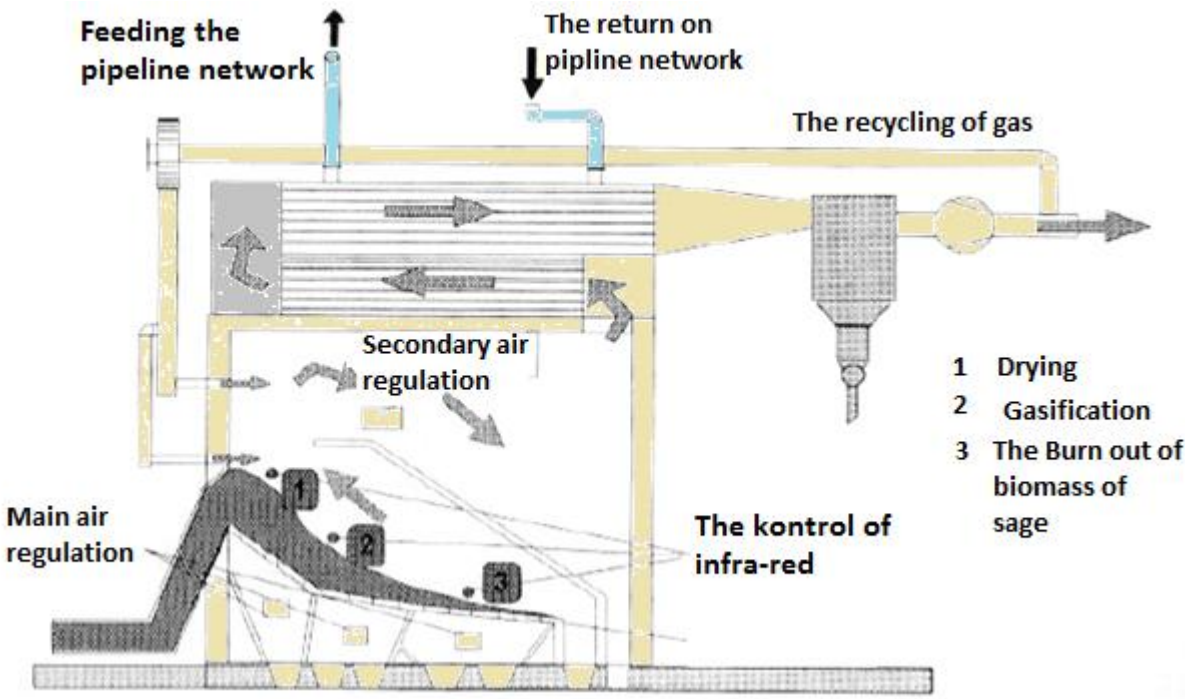

Figure 7: The schematic presentation of the mobile grill with moving forward. (Obernberger, 1998)

3.5 Boliers with food from below

This type of bolier has the combustion zone in the upper part of the base and the first material, such as biomass, is supplied below:

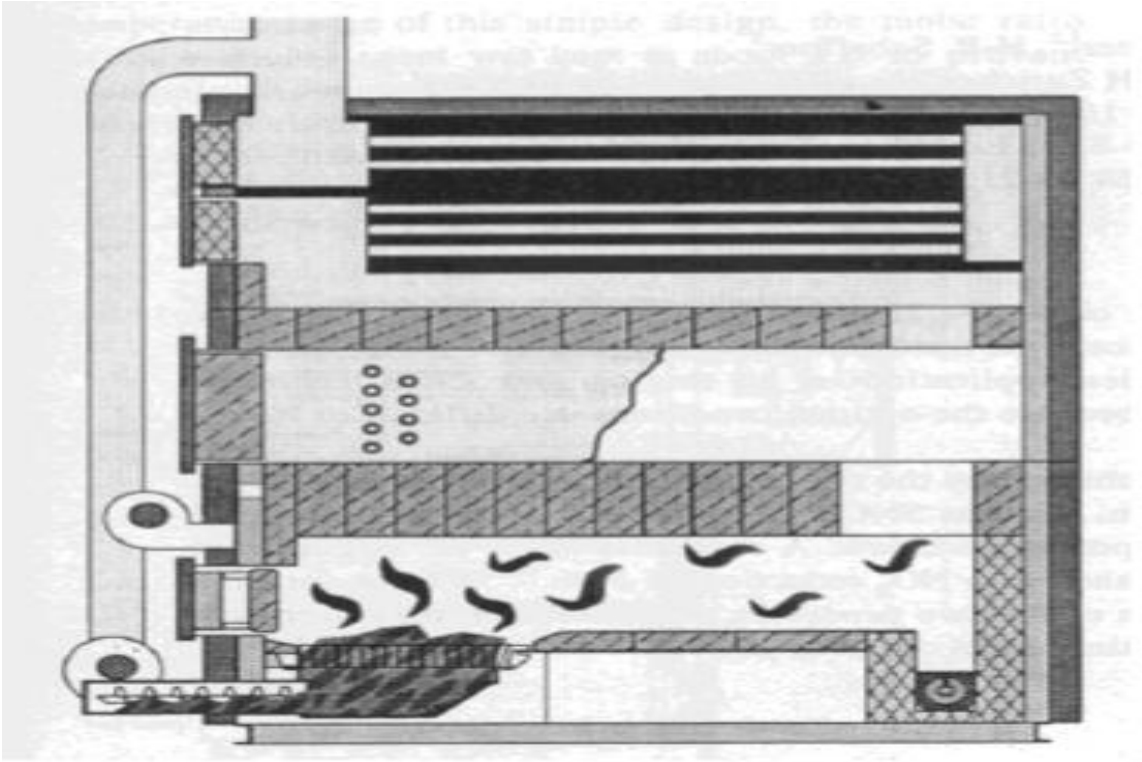

Figure 8: Schematic representation of a new boiler with food from below. (Obernberger, 1997)

These bolier are suitable only for small systems (over a nominal capacity of 6 MWh) and for low-ash

biomass fuels. High percentage of grain biomass requires efficient ash removal systems. *(Obernberger,1997)*

3.6 Fluidized base technology

Depending on the velocity of the air flow through the fluid base, this technology can be divided into the following two categories:

1. With fluctuating fluid slow.
2. With circulating fluidic.

- Fluctuating fluid slow

The air velocity in the dusty base is lower, since the bolier size is in inverse ratio with the air velocity passing through the base, the grille surface and a high power bolier will be high.

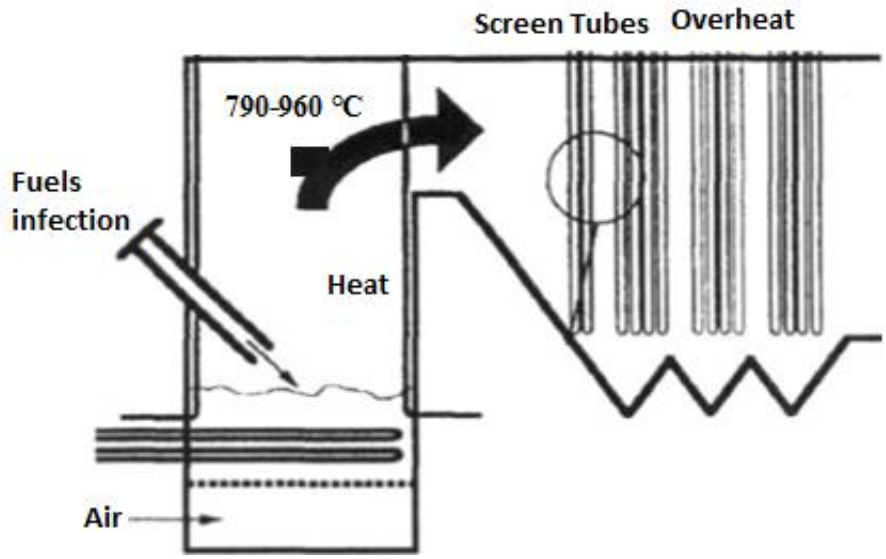

Figure 9: Schematic representation of fluid based boiler. (Obernberger, 1997)

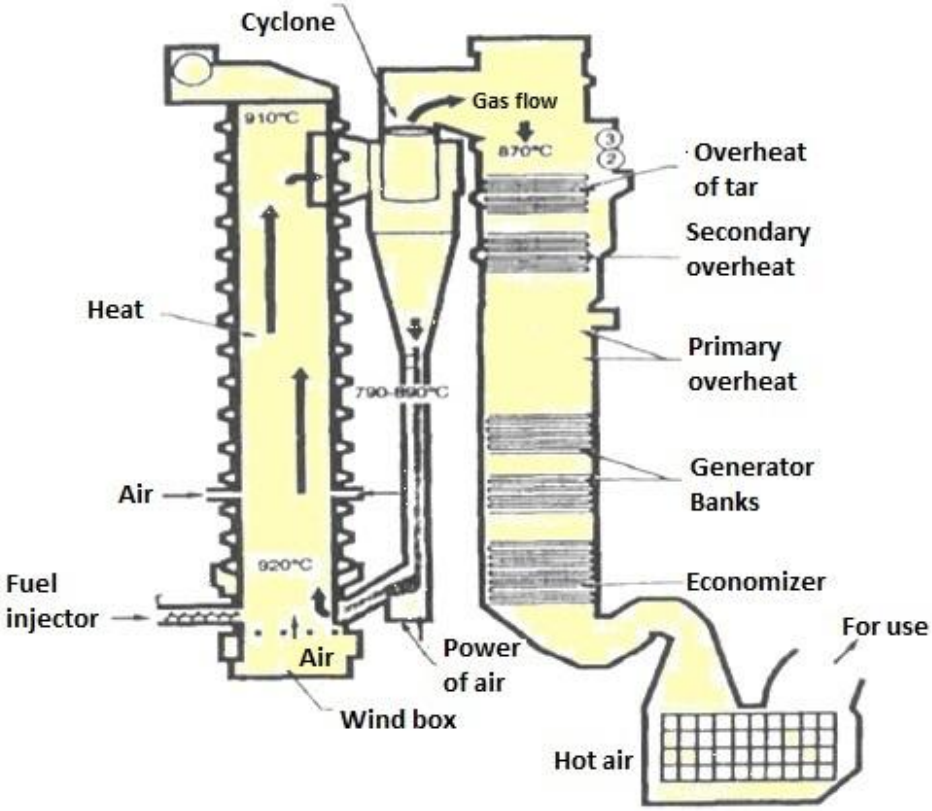

Figure 10: Schematic representation of circulating fluidic Source: (Basu,1991)

This technology is used for plants with a nominal capacity greater than 10 MW. The mainly sandy base is located in the final part of the bolier. The sand is about 1.0 mm in diameter, the air velocity fluctuates between 1.0 m/s and 2.5 m/s. Secondary air is inserted between holes in the shape of horizontal sprays constructed in such a way as to ensure a high efficiency of combustion of combustion during the combustion process.

In this technology, some combustible particles are burned at the reactor base while the rest is burned in the fluid base to keep the temperature at the desired limits of 800°C to 900°C. In most cases it is necessary to place the heat transfer surface on the reactor base.

One of the advantages of this technology is that the amount of ash removed directly from the boiler is bigger than the circulating base boiler. Another advantage of this furnace is their flexibility in terms of particle size and humidity content in biomass. (Obernberger, 1997).

- Circulating fluidic

This combustion technology is widely accepted as an advanced technology for the combustion of various biomass substances in an environmentally acceptable way. (Basu, 1991)

Powder speed 5 m/s - 10 m/s.

Particle size 0.2 mm - 0.4 mm.

The base temperature is around 800-900°C and is controlled by an external heat exchanger by cooling the recycled sand or from the water-cooled walls.

High turbulence of the system leads to transmission

better heat which is the advantage

with regard to the stable conditions of the combustion process

4. Conclusions

Technologies that are used to produce energy from sage biomass are of great interest. Biomass has a number of environmental benefits compared to fossil fuels.

Reducing the temperature in the combustion chamber is necessary to ensure continuous combustion.3. Both of these impacts reduce the temperature in the combustion chamber under the permissible limit, which is necessary to ensure a continuing combustion.

The liquefied base technology is the most appropriate technology in the case of sage biomass.

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RESEARCH ARTICLE **(Open Access)**

Sensitivity of early life stages of *Pelophylax shqipericus* to xenobiotics

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Abstract

The success of assisted reproduction technology applied in amphibians greatly depends on environmental factors. This study evaluated the sensitivity of *Pelophylax shqipericus* embryos to 0.05 and 0.1 mg/L copper exposure for 24h by assessing growth, hematological alterations and possible malformations. Susceptibility increased from fertilization to stage 40 Gosner, inwhich the 24h-LC100 were 0.058 mg/L, with an increased resistance observed from this stage onward. Malformations were observed at all developmental stages observed, with the most common being poor larval development and growth, incurved spinal cords and tails, reduced reaction to different stimuli, loss of equilibrium and shortening of swimming distance. Erythrocyte abnormalities like deformed cells and their nuclei, vacuolated erythrocytes and karryorhectic cells were the most hematological alterations evaluated for both concentrations 0.05 and 0.1 mg/L of copper ($p<0.1$). The high sensitivity of frog embryos to copper and probably to other xenobiotic, make them particularly vulnerable to environmental stress and importantly affect the reproductive success of species. Using frog embryos for acute toxicity tests can provide auseful information on several meaningful endpoints of amphibianreproduction and developmental process.

Keywords:*Pelophylax shqipericus*, embryos, reproduction success, developmental stages, sensitivity, copper.

Introduction

In the last three decades, amphibian populations world-wide are declining at an alarming rate. A number of causes have been suggested to explain global amphibian declinessuch as habitat destruction, environmental pollution, ultraviolet exposure, and pathogenic agents (Collins &Storfer, 2003; Beebee & Griffiths, 2005).

Intense agricultural and industrial production has increased prevalence of heavy metals in surface water. Among other aquatic vertebrates, amphibians are at high risk of heavy metal pollution since they prefer shallow, standing, and temporary ponds around agricultural fields as reproductive habitats (Tyler, 1994; Rowe et al., 2003). The increasing degradation of the aquatic environment by the negative effects of chemical pollution as a result of agricultural activities have been investigated in recent ecotoxicological studies (Van Dam et al., 1998).

Amphibians are extremely sensitive to chemical pollutants since they use both aquatic and terrestrial media throughout their lives(Murphy et al., 2000), and because of their ability to bio-accumulate toxic ions through semipermeable skin and gills, and via food intakes (Zhou et al. 2008). Common heavy metal pollutants, such as lead, zinc, cadmium, mercury and copper, have been shown to accumulate in liver and kidney of amphibians and may adversely affect their growth, development and reproduction, ultimately contributing to population declines (Sharma & Patino 2009; Sun et al. 2018).

Albanian water frog, *Pelophylax shqipericus*, an endemic species of Albania, is included to the IUCN Red List of Threatened Species in 2004, where it has remained since (Uzzell & Isailovic, 2009). Its population is threatened and in decline due to uncontrolled and aggressive collection for commercial purposes, habitat fragmentation by wetland drainage for infrastructure and farming, and pollution from agricultural and industrial run-off (Jablonski, 2011). In Albania to address the problem of extinction of some important amphibian species, assisted

reproductive technology (ART) has been applied successfully (Turani & Aliko, 2015; Turani, Aliko, Faggio, 2018).

Another important contributory factor to the decline of several amphibian species has been determined reproductive failure (Nystrom et al. 2007). For externally fertilizing animals, has been shown that heavy metal stress in water potentially can inhibit amphibian reproduction through oxidative stress, DNA damage in testes and sperm abnormality at extremely low levels (Zhang et al. 2012). Also, may decrease sperm size and normal sperm morphology percentage (Meeker et al. 2008) or/and may directly affect the sperm plasmalemmal structure and hence influence sperm motility and acrosome reaction (Della et al. 2017).

The study of the influence of environmental factors such as heavy metals during various stages development of *P. shqipericus* is of great importance in the successful application assisted reproductive technologies.

In previous studies, it was reported that some pesticides used during agricultural practices caused decreases in amphibian populations (Marco & Blaustein, 1999; Davidson et al., 2001; Sparling et al., 2001; Davidson, 2004; Sayım, 2007; Sparling & Fellers, 2007; Bernabo et al., 2008).

Copper sulfate (CuSO4·5H2O) is used as a fungicide and an algicide in agricultural practices. Furthermore, copper sulfate, being used in various industries such as the textile, leather, electroplating, tree protection, and oil industries, has many opportunities to contaminate the amphibian habitat. Copper sulfate is classified within the pesticides for general use, as defined by the EPA. It is stated that this pesticide, labeled as class I (highly toxic) in terms of toxicity, may have dangerous effects, particularly on species under threat of extinction in aquatic ecosystems, due to its potential risk of being mixed with surface waters (Extoxnet, 1996). This pesticide is used quite frequently in Albania, in agriculture as algaecide and fungicidae.

The effects of copper sulfate on the embryos and tadpoles of *Pelophylax shqipericus* are unknown, although, different study have evaluated susceptibility of amphibians to xenobiotics during various stages of development (Bustuoabad et al., 1977; Pe´rez-Coll &Herkovits 1990; Fort & Stover, 1996; Garcia-Munoz et al., 2009).

The aim of this study was to determine the morphologically and histologically acute toxic effects of copper sulfate on the development, growth, and survival rates of *Pelophylax shqipericus*tadpoles under laboratory conditions.

Materials And Methods

Animals

All sexually mature Albanian water frog *Pelophylax shqipericus,* were obtained from a pond near Scadar Lake (42o10N 19o19E/42.167oN 19.317oE) in the north-western part, Albania, during March-May 2017. Once arrived in laboratory frogs were acclimated for a week until the manipulation moment(in aquarium (40x37x60 cm) separated by sex, with de-chlorinated tap water, temperature 20±1.2ºC, pH 6.5±0.12, 12-h/12-h light/dark cycle). One third of the water in the aquariums was changed every 48h. Animal maintenance and experimental procedures were in accordance with the Guide for Use and Care of Laboratory Animals (European Communities Council Directive 86/609/EEC) and national and institutional guidelines for animal welfare (Act No. 10465, 29/9/2011: “On the Veterinary Service in the Republic of Albania”).

Chemicals

Copper sulphate ($CuSO_4$) is an inorganic salt with wide use as a potent algacidae, fungicidae and molluscicidae. In our study, we used copper sulphate provided by Sigma Aldrich, Germany.

Cyclophosphamide (CP) is one of the most extensively studied mutagens and teratogens. It was used as positive control in our experiments. Different

test solutions were prepared immediately before each experiment.

Experiment Design

After acclimatization in the laboratory, to achieve fertilization, both sexes were subcutaneously injected with approximately 100 ml/10 g bodyweight of the human hormone hCG (Silla et al., 2012) in a concentration of 1 mg/100 ml isotonic saline solution. Squeezed eggs were fertilized *in-vitro* by following the procedure described by Turani & Aliko (2018). The eggs are evaluated as successful fertilized when they reach neural stage (stage 14 according to Gosner) (Gosner, 1960). After this stage, the eggs were transferred from Petri dishes into plastic containers (20 x 11.5 x 7.5 cm) that were filled with aged water to a height of 2 cm. Water in the containers was changed periodically during development of the eggs. All experiments were carried out at a controlled room temperature of 20± 0.58^0C. The counting of formed embryos and unfertilized eggs was done under a stereomicroscope.

In our bioassay,*P. shqipericus*embryos and tadpoles at Gosner stage 2, Gosner stage 21, Gosner stage 40, obtained from two sets of parents were exposed to two low doses of copper sulphate (0.05mg/l; 0.1mg/l) for 24h. In each case, in total 200 *P. shqipericus* embryos and tadpoles were divided into 4 groups with 50 individuals each: (1) negative control; (2) positive control (using cyclophosphamide 5 mg/L); (3-4) treated with different CuSO4 concentrations.

The effects of copper sulphate were observed with a stereoscopic every 24 hours for survival rate and morphologic alterations. The dead tadpoles were counted and LC 100 values for these periods were determined. Also, the behavioral changes observed in all experimental groups were recorded. During the experiment, the water containing the copper compound and the food was not changed.

Morphologic and Hematological Abnormalities

Morphological changes of the*P. shqipericus*tadpoles were studied under a binocular microscope with a Canon Powershot A60 digital camera, andgeneral photographswere made. Twentyfive tadpoles from control and each treatment group, were measured for total body size, width and length, after surviving the 24-h test period.

Furthermore, 5 individuals from control and treatment groups were used to obtain blood samples by cardiac puncture, under a magnifying glass. Peripheral blood smears for each animal were immediately prepared on clean slides, fixed in absolute methanol for 5 minutes and air dried. These slides were stained with 12% Giemmsa solution for 20 minutes. The assay was realized by analysis of 1,000 erythrocytes from each tadpole using 1,000X magnification. The MNA frequency was expressed per 1,000 cells counted. The erythrocyte morphologic abnormalities, due to copper exposure, considered hereby were: swollen erythrocytes, karryorhectic (apoptotic) erythrocytes, and pycnotic erythrocytes.

Statistical Analysis

Data related with morphological measurement were tested for normality using Wilcoxon Signed Rank test, since the data were discrete and of non-normal distribution.The comparison between control and treatment groups in terms of total body size as well as body width and length, was made using the one-way analysis of variance (ANOVA) test. The evaluation of micronuclei frequency, as well as other erythrocyte morphological abnormalities, comparative both positive and negative controls with three exposed groups, was performed using Kruskal-Wallis test. P-values below 0.05 were considered statistically significant. Results were presented as mean ± standard deviation.

Results

According to our lethality data, no deaths were observed among tadpoles in the control group within 24 h of treatment. In one case (stage 40) the first dead tadpoles were detected after 12 h of

exposure, whereas at stage 21, lethality increased proportionally with exposure. More dead individuals were observed at the higher concentration (0.1 mg/l). After 24 h the ratio was 9 dead tadpoles at the higher concentration and none to the others. At the end of the study 9 tadpoles were dead at the 0.05 mg/l and 25 individuals at the 0.1mg/l ($p<0.05$), Figure 1.

After exposure of the tadpoles to the copper sulphate, susceptibility increased from fertilization to stage 40 Gosner, in which the 24h-LC 100 value was calculated as 0.058 mg/L, with an increased resistance observed from this stage onward. According to the results of the ANOVA, this stage is the most sensitive ($p<0.05$) to copper.

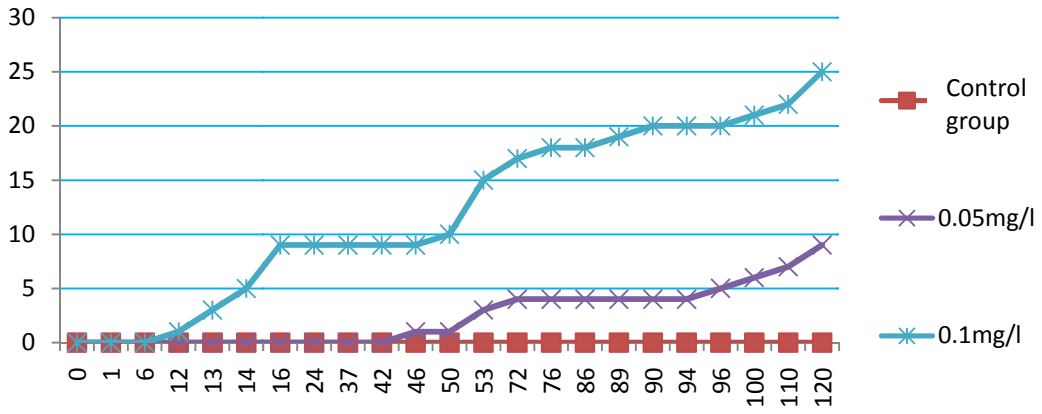

Figure 1. The mortality of *Pelophylax shqipericus* tadpoles in groups of different treatments with copper sulphate during 24 h

Tadpoles exposed to copper expressed malformations and reduced physical activity, especially at highest concentrations of coppersulphate. The most common abnormalities caused by sub-lethal copper concentrations were spinal cord deformity, damaged tails and edema. These effects were usually proportional to the copper concentration. No abnormalities were observed in the tadpoles of the control group. In contrast, these malformations occured mainly in embryos exposed to 0.1 mg/L of copper sulphate, Figure 2. There were differences among the groups according to morphological measurements ($p<0.05$).

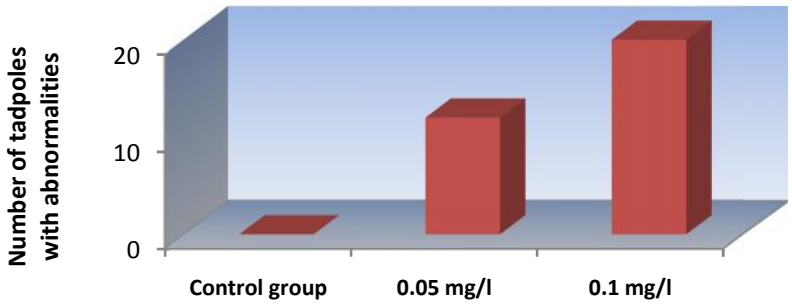

Figure 2. *Pelophylax shqipericus* tadpole's abnormalities in groups of different treatments with copper.

In the tadpoles exposed to copper sulphate in comparison to the control group, were observed some significant changes in their behavior, such as lowering stimulus stimuli, short swimming distance and loss of equilibrium. Also, in the lowest concentrations of copper, were found a higher growth rate than in the higher concentrations. In higher concentrations of copper, the growth rate of tadpoles is nearly zero, Figure 3.

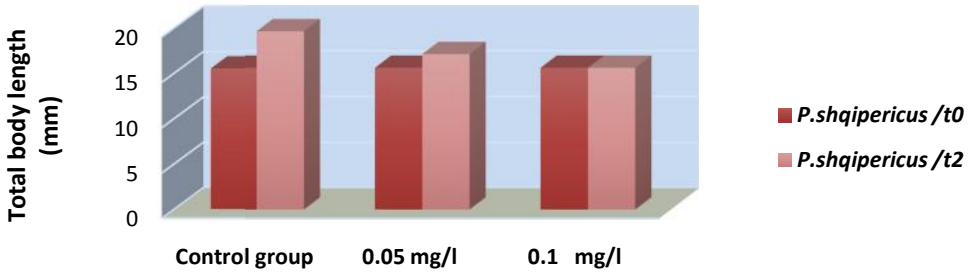

Figure 3. Mean total body length of *Pelophylax shqipericus*tadpoles of control and different treatments at the end of larval toxicity test experimental period

The exposure of *P. shqipericus* tadpoles to copper caused hematological abnormalities. All treated animals were anemic, and were found three damages in tadpole's erythrocyte: (1) swollen erythrocytes, (2) karryorhectic (apoptotic) erythrocytes and (3) pycnotic erythrocytes, Figure 4.There were found also high frequencies of micronucleated erythrocytes. There were no significant alterations in the frequency of micronuclei at the two different concentrations of copper sulphate (p=0.4912).

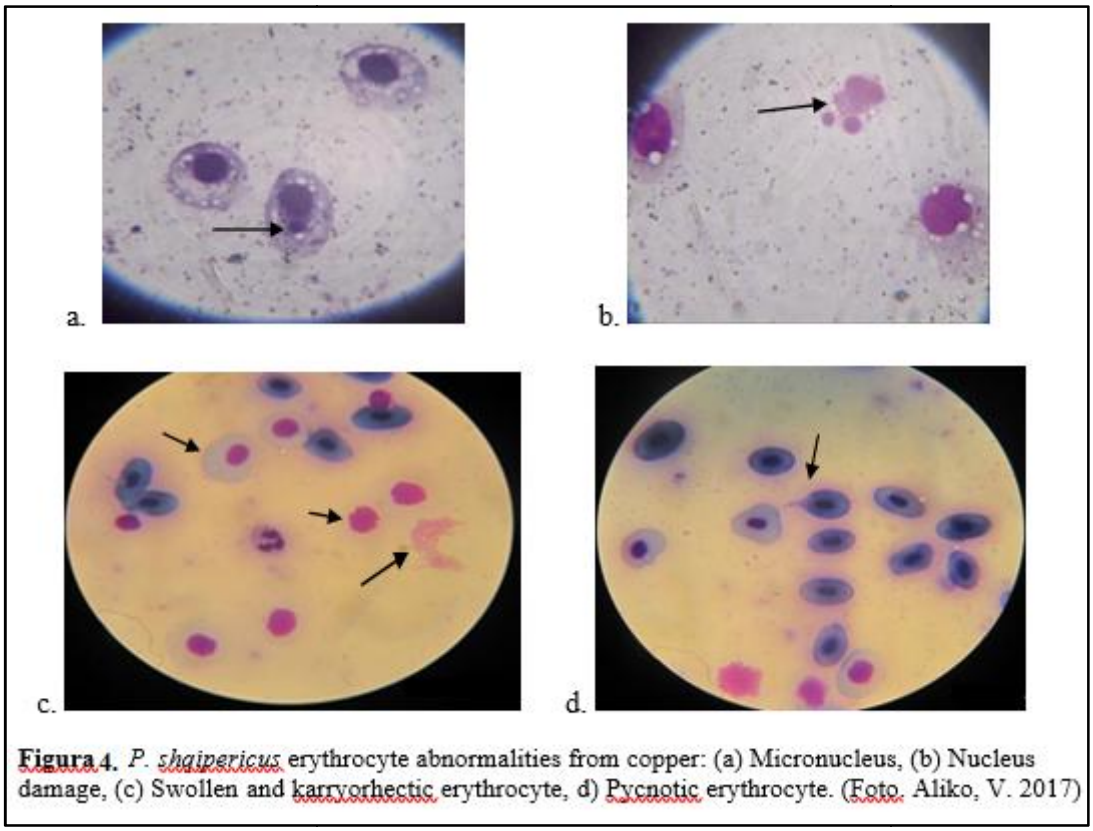

Figura 4. *P. shqipericus* erythrocyte abnormalities from copper: (a) Micronucleus, (b) Nucleus damage, (c) Swollen and karryorhectic erythrocyte, d) Pycnotic erythrocyte. (Foto. Aliko, V. 2017)

Discussions

Our main objective was to examine the effects of copper sulfate on the survival, development, growth, of *Pelophylax shqipericus*. The results obtained in this study show that, copper is a toxic substance that affect growth, morphology and hematology of *P. shqipericus* tadpoles. Intraspecific variation in the susceptibility of amphibian embryos to xenobiotics is well documented (Pierce et al., 1987; Perez-Coll et al., 1988; Tyler-Jones, et al., 1989). The high susceptibility of their embryos to copper (Garcia-Munoz et al., 2009) and other xenobiotics (Bustuoabad et al., 1977; Dumont et al.,

1983;Herkovitsetal., 1990; Perez-Coll et al., 1996) as well as their semiaquatic lifestyle, make amphibians particularlyvulnerable to environmental stress, which could partially explain the worldwile decrease in their populations (Baringa, 1990; Simms, 1969).

It is well known that the toxicity of copper is strongly dependent on several biotic factors (age, stage, size and conditions of organisms) as well as on water quality of the environment (Ossana et al, 2010). In our experimental design those factors were kept constant along the assay, therefore, the observed alterations cannot be attributed to them.

It is clearly proven from the results of this study that, copper sulfate is a pesticide that has the effect of decreasing the survival percentages of *Pelophylax shqipericus* tadpoles.Copper sulfate exposure induces negative effects on development. The increased developing time of tadpoles is a clearly evidence of the role of organism physiological state in facing the toxic effects of water pollution. That effect on growth and development might have important repercussions on the conservation of this species. Susceptibility increased from fertilization to stage 40 Gosner, in which the 24h-LC 100 were 0.058 mg/L, with an increased resistance observed from this stage onward.

Similar acute toxicity studies that investigated the toxic effects of pesticides on toads, have reported that morphological anomalies (head and tail deformations, edema, ventral blistering, crooked body) were observed at the embryonic and larval stages of development (Nebeker & Schuytema, 2000; Ossana et al., 2010). The fact that the morphological anomalies that were seen at the end of the treatment period, such as edema on the necks of tadpoles, were more apparent with increasing doses of copper sulphate suggests the susceptibility of tadpoles of this species to copper sulphate toxicity. Since the negative effects of this toxic substance, may also prevent the development of the skeletal and muskular systems of the tadpoles, the immobility and shortened swimming distances observed in the tadpoles of treatment groups in comparison to the tadpoles in the control group may be attributed to this effect. Similar behavioral changes are reported in previous studies (Garcia-Munoz et al., 2009; Gurkanand Hayretdag, 2012). MNA frequencies in the individuals exposed to copper sulphate were significantly higher than in the negative control. Some authors have suggested that variations in the shape of nucleus could represent an alternative approach for detecting genotoxicity. Cellular and nuclear morphological alterations of tadpole erythrocytes can be considered as cytotoxicity indicators (Aliko & Biba 2011; Cavas et al. 2005).

It is worth to emphasize that the mechanisms that form these cellular and nuclear abnormalities are not yet fully understood (Cavas & Ergene-Gozukara, 2003). We strongly suggest that such morphological alterations could be a manifestation of the combined effects of copper as well as other xenobiotics, potentially found in the water. Finally, since we found more cellular than nuclear abnormalities in the erythrocytes of tadpoles, we can assume that damages caused by copper sulphate are more cytotoxic than genotoxic. At any rate, our results revealed adverse effects of copper on the erythrocyte morphology and tadpole development of amphibians.

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RESEARCH ARTICLE **(Open Access)**

Assessment of physical chemical parameters of leachate from waste landfill in Kosovo

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Abstract

Waste landfill in Kosovo is burdened with mixed (heterogeneous) waste, dominating organic wastes by over 40%. Water drainage streams other than concentrations of organic matter contain many other polluting elements. The purpose of the study is to evaluate some physico-chemical parameters of discharge of landfill streams. In the study are included the landfillsin Peja, Prizren, Podujeva and Gjilan. Organic waste at landfills affect the value of parameters such as pH, total suspended matter (MTS), total organic carbon (TOC), chemical oxygen demand (CHOD), biochemical oxygen demand (BOD_5), total nitrogen (NT) and total phosphorus (PT) and other parameters. High concentrations of these parameters in landfills show high levels of organic waste. Organic waste at the landfill passes into a process of physical, chemical and biological digestion. Treatment of landfills, it reduces polluted emissions in the environment.

Keywords: landfill waste, water discharge pollution

Introduction

Municipalities are responsible for establishing a waste management system for their respective territory, drafting/developing local plans for waste management in line with the national plan[1]there are seven Regional Waste Companies (RWC), operating in seven regions, 26 municipalities where 93 % of the population of the country live. The history of waste management in Kosovo is estimated to have started since the 1970s of the last century [2]. The amount of waste disposed in the Kosovo Landfill Management Companyoperated landfills for 2017 is 280,334.55 ton/year. On the municipality owned landfills operated by the RWC, the amount of waste deposited is approximately 111,951.55 tons/year. This makes 392,286.10 ton/year disposed in sanitary landfills. **[1**]. Landfill sites are constructed by complying with local legislation and international standards. It was found that illiteand bentonite are suitable minerals for ultimate isolation of waste landfills **[3].** Within this framework, an integrated analysis of environmental assessment, including components such as; geological assessment, geo, hydro, impact on the biosphere, and assessments of other impacts to the area**[4].**The waste management sector is characterized by low levels of efficiency, and inadequate experience in the use of modern technology. With the amendment of the Law on Waste in 2012 municipalities are responsible for regulating the responsibilities for waste management **[1].**Decomposition and further degradation of waste in landfills passes through several phases; aerobic, anaerobic phase, initial methanogenic phase and methanogenic stable**[5].**Some of the parameters that are being studied to look at the impact of waste on the environment aretotal suspended matter (MTS), total organic carbon (TOC), chemical oxygen demand (CHOD), biochemical oxygen demand (BOD_5), total nitrogen (NT) and total phosphorus (PT) and other parameters.pH values in industrial and urban

wastewater depend heavily on the amount of free CO_2 in water, sulfuric acid, organic and humic acids **[6].** Suspended substances have negative effects on water because they inhibit the penetration of sun rays and prevents this ecosystem processes in the water. The effect of suspended matter on the aquatic ecosystem depends also on the chemical composition, the concentration of suspended matter, the duration of the exposure **[7]** The presence of CHOD in the water stream shows the serious threat of polluting underground water **[8]**. BOD_5 is an indicator of the required amount of oxygen that needs microorganisms under aerobic conditions to break down organic matter into the water.Therefore, the main focus of water treatment plants is the breakdown of organic matter from microorganisms **[9].** The presence of TOC in water comes from natural organic matter as well as from various synthetic sources. TOC is determined through laboratory analytical method which serves to determine the content of organic substances which originate from urban waste water, waste etc. In case of high contamination of water with organic matter, the TOC values will be presented with high values as well. Total Phosphorus includes all inorganic and organic phosphorus forms in the environment. Phosphorous concentrations depend on the content of natural rocks or the erosion of agricultural lands. The main anthropogenic sources of phosphorus are urban and industrial wastewater, agricultural land, animal and human excrements, detergents. Large concentrations can cause problems in eutrophication of water**[10].**The total nitrogen is the sum of nitrate-nitrogen (NO3-N), nitrite-nitrogen (NO2-N), ammonium nitrogen (NH3-N) and organically bound nitrogen. Total nitrogen is a nutrient essential for plants and animals. However, excessive amounts of nitrogen in a circulating stream can lead to decreased levels of dissolved oxygen which negatively affects the change of plant and animal life. Nitrogen sources include: urban wastewater, chemical fertilizers, animal waste, and industry**[11].**

Material and Methods

Depending on the flow and the distance of the discharge from the landfill, the locations of waste water have been identified and defined site-samplings.The water sampling points were taken for analysis in 4 landfills (Peje, Prizren, Gjilan and Podujeve), their water streams and 2 points in the rivers (before and after the discharge of the river water streams).

Methods according to ISO 5667-10 have been applied for sampling, while methods for ISO-5667-6 standard for sampling in water and rivers have been applied. Samples were taken in different time periods and season during 2015/2016. Sampling is done on average every two months or 6 times within 2015/2016. At each stage the water samples are taken at the same point as previously defined by coordinates. In the Prizren landfill there are 4 points for the sampling of water (a sampling place in the lagoon, a sampling place in the flowing water and two landfills in the Landovica River. In the Podujevo landfill site, water sampling is defined in two monitoring points sampling in the lagoon and a sampling place in the flowing water. In the Gjilan landfill are defined 4 water sampling points (a sampling place in the lagoon, a sampling site in the water stream and two other monuments in the Malisheva River.In Peja's landfill two water monitoring points are defined in the lagoon and the other in the water flow. For the determination of CHOD, BOD_5 and TOC, samples were taken with 100 ml glass bottles and were preserved with 2 ml concentrated H_2SO_4. The temperature, pH, the measurement is done directly at the sampling site. The WTW 3320 wiring device is used. Other parameters are determined in the laboratory as follows; total suspended matter, BOD_5, CHOD, TOC, total phosphorus, total nitrogen, were determined with WTW 6600 UV-VIS spectrophotometer. Standard analytical methods

for the set water parameters are presented in tabular form (see Tabel 1)

Table1. Analytical Methods for the specified water parameters

Symbols	Unit	Type of equipment	The range	Accuracy of measurement	Standardization
pH	0-14	HI98130 pH/Conductivity/TDS	-2 deri 19.99	±0.01	DIN 38404-C5
MTS	mg/L	AADAMLAB-250	0.0001mg-250mg	±0.0005	ASTM 2540 D
CHOD	mg/L	WTW 6600 UV-VIS Spectrophotometry	1 mg/L to 350 mg/L	±0.1	ISO 15705
BOD5	mg/L	Electrocemistry- HI 1946	0.2 mg/L to 350 mg/L	±0.1	ISO 5815-2
TOC	mg/L	WTW -6600 UV-VIS Spectrophotometry	0.5 mg/L to 300 mg/L	±0.1	APHA 5310 D
NT	mg/L	WTW 6600 UV-VIS Spectrophotometry	0.5-15.0 mg/L N	± 0.8	ISO 11905-1
PT	mg/L	WTW 6600 UV-VIS Spectrophotometry	0.002 – 15.3 mg/L	±0.07	ISO 6878

Results and Discussion

The determination of the results of the 7 parameters from the analyzed samples of water from the lagoon, water streams and rivers where the discharge of the polluting waters is carried out, we have obtained the following results; High pH values have been recorded during Phase I and VI in all locations (see Table 2). In lagoon waters, the highest pH values are recorded at the monitoring point (Pd-Llag) at 10.35 and at the monitoring point (Pe-Llag) with max. 9.23. The pH values in the water fluctuate depending on the sampling phase, water volume and meteorological conditions. In the water streams the highest values of pH are recorded at the point (Pd-Rr), at 9.86 and at the monitoring point (Pe-Rr), at 9.56. In both cases the water flow rates were higher than in the lagoon. Lagoon waters in the landfills of Peja and Podujeva, the highest values of pH are recorded during all sampling phases. The higher values of pH in water the landfill streams are recorded in Prizren and Podujevo.

Table 2. pH values in six stages of sample analysis

Fazat	Pe-Llag	Pe-Rr	Pz-Llag	Pz-Rr	Pz-Lmb	Pz-Lp	Pd-Llag	Pd-Rr	GJ-Llag	Gj-Rr	GJ-Lmb	Gj-Lp
F-I	**9.04**	**9.56**	**8.82**	**9.01**	**8.04**	**8.11**	**9.74**	**9.03**	**8.18**	**9.18**	**7.97**	**7.94**
F-II	**9.23**	-	8.97	8.93	7.97	8.04	**10.35**	9.20	7.76	7.78	7.90	7.87
F-III	8.40	8.58	7.91	7.73	7.83	7.85	10.06	9.03	8.38	8.05	7.78	7.80
F-IV	8.34	8.28	8.31	8.00	8.18	8.12	8.83	8.00	7.90	7.53	7.89	7.90
F-V	8.40	8.15	8.04	8.24	8.13	8.18	8.41	8.49	7.96	7.84	7.89	8.31
F-VI	**8.26**	**8.30**	**7.98**	**8.50**	**8.12**	**8.47**	**9.69**	**9.86**	**9.18**	**9.04**	**8.87**	**8.92**

Total suspended matter - (TSM); Total suspended matter resulted to be higher during the II and IV phase for all water sampling sites (see table 3). Measurements have shown that all lagoon waters have very high concentrations of MTS. The highest values were recorded at the monitoring point (Gj-Llag) at 29600 mg / l, and at the point (Pz-Llag), with max values of 15800 mg / l. In the sample analyses at the monitoring points (Pz-Lp and Gj-Lp), it is noticed that the water streams after discharge into the river have caused TSM to rise to higher values. From this it is concluded that the discharge of wastewater from the landfill has a direct impact on the river in raising the TSM values.

Chemical oxygen demand CHOD's highest values are recorded during the II, IV, and VI sampling stages during June, December and March (see table 4).

Table 3. Total suspended particels values in six phases of sample analysis

Fazat	Pe-Llag	Pe-Rr	Pz-Llag	Pz-Rr	Pz-Lmb	Pz-Lp	Pd-Llag	Pd-Rr	GJ-Llag	Gj-Rr	GJ-Lmb	Gj-Lp
F-I	8010	6150	780	2.5	0.85	1.4	290	20.4	5600	31	11.3	12.7
F-II	**3350**	**-**	**7000**	**35**	**11.9**	**18.2**	**1100**	**42**	**29600**	**204**	**158**	**178**
F-III	920	135	3200	246	1.3	4.5	1510	164	11360	184	97	104
F-IV	**8500**	**14800**	**15800**	**60**	**nld**	**14.6**	**1545**	**nld**	**8000**	**21**	**37.5**	**40**
F-V	9900	7800	4050	1170	70.5	164	148	87	6150	26.6	12.7	14.6
F-VI	5200	13400	660	158	1.0	20.4	910	50.5	5800	124	0.3	0.6

Table4. CHOD values in the six stages of sample analysis

Fazat	Pe-Llag	Pe-Rr	Pz-Llag	Pz-Rr	Pz-Lmb	Pz-Lp	Pd-Llag	Pd-Rr	GJ-Llag	Gj-Rr	GJ-Lmb	Gj-Lp
F-I	12400	9100	3500	9.4	6.3	6.8	350	54.4	10700	138	14.2	16.9
F-II	**43500**	*****	**11600**	**45.5**	**44.5**	**44.7**	**1640**	**116**	**36500**	**180**	**100**	**120**
F-III	15200	1470	4500	132	1.6	7.8	1875	282	20600	141	126	138
F-IV	**13800**	**45000**	**31500**	**36**	**nld**	**18**	**575**	**390**	**13400**	**92.8**	**84**	**86**
F-V	15200	14000	5100	1440	87	135	680	152	8500	131	56	57
F-VI	**8700**	**35500**	**3325**	**580**	**1.3**	**37**	**1660**	**128**	**12800**	**208**	**20.8**	**22.2**

* has been no water for sampling

From the lagoon waters, the highest value CHOD is recorded at the monitoring point (Pe-Llag) with a maximum value of 43500 mg / l and point (Gj-Llag), with values up to 36500 mg / l. The lowest values were recorded at the monitoring point (Pd-Llag) at 1875 mg / l.From water streams, the highest values of CHOD are recorded at the point (Pe-Rr), with 45000 mg / l, values many times higher than in the lump, as well as in the point (Pz-Rr), with a value of 1440 mg / l. In the sampling of river monitoring points, such as (Pz-Lp and Gj-Lp), it is noted that the elevated values of CHOD are the result of direct discharge of landfills. Since the values of CHOD have shown a high variability of site sampling, to prove this variability we did statistical analysis with T-student and have shown significant values. From the significant values of GO, we conclude that the averages in points (Pd-Llag / Pd-Rr, GJ-Llag / Gj-Rr, GJ-Llag / GJ-Lmb, GJ- Lmb, Gj-Rr / Gj-Lp, are significant because p-values are <0.05 (see tab 5).

Biochemical Oxygen Expenditures (BOD_5) According to the results of the BOD_5 test, it has

resulted with higher values during the II, IV and VI stages of all time periods of water sampling. High BOD_5 values have resulted high at the same phases with those of CHOD and TOC.

Table 5. Significant values of CHOD, NT, TOC, BOD5 in sample site locations (S student test)

Comparison of sample values	Significance of p values			
	NT	TOC	BOD5	CHOD
Pair 1 Pe-Llag/Pe-Rr	.623	.313	.311	.421
Pair 2 Pz-Llag /Pz-Rr	**.029**	.112	.102	.091
Pair 3 Pz-Llag/Pz-Lp	**.022**	.102	.093	.079
Pair 4 Pz-Rr/Pz-Lp	.151	.158	.148	.178
Pair 5 Pd-Llag/Pd-Rr	.077	**.010**	**.014**	**.019**
Pair 6 GJ-Llag/Gj-Rr	**.026**	**.001**	**.001**	**.010**
Pair 7 GJ-Llag/GJ-Lmb	**.025**	**.001**	**.001**	**.010**
Pair 8 GJ-Llag/Gj-Lp	**.025**	**.001**	**.001**	**.010**
Pair 9 Gj-Rr/GJ-Lmb	.268	**.049**	.060	**.031**
Pair 10 Gj-Rr/Gj-Lp	.258	.058	.067	**.047**
Pair 11 GJ-Lmb/Gj-Lp	.675	**.031**	**.037**	.096

Table 6. BOD_5 values in six stages of sample analysis

Fazat	Pe-Llag	Pe-Rr	Pz-Llag	Pz-Rr	Pz-Lmb	Pz-Lp	Pd-Llag	Pd-Rr	GJ-Llag	Gj-Rr	GJ-Lmb	Gj-Lp
F-I	4950	3050	2280	8.5	3.8	4.2	70	34.4	5150	108	10.42	11.22
F-II	**10900**	*****	**4900**	**11.7**	**16.6**	**16.4**	**575**	**76**	**8100**	**82**	**45.5**	**49**
F-III	6200	760	1340	59	0.6	7.8	415	132	7300	39.5	32	36.5
F-IV	**5500**	**24400**	**16000**	**16**	**nld**	**13.9**	**415**	**244**	**5500**	**70.5**	**67**	**68.5**
F-V	5550	6300	1630	320	29.1	52.6	524	65.5	2420	105	42.5	42
F-VI	**3625**	**20250**	**2600**	**420**	**0.2**	**17.4**	**760**	**79.5**	**7150**	**188**	**14.8**	**18.5**

nld-under the detection limit

In lagoon waters, the highest values of BOD5 are recorded at the monitoring point (Pe-Llag, 10900 mg / l), and in the point (Pz-Llag, 16000 mg / l).

The lowest values of BOD5 are recorded at the point (Pd-Llag), with maximal value up to 760 mg / l.

In water streams, the highest values of BOD5 are recorded at (Pe-Rr, 24400 mg / l), which is above four times higher in the lagoon as well as in the point (Pz-Streams, with a value of 420 mg / l). At river checkpoints as; (Pz-Lp and Gj-Lp), it is noticed that the value of BOD5 has been increased, after discharge of waste streams. From this it is concluded that the

landfills directly affect the increase of the BOD5 level in the river. From the statistical analysis it is observed the significance in the variables of BOD5 values highlighted from one point to another. The significant value of BOD5 resulted in comparative points Pd-Llag / Pd-Rr, Gj-Llag / Gj-Rr, Gj-Llag / Gj-Lmb, Gj-Llag / Gj-Lp and Gj-Lmb / Gj- Lp, where p-values are lower than <0.05.

Total Organic Carbon(TOC) According to the results it is noted that the highest values of TOC have resulted during the II, IV and VI phase of sample analysis (see Table 7). In the analyzed samples of lagoon waters, the highest values of TOC are recorded at the point (Pz-Llag, with a max value of 12,700 mg / l and at the point (Pe-Llag, with a max value of 7900 mg / l). point (Pd-Llag), the TOC had lower values of 580 mg / l. From the water streams, the highest values of the TOC are recorded at the point (Pe-Rr, with a maximum value of 18600 mg / l), higher values than in the lagoon at the same metering stage and at the point of sampling (Pz-Rr, with max values 340 mg / l) of TOC. At the point of sampling site (Pz-Lp), the max values reach up to 29.10 mg / l. At point (Gj-Lp), TOC values are recorded at max with 39 mg / l. Based on this it is found that after the discharge of waste water streams have influenced the increase of TOC values in the river.

Table 7.TOC values in six stages of sample analysis

Fazat	Pe-Llag	Pe-Rr	Pz-Llag	Pz-Rr	Pz-Lmb	Pz-Lp	Pd-Llag	Pd-Rr	GJ-Llag	Gj-Rr	GJ-Lmb	Gj-Lp
F-I	3850	2340	1840	7	2.9	3.8	50	19.8	4050	87	9.5	10.6
F-II	**7900**	*****	**3800**	**8.5**	**6.5**	**7.2**	**440**	**60.5**	**5800**	**61**	**31.5**	**35**
F-III	4800	620	1010	28	0.2	6.3	295	95	5600	29.8	23.4	26.5
F-IV	**4250**	**18600**	**12700**	**10**	**nld**	**2.8**	**338**	**140**	**4300**	**57.5**	**37.5**	**39**
F-V	4300	5000	820	228	28.6	29.1	424	28.6	1820	34.5	34.5	34
F-VI	**2800**	**15250**	**2100**	**340**	**0.2**	**13.7**	**580**	**63**	**5650**	**68**	**9.8**	**11.6**

Based on statistical analysis with Student tests we find significant value in comparing TOC values of water in the lagoons and those of the flows and especially in comparison of these samples Pd-Llag / Pd-Rr, Gj-Llag / Gj-Rr, Gj -Llag / GJ-Lamb, Gj-Llag / Gj-Lp, Gj-Rr / GJ-Lmb and Gj-Lmb / Gj-Lp, (see table XX)

Nitrogen total (NT) - NT values have resulted in high values in all samples at landfill site sampling points. The highest values are measured during the analysis of samples in Phases II, III and VI (see table.8).

Table 8. The values of NT in six stages of analyzing samples

Fazat	Pe-Llag	Pe-Rr	Pz-Llag	Pz-Rr	Pz-Lmb	Pz-Lp	Pd-Llag	Pd-Rr	GJ-Llag	Gj-Rr	GJ-Lmb	Gj-Lp
F-I	163.35	100.65	75.24	0.281	6.13	5.36	2.31	0.805	169.95	18.8	5.34	6.2
F-II	**1560**	*****	**1190**	**3.4**	**1.611**	**1.674**	**68**	**5.3**	**1580**	**5.94**	**3.3**	**4.2**
F-III	**3948**	**97.5**	**421**	**16.7**	**4.25**	**4.61**	**78.7**	**14.67**	**572**	**6.6**	**4.36**	**4.8**
F-IV	224	823	551	4.66	4.16	4.57	63.33	40.65	491	3.1	5.25	3.34
F-V	1012.9	1013.1	344.4	91.5	6.4	7.75	25.73	5.91	330.04	7.12	6.99	7.05
F-VI	**484**	**1486**	**460**	**77**	**4.12**	**5.35**	**208.4**	**28.4**	**739**	**7.11**	**6.64**	**7.47**

The obtained results show that water samples from landfills have the highest values at the point (Pe-Llag) with 3948 mg / l and at the point (Gj-Llag), the maximal NT values were found to be 1580 mg / l. The lowest NT values compared to the other water dump samples are recorded at the point (Pd-Llag), with 208.4 mg / l. Since there are significant changes in the values of the sampling site is doing statistical analysis (Student test). According to comparative data of samples in Pz-Llag / Pz-Rr, Pz-Llag / Pz-Lmb, Pz-Llag / Pz-Lp, Gj-Llag / Gj-Rr, Gj-Llag / GJ-Lmb, Gj-Llag / Gj-Lp, we have significant NT values, where p is less than <0.05 (see tab. Xx).

Total Phosphorus (PT) - The values of PT in the lagoon waters of landfills have resulted in higher values. High PT values are measured during sample analysis in Phase II, IV, V and VI (See Table 9).

Table 9. The values of PT in six stages of analyzing samples

Faza t	Pe-Llag	Pe-Rr	Pz-Llag	Pz-Rr	Pz-Lmb	Pz-Lp	Pd-Llag	Pd-Rr	GJ-Llag	Gj-Rr	GJ-Lmb	Gj-Lp
F-I	217.6	110.9	70.27	0.428	0.189	0.228	5.61	0.639	144.2	3.028	0.421	0.505
F-II	**1229**	*****	**327**	**1.351**	**1.258**	**1.282**	**45.92**	**3.25**	**1022**	**5.04**	**2.8**	**3.36**
F-III	491.45	52.08	161.86	4.05	0.21	0.48	52.69	8.07	54.67	4.17	4.19	4.41
F-IV	**413**	**1275**	**924**	**1.63**	**0.01**	**0.61**	**19.2**	**11.05**	**395**	**2.73**	**3.67**	**3.63**
F-V	**448.42**	**405.04**	**155.84**	**40.48**	**2.46**	**3.42**	**20.99**	**4.419**	**254.3**	**3.73**	**2.64**	**2.79**
F-VI	**261.37**	**1030.19**	**112.66**	**18.2**	**0.28**	**1.57**	**62.78**	**3.95**	**384.97**	**6.16**	**1.21**	**1.66**

From the sampling of wetland sampling, the highest values of total phosphorus have been found at the monitoring point (Pe-Llag) at 1229 mg / l and at point (Gj-Llag), the max values of PT are matched with 1022 mg / l. The results obtained by analyzing the samples of water from the site of the water flow sampling show that the highest values of PT are recorded at Pe-Rr, at 1275 mg / l, when these values at the same stage in the three times smaller, and at the point (Pz-Rr), with max measured values of 40.48 mg / l (see tab 9). It is stated that the water from the Pejë landfill has very high values of PT, as well as water from the Prizren landfill.

4.Conclusion

It has been verified that the pollution of the wastewater from the landfill reaches the impact over 800 m, as is the case at the discharge points (Pz-Lp / Prizren and GjLp / Gjilan)

The results show that the impact of water on landfill pollutants in the environment (land and surface waters), includes a wider area than the study area,

pH as in lagoon waters and in landfill the average pH values were above 8 in the wastewater of Peja, Podujeva and Gjilan. This concludes that the remains of these landfills have concentrations of organic acid content.

According to the most polluted results are the waters of the Gjilan and Podujevo landfills, while the flow of water is the point (Pe-Rr). It is argued that water in the lagoon and water is loaded with suspended matter composed of clay, small particles of organic and inorganic substances, soluble organic compounds and microorganisms.

It was found that the chemical oxygen consumption in all waters of the landfill are raised, but Pe-Llag pointare high compared to other waters. This indicates the concentration of organic matter in these waters is quite high, which comes from organic waste as well as high microbiological activity.

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RESEARCH ARTICLE **(Open Access)**

The effects of in vitro culture on the leaf structure of rootstock CAB 6P (Prunus cerasus)

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Abstract

Stomatal apparatus are epidermal characters that are very important in characterizing the plant species and represent a large variability between in vivo plants growths, in vitro acclimatized.

In our study during 2018 year, epidermal characters are compared (stomata) of the in vivo P. Mahaleb (*prunus cerasus L*) and acclimatized to CAB 6P (*prunus cerasus L*) in vitro.

Epidermal characters which were observed were: distribution and stomatal type, stomatal number (stomatal density) for the whole microscopic field / for leaf surface units in the plantlets of two variants prunus cerasus rootstock CAB 6P studied during the acclimatization of in vitro cultivation and P. Mahaleb in vivo, the leaves are hystomatic with anomocytic stomatal apparatus.

Both variants of in vivo and in vitro cultivation, it turns out that stomata of the down epidermis have elliptical guard cells. Stomatal density is higher in "in vivo" plantlets compared to those of "in vitro" acclimated, due to high air humidity and increased intensity of lighting.

The values of the search indicators are presented with a variable for the respective variants in two rootstocks P. Mahaleb (in vivo) and CAB 6 P (in vitro), respectively: the number of accompanying cells was 5-7 to 5; stomatal length 18.2 to 16.7; stomatal width (μm) 11.3 with 10.4 and average stomatal density (mm2) 39 with 36.

Key word: *in vivo, in vitro, stomata, acclimatization, Prunus cerasus L*

Introduction

The differences that occur between the mother and the plants micro propagated.

Various histological methods are used to understand in vitro systems. Structural analyzes are an important step in the study of organizing and changes in the body of the plant. For a plant species, stomatic structures are controlled by specific genetic mechanisms.

In vitro plantlets, generally grow at low light intensity (1200-3000 lux) and temperature (25 ± 2^{o}C), therefore direct transfer to the wide spectrum of sunlight (4000-12000 lux) and temperature (26-36^{o}C) gives plant changes (Chandra et al., 2010)[1]

Stomatal: the role, changes in culture and their classification

Epidermal Stomatal are very important characters in the characterization of plant species and present great variability among the plants increased in vivo and increased in vitro and acclimated ones. Any stomatal is surrounded from two cells in the form of beans called guard cells which regulate the removal of water, oxygen and carbon dioxide vapor from stomatal by checking the opening and closing of stomatal powder. The stomas are surrounded by another type of cell that is called accompanying or neighboring cells, the number of which varies depending on the species. Stomata along with accompanying cells forms which called stomatal complex or stomatal apparatus

Material and Methods

The in vivo, in vitro and acclimatized CAB 6P (prunus cerasus L) and P. Mahaleb (prunus cerasus L) epidermal (stomatal)

- In vitro materials; high-physiological indicators (1 year old)

- In vivo foliar was obtained from adult tree in May (1 year)

Stomatal density and stomatal height

Plantlets in "in vitro" and in vivo plants, observed in the same time frame.

1. We have available new leaves (in vitro and in vivo) of CAB 6 P and P. Mahaleb.

2. The data table is completed after being performed by 10 microscopic views for each leaf.

3. The obtained epidermis of down periderms of each leaf as follows:

(a) Make a leash for each leaf and analyze 10 views for each piece.

Draw a small leaf area (one square approximately 1-2 cm on one side) with a thin layer of nail cleaner translucent

- Leave for a few minutes to dry well and clean by means of a translucent adhesive scotch, placed on the lamella, labeling the lamella (a thin layer is the best observable).

Be careful not to enter the air down the ribbon of the scotch. (E. Kullaj 2006)[2]

4. Converts each count of stomatal for every viewpoint in stomatal area unit. The surface of a circle is πr^2, and $\pi = 3.14$, while r means the radius, which is equal to half the diameter.

Stomatal density is calculated No stents per mm2, first measuring the surface of leaves (5 leaves for each variant in vitro CAB 6 P; in vivo P. Mahaleb)

Microscopy lams are analyzed and stomatal are identified (a pair of guard cells that surround one but in the center). (Department of Biotechnology AUT, Laboratory of Microbiology Prof. K. Sini). Sliders are then ready for microscopic and photographic exams. Experimental indicators were:

Epidermal Characteristics:

- Distribution and stomatal type

- Number of stumps (stomatal density) for everyone microscopic field / per unit of leaf surface

S = mm^2 = 3.14x 0.145 2 = 0.666 mm^2. The data are processed statistically

Figure 1. Microscopic observation, rootstocks P. Mahaleb (in vivo) and Cab 6 P (in vitro)

Results and discussions

Study of stomatal complex

Types and stomatal distribution

The stomatal apparatus in the leaf epidermis of two in vitro and in vivo variants of CAB 6 P and P. mahaleb, is represented by stomatic cells and associated cells. The accompanying cells are prismatic and strung. The nucleus is clearly distinguished during microscopic observation. Have noted epidermal cells in irregular shape and their number ranges from four to six cells.

In the upper epidermis of leaves of planlets growth in vivo and in vitro acclimatized, the type of stomatal complex is anomocytic (associated cells are radially surrounded by guard cells) (Photo Microscope Prof. Dr. Kristaq SINI).

Planlets of the two categories, differ from the number and shape of the accompanying cells.

Table 1. Stomatal features for different categories (*in vivo*, *in vitro*) of plantlets of CAB 6 P and P. Mahaleb

Indicators	***Above Epiderms of In Vivo***	***Epiderms of In Vitro***
Apparatus stomatal type	Anomocytic	Anomocytic
No of accompanying cells	5-7	5
Stomatal length	18.2	16.7
Stomatal width (µm)	11.3	10.4
Stomatal density (average) mm^2	39	36

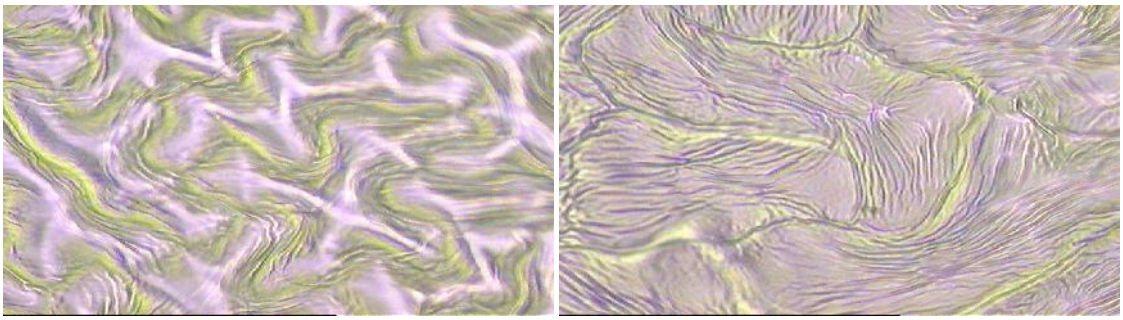

Figure 2. Stomatal apparatus in above epidermis of CAB 6 P *prunus cerasus*

in vitro, (**b**) in vivo

- Pores,
- Stomatal cells,
- accompanying cells (epidermals)

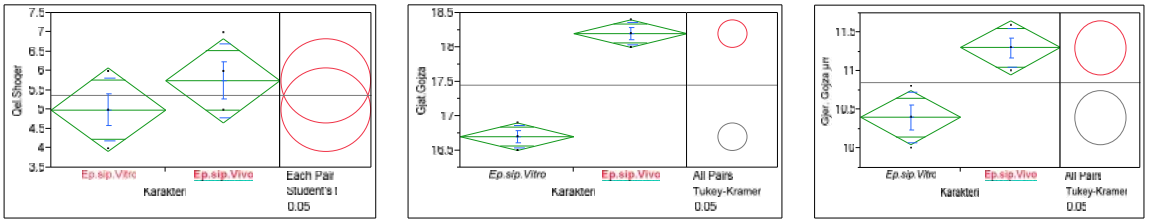

Figure 1, 2, 3. ANOVA accompanying cells, stomatal length and stomatal widthin vivo P. Mahaleb and in vitro of CAB 6 P

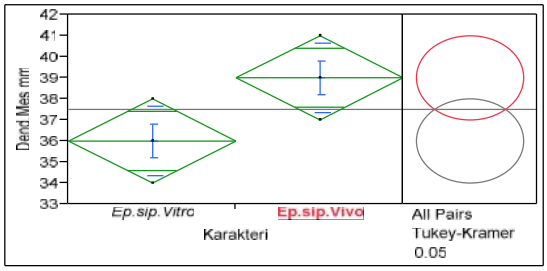

Figure 4. Comparison of two categories in vivo P. Mahaleb and in vitro for stomatal density / mm2 at CAB 6 P

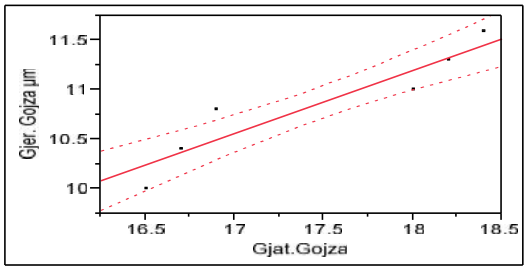

Figure 5. Correlation of stomatal indices in the epidermis of above CAB 6 P and P. mahaleb, in in vivo and in vitro plants a) stomatal length (µm), b) stomatal width

Stomatal width µm = - 0.309013 + 0.639485* stomatal length

- We point out that the leaves of acclimatized plants are treated with controlled temperature and lighting regimes (as mentioned above).
- The type of stomatal apparatus is considered as a key to taxonomy. Stomatal anomycitic types have also been reported in the Compositeae family (Metcalfe & Chalk, 1950, 1979)[4,5,6.7]
- The cultures in the study show significant variation in stomatal density and stomatal length. The variance analysis shows the essential statistical difference (Figure 4). Data on stomatal density in the epidermis results in interesting comparability results. Thus, in both variants there is an average advantage of almost 3 units in in vivo herbs compared to those in vitro
- Stomatal density, in acclimatized plants, is found to be low in comparison to in vivo plants.
- Plantlets growth in vitro has stomatal in the form of loops, while in vivo stomatal with elliptical shape of longer length (Marín *et al.*, 1988, Noé *et al.*, 1996, Tichá *et al.*, 1999).[3,8,9,]
- Standard error is the standard deviation estimated or the measurement of variability in the distribution of values. A low standard error means that there is relatively less distribution in sampling.
- The Bivariate analysis of the two lengths and width gauge indicators in vivo and in vitro is the simultaneous analysis of two variables. Analyses have shown identification of the relationship between the two variables and the importance of the differences between them.

Thus, the endpoints of the search indicators were presented with a variable for the respective variants in two P. Mahaleb (in vivo) and CAB 6 P (in vitro) rootstocks, respectively: the number of the accompanying cells was 5-7 to 5; stomatal length 18.2 to 16.7; stomatal width (µm) 11.3 with 10.4 and mean stomatal density (mm2) 39 with 36.

By comparing stomatal length the variation between the categories is very different. Increased in vivo and acclimatized herbs have prolonged stomata (a form leading to stomatal pore closure) and this parameter, statistically, does not show any variation between these two categories.

The cultures in the study show significant variation in stomatal density and stomatal length.

The variance analysis shows the essential statistical difference (figure 4). Data on stomatal density in the epidermis results in interesting comparability results. Thus, in both variants there is an average advantage of almost 3 units in “in vivo” plants compared to those in vitro

Stomatal density, in acclimatized plants, is found to be low in comparison to in vivo plants.

Conclusions

1. In the plants of both varieties prunus cerasus, rootstock CAB 6P, studied during in vitro acclimatization and in vivo cultivation, the leaves are hypostomatic with anomocytic stomatal apparatus.
2. For both variants of in vivo and in vitro cultivation, it turns out that stomatal above of the epidermis have elliptical guard cells.
3. CAB 6 P in vitro and P. Mahaleb in vivo and stomatal dimensions, have a positive but not highly statistically proven margin difference
4. Stomatal Density is higher in vivo plants compared to acclimatized in vitro plants due to high air humidity
5. Dental density is higher in “in vivo” plants compared to acclimatized ones due to increased intensity of light.
6. The shape and number of accompanying cells in” in vitro” plants varies from those in vivo increased but is a change occurring within the stomatal anomocytic type

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RESEARCH ARTICLE **(Open Access)**

Pomological characteristics and chemical content of the pomegranates (Punica granatum L) in ex situ

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Abstract

Pomegranate (Punica granatum L) is a fruit very important for our life, especially in the last years information for this fruit and content is more increase. This fruit belong the Mediteranean climate and the Mediteranean spread plant, and our country have privilege to have in her land. The varieties were collected in ex situ in UBTirane and the characteristics pomological and chemical are different for each traits. The different varieties are different for different pomological, chemical and morphological traits, the difference are until 5 days for each phase. Fruit weight ranged 250 g to 900 g; aril is red, pink, white and dark red. The number of seed ranged 330 g to 500 g. The weight of seed ranged 120 gr to 462 g. C, chemical traits are different for each trait chemical analyses such are: 1. For Shg 111 – ph is 3.04 when Shg 112 – 3.73 and Shg 113 is 3.02. 2. For Shg 111 – Vit C mg/100 g is 9.2, Shg 112 is 13.6, Shg 113 is 10.15. 3. for Shg 113 - Dry matter is 15.61, 112 is 18.11, Shg 113 is 15.15. For Shg 111 – poliphenol mg/l is 831.29, Shg 112 – 1251.09, Shg 113 691.68. For Shg 111 - antocian is 133.59, Shg 112 is 283.05, Shg 154.46. For at all traits are realize statistical analyses.

Keywords: pomegranate, traits, pomologichal, chemical, diversyty

Introduction

Pomegranate origin is the Near East (Vavilov´s Centre IV), spreading to different regions where its cultivated and where it has a wide genetic diversity as a consequence of the propogation from it seed, which are scattered by man, by birds or other animals andgerminate easily. Punica granatum L is a diploid species whose somatic number is 2n=16 and haploid chromosomes = 8 (westwood, 1982) or 2n = 16 or 18 (Mars, 1998). The aim of this study was to compare and evaluation, characterization and determination mainly traits of germplasm pomegranate in ex situ (field germplasm condition).Pomologichal traits: fruit weight, peel colour, thichness, number of arils, weight of number seed, dimension of fruit (lengthx width), weight of arils, chemical indexs (ph, acidity, Tss Brix x, vitamin c, dry substance, antocian, poliphenol). At all data from this study were analyzed using statistical version.

Materials and methods

This study was carried out in Agriculture University of Tirana, collection Valas. Three varieties, Shg 111, Shg 112, Shg 113, For each varieties was , in samples, five trees of each varieties. All trees were under the same management level. In every tree were collected four orientation(west, east, north, south). For each samples were placing label and transported to lab of Biotechnology and Food Technology Faculty. The data were analysed by analyses of variance (ANOVA) for comparing the main indexs between Shg 111, Shg 112, Shg 113.

Results and discussion

II The evaluation and analysis of the sheep descriptors' characteristics, phenological phases analysis, began with the analysis of flowers, there are

varieties of two types of flowers, one type A flower and one type B flower. Type A condenser, type B fertilizer . The total number of flowers varies depending on variety, age, climate, soil composition, agro-technical services. There are two forms of flowers in the form of bells and bouquets. The petals are red in color. The flower style length observed was determined to be Type A or Type B flowers, or between them. Long-stemmed middle-flowered flowers were found, but this type rarely gives fruit. Long-stemmed strawberry-shaped flowers combine fruits, and they are not scarce in the varieties found in the collection. There are flowers in the shape of the cembalo, with short styles that do not bind the fruit. The bouquet flowers are defined by the length of the style and the shape of the flower, the masculine flowers have a short and undeveloped style. Dominate varieties of flowers of type B, those that link fruits. Female flowers have U-shaped ovaries, and type A are in V form (Wetzstein et al, 2011), this is the way we can determine the type of floral. The development of female flowers influences the productivity of the vegetation. Climate influence and cultural conditions can influence this plant but may be the target of later study. The flowers and the mass of the ovaries show the impact of the fruit and the final mass of the fruit and a greater number of fruits. The age of the fruit plays a role in the fruit. For this it will be a statistical processing to see the correlation.

The fruit has a thick sheet, the cup is not rayed, the tail is short but very elastic that does not stick easily and maintains the weight of the fruit from pasha 150 gr to 800 gr. Within the fruit there are 6 to 12 nests, called the chamber, and the inside are either the seeds or (ariel) colored from white to pink, pink, red and cherry. The fruit juice is found in these seeds, which may be sour, sweet or very sweet, all of these species are found in this collection. The age of fruit plays a role in the fruit size The number of seeds or ariel moves from 150 to 200, 300, to 1200. Each aryl is the result of a pelvic occlusion. The color of the fruit is pink in yellow, red in green, green in yellow, red in red. The seed structure shows the content of the juice.

Table 1: Indexs of morphological pomegranates

Accession	*Color fruit*	*Color arils*	*Weight fruit (gr)*	*Weight arils (gr)*	*Number of seed*	*% of sugar*
Shg1	Yellow to pink	pink	300	145	370	17.5 %
Shg2	Yellow to pink	pink	738	255	217	19 %
Shg3	Yellow to pink	Pink to light	355	170	158	18 %
Shg4	Yellow to pink	Pink to yellow	998	190	462	16.5 %
Shg5	Yellow to red	Red to pink	641	367	400	16 %
Shg6	Red	White to pink	600	246	330	16 %

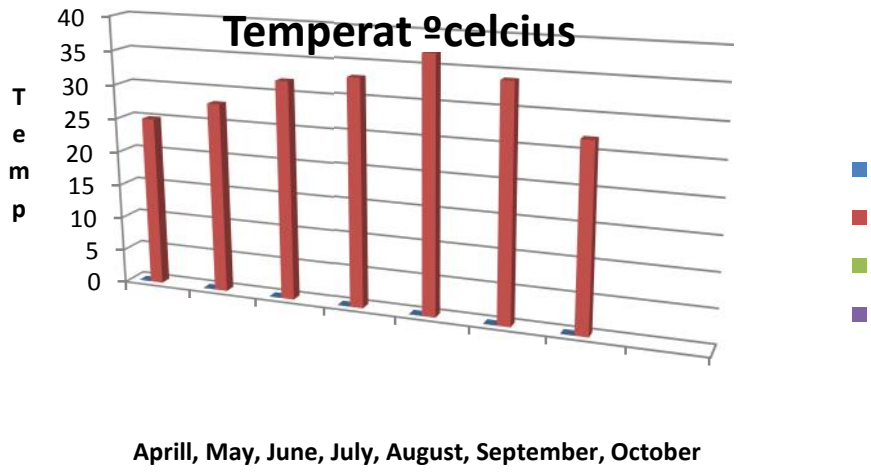


Figure 1: Temperature annual average for 2018

From the temperature annual average for 2018 it is noticed that the temperature for this year has increased by 3•C , there is a shift over the norm. Also the amount of rainfall has been above 70% but of a local character. It has had storms and has caused damage as fruit spoil, and excessive moisture has churned the fruit according to (P.Zorba).

When the weight of one more pomegranate fruit is more than 700 gr, this fruit is classification very large fruit, when the weight fruit is from 300 – 700 gr, this fruit classification large fruit, when the weight fruit is 150 – 300 gr this fruit classification medium fruit and when the weight fruit is < 150 gr this fruit is small and the weight fruit is < 100 gr this fruit is very small. In this study we found medium fruit, large fruit and very large fruit. Average weight was an important indicator to measure the size of the fruit especially for market consume, technique indexs.

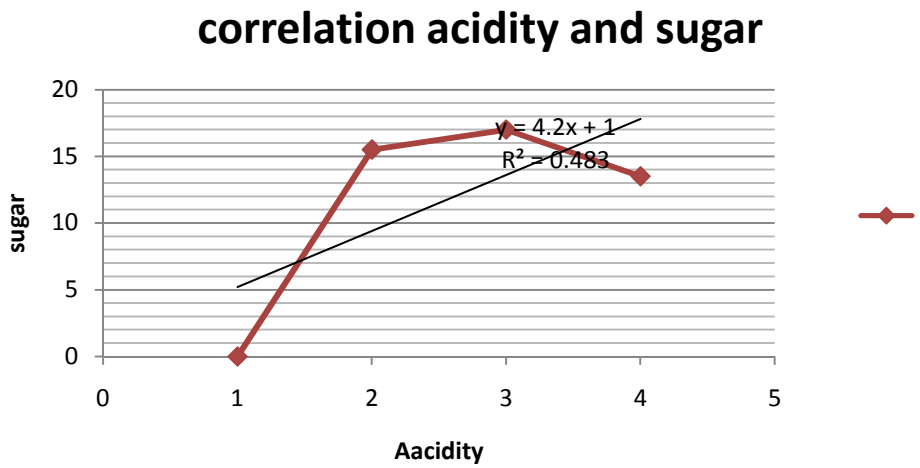
The weight of 100 pomegranate fruit seed, pomegranate fruit was graded into three classes (Sheng, 2008): large – sized grain; grain weight >g; medium – sized grain; between 30 and 50 g; small – sized grain weight < 30 g. Arils are juice containing , is the result fertilization events occurings within a single ovule. Fruit development requires that fertilization is followed by embryo growth and development. Arils have a centrally located seed which contains within it the developing embrio (8).

Table 2: Analyses chemical and physic of pomegranate

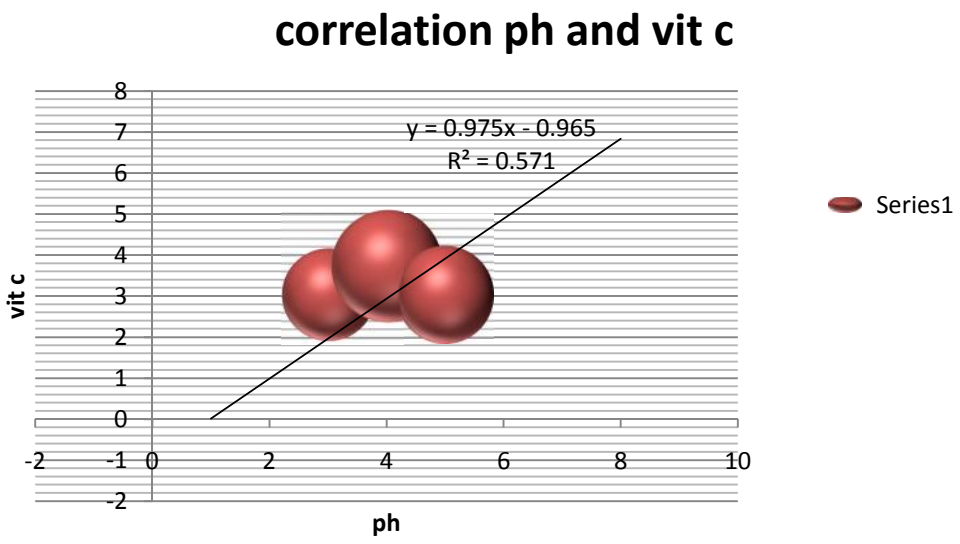
Cod	*ph*	*Acid g/l*	*Tss Brix x*	*Vit c (mg/100 gr)*	*Dry substance*	*Antocyan (mg/l)*	*Polyphenol (mg/l)*
Shg 1	**3.04**	**2.6**	**15.5**	**9.2**	**15.61**	**133.59**	**831.9**
Shg 2	**3.73**	**0.55**	**17.0**	**13.6**	**18.11**	**283.05**	**1251.03**
Shg 3	**3.02or**	**2.9**	**13.5**	**10.15**	**15.15**	**154.46**	**691.68**

The content of acid between Shg 111, Shg 112, Shg 113. Shg 111 and Shg 133 was same for two varieties, content of acid = 2 mg/g is no hight, is reduce but content of acid Shg 112 was only 0.55 mg/g when the fruit is maturity. The content of Brix is different varieties.

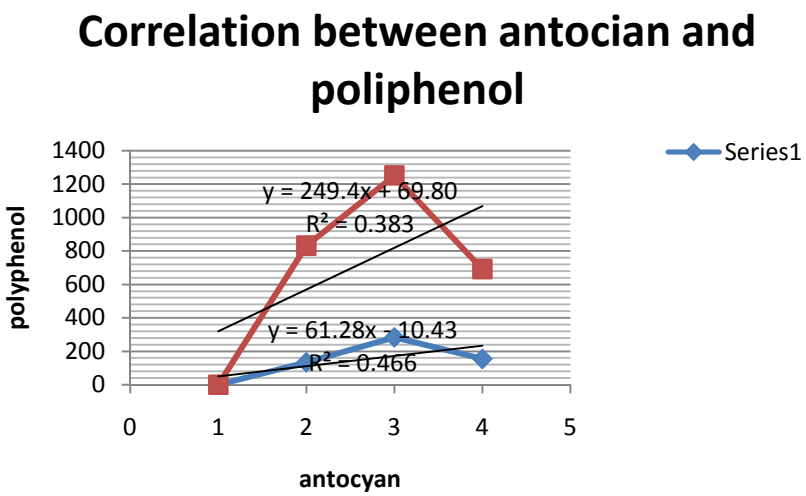
Antocyans are considered as responsible for the red colour of the pomegranate and its seed, which is an important quality attribute. Red depends on the concentracion of the anthocian and on the type of anthocyan. (Du et al 1975). Content antocyan is different for different varieties, moving from 133.59 to 1251.03 to 691.68 for those varieties. The comprehensive comparison between Shg 111, Shg 112, Shg 113 was different. Shg 111 and Shg 113 have few difference, significant difference and more difference than Shg 112. When Polyphenol Shg 111 and Shg 113 was significant difference , few diference and more difference than Shg 112. Antochian and polyphenol are two indicators which are very important for health of peoples. When have genetic diversity we have possibility for more utilization. The health benefits of pomegranate fruit was closely related to soluble phenolic compounds (Li et al , 2009; Seeram et al 2007, et al, 2010, Adams et al, 2010).



Figures 2: Correlation between acidity and sugar



Figures 3: Correlation between ph and vitamin c



Figures no 3: Correlation between Antocyan and Polyphenol

Conclusions

The accession of pomegranate were studied in ex situ is characterized by very good qualitative and quantitative aspects. Genetic diversity is evident in relation to the color of the ariel, the color of the fruit, the taste, etc and in quantitative terms with respect to the fruit weight and the number of seeds in the fruit.

% of sugar increases with rising temperatures. The higher the temperature the higher the amount of sugar but also depends on the variety.

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RESEARCH ARTICLE **(Open Access)**

Potassium Fixation Capacity in some Selected Soils in Albania.

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Abstract

Although a lot of studies are conducted in Albania, little is known and published about soil potassium status. Most of studies have been to know the level of exchangeable forms of potassium. Up to now, there are no publications about the capacity of soils in Albania for K fixation. The objective of this study is to make an assessment of the capacity of some soils for K fixation.

Capacity for K fixation of soils is an important indicator affecting the status of soil K and its availability to crops.

Potassium fixation characteristics were studied in 7 selected soil samples collected in the western regions of Albania. Soils have exchangeable K extracted by 1M NH_4OAc in range from 109 mg K kg^{-1} soil to 238 mg K kg^{-1} soil and nonexchangeable K extracted by 1 N HNO_3 in range from 467 mg K kg^{-1} soil to 830 mg K kg^{-1} soil. To determine K fixation 5 g of each soil sample were weighed into 50-mL plastic bottles and equilibrated for 24 h at room temperature after adding 1000 mg K kg^{-1}soil. A known volume of standard K solution was added to each bottle to make the final soil – solution ratio of 1 : 5. Another set of soil samples was similarly treated with distilled water (control set). After equilibration soil samples were analyzed for K in 1M NH_4OAc extract. The K fixation was calculated as follows:

K fixed = applied - K + NH_4OAc-extractable K of control sample – NH_4OAc-extractable K.

Results shown a K fixation capacity of studies soils in range from 57 mg K kg^{-1} soil to 334 mg K kg^{-1} soil.

Keywords: Potassium, K fixation, Exchangeable and Nonexchangeable forms,

Introduction

Although a lot of studies are conducted in Albania, little is known and published about soil potassium status. Most of studies have been to know the level of exchangeable and nonexchangeable forms of potassium.**[3][9]**Up to now, there are no publications about the capacity of soils in Albania for K fixation.

Fractions of Potassium in soil are (a) total potassium, (b) nonexchangeable (but plant-available) potassium, (c) exchangeable potassium and (d) water-soluble potassium. The most common test for available K^+ is the exchangeable K^+ obtained by extraction with 1M NH_4Cl or NH_4OAc. **[4]**

Potassium availability in soils is regulated by the equilibrium between the forms of potassium and potassium fixation capacity which is further conditioned by the soil characteristics like clay, silt, Organic matter, pH and nature of the exchange complex.**[2]**

When the easily available K is depleted by crop removal or leaching, fixed potassium is released to replenish the labile pool.**[6]**

Capacity for K fixation of soils is an important indicator affecting the status of soil K and its availability to crops.**[2][3]**Among the various factors affecting K fixation, soil clay (quantity and type) constitutes the most important one. It determines the magnitude of the soil fixing capacity and it 2 generally controls the K fixation-release processes **[1]**

Potassium fixation in soils has a direct effect on K availability and on the degree of fertilizer K uptake by plants. Consequently, the study of K fixation problems in soils is of particular agronomic and practical interest. It permits a better understanding

of the soil behaviour to the application of fertilizer and it generally contributes to the more effective evaluation of crop needs for potassium.**[8]**

Hence it is imperative to know the fixation characteristics of soils with a view to making rational recommendations about K-fertilizers.**[8]**

Fixation of added fertilizer potassium is important in the dynamics of soil K and it affects the availability of K to crops. **[7]**Fertilizer recommendation programs must therefore take into account this amount that is initially fixed. In general, periodic evaluation of soil K status is necessary for rational K fertilizer management.**[10]**

Further K fixation and its retention in slowly available form is beneficial especially in light textured sandy soils under high rainfall condition. **[7]**

Exchangeable K^+ ranges between 40 and about 400 mg kg^{-1} soil even more. Concentration of <100 mg K kg^{-1} are frequently in the deficiency range; concentration between 100 and 250 mg K $kg^{-1}$are in the range of sufficiently to well-supplied soils. **[4]**

When the solution and exchangeable pool of K are low to medium status and are enriched with potassium through K-fertilizers, potassium is likely to be fixed with passage of time. **[6]**

The objective of this study is to make a assessment of the capacity for K fixationof some soils selected in the western regions of Albania

The quantity of potassium extracted with 1M NH_4OAc before treatment with KCl represents the total amount of exchangeable forms having the studied soil. The amount of potassium extracted with 1 N NH_4OAc solution after the equilibrium time with the added KCl represents the total amount of exchangeable forms having the soil under study after addition of K to the soil.

Difference before and after treatment with KClas exchangeable potassium that is fixed to the soil.

Material and methods

Soils from seven different sites in the western regions of Albania are used. Soil samples was collected: Two samples from Tirana region (S-TR1 and S-TR2);one sample fromFushë-Kruja region (S-FK); one sample fromLushnja region (S-LU); one sample fromBerat region (S-BR); andtwo samples fromVlora region (S-VL1 and S-VL2).

The soil samples were air-dried, were sieves to pass a 2 mm sieve, and were used for analyzing of physical and chemical properties as:

Soil Texture (Hydrometer method); Organic matter (Potassium dichromate method);pH-H_2O (1:5 soil – water ratio); Total Nitrogen(*Kjeldahl method*); Available Phosphorus. (Mehlich–3 Method); Exchangeable Potassium (Mehlich–3 Method)

Some data on physical and chemical properties of the soil selected for this study are presented in Table 1.

Table 1. Physical chemical properties of selected soils.

	Organic Matter %	pH-H_2O	Total Nitrogen mg kg^{-1}	Available phosphorus mg kg^{-1}	Exchangeable potassium mg kg^{-1}	Sand %	Silt %	Clay %
Minimum	0,5	7,7	590,0	3,0	65,5	6,5	8,6	8,2
Maximum	2,8	8,8	2147,0	36,9	192,3	38,9	58,8	81,2
Average	1,4	8,2	1014,5	16,0	148,9	15,2	42,5	42,3
StDev	0,81	0,42	505,20	11,86	48,94	9,35	23,81	27,50

The data presented in Table 1 show that the lands obtained in this study have a high variability in their physical and chemical properties.

Potassium fixation experiments. For K fixation experiments, 5 g of each soil sample was weighed into a 50 ml plastic bottle and was shaken on a rapid

reciprocating shaker for 24 h at room temperature (20°C) after addition of 25 ml of 200 ppm KCl solution (equivalent to 1000 mg K/kg soil). Another set of soil samples was similarly shaken for 24 h after addition of 25 ml distilled water (control set). Samples were extracted for K three times with 1M NH_4OAc. The extracts were collected in a 100 ml volumetric flask, diluted to volume, and measured for K. **[5]**

The K fixation capacity was calculated as follows:

K fixed = applied - K + NH_4OAc-extractable K of control sample – NH_4OAc-extractable K.

Where:

K-fixed = amount of added K fixed, mg kg^{-1} soil

applied - K = amount of applied-K, mg kg^{-1} soil

Extractable-K= amount of extractable K in K-treated soil after incubation, mg kg^{-1} soil

Extractable-K control sample = amount of extractable K before incubation, mg kg^{-1} soil

In our discussions the quantity of potassium extracted with 1M NH_4OAc before treatment with KCl we will call ***exchangeable – K***, and the quantity of potassium extracted with 1 N NH4OAc solution after the equilibrium time with the added KCl we will call ***extractable – K***.

Results and discussion

Potassium fixation characteristics were studied in 7 selected soil samples collected in the western regions of Albania. Soils have exchangeable K extracted by 1M NH_4OAc in range from 109 mg K kg^{-1} soil to 238 mg K kg^{-1} soil and extractable K extracted by 1 N HNO_3 in range from 467 mg K kg^{-1} soil to 830 mg K kg^{-1} soil.

Exchangeable and extractable potassium in selected soils.

Data of potassium extracted in the studies soils is presented in table 2 and figure 1.

Table 2. Maximum, minimum and main of potassium extracted in the studies soils.

	K extracted by 1M NH_4OAc (mg kg^{-1} soil)			Ratio (%)
	extractable – K	exchangeable – K	fixed – K	fixed-K/extractable-K
Minimum	279.5	109.5	57.5	20,6
Maximum	443.4	237.6	333.9	75,3
Average	374.5	179.2	195.3	49,9
StDev	57.4	50.8	96.9	19,6

In table 1 are presented the maximum, minimum and average values of potassium extracted from selected soils as well as calculation values of fixed – K and ratio between fixed-K and extractable-K.The results show that the exchangeable potassium extracted with 1M NH_4OAc in the control samples is in the range 110-238 mg K kg^{-1} soil, with an average value 180 mgK kg^{-1} soil for all samples. The lowest value was measured in sample S-TR1 and the highest value in sample S-BR (Figure 1) The amount of exchangeable potassium extracted with 1M NH_4OAc in the studied samples after the addition of 1000 mg K kg^{-1} soil as a KCl salt is in the range from 280 to 443 mgK kg^{-1} soil with an average value 375 mg K kg^{-1} soil for all samples. The lowest value was measured in sample S-TR2 and the highest in sample S-TR1 (Figure 1).

Capacity for fixing potassium in selected soils.

The capacity to fix potassium is an important characteristic of the soil and is determined by many environmental factors such as clay fractionmineralogy, organic matter content, cation exchange capacity, soil reaction, etc.**[2][1]**It is also reported that a significant impact on soil capacity to fix potassium has also the sand and silt fractions **[5]**

In other side, capacity for fixing potassiumis a very important indicator to show the potentials of the

soil to adsorb potassium in available forms to plants as is considered extracted potassium from soils with 1M NH_4OAc solution.

In our study we are focused on a quantitative assessment of the capacity of the selected soils to fix potassium.

Results presented in table 2 shown that K fixation capacity of studies soils was in range from 57 mg K kg^{-1} soil to 334 mg K kg^{-1} soilwith an average value 195 mgK kg^{-1} soil for all samples.The lowest value was measured in sample S-TR2 and the highest value in sample S-TR1 (Figure 2).

The ratio between Fixed - K and Extractable K gives an interesting information on the effect that addition of potassium in the soil gives in the amount of extractable potassium. In our study, this contribution is estimated at 20% to 75% with an average rating of 50%. (Table 2)

The lowest value was calculating in sample S-BR and the highest value in sample S-TR1 (Figure 3).

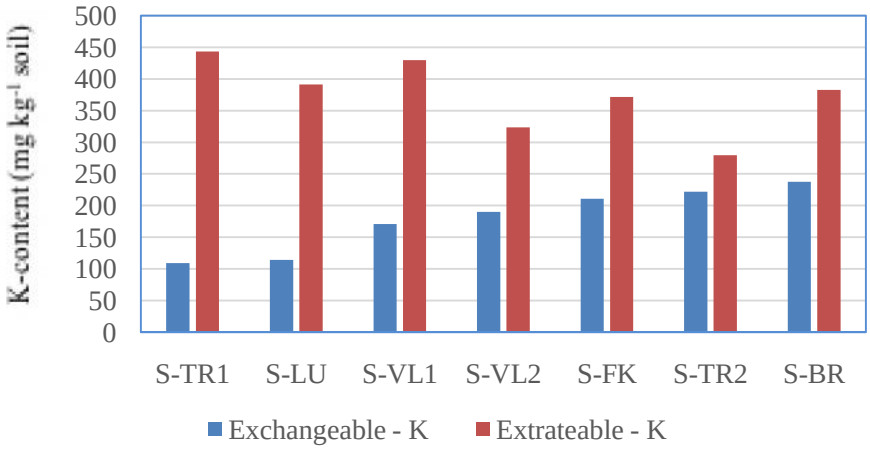

Figure 1. Exchangeable K and Extracteable K in selected soils.

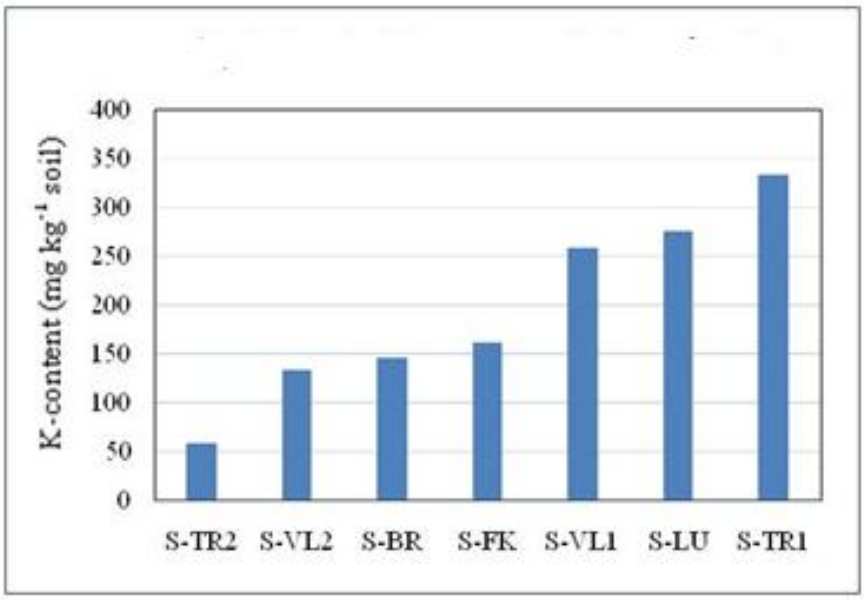

Figure 2: Fixed –K capacity of selected soils

Figure 3: Ratio (%) fixed- K/ Extractable - K

In the presented data we can see that between the amount of potassium fixed in the studied soils and the exchangeable potassium that had before the potassium were added we have a negative correlation.

Thus, the S-TR1 sample where we have measured the smallest amount of the exchangeable potassium, 110 mg K kg^{-1} soil has fixed 334 mg K kg^{-1} soil, creating a new extractable potassium capacity 443 mg K kg^{-1} soil. While the S-BR sample where we have measured the largest amount of the exchangeable potassium, 238 mg K kg^{-1} soil has fixed 145 mg K kg^{-1} soil, creating a new extractable potassium capacity 383 mg K kg^{-1} soil.

To evaluate the trends analysed above, we have calculate the correlations between fixed potassium and the extractablepotassium before and after potassium added to the soil.

Our data indicates that the soil capacity to fix potassium has a good correlation with Extractable potassium before and after K added. (Figure 4 and 5)

The results show that the amount of fixed potassium has shown a goodcorrelation (R^2 = 0.778) with exchangeable potassium in the studied soil, but in negative trend. Soils with large amounts of exchangeable potassium have fixed smaller amounts of potassium added. (Figure 4).

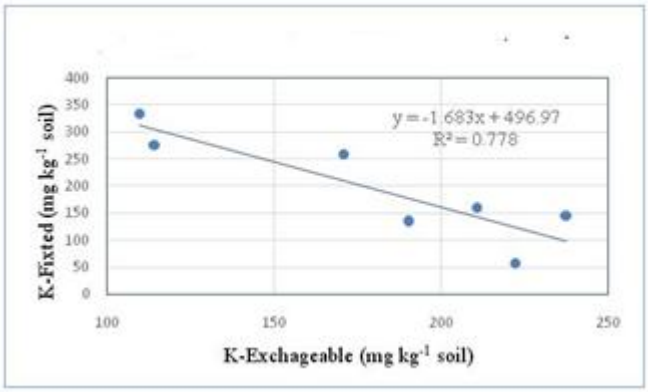

Figure 4: K- exchangeable vs K- fixed in selected soils

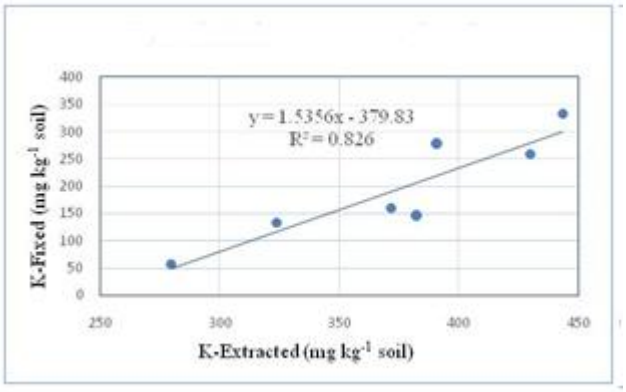

Figure 5: K-extracted vs K- fixed in selected soils

On te other side,amount of the fixed potassium has shown a better correlation (R^2 = 0.826) with extractable potassium in the studied soil, but in positive trend. Soils with large amounts of fixed potassium have fixed larger amounts of extractable potassium. (Figure 5)

The above mentioned data show us that the studied soils require different treatments in the use of potassium fertilizers.

For the studies soils, the contribution of potassium fertilization to increasing the extractable potassium capacity is highest in soils with a low exchangeable potassium compared to highexchangeable potassiumsoils. In the soilswith exchangeable potassium under 180 mg of K kg^{-1} soil is fixed about 2.5 times more potassium than in soils with exchangeable potassium larger than 180 mg K kg^{-1} soil.

In the first group of soils, potassium fertilizer should be used both for plant nutrition and to enrich the soil while in the second group of soils of potassium fertilizers should primarily target plant nutrition.

However, further research is required to study the contribution of different soil factors to capacity of fixation for potassium added to the soil from fertilizers.

Conclusions

K fixation capacity of studies soils was in range from 57 mg K kg^{-1} soil to 334 mg K kg^{-1} soil with an average value 195 mg K kg^{-1} soil for all samples.

The larger amounts of fixed potassium are measured in soils that have the lowest values exchangeable potassium.There is a good correlation between the exchangeable potassium and the fixed potassium with a regression y = -1.683x + 496.97 and R^2 = 0.778

The addition of potassium on the studies soil in the form of KClgave usafter a 24-hour equilibrium a significant increase of the capacity of extractable potassium with 1M NH_4OAc.There is a good correlation between the extractable potassium and the fixed potassium with a regression y = 1.536x - 379.8 and R^2 = 0.826

In the soilswith exchangeable potassium under 180 mg of K kg^{-1} soil is fixed about 2.5 times more potassium than in soils with exchangeable potassium larger than 180 mg K kg^{-1} soil.In the first group of soils, potassium fertilizer should be used both for plant nutrition and to enrich the soil while in the second group of soils of potassium fertilizers should primarily target plant nutrition

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RESEARCH ARTICLE **(Open Access)**

Estimation of Extractable Potassium in some Selected Soils in Albania.

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Abstract

Exchangeable and nonexchangeable forms of potassium are important indicators for assessing the potential of soil for the supply plants with potassium. There are carry out several studies to evaluate the exchangeable and nonexchangeable forms of potassium in some Albanian soils with CAL and EUF extraction methods, as well as the relations between them.

The purpose of this study is to evaluate the exchangeable forms of potassium using different extraction methods as Mehlich-3, Ammonium Acetate and Distilled Water as well as nonexchangeable forms of potassium extracted with 1 N solution of HNO_3.

For this purpose, 8 soil samples were collected in the western regions of Albania. The samples were analysed for the contents of exchangeable and nonexchangeable forms of potassium according to the respective methods. The results show that the soil contains 467.3 – 1002.7 mg K kg^{-1} soil when extracted with 1 N solution of HNO_3. Amount of potassium extracted with the Mehlich-3method was 55,1 – 379.5 mg K kg-1 soil, with Ammonium Acetate was 61.1- 329.0 mg K kg^{-1} soil, and with Distilled Water was 7.6 – 86.0 mg K kg^{-1} soil.

A good correlation exists between the quantity of potassium extracted by Ammonium Acetate and Mehlich-3, but not with water extraction.

Key words: Potassium, Exchangeable and Nonexchangeable forms, extraction methods,

Introduction

Fractions of Potassium in soil are (a) total potassium, (b) nonexchangeable (but plant-available) potassium, (c) exchangeable potassium and (d) water-soluble potassium. The most common test for available K^+ is the exchangeable K^+ obtained by extraction with 1M NH_4Cl or NH_4OAc. **[5]**

Different techniques are proposed in the literature to determine the available K in soils by extraction with diluted salt solutions**.[4][6][7]** The soil testing results are used for K fertilizer recommendations. **[1]**

The neutral normal Ammonium Acetate, and Mehlich-3 solution which extracts both solution and exchangeable K are used for evaluating plant-available soil K in most soil testing laboratories. **[4][6][8]**Also, is suggested the non-exchangeable K extracted by boiling 1 N nitric acid (HNO_3) method as the index of long-term K supplying ability of many soils**[8]**

Exchangeable and non-exchangeable forms of potassium are important indicators for assessing the potential of soil for the supply plants with potassium. Soil test methods should take into consideration the major factors and processes relevant to the availability of a particular plant nutrient**.[5]**The closest correlations between soil tests and plant were found in potassium**.[3]**

There are carry out several studies to evaluate the exchangeable and nonexchangeable forms of potassium in some Albanian soils with CAL and EUF extraction methods, as well as the relations between them**.[2][9]**

The purpose of this study is to evaluate the soluble and exchangeable forms of potassium using different extraction methods as Mehlich-3, 1N Ammonium Acetate and Distilled Water as well as

nonexchangeable forms of potassium extracted by boiling 1 N HNO_3 method.

Materials and methods

Soils from eight different sites in the western regions of Albania are used. The soil samples were air-dried, were sieves to pass a 2 mm sieve, and were used for analyzing of physical and chemical properties as:

Soil Texture (Hydrometer method); Organic matter (Potassium dichromate method); pH-H_2O (1:5 soil – water ratio); Total Nitrogen (Kjeldahl Method); Available Phosphorus. (Mehlich 3 Method)

Some data on physical and chemical properties of the soil selected for this study are presented in Table 1.

Table 1. Physical chemical properties of selected soils.

	Organic Matter %	pH-H_2O	Total Nitrogen mg kg^{-1}	Available phosphorus mg kg^{-1}	Sand %	Silt %	Clay %
Minimum	0,5	7,7	590,0	3,0	6,5	8,6	8,2
Maximum	2,8	8,8	2147,0	36,9	38,9	58,8	81,2
Average	1,4	8,2	1014,5	16,0	15,2	42,5	42,3
DevSt	0,81	0,42	505,20	11,86	9,35	23,81	27,50

In other side, the samples were analysed for the contents of extractable forms of potassium according to the following methods:

Potassium water soluble (K – H_2O), **[7]**

Potassium extracted by NH_4OAc(K – NH_4OAc) **[7]**

Potassium extracted by Mehlich 3 (K – Mehlich 3) **[4]**

and Potassium extracted by Nitric Acid (K – HNO_3)**[7]**

Results and discussions.

Potassium extracted by different methods

The minimum, maximum and average dates about Extractable K content extracted with 1N HNO_3; Mehlich–3; 1N NH_4OAc and Distilled Waterare given in Table 2. From the extraction methods selected in this study, the largest amount of potassium in the soils studied was extracted by1N HNO_3extraction solution. While, the smallest amount of potassium in the soils was extracted during extraction with H_2O solution.The results show that amount of potassium extractedby1N $HNO_3$was 467.3 – 1002.7 mg K kg^{-1} soil,amount of potassium extracted with the Mehlich–3 method was 55,1 – 379.5 mg K kg^{-1} soil,with 1N NH_4OAc was 61.1- 329.0 mg K kg^{-1}soil and with distilled H_2O was 7.6 – 86,0 mg K kg^{-1} soil.

Table 2. Soil Potassium extracted by different methods.

	mg K kg^{-1} soil extracted by:			
	1 N HNO_3	Mehlich–3	1 N NH_4OAc	Distilled H_2O
Minimum	467,3	55,1	61,1	7,6
Maximum	1002,7	379,5	329.0	86.0
Average	701,0	172,8	169,4	49,4
StDev	184,1	100,3	80,2	30,3

The differences in the exchangeable potassium extracted by Mehlich– 3 and NH_4OAc methods are very closed. While the amount of exchangeable potassium extracted with distilled H_2O is about 2-14 time smaller than in the above-mentioned methods.This is true for both extraction methods that we have analysed.

Correlations between different extraction methods.

The different methods of extraction used in this study are correlated with each other and with the clay content. The results are presented in Table 3 and Figures 1 and 2.

Table 3. Thecoefficients of determination (r^2) for the analysed indicators.

	Clay	K-HNO_3	K-Mehlich 3	K- NH_4OAc	K-H_2O
Clay	1				
K-HNO_3	**0,752**	1			
K-Mehlich 3	0,309	**0,589**	1		
K- NH_4OAc	0,417	**0,601**	**0,954**	1	
K-H_2O	0,403	0,293	0	0,015	1

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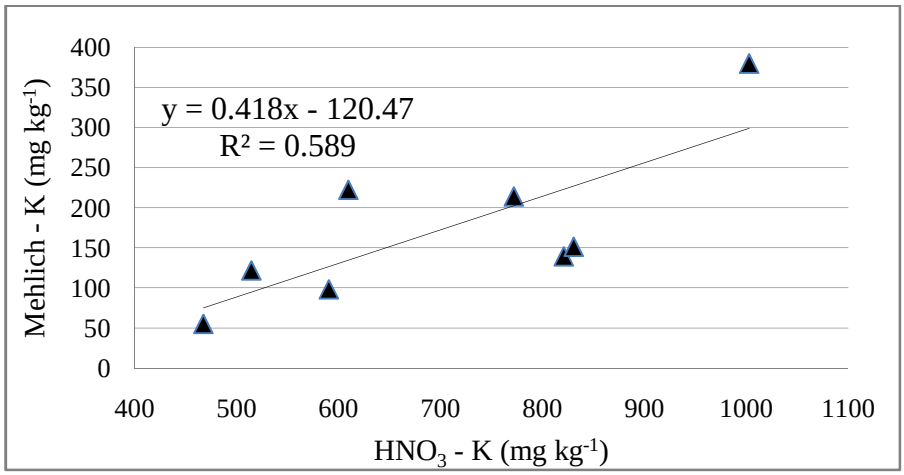

Figure 1. Potassium in soil extracted by HNO_3 and Mehlich 3 methods

A good correlation exists between the quantity of potassium extracted by Ammonium Acetate and Mehlich–3, but not with distilled H_2O extraction

A soil important factor that influence in variability of Kextracted from selected soils is the clay content. The dates indicate strong relationship between clay content in one side and potassium extracted by 1N HNO_3 on the other side.

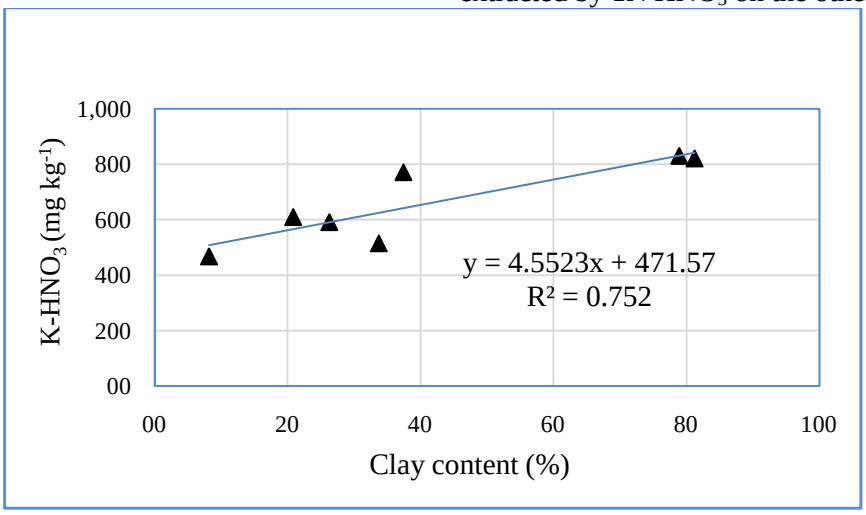

Figure 2: Correlation between clay content and K extracted by 1N HNO_3

Correlations of clay content and potassium extracted by other methods are weak (Table 3)

However, the quantity of exchangeable potassium extracted by Mehlich–3 and 1 N NH_4OAc methods tend to increase related to clay content. While the amount of exchangeable potassium extracted with distilled H_2O has a decreasing trend in relation to clay content.

Conclusions

The content of nonexchangeable potassium in the soil at the range of 460-1000 mg kg^{-1} soil extracted with 1 N HNO_3 method and content of exchangeable potassium in the soil at the range 55-380 mg kg^{-1} soil extracted by Mehlich–3methods and at the range 60-330 mg kg^{-1} soil extracted by 1N NH_4OAc method.

A good correlation exists between the quantity of potassium extracted by 1N NH_4OAc and Mehlich–3, but not with distilled H_2O extraction.

A good correlation exists between clay content in one side and potassium extracted by 1N HNO_3 on the other side.

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RESEARCH ARTICLE (Open Access)

Heavy metal accumulation in liver tissue of two fish species of Adriatic sea

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Abstract

The concentration levels of mercury, lead, cadmium and chrome were measured in liver tissue of two valuable fish species of Adriatic Sea, *Lophius piscatorius* and *Trachurus trachurus.* The level of heavy metals was measured by using atomic absorption spectrophotometer (AAS). Concentration levels of heavy metals in liver tissue of both fish species ranged as follows: Hg 0.05 – 1.45; Cd 0.030 – 0.98; Cr nd – 0.131mg/kg wet weight; Pb resulted always below the detection level (nd) in both liver tissue of fish species. As expected, the levels of heavy metal in liver varied significantly between fish species. The results of our study sustain the fact that liver tissue is an important target location of heavy metals accumulation. Based on these findings, *Lophius piscatorius* and *Trachurus trachurus* should be object of further investigation with the final goal to safeguard the population who constantly consume them.

Keywords: heavy metals, *Lophius piscatorius*, *Trachurus trachurus*

1. Introduction

Marine ecosystems are being contaminated persistently by heavy metals due to the impact of both human and nature activities. These fact have affected and threatened aquatic organisms life for decades. Heavy metals have the ability to accumulate and bioaccumulate in such organisms over the time. Many studies have been conducted recently to detect and evaluate the concentration level of heavy metals in different tissues of fish species with commercially importance [2,3,5,6,7,8,10,11,12,13,15]. Most of these studies were focused mainly in muscle tissue of fish as the edible part of it. But beside these literature have showed that the main important target location of accumulation of heavy metals is liver [4,15]. Liver is the main key organ of metabolisms of lipids, carbohydrates, and proteins. Its function is not limited only on this but its further involved in the detoxification process. Referred to importance of liver as an important biological indicator of heavy metals accumulation the object of our study was to evaluate and monitor the concentration level of mercury, lead, cadmium and chrome in liver of two valuable fish species of Adriatic Sea, *Lophius piscatorius* (Angler fish) and *Trachurus trachurus* (Horse mackerel).

2. Materials and Methods

2.1 Fish sampling

Fish samples namely Lophius piscatorius and Trachurus trachurus (origin Adriatic Sea) were collected randomly during (2010 - 2012). According to the weight the fish samples were divided in two main groups: small fish size (mean weight of 140 g) and large fish size (mean weight 2 kg). The fish samples before they were sent to the laboratory of Toxicology, Institute of Veterinary and Food Safety, Tirana they were first, weighed, catalogued and conserved at - 18◦C. The study included a total of 40 samples of liver tissue, 20 for each fish species and size.

2.2. Determination of heavy metals

A total of 40 samples of liver tissue of *Lophius piscatorius* and *Trachurus trachurus* were evaluated for the concentration level of mercury, lead, cadmium and chrome by using an Atomic Absorption Spectrophotometer (AAS). The liver sample tissue of

the fish species was homogenized in a blender; they were dried at 100 ºC. One g of sample was weighed and then treated with 10 ml of HNO_3 and 5 ml of concentrated H_2SO_4 and let in overnight. The next day they were dried at 150º C for at least, 30 minutes and 50 ml of it were put into a normal flask, and filled with tap water. The heavy metals were measured by ICP-OES, Optima 2100 Dv produced by Perkin Elmer. Heavy metal concentration in fish liver was expressed in mg/kg wet weight (mg/kg ww).

2.3. Statistical analysis

The comparison of the data between two groups was held by using student test, possibilities less than 0.05 was considered statistically important ($p<0.05$). The entire statistic evaluation was carried out by using SPSS 20.0 (Statistical Package for Social Science). (The statistical data on the below table 1 and 2 comprised average, standard deviation, standard error, p value and interval of confidence).

3. Results and Discussion

Concentrations of heavy metals (Hg, Pb, Cd and Cr) in liver tissue of fish species Horse mackerel and Angler fish (*L.piscatorius)* and *T.trachurus*) are given in table 1 and 2 respectively. From the comparison of the data resulted that the mean concentration level of Hg, Cd and Cr varied widely not only among fish species but also between fish size. The order of heavy metal accumulation in both groups of fish species samples followed these range: Hg>Cd>Cr.

Heavy metals in *Lophius piscatorius*: From the comparison data of the study (Tab. 1) resulted that exist an important statistical change according to mean value accumulation of Hg ($p<0.001$), Cd ($p<0.001$) and Cr ($p=0.034$) in liver tissue samples of *L.piscatorius* with different size. The highest concentration value of Hg (mg/kg ww) resulted in large fish size (0.451±0.142) compare to small fish size (0.059±0.011). The same results was obtained in case of Cd in which large fish size (0.981±0.347) revealed higher concentration level of accumulation versus small fish sized (0.030±0.013) of *L.piscatorius*. About Cr (mg/kg ww) the highest concentration level resulted in large fish size (0.131±0.13), in stand of small fish size (0.028±0.027). Based in table 1 lead resulted always below the detection levels (nd) in all liver fish samples.

Table 1. Average mean value concentration of heavy metals in the liver of *Lophius piscatorius* with different weight (small & large fish size) (mg/kg ww)

Species Lophius piscatorius, liver tissue

Metals	weight	Nr	A	SD	SE	t	df	P
Hg	small	10	.0598	.0118	.0037	-9.7276	18	**<0.001**
	large	10	1.4503	.4519	.1429			
Cd	small	10	.0307	.0138	.0044	-8.6518	18	**<0.001**
	large	10	.9810	.3471	.1098			
Pb	small	10	.00					
	large	10	.00					
Cr	small	10	.1318	.1390	.0440	2.2972	18	**0.034**
	large	10	.0289	.0271	.0086			

Heavy metals in *Trachurus trachurus*: From the comparison of the data resulted that exists an important statistical change according to mean value accumulation of Cd ($p<0.001$) in liver tissue of *T. trachurus* in both fish size (small & large fish size). The highest concentration level of Cd (mg/kg ww) resulted in large fish size (0.067±0.009), compare to small fish size (0.0362±0.011). According to Hg ($p=0.08$) the data of the study doesn't showed any relevant statistical change in both fish size of this fish

species. Based on table 2 lead and chrome resulted always below the detection level (nd) in all liver fish samples of *T.trachurus.*

Table 2. Average mean value concentration of heavy metals in the liver tissue of *Trachurus trachurus* with different weight (small & large fish size) (mg/kg ww)

Species Trachurus trachurus, liver tissue

Metals	weight	Nr	A	SD	SE	T	df	P
Hg	small	10	.0828	.0074	.0023	-1.8549	18	.080
	large	10	.1062	.0392	.0124			
Cd	small	10	.0363	.0117	.0037	-6.6112	18	**<0.001**
	large	10	.0677	.0094	.0030			
Pb	small	10	.00					
	large	10	.00					
Cr	small	10	.00					
	large	10	.00					

The entire data of the study shows that liver tissue of both fish species is contaminated with Hg, Cd and Cr, except for Pb which resulted in both species below the detection level (nd). Many studies stated that contamination of fish species with heavy metals in closely connected not only to fish species, age, size, sex but also to other factors such as living environment and feeding behavior [1,3,4,14,16]. The highest concentration level of Hg and Cd resulted in liver tissue of *L.piscatorius* instead of *T.tarchurus* (Tab.1&2). These fish species both originate from Adriatic Sea, but they differ from each other according to size, habitat and feeding behavior. Fish species as *L. piscatorius* with large dimensions who lives into near contact with the sediment are more exposed to heavy metals pollutants than other fish species [13]. *T.trachurus* is a pelagic fish species, with different ecological need and feeding regime. Based on literature pelagic species revealed low concentration levels of heavy metals than benthic fish species [9], which in fact is in accordance with the data of our study. Furthermore it is clear by the study the fact that liver tissue is contaminated by cadmium, mercury and chrome. Organs as liver have the ability to bioaccumulate, biotransform and excrete pollutants as heavy metals. These is the main reason why liver tissue is used as an important indicator of heavy metals pollution in commercially fish species as in fact *L.piscatorius* and *T.trachurus* are for Albanian consumers. Based on consumers preferences liver as internal organ of fish is excluded for food consumption. But Albanians fishery consumers made an exception in which liver of *L.piscatorius* is used for human consumption. These is one of the reason why these commercially fish species and not only should be object of further investigation of heavy metal pollution.

4. Conclusions

The result of the study sustain the fact that liver tissue of *L.piscatorius* and *T.trachurus* are contaminated with dangerous heavy metals such as mercury and cadmium. The contamination of liver tissue of both fish species may serve not only an important indicator of metal pollution of Adriatic Sea but also to commercially fish species who lives on it and are used as food by Albanians consumers.

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RESEARCH ARTICLE **(Open Access)**

Iodine deficiency in Alpine goats in some rural areas of Elbasan district

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Abstract

In the area around the former Metallurgical Combine in Elbasan for the period 1987-2018, the largest pollutant in the country has depleted the lands and has adversely affected the vegetation, animals and human beings. In addition to the high percentage of BPH in humans and animals, there is an increase in the percentage of neoplasms in humans and animals. The focus of our study will be on iodine deficiency in the alpine goats in areas near the metallurgical combine. In the last 4 years, there have been cases of fetal deaths due to iodine deficiency. This phenomenon was encountered in calves, pigs, lambs and goats. Autochthonous races have been more resistant than improved races. Of the most affected species were alpine goats. Percentage of cases of morbidity of this race was about 12% in the villages of Katundi i Ri, Brandashesh, Balldren, Vidhas, Papër, Mjekes, Bujqes, Jogodine and Muriqan. The frequency is proportional to the increase in distance from the metallurgical combine. Case mortality was around 35%. Some of the kids were killed in the first 2-3 months of life, seeing the low profitability of these individuals. Part is carried out in family conditions. These individuals, as in the following pictures, are evidence of iodine insufficiency in food and soil due to pollution and other natural factors, and sometimes the lack of technical assistance for animal nutrition

Keywords: Milk goitre, iodine deficiency, thyroid gland, triiodthyronine, tetraiodtironine, thyroxine

Introduction

The thyroid gland is an endocrine gland. It is composed of stroma and parenchyma(Fig 1). The thyroid gland is wrapped from the outside by a dense capsule of irregular connective tissues which in turn bends into very narrow septa that separate the parenchyma in thyroid follicles[4]. Thyroid parenchyma consists of two types of cells: follicular cells, which surrounds each follicle, and parafollicular cell, which is placed between the adjacent follicles[9].

The follicular cell has squamous shape, cuboid to form high column by thyroid secretion stage. It has a circular core placed in the cell base and is surrounded by acidophile cytoplasm. The cytoplasm contains the extensively extracellular endoplasmic network, Ap Golxhi, and the lysosomal system that interact together to produce tyrosine (T4) and triiodothyronine (T3) hormone.

Hypothyroidism is a disease of iodine deficiency that affects both humans and animals. As its typical clinic has gastri, while subclinical it is more difficult to be diagnosed. Hypothyroidism can be diagnosed as with hormonal blood measurement (serum), but also as a good indicator can be the measurement of iodine eliminated by milk or urine.

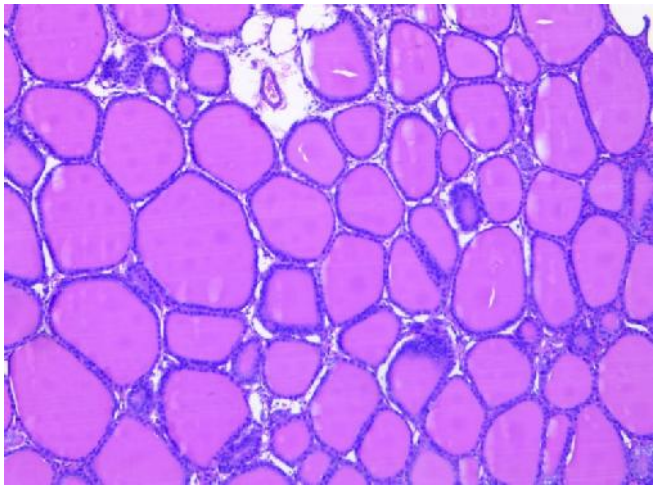

Figure 1 Thyroid follicles(H & E, X 4).

During our research, we were mostly focused on born or congenital hypothyroidism in pigs. Though the mother may not have in the present any signs of morphofunctional thyroid disorder.

Etiology

Among the major pathogens responsible for the development of hypothyroidism, thyroid hyperplasia includes the inadequacy of iodine in food.

Generally hypothyroidism is caused by inadequate iodine in the soil, feeding of animals, water, plants, etc. Sandy lands have low iodine content. The iodine content in plants varies depending on species, climatic conditions and seasonality. So, for example, cereals are poor in iodine, while straw and green meals contain enough iodine content that matches the animal's needs.

Maturing and cutting stages affect the level of iodine in food. Even excessive use of chemicals in agriculture such as DAP reduces iodine recovery[8]. However, these are the primary causes of hypothyroidism.

Secondary causes include proliferative triggers that interfere with thyroxigenogenesis, iodine deficiency in food and genetic enzymatic fission in the thyroid biosynthesis. Even the triggering agents are divided into two subdivisions.

The first subdivision has to do with the fact that with the addition of iodine salts in animal feed, hypothyroidism can be recovered. That being the case, plants such as cabbage, soybeans etc.

The second subsection includes other plants within the Brasiccae spp family such as green cabbage, etc. But unlike the first subdivision, hypothyroidism can not be recovered even with the addition of iodine salts in the food ration because the mechanism of action consists in inhibiting the synthesis thyroid hormone.

All of these factors mentioned above lead to insufficient inhibiting or synthesis of thyroxine and to reduced levels of thyroxine and triiodothyronine. This is caught by the hypothalamus and pituitary that leads to increased secretion of TSH, which in turn leads to follicular cell hyperplasia. The cause of rapid death is due to dyspnea caused by hypertrophic and hypertrophic thyroid gland pressure on the trachea.

It is worth mentioning that from an anamnesis obtained from an animal owner it has been obtained livestock feed in the areas of Lushnja where large amounts of chemical fertilizers were used as DAP.

Materials and methods

Our research methodology consists in analyzing the histopathological changes of the left and right thyroid glands of the dead goats a few hours after birth with the typical swollen gland mark. Thus, in cooperation with the private veterinarian covering the area where the research is being conducted, we have taken thyroid glands and large and small brains in the goats so as to look at the embryonic development disorders that hypothyroidism and changes may have come to histopathologjike. This is because the development of fetal brain is directly dependent on the function of the thyroid gland.

Once samples have been taken, they are stored in refrigerated conditions to be sent to the Food and Veterinary Safety Institute for preparation.

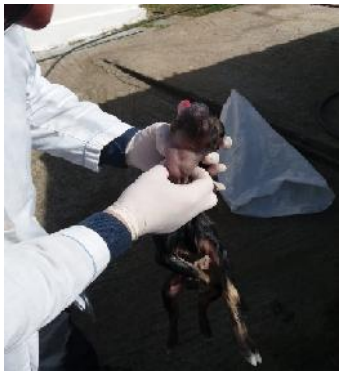

Figure2 Taking thyroid gland for preparation

Figure 2 Length measurement of extra and sinister thyroid glands

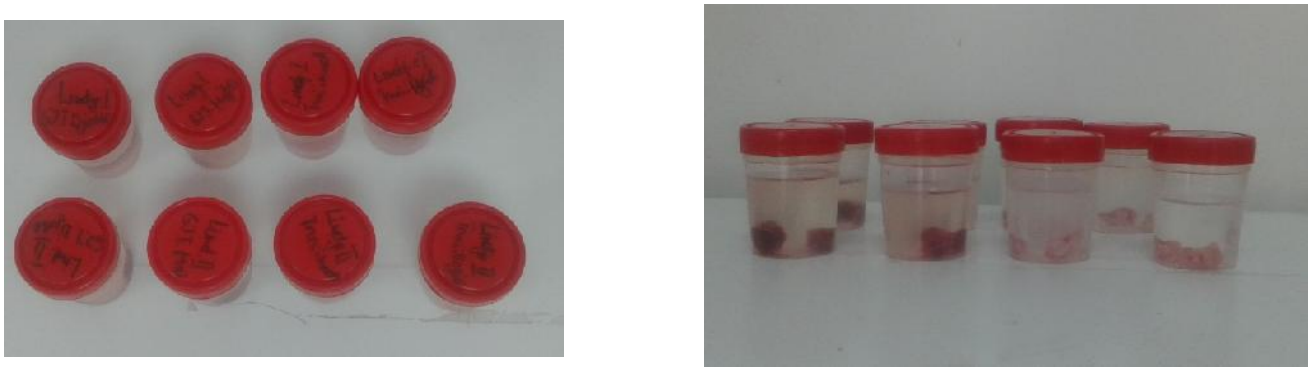

Figure 3 Slides preparation from sample of thyroid glands

Figure 4 A new kid born with congenital goiter

At the Food and Veterinary Security Institute were prepared and colored with Hematoxylin-Eosin. Presently histopathological preparations have been produced in order to make their interpretation.

Clinic

From the newborn goats clinic, it was noted that the period of stroke may be prolonged

considerably, large strains in the fetus can cause distocation and tend to hold the bed. The affected goats are extremely weak and most of them die within a few hours of birth. Thyiroids may be only slightly enlarged. As a consequence of dystocia, even piglets without the sign of swollen thyroid gland[2].

Interpretation and histopathological diagnosis

Diagnosis as mentioned above was decided by the clinic, histopathological changes found in the microscopic images of the thyroid gland preparations. Hyperplastic goitre is a typical sign of hypothyroidism, although the case of kids born from the same goat was seen not all the goats clinically manifested it.

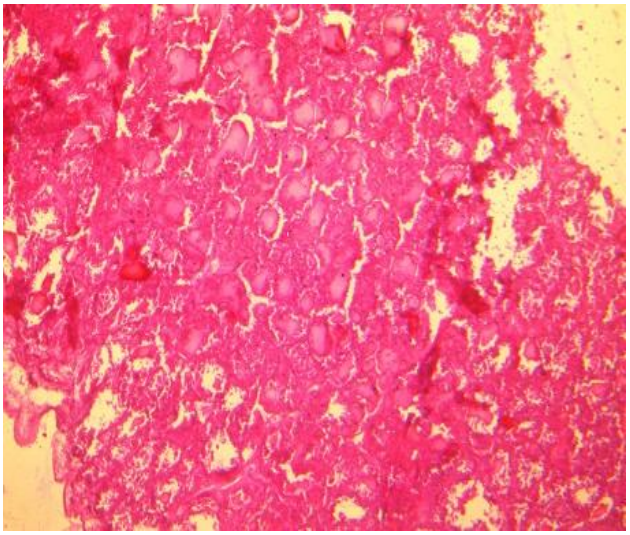

Figure5 Left thyroid gland with partial depletion of follicles and thyroglobuline(H&E X4)

What was noticed was distocia or difficulty in giving birth, generally poorly born, can not drink the colostrum and show no interest in drinking, no hair even though in our cases they have been well developed.

The histopathological changes observed by the microscopic images of the preparations showed signs of hyperplasia of the follicular cells of the thyroid gland(Fig 5). Also in some cases and follicular cell destruction, reductions in colloid.As the follicles were depleted of thyroglobulin, the lining epithelial cells were elongated and moved towards centre of the follicle, giving it a collapsed appearance[1].

Treatment

The surviving newborn goat is treated with proteomyx added in food and intake injections such as Vitamin B12, 2cc, s / c as well as Selenium or E2cc, s / c year.This is the case for a newborn with hyperplastic goiter which was treated last year and that this year has born two kids with the same clinic and also has appeared in the same clinic itself.

Pathogenesis

Congenital hypothyroidism in the goat is almost exclusively associated with the hyperplastic goiter, although the mother may not show any signs of functional thyroid disorder.

Insufficient iodine in foods leading to diffuse thyroid hyperplasia has been severely reduced by the introduction of iodine salts into animal and human nutrition. On the other hand, from the content in foods and triggering agents, this can cause severe thyroid hyperplasia and clinical signs of the gut. In young animals born from mothers who are fed with iodine insufficiency, it is more likely to develop severe thyroid hyperplasia and to show clinical signs of hypothyroidism.

Conclusions and recommendations

Considering the above mentioned it can be said that the causality is multifactorial until the moment when we can not do further hormonal analysis or epidemiological studies aiming at the exclusion of factors. This comes from the fact mentioned above where the cause may be due to iodine insufficiency on the ground, inadequacy in foods, or presence of goitrogene plants or foods.

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RESEARCH ARTICLE **(Open Access)**

Participatory approach for a better Governance of Protected areas: The case of Llogara National Park in Albania

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Abstract

Despite the engagement and involvement of Albania in international conventions related to sustainable development and environmental protection that promote the concept of participatory planning and management of protected areas, it turns out that interest groups and local communities are not adequately and qualitatively involved in decision-making processes, and even have insufficient information. In this paper we : i) analyze the current situation regarding stakeholder participation in decision-making processes in the National Park Llogara; ii) identify the problems faced regarding stakeholder participation in the Park Management; iii) propose actions to involve actors to take part in the consultative dialogue for promoting their role for a more qualitative management of the Park. The analyses is based on a combination of data gathered from the review of recent documentation and publications, meetings as well as data from interviews conducted with representatives of different interest groups. The responses and discussions that led to semi-structured interviews were used for the assessment and analysis of interest groups and at a later stage for Power Influence Mapping Matrix

The analyses shows that local actors are not sufficiently involved in Park management processes, park values are not sufficiently recognized by the actors, public institutions have not followed all the necessary legal and institutional steps to incorporate of actors in Park management. Local actors are ready and willing to set up institutionalized platforms to contribute and influence the park management and governance.

Based on the results obtained, the paper provides recommendations on how further involvement of stakeholders in the Park management processes can be promoted, how to properly enforce the implementation of legal and regulatory framework related to transparency and participatory principles related to decision making that impact the Park management

Key words: protected area, participation, community involvement, governance of natural resources, stakeholders analyses, National Park Llogara

I Introduction: Biodiversity and Protected areas management in Albania

Albania is one of the countries in SEE that has shown a distinguished interest in proclaiming PAs. In 2019, protected areas of different categories cover around 17 % of Albania's land surface. There exist around 800 PAs overall the country (including natural monuments), covering a surface of around 460,000 ha[1][1]. Although, most were originally set up to protect natural habitats, wildlife or more recently biodiversity, they are increasingly expected to provide wider benefits to human society.

The Managment of PAs in Albania is a duty of the National Agency of Protected Areas (NAPA) [2]. The Agency is created by a Government Decree in 2015 [3] [2]. The main aim of the Agency is management, protection, development, expansion and operation of the surfaces of protected areas in our country. NAPA manages the network of protected areas and other natural networks as Natura2000 under management plans drawn up. NAPA operates at national level, but there exists 12 regional agencies at local level, 12 Regional Authorities of Protected Areas, RAPAs) Management and employment for Albania's protected are centralized. Since the creation

[1]Available at: https://www.cbd.int/doc/world/al/al-nr-05-en.pdf

[2] http://www.akzm.gov.al/us/

[3]Available at: https://www.cbd.int/doc/world/al/al-nr-05-en.pdf

of the NAPA (2015), local and foreign citizens are becoming more familiar with the concept of protected areas and are learning more about Albania's protected areas.

II Why a dialogue with stakeholders*?*

The involvment of communitties is very important for an effective manangment of protected aresa. It has to do with processes of active participation in planning, decision-making and local development. This processes are very important for showing respects to values, meets interests and needs of different groups from local community and stakeholders.

Involvement (sometimes also referred to as participation or engagement) and collaboration are vital for successful, adaptive protected area management and governance. Participation makes a basic principle of protected area planning since it has been recognised that without participation by the beneficiaries of the plan, implementation and outcomes will often fail [4] [3]. It requires participatory planning and considering of questions: engaging with whom, why, how and when?

II.1 Participation and equity in global policy documents

The Aarhus Convention promotes the rights of access to information, public participation in decision-making and access to justice in environmental matters (https://goo.gl/mvnHma)). The Convention on Biodiversity – Program of Work in Protected Areas (CBD – PoWPA) and in particular the Programme Element 2: Governance, Participation, Equity and Benefit Sharing (https://goo.gl/tKm3CA) and other relevant documents, strongly promote the concept of participatory planning and collaborative management of protected areas.

Participation of Local stakeholdres in governance of Protected Areas is considered to be important to natural resources managment and biodiversity conservation [5] [6] [4, 5] , Wesselink and al. [6][7], defined participation as any type of inclusion of nonstate actors, both members of the public or organized stakeholdres, in any stage of policy making . Different reasons have been identified for this such as : participations assure legitimity of decision making, increase public credibility, assure access to information, allows contribute of all stakeholdres to decision, imrpve communication among stakeholders, avoid possible conflicts .

After 1980, different studies stressed the inclusion of local stakeholders in natural resources government [7] . The Programme of Work on Protected Areas (PoWPA) of the Convention on Biodiversity has recognized the stakeholdres participation as a key factor of protection of the area [4]. In addition the 2020 Biodiversity of the EU has clearly stressed the importance of stakeholders involvment in natural resources managment and governance [8].

III. Governance of PAs

Governance of Pas plays a *sine qua non* role in the effectiveness for their functioning. Governance identified how the PA is administrated and the associated power and Decision making arrangements. It is about how is making the decision for PA development and management and the way how the decisions for is made. Governance is about how the power is exercised and if and how citizens or the other Stakeholders have their say [9]. The role of the quality of governance in Pas management and development is very well recognized by Global conservation policies as well as by Albanian Government policies and strategies regarding nature conservation [10]. Idea is to make evident if the PA administration incorporates the principle of good governance such as accountability, fairness, equity, participation, effectiveness for achieving biodiversity conservation aims. In this study we tried to analyse the application

[4]Available at: https://www.cbd.int/doc/world/al/al-nr-05-en.pdf

[5]Available at: https://www.cbd.int/doc/world/al/al-nr-05-en.pdf
[6]Available at: https://www.cbd.int/doc/world/al/al-nr-05-en.pdf
[7]Available at: https://www.cbd.int/doc/world/al/al-nr-05-en.pdf

of the principle of participation in the case of NP Llogara.

Governance is characterized based on the key action holding authority and responsibility for the main decisions affecting a PA. There are 4 types of Governance. [11]

i) public governance: PA is governed by the Government (central or local level)
ii) shared governance: various right holders and stakeholders together
iii) private government: governance by private individuals and organizations
iv) governance by local community

Currently, global best practices related to PA governance emphasize the importance of intensive collaboration with all stakeholders. PA managers should involve stakeholders in the governance of protected areas, taking into account that a participatory approach improves the effectiveness of PA governance, creating a favourable environment for improving effectiveness through developing new opportunities and reducing threats.

Participatory approach in Albania

Participatory approach is a relatively new concept for PAs in Albania. All the PAs have been established by government and managed by public institutions with very little participation from other stakeholder groups, especially local ones. There are recently established legal provisions for the participation of stakeholders in the establishment and management of PAs, According to the Law on Protected Areas, national parks are obliged to establish management committees to follow the implementation of management plans of protected areas. Law No 81-2017 recognizes the importance of stakeholder involvement in the management of protected areas. Article 41 of this law requires that a management committee be set up as an advisory body to follow the implementation of the protected areas management plans. In their composition they must be representatives of all stakeholders at central and local level; municipalities, institutions covering agriculture, tourism, etc., CSOs, representatives of businesses, etc.

In Albania participatory processes are in early phase of development. This is a result of a long period of authoritative governance at country level which is reflected in the behaviour of both managers and stakeholders. The capacities of both sides for cooperation and inclusion needs to be developed.

The Management Committees (MC) for Llogara National Park have been established by Government Decrees. It is at the early stages of functioning. The MC is composed of: representatives from the municipality within the administrative territory in which the protected area is found, institutions at regional level institutions with a direct relationship to protected areas (i.e. agriculture, tourism and infrastructure) and CSOs. The challenge is law enforcement.

III Community involvement in National Park Llogara

III.1 The National Park of Llogara

The National Park of Llogara, is the canvas of a beautiful natural mosaic. It has an area of 1010 ha, located in South of Vlora city, traversing the national road Vlore - Saranda. It was declared a National Park in November 1966, and belongs to the II category of protection according to IUCN. This Park is one of the main tourist attractions of Vlora, due to the combination of natural and cultural elements. Visitors along the way to the Park, experience the wonderful feeling that Flora and Fauna of Llogara Park provide, fresh air, wonderful views of coniferous trees, and cold water from the mountains. There is also, a historical site with a panoramic point, called "Caesar's Slopes". Inside the park there is also a Natural Monument of a "Pine Flag". This pine has the shape of the flag, as a result of the action of strong southeast winds and belongs to the Pinus Nigra type. The flora is made up of several species: Macedonian fir, black pine, bush, broomstick and highland. Among the endemic species we can mention: basin flowers (Hypericum haplopylloides), Ionic whale bird (Acis ionica) and chalcedony lily (Lilium calchedonicum L.). Wild Fauna: The Park is the habitat of wolf,

gazelle, rabbit, fox, squirrel and wild boar. Along the territory of the park, there are easily accessible tourist operators who provide hotel and gastronomy services, listing the area with all seasons tourism potential. Extensive tourist information is offered to you by Llogara Visitors Center.

IV Methodology

In order to further analyse the involvment of the stakeholders kin Park Managment we initially conducted a stakeholders analyses as a tool to identify individuals or groups that are likely to affect or be affected by conservation efforts [12].

Secondary data were then collected to ascertain the socioeconomic background of the various stakeholders and to determine their expressed views regarding the PAs.

In order to fulfil the primary of this research, 40 questionnaires are conducted with representatives from each stakeholder group (public institutions, universities, NGOs, residents and business representatives). The questionnaires are used as a guide during interviews to encourage the discovery of unexplored themes. This allowed interviewees to speak freely and raise important issues.

40 interviews were conducted within two months, covering all stakeholder groups. The questionnaire is composed of questions, evaluating the answers in a Likert scale from 5 (completely true) to 1 (completely inaccurate). A dedicated space is used for detailed explanations/comments recorded during the interviews which together with key questions are used as a tool to understand more in depth their role, position and potential engagement. The average time for conducting an interview was about 45 minutes. The interviews are carried out in Vlora, Orikum and Llogara. Face to face interviews are carried out with RAPAs, local government, local community representatives, schools, and businesses representatives living in and around the National Park

The data collected through desk review, documentations, face-to-face interviews and meetings lead to the production and preparation of the Stakeholders Analysis Matrix and Power Mapping (presented in Figure 1). The analyses shows which groups are involved as influential or influenced by decision-making in the protected area. It shows the link and cooperation between the regional, national actors, their interest, their influence and their strength in the National Park Llogara. The group of interest identified are divided into eight main categories: 1. Public Authorities; Regional Agency of Protected Areas (RAPA), 2. Local Governments: Regional Environmental Directorates, Prefectures, Agriculture, Police etc.3. Municipality of Vlora and Administrative Unit Orikum. 4. Local communities of villages around Llogara Park, farmers, residents, etc; 5 Local Businesses: Restaurants, Bars, Hotels, 6. Civil Society Organizations through various causes and sensitive organizations that raises; intellectuals, academics, etc., 7. Media (National or Local Radio Television) as well as Social Networks; 8. Developers or Investors, Donors, Regional Development Agencies, etc.

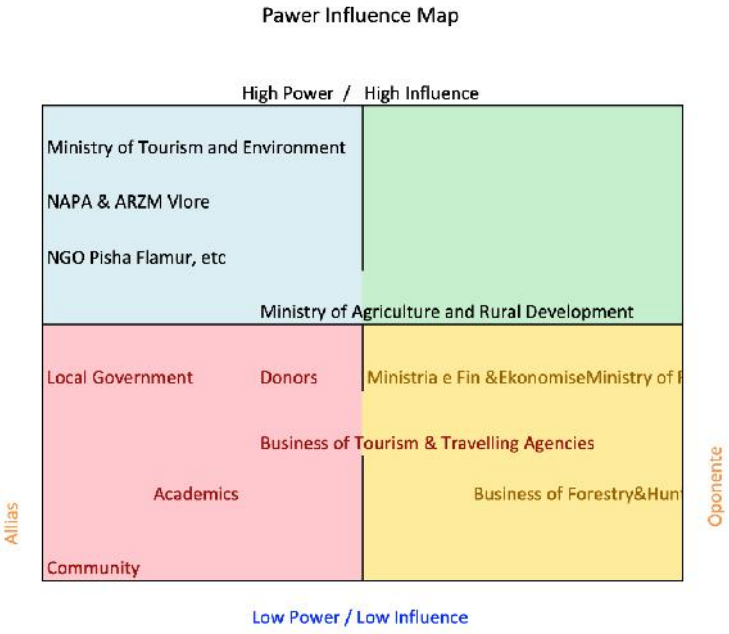

Figure 1: Power Influence Map

The role of stakeholders based on the legal requirements related to participatory approach is shown in the Figure 2.

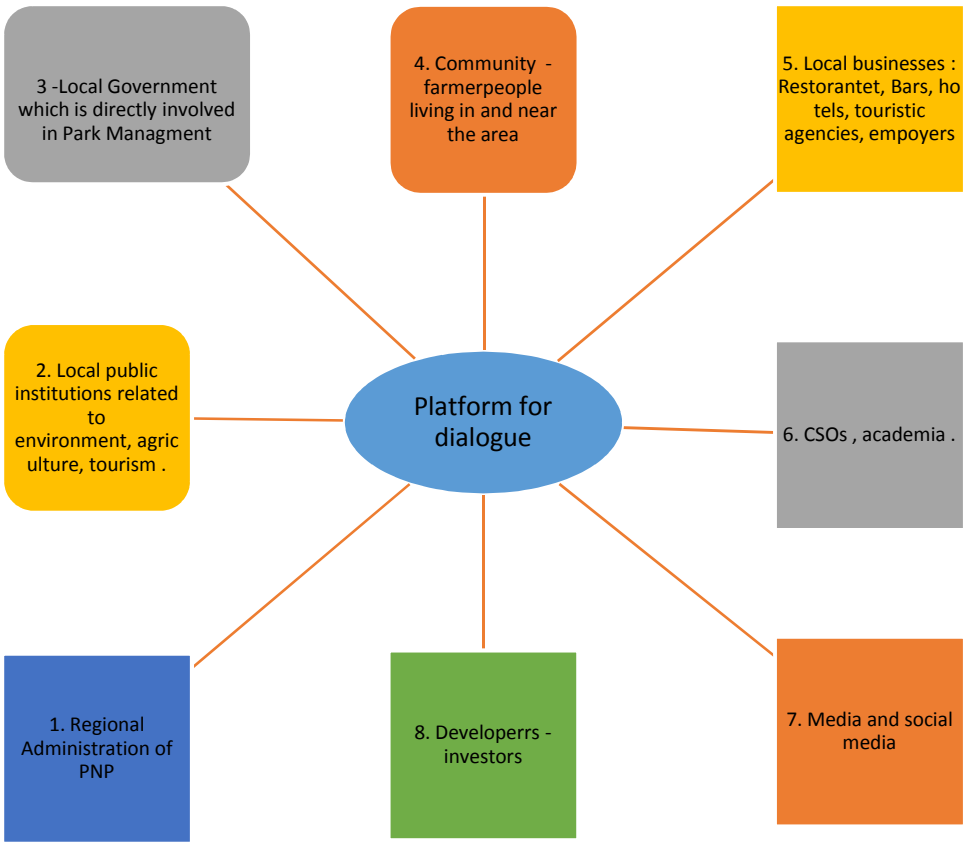

Figure 2: Role of stakeholders in National Park managment

V Data analyses

The questionnaire is developed with representatives of all stakeholders groups. Actually, 8 business persons, 14 public institutions officers, 4 pedagogues, 8 residents in the area and 6 NGOs representatives, Youth Forum for Environment included, were part of the sample.

26 of the interviewees have information about PA legal framework. However, the majority of them represent public institutions (municipality and RAPA), university and local NGOs while businesses and residents seem not to be informed about the respective legislation. Only 5 out 40 interviewees report to have been part of the consolations sessions while drafting the law "On protected areas in the Republic of Albania" all part of public administration. However, 28 of the interviewees report to be informed about the management practices in National Park of Llogara. Only 10 individuals part of public administration and local NGO report to have been invited to participate in public hearings of the Municipality Council with regard to the management of National park of Llogara. More than half of the respondents (26) are not aware about the existence of a forum / committee for the management of the national park of Llogara, but the vast majority of the respondents (33 out of 40) report to have information on values and benefits of the park. Their main source of information with this regard seem to be media and RAPA and local NGOs activities. As for the municipality role in park management, only 8 out of 40 interviewees, all them public institutions representatives, confirm that they do have information about the role of the municipality in the park management. More than half of the respondents think that park activities have not been considered while drafting General Local Plans of Vlora Municipality.

However, despite the low involvement of different categories of stakeholders in the management of national park of Llogara, the vast majority of the interviewees wish to be part of the

management process of National Park of Llogara (Table 1).

The analyses shows that formalized dialogue between the managers of the Protected Area and local communities is not at the required level. It is therefore necessary to increase the awareness of communities on their role in decision-making and management processes and their representation in forums that have to set a dialogue platform with park managers

Table 1: Descriptive analyses estimates

Variable	Category	Frequency
Interviewees categories	Business	8
	Public Institutions	14
	IA	4
	NGO	4
	Residents	8
	Youth Forum for Environment	2
Q1. Informed about PA legal framework	Yes	26
	No	14
Q2. Involvement in Consultation sessions on drafting the law	Yes	5
	No	35
Q3. Informed about Llogara Park management	Yes	28
	No	12
Q7. Invited to participate in public meeting	Yes	10
	No	30
Q8. Knowledge about the forum	Yes	16
	No	24
Q9. Information about the municipality role	Yes	8
	No	32
Q10. Info about values and benefits of the Park	Yes	33
	No	7
Q10.1 Source of information	AZM Activities	16
	Media	6
	NGO's activities	15
	Other	3
Q12. General plans	Yes	22
	No	15
	Don't know	3
Q13.Willingness to be involved	Yes	35
	No	3
	No answer	2

From interviews and meetings with stakeholder representatives, their involvement in the management and influence they had was discussed. Also the power they have in making decisions or how they can exert their influence on a better governance of the Llogara Park. During the meetings and discussions, special focus was also placed on the problems that today are of concern to Llogara Park. As a result of these discussions the following issues were identified: • Illegal logging in the Park, fires, destruction of the landscape, loss of biodiversity. Non-sustainable development in the park (improper

construction, illegal activities), lack of park maintenance, etc.

The main problems of the park as identified by the respondents are:

1. Waste disposal /hygiene
2. Park lightening
3. Lack of waste disposal bins
4. Fires
5. Lack of environmental paths

Other detected problems of less importance include:

- Lack of investments
- Lack of collaboration local government – NGOs
- Lack of an information unit / point functioning all the year long

As perceived by the respondents, RAPA and local NGOs are very much and/or moderately involved in the management process of the park, while municipality, university and inhabitants are slightly or not all involved in the respective process (Figure 3).

About 2/3 of the interviwees (26) evaluate as „very good“ the performance of RAPA implementing the requirements of the law „On protected areas“ while only 10 out of 40 respondents give the same evaluation for municipality. On the other side, no „fair' or „poor“ scores are given to RAPA to this regard, while for the municipality such scores are given by 12 interviewees (Figure 4).

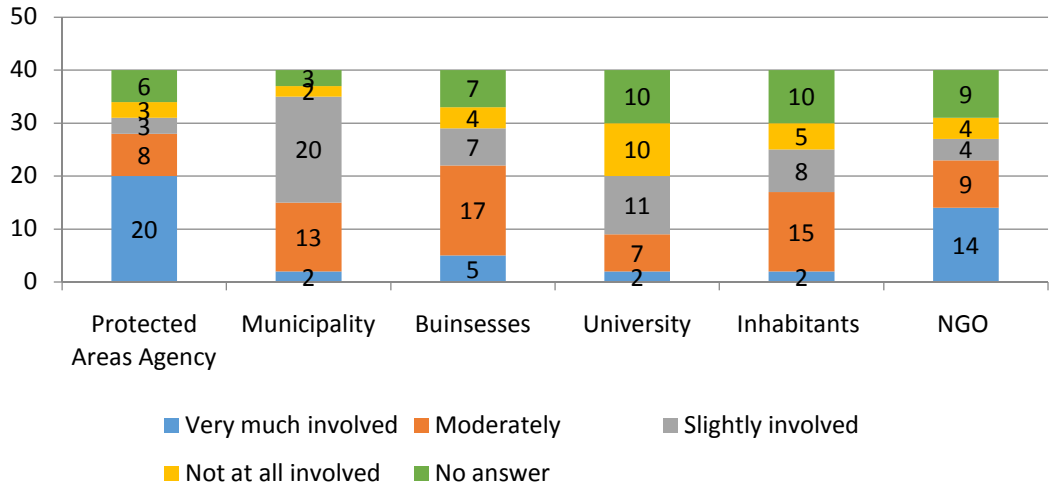

Figure 3: Interviewees' opinion on stakeholders' involvement in Park management

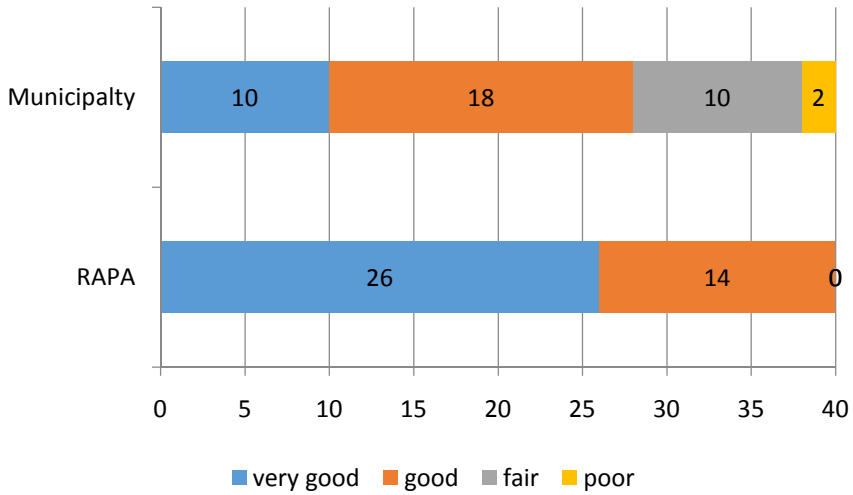

Figure 4: Interviewees' evaluation of the Municipality and RAPA activities

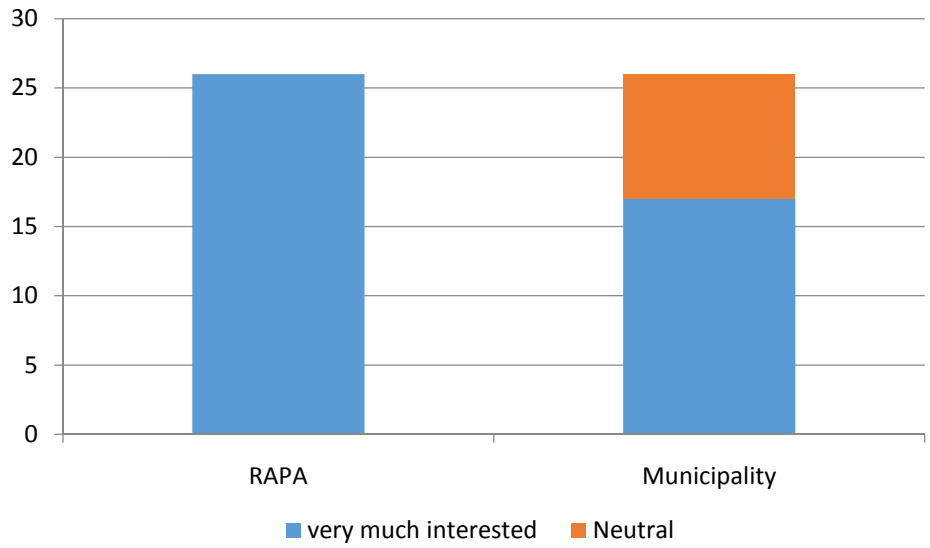

Figure 5: Interest of public institutions to involve other categories of stakeholders in the process of park management

Inhabitants and business, NGOs and university representatives give quite positive scores to the willingness of RAPA and Municipality to involve other stakeholders in the management of the park. All the above mentioned groups representatives see RAPA as very interested to involve them in this process while in case of the municipality 17 evaluate this institution as very much interested and 9 of them are neutral (Figure 5)

The stakeholder's are asked about their involvement in the governance of the NP. The opinion of 56% of them is that there is a need to improve the management approach of the PAs (28% completely true and 28% partially true). Only 9% of the stakeholders consider that the protected areas are managed in an appropriate way. They think that this management approach is relatively new and the positive outcomes will be visible in a more mid and long term time.

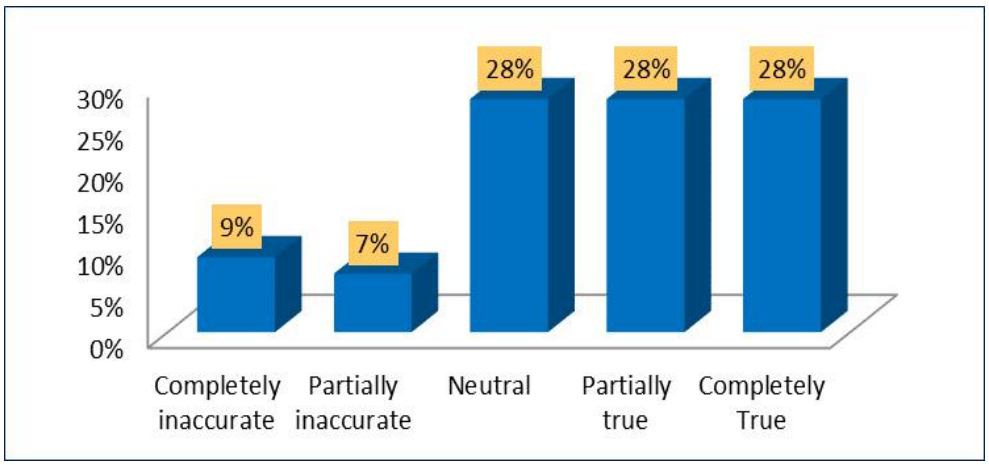

Figure 6: Stakeholders' involvement in NP's governance

VI. Findings and conclusions

Representatives of the community living or working in and around the Llogara National Park area say they would strongly like to see their future and other generations linked to the Park and its development. However, the improvement of standards and a sustainable development in this protected area

presents challenges and opportunities. Actually, the cooperation among stakeholders and the level of involvement of the community in the management of the National Park is not sufficiently developed. The following options for improvement have been identified in the Llogara National Park:

- Creating a forum with the participation of representatives of different interest groups will play the role of a dialogue platform to better manage the area.
- Adoption of the Park Management Plan, for a sustainable development of activities in the area which will improve the residents' livelihood, but at the same time preserve the park's values, maintain it and bring more tourism.
- Increasing citizens' awareness of their power for achieving the goal of being heard and empowered in making decisions or drafting development plans.
- Expanding the number of people engaged to ensure community or other stakeholder representation, using innovative engagement methods focused on reaching contact with young people, women and other vulnerable or disadvantaged groups;
- Increase pressure on the public institutions to ensure participation of Interest Groups in development or physical development plans including the plan for improving the region and surrounding areas of Llogora, roads, schools, social centers, health care and park activities.

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RESEARCH ARTICLE **(Open Access)**

The value of laboratory testing of ceruloplasmin, copper and urinary copper in detection of Wilson disesase.

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Abstract

Wilson disease is a genetically determined copper accumulation disease that usually presents between 6 and 40 years, but it can affect older and younger as well. Disease results from lack or dysfunction of copper transporting ATP-ase. Copper is absorbed and transported to the liver, but the absence of a hepatocellular P-type ATP-ase prevents the incorporation of copper in ceruloplasmin, so copper accumulates in brain, kidney, liver and cornea. Urinary copper is increased as well.Cp is an α2-globulin that contains approximately 95 % of the total serum copper.

Hereby, measurement ofCp, copper and urinary copper are useful test for diagnose and this is the aim of our study.

For a period of ten years, are analyzed 341 patients suspect for Wilson disease from Albania and Kosovo, recommended from gastro-hepatology departaments. Methods used for measurements are immunoturbidimetric for Cp, colorimetry for copper and atomic absorption for urinary copper.

All data are analyzed with SPSS version 20 and Excel 2010.

As results ROC curve with combination of copper and ceruloplasmin increase sensitivity and specificity in detection of Wilson disease and two biomarkers correlate with each- other. The analysis of linear regression showed that decrease of ceruolplasmin increase the possibility to have Wilson disease.

Key words: Ceruloplasmin, copper, urinary copper, regression, analysis etc.

Introduction

Wilsondisease is a genetically determined copper accumulation disease (Tietz et al)[1], leading in copper deposition in target organs like liver, brain, cornea due to the lack of his biliary excretion from the body. Excessive copper deposition in the liver cause hepatic dysfunction from abnormalities in liver test to acute or chronic hepatitis, neurological symptoms in brain, Kaiser – Fleischer rings in cornea etc.

The responsible gene for Wilson disease is ATPB7. It encodes the copper transporting P- type ATP-ase, a very important enzyme for copper transport. In the body ATPB7 play a double role in liver. It participates in copper transporting to the *trans* –Golgi network, where copper is incorporated in ceruloplasmin and in the biliary excretion of copper. Mutations of ATPB7 are closely linked with dysfunction of copper excretion, leading in copper deposition in target organs (Fei Wu et al 2015)[2].

How does it functions copper transport?

Copper is absorbed in the intestine by ATP7A and is delivered to the hepatocyte by a high-affinity copper transport protein HCTR1. Then copper is distributed in other cellular compartments such mitochondria, nucleus and cytosol. Copper binds to Atox1, that transfers copper to TNG and transport it into the lumen with the help of ATPB7. Then copper is incorporated into ceruloplasmin, which is released to blood vessels. Excess copper facilitates ATPB7 trafficking from the TGN to the lysosome and when the level of copper reaches threshold, it is excreted to bile with exocytosis (Hellman N, et al 2002).[3]

Wilson disease results from lack or dysfunction of copper transporting ATP-ase that prevents copper incorporation in ceruloplasmin, leading in accumulation.

The aim of this study is to demonstrate that measurements of ceruloplasmin, copper and urinary copper are useful tests in diagnose of Wilson disease.

Materials and method

For a period of ten years at Intermedica Laboratory are analyzed for ceruloplasmin, copper and urinary copper, 341 patients: 141 females and 200 males suspect for Wilson disease recommended from gastro-hepatology departments in Albania and Kosovo. Those patients did not have liver biopsy or genetic testing. Methods used for measurements are immunoturbidimetric for ceruloplasmin, colorimetry for copper and atomic absorption for urinary copper. Measurements of diagnostic tests are realized using Cobas 6000 analyser and spectrophotometer with atomic absorption. Cut off values for each laboratory testing are:ceruloplasmin 0.2- 0.6 g/L, copper 70-140 µg/dL and 18-35 µg/dL for urinary copper.All data are analyzed using SPSS version 20 and Excel 2010.

Results and discussion

All data that are collected from our sampling, are grouped in tables and processed statistically. First laboratory test analyzed is ceruloplasmin. A patient with Wilson disease is characterized by low level of ceruloplasmin and high level of copper and urinary copper. Literature refers approximately 85 % to 90% of patients with Wilson disease have low levels of ceruloplasmin. Levels less than 0.2 g/L suggest disease, but that does not mean all subjects are sick, because ceruloplasmin levels may decrease even in other clinical situations that results from malabsorbation, liver diseases, hereditary aceruloplasminemia etc (Kelly D. et al)[4]. Based on the copper transport mechanism, the dysfunction of ATPB7 leads in accumulation of copper in target organs, so it is expected that copper levels will be high in the blood. Even for urinary copper are expected high levels as well but not in all cases.

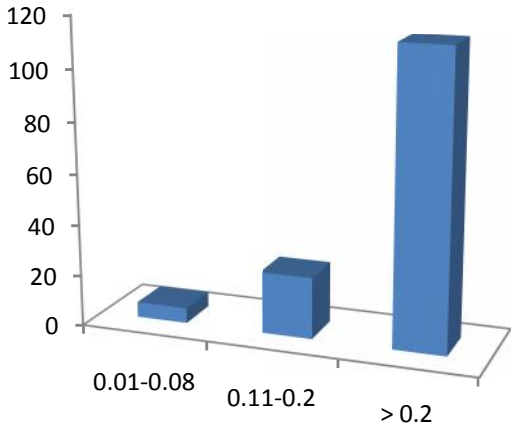


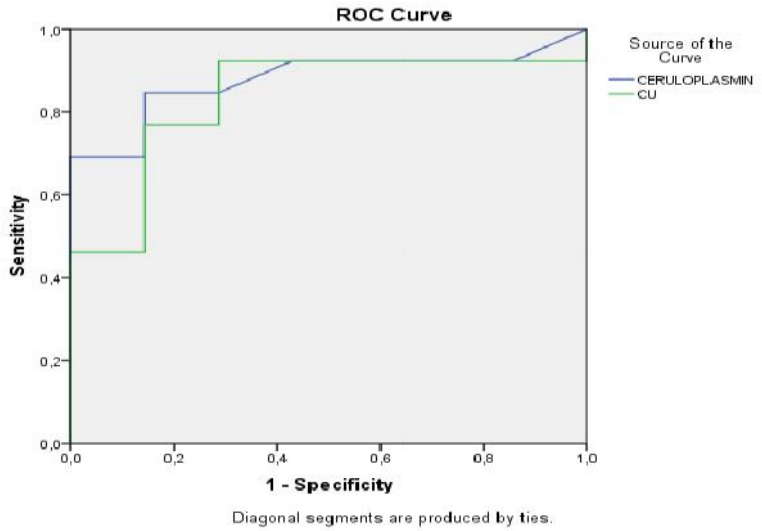
Figure 1. Ceruloplasmin frequency

From our calculation we have obtained graph nr1. In this graph is presented the frequency of ceruloplasmin levels in the entire group of study. We have separated values in three groups. First group with ceruloplasmin levels between 0.01 - 0.08 g/L, second between 0.11 and 0.2 g/L and the third group with values higher than 0.2 g/L. As it shown, first group with lower levels of ceruloplasmin represent the part of population in study with high probability for Wilson disease. Third group include the group with normal or high levels of ceruloplasmin. According our results the population in this study is almost normal, this is shown graphically also. From our study 12 patients, are confirmed with Wilson disease, also by liver biopsy (this is realised later, because at the beginning no one has realised liver biopsy or genetic testing).

To demonstrate ceruloplasmin testing is a useful test in diagnosis of WD we have performed ROC curve using SPSS, between to parameters: ceruloplasmin and copper.

Area Under the Curve

Test Result Variable(s)	Area
CERULOPLASMIN	,879
CU	,835



Sensitivity and specificity are two important statistical concepts for the usefulness of a diagnostic test. Sensitivity is the ability of a test to correctly classify an individual as ′diseased′ and specificity is the ability of a test to correctly classify an individual as *disease- free*. (Parkih et al 2008)[5].

The specificity of a diagnostic test is expressed with area under the ROC curve as it is shown in this graph. From our data analysis Ceruloplasmine has aspecificity 88% and copper 83%. Ceruloplasmine tends to have higher sensitivity than copper, respectively 76 % and 44%. In some studies cerulplasmin is shown to have sensitivity over 98 % and specificity over 55 %.(Mak CM et al.2008)[6] .This study complies with some others written by H. El-Karaksy et al and Chloe Mak et al. Our study varies somewhat in values, but it depends on the size of sample. As I have noticed in the beginning, recommended patients in this study have no liver biopsy or genetic testing and this is another reason that decreases sensitivity.

We have also realised also a linear regression to show how influence ceruloplasmin, copper, urinary copper and age in Wilson disease and the data are as follows.

Table 1. Correlation of parameters in regress

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	,833[a]	,694	,583	,289

In this model of regression we have included these parameters: age, copper, ceruloplasmin, urinary copper and disease like dependent variable. This table provides R and R^2. The R values represent the simple correlation between parameters and it is 0.833 that indicates a high degree of correlation. Around 69 % of patients in this study are explained (from age, Cp, copper and urinary copper) this model of regression that is significant for $P < 0.007$. [7]

Table 2.Anova

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2,082	4	,520	6,235	,007[b]
	Residual	,918	11	,083		
	Total	3,000	15			

Table 3. Coefficients of linear regress

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
1	(Constant)	1,433	,301		4,766	,001
	CERULOPLASMIN	-8,926	2,462	-1,272	-3,626	,004
	Cu U 24 H	,002	,001	,192	1,042	,320
	CU	,005	,003	,489	1,730	,111
	AGE	,008	,009	,255	,951	,362

In the table 3, β coefficients of regress show that decrease of ceruloplasmin levels, increase the probability to have Wilson disease. While other parameters copper, urinary copper and age are with a positive coefficient indicate that their increases, elevates the probability of having WD.

We also have realised a correlation between ceruloplasmin and urinary copper. The coefficient of correlation Pearson is r = -0.49. The negative value of this coefficient indicates that decrease of ceruloplasmin is associated with increase of urinary copper, but since the value is not to close 1, these two parameters do not have a strong relation with each other. So not necessarily decrease of ceruloplasminincrease urinary copper, even though theoretically should be increased, based on the copper mechanism transport. This is shown in graph nr 2.

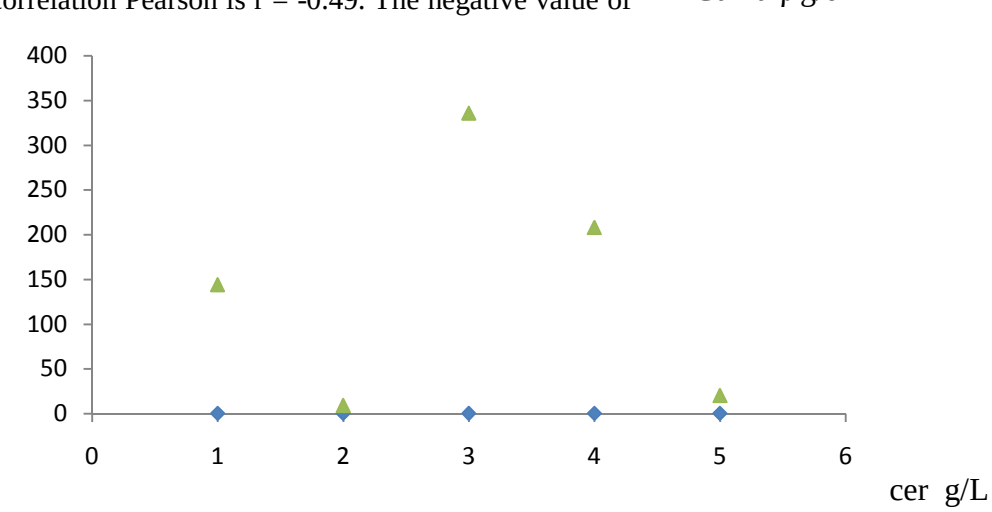


Figure 2. Correlation Ceruloplasmin- Urinary copper

Conclusions

In absence of liver biopsy or genetic testing, ceruloplasmin testing is useful for diagnosis of Wilson disease due to his sensitivity and specificity, (genetic testing for Albania remains still an expensive laboratory testing).

Statistical data analysis of our study showed that copper and urinary copper are auxiliary tests in diagnosis. Liver biopsy still remain as gold standard in diagnose of WD. A combination of biopsy ceruloplasmin,c opper and urinary copper and genetic testing also, are recommendations for a more accurate diagnosis.

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RESEARCH ARTICLE **(Open Access)**

Investigation of green space changes in Tirana-Durres region

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Abstract

Albania is still in a transition period. After 1990 it has gone through very rapid change processes in almost all social-economic aspects of life. An important part of these changes is the rural/urban population structure. In 1989 in urban areas lived just 35.5% of the population while in 2011 it was over 53%. Actually 41.4% of Albanian population lives in Tirana-Durres region. In this area now the main economic operators are concentrated. The urbanization process in many cases has been illegal and has caused serious damages to public spaces and has disturbed the ecosystem services both in urban and rural areas. Green space is a crucial element of sustainable management and should be a key instrument to guide future territory management and planning. Green space types of Tirana-Durres region in 2000, 2006, 2012, and 2018 are investigated based on the elevation data and land use/cover for those years. Spatiotemporal changes of different types of green space were evaluated. The results show that between 2000 and 2018 arable land in west lowland area and forests in hilly and mountain areas were the main types of green space both in Tirana and Durres prefectures. The investigation revealed that large areas of cultivated land in suburban areas of the main cities of the region were changed to other types of land cover, mainly artificial surface. The findings of this research could help the process of green space management and planning.

Keywords: Green space, land cover, Tirana-Durres region

Introduction

At the end of ‘80 Albania used to be the closest and most centralized economy in east Europe but at the beginning of '90 the country jumped from a very isolated economy to a much opened one and was involved in a process of dramatic changes [4, 19]. Albania is still in a transition period. The very rapid change processes have affected almost all social-economic aspects of life. An important part of these changes is the rural/urban population structure. In 1989 in urban areas lived just 35.5% of the population while in 2011 it was over 53%. Actually, 41.4% of Albanian population lives in Tirana-Durres region; Tirana prefecture 895.160 and Durres prefecture 290.126 people [16]. In this area now the main economic operators are concentrated. The urbanization process in many cases has been illegal and has caused serious damages to public spaces and has disturbed the ecosystem services both in urban and rural areas.

In 1984, Roger Ulrich published his paper “View through a window may influence recovery from surgery” [21]. In this paper, based on records on recovery of patients after cholecystectomy, Ulrich studied whether assignment to a room with a window view of a natural setting might have restorative influences. It was concluded that the surgical patients assigned to rooms with windows looking out on a natural scene had shorter postoperative hospital stays, received fewer negative evaluative comments in nurses' notes, and took fewer potent analgesics compared to patients in similar rooms with windows facing a brick building wall. Now days this paper is one of the most cited worldwide. Especially during two last decades there are a lot of studies showing that green spaces play an important role in improving public health, purifying air, regulating climate, reducing noise, increasing biodiversity conservation, etc. [1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 13, 15 and 22]. Different studies have shown that urbanization, especially when it is very quick and not well planned is contributing to the trend of quickly disappearing

green spaces [4, 12, 14 and 20]. Green space is a crucial element of sustainable management and should be a key instrument to guide future territory management and planning. Though there are different definitions of green space, it can be considered as a land surface layer mainly covered with vegetation, including various combinations of natural and semi-natural areas [17]. The loss of green space has not only changed the coverage pattern of the land surface layer, but also directly deprived the habitats for creatures and degraded ecosystem services [9, 11, 13, 17 and 23]. Knowledge of green spaces is essential for maintaining the stable and healthy development of ecosystems.

Two years ago analyses were performed for all public administrative units of Tirana Prefecture and all the municipalities of the country based on the land cover data for 2006 and 2012. Public administration units and municipalities were classified in groups with high and low changes for different land cover categories [12, 20]. In this paper, the distribution of green space changes for 18 years is investigated for Tirana-Durres region. A new category of green space is created. Due to rapid urbanization of this region, green space has been changing from 2000 to 2018. The investigation revealed that large areas of cultivated land in suburban areas of the main cities of the region were changed to other types of land cover, mainly artificial surface. The findings of this research could help the process of green space management and planning.

Materials and methods

Study area

Green spaces of the Tirana and Durres prefecture are investigated in this study. Above 95% of the territory of this region is under plant hardiness zones 8 and 9 [18]. The prefecture of Tirana is the most important one among the 12 prefectures of Albania. Tirana population according to official statistics is 895,160; in an area of 1652 km^2. Tirana city is the capital city of Albania. It has the largest overall population of the region as well as the largest urban population in the country. Since the 2015 local government reform, the prefecture consists of the following 5 municipalities: Kamza, Kavaja, Rrogozhina, Tirana and Vora. The municipalities consist of 38 administrative units and about 250 towns and villages in total. Durres prefecture is composed by three municipalities: Durres, Shijak and Kruje, 21 administrative units and 114 towns and villages. The prefecture has a coast line of 33km. It is bordered by Lezha prefecture in North, Diber prefecture in North East and Tirana prefecture in South. The total area is 766 km^2 and the population 290.126 [16].

Main data resources

The main data used in this study are: (i) CORINE Land Cover (CLC 2000, 2006, 2012 and 2018 data downloaded from https:// land.copernicus.eu/pan-european/corine-land-cover; compiled by the European Environment Agency (EEA) as part of the European Union Copernicus programme and implemented by countries under the guidance and quality protocols of the EEA; (ii) Digital Elevation Model (DEM) data of 30 m resolution for Albania topography and (iii) digital map of the administrative division of Albania provided by the Ministry of Local government.

The layer of CORINE Land Cover Changes (CLCC) is produced since the second CLC inventory (CLC2000). CLCC is derived from satellite imagery by direct mapping of changes taken place between two consecutive inventories, based on image-to-image comparison. Change mapping applies a 5 ha minimum mapping unit (MMU) to pick up more details in CLCC layer than in CLC status layer. Integration of national CLC and CLCC data includes some harmonization along national borders. Two European validation studies have shown that the achieved thematic accuracy is above the specified minimum (85 %). Primary CLC and CLCC data are in vector format with polygon topology. Derived products in raster format are also available. The seamless European CLC and CLCC time series data (CLC1990, CLC2000, CLC2006, CLC2012 and related CLCC

data) are distributed in the standard European Coordinate Reference System defined by the European Terrestrial Reference System 1989 (ETRS89) datum and Lambert Azimuthal Equal Area (LAEA) projection (EPSG: 3035). Results of the CLC inventories can be downloaded from Copernicus Land site free of charge for all users.

CLC data can contribute to a wide range of studies with European coverage, e.g.: ecosystem mapping, modeling the impacts of climate change, landscape fragmentation by roads, abandonment of farm land and major structural changes in agriculture, urban sprawl and water management.

The CORINE Land Cover (CLC) inventory was initiated in 1985 (reference year 1990). Updates have been produced in 2000, 2006, 2012, and 2018. It consists of an inventory of land cover in 44 classes. CLC uses a Minimum Mapping Unit (MMU) of 25 hectares (ha) for areal phenomena and a minimum width of 100 m for linear phenomena. The time series are complemented by change layers, which highlight changes in land cover with an MMU of 5 ha. Different MMUs mean that the change layer has higher resolution than the status layer. Due to differences in MMUs the difference between two status layers will not equal to the corresponding CLC-Changes layer.

The Eionet network National Reference Centres Land Cover (NRC/LC) is producing the national CLC databases, which are coordinated and integrated by EEA. CLC is produced by the majority of countries by visual interpretation of high resolution satellite imagery. In a few countries semi-automatic solutions are applied, using national in-situ data, satellite image processing, GIS integration and generalization. The 2012 version of CLC was the first one embedding the CLC time series in the Copernicus programme, thus ensuring sustainable funding for the future. The 2018 version also funded by Copernicus was produced in less than 1 year (Table 1).

CLC has a wide variety of applications, underpinning various Community policies in the domains of environment, but also agriculture, urban green areas, transport, spatial planning etc.

Identification of Green Space Types

For the purposes of this study, as green spaces are considered the following categories of land cover: Agricultural land, Forestry, Pasture and Semi natural areas. The consideration of agricultural land as one of the types of green space is suggested by different authors [17]. According to H. Tang et al., [17] cultivated land is an important green space and spatial basis for preserving the ecosystem services and social economic development in the southern plain region. This not only has the function of producing food but also serves as the habitats for many species and the leisure space for urban and rural residents.

Topography and land use/cover have an important influence on the characteristics of green space and we followed the model of the above mentioned authors to use these parameters to identify and study the dynamics of green space types. A spatial overlay analysis was performed on the image layers of topography and land use/cover to identify green space types. The topographic image layers were classified using the Digital Elevation Model of Albania (DEM) as follows: Western low plains (0-125m),Western mid plains (126-250m), hills and foothills (251-500m), Intermountain Valleys (501-1000m) and mountains (>1001m). Topographic image layers were classified using DEM elevations, with standard classification: Western low plains (0-125m), Western mid plains (126-250m), hills and foothills (251-500m), Intermountain Valleys or mountain foothills (501-1000m) and mountains (>1001m). The determination of exact green space area for both prefectures we used ArcGIS 10.5 techniques. In this paper seven land cover categories are described: 1. Artificial, 2. Agricultural, 3. Pasture, 4. Forest, 5. Semi natural, 6. Wetlands and 7. Water bodies areas.

After the overlay calculation using the ArcGIS software, 35 types of land-use/cover were identified, and each type was named according to the “land-use/cover type and topographic type”. The names of 35 land cover categories are shown in the legend of figure 1. The first part in each category name

represents the type classified using the land-use/cover data, and the second part represents the type classified using elevation data. So the respective categories are: 5 categories of Artificial areas; 5 categories of Agricultural areas; 5 categories of Pasture areas; 5 categories of Forestry areas; 5 categories of Semi natural areas; 5 categories of Wetland areas and 5 categories of Water bodies, respectively in low plains, mid plains, hills and foothills, mountain foothills and in mountain areas. For Tirana-Durresi region just 27 out of 35 categories are present. So, Artificial mountain areas, Pasture Mountain areas, Water bodies in mountain foot hills and mountain areas and four, out of five, categories of wetland areas are not present.

To analyze the special distribution of green areas changes, the analyses is done at the Public administration Unit level.

Table 1: Evolution of CORINE Land Cover projects for the period 2000-2018

	CLC2000	**CLC2006**	**CLC2012**	**CLC2018**
Satellite data	Landsat-7 ETM single date	SPOT-4/5 and IRS P6 LISS III dual	IRS P6 LISS III and RapidEye dual date	Sentinel-2 and Landsat-8 for gap
Time consistency	2000 +/- 1 year	2006+/- 1 year	2011-2012	2017-2018
Geometric accuracy, satellite data	≤ 25 m	≤ 25 m	≤ 25 m	≤ 10 m (Sentinel-2)
Min. mapping unit/width	25 ha / 100m	25 ha / 100m	25 ha / 100m	25 ha / 100 m
Geometric accuracy, CLC	better than 100 m	better than 100 m	better than 100 m	better than 100 m
Thematic accuracy, CLC	≥ 85% (achieved)	≥ 85%	≥ 85% (probably achieved)	≥ 85%
Change mapping (CLCC)	boundary displacement min. 100 m; change area for existing polygons ≥ 5 ha; for isolated changes ≥ 25 ha	boundary displacement min.100 m; all changes ≥ 5 ha are to be mapped	boundary displacement min.100 m; all changes ≥ 5 ha are to be mapped	boundary displacement min.100 m; all changes ≥ 5 ha are to be mapped
Thematic accuracy, CLCC	not checked	≥ 85% (achieved)	≥ 85%	≥ 85%
Production time	4 years	3 years	2 years	1.5 years
Documentation	standard metadata	standard metadata	standard metadata	standard metadata
Access to the data (CLC, CLCC)	dissemination policy agreed from the start	free access for all users	free access for all users	free access for all users
Number of countries involved	30 (35 with late implementation)	38	39	38 (Turkey to be added in 2019)

Source: Copernicus Land Cover Service; at https://land.copernicus.eu/pan-european/corine-land-cover

Results and their discussion

Types and distribution of green space in Tirana and Durres region during the studied period are shown in Table 2. Between 2000 and 2018, the area of artificial land is increased for all topographic categories, especially it is increased in Western low plains (from 6535 ha to 18444 ha or 182.2%). Agricultural land is reduced in low plains, mid plains and mountains areas, but it is increased in hills and foothills and in mountain valleys by 22.8% and 10.9% respectively. Pastures are significantly reduced also in

hills and foothills and in mountain valleys by 54.9% and 72.9% respectively. Forest areas are reduced in all types of topography. The highest reduction occurred in the category of Agricultural areas in Western low plains, from 77514 ha to 64889 ha, Forest in Western low plains and Western mid plains, respectively 20809 ha to 19584 ha and 24611 ha to 22185 ha, from 2000 to 2018.

Table 2. Main green space changes in the Tirana -Durres region from 2000 to 2018

Land cover category	Area (ha)				Change (%)		
	2000	2006	2012	2018	2000-2006	2006-2018	2000-2018
Artif. lowplains	6535	17599	18265	18444	169.3	4.8	182.2
Artif. midplains	1212	2585	2958	2958	113.3	14.4	144.1
Artif. hills&foothills	331	449	403	403	35.6	-10.2	21.8
Artif. moun.fhills	120	120	126	126	0.0	5.0	5.0
Agric. lowplains	77514	67988	64909	64889	-12.3	-4.6	-16.3
Agric. midplains	16080	15111	14892	14989	-6.0	-0.8	-6.8
Agric. hills&foothills	8464	8129	10392	10392	-4.0	27.8	22.8
Agric. moun.foothills	5539	5502	6140	6140	-0.7	11.6	10.9
Agric. mountains	707	680	435	435	-3.8	-36.0	-38.5
Pastu. lowplains	3978	3500	3915	3866	-12.0	10.5	-2.8
Pastu. midplains	893	869	930	930	-2.7	7.0	4.1
Pastu. hills&foothills	523	487	236	236	-6.9	-51.5	-54.9
Pastu. moun.foothills	203	203	55	55	0.0	-72.9	-72.9
Forest lowplains	20809	20220	19552	19584	-2.8	-3.1	-5.9
Forest midplains	24611	24188	24373	22185	-1.7	-8.3	-9.9
Forest hills&foothills	24605	24630	21348	21278	0.1	-13.6	-13.5
Forest moun.foothills	20588	20666	18985	20009	0.4	-3.2	-2.8
Forest mountains	19454	19488	18520	19260	0.2	-1.2	-1.0
S.natu. lowplains	1557	1525	3973	4126	-2.1	170.6	165.0
S.natu. midplains	606	643	2222	2251	6.1	250.1	271.5
S.natu. hills&foothills	1803	1594	3000	2993	-11.6	87.8	66.0
S.natu. moun.foothills	2004	1963	2004	2123	-2.0	8.2	5.9
S.natu. mountains	1607	1600	2088	2073	-0.4	29.6	29.0
Wetland. lowplains	466	459	459	454	-1.5	-1.1	-2.6
W.bod. lowplains	1829	1396	1447	1409	-23.7	0.9	-23.0
W.bod. midplains	107	112	111	111	4.7	-0.9	3.7
W.bod. mountains	0	436	402	422		-3.2	

The types and distribution of green spaces in the Tirana-Durres region during the periods 2000-2006, 2006-2012 and 2012-2018 are illustrated in Figure 1. From this figure it can be seen that the main changes in artificial urban areas have happened during the period 2000-2006, although the trend of artificial area increasing and green space decreasing continued during all the study period. These changes are concentrated in Western low plains and in Western mid plains.

To see the transformations and the size of the transition area the transition matrix are prepared and shown in Tables 3-6 for the time period 2000-2006, 2006-2012, 2012-2018 and the whole period of study 2000-2018. To highlight the highest changes in land cover categories the table background is marked by color where red indicates a sharp change; gray

indicates a small change and white indicates no change, while blanks indicate that this type of land use/cover does not exist.

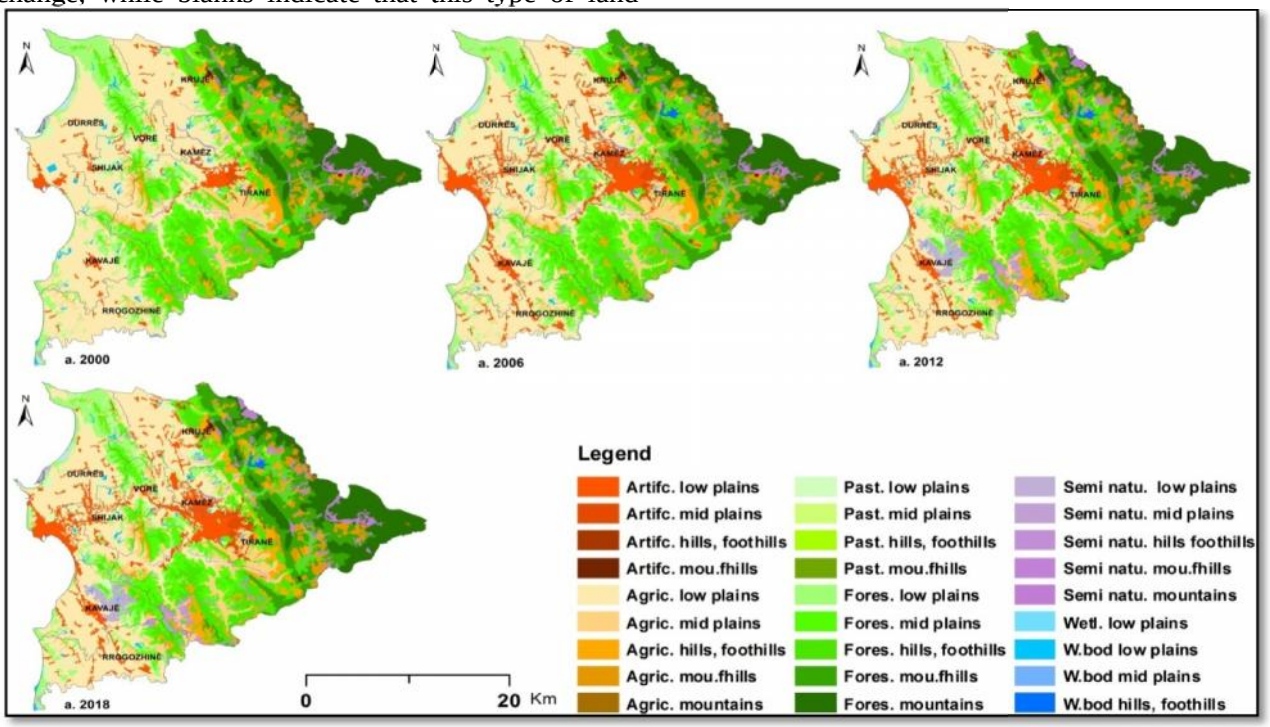

Figure 1 The types and distribution of green spaces in the Tirana-Durres region during the study periods

Table 3. Transition matrix of land cover in the Tirane-Durres region from 2000-2006 (ha).

Topography	2006 / 2000	Agricultural	Forestry	Pasture	Semi natural	Water Bodies	Artificial	Wet land
Western low plains	Agricultural	66718	174	87	60	111	10365	
	Forestry	180	19860	90	20	142	510	7
	Pasture	544	33	2958	6	27	409	
	Semi natural	34	73	0	1318	22	110	
	Water Bodies	23	213	362	120	105	1005	
	Artificial	310	29	3	0	1	6192	
	Wetland		28		2	436		
Western mid plains	Agricultural	14732	130	141	8	9	1061	
	Forestry	246	24015	42	14	6	288	
	Pasture	67	31	683	23		90	
	Semi natural	2	6	0	598		0	
	Water Bodies	1	6	3			98	
	Artificial	57	11	0			1144	
Hills and foothills	Agricultural	8017	232	0	11	134	70	
	Forestry	69	24261	4	0	179	92	
	Pasture	0	10	483		27	3	
	Semi natural	0	125	0	1583		95	
	Artificial	29	17	0			285	
Inter mountain Valleys	Agricultural	5499	39	0	0		0	
	Forestry	0	20588	0	0		0	
	Pasture	0	0	203				
	Semi natural		41	0	1963		0	
	Artificiale	0	0		0		120	
Mountains	Agricultural	680	27		0			
	Forestry	0	19454		0			
	Semi natural		7		1600			

Table 4. Transition matrix of land cover in the Tirane-Durres region from 2006-2012 (ha).

Topography	2012 / 2006	Agricultural	Forestry	Pasture	Semi natural	Water Bodies	Artificial	Wetland
Western low plains	Agricultural	60078	3588	1150	1567	63	1542	0
	Forestry	2645	15656	558	1217	40	102	2
	Pasture	1035	188	2170	26	18	62	
	Semi natural	55	164	0	1110	117	80	0
	Water Bodies	65	58	23	61	1183	5	0
	Artificial	1031	74	15	1	4	16475	
	Wetland	0	0		0	3		457
Western mid plains	Agricultural	12534	2013	147	141	0	275	
	Forestry	2156	20198	89	1569	2	175	
	Pasture	126	19	689	5	0	30	
	Semi natural	22	103	0	519		0	
	Water Bodies	1	2	1		109	0	
	Artificial	52	50	5	0	0	2478	
Hills and foothills	Agricultural	6939	1083	1	88	0	19	
	Forestry	3159	19859	77	1490	4	41	
	Pasture	176	83	159	69		0	
	Semi natural	37	306	0	1251	0		
	Water Bodies	1	7		10	418		
	Artificial	80	26	0	0		343	
Inter mountain Valleys	Agricultural	4394	950	31	126		0	
	Forestry	1544	18528	16	536		42	
	Pasture	102	47	7	47			
	Semi natural	65	516		1382			
	Artificiale	36	0		0		84	
Mountains	Agricultural	388	281		11			
	Forestry	40	18738		709			
	Semi natural	7	240		1352			

Table 5. Transition matrix of land cover in the Tirane-Durres region from 2012-2018 (ha).

Topography	2018 / 2012	Agricultu	Forestry	Pasture	Semi natural	Water Bodies	Artificial	Wetland
Western low plains	Agricultural	64750	0		0		159	
	Forestry	100	19499		120	0	9	
	Pasture	39		3866			10	
	Semi natural				3985			
	Water Bodies	0	0	0	23	1404		
	Artificial	0	0				18265	
	Wetland					5		454
Western mid plains	Agricultural	14892	0					
	Forestry	97	22270		18		0	
	Pasture			930				
	Semi natural				2233			
	Water Bodies					111		
	Artificial		0		0		2958	
Hills and foothills	Agricultural	10392						
	Forestry		21278		85			
	Pasture			236				
	Semi natural				2908			
	Water Bodies					422		
	Artificial						403	
Inter mountain Valleys	Agricultural	6140	0		0			
	Forestry		20009		33			
	Pasture			55				
	Semi natural				2090			
	Artificiale						126	
Mountains	Agricultural	435						
	Forestry		19260		0			
	Semi natural				2073			

Table 6. Transition matrix of land cover in the Tirane-Durres region from 2000-2018 (ha).

Topography	2012 \ 2018	Agricultural	Forestry	Pasture	Semi natural	Water Bodies	Artificial	Wetland
Western low plains	Agricultural	59621	3681	1135	1630	120	11328	0
	Forestry	2865	15228	643	1346	127	592	9
	Pasture	1372	228	1837	33	40	469	
	Semi natural	104	209	0	975	59	193	18
	Water Bodies	308	73	235	143	1052	18	0
	Artificial	619	53	17	0	1	5844	
	Wetland	0	28		0	10		427
Western mid plains	Agricultural	12290	2077	280	152	8	1272	
	Forestry	2427	19982	131	1597	6	469	
	Pasture	186	51	508	27	0	121	
	Semi natural	27	102	0	474		2	
	Water Bodies	7	1	3		96		
	Artificial	51	57	9			1094	
Hills and foothills	Agricultural	6947	1248	1	92	134	42	
	Forestry	3153	19479	81	1579	166	146	
	Pasture	176	93	155	69	27	3	
	Semi natural	39	419	0	1252	95	0	
	Artificial	77	40	0	2		212	
Inter mountain Valleys	Agricultural	4414	967	31	126		0	
	Forestry	1521	18440	16	568		42	
	Pasture	102	47	7	47			
	Semi natural	67	554		1383			
	Artificiale	36	0		0		84	
Mountains	Agricultural	388	308		11			
	Forestry	40	18706		708			
	Semi natural	7	246		1354			

Transition matrix tables illustrate in details what changes happened in four different periods of time for all land cover categories and green space types. Table 3 shows that the main change that happened during the period 200-2006 was that 10365 ha of agricultural land were transformed to urban artificial land. During the period 2006-2012 there are some significant transitions among different green space types. So in Western low plains 3588 ha of agricultural land was transformed to forestry, while 2635 ha of forestry were transformed to agricultural land. Similar changes between agricultural areas and forestry areas happened also in other topographic categories maintaining equilibrium between these types of green space for this period. During the period 2012-2018 the changes are much smaller (Table 5). Table 6 gives a summary of all land cover and green space type changes for 18 years period 2000-2018.

To analyze the spatial distribution of green space changes the investigation was done for all Public administrative units that belong to Tirana-Durres region. So, 45 administrative units were investigated. The result of this investigation showed that during the 18 years period 2000-2018 just 11 administrative units out of 45 increased slightly the agricultural areas, while almost all of them (excluding just one) increased the artificial areas. The administrative units close to the prefectures city centers Tirane and Durres like Farkë, Sukth, Golem, Xhafzotaj, Paskuqan, Kashar, Rrashbull, Durrës, Kamëz etc. showed more clearly this trend. During this period just 13 administrative units out of 45 increased the forestry area, 27 administrative units had significant reduction of forestry areas and 5 of them maintained equilibrium.

Conclusions

In this paper types and distribution of green spaces in Tirana and Durres region during the period 2000-2018 are investigated. . The investigation revealed that large areas of cultivated land in suburban areas of the main cities of the region were changed to other types of land cover, mainly artificial surface. The study found that during this period, the area of

artificial land is increased for all topographic categories, especially in Western low plains (from 6535 ha to 18444 ha or 182.2%). Agricultural land is reduced in low plains, mid plains and mountains areas, but it is increased in hills and foothills and in mountain valleys by 22.8% and 10.9% respectively. Pastures are significantly reduced also in hills and foothills and in mountain valleys by 54.9% and 72.9% respectively. Forest areas are reduced in all types of topography. The highest reduction occurred in the category of Agricultural areas in Western low plains, from 77514 ha to 64889 ha, Forest in Western low plains and Western mid plains, respectively from 20809 ha to 19584 ha and from 24611 ha to 22185 ha. The main changes in artificial urban areas have happened during the period 2000-2006, although the trend of artificial area increasing and green space decreasing continued during all the study period. These changes are concentrated in Western low plains and in Western mid plains. During the period 2006-2012 there are some significant transitions among different green space types. From 2012 to2018 the changes are much smaller. The administrative units close to the prefectures city centers Tirana and Durres showed more clearly this trend. The findings of this research could help the process of green space management and planning.

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RESEARCH ARTICLE (Open Access)

In silico analysis of osteocalcin gene in Cyprinidae

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Abstract

Cyprinidae family includes a large number of fish species, where the most popular are zebrafish (*Danio rerio*), grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*). In this study, osteocalcin in *Cyprinus carpio* was investigated, regarding physicochemical characteristics, structural properties using several available bioinformatic tools. Sequences were retrieved from Genbank. Sequence alignment of the gene and protein sequence were done with ClustalW. The 3D structure of protein was predicted by SWISS-Model softare andwas checked by Z-Score using Qmean server, ERRAT, and Rampage Ramachandran plot analysis. The information provided here is a theoretical overview that will help to get an idea about the predicted protein structure.

Keywords: osteocalcin, bioinformatic tools, sequence alignment

Introduction

The Cyprinidae is the second largest fish family in the world and one of the most widespread in freshwater. [1]. Cyprinidae family includes a large number of fish species, where the most popular are zebrafish (*Danio rerio*), grass carp (*Ctenopharyngodon idella*) and common carp (*Cyprinus carpio*). In this study, osteocalcin in *Cyprinus carpio* was investigated by the use of bioinformatic tools. Osteocalcin (bone Gla protein) is an extracellular matrix protein synthesized by osteoblasts that is a marker of bone. Nishimoto et al., 1995 [2] have characterized osteocalcin from Cyprinus carpio for aminoacid sequence and extent of secondary structure. Carp osteocalcin is a polypeptide of 45 amino acids and an abundant component of carp rib bone comprising over 35% of the total extractable proteins [2]. Actually a large number of computational tools are available from different sources and help researchers to analyze the properties of different proteins. The aim of this study is to select homologous sequences related to bone Gla protein [Cyprinus carpio 'color'] (AIT51847.1) and analyze the physico-chemicals properties of selected proteins by *in silico* methods.

Material and methods

The nucleotide and protein NCBI databases were used to retrieve in FASTA format the target nucleotide and protein sequences of *Cyprinus carpio*. The Basic Local Alignment Search Tool (BLAST), P-suite was used to find the regions of similarity between sequences. Multiple sequence alignment (MSA) is carried out by Clustal Omega.

Primary sequence analysis

Pepstats analysis tool (http://www.ebi.ac.uk/Tools/seqstats/emboss_pepstats/), available at EMBl-EBI website is used to calculate the statistics of protein properties. ProtParam [3] online tool (http://web.expasy.org/protparam/) available at ExPASy server compute physicochemical properties like theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient,

instability index, aliphatic index and grand average hydropathy (GRAVY).

Secondary sequence prediction

In order to calculate the secondary structural features Self Optimized Prediction Model (SOPMA) online tool [4] was used. It predict the quantitative values of alpha helix, beta sheet and coils within the protein sequence.

PSIPRED (Protein Sequence Analysis Workbench) (http://bioinf.cs.ucl.ac.uk/psipred/) predict secondary structure from primary sequence and a graphical representation of the secondary structure is obtained. CYS_REC (http://www.softberry.com/berry.phtml) online tool is used to identifies the position of cysteins, total number of cysteins present and pattern, if present, of pairs in the protein sequence.

Subcellular localization was predicted by the use of CELLO v.2.5 [5] 1.1 server (http://www.cbs.dtu.dk). Motif Scan [6] server (http://myhits.isb-sib.ch/cgibin/motif_scan) was used to identify known motifs in the sequence. Furthermore, Pfam server (http://www.sanger.ac.uk/software/pfam/search.html) was used for domain analysis.

Tertiary structure prediction and model evaluation template.

Computational prediction of three dimensional (3D) structure of the protein was performed by using Swiss-Modeler (http://swissmodel.expasy.org/) program [7]. After modeling, the quality and validation of the model was evaluated by several structure assessment methods, containing Z-Score by using QMEAN [8], Rampage Ramachandran plot analysis (http://mordred.bioc.cam.ac.uk), and ERRAT [9].

Result and discussions

The protein sequence (AIT51847.1) was blasted and the list of five protein sequences with significant alignment were retrieved and the detailed information is given in table 1. Multiple Sequence Alignment (MSA) of selected sequences, that was performed by Clustal Omega is given in figure 1, where the shaded regions indicate similar residues.

Table 1: List of protein sequences selected according to species

Organisms	*Accession ID*	*Gene Accession*	*Protein name*
Cyprinus carpio 'color'	AIT51847.1	KF876170.1	bone Gla protein
Carassius auratus	BAM62632.1	AB685220.1	osteocalcin
Carassius auratus	BAL45872.1	AB606419.1	bone gamma-carboxyglutamate protein
Danio rerio	NP_001077326.1	NM_001083857.3	osteocalcin precursor

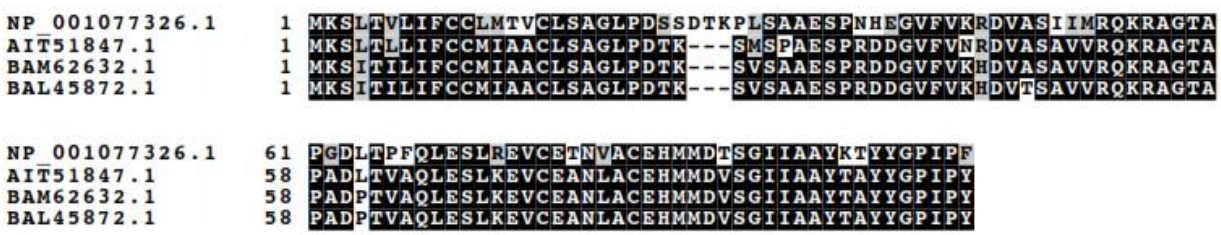

Figure 1: Multiple Sequence Alignment of selected protein sequences.

Aminoacid composition for each of four selected proteins computed by PEPSTATS is given in table 2. The results show that the most abundant aminoacid is Ala. The least common

aminoacid are His, Asn, Gln. The cysteine residues take part in disulphide bonds formation, that play an important role in folding and stability of a protein [10]. The results of table show a low level of cysteine residues, therefore the stability of the protein is not dedicated to the formation of disulphide bonds. The physical and chemical parameters of selected proteins are computed by ProtParam and are shown in table 3. The isoelectric point (pI) varies from 4.16 to 5.56, indicating that proteins are considered as acidic. The Instability index (Ii) is lower than 40, except of *Cyprinus carpio*, therefore these proteins are considered as stable. If the instability index is higher than 40 the protein may be unstable [11]. The GRAVY scores are positive, indicating that all proteins are hydrophobic. Aliphatic Index have very high values in all proteins, indicating that proteins are thermostabile [12].

Table 2: Aminoacid composition obtained by PEPSTATS analysis tools

Organisms	*Most abundant aminoacid*	*Mole (%)*	*Least common amino acid*	*Mole (%)*	*Mole (%) of Cys residues*
Cyprinus carpio 'color'	Ala	14,851	His	0.990	4.950
Carassius auratus	Ala	15.842	Asn	0.990	4.950
Carassius auratus	Ala	14,851	Asn	0.990	4.950
Danio rerio	Ala	8.654	His, Asn, Gln	1,923	4.808

Table 3: Physical and chemical parameters of selected proteins computed by ProtParam

Accession Number	*Sequence length*	*M. W.*	*pI*	*-R*	*+R*	*EC (Cys residues non reduced)*	*EC (Cys residues reduced)*	*II*	*AI*	*Gravy*
Cyprinus carpio 'color'	101	10745.48	4.90	11	8	6210	5960	45.24	91.88	0.286
Carassius auratus	101	10666.36	5.16	11	8	6210	5960	36.26	91.88	0.312
Carassius auratus	101	10696.39	5.16	11	8	6210	5960	35.42	90.89	0.287
Danio rerio	101	11288.12	5.56	11	8	4720	4470	37.24	84.42	0.128

M. W. : molecular weight; -R negatively charged residues; +R positively charged residues; EC. extinction coefficients; II Instability Index; AI Aliphatic Index; GRAVY: Grand average of hydropathicity

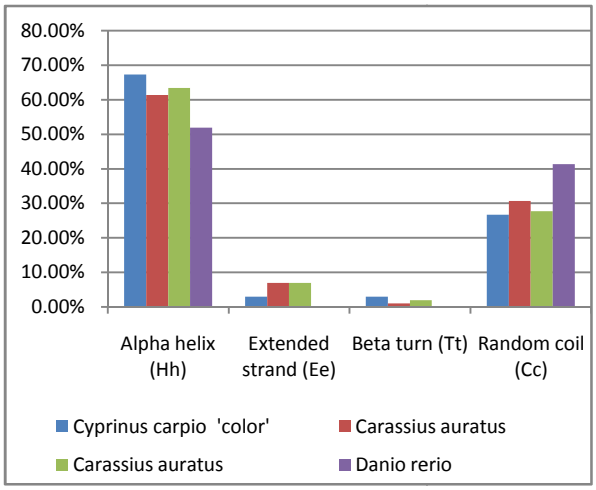

Figure 2: Graphical representation of percentage of helixes, sheets, turns and coils of osteocalcin of different cyprinidae species.

The analysis of disulphide bonds/bridges showed the absence of disulphide bonds between cysteine residues. The results given in figure show that alpha helix dominated followed by random coil, extended strand and beta turn for all four sequences. Alpha helices and beta sheets are secondary structure elements. Hydrogen bonding is a well known feature of alpha helix, that play role in folding and stabilization [10]. Beta sheets play role in biological activities of proteins [10]. Also, the beta turns play an important role in protein stability. [10]. Random coils have important functions like for flexibility and conformational changes of proteins [13]. As it is shown in the figure 3, the confidence of prediction observed throughout the predicted secondary structure was quite high.

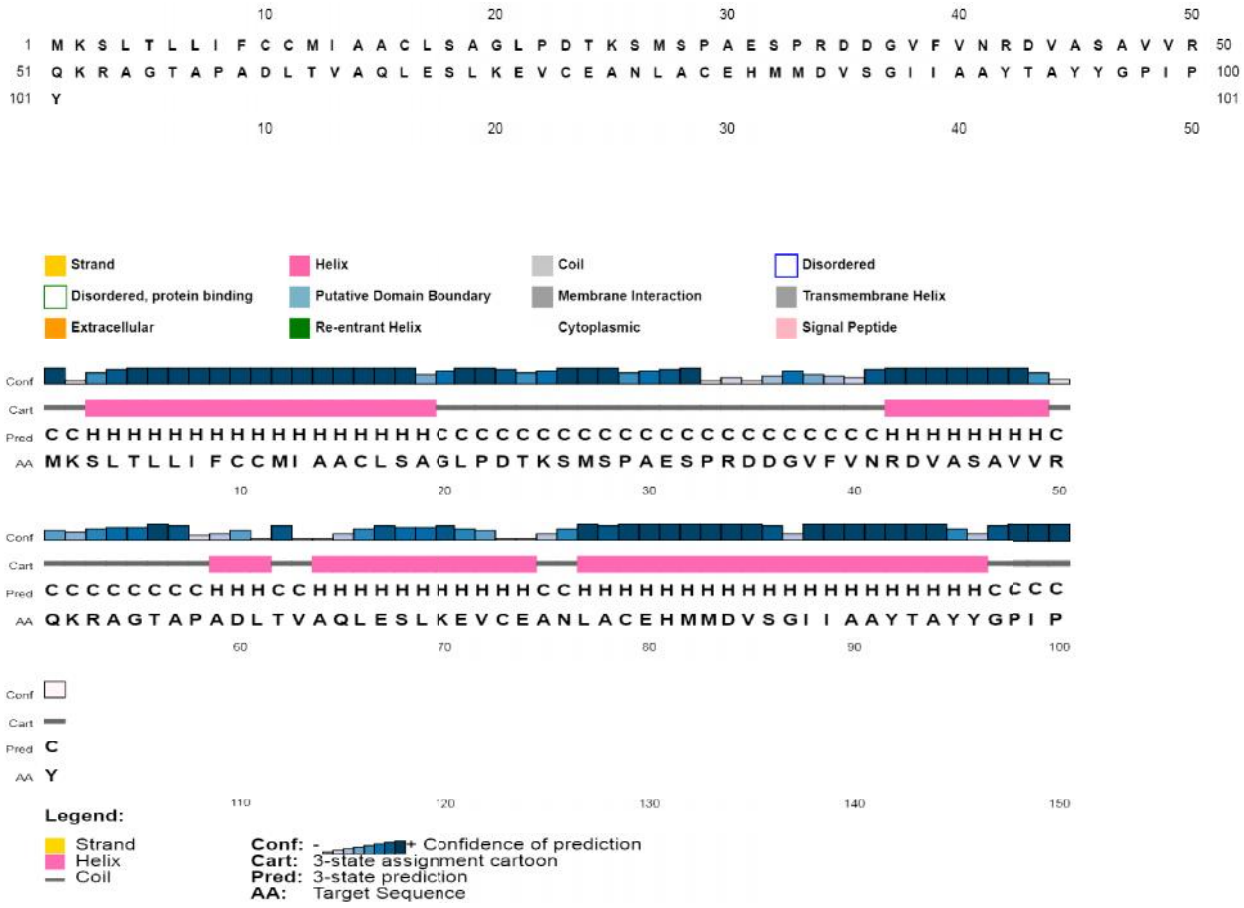

Figure 3: Graphical representation of the predicted secondary structures present within the target protein osteocalcin (`AIT51847.1`)

Subcellular localization prediction was performed by the use of CELLO v 2.5 and osteocalcin was localized in the extracellular matrix. According to Nishimoto et al., 2003, [2] Osteocalcin (bone Gla protein) is an extracellular matrix protein synthesized by osteoblasts,

There were determined three types of motifs, by Motif scan tools (Table 4). The highest number of motifs was N-myristoylation site, with four times. Protein N-myristoylation is a cotranslational lipidic modification of many eukaryotic proteins consisting on the attachment of myristic acid to the N-terminus that is catalyzed by the ubiquitous eukaryotic enzyme, N-myristoyltransferase (NMT) [14].

Table 4 : The motifs of osteocalcin in C. carpio by motif Scan

Motif information	*No of sites*	*Aminoacid residues*
Casein Kinase II pohosphorylation site	3	28 - 31; 32 - 35; 8 - 71
N-myristoylation site	4	20 - 25; 37 - 42; 55 - 60; 87 - 92;
Protein kinase C phosphorylation site	2	32 - 34; 68 - 70

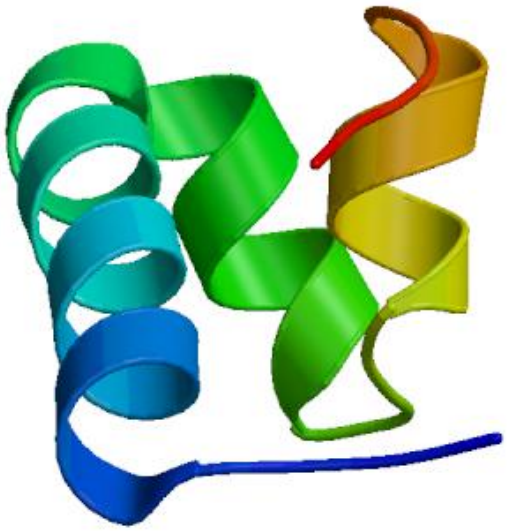

Figure 4. The predicted three-dimensional structure of osteocalcin by modelled SWISSMODEL

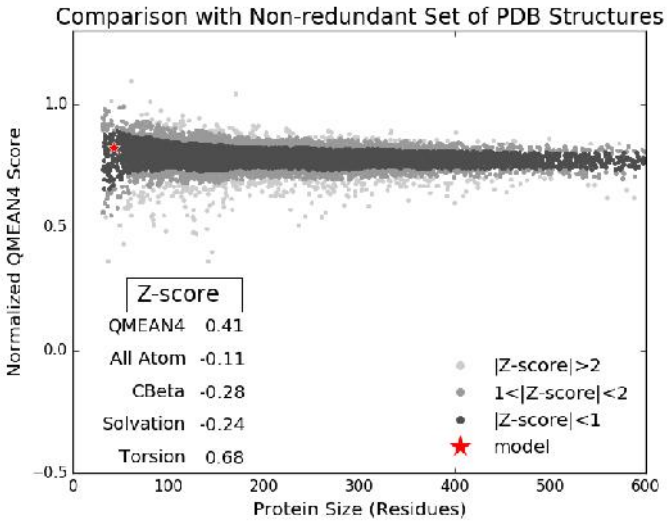

Figure 5:Z-score of query protein using QMEAN server

The three dimensional structure of osteocalcin was predicted by SWISS MODEL homology modeling program. The results are shown in figure. PDB 1vzm.1.A was selected as template with 61.36% sequence identity to query sequence (AIT51847.1).

The structure was validated validated through energy minimization with Z-Score by the use of Qmean server, ERRAT, and Rampage Ramachandran plot analysis. The Z-score was found as 0.41 (Figure 5). The overall quality factor evaluated by ERRAT was found as 97.143 which is very good quality (Figure 6) Ramachandran plot analysis (Figure 7) showed 97.3% residues in most favored region, 2.7% residues in additonal allowed region. More than 90% of residues reside in the favored region, which imply a good quality model [15].

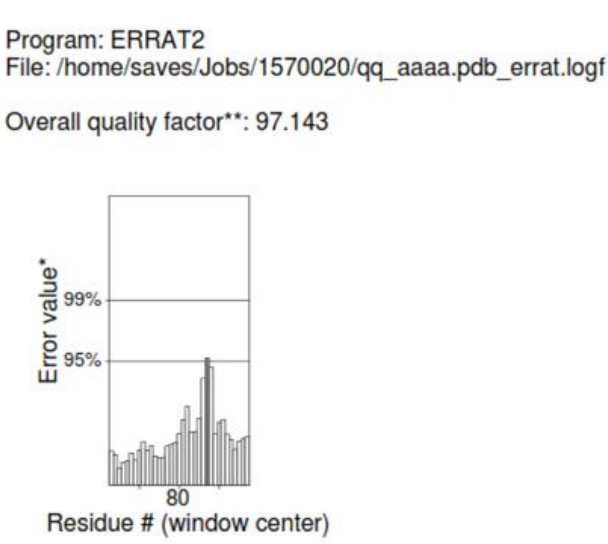

Figure 6: Overall quality factor evaluated by ERRAT

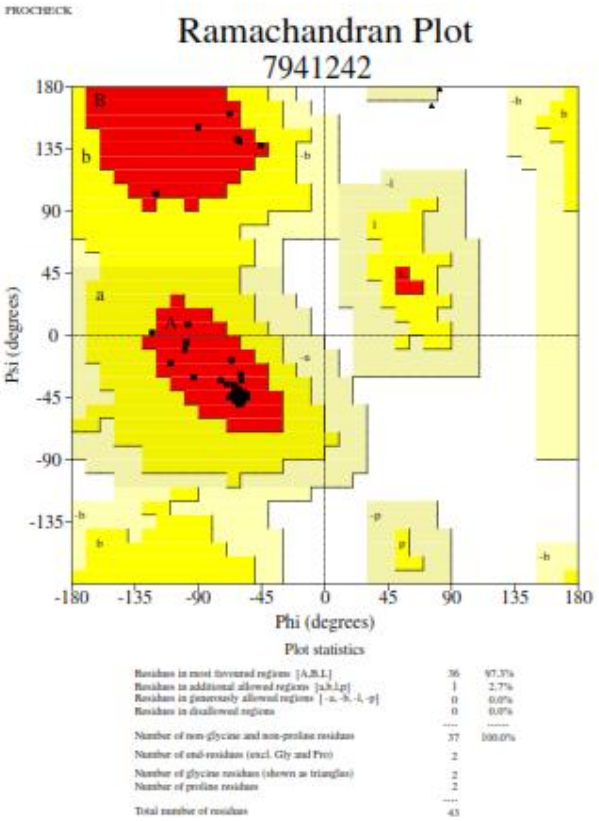

Figure 7: Graphical representation of Ramachandran Plot by ProCheck

Conclusions

In this paper we tried to analyze structural properties and predict 3D structure of osteocalcin in *Cyprinus carpio*. The analysis by the use of different online softwares showed that it is an extracellular protein, thermostable with a molecular weight of 10.75 kD. The amino acid composition reveals the abundance of Alanine amino acid in all the Cyprinidae studied species. Secondary structure analysis revealed that alpha helix were more abundant towards other secondary structure elements like random coil, extended strand and beta turn.

The modelling of 3D structure of osteocalcin was performed by Swiss Model and was validated with Z-Score by the use of Qmean server, ERRAT, and Rampage Ramachandran plot analysis

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RESEARCH ARTICLE (Open Access)

Genetic diversity of Cyprinus carpio from two hatcheries in Albania

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Abstract

The genetic differentiation of two farm carp populations of Tapiza and Klosi is analyzed by the use of five microsatellite markers. A total of 60 individuals sampled from these farms were investigated. In both population a high number of alleles per each locus is observed. Observed and expected heterozygosity values at the level of population are 0.542 and 0.886 respectively. NJ tree based on Allele Sharing Distance (ASD) and Factorial Correspondence Analysis (FCA) were used to analyze population differentiation and they both show two clear groups, implying that both populations are differentiated.

Keywords: microsatellite, heterozugosity, Factorial Correspondence Analysis,

Introduction

Cyprinus carpio is an important fish species in Albania. It is found in natural lakes of Albania and is cultivated in a semi intensive way in different private farms. In this study we intend to characterize the genetic diversity of carp from two hatcheries of Klosi and Tapiza, which produce fingerlings that are use for stocking different ponds and reservoirs in the country. A description of these hatcheries is carried out by Bixheku et al.,2017 [1] .

We are using five microsatellite markers that are used also for the characterization of different carp population in the world, but also for the carp from our native lakes [2] . Microsatellites are markers that are widely used for the genetic characterization of several species and carp as well [3, 4, 5] .

The results that are provided here can be used for organizing breeding strategy and programs for this specie.

Material and methods

Samples were collected from two carp farm populations of Tapiza and Klosi. A total of 60 individuals were selected. Microsatellite were analyzed as previously described by [6] .

Population heteozygosity were analysed by Genalex software [7] . Allele sharing distance, was calcutated by Genepop software [8] and a Neighbour Joining (NJ) tree based on this matrix was constructed by treeview [9] . Factorial Component analysis was performed by genetix software and Principal component analysis, was carried out by Genalex software [10] .

Result and discussion

In figure are shown allelic frequencies per each locus. The allele numbers obtained per each locus is high. High values are obtained for the same markers by Biba et al, 2015 [2] , for the carp from natural lakes of Albania, or Tomiljanovic [4] from carp croatian ppulation (Tomljanovic et al 2013). In tables 1 and 2 are given the observed and expected heterozygosity values per locus and population. Per each locus are observed high expected and observed heterozygosity values. Observed and expected heterozygosity values at the level of population are 0.542 and 0.886 respectively

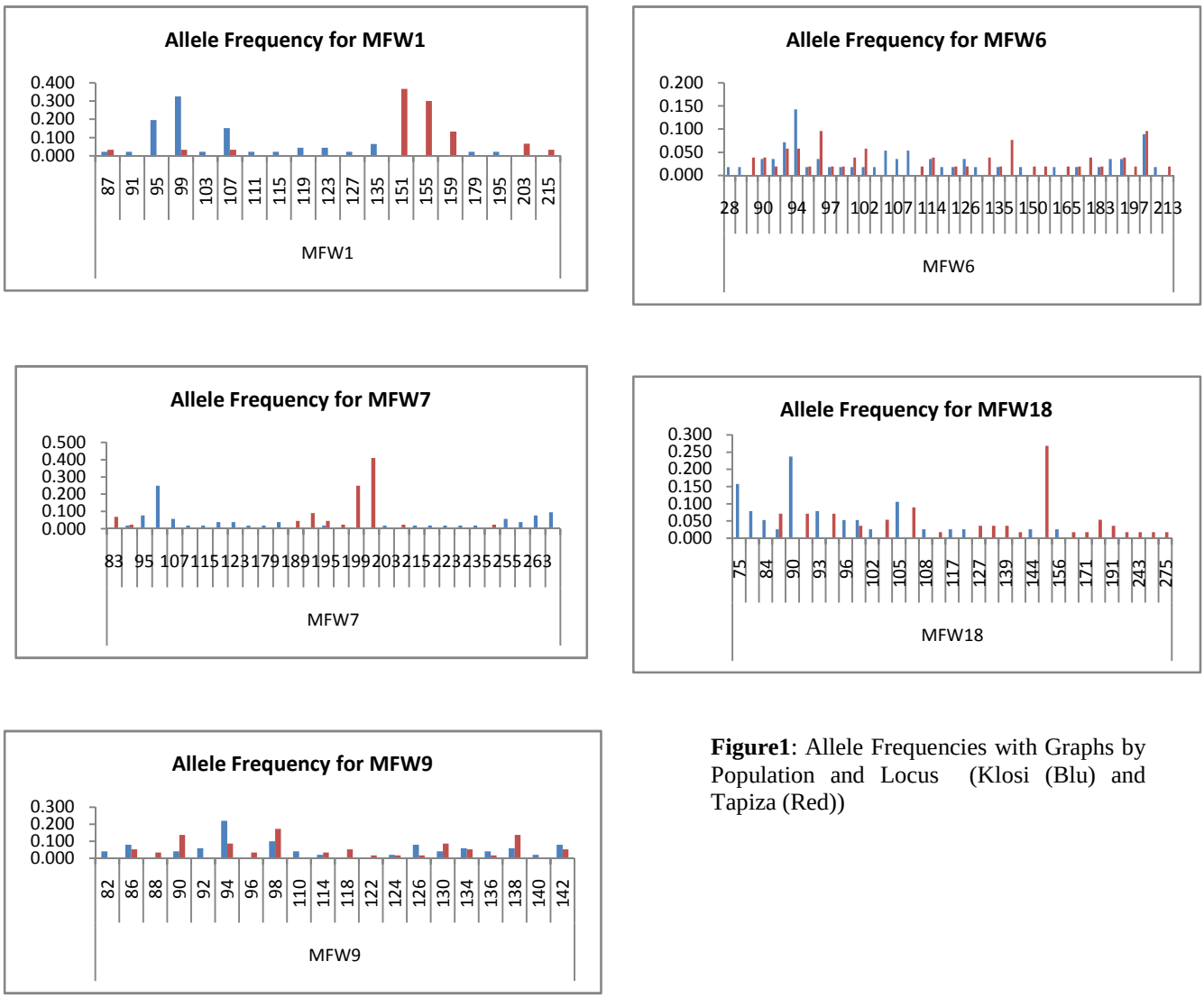

Figure1: Allele Frequencies with Graphs by Population and Locus (Klosi (Blu) and Tapiza (Red))

Table 1: Observed heterozygosity values (Ho) per population and locus,

Pop	*MFW1*	*MFW6*	*MFW7*	*MFW18*	*MFW9*	*Average*	*StDev*
Klosi	0.522	0.821	0.462	0.632	0.640	0.615	0.062
Tapiza	0.333	0.808	0.364	0.429	0.414	0.469	0.086
All Populations						0.542	0.056

Table 2: Expected heterozygosity values (He) per population and locus,

Pop	*MFW1*	*MFW6*	*MFW7*	*MFW18*	*MFW9*	*Average*	*StDev*
Klosi	0.820	0.945	0.899	0.882	0.902	0.890	0.020
Tapiza	0.749	0.950	0.751	0.890	0.902	0.848	0.041
All populations						0.886	0.014

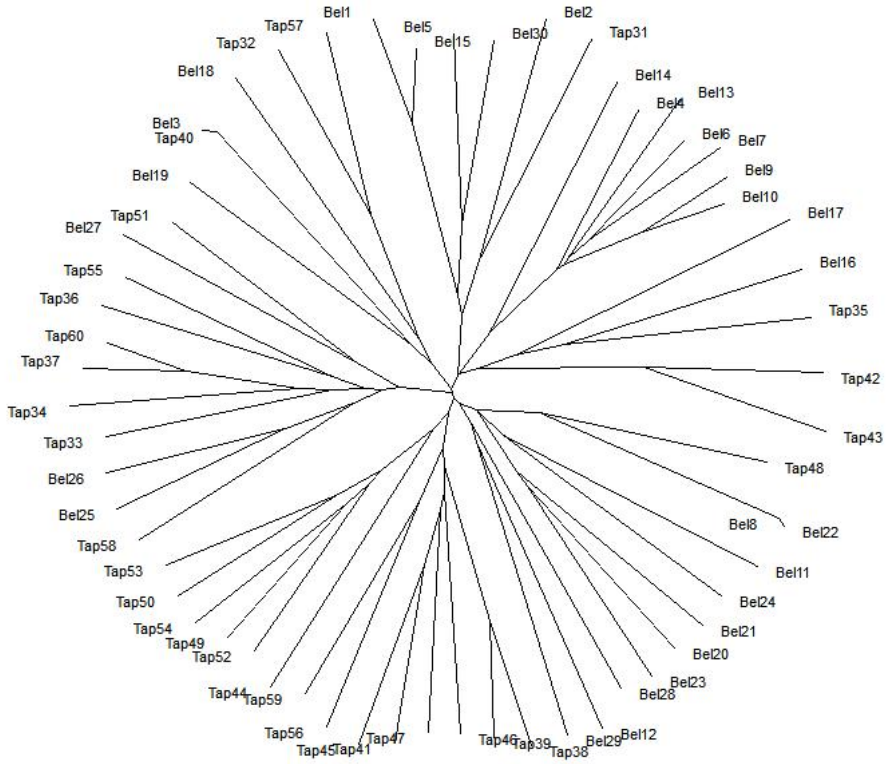

Figure 2:. The NJ tree constructed based on ASD between the individuals from two farm populations

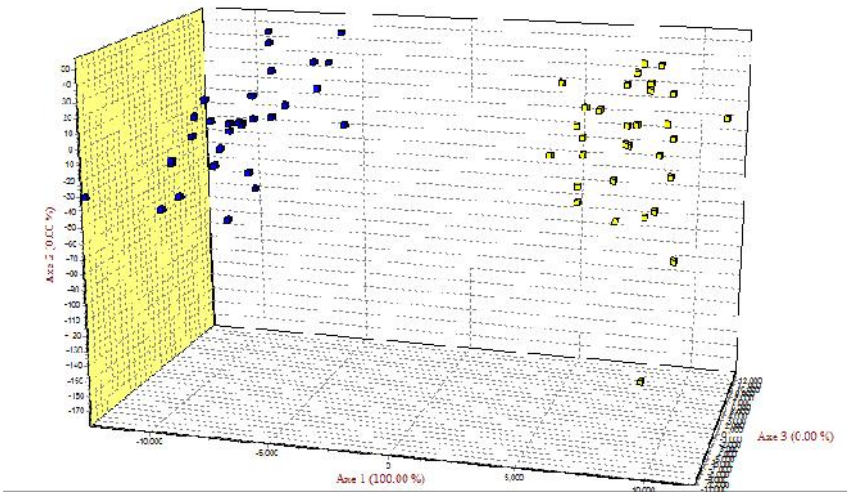

Figure 3: FCA displaying the relationship between individuals from two carp farm populations.

In figure 2 is given the Neighbour Joining, with a star like shape, tree based Allele Sharing Distance (ASD). This distance is based on idea that the common alleles at all samples of the same species have existed before the samples split. Therefore these alleles are more frequent that the new ones. The proportion of shared alleles increases with increasing the genetic similarity of samples

The 3 D graphic of Factorial Correspondence Analysis (FCA) is presented in figure 3. As can be seenthere are two clear grouping.

The genetic characterization of carp from these two hatcheries is carried for the first time and is of great importance, since both hatcheries produce fingerlings that are used for population of ponds and other fish farms in Albania.

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RESEARCH ARTICLE **(Open Access)**

Albanian forest role to the climate change mitigation and national economy

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Abstract

Albania has ratified the Kyoto Protocol in 2005 and recently has prepared the third National Communication to Mitigate the Greenhouse Gases (GHG). The main contributors in terms of GHG emissions (CO2 eqv.) according to this report were; (i) Energy sector (47.9%), (ii) Agriculture (14.26%), (iii) Industry (11.37%),(iv) Land Use Change and Forestry (20.64%) and (v) Waste (6.64%). Forestry sector has e great potential to GHG emissions by changes in management practices and wood use. The total estimated wood fuel consumption is 2.658.268 m3 equivalent with 651.4 ktoe or 7,577000 MWh energy (FAO, 2016). This amount of energy might be provided also from other sources like electric energy, natural gas etc and the economic value is estimated around 387 million euro (EUR) with a 3.45 % contribution in total GDP of Albania. Woodfuel use for energy purposes, significantly contribute to offsetting GHG emissions, therefore good practices in forest resource management and wood use must be implemented. To mobilize more wood from the forest sources at national level, efficient and effective measures need to be developed and applied. The objective of this study is to highlight the efficient and effective measures developed in Albania, to increase the role of forestry sector to meet energy demand and mitigate GHG.

Keyword: climate change mitigation, wood fuel consumption, contribution, national economy.

1. Introduction

Historically, forests in Albania has been used as a primary energy source for heating, cooking and boiling water, particularly in rural areas. Today, the demand for fuelwood remains high. As late as 2012, the Living Standards Measurement Survey (LSMS) showed that 63% of all households surveyed used wood as their main source of energy for heating.

While forests also offer other services such as biodiversity conservation, land protection from erosion,by winds, floods, protect watersheds, mitigating climate change through carbon sequestration, etc.. [3].

Albania is playing a leading part in international efforts to stop global warming and limit damaging climate change, which particularly threatens developing countries and the poor and vulnerable [5].

Albania has ratified the UNFCCC in 1995 and it's Kyoto Protocol in 2005. As a party to UNFCCC, Albania has prepared national communications to the Convention. This process has helped to bring climate into the national development agenda, thus in principle Albania was eligible to enter into the carbon trading market since 2005.

The forest sector appears to be a net emitter of GHGs, due to the fact that the level of annual forest cutting is higher that the forest's natural growth [7]. The most part of CO2 emissions from the Land Use Change and Forestry (LUCF) sector originate from carbon stock changes.

A good example on contribution of forestry sector to the climate change was the implementation of Carbon Sequestration Project [2]. Albania, has implemented the carbon sequestration component (Clean Development Mechanism) in frame of the Natural Resources Development Project within the

years 2005 -2012 through the Ministry of Environment. The objective of carbon sequestration project in Albania was reducing soil degradation, conserve biodiversity and enable GHG emission reduction.

The assisted natural regeneration project aimed to restore the vegetation on degraded lands distributed in 24 poorest communes over five regions of the country. This project was implemented in 3.990.45 ha and the total GHG over the monitoring period amount of tCERs to 128.757,50 ton CO2 e [8]. The implementation of carbon sequestration enables Green Houses Gasses emission reduction and in other side was a tool to get financial resources, to be used for improvement of forests and pastures [2].

Although Albania is a country with a low-carbon economy, it is committed to reduce the carbon dioxide emissions by 11.5 % as compared to the baseline scenario for the period 2016 – 2030 [7]. The main mechanisms of achieving this objective were related to maintaining the low level greenhouse gases emissions from energy production sector and developing low carbon policies in order to prevent the increase of greenhouse gases emissions from other sectors of the economy.

The development and publishing of the Third National Communication of Albania to the United Nations Framework Convention on Climate Change in the year 2016, has been a joint undertaking by the Ministry of Environment (MoE) and the United Nations Development Program (UNDP) Albania.

According to TNC, the main contributors in terms of GHG emissions (CO2 eqv.) for the year 2005 is the Energy sector (47.9%), followed by Agriculture (14.26%), Industry (11.37%), Land Use Change and Forestry (20.64%) and Waste (6.64%) [1].

GHG emissions from LUCF have decreased in the inventory period (2000-2009) even if the forestry sector is still a **GHG emitter** because of the higher use of biomass respect to the annual natural increment of forests.

Total GHG emissions from 1990 to 2009 according to the Albanian TNC[1]

Sector	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Energy	4.116,93	4.151,67	4.274,11	4.447,34	4.698,02	4.633,37	4.671,28	4.721,45	4.778,87	5.116,04
Industrial Processes	520,00	852,00	806,00	966,00	1.043,00	1.118,00	1.195,00	1.470,00	1.599,00	1.701,12
Agriculture	1.552,48	1.510,27	1.464,28	1.475,62	1.464,28	1.402,96	1.374,82	1.283,26	1.204,51	1.130,80
LUCF	2.295,00	1.855,00	1.391,00	1.054,00	1.123,00	1.056,00	860,00	970,00	530,00	213,00
Waste	590,64	592,74	591,69	601,35	647,29	652,96	723,10	730,66	697,48	827,68
TOTAL	***9.075,00***	***8.961,70***	***8.527,10***	***8.544,30***	***8.975,60***	***8.863,30***	***8.824,20***	***9.175,40***	***8.809,90***	***8.988,60***

Source: National Climate Change Strategy and Plan for Albania (2016)

[1]National Climate Change Strategy and Plan for Albania (2016). The project; Technical assistance for institution building of the Ministry of Environment in enforcing environmental and climate Acquis. Europe Aid/135700/DH/SER/AL. Project acronyms IBECA. Agrotec SpA – Italy.

Based on Third National Communication of the Republic of Albania under the United Nations Framework Convention on Climate Change (2016): related to the Projected CO2 emissions from forests (in Gg), during the period 2015-2050, adopting the selected mitigation measures can reduce the amount of emissions from forests[2] from 1,130.7 Gg of CO2 in 2015 to 663.2 Gg in 2030 [7].

Thus, a reduction of GHGs emissions by almost 60% for a period of 15 years can be achieved. On the other hand, the proposed technological measures do not provide any further reduction between 2030-2050, since their efficiency will peak in the year 2030, and the forest sector will continue to bean emitter of GHGs.

Albania's National Strategy for Development and Integration (NSDI I) 2007-2013 (DCM No. 342) of 12/03/2008 was a key national strategic document that states Albania is a small actor in the global environment due to its low per capita GHG emissions. Nevertheless, the strategy outlined measures for mitigation and adaptation to climate change, although the overall goal of these measures is not explicitly related to climate change.

The NSDI focused on energy efficiency improvement in all sectors, with a view to reduce energy consumption and GHG emissions. In the context of the EU Accession process and the Energy Community Treaty, and in line with the EU 20-20-20 objectives, Albania has already set Quantified Objectives related to energy efficiency (9%) and renewable energy sources (38%) in 2018 and 2020 respectively compared to 2009.

Woodfuel use for energy purposes, significantly contribute to offsetting GHG emissions, therefore good practices in forest resource management and wood use must be implemented. To mobilize more wood from the forest sources at national level, efficient and effective measures need to be developed and applied.

The objective of this study is to highlight the efficient and effective measures developed in Albania, to increase the role of forestry sector to meet energy demand and mitigate GHG.

In order to assess the contribution of forests role to climate change and mitigation and economy, it has to be noticed that all calculations were done based on the estimation wood fuel energy (according Fao 2016).

2. Materials and methods

The data used for the study were provided by the study " Woodfuel consumption in Albania" carried out in the framework of the FAO Project: "Technical assistance for using wood energy to improve sustainable economic rural development and meet the 2020 renewable energy targets for the Western Balkans" (TCP/RER/3502) in cooperation with the Ministry of Infrastructure and Energy, financed by FAO in 2017.

The methodology used consisted on face to face interview done with various stakeholders like households (HHs), enterprises and public buildings (schools and kindergartens). A two - Stage Sampling Random Procedure was used for the survey of households. On the first step were selected the primary sample units and the list of the households was selected randomly on the second stage. The total number of the interviewed households was 5339 of which 1603 in urban areas and 3736 in rural areas [6].

The sample of enterprise was selected based on the systematic random sampling stratified according to economic activity and size. In total were interviewed 469 enterprises (business sector), meanwhile that in pubic sector (were interviewed 327 facilities in total [6].

The objective of this study is to highlight the efficient and effective measures developed in Albania,

2Third National Communication of the Republic of Albania under the United Nations Framework Convention on Climate Change (2016):

to increase the role of forestry sector to meet energy demand and mitigate GHG. In order to do the assessment of Albanian forest role to the climate change mitigation and national economy, in frame of this paper we were focused only to the part of estimated woodfuel energy used for heating by households, public sector and business sector.

Based on the assumption that the amount of woodfuel energy can be provided also by other energy sources like electricity or natural gas. For this reason, it was calculated how much money it is needed to buy electric energy compared with the same amount of woodfuel energy estimated by FAO survey (2016). Through such comparisons, we want to know the weight of estimated woodfuel energy based on the total GDP, or the share of wood-based energy in total GDP.

On the other side, in order to assess the woodfuel contribution to climate change mitigation and economy we assume that intead of woodfuel, we are going to use natural gas. For this reason, it was calculated the quantity of T CO2 eq can be emitted in atmosphere, when it is used natural gas for energy. As it is known, the use of woodfuel energy reduces or prevents CO2 emissions. Keeping in mind this fact, based on the the price of CO_2 eqv in the current market, it was calculated how much incomes can be generated through using woodfuel energy.

3. Results and discussion

The processing data carried out in frame of the survey "Woodfuel consumption in Albania" show that the total estimated consumption in households, public buildings and in part of bussiness sector was 2,658,268 m3 or 7,575 TWh or 651,388 ktoe [6]. The detailed woodfuel consumption according to households, public sector and business sector is given on the Table 2.

Table 2: Estimated consumption of woodfuels in Albania

Wood fuels	*Estimated consumption*		
	m3	TWh	ktoe
Households	2.573.627	7,333	630,65
Public sector*[1]	37.084	0,106	9,086
Business sector*[2]	47.557	0,136	11,652
Total	**2.658.268**	**7,575**	**651,388**

Source: Woodfuel consumption in Albania (FAO, 2016)

Comments:

*[1]: Only part of educational facilities and selected part of other public building are included

*[2]: Only part of bussines sector was included in the survey

As it was shown on the above table (Table 2), the total estimated woodfuel consumption (based on survey FAO 2016), is given m^3, TWh, in ktoe. That energy which comes from woodfuel might be provided also from other sources like electric energy, natural gas etc.

It was mentioned in the methodology, it has to be noticed that the amount of woodfuel energy can be provided also by other energy sources like electricity. Based on that, the calculation is how much money it is needed to buy electric energy compared with the same amount of woodfuel energy estimated by FAO survey (2016). Through such comparisons, we try to assess

the weight of estimated woodfuel energy based on the total GDP as it shown in the table 3.

Table 3 Contribution of Wood energy in Total GDP of Albania

Wood fuels	Estimated consumption			Wood energy Content (MWh)	Price of electric energy	Annual Revenues (euro)	GDP (Billion Euro)	Share %
	m3	TWh	ktoe					
House-holds	2.573.627	7,333	630,65	*7333000*	*51.1** Euro/ MWh*	*374716300*		
Public sector*[1]	37.084	0,106	9,086	*106000*		*5416600*		
Business sector*[2]	47.557	0,136	11,652	*136000*		*6949600*		
Total	**2.658.268**	**7,575**	**651,388**	***7577000***		***387082500***	**11. 21***	**3.45**

*The figure 11.21 Billon Euro represents the GDP of Albanian for the year 2016 (According to World Bank, GDP in Albania for the year 2016, was 11.93 billion USD, or 11.21 billion Euro (Based on the Yearly Average Exchange rates for the year 2016; 1 USD = 0.940 Euro)

** The 51.1Euro/MWh – represents the price of electric energy that Albanian Power Corporation (KESH) has bought in the international market for the year 2017.

If the amount of energy would be provided by electric energy the Albanian Power Corporation should spend around 387 million EUR, (or respectively 387,082,500 Euro) to buy such amount of energy in international market. Current use of wood energy substitutes imports of electric energy in the value of 387 million EUR.

In order to calculate how big is the contribution of wood energy in total GDP in Albania, we compare the amount of money to be used for buying of electric energy in international market with total GDP in Albania. Taking into consideration that the last GDP in Albania was 11, 93 billion USD (or converted in 11.21 billion Euro), we calculated that contribution of wood energy in total GDP in Albania is 3.45 %. So, the conclusion related to the share of wood-based energy is around 3.45 percent of total GDP in Albania.

In order to calculate the wood fuel contribution to climate change mitigation and economy, we assume that the amount of wood energy is provided by natural gas. As we know, use of wood fuel for energy reduce or prevent the emitting of CO2 in atmosphere.

Table 4 Ecological and economical contribution in GHG (Albania)

Wood fuels	Estimated consumption			Wood energy Content (MWh)	C02 Kg/MWh	Quantity T CO2eq	Price for CO2eq	Incomes (euro)
	m3	TWh	ktoe					
House-holds	2.573.627	7,333	630,65	*7.333.000*	*257.72*	*1.889.860,8*	6,77 €	*12.794.357,6*
Public sector*[1]	37.084	0,106	9,086	*106.000*		*27.318,32*		*184.945,03*
Business sector*[2]	47.557	0,136	11,652	*136.000*		*35.049,92*		*237.287,96*
Total	**2.658.268**	**7,575**	**651,388**	***7.577.000***		***19.522.229***		***132.165.490,3***

- *The figure 257.72 represents the quantity in kg of CO2 emitted in atmosphere, when we produce 1 MWh energy from natural gas.*

- *The Price for CO2 eq is taken 6.77 Euro (referring to the current market price in October 6, 2017)*

Use of wood fuel for energy prevented emission of 19.522.229 tones of CO_2 from the fossil fuels, which estimated in economical point of view is EUR 132.165.490,3. The theoretical value of these carbon emissions in economical term currently value would be around 132 million EUR.

4. Conclusions

- Based on the calculations carried out for the estimated woodfuel energy (based on the FAO survey 2016) and comparing with the electricity energy show that the contribution of woodfuel energy in total GDP in Albania is 3.45 %. This conclusion represent the share of wood-based energy is around 3.45 percent of total GDP in Albania.
- Current use of wood energy substitutes imports of electric energy in the value of 387 million EUR.
- Use of wood fuel for energy prevented emission of 19.522.229 tones of CO_2 from the fossil fuels, which estimated in economical point of view is EUR 132.165.490,3.
- The theoretical value of these carbon emissions in economical term currently value would be around 132 million EUR.

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