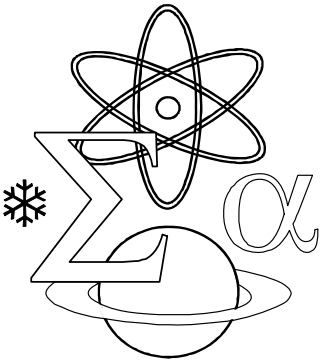
PUBLISHED BY THE ACADEMY OF SCIENCES OF ALBANIA

JNTS

JOURNAL OF NATURAL AND TECHNICAL SCIENCES



2014, Vol. XIX (3)

Aims and Scope
This Journal is a multidisciplinary publication devoted to all field of Natural and Technical Sciences. The Editor of JNTS invites original contributions which should comprise previously unpublished results, data and interpretations. Types of contributions to be published are: (1) research papers; (2) shorts communications; (3) reviews; (4) discussions; (5) book reviews; (6) annonuncements.

ISSN 2489-0484

Graphic by: Enkelejda Misha, Printed by “Kristalina KH”

AN IMPROVEMENT OF MARKOV'S MODEL WITH NON-STATIONARY PROBABILITIES OF LIN AND YANG AND ITS IMPLEMENTATION THROUGH PROGRAMMING IN C

Romeo MANO
University, Eqerem Çabej, Faculty of Natural Sciences, Department of Mathematics and Informatics, Gjirokastra, Albania
Endrit XHINA
University of Tirana, Faculty of Natural Sciences, Department of Informatics, Tirana, Albania

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ABSTRACT

The final version Bassel II which was approved in January 2005 (ECB, 2005) recommended: i) adequate techniques for the creation and management of credit risk models, fundamental for financial institutions of risk management and, ii) creating models for calculating the operational risks, in particular for the construction of calculation techniques to provisions in for a better management of reserves for losses from loans at risk. The paper aims at presenting and implementing a multivariate probability model based on the treatment of credit portfolio with regard to the number of loans as a random process of homogeneous Markov's chain type offered by Lin and Yang. Provisional classification of the loan portfolio by Central Banks and unconditional probabilities of CR + were used. In the commonly used techniques for the management of risk models it has been noticed a high level of complexity not only by theoretical definition but also by calculating techniques for finding the joint probability multivariate distribution of loans at risk. In most cases, this complexity occurs due to the insufficient information provided by banks. The importance of the model presented here lies in: i) the modelling probability of the portfolio classification with regard to the observance of the repayment term and, ii) the elimination of the complexity of calculation techniques by applying simple facilitating applications in computer programming.

Keywords: portfolio at risk, provisions, random processes, Markov's models, multivariate distribution, C #

1. INTRODUCTION

Creating techniques that foresee the losses in value as a result of their exposure to the credit market is of primary importance for any financial institutions (hereinafter FI's), i. e., building predictive models of potential losses referring to monetary values created mostly from crediting in the form of loans within a predetermined time period. The amount of monetary assets lent to clients constitutes what is called the loan portfolio. The value of this portfolio which does not return at the predetermined time constitutes the loan portfolio at risk. The entirety of the loan portfolio of the FI's consists of individual loans disbursed to each client of the IFs. So, a loan portfolio is truly an amount in cash value, but simultaneously this portfolio is also a set of borrowers. Experience of certain units of FI-s (i.e., Second Level Banks) on the assessment of the loan portfolio lets us understand that the assessment of credit risk portfolio is made by using both approaches of credit portfolio assessment: i) monetary value and, ii) number of loans/borrowers. It has been noticed from the study of models offered that they focus only on the treatment of a database for monetary value and not the number of loans/borrowers. Therefore, the aim of this paper is to offer the possibility of implementing a probability model on a portfolio in the number of loans by means of programming in C #.

2. Literature overview

Technologic development has a long history in the realm of theoretical models of credit risk. As a result, the two theoretical models of credit risk are the credit assessment models comprising four subgroups and the models of portfolio credit risk.

The classification into subgroups was based on the LGD (Loss Given Default) considerations and the relationship between PD (Probability of Default) and RR (Recovery Rate).

In the first subgroup, known as first generation of structural models, PD and RR are the functions of the structural characteristics of companies. In these models, the RR is considered as an endogenous variable. PD and RR are two "reverse" functions, i.e., as PD increases, RR decreases. Here, the models were introduced by Merton (1974) and Geske (1977).

In the second subgroup, known as the second generation of structural models, RR is considered as exogenous variable and independent from the values of the assets of the company. In general, the RR is defined as a fixed rate of current debts of the company. Consequently, it is independent from the PD. Here, the models were introduced by Kim *et al*., (1993) and Longstaff and Schwartz (1995).

The third subgroup comprises models reduced in form, where in most of the cases, the RR is assumed to be an exogenous constant independent of the DP. Innovation in some of these models stands in the RR assumed to be a stochastic variable (random variable) independent of the PD. Here, the models were introduced by Litterman and Iben (1991) and Duffie (1999).

Recent contributions to the construction of models that have at their core the relationship between PD and RR, are all involved in a subgroup (fourth subgroup). Their characteristic is that the DP and RR are treated as stochastic variables. These variables are dependent on common factors of systemic risk which determine the general economic situation. In these models, PD and RR are negatively correlated. In macroeconomic concept, negative correlation is a result of mutual dependence of PD and RR from the same simple systematic factor. While in the concept of microeconomic approaches, the correlation negativity derives from the levels forecast-demand offers of securities. The presentation of these models belongs to the beginnings of our century and it is included in the studies made by: (Frye, 2000; Jarrow 2001; Carey and Gordy, 2003; Altman *et al*., 2005; Acharya *et. al*., 2007; Miu and Ozdemir, 2006).

The second group of risk models dates since the second half of the twentieth century. Banks and their technical consultants developed a new way of modeling credit risk focusing on estimating potential losses with a predetermined level of security for a loan portfolio exposure within a certain period of time, usually equal to a financial year. These models were motivated by the growing importance of credit management and they have in the center of attention the values at risk (VaR) of lending financial companies Assets. VaR models include: the CreditMetrics + model of Morgan (Gupton *et al*., 1997), Credit Suisse Financial Products otherwise known as CreditRisk + (Products, 1997), CreditPortfolioView + of McKinsey (Wilson, 1998), CreditPortfolioManager+ of Moody and the Risk Manager + of Kamakura (Crosbie, 1999).

We can classify the VaR models into the two following main categories: i) the models Default Mode (DM) and ii) the models Mark-to-Mark (MTM). In these models, the credit risk is identified as risk of loss and is used the binomial distribution with only two possible probability events— payment on time and non-payment on time—is used. In the DM models, loan losses are considered only if the event "not paying on time" occurs. While the MTM models are multinomial considering as loss not only the failure but also the negative situation of the credit. Credit risk models aim at determining probability distribution function of expected losses on the loan portfolio. With these models it begins to be used in determining way the probability concepts of the mathematical expectation and of the dispersion of distribution. Thus, the potential losses on financial concept are equal to the mathematical expectation of the probability distribution of loss as random variable. This

mathematical expectation value expresses the amount that investors expect to lose over a predetermined period of time. On the other hand, unforeseen losses express the deviation from the expected losses and thus, they indicate the value of the actual portfolio at risk. Put in terms of probability, unexpected losses can be calculated as standard deviation of probability distribution of losses. Common Specific of all VaR models is that RR and DP are considered as independent random variables and except the case of CR + model, they are considered as stochastic variables. Detailed information about CR + following (Gordy, 2000) is in the forthcoming paragraph reported.

Detaching from the standard classification of credit risk, many researchers in the field of credit risk models have achieved satisfactory results which are continuously used to improve these models. Chin-Tsai and Shih-Yu (2003), offered a predictive model using non-stationary probabilities of Markov's chains (Smith and Lawrence, 1995) and Gray model (Lin and Yang, 1999). For the first time, their model used the classification of the loan portfolio in financial states and it was limited to seven financial states. Communication of these states according to Lin and Yang's model is in Figure 1 depicted. Using non-stationary probabilities of Markov's chains, Lin and Yang concluded that the probability of the state for the period ahead is expressed by the equation:

$$P_j(t+1) = \sum_{i=1}^{6} P_i(t) P_{ij}(t) \tag{2.1}$$

wherein:

$P_i(t)$ is the probability that the loan is in state *i* of the period (time) *t*;

$P_j(t+1)$ is the probability that the loan is in state *j* at time *t* + 1;

$P_{ij}(t)$ is the probability of transition from the state *i* of the period *t* in state *j* in the following period *t* + 1;

Taking the random variable $X^{(1)}(t)$ in period *t* = 1 with distribution given by equation (2.1) and by applying the differential equation of first grade of GM (General Models) (Deng, 1989) Lin and Yang presented the following model:

$$\frac{dX^{(1)}(t)}{dt} + aX^{(1)}(t) = b \tag{2.2}$$

For calculating the parameters *a* and *b*, this model employs a database of monetary value to make the classification of seven financial states. In this model, the family of initial distribution of the Markov process has not been defined, but it has been assumed as known.

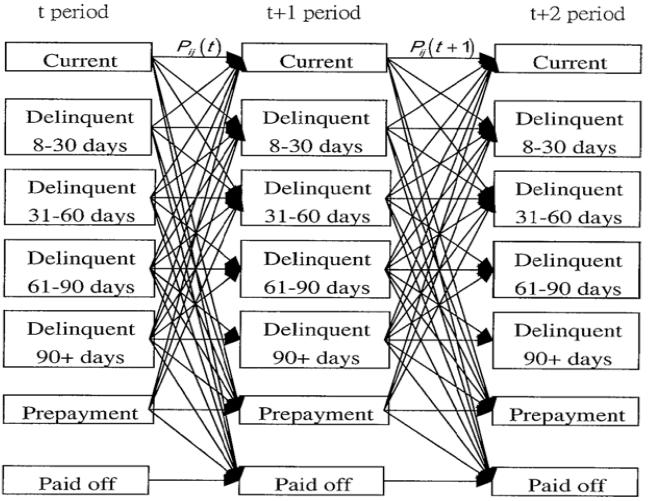

Fig. 1. The communication graph of states by Lin and Yang.

One of the VaR models, namely CR + (Gordy, 2000) offers a definition of these probabilities by means of econometric considerations for determining risk factors.

CR + is a risk model of default for an obligation that stems from borrowing. Each borrower has only two possible situations at the end of the period of default, "delayed" and "non-delayed". In case of default, the lender has a loss of physical size, which is the exposure of the lender to the market.

The conditional probability $p_i(x)$ of having a "delay" for debtor of *i*-t is a function of the type of borrower class $\xi(i)$, which carries the risk factors x, and charger factors $\left(w_{i_1},\ldots,w_{i_k}\right)$, which measure the sensitivity of borrowers to *i*-t in each of the risk factors. In this way, CR + specifies this density function:

$$p_i(x) = \overline{p}_{\xi(i)}\left(\sum_{l=1}^{k} x_k w_{i_k}\right) \tag{2.3}$$

where $\overline{p}_{\xi(i)}$ is the unconditional probability of the borrower class ξ to which the following condition must be met:

$$E\left[p_i(x)\right] = \overline{p}_{\xi(i)} \tag{2.4}$$

As the basic reference for the results of this paper serve the following premises: i) treatment of the process "delay" of a loan as a Bernoulli random variable and, in general, of the loan portfolio as a random vector with Bernoulli's margins, ii) equation (2.3) that gives unconditional Bernoulli's

probability to have a "delay" and, iii) treatment of the loan portfolio as a Markov chain with non-stationary probabilities of type (2.1).

By treating the loan portfolio with regard to the number of loans/borrowers and not their value, the present paper aims at: i) modifying the model provided by Lin and Yang in terms of redesigning the classification of the portfolio by Central Banks (case the Bank of Albania), offering a more comfortable and clearly perceptible process graph, ii) defining the initial distribution of the process by means of its point estimation, iii) using of unconditional probabilities of CR# in model, iii) constructing Markov type model probabilities through the probabilities of univariate and multivariate distribution of the portfolio and, iv) presenting a possible implementation of the model by means of a programming language (C #).

3. Classification of the portfolio at risk

Referring to the Bank of Albania Regulation "On credit risk management", dated 27.12.2006, bad loans are classified into groups based on delays of monthly instalments. On the basis of these groups is defined the provisioning percentage value of loans at risk, as shown in the table below.

Table 1. Classification of loans by the number of delayed instalments and the measure of their provisioning

Group	Installments on delay	Percentage of provisions
Group I	0 days=0 installments	0%
Group II	1 - 30 days= 1 installments	1%
Group III	31 - 90 days=2 - 4 installments	5%
Group VI	91-180 days= 5 - 7 installments	20%
Group V	181-365 days=8-12 installments	50%
Group VI	Over than 365 days=over than 12 installments	100%

The table shows that each loan is classified into one of six groups as given in the first column regardless of correctness towards the bank with regard to payment of instalments. Each state classification is determined by the number of delayed instalments, as shown in the second column, whereas provisioning is determined at the rate specified in the third column. At the end of each financial month banks make reclassification of its credit portfolio. For instance, a loan falls into the Group III when the borrower has 31 days to 90 days in arrears. In other terms, the borrower has not paid one, two or three monthly obligations from its obligation towards the bank in the period between the two reclassifications. Also, the borrower can make the payment of one, two or more instalments in the span of a financial month, which makes

the borrower acquire a better status in classification with more than one unit, for example, by paying a number of instalments he passes from Group III to Group I. However, he cannot "go down" more than one unit i.e. he cannot go from Group II to Group IV at the time of a monthly reclassification due to the fact that the classification is determined by the delayed days, which means that a credit that is 30 days in arrears may not exceed the end of the month following 91 days in arrears (!). The probabilities of type given by the equality (2.1), does not foresee the practical restrictions that exist in the process of periodic classification of portfolio. This is the reason for the complexity of the graph of process presented by Lin and Yang (Fig.1)

Loans that are past due over 12 monthly instalments fall into Group VI which in bank terminology are called loans with written-off status, which they preserve along all the financial year and are provisioned by 100%. They do not change this status even if in the coming period the borrower makes the payment of instalments, which can practically reduce the loan below the 12 months delay.

Since for any loan a decision is conditioned by a return probability and each month the quality of this reversibility is checked, then the above bank practices can be justified by treating the loan portfolio by CR+ as a random vector with marginals of the type of Bernoulli random variable.

3. Construction of the model and its implementation on a database

Let's have a loan portfolio, with a total volume of loans equal to *n* creditors, which consists of loans that have a state status, defined by *Tab. 1*.

If we denote with Y_i, $1 \le i \le n$ each of the loans, then the random vector $Y = (Y_1, \ldots, Y_n)$, represents the loan portfolio. Marginals of the vector Y are random variables, which can be seen as a Bernoulli random variable with probability of "success" equal to $1-\pi$, where π denotes the probability given by the equality (2.3) (Note that "success" is considered the monthly instalment payment).

Let's suppose that the loan Y_i is in arrears by j_i instalments.

Let we consider a random variable X_i that expresses the number of successes in $j_i + 1$ independent tests of random variable Y_i.

In this way, we take the random vector $X = (X_1, \ldots, X_n)$, whose marginals are random binomial variables with parameters $j_i + 1$ and $1-\pi$, $\forall i = 1, ..., n.$ As a bank practice, any loan which exceeds 365 days in arrears, i.e. over 12 instalments in arrears, is extracted from loan portfolio with the status "written-off "and becomes provisional with 100% of the

amount in arrears (Group VI, Table 1), then the values that will take the delay j_i will be the community $E=\{0,1,...,13\}$, where state 13 represents the status condition "written-off". On the other hand, testing the classification of the loan portfolio according to Table 1 is done at the end of each financial month, which will determine the time *t* of each test, thus $T=\{0,1,...,12,...\}$. $t=0\in T$ expressing the moment of the beginning of the process and $t=12\in T$ expresses "the last" moment of financial year. Theoretically, the process continues for an infinite discrete time, but IFs are interested in what happens with the loan portfolio at the end of the financial year.

Denote with $Z_{i,t}$ the random variable that expresses the state of the loan classification Y_i at the moment $t\in T$. If $Z_{i,t}=j_i$ at the moment $t\in T$, and Y_i is j_i instalments in arrears, then at the moment $t+1\in T$ we have $Z_{i,t+1}=j_i+1-X_i$.

This means that events $\{Z_{i,t+1}=j_i+1-k/Z_{i,t-1}=j_i\}$ and $\{X_i=k\}$ are probability equivalent.

Therefore,

$$P\left(Z_{i,t+1}=j_i+1-k/Z_{i,t-1}=j_i\right)=P\left(X_i=k\right) \tag{4.1}$$

Since X_i has binomial distribution with parameters j_i+1 and $1-\pi$, $\forall i=1,...,n.$, then

$$P\left(Z_{i,t+1}=j_i+1-k/Z_{i,t-1}=j_i\right)=P\left(X_i=k\right)=C_{j+1}^{k}\left(1-\pi\right)^{k}\pi^{j+1-k} \tag{4.2}$$

Since family $\left(Z_{i,t},t\in T\right)$, expresses development in time of random variable Y_i, then it is a random process with discrete space of states $E=\{0,1,...,13\}$ and discrete time space $T=\{0,1,...,12,...\}$

Possible values that $Z_{i,t+1}$ can take, if $Z_{i,t}=j_i$, depending on the values X_i, will be:

$$Z_{i,t+1}=j_i-\left(k-1\right)=j_i+1-k;\ \ 0\le k\le j_i+1 \tag{4.3}$$

which means that for the loan of *i*-s it is paid not only the current instalment but also $k-1$ of the other instalments in arrears, i.e. $X_i = k$

For the specific case when $Z_{i,t} = j_i = 13$, any value X_i will not change the situation "written-off" of the loan and therefore $Z_{i,t+1} = 13.$ In the terminology of random processes, this situation is called the absorbing state and by the probability it means that:

$$P\left(Z_{i,t+1} = 13 / Z_{i,t-1} = 13\right) = 1 \tag{4.4}$$

Every other case is an impossible event (!).

The above communication can be shown in a graph, as in Fig. 2. It is obvious that this reconsideration of portfolio produces a communication of states clearer and more coherent with regard to the classification of the portfolio according to the Central Banks.

With reference to equations (4.2), (4.3) and (4.4), we may propose as one-step transition probabilities for each pair of states from space $E = \{0,1,...,13\}$ and $\forall \mathrm{i} = 1,2,...,n$, the following sizes:

$$p(j,l) = \begin{cases} C_{j+1}^{(j+1)-l}\left(1-\pi\right)^{(j+1)-l}\pi^{l} & \text{per } 0 \le l \le j+1 \text{ dhe } 0 \le j \le 12 \\ 0 & \text{per } j+2 \le l \le 12 \text{ dhe } 0 \le j \le 12 \\ 0 & \text{per } l < j \text{ dhe } j = 12+1 \\ 1 & \text{per } l = j \text{ dhe } j = 12+1 \end{cases} \tag{4.5}$$

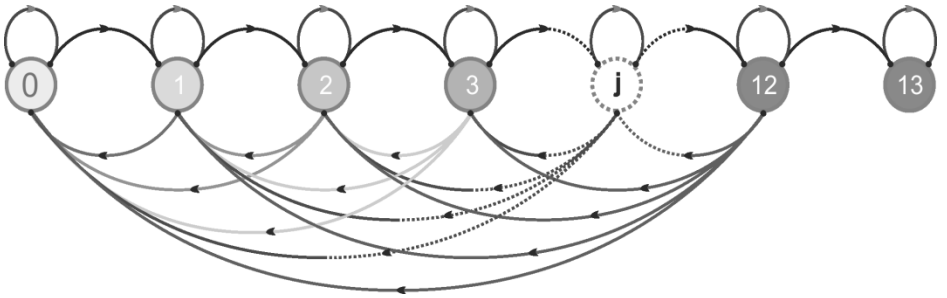

Fig. 2 Graph of the random process.

If the elements of the equation (4.5) are expressed in the form of a matrix, the one-step transition matrix M is obtained, as shown in Tab.2 that follows:

Table 2. Matrix of the one-step transition of the loan Y_i

$$M=\begin{pmatrix} 1-\pi & \pi & 0 & \cdots & 0 & \cdots & 0 & 0 \\ (1-\pi)^2 & 2\pi(1-\pi) & \pi^2 & \cdots & 0 & \cdots & 0 & 0 \\ (1-\pi)^3 & C_3^2\pi(1-\pi)^2 & 3\pi^2(1-\pi) & \cdots & 0 & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \cdots & \vdots & \cdots & \vdots & 0 \\ (1-\pi)^{j+1} & C_{j+1}^{j}\pi(1-\pi)^{j+1-3} & C_{j+1}^{3}\pi^2(1-\pi)^{j-3} & \cdots & C_{j+1}^{l}\pi^l(1-\pi)^{j+1-l} & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \cdots & \vdots & \cdots & \vdots & 0 \\ (1-\pi)^{13} & C_{13}^{12}\pi(1-\pi)^{12} & C_{13}^{11}\pi^2(1-\pi)^{11} & \cdots & C_{13}^{l}\pi^l(1-\pi)^{13-l} & \cdots & 13\pi^{12}(1-\pi) & \pi^{13} \\ 0 & 0 & 0 & \cdots & 0 & \cdots & 0 & 1 \end{pmatrix}$$

The lending FIs collect data on the history of credit risk, therefore they normally collect data about the classification of their portfolio in different time periods. Data records can be short-term and long-term. It is clear that the ratio of the number of credits to a particular group with the total number of loans portfolio gives the relative density of this group.

Lin and Yang assumed as known the initial distribution of the process, allowing the FI-s to select an appropriate initial distribution for implementation of the model offered by them. We propose as a family of initial distribution of the process its point estimation based on the statistical history of the classification of the loan portfolio in the number of borrowers. In this way we combine historical data with financial market expectations according to probability of return offered by CR +. In concept of point estimation of probability, the relative density is a good estimation of the probability.

Thus, the numerical values $d_n(j)=\frac{n_j}{n}$ of relative frequency gives the relative density of class of loans that are *j* instalments in arrears, where n_j is the number of loans that are *j* instalments in arrears at the end of the previous year, while *n* is the total number of loans portfolio of the same year. Then, the family $\left[d_n(j), j\in E\right]$ is a good point estimation of the family $\left[f_0(j), j\in E\right]$ that shows the initial distribution of the process.

Table 3 Classification at the end of 2008

Number of overdue instalments	No Credit	Relative frequency
0	1524	0.762
1	243	0.1215
2	39	0.0195
3	26	0.013
4	14	0.007
5	20	0.01
6	23	0.0115
7	15	0.0075
8	7	0.0035
9	11	0.0055
10	10	0.005
11	13	0.0065
12	9	0.0045
w-off	46	0.023
total	2000	1

Table 4 Forecast for the end of 2009

Number of overdue instalments	No Credit	Relative frequency
0	1959	0.800572
1	181	0.073968
2	37	0.015121
3	29	0.011851
4	27	0.011034
5	20	0.008173
6	14	0.005721
7	11	0.004495
8	21	0.008582
9	11	0.004495
10	7	0.002861
11	10	0.004087
12	8	0.003269
w-off	112	0.04577
total	2447	1

Probabilities of type (4.5), the one-step transition matrix M and the above initial distribution of the process make implementation of Markov's univariate and multivariate models (Mano, 2010) possible:

$$p_t(l) = P\left(Z_{i,t} = l\right) = \sum_{j=0}^{13} f_0(j)\, p^{(t)}(j,l) \qquad (4.6)$$

$$p_t(l_1,...,l_n) \cong \sum_{j=0}^{13} d_n(j)\, p^{(t)}(j,0) \times \prod_{r=0}^{13}\left[\sum_{j=0}^{13} d_n(j)\, p^{(t)}(j,l_r)\right]^{n_r} \qquad (4.6')$$

Implementation through a program in language C # of this model (Mano *et al*., 2011) does not require special specifications of information and do not endanger the principle of banking confidentiality, but the information that banks are obliged to declare is sufficient.

Tables 3 and 4 report upon the classification of the number of credit portfolio for 2008 and 2009, as declared by one of the commercial banks operating in the Albanian market.

Implementation of data of tables 3 and 4 models (4.6) and (4.6 ') and the execution of this model by the code in C# helps obtain the results of models which are indicated by the output of Fig. 3. In section A of this output, it is given the multivariate probability according to equality (4.6'), while in Section B it represents the univariate probabilities according to equality (4.6).

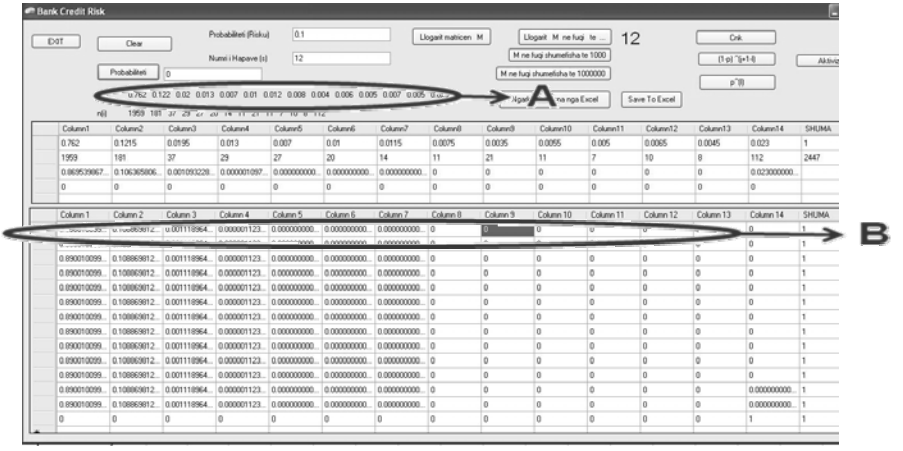

Fig. 3 Table of results for data tables 3 and 4

4. CONCLUSIONS

Reconsideration of the classification of the loan portfolio by the number of loans according to the Central Banks and the use of the relative density of this classification as a point estimate for the initial distribution of the process enables an improvement in result and application of the model offered by Lin and Yang. Thus, perceiving probabilities of type (2.1) under the restriction that represents the periodic classification (monthly) of the portfolio loan in number of loans, we offered probabilities of the form (4.5) which allowed us to treat the periodic classification process of the portfolio through a random process of homogenous Markov chain type with the one-step transition matrix M. Using unconditional probabilities of CR + in the binomial probabilities of the model realizes the effect of the impact of financial factors of the result of Markov's univariate and multivariate models. Both of the results above and the possibility of algorithmic implementation of the model through programming in C #, create the possibility of their application on simple and easily accessible databases.

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ASSESMENT OF WATER QUALITY OF THE MAIN PORTS IN VLORA CITY, ALBANIA

Sonila KANE
University Ismail Qemali, Faculty of Technical Sciences, Department of Chemistry, Vlora, Albania
Pranvera LAZO
University of Tirana, Faculty of Natural Sciences, Department of Chemistry, Tirana, Albania
Flora QARRI
University Ismail Qemali, Faculty of Technical Sciences, Department of Chemistry, Vlora, Albania

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ABSTRACT

Anthropogenic impact is of great concern for the water quality in port areas. The present paper investigates on the seawater quality in Commercial Port and Fishing Port (Triport), Vlora, Albania as here the waters are subject to a variety of potential pollutant sources including boat and ship antifoulants, boat hull cleaning, and other releases and discharges from boats, wastewater discharges from municipalities and industrial facilities etc. Samples were collected from seven sampling sites and physico-chemical parameters of water such as temperature, pH, dissolved oxygen (DO), biological oxygen demand (BOD), particulate material (TSS and TDS), nutrients (N/NO_2^-, N/NO_3^-, P/PO_4^{3-}) were determined. One water sample was collected from one site, in the Vlora Bay (Orikum), 500 m from the shore. Once collected, the sample was analyzed and used as a comparative sample. Sample stations were chosen carefully in order to assess the level of the water contamination and evaluate the possible polluting sources of these two portal areas. Nutrients content was found at higher levels in both ports, but still within levels permitted under Chinese State Standard of Seawater Quality GB3097-1997 and EC Directive CEE 78/659.

***Keywords*:** ports, Vlora, seawater quality, physico-chemical parameters, nutrients, pollutant sources

1. INTRODUCTION

Good environmental quality is essential for sustaining coastal and marine ecosystems, commercial and recreational fisheries, and economic growth in

coastal regions (Vandermeulen and Cobb, 2004). Marine ports are significant hubs of economic activity and major sources of pollution (Bird, 1971; Chubarenko, 2008). Their waters are subject to a variety of potential pollutant sources including boat and ship antifoulants, boat hull cleaning, and other releases and discharges from boats, wastewater discharges from municipalities and industrial facilities, storm water runoff from municipal, industrial (including shipyards) and agricultural activities, groundwater and the atmosphere. These sources of potential pollutants can degrade the water quality, beneficial uses of the waters through causing aquatic life toxicity and excessive food web bioaccumulation, as well as causing other impairments of the waterbody's beneficial uses (Lee and Jones-Lee, 2003). Multiple contaminants from ports disperse in the surrounding water basins and may seriously affect the condition of river and sea water ecosystems (Galkus and Joksas, 1997; Joksas *et al*., 2003; Chubarenko, 2008). Every port is distinguished for specific natural and anthropogenic factors responsible for water contamination levels, spectrum of pollutants, and dynamic patterns (Galkus and Joksas, 1997; Joksas *et al*., 2003; Chubarenko, 2008). The Vlora Bay is one of the most attractive coastal zones of Albania, and it has been defined as a top-priority tourism area (Rivaro *et al*., 2011). It presents quite suitable conditions to accommodate vessels of various types. Two of the most important ports of Vlora city, used for this purpose, are the Commercial Port and Fishing Port (Triport). Human activities in port areas cause significant water pollution. Water exchange in bays is often limited and shipping activities introduce contaminants, which include oil, trace metals, nutrients and organochlorine compounds (UNEP, 1990). In addition, during the period of port operation, routine pollution from ships – oil, litter, gaseous air pollutants, dust, hazardous materials etc. are often significant (Stoyanov *et al*., http://www.pse.ice.bas.bg). The natural composition of the waters could be affected by human pollution through water discharges from the surrounding areas (Tursi *et al*., 2011). One of the most common causes of water pollution in harbours is the dumping of untreated sewage. Close to the anchor gate of commercial port, the untreated urban wastes of Skela area are discharged through a collector directly into the sea. Beside the other things these wastes can cause an increase in the content of inorganic compounds of nitrogen and phosphorus. When a water body is overloaded with nitrogen and phosphorus, a process of euthrophication can rapidly increase and become a serious pollution problem (Chubarenko, 2008). Finally, the natural composition of the waters in ports could be affected by the wastes discharged from transport ships and ferry anchored in the commercial port of Vlora or fishing vessels in Fishing Port (Triport).

The present paper aims at assessing the quality of seawater in the Commercial Port and Fishing Port, Vlora, Albania. A single expedition to

collect water samples in these ports was carried out and seawater quality assessment was based on the physico-chemical parameters of water such as temperature, pH, dissolved oxygen (DO), biological oxygen demand (BOD), particulate material (TSS and TDS), nutrients (N/NO_2^-, N/NO_3^-, P/PO_4^{3-}).

2. MATERIALS AND METHODS

Studied area

Vlora Bay is located in southern Albania, at the intersection of the Adriatic and Ionian seas. It is a semi-closed bay and has restricted water exchange with the Adriatic Sea throughout the mezocanal inlet. The bay is one of the most attractive coastal zones of Albania, and has been categorized as a top-priority tourism area. Unfortunately, the area has suffered from significant population growth and increase in human activities in recent years (Rivaro *et al*., 2011). Vlora Bay presents quite suitable conditions to accommodate vessels of various types.

Fig. 1. Map of sampling stations in: a) Fishing port; b) Commercial port.

Two of the most important ports of Vlora city, used for this purpose, are the Commercial Port and Fishing Port (Triport). The Commercial Port of Vlora, situated in the South-West of Albania, on the Adriatic Sea and is the second port in importance (after port of Durrës), for the severity of capacity, and the largest port of South Albania serving whole surrounding region for the transport of goods and passengers. For the features it is a straightforward port consisting of two major moths, holding vessels such as cargo ship and ferries. There are no strong currents and fluctuations that may affect navigation tools, so it can ensure a safe mooring beside him, outside the system of quays and a place that protects ships thanks to its geographical position. Fishing Port

known as Triport is situated in the north-west of the Commercial port of Vlora. This port is mainly used for the anchor of fishing vessels.

Sampling Stations

Sample sites are shown in Figure 1, with reference number given for each location and reported in Table 1, including Commercial Port of Vlora and Fishing Port (Triport). Five sampling sites are located in the Commercial port of Vlora (two sample sites in the anchor quay of the commercial vessels, two sample sites in ferry anchor quay and one sample site between the two quays) and two sampling sites in the Fishing Port (Triport).

One water sample was collected from the site in Vlora Bay (Orikum), 500 m from the shore. Once collected, the sample was analyzed and used as a comparative sample.

Table 1: Sampling points of water samples

Sample number	Sample type	Latitude	Longitude	Collection place
St 1	Water sample	40°27'0.19"N	19°29'7.41"E	Commercial port
St 2	Water sample	40°27'2.49"N	19°29'6.97"E	Commercial port
St 3	Water sample	40°27'3.41"N	19°29'1.19"E	Commercial port
St 4	Water sample	40°27'4.25"N	19°28'53.15"E	Commercial port
St 5	Water sample	40°26'57.45"N	19°28'49.41"E	Commercial port
St 6	Water sample	40°28'57.61"N	19°25'37.29"E	Fishing port
St 7	Water sample	40°29'4.77"N	19°25'49.08"E	Fishing port
St Orikum	Water sample	N 40 28 269	E 19 27 833	Vlora Bay (Orikum)

Samples were collected in June 2014, 50 cm deep and 50-100 m from the shore. Sample stations were chosen carefully in order to assess the level of the water contamination and to evaluate the possible polluting sources of these two portal areas. Sample collection, transport and conservation was in line with recommendations made by APHA (1998).

Water samples treatment

Water samples were collected in 1.5 litre PET bottles and transported the same day to the laboratory using refrigerated containers stored below 4^{o}C. Once transported to the laboratory, physico-chemical parameters of water were determined. Surface water temperature, pH and redox potential were measured using a pH meter (Model pHS-3BW). Electrical conductivity (EC) and total dissolved solids (TDS) were measured using a conduct meter (Model DDSJ 308A). Total suspended mater (TSS) was determined by pouring one litre volume of water through a pre-weighed filter of 0.42 μm pore size, and

weighing the filter again after drying it at 105°C for 2 hours to remove all the water. The concentration of dissolved oxygen (DO) and biological oxygen demand (BOD) was determined applying the Winkler method. Nutrients were measured using UV 2401 PC, spectrophotometer for nitrogen compounds and PYEUNICAM SP-9 spectrophotometer for phosphate determination and in line with the APHA (1998) standard procedures.

3. RESULTS AND DISSCUSIONS

Diagrams of figure 2 and 3 plot the physico-chemical parameters of the waters in the Commercial and Fishing ports of Vlora.

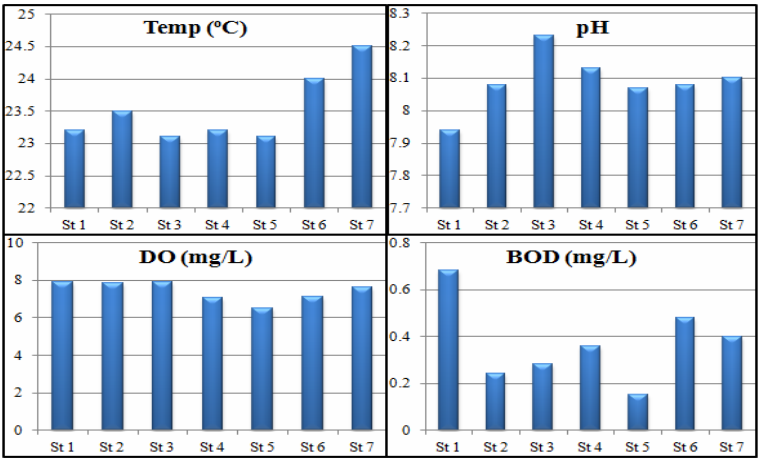

Fig. 2: a) temperature, b) pH, c) dissolved oxygen d) biological oxygen demand level in water of ports

Temperature

The water temperature is the best index of seasonal fluctuations (Stakeniene *et al*., 2011). It depends on weather conditions, removal of shading stream bank vegetation and storm water. Temperature of surface water of the two ports varying from 23.1 to 24.5°C is expectable and normal for the month of June, the sampling period.

pH

The pH of aquatic ecosystems depends on the chemical and biological activity of water. Changes in pH can be indicative of an industrial pollutant, photosynthesis or the respiration of algae that is feeding on a contaminant. Most ecosystems are sensitive to changes in pH and the its monitoring has been incorporated into the environmental laws of most industrialized countries (Bellingham, http://www.stevenswater.com). Figure 2/b depicts the

pH values varying from 7.94 (station 1) to 8.23 (station 3) (the commercial port of Vlora), respectively.

Dissolved oxygen

Dissolved oxygen (DO) is an important environmental parameter needed for a good quality of surface water. In general, it is available as dissolved state at water bodies (PHILMINAQ, aquaculture.asia). The fluctuations in the dissolved oxygen levels in water can be caused by aquatic vegetation and anthropogenic status of the water. It is well known that the temperature and salinity affect the dissolution of oxygen in water (Saravanakumar *et al*., 2008). DO in waters originates from the air dissolution and the process of photosynthesis that occurs during daylight. At high temperature, which is usually observed in dry season, the solubility of oxygen decreases while at lower temperature (wet season) it increases (Plimmer, 1978; McNeely *et al*., 1979). Dissolved oxygen in ports water varied (see Fig. 2/c) from 6.48 mg/L (station 5) to 7.92 mg/L (station 1).

Biological oxygen demand

The determination of the biological oxygen demand (BOD) revealed that the highest concentration (0.68 mg/1) was found in staion 1 and the lowest was (0.15 mg/l) in station 5 (Figure 2/d) with an average value 0.37 mg/l. BOD levels serves as an indicator of organic pollution of water (Nemerow, 1974; Tchobanoglous and Schroeder, 1985)—higher the BOD, higher the amount of pollution in the test sample. A marine environment that presents high levels of BOD is not appropriate for the life of species that require oxygen.

Red/ox Potential

Redox potential is the activity or strength of red/ox processes in solution and its values varied from -82.4 mV to -65.4 mV. Negative values of the redox potential in all stations indicates the reducing properties of seawater (Bellingham, stevenswater.com)

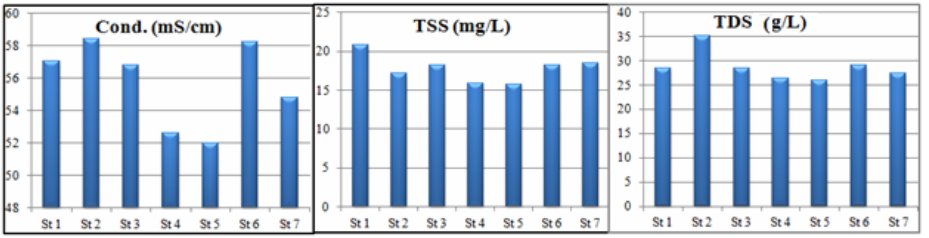

Fig.3: a) conductivity, b) TSS, c) TDS level in water of ports

Conductivity, TDS and TSS

Conductivity, TDS and TSS were found at higher levels in the anchor quay of the commercial vessels in Commercial port area. Conductivity varied from 52.013 mS/cm (station 5) to 58.413mS/cm (station 6). The fluctuation in electric conductivity is dependent on fluctuations of TDS (Boid, 1981). Total solids (TS) refers to any matter either suspended or dissolved in water. High concentrations of TSS have several negative effects, such as decreasing the amount of light that can penetrate the water, thereby slowing photosynthetic processes which in turn can lower the production of dissolved oxygen; high absorption of heat from sunlight, thus increasing the temperature which can result to lower oxygen level. (PHILMINAQ, http://www.aquaculture.asia) TSS content in ports water varied from 15.7 mg/L (station 5) to 20.8mg/L (station 1). TDS concentration was found at lower level in station 5 (26.00 g/L) and higher level at station 1 (35.04g/L).

Descriptive statistics was applied to the results obtained for the physico-chemical parameters. Minimal, maximal and mean values are in Table 2 reported and in Figure 4 depicted.

Table 2. Physico-chemical parameters of Commercial Port and Fishing Port of Vlora City

	pH	E (mV)	T (°C)	TSS (mg/l)	TDS (g/L)	DO (mg/L)	BOD (mg/L)	Cond. (mS/cm)
Mean	8.09	-71.929	23.514	17.757	28.684	7.414	0.37	55.698
Stand.Error	0.032	2.119	0.204	0.658	1.147	0.207	0.066	0.982
Median	8.08	-72.3	23.2	18.2	28.406	7.6	0.36	56.813
Standard deviation	0.086	5.606	0.540	1.740	3.034	0.549	0.174	2.600
Sample variance	0.007	31.432	0.291	3.029	9.206	0.301	0.030	6.758
Kurtosis	2.211	1.684	0.524	0.496	4.103	-0.542	0.685	-1.573
Skewness	-0.211	-0.893	1.287	0.585	1.863	-0.799	0.772	-0.539
Minimum	7.94	-82.4	23.1	15.7	26.006	6.48	0.15	52.013
Maximum	8.23	-65.4	24.5	20.8	35.048	7.92	0.68	58.413
Sum	56.63	-503.5	164.6	124.3	200.79	51.9	2.59	389.891
Confid.level (95.0%)	0.079	5.185	0.499	1.610	2.806	0.507	0.161	2.404

Chemical-physical parameters presented low values of variation and St.Dev/Mean ratios, i.e., a stable condition in different parts of Vlora portal area. The values of skewness (close to 0) and kurtosis (close to 3) indicate more or less a normal distribution of these parameters.

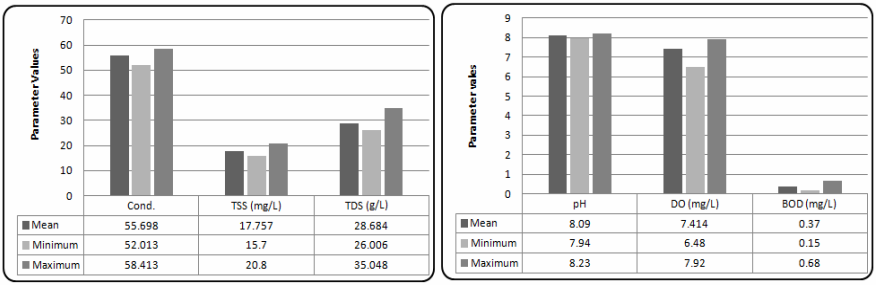

Fig. 4: Statistical data treatment of physical-chemical parameters.

TSS (recommended value is (≤25mg/L) values and pH values (6-9) were within limits permitted under the EC Directive CEE 78/659 (EC Fish Directive 44, 2006). Compared to the correspondent values for the resulted temperature (for the temperature 23-24^{o}C, the amount of DO should be about 8.5 mg/L), DO was found in slightly lower levels in all analyzed samples Sources can be discharges of the untreated wastes from the surrounded urban area and the ships anchored at these ports. DO content was within levels permitted under the EC Directive CEE 78/659 (EC Fish Directive 44, 2006) (≥ 8mg/L). According to the Chinese State Standard of Seawater Quality GB 3097-1997 (Wang *et al*., 2011) for DO and BOD content in water (respectively ≥ 6 mg/l and ≤2mg/L), water of both ports were clean.

The figure 5 depicts the nutrient content (nitrites, nitrates and phosphates) in Comercial and Fishing Port of Vlora.

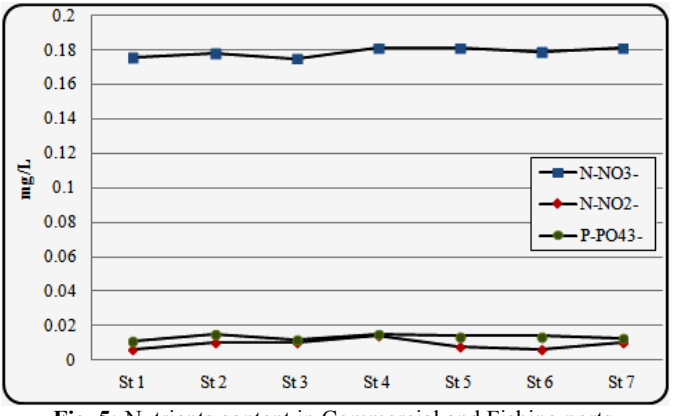

Fig. 5: Nutrients content in Commercial and Fishing ports.

In Table 3, the descriptive statistics for the results of nutrients in seawater samples are reported. The minimal, maximal and mean values in Figure 6 depicted.

Table 3. Physico-chemical parameters of Commercial Port and Fishing Port of Vlora City

	$N\text{-}NO_3^-$ (mg/L)	$N\text{-}NO_2^-$ (mg/L)	$P\text{-}PO_4^{3-}$ (mg/L)
Mean	0.179	0.009	0.013
Standard Error	0.001	0.001	0.001
Median	0.179	0.01	0.014
Standard Deviation	0.002	0.003	0.002
Sample Variance	0.000	0.000	0.000
Kurtosis	-1.481	0.377	-0.809
Skewness	-0.535	0.566	-0.620
Range	0.006	0.008	0.004
Minimum	0.175	0.006	0.011
Maximum	0.181	0.014	0.015
Sum	1.251	0.064	0.094
Confidence Level(95.0%)	0.002	0.003	0.001

Chemical-physical parameters present low values of St.Dev/Mean ratios, i.e., stable condition in different areas of the port. The values of skewness (close to 0) and kurtosis (close to 3) indicate more or less a normal distribution of these parameters.

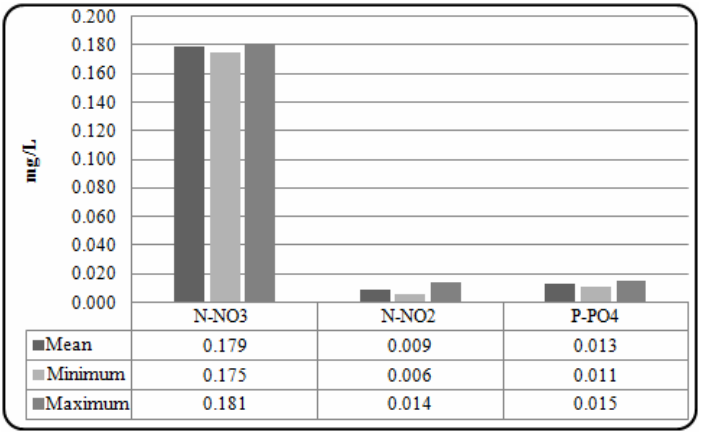

Fig.6: Statistical data treatment of nutrients.

Nitrates concentrations in all samples collected from the two ports varied from 0.175 to 0.181 mg/l. High content of nitrates in seawater is not

concerning as they are the main nutrient for the aquatic biota and its intensive development causes rapid reduction of nitrates.

Nitrites ($N\text{-}NO_2^-$) in sea water are found as intermediate compounds obtained from microbial reduction of nitrates or ammonium oxidation. Their level varied from 0.006 to 0.014 mg/l. Another possible source of nitrites might be phytoplankton and wastewater discharges. The ideal and normal measurement of nitrite is zero in any aquatic system. According to EC Fish Directive 44 (2006) recommended values for nitrites are <0.03 mg/l (*Cyprinid* waters). Nitrites content was within permitted level in both ports.

Almost all of the phosphorus (P) present in water is in the form of phosphate (PO4) and it is an essential plant nutrient as it is often in limited supply and stimulates plant (algae) growth and its role for increasing the aquatic productivity is well recognized. High levels of both phosphate and nitrate can lead to eutrophication, which increases algae growth and ultimately reduces dissolved oxygen levels in the water (Murdoch *et al*., 2001). Phosphates in both ports was found at low levels; from 0.011 to 0.015 mg/L. The mean value was 0.013 mg/L (Figure 6), lower than EC guide (≤ 0.4 mg/L for Cyprinid waters).

Kane *et al.,* (2013) have previously reported that content of nutrients in other areas of Vlora Bay (Orikum) was as following: nitrates <0.018 mg/L; nitrites <0.005 mg/L; phosphates <0.004 mg /L. In the present investigation the nutrients content was found at higher levels, in both ports. The sources can be urban wastes discharged from the surrounding area and untreated sewage discharged from the vessels. Higher content of nutrients generally were found in station 4 situated near the ferry anchor quay of Commercial Port.

4. CONCLUSIONS

Vlora Bay presents quite suitable conditions to accommodate vessels of various types. Two of the most important ports of Vlora used for this purpose are the Commercial Port and Fishing Port (Triport). Sample stations were chosen carefully in order to assess the level of the water contamination and evaluate the possible polluting sources of these two portal areas.

The physical-chemical parameters of water including dissolved oxygen (DO) and biological oxygen demand (BOD) were within levels permitted.

Compared to the content of nutrients in other areas of Vlora Bay (Orikum) (nitrates <0.018 mg/L; nitrites <0.005 mg/L; phosphates <0.004 mg /L), the content of nutrients in sea water samples taken in both ports resulted in higher levels. Sources can be discharges of the untreated wastes from the surrounded urban area and the ships anchored at these ports. Nutrients content was found at higher levels in station 4 situated near the ferry anchor quay of Commercial Port, but still within levels permitted under the EC Directive.

The seawater at both ports was oligotrophic as indicated by inorganic nutrients content. Given the importance of the bay and the ports, constant monitoring of the physical and chemical parameters in water of these main ports of Vlora is crucial and unavoidable, due to continous anthropogenic activities around the area and the inappropriate discharges of oil wastes or other solid and liquid wastes from the ships anchored in these ports.

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VARIABILITY IN ESSENTIAL OIL COMPOSITION OF *ROSMARINUS OFFICINALIS* L. FROM ALBANIA

Vilma PAPAJANI
Faculty of Pharmacy, University of Medicine, Tirana, Albania
Entela HALOÇI and Klodiola DYLGJERI
Faculty of Medicinal Sciences, University Aldent, Tirana, Albania

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ABSTRACT

Rosmarinus officinalis L. (Lamiaceae), commonly known as rosemary**,** is widely used as a culinary herb, flavoring substances of food products also in pharmaceutical and cosmetic industries. It is one of the important aromatic medicinal plants exported from Albania. The objectives of this study were: a) to analyze the chemical profile of essential oils from Albanian rosemary populations; b) to evident the phenological variations in essential oils of rosemary, harvested at two different vegetative stages. Aerial parts of rosemary plants were collected, at flowering, in different areas of southern and central Albania (5 locations) in order to determinate the chemical composition of essential oils. Also samples from two southern regions were harvested in pre-flowering and flowering phenological stages. The chemical composition of essential oils, obtained by hydrodistillation, was analyzed by gas chromatography and gas chromatography-mass spectrometry. In order to interpret the results obtained by GC, GC/MS analysis of essential oil, a statistical Cluster analysis, based on Euclidean distances, was applied. The essential oils content and composition changed according to the collection areas and phenological stages of plant samples. Essential oil yields ranged from 0.94 to 1.82 % of dried weight. The major compounds identified were 1,8 cineole (12.04-16.67%), camphor (9.91-16%), verbenone (6.8-12.59%), α-pinene (9.55-17.65%) etc. Cluster analysis resulted with three different clusters by grouping the analyzed essential oils in three groups based on similarity of their chemical profiles. The average quantity of oxygenated monoterpenes, as the main group of compounds of rosemary essential oils, ranged from 44.43% (pre-flowering) to 54.69% in full-flowering stage. The quantity of 1-8 cineole was lower in pre-flowering and increased in full flowering in contrast with the content of α-pinene that decreased in full-flowering. The results confirm the influence of environmental conditions and phenological stages on the chemical composition of rosemary essential oil.

***Keywords**: Rosmarinus officinalis*, essential oils, chemical composition, phenological stage, chemotypes, 1,8 cineol.

1.INTRODUCTION

Rosmarinus officinalis L. (Lamiaceae), commonly known as rosemary, is native to Mediterranean regions and is widely cultivated elsewhere in herb garden and as an aromatic ornamental (Trease and Evans, 2009). It is an evergreen shrub with fragrant needle-like leaves (Bousbia et al., 2008) widely used as a household spice and as a flavoring agent of food products also in pharmaceutical and cosmetic industry (Pintore et al., 2002). Rosemary leaves and essential oil have many traditional uses based on their antibacterial, carminative, spasmolytic, antioxidant action (Trease and Evans, 2009; Wang et al., 2008). The essential oil of *Rosmarinus officinalis* is very important for its medicinal uses due to its antibacterial, cytotoxic, antioxidant, antiphlogistic and chemopreventive properties (Celiktas and al., 2007; Gachkar et al., 2007; Wang et al., 2008; Kadri et al., 2011). Recent studies demonstrated the antibacterial and antifungal activity of rosemary oil (Delcampo et al., 2000; Pintore et al., 2002; Oluwatuyi et al., 2004; Rozman and Jersek, 2004; Ozcan et al., 2008; Moghtader and Afzali, 2009).

The biological activities of the essential oils are correlated to the chemical composition of the oils and to the presence of specific chemical compounds. The chemical profiles of *Rosmarinus officinalis* essential oils from different regions of the world have been reported in several studies. The major compounds described for the oil are α-pinene, 1,8-cineole, camphor, bornyl acetate (Bauer et al., 1997; Pino et al., 1998; Viuda et al., 2007) which are also the main components responsible for the antimicrobial activity of rosemary essential oil (Pintore et al., 2002; Delcampo et al., 2000). Significant variations in the chemical profile of essential oil have been reported and different chemotypes were evidenced according to the geographical area (Chalchat et al., 1993; Lawrence, 1995; Dellacasa et al., 1999; Valera et al., 2009). Also differences in content and chemical composition of essential oils of rosemary were reported with relation to the phenological stages. (Salido et al., 2003).

Rosmarinus officinalis L. (rosemary) is cultivated in Albania many years ago and is widespread in southern and central regions. It is one of the important aromatic plants exported from Albania (Asllani, 2004). In the present study we aimed to analyze the chemical profile of essential oils from Albanian rosemary populations as a contribution to the characterization of the geographical and biochemical variability and also to evident the phenological variations in essential oils of rosemary, harvested at two different vegetative stages.

2. MATERIALS AND METHODS

Aerial parts of rosemary plants were collected at full flowering period, in different areas of southern and central Albania respectively from Fieri, Vlora, Berati and Durrësi, Tirana; also samples from two southern regions (Fieri, Vlora) were harvested in pre-flowering and flowering stages. Voucher specimens of populations are deposited at the Herbarium of Faculty of Pharmacy, University of Medicine, Tirana, Albania. The air-dried plant materials were subjected to hydrodistillation using a Clevenger-type apparatus according to standard procedures (European Pharmacopoeia, 2004). The oil yield was recorded for each sample and calculated on dried weight.

The essential oils were analyzed using a Shimadzu GC-14B with a flame ionization detector (FID), fitted with a fused silica column HP-5MS (30m x 0.25mm, film thickness 0.25μm). The column temperature was programmed from 60°-240°C at a rate of 3°C/min; using helium as the carrier gas with a flow rate of 1.1 mL/min, sample injection 1 μL. GC/MS analyses of the oils were run on a Shimadzu GCMS-QP2010S gas chromatograph fitted with a fused silica HP-5 (5% phenyl methyl polysiloxane) capillary column (30m x 0.25mm, film thickness 0.25μm). The column temperature was programmed from 60°-240°C at 3°C/min using helium as the carrier gas with a flow rate of 1.1 mL/min. Other operating conditions were as follows: inlet pressure 9.43 psi, injector temperature 250°C, detector temperature 280°C, split ratio 1:25, injection volume 1 μL. Ionization of the sample components was performed in the EI mode (70 eV) with scan range 40-450 amu.

Qualitative and quantitative analysis: The linear retention indices, RI, for all compounds were determined by injection of the hexane solution containing the homologous series of C_8-C_{26} *n*-alkanes (Van den Dool and Kratz, 1963). The identification of the volatile constituents was accomplished by comparing their retention indices and mass spectra with literature data (Adams, 2007), by computer library search (Wiley library, NIST/EPA/NIH Mass Spectral Library 2.0 and Mass Finder 4 Computer Software) and in the laboratory own database. The percentage composition of the essential oils was computed from GC/FID peak areas without correction factors.

In order to interpret the results obtained by GC, GC/MS analysis of essential oils, a statistical Cluster analysis (based on Euclidean distances) was applied by using the software SPSS 12.0.

3. RESULTS AND DISCUSSION

The essential oils isolated by hydrodistillation were obtained in yields (Table 1) that vary between 0.94 to 1.82% (volume/dry weight of plant

samples). The oil contents during two phenological stages (Table 2), ranged from 0.65% (pre-flowering) to 1.2% (full flowering).

Table 1. Essential oil yields (%, v/w) of *Rosmarinus officinalis* samples collected in different areas

Areas of collection	Vlora	Berati	Fieri	Durrësi	Tirana
Yield of essential oil (%)	0.94	1	1.2	1.82	1.7

Table 2. Phenological variations of essential oil yield (% v/w)

	Vlora		Fier	
Vegetative stage	Pre-flowering	Full flowering	Pre-flowering	Full flowering
Yield of essential oil (%)	0.65	0.94	0.8	1.2

Chemical constituents of *Rosmarinus officinalis* L. essential oil samples, collected in different areas of Albania, are presented in Table 3. Twenty two components were identified representing until 89% of the oil. The major constituents identified were 1,8 cineole, α-pinene, camphor, verbenone, borneol, linalool. The data showed that oxygenated monoterpenes represented the most abundant fraction in the rosemary oil that ranged from 46.25% (Vlora) to 63.14% (Fieri). The monoterpene hydrocarbon fraction ranged from 15.1% (Vlora) to 30.94% (Durrësi). In contrast, the aromatic compounds and sesquiterpene fraction represented lower percentage in the oil, respectively: 1.48-4.52% and 1.83-6.49%. The major components of essential oils varied depending the harvest locations of plant samples as follows: 1,8 cineole from 12.04% (Vlora) to 16.67% (Tirana); camphor from 9.91% (Vlora) to 16% (Fieri); verbenone 6.8% (Durrësi) to 12.59% (FIeri), α-pinene 9.55% (Vlora) to 17.65% (Durrësi).

According to the statistical Cluster analysis, three clusters have resulted (Fig. 1) by grouping the analyzed essential oils of *R. officinalis* in three groups based on similarity of their chemical profiles. Samples from Berati and Fieri were grouped in the first cluster based on the similarity in camphor, 1,8 cineole, verbenone, borneol, content (Table 3); samples from Durrësi and Tirana were grouped in the third cluster based on the similarity in monoterpenes content represented by α-pinene also by other oxygenated compounds like 1,8 cineol, camphor, verbenone (Table 2). The sample from Vlora was grouped in a separated cluster because of its lower content of monoterpenes and oxygenated monoterpenes in comparison with other samples and also, by higher content of β-caryophyllene (5.56%). As indicated

above, essential oils obtained from rosemary samples showed significant variability in their chemical composition.

Based on the literature, oxygenated monoterpenes are the main compound group of *Rosmarinus officinalis* essential oils **(**Chalchat et al., 1993; Arnold et al., 1997; Pintore at al., 2002). Rosemary originated from Turkey resulted with p-cymene (44.02%), linalool (20.5%), γ-terpinene (16.62%), thymol (1.81%), β-pinene (3.61%) as main constituents (Ozcan et al., 2008). Depending on the geographical locations of where the plant grows, the essential oil of *Rosmarinus officinalis* from Spain resulted with α-pinene (24.7%), 1,8-cineole (21.8%), camphor (18.9%) and borneol (4.5%) (Chalchat el al., 1993), also with camphor (32.33%), 1,8-cineole (14.41%) and α-pinene (11.56%) as the main components (Tomei et al., 1995; Santoyo et al., 2005). Compared with other rosemary oils, Albanian essential oils were more similar to those of Italian origin due to their 1.8 cineole, camphor, α-pinene contents (Reverchon and Senatore, 1992; Arnold et al., 1997).

Table 3. Chemical composition of *Rosmarinus officinalis* L. essential oils from different areas

Compound (%)	RI	**Vlora**	**Berati**	**Fieri**	**Durrësi**	**Tirana**
α-pinene	896	9.55	10.37	9.64	17.65	17.19
Camphene	911	0.21	2.05	1.97	3.83	2.90
β-pinene	937	0.69	0.86	0.53	0.96	0.75
Myrcene	950	0.29	1.13	1.04	1.52	1.26
Phellandrene	970	0.99	1.32	1.07	0.75	0.74
α-terpinene	979	0.22	0.31	0.16	0.61	0.34
p-cymene	991	0.77	1.95	1.49	1.78	0.38
Limonene	993	0.37	3.48	3.29	4.29	2.94
1,8- cineol	998	12.04	13.43	12.87	16.01	16.67
γ-terpinene	1033	0.25	0.13	0.22	0.54	0.72
Terpinolene	1059	2.53	0.6	0.79	0.79	1.17
Linalool	1076	4.84	5.32	4.22	3.25	3.08
Camphor	1120	9.91	15.34	16.01	15.46	12.92
Borneol	1142	5.37	9.43	9.80	5.21	8.06

terpinen-4-ol	1153	0.71	2.26	3.91	1.16	2.38
α-terpineol	1166	1.83	2.43	2.46	2.05	1.94
Verbenone	1172	10.04	11.07	12.59	6.80	8.29
bornyl acetate	1263	1.51	0.76	1.28	2.34	3.15
Thymol	1274	2.03	0.95	1.20	0.62	0.51
Carvacrol	1300	1.72	1.46	1.66	0.78	0.59
β-caryophyllene	1402	5.56	2.67	1.98	1.83	1.99
α-humulene	1433	0.93	0.12	0.10	-	-

RI- Retention indices calculated on HP-5 column.

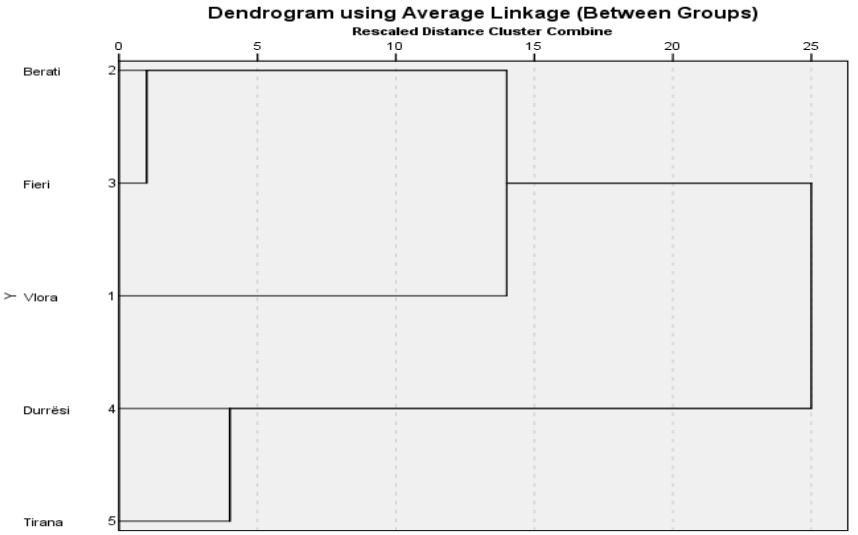

Fig. 1. Dendrogram obtained by cluster analysis, based on Euclidean distance, performed on the essential oil compounds of *Rosmarinus officinalis* populations

Table 4. Chemical composition of *Rosmarinus officinalis* essential oils during two phenological stages

Compounds (%)	RI	Vlora		Fieri	
		Pre-Flowering	Full Flowering	Pre-Flowering	Full Flowering
α-pinene	896	13.20	9.55	14.0	9.64
Camphene	911	2.30	0.21	2.40	1.97

β-pinene	937	1.05	0.69	1.14	0.53
Myrcene	950	1.39	0.29	1.39	1.04
Phellandrene	970	1.10	0.99	1.92	1.07
α-terpinene	979	0.26	0.22	0.54	0.16
p-cymene	991	0.65	0.77	1.12	1.49
Limonene	993	3.30	0.37	3.46	3.29
1,8- cineol	998	9.52	12.04	9.54	12.87
γ-terpinene	1033	2.08	0.25	2.22	0.22
Terpinolene	1059	1.76	2.53	1.53	0.79
Linalool	1076	3.22	4.84	3.12	4.22
Camphor	1120	12.60	9.91	14.11	16.01
Borneol	1142	3.42	5.37	5.22	9.80
terpinen-4-ol	1153	1.05	0.71	1.98	3.91
α-terpineol	1166	1.90	1.83	2.01	2.46
Verbenone	1172	9.02	10.04	10.13	12.59
bornyl acetate	1263	0.98	1.51	1.04	1.28
Thymol	1274	0.41	2.03	0.32	1.20
Carvacrol	1300	0.72	1.72	0.74	1.66
β-caryophyllene	1402	4.75	5.56	1.54	1.98
α-humulene	1433	0.88	0.93	0.32	0.10

RI-Retention indices calculated on HP-5 column.

Variations in the chemical composition of the essential oil of rosemary, harvested at two different phenological stages (pre-flowering and flowering), are presented in Table 4. The examination of the data demonstrated that the average quantity of oxygenated monoterpenes, as the main group of compounds of rosemary essential oils, ranged from 44.43% (pre-flowering) to 54.69% full-flowering stage in the contrast with the quantity of monoterpenes that decreased from 27.52% to 16.9% at full flowering. The quantity of 1-8 cineole was lower in pre-flowering (9.52%) and increased in full flowering (12.87%) in contrast with the content of α-pinene that decreased in full-flowering (from 14 to 9.55%). The significant differences were also observed in quantity of borneol. Flowering stage gave more oil content compared with pre-flowering stages (Table 2). It is evident that content and composition of rosemary oil are influenced by plant vegetative stages (Salido et al., 2003; Emadi et al., 2007).

4. CONCLUSIONS:

Rosmarinus officinalis L. from Albania resulted with high content of essential oil. The highest oil yields (1.2-1.8%) were recorded during the full flowering period. The oxygenated monoterpenes represented the most abundant fraction in the rosemary essential oils, with highest quantity at full-flowering stage. Close examination of the GC and GC/MS data reveals that

rosemary oils from central areas of Albania possessed higher quantity of monoterpenes and lower quantity of oxygenated monoterpenes in contrast with the oils from southern areas that possessed higher quantity of oxygenated components.

Based on chemical compounds, the populations of *Rosmarinus officinalis* were represented by camphor/1,8-cineole/verbenone/α-pinene/borneol, α-pinene/1,8-cineole/camphor and 1,8-cineole/camphor/verbenone/β-caryophyllene chemotypes. The observed variations in the chemical profile of rosemary essential oils confirm the influence of geographical, environmental conditions and phenological stages on the nature of plant chemical composition.

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CALCIUM AND MAGNESIUM CONCENTRATRION IN BLOOD SERUM OF FEMALE PEOPLE IN ALBANIA

Marjena BIXHI
Clinic of Gynecology, New Life, Durrës, Albania
Pranvera LAZO and Joana GJIPALAJ
Department of Chemistry, Faculty of Natural Sciences, University of Tirana, Albania

ABSTRACT

The present investigation aims to: i) determine magnesium and calcium content in blood serum of pregnant and non-pregnant women, ii) find a possible correlation in the measurement between these two electrolytes and, iii) show the effectiveness of therapy with substitute of these elements. 77 blood samples were collected from February to March 2013 in line with the World Health Organization (WHO) protocol. The serum samples were prepared using the high speed centrifuge (3600 rpm, in gel tubes) of blood samples diluted to 1:10 ratio with de-ionized water that contains 0.25% Triton X-100. The analysis of serum samples were carried out via Atomic Absorption Spectroscopy (AAS) with acetylene-air flame measuring absorbance of magnesium and calcium in serum samples. Result reported that the normal values were higher in pregnant women who were treated with substitute of calcium and magnesium, demonstrating the effectiveness of therapy with substitute of these elements.

Keywords: magnesium, calcium, female, pregnant female, blood sample, AAS with flame

1. INTRODUCTION

Electrolytes are important for the regulation of some essential processes in the body, among which the most important are: i) regulation of water balance and its distribution in the body;Regulation of osmotic equilibrium; ii) regulation of acid-alkaline balance through plasma bicarbonates management; iii) regulation of electrical potential of cell membranes and, iv) impact on neuromuscular activity (Brommer and Coburn 1981; Dubose Jr 2008).

The concentration of electrolytes plays an important role not only in maintaining osmotic pressure, but in moving water in different compartments of the body.

The role that electrolytes play in the human body makes them of fundamental importance for the biological and medical sciences (Zilva and Pannal 1979; Denney. 1987; Buzo, 1993; Kopelkaj and Buzo 2007).

Information about the concentration of calcium and magnesium in the serum for pregnant and non-pregnant women and the correlation between them is here reported.

1.1 Calcium

Calcium is the most important mineral substances for the organism. On average, an individual adult body contains about 1200 g calcium. About 1% of the amount of calcium in the blood is in the plasmatic water space. The rest, about 99% is deposited in bone tissue and located in a dynamic balance with the amount of calcium present in the plasmatic water space (Barnett *et al*., 1973). Chemically, calcium in the blood plasma could be in three different forms:i) connected to plasma proteins, mainly with albumins. In this form is on average 40-50% of the total amount of calcium present in blood plasma; ii) free ionic form, Ca+2. It is this amount that represents the chemically active calcium, able to exert physiological actions and participate in biochemical processes. The concentration of free calcium ions in blood plasma, in medical practice is called ionized calcium, and, iii) chemical complexes with citrate, phosphates. In blood plasma, calcium which is bound to citrate and phosphates represents 5-10% of the total amount of calcium contained in the blood plasma (Gindler and King, 1972; Bagrnski *et al*., 1973, Brommer and Coburn 1981; Robertson and Marshal 1981).

The daily average calcium intake for adults is 700 mg.During pregnancy and the period of lactation, the daily calcium intake goes up to 1000-1500 mg.

It has been reported that the normal values for calcium are 9 - 11 mg/dl or 2.25 - 2.75 mmol/l for children and 9 -10.7mg/dl or 2.25 - 2.67 for adults (WHO, 2013).

Poor maternal and newborn health and nutrition remain significant contributors to the burden of disease and mortality. Calcium supplementation has the potential to reduce adverse gestational outcomes, in particular by decreasing the risk of developing hypertensive disorders during pregnancy, which are associated with a significant number of maternal deaths and considerable risk of preterm birth, the leading cause of early neonatal and infant mortality (WHO, 2013).

Table 1 The suggested scheme for calcium supplementation in pregnant women (Resolution WHA65.11. 2012)]

Dosage	1.5–2.0 g elemental calcium/day (1 g of elemental calcium equals 2.5 g of calcium carbonate or 4 g of calcium citrate)
Frequency	Daily, with the total daily dosage divided into three doses (preferably taken at mealtimes)
Duration	From 20 weeks' gestation until the end of pregnancy
Target group	All pregnant women, particularly those at higher risk of gestational hypertension
Settings	Areas with low calcium intake

1.2 Magnesium

Magnesium is an important mineral element of the human body. This element is part of the most important group of cations that are in the human body, preceded by: sodium, potassium, calcium.

The plasmatic concentration of magnesium is 1.7- 2.1 mg / dL (0.6- 15.1 mmol / l). 55% magnesium could be found in the skeleton with the remainder located in the space within the cell. Magnesium and calcium are the two most important cation of the body. Only 1% of magnesium in the body is actually found in blood. In blood serum, approximately 55% of the magnesium is in the form of free Mg 2+ ions, 30% is associated with proteins (mainly with albumins) and 15% forms complexed with phosphates (PO43-), citrate and other anions.

Reduction of magnesium in the blood is caused by renal losses, gastrointestinal disorders and certain therapeutic treatments. The absence of magnesium in the body causes neuromuscular hyper-excitation, disorders cardiac rhythm, etc.

Increasing magnesium content beyond the allowed values is caused by renal insufficiency, by hemolytic anemia, and certain therapy which contain magnesium. One of clinical signs of increased values of magnesium is the neuromuscular depression (Elin, 1988; Fawcett *et al*., 1999).

As potassium, magnesium is more concentrated in the area within cells.30% of magnesium get through diet absorbed in the intestinal level, as well as calcium. 70% of plasmatic magnesium, is filtered at the glomerular space, re-absorbed in proximal tube and then actively secreted in the distal level of tubulin.

A large number of studies have shown that the absence of Mg is related to Ca disorders, phosphates and potassium during cardiac disorders, especially in the ventricular arrhythmias which is resistant to the classic treatment (Farrell, 1984; Fawcett *et al*., 1999).

2. MATERIALS AND METHODS

2.1. Blood Sampling

Blood samples were collected from February to March 2013 in line with the World Health Organization protocol: i) extending the patient's arm and inspect the antecubital fossa or forearm, ii) locating a vein of a good size that is visible, straight and clear, iii) applying the tourniquet about 4–5 finger widths above the venepuncture site and re-examine the vein, iv) disinfecting the site using 70% isopropyl alcohol for 30 seconds and allow to dry completely (30 seconds), v) anchoring the vein by holding the patient's arm and placing a thumb below the venepuncture site, vi) entering the vein swiftly at a 30 degree angle, vii) once blood is sufficiently collected, tourniquet could be released before withdrawing the needle and, vii) withdrawing the needle gently and then give the patient a clean gauze or dry cotton-wool ball to apply to the site with gentle pressure (WHO, 2010).

2.2 Blood Serum Preparation

Blood serum is the present investigation used to the concentration of Ca and Mg in women. Procedures followed to prepare blood serum are: i) 5 ml of blood obtained by venous puncture were added to the tube with gel, ii) blood was allowed to clot by leaving it undisturbed at room temperature for 5 min. The tube was put in thermostat with temperature 37© to accelerate the degradation of fibrinogen for 4 min and, iii) Once the tube was put in the thermostat and the degradation of fibrinogen was accelerated, the clot was removed via centrifugation at 3600 rpmin a refrigerated centrifuge.

At the end of this process were obtained the blood serum in light yellow color (WHO, 2010). The serum samples were diluted to 1:10 ratio with de-ionized water comprising 0.25% Triton X-100.

2.3 Methods

The analysis of serum samples (absorbance of Mg and Ca in serum samples) was carried using AAS involving acetylene-air flame. First, measurement conditions were optimized. To realize the procedure of the control analysis, in the absence of certified samples CRM, we have used the calibration method, measuring the blank sample during measurement, the discontinuation of the apparatus after each measurement, the control of one of the standard solution in every 10 measurements, and analysis of several parallel samples (5% of the total number of samples).

Fig.1: AAS with flame equipment.

2.3.1 The preparation of standards solution

A series of standard solutions for each element were prepared in 50ml tubes and standard solutions with known concentrations of 1000 mg / L were used. The concentration of standard solutions for each of them was determined based on sensitivities of the elements.

2.3.2 Optimization

First, the lamp energy through relevant parameters (wavelength, position of the lamp, the lamp currents, cracks in monochromator) was optimized. Once the lamp energy was optimized, atomization of parameters (type of flame, the acetylene / air ratio, measurement time as well as the number of parallel measurements) was carried out.

2.3.3 Quality control analysis

Calibration method was applied for the quality control analysis because CRM certified materials lacked. The method involved measuring the blank test during the measurement of the samples, the discontinuation of the apparatus after each measurement, control one of the standard solutions in every 10 measurements and, analysis of several parallel samples (5% of the total number of samples).

2.3.4 The calibration method

Figure 2 and 3 depict the calibration curves and some of the parameters for each of the two elements under investigation.

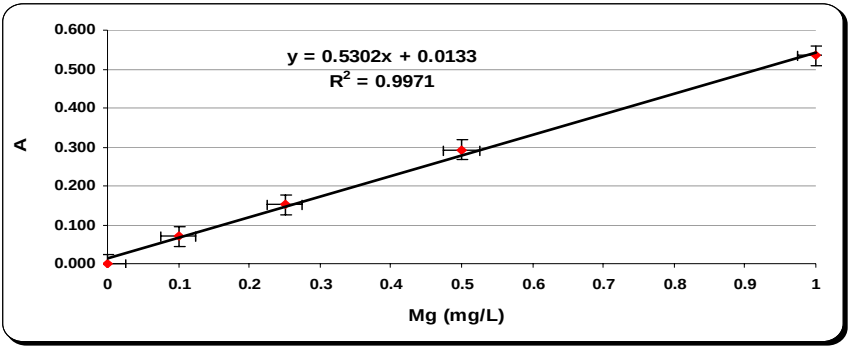

Fig. 2: The calibration curve of Magnesium

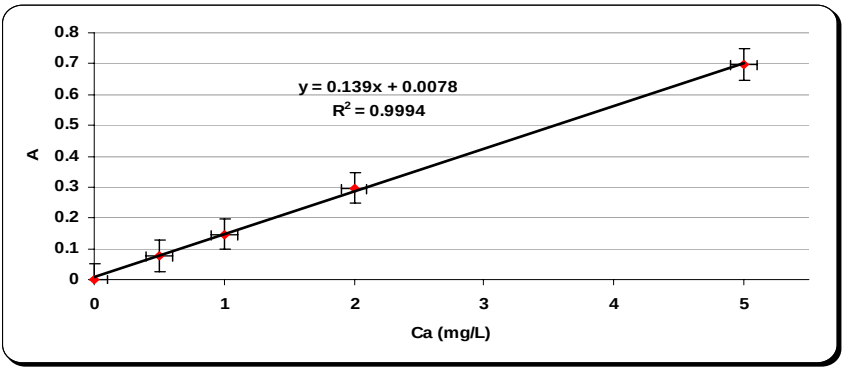

Fig. 3: The calibration curve of Calcium.

Table 2 The parameters of the performance of the analysis

Elements	Σ	AL*	CL	Cp	RSD% mes	Rec% mes
Mg	0.000577	0.000732	0.01	0.02	2.01	103.5
Ca	0.000577	0.002232	0.03	0.1	3.8	100.8

*Note: When we use Atomic Absorption Spectroscopy for the measurement, the minimum absorption signal value is taken A≥0.0044 and in this case, for the values of AL less than 0.0044, the theoretical value:

AL = 0.0044 is used for the further calculation.

3. RESULTS AND DISCUSSIONS

Figure 4 depicts calcium and magnesium concentration in the blood samples and the levels permitted. Figure 5 depictsCa/Mg ratio in blood samples of the group of women here investigated.

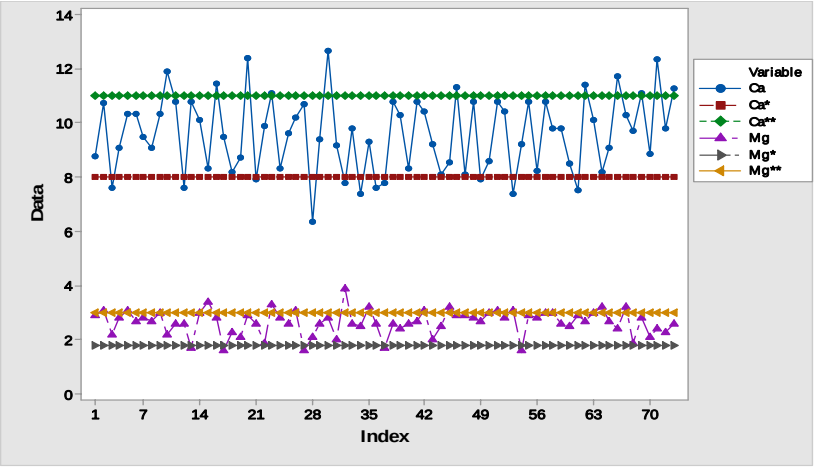

Fig.4: Ca and Mg concentration (mg/dL, given at logarithmic scale) in the blood samples: * - minimal permitted level; ** - maximal permitted level

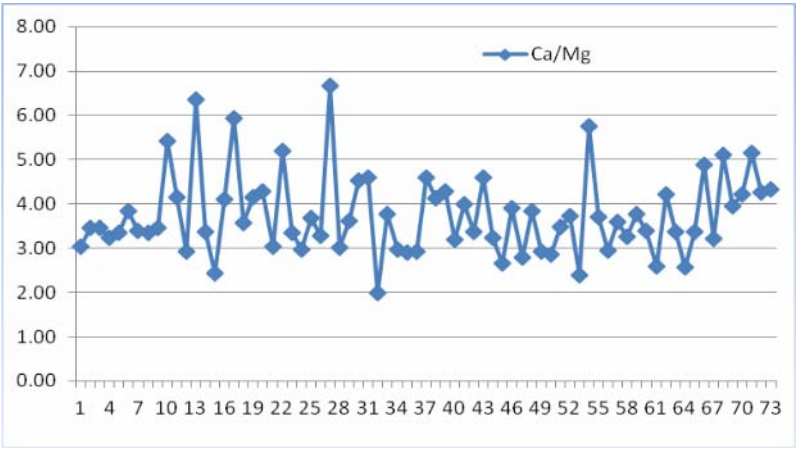

Fig. 5: Ca/Mg ratio in blood samples of female people under investigation (N=75,the min value = 2.0, max value = 6.7, mean concentration = 3.0).

Most of the samples had the normal range of concentration in blood serum for both elements. The concentrations of the two elements of pregnant women are comparable with those of non-pregnant women. The concentration of these elements was found at a lower level than the level permitted only in a small group of blood samples.

The data for each element have been subject to the descriptive statistics and the results are depicted in the Figure 6 and 7.

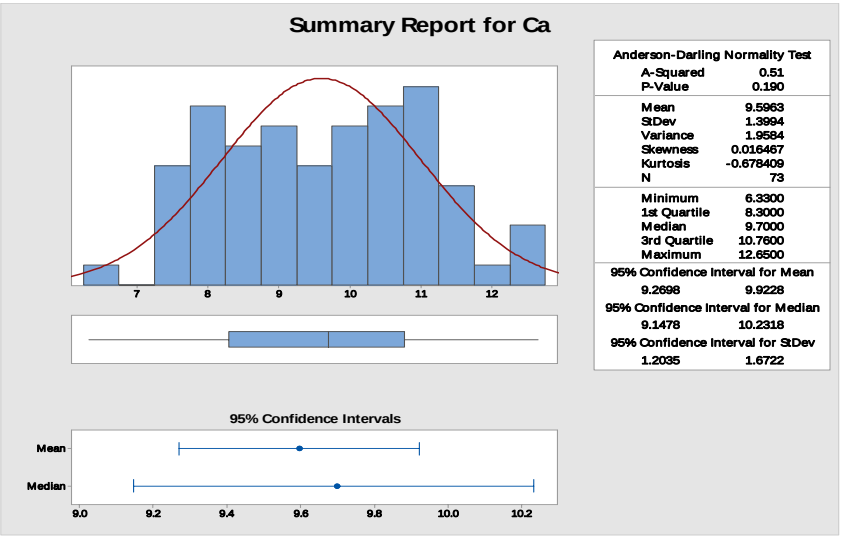

Fig. 6: Descriptive Statistics of measurements obtained by this study for Ca

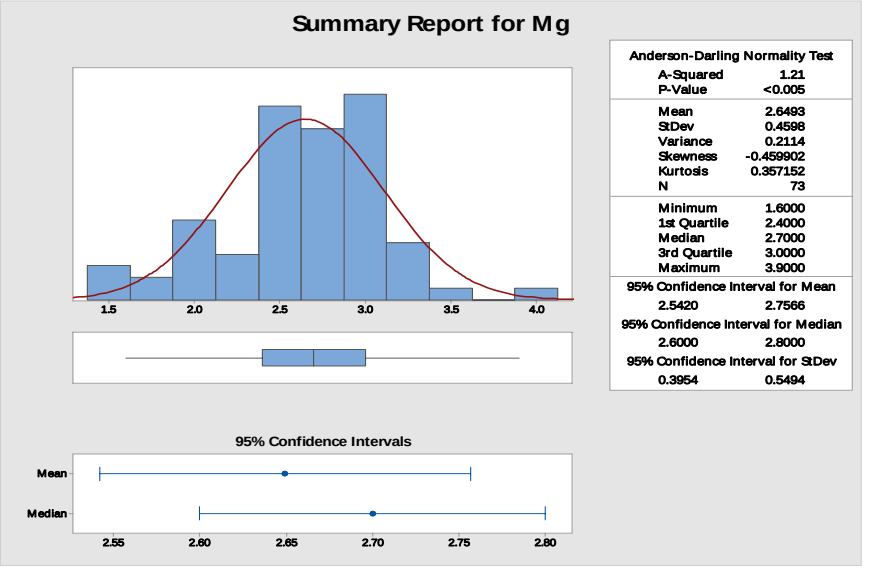

Fig. 7: Descriptive Statistics of measurements obtained by this study for Mg

The Mg content in blood samples was characterized by high positive values of skewness (4.25) and negative value of kurtosis (-1.8). The high negative values of the skweness indicate that the data are negatively skewed and are affected by complicated factors (kurtosis value>3). The mean and the median

values of both parameters are positioned left (close to minimum values). Pearson correlation analysis and multivariate analysis were carried out for an accurate interpretation of the data.

Table 3 The results of thePearson correlation analysis: Ca, Mg, Ca/Mg

	Ca	**Mg**	**Ca/Mg**
Ca	1		
Mg	0.047	1	
Ca/Mg	**0.540** (P=0.000)	**-1.778** (P=0.000)	1

Pearson correlation analysis reported a positive correlation between Ca and Ca/Mg ratio: high values of Calcium indicate high values of this ratio (tab.3). On the other hand, it showedthe negative correlation between Mg and Ca/Mg ratio: high values of Magnesium indicate low values of this ratio.

Cluster Analysis of Observations: Ca, Mg, Ca/Mg
Euclidean Distance, Complete Linkage
Amalgamation Steps
Final Partition: Number of clusters: 5

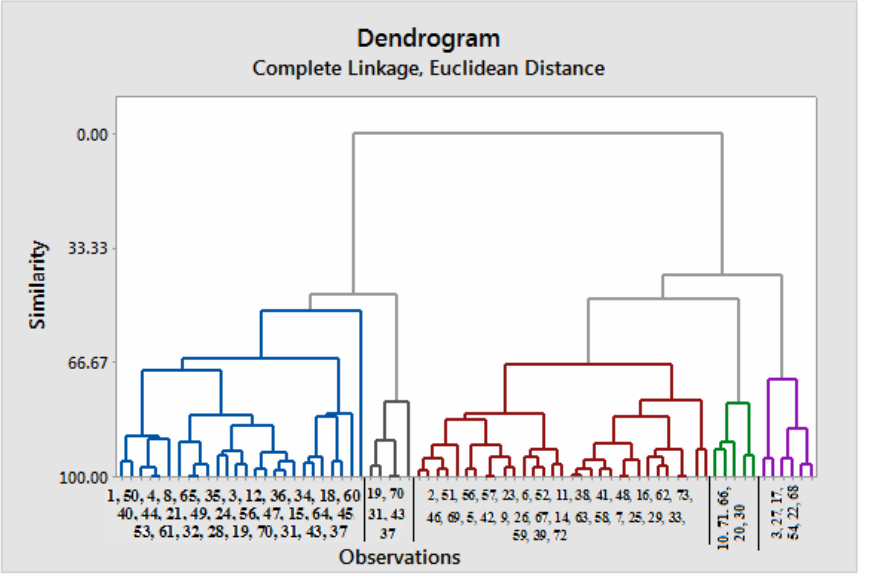

Fig. 8. The dedrogram of Cluster analysis of the people involved in the investigation.

Based on the dedrogram of Cluster analysis (fig. 8) the 77 women under investigation can be devided into 5 Clusters:

Cluster 1: (31 cases) - 1, 50, 4, 8, 65, 35, 3, 12, 36, 34, 18, 60, 40, 44, 21, 49, 24, 56, 47, 15, 64, 45, 53, 61, 32, 28, 19, 70, 31, 43, 37

Cluster 2: (5 cases) -19, 70, 31, 43, 37

Cluster 3: (31 cases) -2, 51, 56, 57, 23, 6, 52, 11, 38, 41, 48, 16, 62, 73, 46, 69, 5, 42, 9, 26, 67, 14, 63, 58, 7, 25, 29, 33, 59, 39, 72

Cluster 4: (5 cases) – 10, 71, 66, 20, 30

Cluster 5: (6 cases) – 3, 27, 17, 54, 22, 68

Cluster 1 includes pregant women, cluster 3 includes non-pregnant women and cluster 2, 4 and 5 are dividedbased on pregnancy stages and age.

The factor analysis with varimax rotation was applied and the number of the most important factors was determined for an accurate interpretation of the data.

Factor Analysis: Ca, Mg, Ca/Mg

Principal Component Factor Analysis of the Correlation Matrix
Varimax Rotation

Variable	**Factor1**	**Factor2**	Factor3	Communality
Ca	0.046	**0.998**	0.043	1.000
Mg	**-0.995**	0.091	0.043	1.000
Ca/Mg	**0.837**	**0.493**	0.237	1.000
Variance	1.6932	1.2470	0.0598	3.0000
% Var	0.564	0.416	0.020	1.000

Two main factors were extracted from factor analysis.

Factor 1 is an important factor that represents 56,4% of the total variance of the data. Characterized by high negative loads of Mg and high positive loads of Ca/Mg ratio, the factor 1 reduces Mg contraction in blood during first months of pregnancy. The lower the concentration is the higher the Ca/Mg ratio, as it does not depend on Ca.Consequently, high positive loads of Ca/Mg ratio could be expected.

Factor 2 is another important factor that represents 41.6% of the total variance of the data.Characterized by high positive loads of Ca (0.998) and moderate positive loads of Ca/Mg ratio, it increases of Ca in blood that is a related to the rich diet and the prenatal vitamin supplements (containing large quantities of Ca than Mg) uptake. As it does notdepend on Mg, the higherCavalues, thehigher the Ca/Mg ratio is.Consequently, positive loads of Ca/Mg ratio could be expected.

4. CONCLUSIONS

The results reported that the most part of the samples has the normal range of the concentration of Ca and Mg in blood serum of females under investigation. Only a small group of blood samples included in the study resulted in lower values than the Ca and/or Mg permitted levels.

The concentrations of the two elements of pregnant women are comparable with those of non-pregnant women showing the effectiveness of prenatal vitamin supplements.

The contraction in the first months of pregnancy reduces the amount of Mg in the blood. This factor does not depend on Ca. Consequently, as Mg reduces the Ca/Mg ratio increases.

The rich diet and the prenatal vitamin supplements (containing larger quantities of Ca than Mg) increase the amount of Ca in blood. Consequently, the Ca/Mg ratio is increased.

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THE USE OF VASCULAR PLANTS AS BIOINDICATORS OF AIR POLLUTION IN TIRANA, ALBANIA

Rudina TRIKSHIQI, Pranvera LAZO and Mimoza REXHA
Department of Chemistry, Faculty of Natural Sciences, University of Tirana, Albania

ABSTRACT

The leaves of vascular plants (*Ligustrum lucidum*, Fam: *Oleaceae* and leandro plant, *Nerium oleander*, Fam: *Apocynaceae*) were collected from 23 sampling sites along the streets of Tirana, Albania in March 2013. The sites suffered from anthropogenic impact. A composite sample was prepared for each sampling site and each species was investigated. Wet digestion technique was applied for sample digestion in half pressure Teflon tubes. Heavy metals (Cu, Zn, Pb, Mn and Fe) in leaves samples were determined via Atomic Absorption Spectrometry (AAS). Once obtained, analytical data were statistically investigated using EXCEL and MINITAB-15 Package Programs. The Pearson Correlation analysis ($p<0.05$) was carried out on the data set of heavy metals to describe their behaviour and association. No high correlation was found among the heavy metals. Multivariate analysis (Cluster Analysis and Factor Analysis) helped define the groups of samples with similar patterns of element concentrations and the number of the groups and most important factors are here discussed. Based on the similarity of the distribution of the elements at each sampling site, two main groups of elements were extracted. The data were also processed with factor analysis (FA) in order to identify the main source categories of the samples with regard to site contamination and elements distribution. Results reported that plants species and samples position are of great impact for the distribution of trace metals. In addition, the concentration of heavy metals was variable due to sampling site and the plant species.
Keywords: vascular plants, heavy metal, furnace AAS, Urban environment, air Pollution

1. INTRODUCTION

Tirana, the capital of Albania with an area of 1238.5 km² is located in the heart of the country.

314.5 km² out of 1238.5 km² is arable land. The climate is Mediterranean. The mean annual rainfall is 1015.6 mm and the temperature 15.7 ºC. The potential evaporation is 916 mm. This region has a dissected topography and contains a variety of landforms like high mountains, hills, valleys and plains.

The use of vascular plants as bioindicators of the air pollution in Tirana city is because these plants are stationary, practical, reflect conditional sites and can assess pollutant level (Brodribb and McAdam, 2010**).** Also they can absorb heavy metal from the ground and air (Banks and Nishiyama, 2011). Absorption of heavy metal depends on movement of elements from the soil to the roots, by the membrane of the epidermal cells of roots. Types of vascular plants are: *Ligustrum lucidum*, Fam: *Oleaceae* and leandro plant, *Nerium oleander*, Fam: *Apocynaceae*. Soil parent materials are generally composed of Mesozoic limestone in the mountain zone, Tortonian molasse formations, Paleogene flysch and Cretaceous limestone in the hilly zone, and the Quaternary deposits in the plain zone (Alloway, 1995). Air pollution is of the greatest concern mostly for the population living in urban areas, especially in the metropolitan areas. These areas are usually characterized by densely populated, heavy traffic rates and the presence of many industrial plants on their suburbs. Problems regarding air pollution on these areas are more evident over metropolitan areas in developing countries. Air pollution has a bad impact on humans' health, causing respiratory and cardiovascular diseases (Mandija and Zoga, 2012).

The present paper aims to investigate the level of heavy metals (Cu, Fe, Pb and Zn) in vascular plants in Tirana. Different species of vascular plants that are able to accumulate heavy metals were in the present investigation involved. Bhandari and Bauthiyal, (2013) said that the concentration of copper (Cu) in low levels remove (Weisberg *et al*., 2003) copper zinc-superoxide dismutase (CuZn–SOD), cytochrome c, and plastocyanin enzymes very important for the normal growth of plants. At high concentration, Cu can become toxic and cause symptoms such as chlorosis and necrosis, stunting, leaf discoloration and inhibition of root growth (van Assche and Clijsters, 1990; Marschner, 1995). Iron (Fe) is an important element in a large number of metabolic routes in plants (Nordbeg *et al*., 2007; Turksis and Zavadskas 2010), but in high concentration it has phytotoxic effects on plants and air (Sandman and Boger, 1980; Palma *et al*., 1997). Plants can accumulate heavy metals in leaves and roots, causing physiological disturbances in many processes including growth, water relations, photosynthesis, nutrient uptake, allocation and assimilation (Stephan *et. al*., 1994). The atmosphere is one of the most important pathways for the transport of heavy metals.

Urban atmosphere is subject to pollutants (Connolly and Gueriot, 2002; Azima *et al*., 1994-2002) of anthropogenic origin such as industries and residential heating and mobile sources (road traffic) (Baker 1998; Cail *et. al*., 2005). According to their different physical and chemical properties, the size and composition of the source particles, these pollutants are partitioned between liquid and vapour phases and are subsequently transported in the air (Akosy and Sahin, 1999; Huang *et. al*., 1994).

2. MATERIAL AND METHODS

Sampling

Sampling was carried out in the last 10 days of March 2013, at 20 stations in Tirana city, about one week after rainy days. The monitoring stations were located along the main streets of the city. A composite sample was prepared to investigate each sample. In the present investigation *Ligustrum lucidum*, Fam: *Oleaceae* and leandro plants, *Nerium oleander*, Fam: *Apocynaceae* were involved for the monitoring purposes.

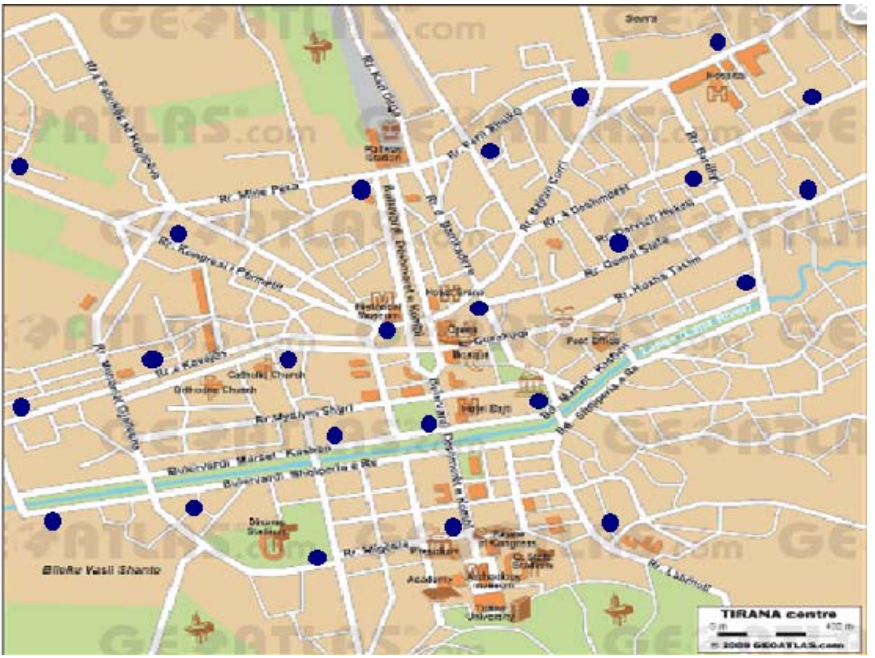

Fig. 1: Map of Tirana city, where are taken the sample of vascular plant.

The leaves of ligustra, *Ligustrum lucidum* (station 1, 2, 3, 4, 6, 9, 10, 12, 18, 19, 20) *Pitospora tobira* (station 3, 4, 7, 8, 9, 14, 17, 18) and leandro, *Nerium oleander* (station 1,2,3,5,7,8,9,11,12,13,15,16,17,18,19) tree species were collected from most of the sampling sites. Table1 reports the other tree species.

Table 1. The description of some species of different monitoring sites

Station	Species
1	*Laurus nobilis*
2	*Hedera helix*
4	*Ligustrum vulgare*
5	*Eucaliptus globulus*
6	*Jasminium fruticans*
8	*Hedera helix, Jasminium fruticans*
10	*Ligustrum vulgare*
11	*Jasminium fruticans*
12	*Jasminium fruticans*
14	*Ligustrum vulgare, Laurus nobilis*
16	*Jasminium fruticans, Laurus nobilis*
17	*Ligustrum vulgare*
20	*Eucaliptus globulus*

Preliminary treatment of the samples

Once transported at the laboratory, the leaves samples were dried at ambient air on sheet papers until a constant weight was reached. Once, the constant weight was reached, the leaves were homogenized to a fine material and crushed by hand wearing laboratory polyethylene gloves without powder.

Sample digestion

Dried plant samples were digested with nitric acid (ultra pure, 65%) and deionised water (9:1) in half pressure Teflon tubes. The experiment dishes were cleaned with dilute nitric acid and washed with distilled water. Elements standard solutions used for calibration curves were prepared by dilution of base standard liquids of 1000 mg / L.

0.5 g dry leaves of each sample were digested with 10 mL of nitric acid HNO3 (9:1). Teflon tubes were closed and left at room temperature for 48 h. Once left at room temperature for 48 hours, the leaves were digested for 3 hours at 80-90 ° C. The temperature was subsequently increased to 200° C for 1 hour for full digestion purposes. Once cooled up, the Teflon tubes were opened and left for the evaporation of nitric acid to small volumes. Once nitric acid was evaporated to small volumes, the samples were diluted with deionised water to a total volume of 50 mL.

The vascular plant digests were analysed for Cu, Fe and Zn using flame AAS Atomic Absorption Spectrophotometry by using air-acetylene flame. Pb

was determined using AAS with electro-thermal atomiser with graphite furnace.

Calibration method

Linear calibration method that find the link between the analytical signal and the measured analyse concentration were used for obtaining the calibration curves of each element. Blank solution of each element was used. Heavy metal concentration in samples was calculated based on their analytical signal (value of absorbance and the relevant equations of calibration curves).

3. RESULT AND DISCUSSION

Reported on table 1, the descriptive statistic analysis was carried out on the data sets for a rapid and preliminary screening of heavy metals concentration. The trace metal contents were expressed as arithmetic means ± 2Stdev. The analytical data were statistically investigated using EXCEL and MINITAB-15 Package Programs. Table 2 reports the distribution tendency of the heavy metals listed in an ascending order of accumulation factor.

Table 2: Results of statistical data processing (EXCEL, Descriptive Statistic) (mg/kg, DW).

Parameters	Fe	Pb	Zn	Cu	Mn
Mean	528.97	3.82	38.48	11.30	216.43
Median	451.09	3.01	34.68	9.64	80.95
Minimum	192.93	1.76	11.11	3.61	10.48
Maximum	1369.57	13.60	141.41	24.10	347.10

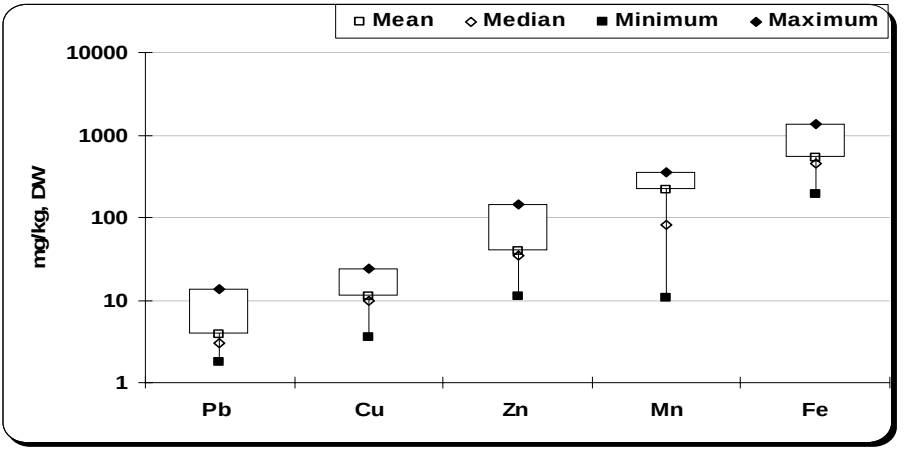

Fig. 2 The box-plot of the distribution of the elements (log-normal scale).

The distribution of each element follows the trend: Pb<Cu<Zn<Mn<Fe. Here, the changes in concentration of different elements in various monitoring site is clearly noted.

Table 3: Presentation of the reports Mean/Median and Max/Min

Parameters	Fe	Pb	Mn	Cu	Zn
Mean/Med	1.17	1.27	**3.02**	1.11	**2.67**
Max/Min	7.10	7.71	**937.50**	12.73	**331.32**

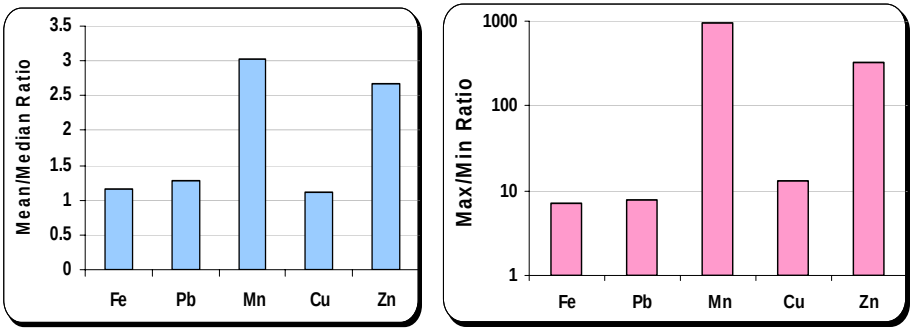

Fig.3. The graphical presentation of the reports of the (a) Mean/Median and (b) the Max/Min ratios

Figure 3 reports that the area under investigation is mainly polluted by Mn and Zn. As, their concentration data are not normally distributed (mean>>median) and represent very (high values of Max/Min values), cluster analysis (correlation analysis and analysis of elements in groups) was carried out to define the natural or anthropogenic origin of elements in leaves samples.

Correlation analysis ($p<0.05$) was carried out on the data set of heavy metals to describe their behaviour and association.

Table 4.Correlations between elements Cu, Pb, Zn, Mn, Fe

	Cu	Pb	Zn	Mn	Fe
Cu	1				
Pb	-0.155	1			
Zn	0.335	0.058	1		
Mn	0.095	-0.162	0.083	1	
Fe	0.573	-0.127	0.181	0.25	1

The Multivariate analysis (Cluster Analysis, CA) was carried out to detect the groups of samples with similar patterns of element concentrations and the number of the groups is here discussed.

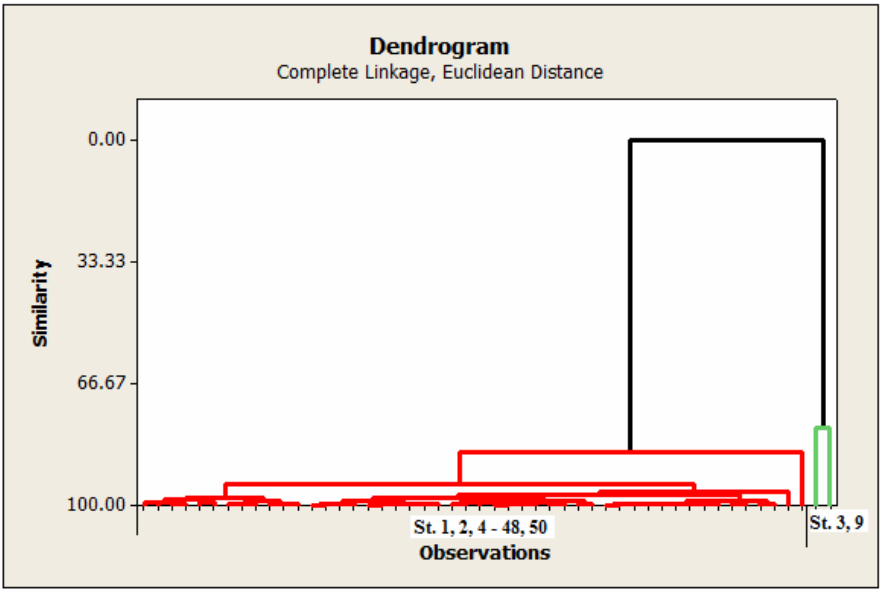

Fig. 4. The distribution of the stations classified on their similarity, Euclidean Distance, Complete Linkage, Amalgamation Steps; Final Partition: Number of clusters: 2

Cluster 1 comprises the following stations 1, 19, 6, 11, 22, 48, 14, 34, 44, 26, 33, 42, 2, 32, 27, 18, 4, 23, 5, 7, 8, 20, 12, 43, 24, 17, 46, 28, 31, 21, 25, 32, 39, 9, 13, 30, 29, 36, 45, 47, 16, 38, 35, 40, 10, 41, 15, 50 where *Ligustrum lucidum*, Fam: *Oleaceae* prevail. They have similar accumulation abilities under similar environmental conditions of heavy metals.

Cluster 2 comprises the station **3** and **49** where *Laurus nobilis* and *Eucaliptus globulus* plants prevail. Here, traffic emission is of great impact. The data were also processed with factor analysis (FA) to identify the main source categories of the analysed samples regarding site contamination and elements distribution.

Factor analysis was carried out for a better interpretation of the results:

Factor Analysis: Cu, Pb, Zn, Mn, Fe
Principal Component Factor Analysis of the Correlation Matrix
Rotated Factor Loadings and Communalities
Varimax Rotation

Variable	Factor1	Factor2	Factor3	Communality
Cu	**0.882**	-0.097	-0.009	0.787
Pb	-0.132	**0.896**	-0.011	0.820
Zn	**0.624**	**0.466**	-0.044	0.608
Mn	0.087	-0.010	**0.979**	0.966
Fe	**0.746**	-0.199	0.294	0.683
Variance	1.7483	1.0691	1.0473	3.8648
% Var	0.350	0.214	0.209	0.773

Results reported three factors effecting the differences in trace metals distribution in plant species.

Explaining most of the variance (35%), **factor 1** has high loads of Cu, Zn and Fe. The source is the anthropogenic factor of brake lining, tire wear particle and car brake and soil dust.

Explaining 21.4 % of the total variance, **factor 2** has high loadings of Pb and Zn, as typical elements with anthropogenic input from traffic emission. Pb acts as the marker element for motor vehicle emissions (Caille *et al*., 2005).

Explaining 20.1 % of the total variance, **factor 3** has high loading of Mn which is mainly of geochemical origin of wind soil dusts and the feedings of the plants from their roots system.

4. CONCLUSION

In the present investigation vascular plants like *Ligustrum lucidum*, Fam: *Oleaceae* and leandro plant, *Nerium oleander*, Fam: *Apocynaceae* were involved. The concentration of elements in vascular plants collected from polluted areas was found at higher levels than in the areas distant from crossroads or dense traffic. The distribution of each element followed the trend: Pb<Cu<Zn<Fe. Here, the changes in concentration of different elements in various monitoring stations are obvious. Correlation analysis and analysis of elements in groups (Cluster Analysis) reported the similar accumulation abilities under similar environmental conditions of heavy metals in the plants under investigation. Factor analysis reported that traffic emission is the main source of pollution.

Using different plants in same or different streets results reported that *Ligustrum lucidum*, Fam: *Oleaceae* is a vascular plant that can absorb more easy and fast heavy metals, so we can suggest to have more of this plant in our city.

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EVALUATION OF OVARIAN TUMOR BIOMARKERS HE4 AND CA125 IN WOMEN ACCORDING TO AGE

RIDVANA MEDIU
Clinical-Biochemical Laboratory, General Hospital, Kavajë, Albania
Elda MARKU, Pranvera LAZO
Department of Chemistry, Faculty of Natural Sciences, university of Tirana, Albania
Naim MEDIU
Department of Surgery, General Hospital, Durrës, Albania

ABSTRACT

A variety of biomarkers have been developed to monitor the growth of ovarian tumorand detect the disease at early stage. Tumorantigen 125 (CA125) is a protein found on the surface of many ovarian tumor cells. It also can be found in other tumors and in small amounts in normal tissue. A CA125 test measures the amount of this protein in the blood.CA125 (MUC16) has provided a useful serum tumor marker for monitoring response to chemotherapy, detecting disease recurrence, distinguishing malignant from benign pelvic masses, and potentially improving clinical trial design.Human epididymis protein 4 (HE4) is a relatively new marker for ovarian carcinoma.He4 measurements in serum have been proposed for improving the specificity of laboratory identification of OC.The paper aims at evaluatingthe diagnostic performance of both CA125 and He4 in discriminating benign ovarian tumorsfrom other benign gynecologic diseases. In the present investigation 139female outpatients aged between 11 and 72 years old (mean age 39.7 years) were involved afterbeing diagnosed with an ovarian cyst during a visit to a gynecologist or investigation of unknown abdominal pain by computer tomography. Fasting blood samples were collected and centrifugedfollowing the standardized procedure.All the analyses were performed in plasma. CA125 and He4 plasma concentrations were determined using automated analyzerCobas®6000 (Roche Diagnostics). The data were statistically treated by using Descriptive Statistics for Windows. CA125 values exceeded the upper limit (cut-off value 35 U/ml) in 19.05% of serum samples. He4 values exceeded the upper limit (cut-off value150pmol/l) in only 2.38% of serum samples. In these samples, CA125 values were above the limit, as well.In order to have better information regarding the correlation between the two parameters, the data were divided in three subgroups based on patient's age. For all subgroups descriptive statistics including mean, standard deviation, minimum and maximum

values were obtained.Measuring serum HE4 concentrations along with CA125 concentrations may provide higher accuracy for detecting ovarian tumor.
Keywords: He4, CA125, tumor marker, ovarian

1. INTRODUCTION

The ovarian tumor (OC) accounts for about 3% of tumors among women, but it causes more deaths than any other tumor of the female reproductive system(www.tumor.org).Ovarian tumor is diagnosed annually in more than 200,000 women worldwide, with the greatest incidence in the US and Northern Europe (Sandri*et al.,* 2013).However, fewer than 30% of all ovarian tumors are diagnosed in stages I/II and worldwide mortality from ovarian tumor has decreased only by 12% since 1973 (Ferlay*et al*., 2008).The 5-year survival rate is less than 20% for women diagnosed at the late stage, whereas it is 90% if detected in the early stage(Schollerand *etal*., 2007), (Zhang*etal*., 2011).Ovarian tumor accounts for about 4% of tumors cases that affect womenin Albania. Risk factors affecting the proliferation of these diseases are genetics, family history, age, race, infertility, diet, lack of physical activities and obesity (Jorgoni2013).The main challenge for laboratory biomarkers of OC diagnosis is to allowthe accurate detection of malignancy as early as possible to improve clinical outcome and survival of patients (Moore*et al.*2007). CA125(carcinoma antigen 125, or carbohydrate antigen 125) is aproteinthat in humans is encoded by the*MUC16*gene which is a member of themucinfamilyglycoproteins. It is commonly referred to as a "biomarker" or "tumor marker", because it provides information about the biological state of an ovarian tumor(Köbel*et al.*2008).Therefore, early detection may result in better outcomes. CA125 is the most widely used serum marker in the detection and management of the disease.It was first identified in the early 1980s and can be helpful in determining whether an ovarian mass is malignant or not. As CA125 effectiveness in the identification of the malignancy is threatened by its low diagnostic specificity, human epididymis protein 4 (He4) measurements in serum have been proposed for improving the specificity of laboratory identification of OC (Rosen*et al.*2005).The WFDC2 (He4) gene is a member of a family of stable 4-disulfide core proteins that are secreted at high concentrations (Hellström*et al*., 2003). It is amplified in ovarian carcinomas,whereasits expression in normal tissues, including ovary, is low (Havrilesky*et al*.,2008).Serum concentrations of He4 are less affected by menstruation, ovulation and other benign ovarian conditions (e.g.endometriosis) compared with CA125 (Ono*et al.* 2000). Also the He4 assay may have an advantage over the CA125 assay as it is less frequently positive in patients with nonmalignant disease (Hellström*et al*.2003).The combination of CA125 and He4 is a more accurate predictor of malignancy

than either marker alone (Moore *et al.*2008). The women with a gynecological disease and increased concentrations of He4 and CA125 are at higher risk for malignant pathologies,as expected from immunohistochemical data (Rosen*et al.*2005). He4 is found in high concentrations in the serum of women with serous epithelial ovarian tumor.

2. MATERIALS AND METHODS

Study of population

In the present investigation 139female outpatients were involved after being diagnosed with an ovarian cyst during a visit to a gynecologist or during investigation of unknown abdominal pain by computer tomography. The inclusion criteria were the availability of complete clinical records and the consent to have additional testing for new markers for ovarian tumor. The exclusion criteria were pregnancy and significant concomitant disease. The mean age was 39.7 years old (range 11-72). The study population was divided into three groups according to age : <35 (42 patients-subgroup I), 35-45 (38 patients-subgroup II) and 46-55 (33 patients-subgroup III). The small number of patients older than 55 years old (only 26) and the huge heterogeneity among studies did not allow any data pooling and further statistical elaboration.

Sample collection

Venous blood samples were collected following an overnight fasting (serum and EDTA samples) in line with the standardized procedures for collection and storing. All analyses were performed on plasma. Fasting blood samples were collected, centrifugedand stored following the standardized procedures.

He4 and CA125 analysis

Serum tumor markers' concentrations were determined using respectively Coobas e601 automated analyzer for Ca125 and Elecsys2010 automated analyzer for He4(Roche Diagnostics), which are based on electrochemiluminesence immunoassay ECLIA. Results were measured via a calibration curve which is instrument- specifically generated by 2- point calibrations and a master curve provided via the reagent barcode. The resulting chemiluminescent reaction was measured as relative light units. A direct relationship exists between the concentration ofHe4 and CA125 respectively antigen in the sample and relative light units.

Statistical Analysis

Descriptive Statistics were applied to each data set in order to analyze and interpret the results.The mode of the data distribution was discussed according to kurtosis and skewnesstest. Minimum and maximum values obtained for each parameter was estimated, as well.A new parameter (CA125/ HE4 ratio) was used, in order to obtain new information for the possible correlation between the two tumor markers.Factoranalysis was used to indicate important implicit features. MINITAB 17 software package was used for data analysis.

3. RESULTS AND DISCUSSIONS

Tumor marker concentrations in all patients (N=139)

The analytical values of CA125 in 139 patients' serum samples are in Figure 1 depicted. We have considered the concentrations 35U/ml and 150pmol/l as the upper limits (cut-off values) of normality for CA125 and He4, respectively.In 21.58% of serum samples CA125 concentrations exceeded the cut-off value. He4 concentrations exceeded the cut-off value in only 2.16% of serum samples. In these last samples, CA125 values were above the limit, as well.

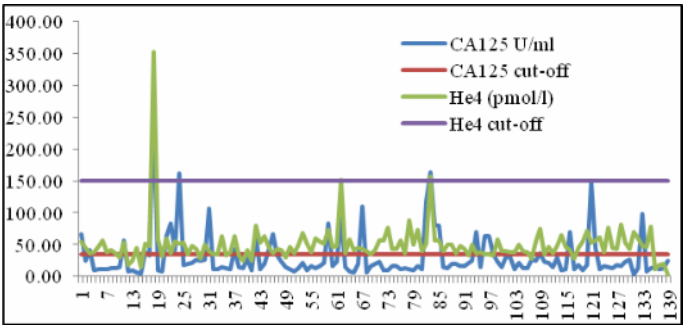

Fig.1: Distribution profile of CA125 and He4 concentrations in all serum samples compared with cut-off values (35U/ml for CA125 and 150pmol/l for He4).

The ratio CA 125/He4 was a means to address the correlation between the two tumor markers.Descriptive Statistics for CA125, He4 and CA125/He4are presented in Table 1.

Table 1. Descriptive Statistics for CA125, He4 and CA125/He4valuesin all serum samples

Variable	Mean	Median	StD	Variance	CV %	Min	Max	Skewness	Kurtosis
CA125	30.58	16.53	36.44	1328	119	3.52	268	3.39	15.05
He4	49.62	44.73	32.22	1038	65	3.51	352	6.54	57.47
CA125/He4	0.67	0.42	0.79	0.620	118	0.05	6.85	4.41	28.09

The analytical results for CA125 and He4 reported different variability. CA125 values had a high variability (CV=119%), He4 values had an average variability (CV= 65%), while CA125/He4 had high variability as well (CV =118 %). High disparity is evident for the analytical data of both parameters which are characterized by high values of skewness (>0) and kurtosis (>3) which indicate an asymmetrical distribution ofCA125 and He4, as well as the influence of complicated factors. The histograms of the data distribution of the three parameters are in Figure 2 - 4 depicted.

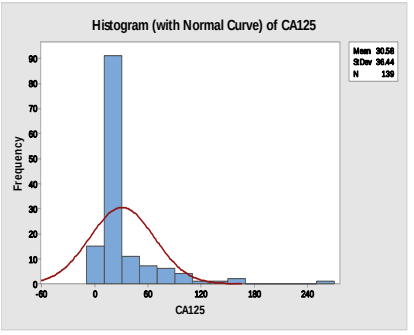

Fig. 2. Histogram of normal distribution of CA125.

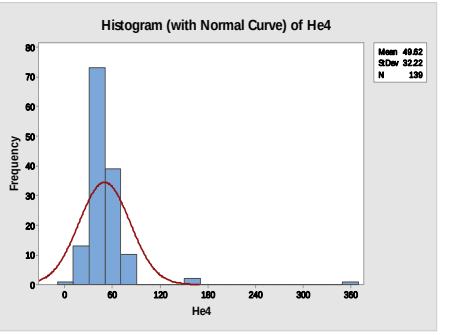

Fig. 3. Histogram of normal distribution of He4.

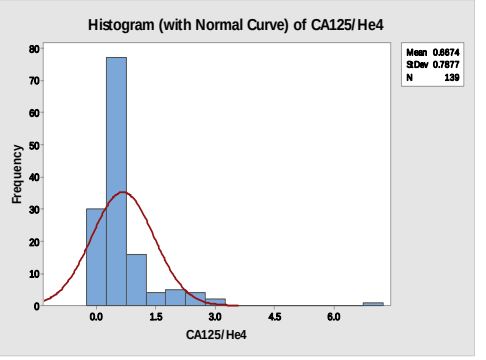

Fig. 4. Histogram of normal distribution of CA125/He4.

The three parameters were characterized by high values of skewness (3.39 for CA125, 6.54 for He4 and 4.41 for CA125/He4) and kurtosis (15.05 for CA125, 57.5 for He4 and 28.49 for CA125/He4). These high positive values indicate that the data are positively skewed and affected by complicated factors (skewness value > 0 and kurtosis value>3).The high variance of CA125, He4 and Ca125/He4 values is shown in boxplots of the Figure 5.

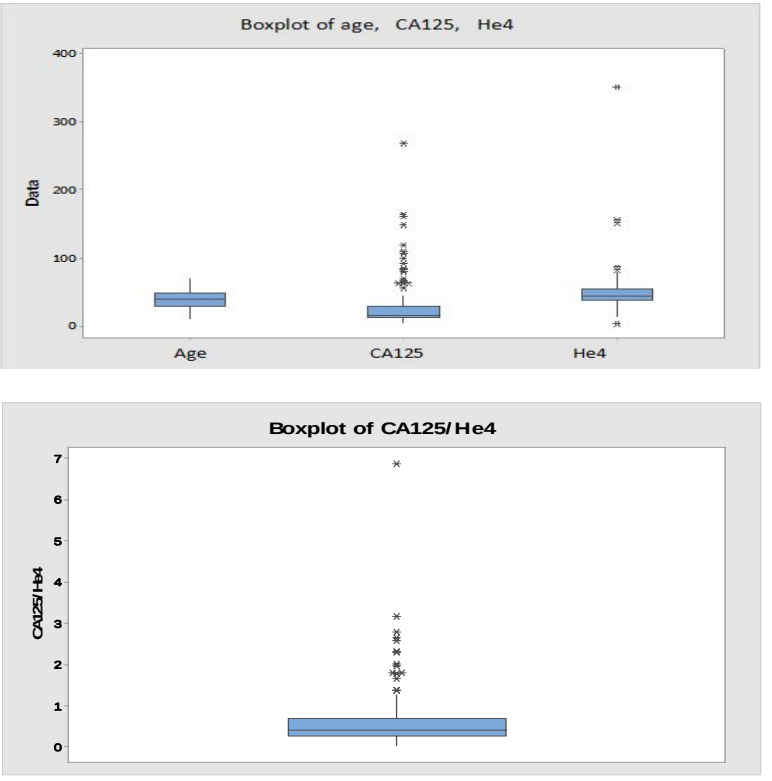

Fig. 5. Boxplots of CA125, He4 and Ca125/He4 values for all samples.

The data correlation analysis between three parameters (Figure 6) showed that CA125, He4 and CA125/He 4 ratio do not obey the normal distribution (p-value <0.005).

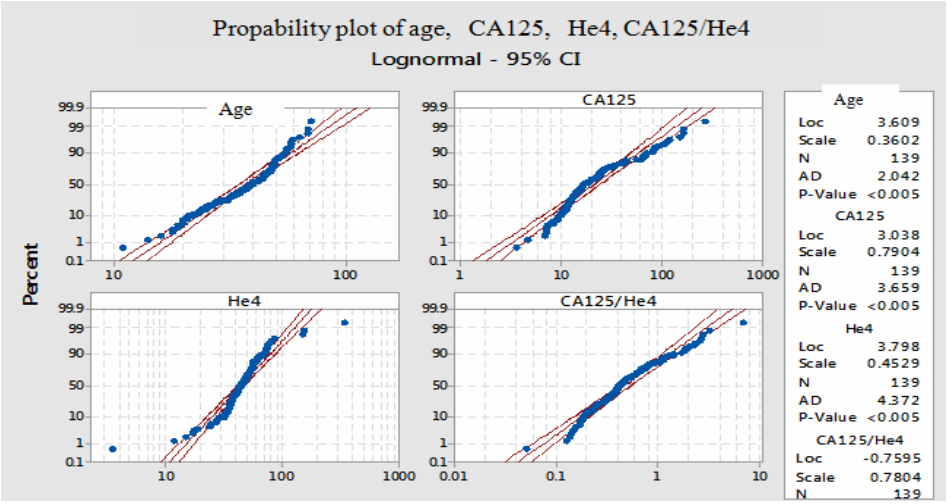

Fig. 6. Probability plot of age, CA125, He4 and CA125/He4 for all samples.

For a better interpretation of factors influencing the association and distribution of the studied parameters in blood serum samples, factor analysis with Varimax Rotation was used.

Three main factors were extracted (Eigen values >1) and the minimum number of 3 factors was selected. The results of factor analysis are in Table 2 reported and Figure 7 depicted.

Table 2. The results ofFactor Analysis of the Correlation Matrix of CA125, He4, CA125/HE4 in 139 blood serum samples (Rotated Factor Loadings and Communalities, Varimax Rotation)

Variable	Factor1	Factor2	Factor3	Communality
Age	0.080	-0.041	-0.995	0.999
CA125	0.773	0.586	0.068	0.946
He4	0.963	-0.122	- 0.147	0.964
CA125/He4	-0.006	0.986	0.038	0.974
Variance	1.5312	1.3332	1.0180	3.8824
% Var	0.383	0.333	0.255	0.971

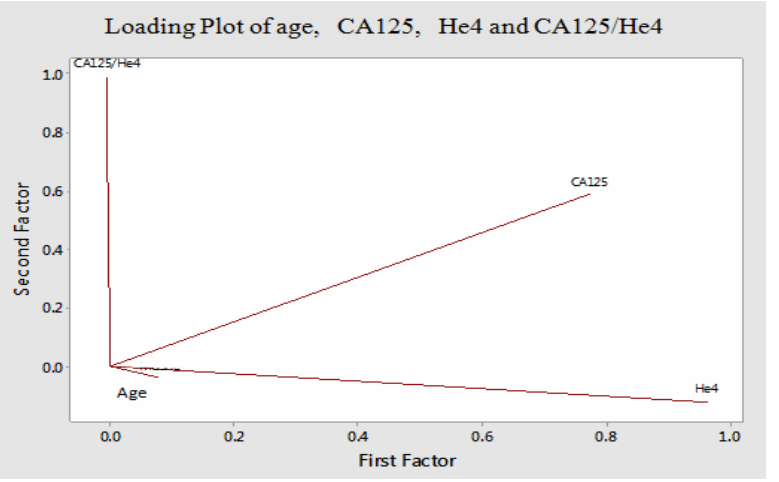

Fig. 7: Loading Plot of Age, CA125, He4 and CA125/He4ratio.

Three main factors were identified effecting the differences in the distribution of these parameters:

Explaining most of the variance (38.3%), **factor 1** has high loadings of CA125 (0.773) and He4 (0.963). Consequently, that both tumor markers represented positive values in cases with suspect an OC. In addition, it is a means to address further examinations.

Explaining 33% of the total variance, **factor 2** has high loadings of CA125 (0.586) and CA125/He4 ratio (0.986), because in different benign gynecologic conditions in premenopausal women, the elevated values of CA125 are more frequent than He4 ones. Consequently, He4 can be a good complement to distinguish CA125 false positives from true positives.

Explaining 25.5% of variance,**factor3** has high negative loading for age (-0.995). (Van *et al*. 2011) reported that compared with CA125, HE4 is inversely influenced by age.

Tumor markers in subgroup I

The data classification into three subgroups based on age was a means to address correlation between the CA125 and He4. Descriptive statistics, including mean, standard deviation, minimum and maximum values were calculated for the three subgroups.

Table 3 reports that the mean value ofCA125 serum concentrations in the first subgroup is 25.55U/ml— lower than the cut-off value. The minimum value is 6.71U/ml. The maximum value is 149 U/ml.Here Ca125 serum concentrations do nothave a normal distribution and were positively skewed

(skewness values >2 and kurtosis values >3). For the He4 tumor marker the mean value is 41.37 U/ml— lower than the cut-off value 150 U/ml.The analytical results for CA125 and He4 represent different variability. CA125 values had a high variability (CV=113%), He4 values have a low variability (CV= 25%), while CA125/He4 parameter had a high variability (CV =95 %). In this subgroup He4 serum concentrations hada normal distribution(skewness values -0.08 and kurtosis values 0.09) and p-value= 0.2(p>0.05), due to the young age of patients in this subgroup involved and the lower effect of menstruations, ovulation and other benign ovarian conditions compared to CA125.

Table 3. Descriptive Statistics for CA125, He4, CA125/He4 in first subgroup (<35 years old)

Variable	Percent	Mean	Median	StD	Variance	CV %	Min	Max	Skewness	Kurtosis
CA125	100	25.25	15.98	23.39	805.91	112.45	6.71	149.20	3.07	9.91
He4	100	41.37	39.66	10.31	106.28	24.92	17.60	62.80	-0.08	0.09
CA125/He4	100	0.60	0.42	0.57	0.33	96.50	0.13	2.80	2.81	7.97

The distributions of the experimental data and the probability plots of Ca125, He4 and their ratio for subgroup I, are in Figure 8 and 9 depicted, respectively.

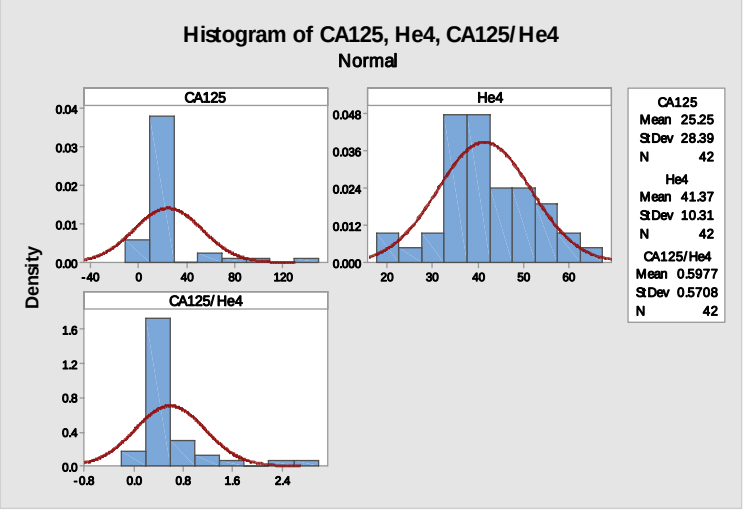

Fig.8. Histograms of CA125, He4 and CA125/He4 ratio values in subgroup I.

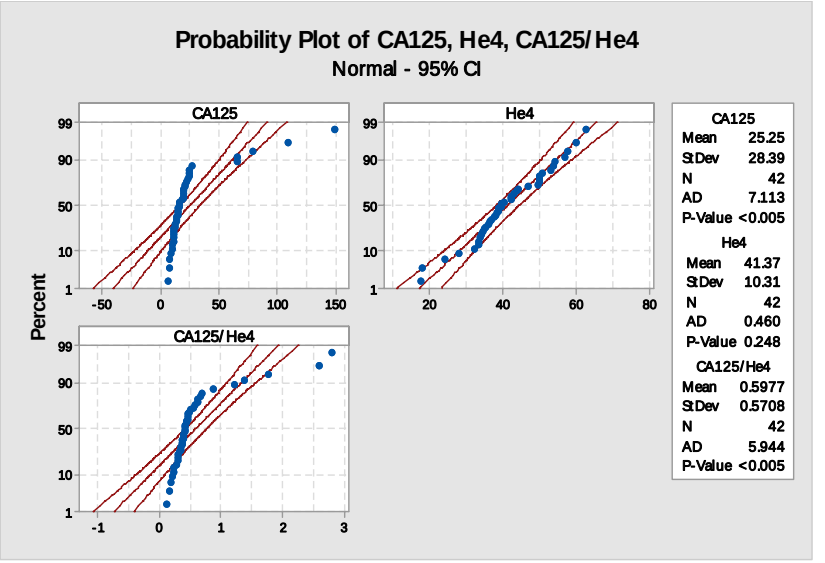

Fig. 9. Probability plot of CA125, He4 and CA125/He4 ratio values in subgroup I.

Tumor markers in subgroup II

For subgroup II patients (35-45years old),descriptive statistics including mean, standard deviation, minimum and maximum values of CA125 and He4 measured in 38 patients' serum samples are presented in Table 4.

Table 4. Descriptive Statistics for CA125, He4, CA125/He4 in the second subgroup (35-45 years old)

Variable	Percent	Mean	Median	StD	Variance	CV %	Min	Max	Skewness	Kurtosis
CA125	100	33.01	16.50	35.43	1255.00	107.33	3.52	163.80	2.00	4.08
He4	100	47.01	42.13	22.64	512.46	47.75	12.01	156.70	3.18	14.49
CA125/He4	100	0.72	0.41	0.67	0.44	92.73	0.05	2.66	1.49	1.34

Table 4 reports that the mean value ofCA125 serum concentrations is 33.01U/ml— lower than the cut-off value. The minimum value is 3.52 U/ml. The maximum one value is163 U/ml.In this subgroup Ca125 serum concentrations do not to have a normal distribution and are positively skewed (skewness values=0 and kurtosis values >3). He4 tumor marker mean value is 47.01U/ml—lower than the cut-off value 150 U/ml.The analytical results for CA125 and He4 show different variability. CA125 values have a high variability (CV=107.33%), He4 values have a moderate variability (CV=

47.75%). CA125/He4 parameter has a high variability (CV =92.73 %). Analytical valuesof CA125and He4 are positively skewed and not normally distributed, p-value<0.005. CA125/He4 ratio represents a normal distribution, p-value 0.028. The distributions of the experimental data and the probability plots of Ca125, He4 and their ratio for subgroup II are in Figures 10 and 11 depicted, respectively.

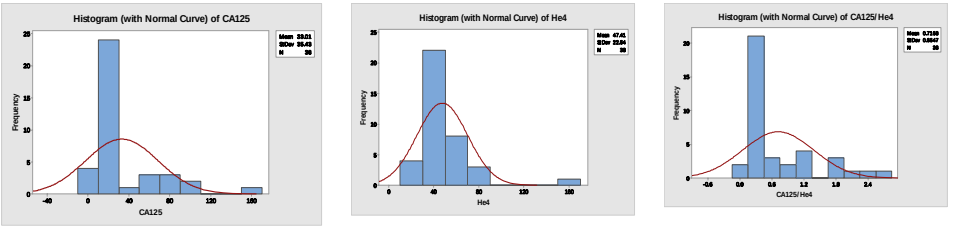

Fig. 10. Histograms of CA125, He4 and CA125/He4 ratio values in subgroup II

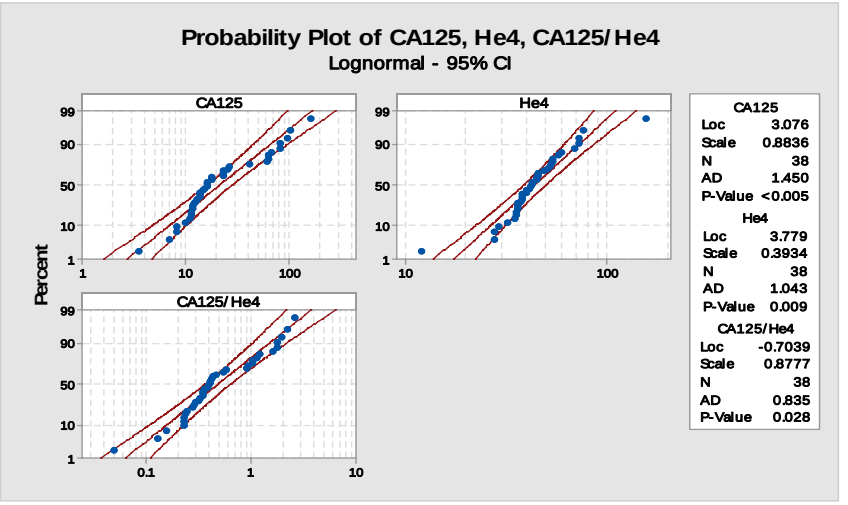

Fig. 11. Probability plot of CA125, He4 and CA125/He4 ratio values in subgroup II

In subgroup II patients the mean value was increased for both tumor markers CA125 and He4. He4 values had a small variability, suggesting that He4 is a good complement to distinguish CA125 false positive from true positive,especially in this age group women. A good correlation was found between age and CA125. CA125/He4 ratio represented low variability of its mean value, as well.

Tumor markers in subgroup III

For subgroup III patients (46-55years old),descriptive statistics including mean, standard deviation, minimum and maximum values of CA125 and He4 measured in 38 patients' serum samples are in Table 5 reported.

Table 5. Descriptive Statistics for CA125, He4, CA125/He4 in third subgroup patients (46-55 years)

Variable	Percent	Mean	Median	StD	Variance	CV %	Min	Max	Skewness	Kurtosis
CA125	100	29.29	19.30	44.72	2000.11	152.68	7.11	268.20	5.04	27.42
He4	100	56.59	48.10	54.99	3023.54	97.16	3.51	352.00	5.11	28.16
CA125/He4	100	0.48	0.51	0.29	0.08	58.83	0.14	1.08	0.34	-0.98

Table 5 reports that the mean value ofCA125 serum concentrations is 29.29 U/ml lower than the cut-off value. The minimum value is 7.11U/ml.The maximum value is268U/ml.Inthis subgroup Ca125 serum concentrations do not to have a normal distribution and are positively skewed (skewness values=0and kurtosis values >3). He4 mean value is 56.59U/ml—also lower than the cut-off value150U/ml.The analytical results represent high variability (CV=152.68%for CA125, CV=97.16% for He4) and moderate variability for CA125/He4 ratio (CV=58.53%). The analytical values are positively skewed and not normally distributedfor all parameters. The distributions of the experimental data and the probability plots for subgroup III are in Figure 12 and 13 depicted, respectively.

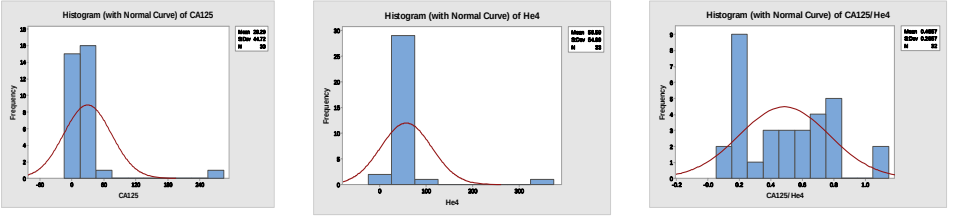

Fig. 12. Histograms of CA125, He4 and CA125/He4 ratio values in subgroup III.

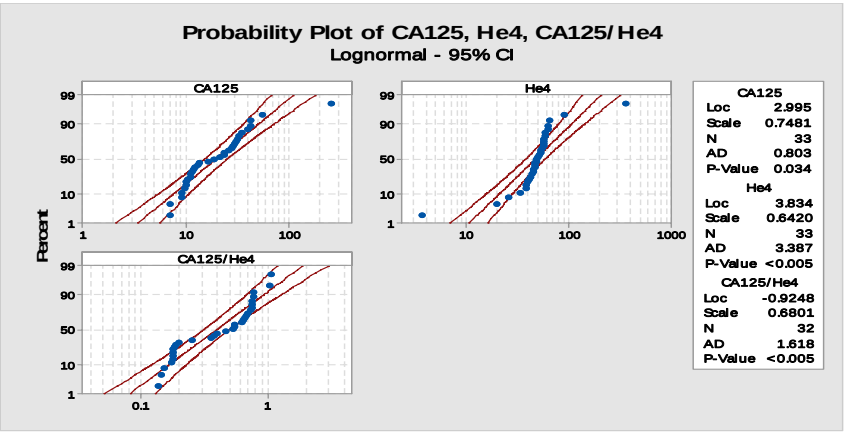

Fig. 13. Probability plot of CA125, He4 and CA125/He4 ratio values in subgroup III.

All analytical results reported that the highest concentrations for both studied tumor markers are found in subgroup III (268 U/ml for CA125 and 352pmol/l for He4 confirming the presence of a higher risk for malignancy for women patients of this age with gynecological disease and increased concentrations of He4 and CA125. In particular the risk for OC increases significantly for patients with He4 positive results. In healthy premenopausal women CA125 is often more elevated than He4 in different benign gynecologic conditions (Andersenet al. 2010). This is also true for our set of data, suggesting that He4 is a good complement to distinguish CA125 false positive, from true positive cases, especially in fertile women.

4. CONCLUSIONS

The analytical results for CA125 and He4 reported different variability. The mean and the median values of both parameters are positioned close to the minimum values. Both tumor markers represent positive values in cases of suspected OC and are a means to address further examinations. In healthy premenopausal women, CA125 is often more elevated than He4 in different benign gynecologic conditions. This is also true for our set of data, suggesting that He4is a good complement to distinguish CA125 false positive, from true positive cases,especially in fertilewomen. We took different values for CA125 and He4 tumor markers according to age. The highest concentrations of both studied tumor markers were found in subgroup III(46-55 years old). Compared with CA125, HE4 seems to be inversely influenced by age.The results confirmed that combining CA125 and He4 measurements, we can improve the diagnostics performance for OC. The use of this combination can

improve the detection of ovarian tumor, as compared with use of either marker alone for the discrimination of benign from malignant ovarian lesions.Due to the high prevalenceof OC in the post –menopausal women and the need for data related to early tumor stages, morestudies tailored on this specific subgroup (subsets) are needed.

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GEOPHYSICAL OVERVIEW ON SHKODËR-PEJË DEEP TRANSVERSAL FRACTURE[1]

Alfred FRASHËRI
Faculty of Geology and Mining, Polytechnic University of Tirana, Albania
Salvatore BUSHATI
Academy of Sciences of Albania
Neki FRASHËRI
Faculty of Information Technology, Polytechnic University of Tirana, Albania
Shpresa DEMA
Albanian Geological Survey

ABSTRACT

Integrated geophysical data such as gravity, magnetic, paleomagnetic, geothermal, seismological and remote sensing images of the zone crossing the deep transversal fracture Shkodër–Pejë are here overviewed. In the present investigation, regional geological setting data of Shkodër-Pejë sector of the Mediterranean Alpine Folded Belt, which represents the existence of an important disjunctive deep tectonic element, were used to analyse the geophysical data. Geological mapping at the scale 1:25.000 and the regional maps at the scale 1:200.000 could not trace the outcrop of this thrust at the Earth's surface. Consequently, different concepts about have arisen due to different situations regarding the geological setting of the Albanides. In 1901, the outcrop was called "scharung" and from1970 to 2012 it was called "transform transversal fault". In addition, efforts have been made either to silence about its existence or to deny it. The integrated geophysical information provides detailed information about the depth of the area around the transverse Shkodër-Pejë which represents a deep transverse vertical fracture affecting the Moho Discontinuity.
***Keywords*:** Mediterranean Alpine Folded Belt, Shkodër-Pejë transversal, geophysical anomalies, deep fracture, gravity anomalies inversion

[1] This paper is a revised version of the paper "*Geophysical overview on Shkodër-Pejë deep transversal fracture"* presented at the XX CBGA Congress in Tirana, Albania, from 24 to 26 September 2014

1. INTRODUCTION

Historic review

Hellenides-Albanides-Dinarides, branch of the Mediterranean Alpine Folded Belt, are interrupted by a deep transversal tectonic fracture in the Shkodër-Pejë segments. Figure 1 depicts this fracture correlated with contact between Eurasian and African Plates in Drini Bay, in the Adriatic Sea. Cvijich (1901) described this fracture for the first time calling it "scharung". The fracture was called "deviation" by Bourcart 1919; 1925; Kossmat 1924; Koberh 1929; Nopca 1929; Novack, 1929; Zuber, 1940, "accident" by Aubouin and Ndojaj 1964. Aubouin *et al*., (1970) stated that the fracture dates since the Tethyan Ocean paleogeography. In the end, Çollaku and Cadet 1991 called it "Faille transversale Scutari-Peć".

As the Albanides geological setting and its geologic history has been based on geosynclines' theory, this fracture has not been mentioned in the Albanian geological studies for several decades (Geology of Albania, 1970). Only later, some authors, who admit the opening of the Mirdita Ocean, interpreted this transversal as

oceanic transform fracture. Shkodër-Pejë transform fault is represented by the north-western front of the ophiolitic belt (Kodra *et al*., 1994; Melo, 1986; Melo *et al*., 1991; Peza *et al*., 1971; Xhomo *et al.,* 2002), or by the northern border of Cukali subzone as natural geological border between Alps Zone and Cukali ones (Papa *et al*., 1985; Papa, 1991: 2003). Qirinxhi *et al*., (1983) and Qirinxhi *et al*., (1991) stated that the Shkodër–Pejë thrust and its position are unobservable and could not be mapped during geological field surveys. However, it might exist in the depth. Different seismological studies provide information about an active fault zone according to the well-known Shkodër-Pejë direction (Sulstarova *et al*., 1972; Aliaj, 1988; Muço *et al*., 2001). Aliaj (1988; 2006) stated that the Shkodër-Pejë transverses fault divides Dinarides from Hellenides. These interpretations addressed new geological interpretation with regarding its position. The above interpretations regarding the Shkodër-Pejë fracture also have resulted in an alternative geological opinion concerning its position. Recently, the transversal tectonic fracture in the Shkodër-Pejë segment has oceanic transform fracture. Shkodër-Pejë transform fault is represented by the north-western front of the ophiolitic belt (Kodra *et al*., 1994; Melo, 1986; Melo *et al*., 1991; Peza *et al*., 1971; Xhomo *et al.,* 2002), or by the northern border of Cukali subzone as natural geological border between Alps Zone and Cukali ones (Papa *et al*., 1985;

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In the last 50 years, remote sensing data have revealed some tectonic lineaments along the Shkodër-Pejë transversal zone (Chorowich *et al*., 1981).

[2] The papers were introduced at the XX CBGA Congress in Tirana, Albania, from 24 to 26 September 2014.

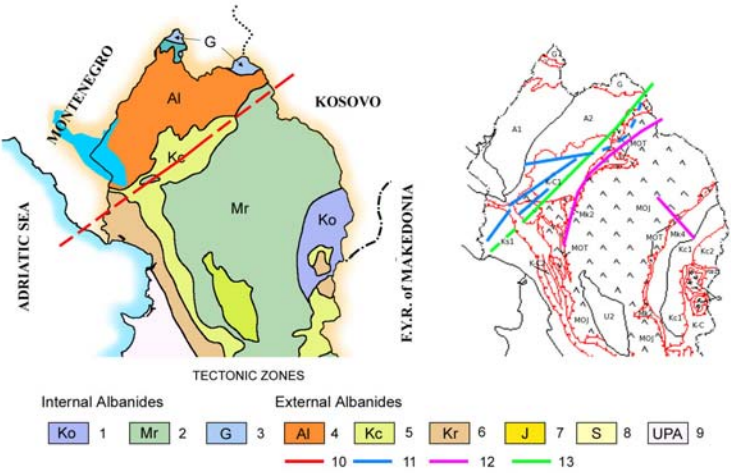

Fig. 1. Tectonic scheme of the Albanides (a) and scheme of the active tectonics system of the Albanides [Xhomo *et al*., 2002] (b) completed by traces of the Shkodër-Pejë deep transversal fracture after geophysical and remote sensing data.1- (Ko) Korabi zone: 2- (Mr) Mirdita zone; 3- (G)-Gashi zone; 4- (Al) Alps zone; 5- (K-C1) Krasta- Cukali; 6- (Kr) Kruja zone; 7 (J) Ionian zone; 8 (S) Sazani zone; 9 (UPA) Peri Adriatic Depression; 10- The trace of the deep transversal thrust Shkodër-Pejë after geophysical and remote sensing data; 11- Fractures after remote sensing data (Chorovitch *et al*. 1981); 12- Thermal anomaly axis of the Heat Flow Density; 13- Fracture Shkordër-Pejë thrust (Çollaku and Cadet, 1991); G-Gashi zone; MOJ- Mirdita ophiolites; MOT- T2-J3 Ophiolites (Efusive-sedimentary formation): Ko, Ko1, Ko2-subzones of Korabi zone; MK4 Gjallica subzone; Ao, A1, A2 subzones of Alps zone; K-C1- Cukali subzone; K-C2- Krasta subzone; Kr1- Dajti subzone of Kruja zone.

2. MATERIALS AND METHODS

The regional geological maps of Albania by Nopcsa, (1929), Nowack, (1929), Zuber, (1940), geological maps of Albania at the scale 1:200.000 (1967; 1983; 2002), the Tectonics Map at the scale 1:200.000 (Fig. 1), and the neotectonics maps of Albania (Sulstarova, 2009) illustrate the geologic-tectonic settings of Shkodër-Pejë region. In addition, the aforementioned information is reported in (Sulstarova *et al*., 2011). Detailed geological setting of the Kiraj-Ndreaj-Brashtë in Cukali zone is illustrated in the Geological Map at the scale 1:25.000 (Qirinxhi *et al*., 1983). Frashëri *et al*., (2009) carried out integrated surveys and studies including Shkodër-Pejë fracture zone.

Seismicity of Albania was studied based on historical and instrumental data and the distribution in time and space of the seismic activity in the country and surrounding areas for a period of about 2000 years (Aliaj 1988; Tagari, 1993; Muço, 1994; Sulstarova *et al.,* 2011). The seismic hazard estimation of

Albania which is part of seismological studies provided important geological information about the structure of the Earth crust of Albanides.

The map of gravity Bouguer Anomalies of Albania (Bushati, 1988) and the map of total magnetic vector (T) of Albania (Bushati, 1998) at the scales 1:200.000 helped observe in details two profiles of Shkodër-Pejë. Gravity and magnetic surveys providing information about the density and magnetic properties of the rock were carried out along with detailed geological studies (Qirinxhi *et al*., 1983).

Collected from the paleomagnetic studies in Albania from 1991-1995(Mauritsch *et al*., 1991; 1994; Kissel *et al*., 1992; 1994, and 1995, Mauritsch *et al*., 1995; Speranza, 1995; Frashëri and Bushati 1995), paleomagnetic data show the dynamic evolution of the Albanides.

Geophysical investigation (Aliaj, 2006; Frashëri *et al*., 1991; Frashëri *et al*., 2004; Frashëri *et al*., 2010; Bushati, 1988; 1998; Koçiu, 1989) provided some information about the crystal basement of Albanides. Frashëri *et al*., (2004) investigated the propagation of the thermal field of earth. Marine currents, waves, water salinity and temperature at various depths and heat flow density of earth at the sea bottom in the offshore of Drini Bay, at the south-western part of Shkodër-Pejë fracture zone are reported in (Frashëri *et al*., 2011, Geothermal Atlas of Europe, 1992).

Remote sensing based on LANDSAT and MODIS data was used to identify some regional geological features and analyse the ground temperatures.

3. RESULTS AND DISCUSSION

Applied in north Albania and in the Adriatic Sea, the complex methods are of great value for the depth of Albanides, as it helps to further understand the fracture zone in Shkodër - Pejë region. Figure 2 depicts the block character of the crystal basement of Albanides (Bushati 1988; 1998, Koçiu, 1989; Frashëri *et al*., 1991; 2010). Thickness location of these blocks is shallower in Mirdita Tectonic Zone. The crust construction and their dynamics are reflected in the geological setting of Albanides tectonic zones and their tectonic characteristics. Block structure is controlled both by a system of NW-SE longitudinal faults and transverse faults. Earths local heat makes the transversal faults evident. Figure 1 depicts geothermal energy related to a great heat flow through the aforementioned fractures (Frashëri *et al*., 2004).

In addition, the figure 1 depicts the folded belt of Dinarides-Albanides-Hellenides divided transversally into two parts. The northern part is represented by the western-northern edge of Kruja tectonic zone, the northern part of Cukali zone, the Albanian Alps and Gashi zones where the Dinarides tectonic zones: Budva, High Karst, Dalmate, Durmitor, Serbian, and Golia

zone follow. The southern part is represented by Sazani and Ionian tectonic zones, the southern part of Cukali zone and the Krasta-Cukali, the Mirdita and Korabi tectonic zones which are followed by the Hellenides. This deep fracture is generally considered a multi phase feature of the transversal tectonic faults zone, with a north-eastern extension of about 30^{o}. Consequently, Shkodër-Pejë deep fracture divides two big areas which have different geological settings and background with regard to the continent and orogenic front in the Adriatic Shelf.

The figure 4 depicts the Albanian orogenic thrust front cut and displaced by the Othoni Island-Dhërmi—the north of Sazani Island— and Bay of Drini-Lezha strike-slip faults (Aliaj, 2006). The orogenic front, north of the Drini Bay-Lezha city strike-slip fault, in the Adriatic offshore, belongs to the Kruja zone. In the Tertiary phase, this strike-slip fault functioned as a right pushing, shifted towards the inner zones in the south-west (Sulstarova *et al*., 2011). Ophiolitic belt in the Mirdita tectonic zone is displaced more than 100 km in the south - west direction. Cut and displaced, the front thrust of the orogen was identified in the outer zones, at the transversal Drini Bay - Lezha city (Sulstarova *et al*., 2011).

Offshore seismic surveys carried out at the Montenegrin Adriatic shelf and deep wells (Dragasević, 1983; Picha, 2002) provided further information about the geological setting occurred at the depth on the western edge of Shkodër-Pejë deep thrust in the Adriatic shelf.

Onshore geological surveys which were carried out in the Adriatic coast line Shkodër-Cukal-Tropoja mapped two regional disjunctive tectonics; one regional disjunctive tectonic in the north and one regional disjunctive tectonic in the south borders of Cukali subzone. The later was separated from the Alps and Mirdita zone, respectively (Fig. 1 and 2). Here, the fault zone could be considered as a transformed transversal fault, aligned with the overthrust tectonic in the northern boundary of the Mirdita zone (Kodra *et al*., 1994, Xhomo *et al*., 2002), as a tectonic border between Cukali and Alps zone, or as natural geological border between them (Papa *et al*., 1991). Intensively folded structure of the Cukali subzone has northwest-southeast strike. Surface geological data collected from the Permian rocks of Albanian Alps tectonic zone, to the Upper Triassic - Maastrichtian- Paleocene - Eocene rocks in Cukali subzone, and all magmatic and sedimentary rocks that spread of the Mirdita zone at the surface could not prove the presence of the Shkodër-Pejë transversal fault at the surface of the area (Qirinxhi *et al*., 1983). On the other hand, geophysical studies provided information on the presence of the regional transversal deep fracture through Cukali subzone.

3.1. Gravity and magnetic data

Scattering of gravity and magnetic fields has very well contoured the inner Albanides, particularly the ophiolitic belt. Bouguer gravity anomaly and magnetic anomalies have common peculiarities: i) epicentres located over the eastern belt of the ophiolites; ii) separated by flyschoid corridor, ophiolitic belt is represented by the north and south part and, ii) very intensive gravity and magnetic anomalies compared with Internal Albanides. These peculiarities and anomaly configuration occurred due to the nape character of the ophiolitic belt (Frashëri *et al*., 2010).

Gravity and magnetic surveys carried out by Bushati (1988) reported that the Alps zone is represented by a minimum of Bouguer gravity anomaly in general and a particularly small amplitude anomaly in southern part of the Alps' zone (Fig. 2). In the north, increasing of the intensity of magnetic anomaly is observable. This pecularity of the magnetic anomaly is a means to address the overthrust character of Albanian Alps. Over the Cukali subzone a linear upward trend of the intensity of the Bouguer anomaly toward the Mirdita zone due to vertical deep tectonic fracture could be observed.

Once the inversion model (traditional ones) was applied, fracture level with amplitude of about 4km in the Moho Discontinuity was detected (Fig. 2). Geological mapping illustrates fracture amplitude gradually reduced towards Earth surface where it almost extinguishes. This deep disjunctive tectonic represents Shkodër-Pejë fracture. Similarly, investigations with regard to the deep strike-fault of the Adriatic shelf crust report that the Shkodër-Pejë deep fracture at the depth might consist of several branches, occupying a wide zone of influence and action. Shkodër-Pejë fracture is parallel with other transversal deep fracture of the Albanides such as the Dibër-Elbasan-Vlorë transversal deep fracture.

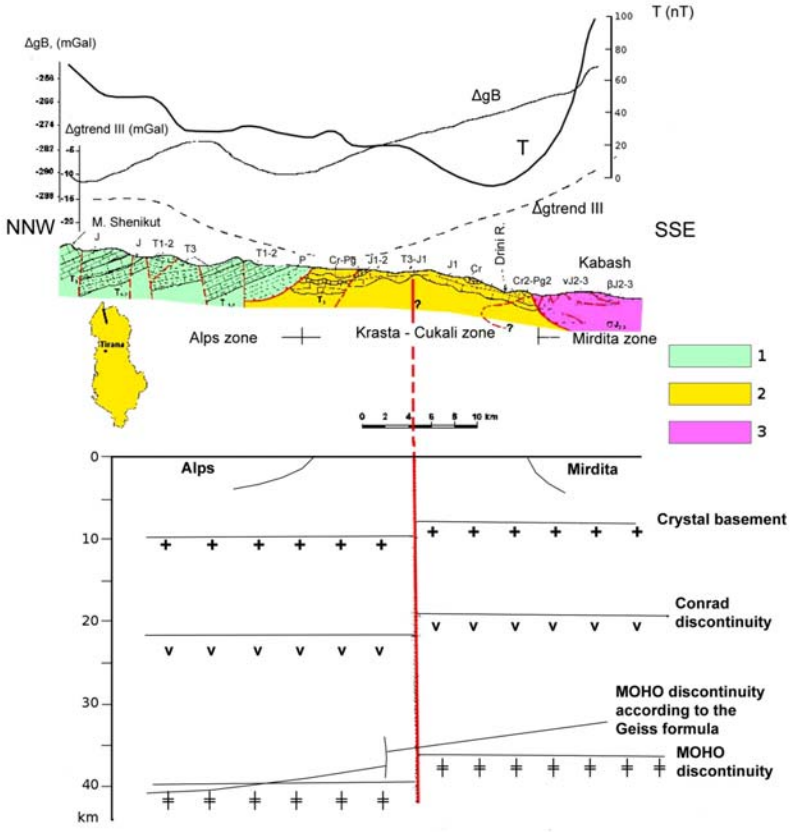

Fig. 2. Gravity Bouguer Anomaly (Δg) and Magnetic Anomaly (T) profile I-I, Mountain Sheniku at the Albanian Alps-Kabash in Mirdita zone, and gravity inversion results.

3.2. Paleomagnetic surveys - an important indicator of the presence of Shkodër-Pejë deep fracture and its geodynamic evolution role in Alpine Mediterranean Folded Belt Hellenides-Albanides-Dinarides

Dynamic evolution of the North Albanides has been of great impact for paleomagnetism of the north region. Many paleomagnetic studies have been carried out in Albania (Mauritsch *et al*., 1991-1995; Kissel *et al*., 1992; Muttoni *et al*., 1940; Frashëri and Bushati 1995; Frashëri *et al*., 1995; Kissel *et al*., 1995; Mauritsch *et al*., 1995; 2006; Mauritsch, 2000; Muttoni *et al*., 1994; Speranza 1995). Paleomagnetic studies reported that, located in the south side of the Shkodër-Pejë transversal zone, Ionian and Kruja tectonic zones have supported a joint clockwise rotation, with an angle of about 45-50^{o} during and after Eocene-Oligocene period. This rotation occurred in two

phases; by 25^{o} in each phase in the middle Miocene up to Plio-Pleistocen. Ionian and Kruja zones don not have any different rotation between each-other. Kçira site is characterized by a 40^{o}-45^{o} clockwise rotation occurred since Early-Middle Miocene. For this rotation, the Kçira pole acquired a West Gondwana affinity (Muttoni *et al*., 1996). A large Neogene clockwise rotation, D=40^{o}, I=3.8^{o} has occurred in: i) the Mirdita zone of Inner Albanides, ii) southern Albania and, iii) in north of Albanian-Greek border (Mauritsch *et al*., 1994).

Eocene limestone anticlines of the Renz and Kakariq area, which are located in the south of the Shkodër-Pejë transversal zone, have a rotation of about 31^{o}. Consequently, these two anticlines have a declination with 18^{o} smaller than the declination of the Eocene limestone in the Central Albania. These two anticlines might have superposition of two rotations with inverse sense: clockwise rotation of 50^{o}, which has been subdued all External Albanides structures and local counter clockwise rotation by 25^{o}, which has rotated only these two anticlines that have a Dinarides strike.

Declinations showing the 82^{o}-140^{o} clockwise rotation have been observed at the Komani area, in the southern side of Shkodër-Pejë transversal, at north-western edge of the ophiolitic belt of Mirdita zone. The same 28^{o}-57^{o} clockwise rotation is observed for ultra basic rocks of western edge of the Gomsiqe massive, in the south-western direction from Komani (Fig. 3). Gabbro massive at Bozhaj, in the south of Korça, have a magnetization vector with declination Do=282^{o} and inclination Io=60.9^{o}, the same direction as in the Khalkidhiki in Greece (D=240^{o}-312^{o} and I=30^{o}-68^{o}) demonstrating an Upper Cretaceous anti-clockwise rotation and Upper Tertiary clockwise rotation of the Khalkidhiki (Feinberg *et al*., 1996).

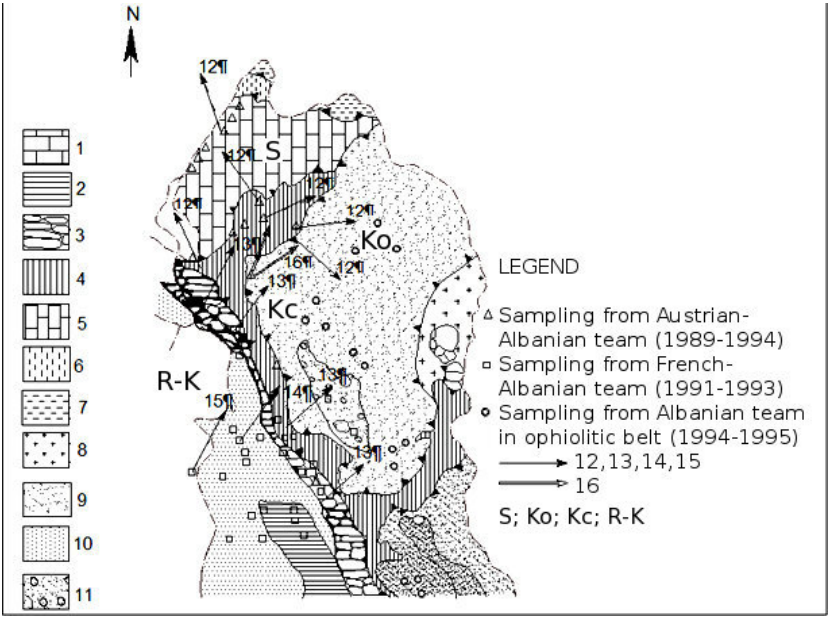

Fig. 3. Scheme of the paleomagnetic echantionage in Northern Albania, during 1989-1994.1-Sazani Zone; 2-Ionian Zone; 3-Kruja Zone; 4-Krasta-Cukali Zone; 5- Albanian Alps Zone; 6-Vermoshi Zone; 7- Gashi Zone; 8-Korabi Zone; 9-Mirdita Zone; 10- Periadriatic Depression; 11-Mollasic depressions; 12- Jurasic limestone; 13- Upper Cretaceous-Eocene limestone: 14- Middle Neogene molasses: 15- Pliocene formation: 16- Ophiolite.

Limestone samples from Albanian Alps at Selca area, in the north of Shkodër - Pejë transversal, shows a counter clockwise rotation of 20^{o} in relation with the actually north direction, the same value as in south of Dinarides‘ structures. In addition to the Selca area, analogue counter clockwise rotation occurred in the Jurassic limestone, at the south of Shkodra lakeshore, showing that both sections belong to the same tectonic zone—the north of Shkodër-Pejë transversal area.

The surface area from Kotorr to Split, (which belongs to the Dinarides in Dalmatia in the north of Albanides) has been almost immovable, since Eocene. In the north, Dinarides’ orogen is characterized by regional direction of the structures N12^{o}, unlike the Albanides orogen structures with a direction N150^{o}, such as Hellenides in the south (Kissel, 1995; Mauritsch *et al*., 1993; Mauritsch *et al*.,1995; Mauritsch *et al*.,2006). Paleomagnetic studies carried out in the external Dinarides reported that this orogen has no significant counter-clockwise rotation in relation to Africa which dates since Eocene. Such a small rotation is observed in the limestone of the Albanian Alps, in the south shore of Shkodra Lake.

Paleomagnetic directions reported a strong tectonic disturbance in the centre of Shkodër - Pejë zone. A country-clockwise rotation of about 45^o could be observed at the Jurassic limestone, in the north of Cukali subzone located 4 km in the northwest direction of Prekali village. Only in less than 2 km south, a 60^o clockwise rotation is observed. As a result, a strong tectonic impact in the transversal thrust zone could be met.

Paleomagnetic studies reported that the Shkodër-Pejë belt represents a transition zone between counter-clockwise rotation in the north, and clockwise rotation in the south (Fig.3). Consequently, tectonic influence over Cukali subzone is substantial. Thus, Shkodër-Pejë lineament defines a transition zone which separates the Albanian Alps and Dinarides (counter clockwise rotation) from Albanides and Hellenides (clockwise rotation). For the rotation pole located at Shkodër-Pejë transversal fracture, southern Albania has undergone a horizontal displacement of about 173 Km (Speranza, 1995).

3.3. Seismological peculiarities related to Shkodra-Peja seismo-active transversal fault zone

Seismological studies carried in the last 50 years in Albania have mapped seismoactive longitudinal and transversal deep tectonic fault zones of the Albanides such as the Shkodër-Pejë transversal fault zone. (Aliaj, 1988; Muço, 1984; 1991, Sulstarova *et al*., 2011). which divided the northern part of the Ionian-Adriatic thrust fault zone. The later represented the longest fault zone along the Adriatic and Ionian coast comprising two segments. Based on focal mechanism solution, in northern littoral side of the Shkodër-Pejë fault zone, the compression strain has a P=16^0 NE-W strike, and 10^0 plunge, while the axis of expansion T = 124^0 and 79^0 dip. In the south of the Shkodër- Pejë fault zone, the compression strain has a P=274^0 E-W strike, and 10^0 dip, and the axis of expansion T = 164^0 SE-NW and 64^0 dip (Sulstarova, 1988; Tagari., 1993). In the internal area, the Vau i Dejës-Pukë-Tropojë region and the tensional stress regime has a NW-N extension (Sulstarova, 1988; Muço, 1994).

Offshore geophysical explorations revealed a zone of deep fractures in the Adriatic Sea located between Buna River discharge and Kotorri estuary, approximately 15-20 km from coastline and with a NW strike direction. The focus of the earthquake occurred in April 15, 1979 was located in this Adriatic offshore area, near the Albanian-Montenegrin border, at the northern the Shkodra-Peja fault zone (Sulstarova *et al*., 2011). The analysis of the seismic P waves' velocities (Vp) and Vp/Vs ratio reported the difference between the upper parts of the Earth crust (0-30 km), in the both sides of the Shkodra-Peja fault zone (Ormëni, 2010).

The stress field, the fault system and the spatial distribution of the seismic activity show that the Albanian territory and the surrounding areas are constructed by many blocks which move relatively towards each-other due to the collision of the Eurasia and African great plates in the region of the Adriatic promontory of African plate (Sulstarova *et al*., 2011). All the aforementioned seismological features prove that Shkodra-Peja transversal presents a deep fracture, a relatively wide active fault zone that separates the paleogeographic units with different geological settings and the dynamic of their development as well.

3.4. Heat Flow density anomalies

Geothermal studies at Northern Albania have been performed in 19 boreholes and one deep well. Figure 1-b depicts the axes of the anomalies of heat flow density. Epicentres of the two anomalies are located on the same axis, which have a northeast - southwest extension on the overthrust tectonics in the north border of the ophiolitic belt. The third axis is located in Keçel village in the east of Kukësi ultrabasic massif. The heat flow density values go up to 60-70 mW/m^2. Radiogenic heat generation of the ophiolites is very low. Under these conditions, increase of heat flow in the ophiolitic belt relates to the heat flow transmitted from the depth. Having radiogenic heat generation, granites of the crystalline basement, represent the heat source. Heat flow anomalies are conditioned by intensive heat transmitted via deep and transversal fractures. Comparing these characteristic of the thermal field and the geological setting of the zone with the structure of the strike-fault system in southwest of the Shkodër-Pejë transversal in Adriatic Shelf, this thermal anomaly might be associated with the branches of the transversal fracture.

Figure 1-b depicts the thermal anomaly over the Adriatic shelf area occurred at the epicentre of the Drini Bay (Geothermal Atlas of Europe, 1992), over the prolongation inside the Albanian Adriatic Shelf of Shkodër-Pejë transversal deep fracture proving that the Shkodër-Pejë transversal deep thrust continues even in the Adriatic Shelf where it is interrupted by the Adriatic longitudinal strike-slip fault system.

3.5. Remote sensing and GIS information

First investigations involving Landsat imagery presented a sketch of tectonic thrust of the northern Albania, which made the main segments of Shkodër-Pejë transversal fault distinguishable (Chorowich *et al*., 1981).

GIS and seismological investigations, determined the velocity of microplates movement in the Adriatic Sea. Nubia (Africa) microplate is moving NW with respect to Eurasia with a velocity of 6 mm/yr, while the

Adria microplate moves NE at a rate of 4-5 mm/yr (Battaglia *et al*., 2004). Adria is considered as an independent microplate within Nubia–Eurasia plate boundary one (Nocquet *et al*., 2001) which is divided by the Gargano-Dubrovnic fault into two blocks (Oldow *et al*., 2002). Here, defining the position of the NW edge of Otranto channel which is located in the direction of the Shkodër-Pejë deep transversal fault in the eastern Adriatic Shelf that resulted parallel with Gargano–Dubrovnic fault zone.

Vegetation coverage of the area and soil coverage of rocks were barriers for the identification of geological structures via satellite imagery. However, combining different bands from Landsat helped distinguish two areas separated during the same delineation of Shkodra–Peja fault (Figure 4).

Ground temperature profile obtained from MODIS images was analysed calculating the average temperature for day and night from 24 images spanned in the period 2004–2006. A "bridge" of relatively higher temperature delineated between Shkodra and Peja could be met.

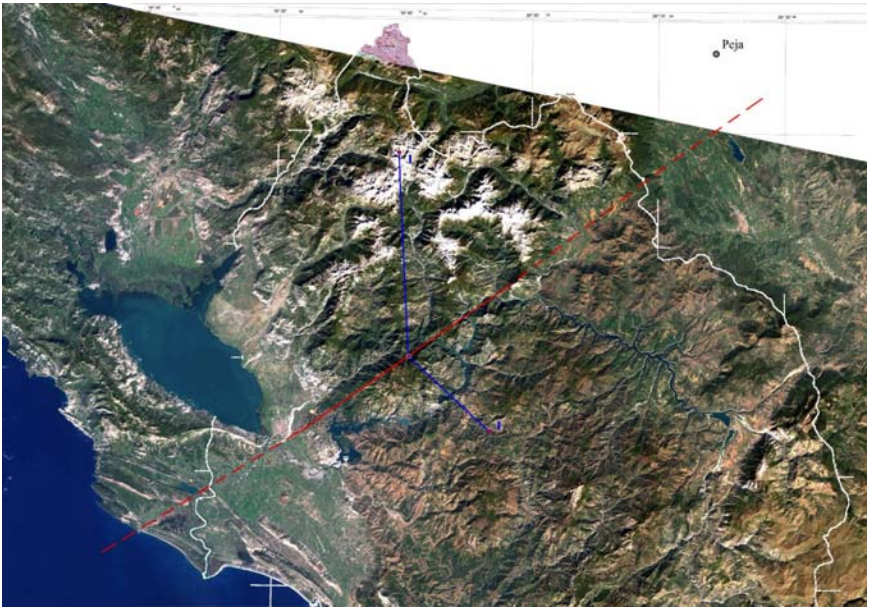

Fig. 4. Satelite image of Shkodër-Pejë transversal fault at Northern Albania.

4. CONLUSIONS

Earth Crust of the Albanides exhibits a block structure controlled by a system of NW-SE longitudinal faults as well as transverse ones. The blocks have different thickness.

The inversion of gravity data reported that Shkodër-Pejë zone presents a vertical deep transversal fracture which separates the two Earth crust blocks from each-other. The fracture which represents a seismically active belt interrupts the MOHO Discontinuity with amplitude of about 4 km that decreases towards the Earth surface. Satellite images reported that this vertical fracture outcropped throughout the Cukali subzone.

Paleomagnetic studies reported that margin assemblage in the Albanides encountered a clockwise rotation of about 45^{o}, after upper Oligocene. Shkodër-Pejë transverse fault represents a transition zone between the clockwise rotation of the Albanides and Hellenides and the counterclockwise of Albanian Alps and Dinarides. Regarding the rotation pole located at Shkodër-Pejë transverse fault, the horizontal displacement is about 173 Km in southern Albanian border.

Shkodër-Pejë fracture of the Albanian Adriatic shelf in Drini Bay continues over the epicentre of the heat flow. This correlation argues relation of the geothermal anomaly with depth fractures of the Earth Crust in Adriatic Shelf.

Shkodër-Pejë deep transversal fracture; represent the contact between the African and Eurasian plates, which interrupts transversally territory the Albanides folded belt.

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APPLICATION OF DPSIR FRAMEWORK TO A MEDITERRANEAN TRANSITIONAL WATER BODY: THE BUTRINT LAGOON, SOUTHWEST ALBANIA

Lavdie MOISIU
Albanian Geological Survey, Tirana, Albania
Vasilios KAPSIMALIS
Hellenic Centre for Marine Research, Institute of Oceanography, Greece
Çerçiz DURMISHI
Polytechnic University of Tirana, Faculty of Geology and Mining
Christos ANAGNOSTOU
Hellenic Centre for Marine Research, Institute of Oceanography, Greece

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ABSTRACT

Tectonic in origin, Butrinti Lagoon is of an ecological and economic importance (Ramsar Site). In addition, it is surrounded by forested hills, mountains, freshwaters and brackish marshes (Vurgu field in the north, Cimikos hill in the north-east, Bufi hills and Bufi Lake in the east, and Butrint and Ksamil National Park in the west). Sometimes it is called Lake Butrint due to its large surface area and its depth (maximum, 22 m; average, 14 m). Unfortunately, the Lagoon suffers from anthropogenic impact. In the present investigation, a new Drivers-Pressures-State-Impacts-Responses (DPSIR) model was applied to provide a holistic analysis of the cause-effect relationship between the components, which interact in social, economic and environmental structures and functions. Much important research has been carried out in the area with subsequent publication of many noted papers very helpful for the present investigation. The information provided was re-arranged into the DPSIR components. Results reported wetland reclamation; fishery, commercial aquaculture farming, and water pollution reduced physical, chemical, and biological properties of the Lagoon. Consequently, negative ecological and social economic impacts, i.e., dystrophic crises, reduction of fishery and mussel production, ecological quality degradation, biodiversity loss and reduction of aesthetic value of the landscape were unavoidable. Given the importance of the Lagoon and the consequences of anthropogenic impact, friendly environmental solutions like a reduced use of fertilizers and pesticides, adoption of biological practices on agricultural crops, discharge into the lagoon of well-oxygenated water of adjacent rivers, preservation of

the Butrint marshlands, and control the unregulated urban construction are crucial and of immediate importance along with public awareness.
***Keywords*:** environmental management; DPSIR approach; Butrinti Lagoon; Mediterranean

1. INTRODUCTION

Over the last decades, significant deterioration of coastal areas has been accomplished due to increasing human intervention (GESAMP, 1987; Islam and Tanaka, 2004). Intensive agricultural, industrial and urban activities supply the coastal and aquatic systems with contaminants, such as persistent organic pollutants, nutrients, hydrocarbons, radionuclides, heavy metals, debris etc. (Driscoll *et al*., 2003). These releases damage the quality of surface water (rivers, lakes, lagoons etc.), groundwater (aquifers) and aquatic life. Consequently, they threaten the biodiversity of the Lagoon.

Accurate control of good environmental quality is considered as a high priority for conservation and sustained management of the existing natural resources (Elliott, 2002; Scheren *et al*., 2004). Nevertheless, complicated interactions, conflicts and constraints between stakeholders do not allow the application of effective management strategies (Borja *et al*., 2006). Moreover, gaps in scientific knowledge regarding local ecosystem processes and inappropriate policies have been a continuous obstacle for appropriate environmental solutions.

In Albania, natural resources have been extensively exploited from 1950 to 1990. No environmental criteria for preservation, maintenance and balanced utilization of natural resources and biological diversity, in accordance with the principles of sustainable development was followed. Consequently, 30% of the country's area deteriorated due to agricultural waste, industrial effluents and urban sewage. In the early 1990s, Albanian environmental legislation law was amended. However, environmental situation couldn't improve and in some cases it aggravated (REC Country Office in Albania, 2000). Environmental issues such as inland waters, waste treatment, biodiversity conservation, forest protection and coastal zone development remain top-priority of all the institutions involved in the area.

The present investigation aims at providing information about effective management strategies for the Butrinti Lagoon as they are a means to address improvement of environmental situation and the sustainable development of the Lagoon and surrounding area. Pano *et al*., (1984) provided information about the biogeochemical processes characterizing the Lagoon. Koutsouris *et al*., (2004) investigated the socio-economic parameters. Martin (2001) and ASPBM (2010) have currently drafted two Management Action Plans for the development of the Butrint National Park. Recently, the Ministry of

Environment with its Decision 404/20.06.2012 has approved a new Management Plan and has appointed as responsible for the implementation of Plan the Administration and Management of Butrint Office. One of the most urgent requirements, pointed out by the Management Plan, is the identification and application of detailed management strategies for each habitat type (alluvial plains, upland and foothill areas, marshlands and coastal areas). The sustainable rehabilitation of wetlands and forest areas is of immediate importance for the environment.

The present paper evaluates all the information provided with regards the Butrint Lagoon and its surrounding wetlands, and re-arranges it according to the holistic Driver-Pressure-State-Impact-Response (DPSIR) approach identifying indicators for habitat enhancement of the Lagoon.

2. The Study area

The Butrint Lagoon is an elongated basin with an average length of 7 km and width of 3 km. The surface is some 1600 ha and the maximum depth reaches 22 m. It joins Ionian Sea (eastern Mediterranean) through the nature channel of Vivari, which is 3.6 km long, 100 m wide and 6 m deep. The lagoon is surrounded by the Vurgu Plain to the north, the Mile Mountain to the east, the Vrina Plain to the south and the mountainous Ksamili Peninsula to the west (Figure 1).

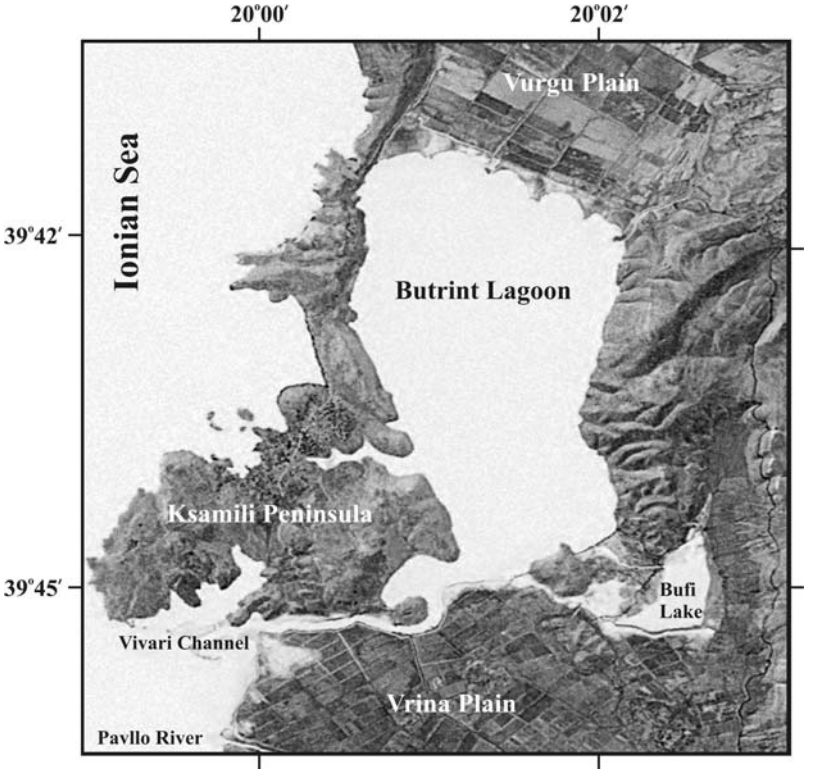

Figure 1 Location map of the Butrint Lagoon.

The climate is characterised by dry summers and mild winters, whilst the average rain precipitation is approximately 1500 mm/yr. The Butrint Lagoon is characterised by low tide amplitude (<30 cm) and the water regime is typical of coastal lagoons (Negroni, 2001). Freshwater enters the Lagoon from the Bristica and Kalasa Rivers located in the northern part, and the Pavllo River and the Bufi (or Rreza) Lake located in the southern part.

Butrinti is mentioned for its outstanding biodiversity, including a plenty of plants, insects, fishes, amphibians, birds and mammals that are considered as endangered, rare or insufficiently known species (Miho *et al*., 2013). The Lagoon and the Vivari Channel support extensive reed beds, saltwater marshes and riparian woodland (ASPBM, 2010) (Figure 2).

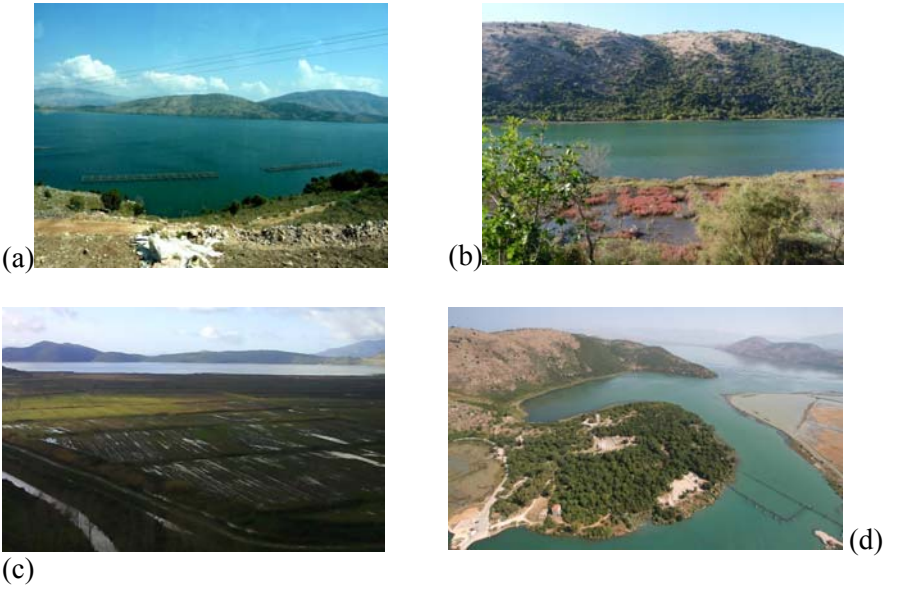

Figure 2 Photographs from the Butrint Lagoon and its surrounding areas showing: (a) the landscape of the lagoon with mussel beds; (b) wetland vegetation; (c) the irrigation network of Vurgu Plain; and (d) the irrigation network of Vrina Plain discharging into the Vivari Channel

Butrint is an area of high archaeological importance. In the south-western shore of the Lagoon, there are the ruins of Buthrotum, one of the main maritime and commercial centres of the ancient world. Another two important archaeological sites (Diaporit and Kalivo) are located in the coastal zone of the Lagoon (Martin, 2002). In addition, submerged remains have been reported on the bed of the Lagoon and Vivari Channel (Hounslow and Chroston, 2002; Hounslow and Chepstow-Lusty, 2004).

3. MATERIALS AND METHODS

The DPSIR framework is a conceptual model for analysing environmental problems, with regards to sustainable development. It provides helpful insights on the relationships between the origins and consequences of environmental problems and, at the same time, helps to understand their dynamics by addressing the links between D-P-S-I-R elements (Figure 3).

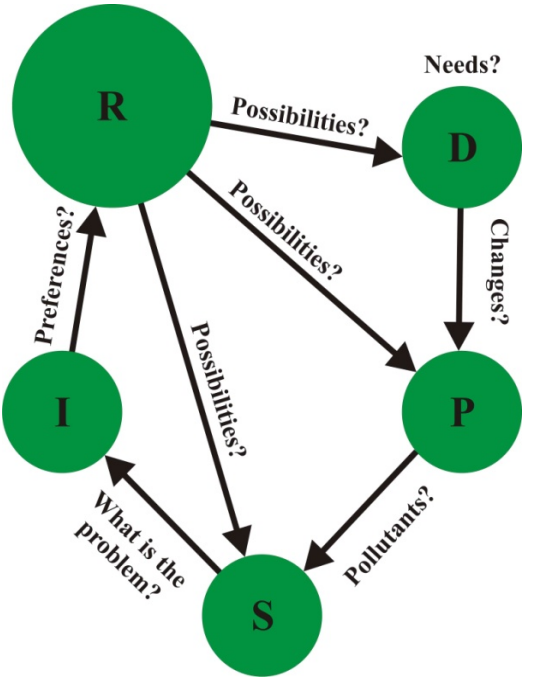

Figure 3 Components of the DPSIR framework and their interrelationships (modified from EEA, 1999)

The DPSIR scheme adopted by the European Environmental Agency (EEA, 1999) is the refined and extended version of the Pressure-State-Response (PSR) model developed by the Organisation for Economic Co-operation and Development (OECD, 1993). Turner *et al*., (1998) and Elliot (2002) developed further the DPSIR to the concept of coastal zone and marine management, respectively.

The driving forces represent large-scale human needs (e.g. for energy, fisheries, land use, transportation) and natural factors (e.g. climate, topography) that in turn cause pressure on the environment (e.g. land conversion and reclamation, wastes disposal). These pressures change the state of the environment (e.g. water quality, nutrient concentration, habitats) that has a significant impact on human welfare (e.g. water-born diseases, decrease of fisheries productivity). The adverse impacts can be mitigated by responses or actions that control socio-economic drivers and consequent environmental pressures creating a continuous and dynamic cycle with feedback loops (Ledoux and Turner, 2002; Walmsley, 2002).

4. DPSIR framework analysis for the Butrinti Lagoon

The main socio-economic activities, associated with the Butrinti Lagoon and its coastal zone, are mainly tourism, aquaculture, fishing and mussel cultivation.

4.1. Driving forces

The total population of the Butrint region numbers approximately 26,000. Butrinti Lagoon is surrounded by Saranda, the largest city and the villages of Ksamil, Blerimasi, Pllaka, Dritasi, Shën Delli, Vrina, Xarra and Mursia. Over the last three decades, a rapid increase of population growth occurred in the area, mainly due to internal immigration. With a population of 14,500 inhabitants; 30% more than 35 years ago (10,900, in 1979), is the economic and commercial centre of the region with an international port that links the city with the Corfu Island (Greece). It is an important holiday destination for national and foreign (mainly Greeks and Italians) visitors. The village of Ksamili was established in the 1970s in order to host workers of the local co-operative farms. Today, there are 4000 permanent inhabitants, employed mainly, with tourism, fishing and mussel cultivation.

An important sector of the local economy is tourism (Bego and Malltezi, 2011). The archaeological site of Butrint is an attractive destination for tourists. Since 2000, about 20,000 people per year visit the area, mainly, between April and October. In addition, eco-tourism is another developing activity, focusing on bird watching, flora tours and guided walks. Albanian state institutions give special emphasis on the tourist utilization of the region, encouraging small and medium private enterprises to invest in facilities and services. Although the main tourism services (hotels, restaurants, tour agencies) are focused in Saranda, Ksamili provides a sufficient tourism infrastructure, providing an alternative holiday residence for domestic tourists.

Agriculture and livestock rising, although less intensive than in the past, still remains important activity of the local economy. Crops, olive grooves, citrus, vegetables and vineyards are the main agricultural products of the region, cultivated on the Vurgu and Virna Plains. The northern and southern flatted areas of the Lagoon coastal zone are used as pasture, which has been overgrazed partly by goats, cows and sheep.

Fishery is an important activity in the Lagoon and the Vivari Channel. The main commercial fish are grey mullets (*Chelon lobrosus* Risso and *Mugil cephalus* L.), eels (*Anguilla anguilla*) and see breams (*Sparus aurata* L., *Sparus pagrus* L. and *Sparus caeruleostictus* Valenciennes). The fleet consists of 20 small boats, but many fishermen work without a licence. The annual

fish catch ranges between 65 tonnes (in 1986) and 120 tonnes (in 1990). 40 percent of fish is exported to Greece and Italy (Peja *et al*., 1996).

Bivalve farming started in the early 1960s for the production of the mussels (*Mytilus galloprovincialis*). In 1980s, a profitable aquaculture industry was established with more than 250 employees (most of them settled in Ksamili). Fixed mussel beds were constructed in the southern and western part of the Lagoon, covering an area of some 10 ha. In 1989, the mussel production reached a maximum of 5000 tonnes/year. However in 1990s, it fell down because of a ban on exports imposed by the EU. Currently, sporadic efforts have been made to put into operation a part of the mussel beds, mainly for the local market.

4.2. Pressures

During the last decades, Saranda and Ksamili have been rapidly expanded. The new built areas have a chaotic structure, with the basic urban infrastructure (street-plan, electricity, water supply and sewage network) not to be priority. In particular, Manastiri – Ksamili segment has expanded without any infrastructure plan onto the hilly coastal zone of the western part of the Lagoon. A few years ago, a significant amount of the domestic sewages were discharged directly into the lagoon, whilst solid waste was dumped just outside of the village. In addition, litter from the surrounding villages and agricultural activities are dumped untreated in the Vurgu and Vrina Plains.

The development of the cultural- and eco-tourism does not have any significant impact on the environment of the Lagoon. However, uncontrolled and illegal construction of tourist settlements in Manastiri –Ksamili has damaged the landscape and degraded the aesthetic values of the western coastal zone of the Lagoon. In addition, some sport activities, such as water skiing and jet skiing, disturb the sensitive habitats of the ecosystem.

Illegal and uncontrolled pastures, intensive quarrying, intentional or unintentional fires of forests and agricultural residues produce emissions of organic or inorganic contaminants entering by the atmosphere, runoff and groundwater pathways into the lagoon (ASPBM, 2010).

In 1959, an extended irrigation network was developed on the Plains and the Bistrica River, flowed previously into Butrint Lagoon, was diverted into the Saranda Bay. As a result, an uncultivated area of some 3000 ha, including 1300 ha of wetlands, was reclaimed for agricultural purposes, i.e. crop production and grazing.

Before the 1990s, excessive use of pesticides and fertilizers lead to significant concentration of pollutants in the alluvial plains and lagoon waters. Nevertheless, over the past decade, fertilizer and pesticide consumption have seriously declined; in particular, the average fertilizer use was 158 kilograms of active ingredients per hectare in 1989, 135 kg/ha in 1990, 38 kg/ha in 1991,

and 13 kg/ha in 2000, whilst the average pesticide use dropped approximately 60%.

Recent dredging activities within the irrigation channels, which discharge freshwater into the Lagoon, were undertaken after siltation of their mouths. In addition, routine dredging of the Vivari Channel allows a steady exchange of water masses between the Ionian Sea and the Butrint Lagoon.

Although the formal use of fishing methods does not have affect negatively the state of the lagoon system, illegal methods, such as poison and dynamite, damage the quality of the water and diminish the fishing resources. Leakage of fuel and oil of fishing boats are significant pressures that aggravate the Lagoon.

From 1970s to 1980s, mussel cultivation suffered from increased consumption of diluted oxygen and significant accumulation of organic matter within the lagoon. In addition, construction of cement mussel beds (some 10 ha) and fixing of their bases on the lagoon bed is likely to disturb the benthic community and the surface sedimentary pattern (Figure 4).

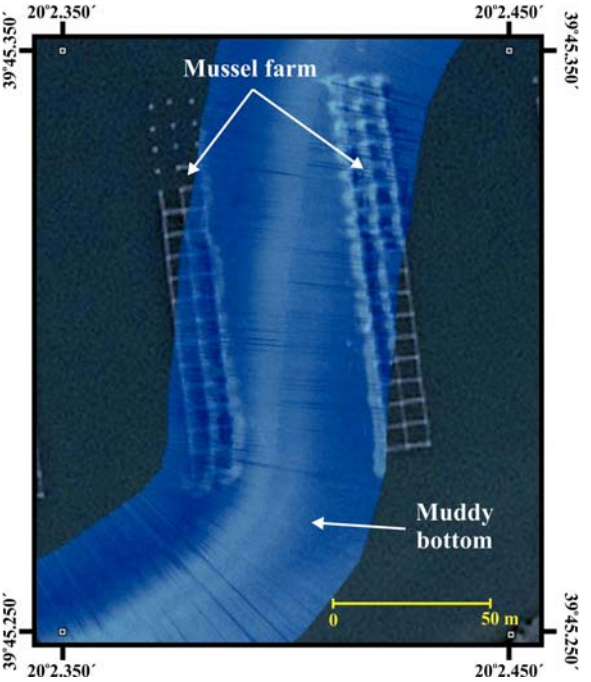

Figure 4 Side scan sonar imagery derived by the southern part of Butrint Lagoon during July 2012 showing the disturbance of soft bottom habitat caused by bases of mussel beds. The background image comes from Google Earth

Since the early 1990s, many of mussel bed structures have been abandoned due to a lack of market demand (Martin, 2002), degrading visually the landscape (Figure 2a). Accumulation of mussel shell debris on the coastal zone, as a result of mussel treatment by individual farmers, has caused an artificial progradation of the shoreline.

4.3. State

Lagoon waters are characterised by mesotrophic conditions, with some seasonal eutrophic peaks (Bushati *et al*., 2010, 2012; Osmani and Peja, 2010; Çako *et al*., 2013; Kolitari *et al*., 2013). The water column is divided into two layers: i) the upper layer, located at depths from 0 to 7.5-8 m, which is characterized by relatively high dissolved oxygen concentrations (2-9 mg/l) and can be considered as oxygenated water body and, ii) the lower layer, in which oxygen is negligible or absent. Consequently, hypoxic or anoxic conditions are dominant. The absence of dissolved oxygen from deeper waters is accompanied by the production of H_2S, with concentrations varying between 10 mg/l and 35 mg/l. The salinity fluctuates from 18.00 ppt in winter to 35.00 ppt in summer, whilst water temperature varies from 14ºC in winter to 25ºC in summer. The organic matter is about 2-10 mg/l (Pano *et al*., 1984; Peja *et al*, 1996). The concentrations of Cd, Cr, Cu, Hg and Pb in the lagoon water are relatively low, but in some locations Cd and Pb exceed the EU standards due to agricultural activities and urban effluents derived by Ksamili (Topi *et al*., 2012; 2013). The presence of organochlorine pesticides (OCPs) in the water is also relatively low with values ranging from 7.3 ng/l to 30.7 ng/l (Nuro and Marku, 2011)

The surficial sediments are characterised as clays in the lagoon (Tsabaris *et al*., 2007; Ariztegui *et al*., 2010), and muddy sands in the Vibari channel. The maximum concentrations of some heavy metal in sediments are relatively high (e.g. Cd is 0.443 ppm, Cr attains 145.4 ppm and Pb reaches 63.4 ppm), implying human-derived contamination (Topi *et al*., 2012).

The Butrint Lagoon and its adjacent wetlands are mentioned for the rich flora and fauna including a large number of globally endangered species (Bego and Malltezi, 2012). The area shelters the highest biodiversity in Albania with approximately 17% of the country's species richness. Butrint wetland is an important bio-geographic region for amphibians, reptiles, bird and mammals. In the lagoon, 49 taxa of benthic macroinvertebrates have been detected and its ecological quality is characterized as "moderate" (Boncagni *et al*., 2010-2011). Phytobenthos communities are limited and grow in shallow water areas near the Vurgu and Vrina plains, in the northern and southern part of the lagoon, respectively, as well as in the Ksamili bay in the western part of the lagoon (Miho *et al*., 2013).

4.4. Impact

The geochemical and ecological conditions of the lagoon have changed significantly due to human activities over the last 50 years. As a result of wetland reduction, a number of Globally Endangered Species have been lost or became very rare (Bego *et al*., 2012). In addition, the water and bottom sediments of lagoon as well as soil of arable plains were enriched by pollutants, which were derived by the overuse of pesticides and fertilizers (Topi *et al*., 2012).

Once the Bistricë River diverted, the salinity in lagoon waters increased. Although this increase led to a considerable biodiversity loss, a great development of mussel cultivation was favoured (Demiri *et al*., 2014). However, the limited freshwater discharges in the lagoon and the intensive farming of mussels caused a significant increase of the organic matter. The surplus of organic matter is decayed by sulphobacteria producing high levels of sulphuric gas (H_2S) and consuming the available diluted oxygen. Such conditions create anoxic episodes, which are resulted in massive death of aquatic organisms. These crises in mussel farming and fish stock were observed during summers of 1979 to 1983, 1987, 1992, 1994 and 2012 (Miho *et al*., 2013). Particularly, in 1992, an anoxic episode destroyed the entire mussel production and in 1994, the EU imposed a ban on exports for sanitary reasons. Except of the above dystrophic crises, the discharge of wastewater from urban and rural areas into the lagoon favours the presence of *Escherichia coli* in lagoon water (Sulaj *et al*., 2009) as well as *Listeria monocytogenes* which detected in live mussels for first time in 2008 (Memoci and Sulaj, 2011; Sulaj *et al*., 2012).

4.5. Response

The highest governmental body responsible for environmental issues of Albania is the Council of Ministers. The functional jurisdiction of Butrint lagoon is divided between different public administrative bodies such as the Ministry of Urban Development and Tourism; Ministry of Environment; and Ministry of Agriculture, Rural Development and Administration of Waters. A very important decision-making body joining representatives of the above ministries, international organizations and NGOs is the National Board of Butrint Park.

The National Environmental Action Plan (1993) was the first step towards an integrated environmental strategy and protection measures in Albania. In addition, the Integrated Water and Ecosystem Management Project (2003) proposed feasible policies for the reduction of sewage pollution, and the protection and restoration of endangered coastal and marine habitats. In cooperation with the national policy, international organizations (United Nations, European Union, World Bank etc.) have financed many programs on

the Albanian environment. For the Butrint National Park, focused Management Plans (ASPBEM, 2010) were drawn up identifying the main issues that threaten the area and still hamper effective management.

Responses taken for the conservation and/or management of the Butrint are dated since 1948, when the ancient city of Buthrotum was designated as a Cultural Monument. In 1992 and 1999, UNESCO declared the Butrint as a Word Heritage Site; whilst in 2000, the Albanian Government characterised an area of 2900 ha as a National Part. In March 2003, Butrint became a designated site (No 1290, Wetlands International Site Reference: No 3AL002; total site area: 13500 hectares; Ramsar criteria: 1, 2, 3, 8). In July 2005, UNESCO at its 29th session of the World Heritage Committee decided to remove Butrint from the List of World Heritage in Danger (Decision 29 COM 7A.27). In 2013, the Albanian Council of Ministries with its Decision extended the boundaries of the Butrint Natural Park to a total surface area of 9424 ha. However, more efforts should be undertaken by stakeholders, e.g. politicians, users, scientists and NGOs, to solve the problems identified by the assessed impacts (Bego *et al*., 2012). Any decision taken should be environmental sustainable, technologically feasible, economically viable, socially desirable, legally permissible and administratively achievable (Elliot, 2002). Under these requirements, we recommend, indicatively, the following policy measures and planning actions, which could mitigate or even reverse the adverse effects on the environment of the Butrint Lagoon: The reduction of contaminants entering the lagoon could be accomplished by minimizing the use of fertilizers and pesticides for agricultural purposes, and/or cultivating biological crops on the Vurgu and Vrina Plains. In addition, proper treatment and management of urban waste should be realized by the communes of Ksamili, Xarrë and Aliko.

Dystrophic crises in mussel farming and fish stock could be eliminated by the controlled discharge into the lagoon of well oxygenated water of rivers Bistrica and Pavllo. Moreover, restrict regulations, in accordance with the provisions of the Law on “Fisheries and Aquaculture”, on mussel cultivation and fishing should be established.

Biodiversity loss is likely to slow down or even reversed by the maintenance of fresh and brackish water marshland surrounding the lagoon. However, this policy should be acceptable by local stakeholders, and thus a great effort is required by the authorities to inform and induce people (e.g., farmers, landowners, inhabitants, etc.) for the necessity of the wetland preservation.

The aesthetic enhancement of the Lagoon could be come through the removal of the majority of hard constructions, i.e. the mussel beds from the lagoon, and the controlled expansion of the village of Ksamili through an official town plan. Nowadays, a coordinated action was undertaken by the

Albanian government and local authorities for demolishing about 200 illegal constructions. This project is still in process for all objects constructed after the entry into force of the Law on legalization of illegal buildings. The success of this effort depends on the willing of local municipalities and the respect of users in the law. Urban Territorial Plan remains the main legal instrument that can result in the sustainable development of the area.

5. CONCLUSIONS

The DPSIR conceptual model is used in the Butrint Lagoon and its catchment in order to recognize, quantify and predict the effects of human activities on the "naturalness" of the system.

After the Second World War, the need for economic self-sufficiency, exploitation or production of natural sources, and social cohesion was the main driving force, which prompted the previous regime to interfere on the Butrint wetland. In the 1950s, a large-scale reclamation project was realized on the Vurgu and Vrina marshlands creating about 28 km^2 new agricultural lands. The main rivers discharging into the lagoon were diverted and directed to the Ionian Sea. In addition, over-intensive pattern of cultivation resulted to enter into the lagoon large quantities of organic and inorganic contaminants causing strong pressure on the local environment. The main environmental problems facing Butrint Lagoon are: i) pollution of waters and surficial sediments derived by diffuse sources, ii) anoxic conditions of the deepest waters, iii) reduction of brackish and freshwater marshland and, iv) aesthetic degradation of the landscape. All the aforementioned environmental issues could be mitigated or eliminated by the implementing appropriate policies and planning actions decided jointly by all involved parties. Here, reducing the use of fertilizers and pesticides, adopting biological practices on agricultural crops, discharging into the lagoon of well oxygenated water of rivers Bistrica and Pavllo when necessary, preserving the Butrint marshlands, and finally controlling the unregulated urban construction is fundamental.

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The National Environmental Action Plan (1993)

DIVERSITY OF *PLUM POX VIRUS* (PPV) STRAINS IN ALBANIA AND KOSOVO

Arben MYRTA
Certis Europe, Brussels, Belgium
Francesco PALMISANO
Centro di Ricerca, Sperimentazione e Formazione in Agricoltura, Locorotondo, Italy
Latif SUSURI
Academy of Sciences and Arts of Kosovo,
Angelantonio MINAFRA and Donato BOSCIA
Istituto per la Protezione Sostenibile delle Piante, CNR, Bari, Italy

ABSTRACT

PPV typing in Albania and Kosovo was carried out by DASI-ELISA with strain-specific monoclonal antibodies (MAbs) and, in some cases, also by RT-PCR and sequence analysis. Two field surveys were carried out in Albania and 59 PPV isolates were collected from eight regions of the country. In Albania, strains PPV-M (24) and PPV-D (13) were more frequently found than PPV-Rec (7), PPV-T (1) and PPV-An (1). In the first survey, mixture of strains D and M was detected in 13 out of 48 samples by Mabs. Occasional infections by PPV-T or PPV-An were found during the second field survey. In Kosovo, 26 viral isolates were selected and subjected to serological and molecular typing. PPV-Rec (23 out of 26) largely prevailed over PPV-M and PPV-D. PPV-Rec occurred in all areas in old and young orchards, indicating to be endemic in the country. The diversity of PPV isolates was much higher in Albania than in Kosovo, likely because of the longest history of PPV presence in Albania and of the most frequent introduction of propagation material from abroad. Our data rank Albania among the Eastern European countries with the highest biodiversity of PPV isolates, and as the potential area of origin of PPV-M, PPV-Rec and PPV-T.

1. INTRODUCTION

Sharka (plum pox) is the most important virus disease of stone fruits in Europe, where millions of trees are infected. It causes heavy yield losses to susceptible cultivars because of the reduced quality and premature dropping of the fruits (Cambra *et al.*, 2006). This disease was first described on *Prunus*

domestica cv. Kjustendil in Bulgaria early in the last century (Atanassov, 1932), from where it spread to most of the European and to several Asian and American countries (Barba *et al*., 2011). Eastern Europe, and the Balkans in particular, are the most affected areas.

Plum pox virus (PPV), the causal agent of the disease, occurs as nine distinct strains. Most of the PPV isolates were in the past assigned to one of two major strains or types, PPV-M (Marcus) and PPV-D (Dideron) (Bousalem *et al.*, 1994, Candresse *et al.*, 1998), accompanied by two minor strains, one of which infects naturally cherry (PPV-C), whereas the other is denoted El Amar (PPV-EA) for its site of origin in Egypt (Wetzel *et al.*, 1991, Nemchinov and Hadidi, 1996; Myrta *et al.*, 2006). Later findings were the recombinant strain PPV-Rec (Glasa *et al*., 2002), and the four molecularly divergent isolates PPV-W (Winona), PPV-T (Turkey), PPV-CR (Cherry Russian) and PPV-An (Ancestral) reported, respectively, from Canada (but most likely originated from Russia), Turkey, Russia and Albania (James and Varga, 2005, Ulubas-Serce *et al.*, 2009; Glasa *et al.,* 2011; 2013).

The distribution of Sharka in Albania and Kosovo has been investigated in the last two decades. The disease proved to be widespread in Eastern Albania, especially in European plums (Myrta *et al*., 1994), and in Kosovo, where it was found in most of the territory, prevailing again in European plum (Myrta *et al*., 2011). Preliminary information on the type of PPV isolates from both countries has been reported in (Myrta *et al*., 1994; 2011). The present paper provides detailed information about the biodiversity of the Albanian and Kosovo PPV strains.

2. MATERIALS AND METHODS

Sharka-symptomatic trees were sampled in both countries and analysed either for the presence of PPV and strain identification. Samples collected in seven locations each in Albania and Kosovo were tested by double antibody sandwich indirect – enzyme linked immunosorbent assay (DASI-ELISA), using universal monoclonal antibody 5B-IVIA (Cambra *et al*., 1994), and samples that gave doubtful responses were analyzed by reverse transcription - polymerase chain reaction (RT-PCR) (Wetzel *et al.* 1992). Two field surveys have been carried out in Albania, in 2000 and spring 2010, respectively. In Kosovo the survey was carried out the same year with the second survey in Albania.

The characterization of PPV strains from the early 2000s collection relies mostly on serological tools (DASI-ELISA), using strain-specific monoclonal antibodies (Mabs): Mab4DG (PPV-D specific) (Cambra *et al*., 1994), MabAL (PPV-M specific) (Boscia *et al*., 1997), MabEA24 (PPV-EA specific) (Myrta *et al*., 1998) and MabAC (PPV-C specific) (Myrta *et al*., 2000). Furthermore,

RT-PCR followed by the restriction fragment length polymorphism (RFLP) analysis targeting the CP and P3-6K1 genomic regions was done (according Candresse *et al*., 1998) on 10 PPV-M isolates typed by Mabs, to check for the presence of PPV-Rec.

Molecular typing consisting of a two-step RT-PCR amplification of two different genomic regions (CP and P3-6K1) and sequence analysis were carried out on samples collected in the second survey (2010). Amplification of the (Cter) CP was done using primers P4/P3M, P4/P3D (Dallot *et al*., 2008), while the primers PCI and PP3 (Glasa *et al.,* 2002) were used for the amplification of the region (Cter) P3-6K1-(Nter) CI. The uncloned PCR products were directly sequenced on both strands (Macrogen, NL) to identify the master (prevailing) sequence of the isolate. Those sequences were compared with the following reference PPV isolates: Ab-Tk (GenBank accession No. EU734794, T strain), BOR-3 (AY028309, Rec strain), PS (AJ243957, M strain), SK68 (M92280, M strain), W3174 (AY912055, W strain), D (X16415, D strain), SoC (AY184478, C strain), SwC (Y09851, C strain) and EA (AM15175, EA strain). Basic sequence comparison was performed by BLAST analysis (Altschul *et al*., 1990), multiple alignment (Clustal X; Thompson *et al*., 1997) and a phylogenetic tree was drawn by Neighbor-Joining method applying the MEGA5 program (Tamura *et al*., 2011).

3. RESULTS AND DISCUSSION

Typing of Albanian PPV isolates showed the presence of a large variability among them in every visited region and within each of the surveyed orchards. In the early 2000s, the virus strains identified by Mabs were PPV-M (23) and PPV-D (12), plus 13 isolates supposed to be natural mixtures of the two strains, as they were positive to both PPV-M and PPV-D-specific Mabs (Table 1). Followed by RFLP, the PCR analysis of the P3-6K1 region showed that three of the ten isolates from Korçë and Elbasan, that had serologically been typed as PPV-M, were in fact PPV-Rec (Table 1). Summarizing, the identified viral isolates were: 20 PPV-M, 12 PPV-D, 3 PPV-Rec and 13 PPV-M+PPV-D. However, based on the results of the second survey in Albania (Table 2), viral isolates identified by serology as PPV-M, could be further distinguished by molecular tools such as PPV-M, PPV-Rec, or PPV-T (molecular typing for the latter strain was not done during the first survey). Similarly, plant samples that reacted with both Mabs specific to PPV-D and PPV-M could host either a mix of PPV-D and PPV-M, or PPV-T or -An strains.

Table 1. *Plum pox virus* strains identified in Albania in the early 2000s. Specification about MAbs and related bibliography are reported in the text.

Origin of isolates (nr.)	No. of isolates	Typing by MAbs 5B	AL	4DG5	AC	EA24	Molecular typing **by RFLP** P3-6K1-CI	Virus strain
Korçë (21)	8	+	+	+	-	-	n.t.	M+D[1]
	6	+	-	+	-	-	n.t.	D[1]
	5	+	+	-	-	-	M	M[2]
	2	+	+	-	-	-	Rec	Rec[3]
Pogradec (8)	4	+	+	-	-	-	n.t.	M[1]
	4	+	-	+	-	-	n.t.	D[1]
Elbasan (3)	2	+	+	-	-	-	M	M[2]
	1	+	+	-	-	-	Rec	Rec[3]
Librazhd (10)	5	+	+	+	-	-	n.t.	M+D[1]
	3	+	+	-	-	-	n.t.	M[2]
	2	+	-	+	-	-	n.t.	D[1]
Tiranë (4)	4	+	+	-	-	-	n.t.	M[1]
Peshkopi (2)	2	+	+	-	-	-	n.t.	M[1]
	48							

[1]Strain typing done only by ELISA; [2]PPV-M identified by ELISA and confirmed by RT-PCR; [3]isolates identified as PPV-M by ELISA but as PPV-Rec by RT-PCR; n.t.= not tested

In the survey of 2010, the 11 tested viral isolates were typed as PPV-D (1), PPV-M (4), PPV-Rec (4), PPV-T (1) and PPV-An (1) (Table 2). The detection of PPV-T represented the first report of this strain in Albania, while that of PPV-An was a further confirmation to the presence of this recently discovered strain in the same country (Palmisano *et al*., 2012). Isolates of a new sub-group of PPV-M, named "atypical Marcus Albania", were also recently reported in Albania (Palmisano and Minafra, personal communication), besides to PPV-Ma (the isolates from Mediterranean) and PPV-Mb (the isolates from central and eastern Europe) (Dallot *et al*., 2011).

Table 2. *Plum pox virus* strains identified in Albania in 2010. The molecular discrimination consisted in a phylogenetic attribution of the obtained isolate sequence.

Origin and number of isolates	No. of isolates	PPV typing					Strain
		Serological			Molecular		
		5B	AL	4DG5	Region P3-6K1-CI	Region NIb-CP	
Durrës (1)	1	+	+	-	M	M	M
Tiranë (1)	1	+	-	+	D	D	D
Korçë (4)	2	+	+	-	M	M	M
	1	+	+	-	Rec	Rec	Rec
	1	+	+	+	An	An	An
Elbasan (5)	1	+	+	-	M	M	M
	3	+	+	-	Rec	Rec	Rec
	1	+	+	+	T	T	T
Total	11						

Collectively, data from the two Albanian surveys indicate that the frequency of PPV-Rec compared to that of PPV-M was 7:11 (out of 18 tested isolated). In the testing of early 2000s there were several samples, reacting with both monoclonal antibodies specific to PPV-D and PPV-M, that may have been natural mixtures of the two strains or PPV-T or PPV-An (whose coat proteins behave serologically as M strain), as found in the last study.

An Albanian isolate from Çerravë (AL-11pl) showed properties expected for a strain candidate to be the PPV-M ancestor in one of the evolutionary scenarios described by Glasa and Candresse (2005). Thus, AL-11pl can be regarded as the representative of a further PPV strain, for which the name PPV-An was proposed (Palmisano *et al*., 2012). The presence of a PPV-T isolate in the same Albanian region where AL-11pl was discovered, and the hypothesis that this latter isolate could be the potential ancestor of all the known PPV 'M-type' strains of Eastern Albania, candidate this area as the putative center of speciation of PPV-M. Based on these findings, Albania appears as the Eastern European country with the highest biodiversity of PPV isolates, and the potential area of origin of PPV-M, PPV-Rec and PPV-T.

The presence of diverse PPV strains in Albanian stone fruit orchards has been already reported (Myrta *et al*., 2001; Stamo and Myrta, 2006). The present investigation confirms and extends this finding by analyzing a higher number of isolates (59) coming from different regions (8) and host species (plum, apricot and peach). Moreover, a phylogenetic analysis performed on both domains, from isolates sequenced in 2010, perfectly confirmed the belonging of all isolates to the typed strains (data not shown).

As mentioned, in Kosovo a total of 26 PPV isolates were selected and analyzed for strain typing. Serological and molecular testing disclosed the predominance of PPV-Rec (23 out of 26) over PPV-M and PPV-D (Table 3). PPV-Rec appeared to be endemic in the country as it occurred in all areas, irrespective of the age of the orchards. These data are in line with those from the neighboring Serbia, where PPV-Rec prevails (Glasa *et al*., 2005; Dulic-Markovic and Jevremovic, 2006), and can be explained by the common origin of the propagating materials. Despite to the high inoculum pressure of PPV-Rec, the only peach sample analyzed hosted PPV-M, thus confirming the difficulty of PPV-Rec to naturally infect peach trees.

Table 3. *Plum pox virus* strains identified in Kosovo[1]

Origin of the isolates	No. of isolates	PPV typing					
		Serological			Molecular		Strain
		5B	AL	4DG5	Region P3-6K1-CI	Region NIb-CP	
Ferizaj	2	+	+	-	Rec	Rec	Rec
Ferizaj	1	+	-	+	D	D	D
Viti	1	+	+	-	Rec	Rec	Rec
Dardani	2	+	+	-	Rec	Rec	Rec
Malishevë	2	+	+	-	Rec	Rec	Rec
Klinë	2	+	+	-	Rec	Rec	Rec
Ferizaj	3	+	+	-	Rec	Rec	Rec
Viti	3	+	+	-	Rec	Rec	Rec
Gjilan	6	+	+	-	Rec	Rec	Rec
Gjilan	1	+	+	-	M	M	M
Prishtinë	2	+	+	-	Rec	Rec	Rec
Prishtinë	1	+	+	-	M	M	M
	26						

[1] The majority of the samples (85%) were European plums

The results reported that the diversity of PPV isolates is much higher in Albania than in Kosovo, as a consequence of the most ancient presence of PPV in the former country and of the most frequent introduction of propagation material from abroad. Likely, these conditions allowed a high frequency of mutations and recombination events that justify the presence of the high variability intra- and inter-strains. Further and more extensive investigations on the genetic variability of a larger number of Albanian isolates would be desirable, for deepening the knowledge of the evolutionary history of PPV.

ACKNOWLEDGEMENTS

The authors owe a debt of a special gratitude to Prof. Giovanni P. Martelli at University of Bari, for his critical reading and suggestions made for the improvement of this manuscript. The sequencing of Albania and Kosovo PPV isolates was supported by SharCo project (*Containment of Sharka virus in view of EU-expansion*) in the 7th EU FP.

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THE GENETIC POLYMORPHISM OF HUMAN LEUKOCYTE ANTIGEN SYSTEM IN THE ALBANIAN POPULATIONS OF ALBANIA AND KOSOVO AND THEIR COMPARISON WITH THE OTHER POPULATIONS

Erkena SHYTI and **Genc SULÇEBE**
Laboratory of Immunology and Histocompatibility, Faculty of Medicine, University of Medicine, Tirana, Albania

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ABSTRACT

Human leukocyte antigen (HLA) polymorphism is a means to address the genetic relationships among populations. Investigating these relationships is of particular importance for anthropologic studies and designing common international transplantation programs and unrelated donor registry networking. In the present investigation results on the HLA genetic polymorphism in the Albanian populations from Albania and Kosovo is reported and compared with neighboring and other European populations. HLA allele genotyping has been conducted through molecular methods applied in the DNA isolated from the blood of sampled individuals of Albanian origin from Albania and Kosovo. HLA allele and haplotype frequencies have been estimated using computer tools adapted to HLA data. Population differentiation was studied by computing Nei's genetic distances based on the frequencies of HLA-A, -B and -DRB1 alleles. Results on genetic distance analysis reported that the Albanians from Kosovo share a very close HLA-based genetic relationship with the Albanians from Albania, followed by Bulgarians, FYROM Macedonians and Greeks. The Croatian, Bosnia-Herzegovinian and Serbian populations are more distantly located. The genetic distances to other European populations are proportional to their geographic distances. Consequently, HLA-compatible unrelated hematopoietic stem cell donors for Albanian patients could be found with a higher probability not only among the Albanian population in Albania and Kosovo, but also in donor registries of populations more closely related HLA genetically with Albanians such as Macedonians, Bulgarians or Greeks, not excluding here other populations living in the Balkan area.

Keywords: population genetics, human leukocyte antigen, genetic relationships, Albanian population, allele polymorphism

1. INTRODUCTION

The human leukocyte antigen (HLA) system, a gene complex situated in the short arm of chromosome 6 (6p21.3), is the most polymorphic gene system in the human species (Janeway *et al.,* 2001). It is responsible for the huge variety of the immune response in humans and for the discrimination of self from non-self (Ferrer *et al.,* 2005).

Taking into account the considerable allelic HLA diversity, the knowledge about the HLA polymorphism of different populations or ethnic groups is especially important (Maiers, *et al.,* 2007). HLA polymorphism has been widely used in anthropological studies (Bodmer, 1997; Sanchez-Mazas *et al*., 2012) and furthermore, the HLA genetic diversity data from different populations are very useful for medical applications. In this regard, the knowledge about HLA allele and haplotype frequency rates in various populations is of primary importance in the field of unrelated donor hematopoietic stem cell transplantation in order to both optimize the recruitment of donors in registries and also to improve searching strategies for unrelated donors (P□ron, *et al.,* 2006).

The Balkan Peninsula is located in the Southeast Europe and social cultural and religion diversity has been of the greatest concern and source of wars and conflicts. Consequently, population admixtures have hardly occurred among these nations. However, ethnic minorities from all these populations have always lived in the territories actually populated by Albanians. So, investigating the genetic relationships among the populations living in the Balkan Peninsula is of great interest.

The HLA allele and haplotype frequency in Albanian population of Albania and Kosova has been separately investigated and the results are already reported (Sulçebe *et al.,* 2009; Sulçebe *et al.,* 2013). This is a comparative genetic distance investigation of Albanian populations of Albania and Kosovo with other European populations which aims to provide a more detailed analysis about genotypic HLA allele and haplotype frequency similarities between the Albanian populations of Albania and Kosovo and to compare them with other populations from all Europe and particularly with the South-East and Balkan populations, in order to define more precisely the respective HLA-based genetic relationships.

2. MATERIALS AND METHODS

Sample populations

The 280 healthy unrelated Albanian individuals from Albania and Kosovo are in the present investigation included. They were all students from the University of Medicine of Tirana, Albania and blood donors of the Blood Bank Centers of the University Hospital Centers of Tirana and Prishtina, Kosovo. In addition, they originated from all regions of Albania and Kosovo and selected after informed consent and being declared of Albanian origin from at least 3 generations.

HLA Typing

HLA typing of all the individuals studied was carried out at the Laboratory of Immunology and Histocompatibility of the University Hospital Center, Mother Teresa, Tirana, Albania.

Genomic DNA was extracted using QIAamp DNA Blood Mini Kit based on manufacturer's instructions (Qiagen, Hilden, Germany). Genomic DNA typing of 2-digit HLA-A, -B, and -DRB1 allele-groups was carried out applying the polymerase chain reaction sequence specific priming (PCR-SSP) method (One Lambda Inc, Canoga Park, USA) and using a Luminex based, sequence specific oligonucleotide (PCR-SSO) method (Tepnel Lifecodes, Stamford, CT, USA).

Statistical analyses and inter-population comparisons

HLA allele and haplotype frequencies were estimated using Arlequin population statistics software package (Excoffier *et al*., 2005) by a maximum-likelihood analysis from unknown gametic phase genotypic data through an expectation-maximization (EM) algorithm (Excoffier and Slatkin, 1995).

Population differentiation was studied via Nei's genetic distance measurement implemented in the Joe Felstenstein's Phylip phylogeny program package (Felsenstein, 1989). This calculation makes the assumption that the different populations evolve independently, each gene frequency changes by genetic drift and new mutations can also occur.

Genetic distances were calculated from the frequencies of 14 HLA-A, 22 HLA-B and 12 HLA-DRB1 2-digit alleles tested simultaneously in all the populations studied. The data used for inter-population allele frequency comparisons were taken from D. Middleton's "http:// www.allelefrequencies.net" web-site (Gonzalez-Galarza *et al.,* 2011). These data were compared with the corresponding published data when available.

The multidimensional scale (MDS) graphical presentation of the genetic distances between the Kosovo Albanian population and other 24 sample populations, was carried out through an Euclidean distance model method included in the SPSS 18 statistics software package. The unrooted phylogeny tree was constructed with the Neighbor-Joining method implemented in the Phylip software package (Saitou and Nei, 1987).

3. RESULTS

The allelic frequency distributions of HLA-A, -B, -C, -DRB1 and -DQB1 loci in the Albanian populations from Albania and Kosovo are shown in the respective charts of Figures 1- 5. All the allele frequencies presented in these charts are higher than 1%. The numbers of 2-digit alleles detected in the Albanian individuals from Albania were 17 HLA-A*, 30 HLA-B*, 12 HLA-Cw*, 13 HLA-DRB1* and 5 HLA-DQB1* allele-groups. Sixteen HLA-A, 22 HLA-B, 12 HLA-Cw*, 13 HLA-DRB1 and 5 DQB1 allele groups were detected in the Albanian individuals from Kosovo.

The five most frequent HLA-A allele-groups found in both groups of individuals are HLA-A*01, HLA-A*02, HLA-A*03, HLA-A*11 and HLA-A*24, followed by the other HLA-A allele-groups with lower frequency (Fig.1).

HLA-B allele-groups most frequently found in both groups studied are HLA-B*18, HLA-B*51, HLA-B*35, HLA-B*44, HLA-B*07, HLA-B*40 and HLA-B*08 (Fig. 2).

At the HLA-Cw locus, the most frequent HLA-Cw allele-groups in both groups are: HLA-Cw*07, HLA-Cw*12, HLA-Cw*15, HLA-Cw*04, and HLA-Cw*06 (Fig. 3).

As far as concerns the HLA-DRB1 locus, the most frequent HLA-DRB1 allele-group found in both study groups is HLA-DRB1*11, followed by HLA-DRB1*16, HLA-DRB1*13, HLA-DRB1*14 and HLA-DRB1*15 (Fig. 4).

The five HLA-DQB1 allele-groups have also similar frequency rates in both groups of Albanian individuals. The most frequent allele-group at this locus is HLA-DQB1*03, followed by HLA-DQB1*05, HLA-DQB1*06, HLA-DQB1*02 and HLA-DQB1*04 (Fig. 5).

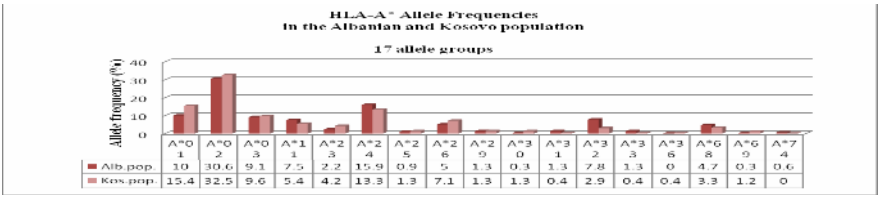

Fig.1. HLA-A* allele-groups with the respective frequencies (%) in the Albanian and Kosovo populations.

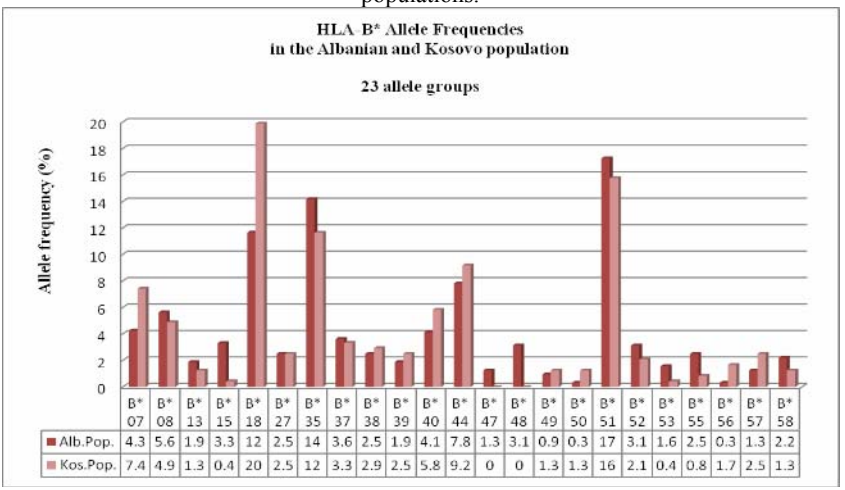

Fig. 2. HLA-B* allele-groups frequencies (in %) at the Albanian and Kosovo populations.

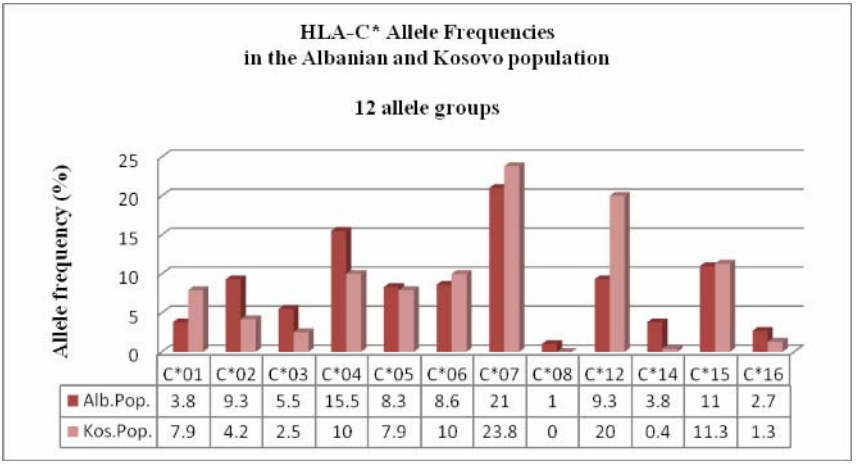

Fig. 3. HLA-C* allele-groups with respective frequencies (in %) in the Albanian and Kosovo populations.

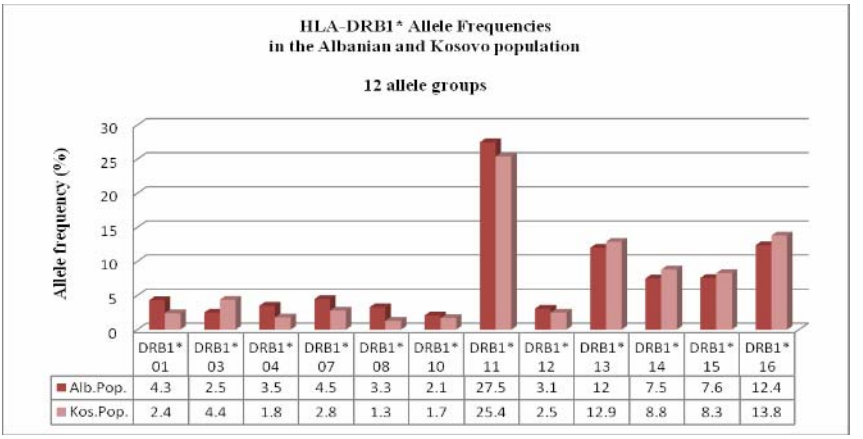

Fig. 4. HLA-DRB1* allele-group frequency rates (in %) in the Albanian and Kosovo populations.

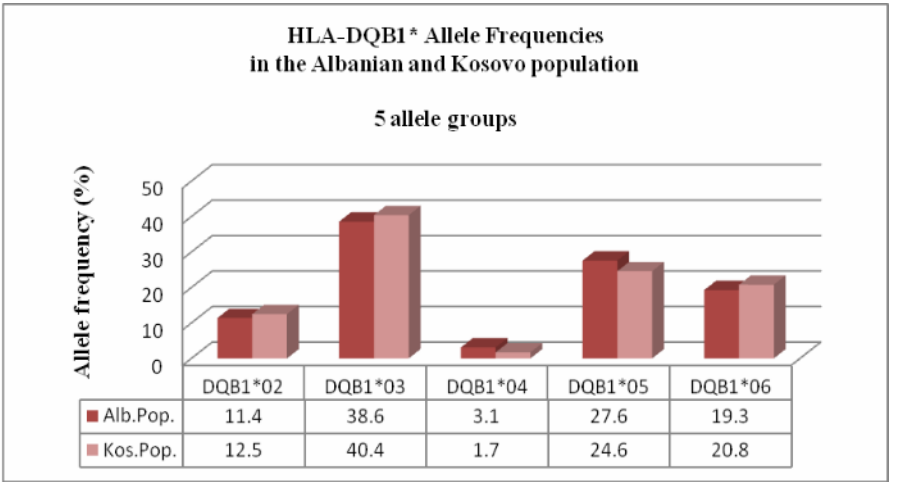

Fig. 5. HLA-DQB1* allele-group frequency rates (in %) in the Albanian and Kosovo populations.

Haplotype frequencies

Haplotype frequencies were estimated by considering 5 loci (HLA-A-B-C-DRB1-DQB1) extended haplotype combinations. The most frequent haplotypes in both groups of individuals are: A*02-B*18-Cw*07-DRB1*11-DQB1*03 with a frequency of 4.3 % in the Albanian group from Albania and 7.3 % in the Albanian group from Kosovo. It is followed by the haplotype A*01-B*08-Cw*07-DRB1*03-DQB1*02 with a frequency of 3.1% in the Albanian group from Albania and 2.5% in that from Kosovo.

Population comparisons

The HLA-based populations were compared via Nei's genetic distance measurement. Genetic distances were calculated from the frequencies of 14 HLA-A, 23 HLA-B and 12 HLA-DRB1 allele-groups tested simultaneously in all the populations studied (Table 1).

Table 1. Nei's genetic distances listed in an increasing order, among 24 populations compared to the Albanian populations from Kosovo and Albania, calculated on the basis of HLA-A -B and -DRB1 allele-groups frequencies tested simultaneously.

No.	Populations	Genetic Distance
1	Kosovo Albanians	0.000000
2	Albania Albanians	0.028972
3	Macedonians	0.047231
4	Greeks (North)	0.050845
5	Greeks (All)	0.052661
6	Bulgarians	0.059775
7	Croatians	0.073278
8	Bosnia/Hercegovina	0.076104
9	Serbians	0.080415
10	Cretans	0.102184
11	Italians	0.120449
12	Romanians	0.125871
13	Austrians	0.152119
14	Turkey	0.154423
15	Russians (North/west)	0.158185
16	Portuguese (Center)	0.158719
17	Polish	0.159820
18	French (South/East)	0.184769
19	Czech	0.191666

20	Germans (Essen)	0.203355
21	Spain (Ibizans)	0.239802
22	Welsh	0.278307
23	Scottish (Orkney)	0.316043
24	Spain (Basque)	0.368928
25	Pakistanians (Burushi)	0.413339
26	Chinese (Shanghai)	0.511308

A multidimensional scaling analysis based on Nei's genetic distances computed for the 3 loci HLA-A, -B and –DRB as shown in Table 1 is presented graphically in Figure 6. Albanians from Albania and from Kosovo appear to be HLA-based genetically close to Macedonians, Bulgarians and Greeks, followed by the other populations from South-East Europe (populations from Crete, Serbia, Croatia, Romania and Bosnia-Herzegovina). The general genetic pattern observed for Europe suggests a geographic differentiation, with Western/Northwestern populations projected on the upper side and Eastern/Southeastern populations on the lower side. We also note a pronounced differentiation of Spanish Basques on the upper right side and of Pakistani and Chinese population along the lower right side.

A neighbor-joining unrooted phylogeny tree, computed from the genetic distances of 13 sample populations from Central and South-East Europe compared to the Albanian populations from Kosovo and Albania is in Figure 7 depicted.

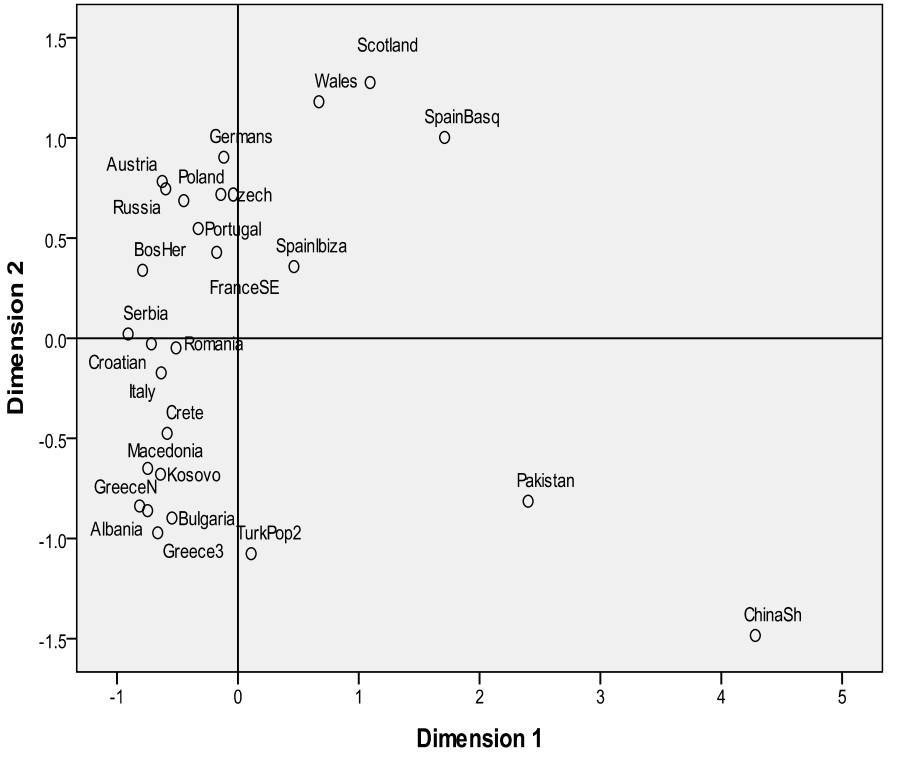

Fig.6. Multidimensional scale presentation of genetic distances of 24 populations compared to the Albanian populations from Kosovo and Albania, calculated on the basis of HLA-A -B and -DRB1 allele-group frequencies.

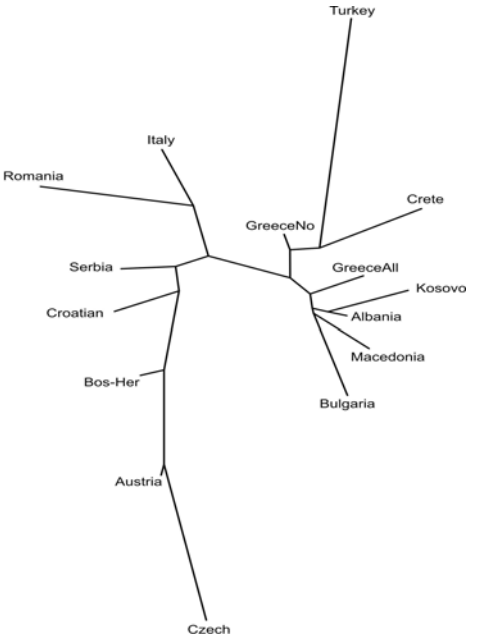

Fig. 7. Unrooted phylogeny tree calculated on the basis of the genetic distances between the Albanian populations from Albania and Kosovo compared to other 13 Balkan Peninsula and Central European populations.

4. DISCUSSION

The Balkan Peninsula is located in the Southeast Europe and social cultural and religion diversity has been of the greatest concern and source of wars and conflicts. Consequently, population admixtures have hardly occurred among these nations. As far as concerns the Albanians, it is worth mentioning that due to the historical separation of the Albanian populations of Albania and Kosovo into different political states, no significant admixtures have occurred also between these populations since at least one century.

Therefore, investigating HLA-related genetic relationship between these two populations and between them and other Balkan and European populations is of great interest.

Nei's genetic distances was calculated using 2-digit allele-group frequencies of HLA-A, -B and -DRB1 loci studied simultaneously in these populations in order to compare genetically both groups of Albanian populations to the other European populations. Once calculated, the distances were used to plot a graphical analysis through a multidimensional scale and also a neighbor-joining unrooted phylogeny tree presentation.

No significant difference between the Albanian populations of Albania and Kosovo was found at the allele frequency rates for all the HLA loci studied. The results clearly reported an evident common genetic background between the Albanian population between the two countries, proving a common genetic pool between these two populations. Apparently, this common genetic background has resisted to historical consecutive invasion and immigration waves such as those from Roman, Slavic populations or others.

According to Nei's genetic distance analysis we can also conclude that the Albanians from Kosovo and Albania share a very close HLA genetic relationship with Bulgarians and FYROM Macedonians, and also with Greeks, while the Serbian, Croatian, Bosnia-Herzegovinian, Romanian and Italian populations are more distantly located. These data are in agreement with the Fst-based statistical analysis performed by our group on the HLA-B and -DRB1 loci tested separately, where a clear significant statistical difference is found between the Albanian populations of Albania and Kosovo on the one side and Serbian, Croatian, Bosnia-Herzegovinian, Romanian and Italian populations on the other side, but not with Bulgarians, Macedonians and Greek populations (Sulçebe *et al.,* 2013).

It is evident that a high HLA genetic relatedness is found not only between the Albanians from Albania and Kosovo but also between them and the Macedonian, Bulgarian and Greek populations living in the South and South-East of Balkan Peninsula. They clearly differentiate from the Serbian, Croatian, Bosnian and Romanian populations living further north of Balkans. These results have been recently confirmed by (Ralph and Coop, 2013).

Other studies have reported about HLA genetic relationships of the populations of Southeastern Europe and Balkan Peninsula, but not all populations living in this area have been included in these studies (Ivanova *et al*., 2004; Petlichkovski *et al*., 2004). To our knowledge, this is the first HLA-based genetic distance population study in which all the populations living in the Balkan Peninsula region are compared using multiple HLA loci allele groups tested simultaneously.

The other populations studied, such as Austrians, Turkish, Russians (North/West), Portuguese, Polish, French (South/East), Czech, Germans, Spanish (Ibiza), Welsh, Scottish (Orkney), and Basque, are clearly more distantly located from the Albanian population, and their genetic distances are generally proportional to the corresponding geographic distances.

A geographic pattern clearly appears from this genetic distance data analysis, showing that the populations living in the Balkan area differentiate from those living in Central, Northern and Western parts of Europe. In addition, a Northwest to East/Southeast genetic differentiation appears in Europe (Nunes *et al.,* 2014).

Starting from these results, we can conclude that unrelated hematopoietic stem cell donors for Albanian patients can be more easily found in donor registries of those populations that are HLA genetically more closer with the Albanian population such as Greeks, Macedonians and Bulgarians but also of other populations living in the Balkan area, which are apparently more closer HLA genetically than Western or Northern Europe populations. The practical application outcome of these findings is the utility to implement common HSC donor registries and transplantation programs between countries with most closed HLA genetic relatedness.

ACKNOWLEDGMENTS

The authors owe a debt of a special gratitude to the Albanian Agency for Research, Technology and Innovation (ARTI) for its financial support. The present investigation was carried out under the framework project European COST Action BM0803. The authors would like also to thank Dr. Artan Simaku for kindly performing the Multidimensional Scale Analysis.

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TECHNOLOGIC DEVELOPMENT AND ITS BENEFITS TO THE CUTTING SECTION-A STUDY CASE

Elmira DUMISHLLARI
Department of Textile and Fashion, Polytechnic University of Tirana, Albania

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ABSTRACT

Currently, a new technology has been applied in cutting section and the results are here reported. The technological cutting process is the first stage in the garment manufacturing process and it makes about 20% of the total time of the garment production. The results reported that new technology is economically efficient.
Keywords: economization, apparel production, working time, raw material, new technology.

1. INTRODUCTION

Financial and economic crisis has been of bed impact on the different companies involved in clothing manufacturing. Better management of production factories by undertaking investments which will ensure economization and product quality of irreplaceable importance (CheckPointSystem, 2011).

The technological cutting process is the first stage in the garment manufacturing process and it makes about 20% of the total time of the garment production (Kirin and Dragčević 2012).

The impact of Kuris Pattern Design (KPD) cutting robot used in the cutting room for an efficient production process and product quality is here reported.

The present study was carried out for three months in a clothing manufacturing factory in Tirana, for sports apparel for disciplines such as football, volleyball, cycling, basketball and other orders that might come. This company operates full package, realizing complete production package, such as the purchase of raw and auxiliary materials and the sending to the client (Gereffi and Frederick 2010).

2. MATERIALS AND METHODS

The study relays on the traditional (manual) and a new technology which involves the KPD cutting robot.

Indicators such as working time for each process (Gulnaz, 2011), the number of employees, consumption of raw materials and other consumptions for the application of each method were here compared and the results are reported.

2.1 KURIS Pattern Design cutting robot

Produced in 2012, the main function of this robot is pattern computerized marking and cutting. This investment cost € 120,000.

The robot enables the laying of markers, the best use of fabric, simultaneously cutting of some patterns, and fixed cutting of them, thus improving the quality of the pattern.

For the production of cutting robot, the apparel company requested the following parameters: i) robots table length 5 m, as it is sufficient for cutting two mattresses laid simultaneously one after the other), ii) width of robot's table, 2 m (maximum width of fabrics used in this company goes up to 1.8 m), iii) programs for cutting polyester fabrics, polyamide etc. and, iv) programs for cuting mattresses up to 25 layers with fabric of various thicknesses.

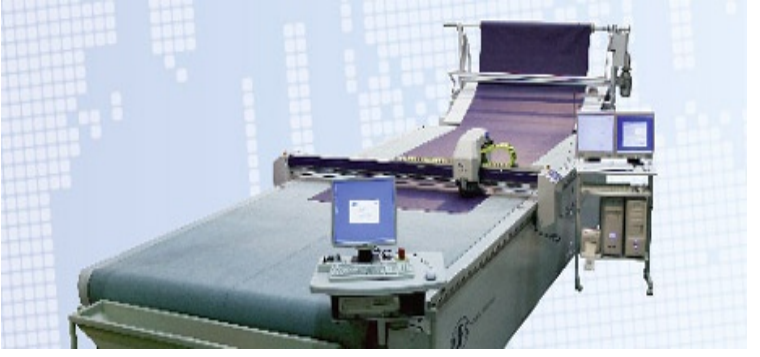


Fig. 1. KURIS Pattern Design cutting robot.

KPD cutting robot comprises (KPD, 2008): the table, two rubber cylinders for fabric ball placement, hand lever for rotation of the fabric ball, saw for cutting fabric sheets after spread of the mattress, two metal cylinders parallel to each other (in the first is set cutting room plastics and in the second paper of cutting room), head equipped with a thin saw and a red laser, two computers connected to each other. The first computer is used for marking and storing purposes. The second computer is used for carrying the charts in the appropriate folders and giving commands for cutting charts. KPD is equipped with a software which is used for marking purposes (fig.1).

Patterns marking, graph length and efficiency of the fabric, expressed as a percentage, appear on the screen of the computer (fig.2).

Fig.2. Marking process (on the left) and commands for cutting (to the right).

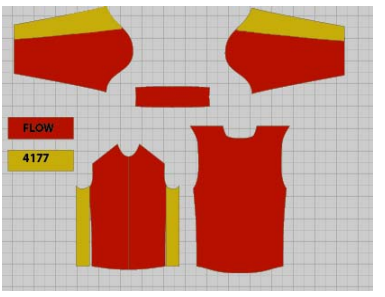
2.2 Comparison of two cutting methods used

The present paper compares the production processes applying the manual cutting (M_1) and the KPD cutting robot (M_2), respectively.

Here, an order of 60 sport jackets of the small (10 pairs), medium (15 pairs), large (30 pairs) and extra large measures(5 pairs) are involved (Coelho 2012).

The fabric used is called flow and consists of synthetic fibers.

Chest, back, sleeves, collar, two pocket chains of 20 cm, chain of 70 cm placed in front of jacket and elastic lace are the integral parts of the jacket.



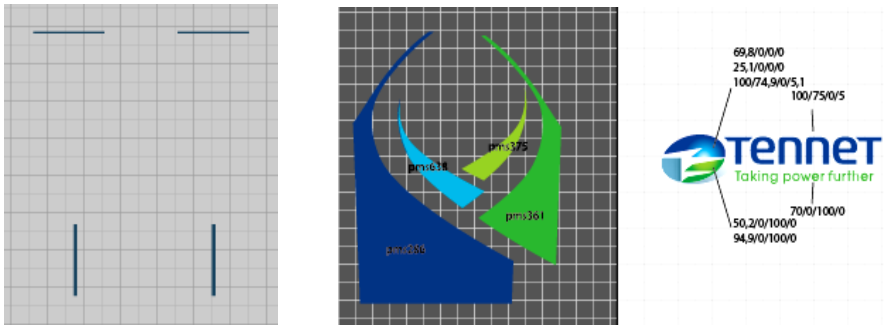


Fig. 3. Task order.

The procedures required for the final product are in the figure 4 shown (Glock and Kunz 2005).

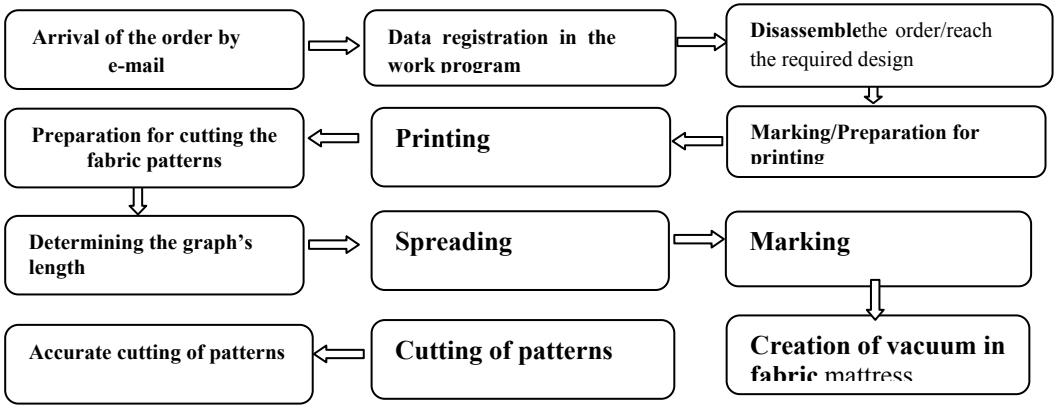


Fig 4. The step by step process of garment production.

The methods have the same production stages until " patterns printing". The forthcoming paragraph provides information about the procedures that differ for each method. Once the methods are compared, economization rate could be obtained.

Preparation of graphs for cutting the fabric patterns

In the first method, jacket patterns were printed on the paper of the printer. The large size has 30 pieces with 3 patterns per each to be printed, i.e, an economic use of paper and printer. A mattress of 10 sheets of fabric has been prepared. The length of the three patterns goes up to 3.71 m. Marking is done by the employee who measures the width of the graph which is the same as

the printer's paper, 1.56m. This process previously accomplished only by an employee.

The second method involving the KPD software is applied to the same number of patterns for the same size (L). The number of patterns and the width of the fabric are the only components that have to be set up in the software as marking is done automatically by the program.

The length of the graph for the three patterns is 327 cm. The graph is 2 cm less wider than the fabric.

This process is carried out only by an employee.

The program makes automatic positioning of patterns and automatically displays the spreading length and efficiency.

In the end these graphics are saved in the computer which is connected to the robot.

Determining the length of the graph

In the first method the operator measures the fabric and determines the length of the graph himself.

In the second method, the operator again determines the length of the graph with the help of a meter and spreads the paper of the cutting room.

In both cases, this is an economically inefficient.

Spreading on fabric sheets

In the first method the fabric ball was placed on an iron rod and fixed at a certain height to facilitate the process of fabric rotation. The operators pull the fabric to the length of the graph. Once the fabric is pulled, the fabric mattress is cut using an electric saw.

In the second method, the ball of fabric self-rotates on the surface of the desk, enabling the creation of fabric mattress.

Positioning of patterns (Marking) (Ambastha, 2013)

In the first method, marking is done for a better exploit of fabric. The process is carried out in 6 minutes.

In the second method, the process is computerized.

Creation of vacuum in fabrics' mattress

In the first method, the fabric mattress was ironed to fix the paper patterns. The process lasted 3 minutes.

In the second method, this process is not carried out, but the plastic passes over the created mattress in 30 seconds.

Cutting

The first method involves an electric saw to cut the patterns which could not be cut fixed and the fabric mattress could be badly cut. This process was carried out in 9 minutes.

In the second method, the laser ray placed in the robot's head was used to determine the length of the mattress. Once the length was determined, the command for cutting was given. The process was carried out in 4 minutes.

Accurate cutting of patterns

In the first method, the patterns are not cut fixed. There was a tolerance of 1 cm in length in two sides of the pattern. 3 m of fabric were used for the 30 pairs. The process was carried out in 10 minutes.

In the second method, the process was carried out by the robot.

3. RESULTS AND DUSCUSSIONS

This is a case study that provides information about the application of two technologies; the manual and KURIS Pattern Design cutting robot (KPD) in the area of textile industry. A range of indicators for each method such as time of processing (Sarkar, 2011), number of employees, the first raw material consumption and other consumption was analyzed. In the present study, 60 pairs of sport jackets from different disciplines were involved. The samples were collected from a manufacturing company operating full package in Tirana, Albania, in a period of three months. The collection and measurement of work time for each process are based on operator's performance sheets (Tab 1).

Results reported i) a faster cutting process for fabric patters (35 min for M_1 and 8 min for M_2, i.e., saving 27 minutes from the working hours, ii) one operator carrying out the processes in both cases, iii) lower price for the printing paper (€ 4.23 or 600.58 ALL) as the cost of a paper per meter is € 1.14, iv) a faster spreading process (12 minutes faster) as in the first method it occurred in 19 min and in the second in 7 minutes, v) marking process occurred in 6 minutes from two operators. In the second method it was not carried out, vi) a faster process of creating vacuum in fabric's mattress (2.5 minutes); 3 minutes via the first method and 30 sec. via the KPD cutting robot, vii) a 5min faster cutting process carried out by one operator (9min carried out by two operators when applying the first method and 4min when applying the second), viii) accurate cutting of patterns carried out in 10 minutes by one operator, in the manual cutting process. In the second method the process is not carried out and, ix) a tolerance of 2 cm of fabric for every pattern (M_1). Our graph consists of 15 patterns (back – 3, front – 3, collar – 3 and sleeve – 6), consequently will have 0.3 m for each sheet of fabric. Our

mattress consists of 10 sheets of fabric, so will spent 3 m of fabric. The cost of 1 m of fabric is 4.9 euro. Consequently, there is cut of the cost with 14.7 Euros of the cost. Finally, in this process are saved 10 min and 2118.65 ALL.

New technologies such as KPD cutting robot are are of great economic benefit for the cutting section, as patterns could be cut precisely maintaining exactly any shape or curvature (Rahman *et al*., 2014).

Table 1. The results of the processes leading to retrench, obtained during the study of two methods, used for cutting patterns

Process	Time of work (min)		No. of operators		Fabric (m)		Paper (m)	
	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
Graph preparation	35	8	1	1	0	0	3.71	0
Spreading	19	7	2	2	0	0	0	0
Marking	6	0	2	0	0	0	0	0
Creation of vacuum	3	0.5	2	2	0	0	0	0
Cutting	9	4	2	1	0	0	0	0
Accurate cutting	10	0	1	0	3	0	0	0
Sum	82	19.5	6	3	3	0	3.71	0

4. CONCLUSIONS

Technologic development is of great economic benefit for the textile industry for the processes are well-timed and qualitative. The number of operators is reduced for graphics could be simultaneously cut, costs for salaries are halved, coefficient of utilization of the fabric is increased and saving of the fabric is about 15%, the production rate is approximately tripled.

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PRICE BEHAVIOR IN THE ALBANIAN FOREX MARKET ESTIMATED THROUGH HURST EXPONENT

Luis LAMANI
Polytechnic University of Tirana, Albania

ABSTRACT

Albanian financial sector hardly provides sufficient information on strategies related to the market which is considered as a controlled financial system. Banks, financial companies, individual speculators and import-export companies play an important role in the area of finance. In the present paper, Hurst exponent - behavior of prices in Albanian forex market is reported.
***Keywords*:** Albania forex market; Fractal markets; Hurst exponent; Price prediction; R/S analysis; Standard Deviation

1. INTRODUCTION

Predicting the performance of financial markets over a period of time is both interesting and challenging. Financial markets provide continuously information about demand-supply relationship, prices of different products at different periods of time. Foreign exchange prevails in the Albanian financial system and provides appropriate financial data. There is no stock exchange.

Albanian financial sector hardly provides sufficient information on strategies related to the market which is considered as a controlled financial system. Lack of appropriate data and real estimation make analyzing the exchange rate of EUR/ALL, USD/ALL challenging (Lamani and Baci, 2014). The data produced are a simple average of the exchange rate values obtained from some banks and other financial institutions. Beyond the extent there is a certain degree of a predictable system, based on chart analysis (Lamani and Baci, 2014). Information is reflected in the exchange rate values.

In terms of volume of trading, the forex market is the most fluid and dynamic market in the world. Banks, financial companies, individual speculators and import-export companies play an important role in the area. The present paper empirically investigates the Hurst exponent behavior of prices in Albanian forex market relationship. Hurst estimates the persistency of the market. The latter part of the paper reports the value of Hurst showing different behaviors of the data series.

A review of Hurst exponent

The origin of studies involving the Hurst exponent lie in hydrologic studies where it was used to determine the optimum dam sizing for the Nile river's volatile rain and drought conditions observed over a long period of time.

Then he looked at the peak value and compared it to the lowest level reached. He called the difference, Range or just R. His formula gives the value of R, which indicates how big the reservoir should be to avoid floods or droughts downriver. It is determined by σ , the standard deviation of the discharges from one year to the other; N, the number of years under study; and α, the power-low exponent that drives the whole equation. Hurst, using logs, used the equation:

$$\log\left(\frac{R}{\sigma}\right) = K \log\left(\frac{N}{2}\right)$$ (Mandelbrot *et al*, 2008)

where K = 0.73, Standard deviation σ = 0.09 and 0.46 ≤ R ≤ 0.96.

Hurst (1951) revealed that many naturally occurring empirical records appeared to be well represented as a generalization of Einstein's equation (1905):

$$E\left[\frac{R}{S}(n)\right] \sim cn^{H}, c \rightarrow positive\ constant, n \rightarrow \infty$$

According to the original proposition, Hurst exponent (H) = 0.5 would represent a random walk, a Brownian motion, in which the current value of the series would not be dependent upon past values of the series. Varying from 0 and 0.5, the Hurst value reports the anti-persistent behavior of the series which display the "mean-reverting" characteristics. Varying between 0.5 and 1, the Hurst value indicates a persistent behavior of the time series, i.e., unexpanded series in a random walk. However, while examining Nile River overflow, Hurst found the value of H-exponent was much larger at 0.91.

2. MATERIALS AND METHODS

The exchange rates of EUR/ALL and USD/ALL are a means to address the real condition of Albanian financial system[3]. The daily returns from the time series of EUR/ALL from January 1999 to December 2013, and of USD/ALL from January 1994 to December 2013 are used to calculate the Hurst exponent. The calculation for USD/ALL is divided into two phases: i) the period from January 1994 to December 2009 and, ii) the period from January 1999 to December 2013.

A statistical test called rescaled range analysis was developed by Mandelbrot and Wallis (1969). The H was calculated through rescaled range analysis (R/S analysis, - short for range divided by standard deviation) (Mandelbrot, 2004). The R/S is widely used for testing whether long-term dependence is present in a series of data. It is known by statisticians as "non-parametric" tests that make no assumptions on data organizing (Mandelbrot *et al*., 2008).

The R/S formula simply measures whether, over varying periods of time, the amount by which the data vary from maximum to minimum is greater or smaller than what to expect if each data point were independent of the last (Mandelbrot, *et. al*., 2008). Mandelbrot further explained if different from expectations, then the precise sequence of the data must be important: a "run" of gains and losses must be pushing the extreme values farther than they would otherwise go by pure chance.

For a given set of observations $X_i, i \geq 1$, with partial sum:

$$Y_n = \sum_{i=1}^{n} X_i, n \geq 1$$

and sample variance

$$S_n = \sqrt{\frac{1}{n}\sum_{i=1}^{n} [(X]_i - \frac{1}{n}Y_n)^2}, n \geq 1$$

the R/S statistic is defined as:

$$\frac{R}{S_n} = \frac{max_{0 \leq t \leq n}\left(Y_t - \frac{t}{n}Y_n\right) - min_{0 \leq t \leq n}\left(Y_t - \frac{t}{n}Y_n\right)}{S_n}, n \geq 1$$

[3] Source: Bank of Albania

X_t iX_t s the daily return series of EUR/ALL and USD/ALL.

In practice, classical R/S analysis is based on a heuristic graphical approach, originally developed by Mandelbrot and Wallis (1969a- c).

3. THE CALCULATIONS

Rescaled range analysis depends on multiple lengths of time to be analyzed. Hurst exponent is calculated as the slop of the line generated by taking log on *x* axis and log(R/S) on *y* axis. Series of rescaled range is calculated separately for different sample size. EUR/ALL, USD/ALL price series from January 1999 to December 2013 include 3747 daily returns. For the first phase of USD/ALL price series from January 1994 to December 2009 include 3747 daily returns.

The following ranges of daily returns are recorded: 1 range of 3747 daily returns, 2 ranges of 1874 daily returns, 4 ranges of 937 daily returns, 8 ranges of 468 daily returns and, 16 ranges of 234 daily returns. The last size of each range is 1/32 with 32 ranges of 117 daily returns. For EUR/ALL and USD/ALL are calculated 63 arithmetic means. Given the six categories of ranges, six ranges of deviations from the mean would be expectable. Next we calculate the total running of deviations from the mean, the cumulative deviate series.

Once the cumulative deviate series is calculated, the calculation of the maximum and minimum values in the series of deviations for each range follows to take the difference between the maximum and minimum values and the widest difference could be considered. There are 63 calculations, one for each of the 63 ranges, and 63 standard deviation series.

$$S_n = \sqrt{\frac{1}{n}\sum_{i=1}^{n}[(X]_i - \frac{1}{n}Y_n\Big)^2}, n \geq 1$$

The rescaled range for each range in the time series is calculated to the width of the range measured in standard deviations.

$$rescaled\ range = \left(\frac{R}{S}\right)_n = \frac{R_n}{S_n}, n \geq 1$$

Now, the Hurst exponent could be calculated by taking the average rescaled range (R/S) values for each of the region size.

The Hurst exponent could be estimated via R/S analysis. The Hurst exponent is the slope of the logarithmic values for the size of each region and for each region's rescaled range.

The Hurst exponent is also related to the fractal dimension, which gives a measure of the roughness of a surface. The relationship between the fractal dimension, D, and the Hurst exponent, H, is $D = 2 - H$

4. RESULTS

Table 1 reports the USD/ALL 1994 – 2009 Hurst exponent calculation values.

Table 1. USD/ALL 1994 – 2009 Hurst exponent calculation values

Region Size	R/S	Log Size (x)	Log R/S (y)
3747	108.07	3.57	2.03
1874	65.81	3.27	1.82
937	41.69	2.97	1.62
468	26.82	2.67	1.43
234	25.93	2.37	1.41
117	13.54	2.07	1.13

Table 2 reports the USD/ALL 1994 – 2009 Hurst exponent calculated for full series, 1/2, 2/2, 1/3, 2/3, 3/3 of the Log Size(x) and Log R/S(y) values of the table 1.

Table 2. USD/ALL 1994 – 2009 Hurst exponent calculated for full series, 1/2, 2/2, 1/3, 2/3, 3/3 of the Log Size(x) and Log R/S(y) values in table 1.

	Full series	1st half	2nd half	1st third	2nd third	3rd third
Intercept	0.01	0.42	0.16	0.52	0.27	0.81
R-sq.	0.97	1.00	0.79	1.00	1.00	1.00
Slope (Hurst exponent)	0.56	0.69	0.49	0.72	0.64	0.94
Fractal dimension (D)	1.44	1.31	1.51	1.28	1.36	1.06

Table 3 reports the USD/ALL 1999 – 2013 Hurst exponent calculation values.

Table 3. USD/ALL 1999 – 2013 Hurst exponent calculation values

Region Size	R/S	Log Size (x)	Log R/S (y)
3747	81.80	3.57	1.91
1874	57.30	3.27	1.76
937	33.22	2.97	1.52
468	23.80	2.67	1.38
234	24.31	2.37	1.39
117	13.26	2.07	1.12

The table 4 reports USD/ALL 1999 – 2013 Hurst exponent calculated for full series, 1/2, 2/2, 1/3, 2/3, 3/3 of the Log Size(x) and Log R/S(y) values of the table 3.

Table 4. USD/ALL 1999 – 2013 Hurst exponent calculated for full series, 1/2, 2/2, 1/3, 2/3, 3/3 of the Log Size(x) and Log R/S(y) values of the table 3.

	Full series	1st half	2nd half	1st third	2nd third	3rd third
Intercept	0.12	0.40	0.30	0.08	0.09	0.69
R-sq.	0.95	0.99	0.72	1.00	1.00	1.00
Slope (Hurst exponent)	0.49	0.65	0.42	0.51	0.48	0.87
Fractal dimension (D)	1.51	1.35	1.58	1.49	1.52	1.13

Table 5 reports the EUR/ALL 1999 – 2013 Hurst exponent calculation values.

Table 5. EUR/ALL 1999 – 2013 Hurst exponent calculation values

Region Size	R/S	Log Size (x)	Log R/S (y)
3747	62.94	3.57	1.80
1874	49.50	3.27	1.69
937	34.50	2.97	1.54
468	24.11	2.67	1.38
234	19.97	2.37	1.30
117	12.66	2.07	1.10

Table 6 report the EUR/ALL 1999 – 2013 Hurst exponent calculated for full series, 1/2, 2/2, 1/3, 2/3, 3/3 of the Log Size(x) and Log R/S(y) values of the table 5.

Table 6. EUR/ALL 1999 – 2013 Hurst exponent calculated for full series, 1/2, 2/2, 1/3, 2/3, 3/3 of the Log Size(x) and Log R/S(y) values of the table 5.

	Full series	1st half	2nd half	1st third	2nd third	3rd third
Intercept	0.18	0.26	0.16	0.56	0.00	(0.26)
R-sq.	0.99	0.99	0.95	1.00	1.00	1.00
Slope (Hurst exponent)	0.46	0.43	0.46	0.35	0.52	0.66
Fractal dimension (D)	1.54	1.57	1.54	1.65	1.48	1.34

The H results of the table 2, 4, and 6 are in the following charts compared. The slope is an estimate of the Hurst exponent.

Fig. 1. Slope (Hurst exponent) - USD/ALL and EUR/ALL

Figure 2-4 show the charts of the R/S analysis for the two phases of USD/ALL and EUR/ALL obtained by plotting the logarithm of the size (x axis) of each series versus the logarithm of the rescaled range (y axis).

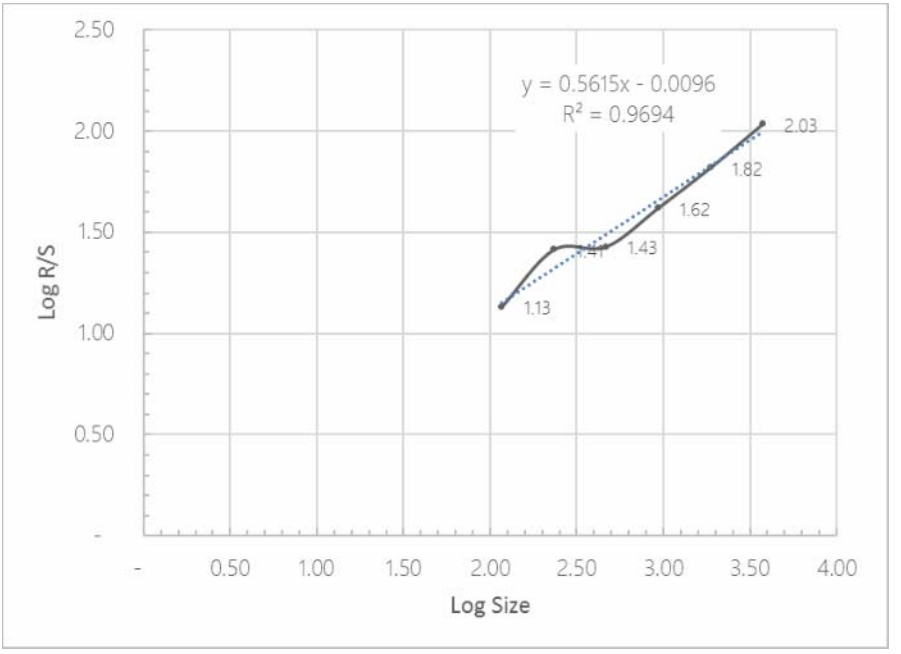

Fig. 2. Rescaled Range Analysis of the USD/ALL, 5 January 1994 to 8 December 2009

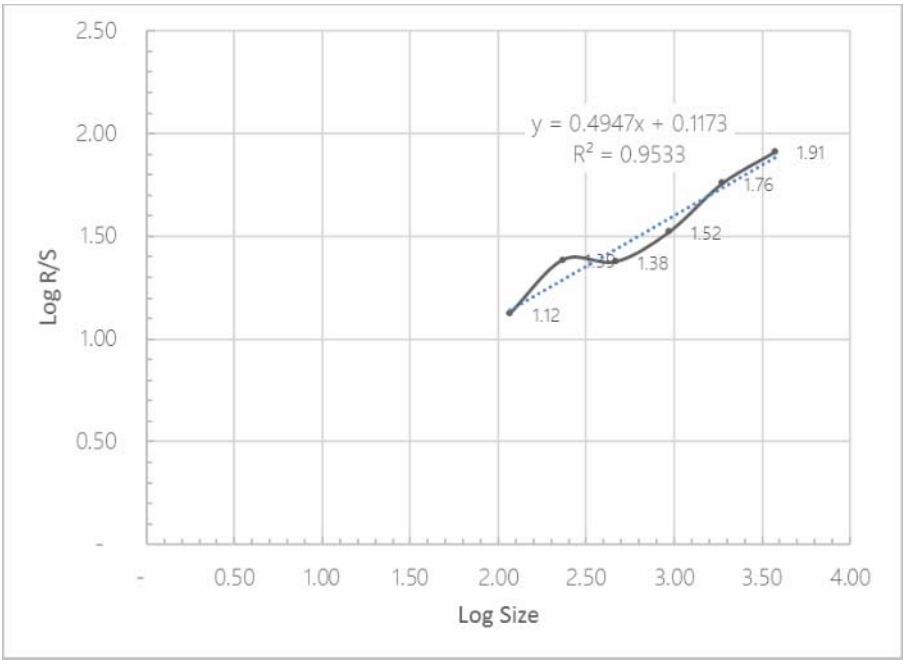

Fig. 3. Rescaled Range Analysis of the USD/ALL, 5 January 1999 to 31 December 2013.

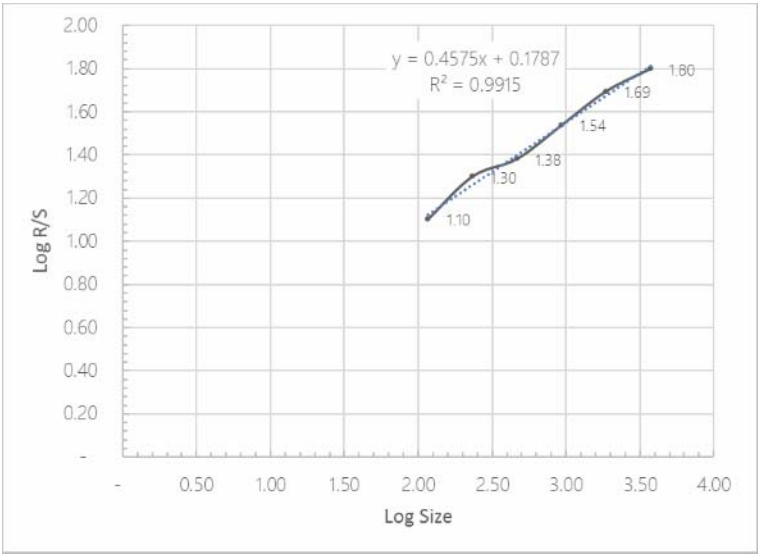

Fig. 4. Rescaled range analysis of the EUR/ALL, 5 January 1999 to 31 December 2013.

To simplify, original Brownian motion is considered. Einstein (1956) said that the displacement of a Brownian particle is not proportional to the elapsed time, but rather to its square root. The Brownian square root rule, mentioned earlier, applies: if the molecule wanders 100 seconds will get around 10 times farther than one that travels just one second (Mandelbrot et al., 2008).

In finance this tell us how far, in any given holding period, the price may rise or fall and how much it is likely to fluctuate within that broad band (Mandelbrot et al., 2008). A rate move in one direction will tend to continue on the next day, and few days later. The trend is proportional to some power, any fraction between 0 and 1, of the time elapsed. Each fraction of the power produces a totally different type of price series.

The Hurst exponent is estimated by a linear regression line through the points of Log Size (x) and Log R/S (y). A line has the form $y = ax + b$, where b is the y-intercept and a is the slope of the line. A linear regression line for USD/ALL first phase (fig.2) calculated through the points in table 1 results in a y-intercept of 0.0096 and a slope of 0.5615. A linear regression line for USD/ALL second phase (fig.3) calculated through the points in table 3 results in a y-intercept of 0.1173 and a slope of 0.4947. A linear regression line for EUR/ALL (fig.4) calculated through the points in table 5 results in a y-intercept of 0.1787 and a slope of 0.4575.

4. CONCLUSION

For the first phase of USD/ALL that covers the period from January 1994 to December 2009, an H bigger than 0.5 is estimated. Prices have a tendency to keep going in the same direction, thus maintaining the strong bullish trend that has been in place since ’94 followed by a long bearish trend up to 2008.

Fig. 5. EUR/ALL chart (left) and USD/ALL chart (right)

For the USD/ALL exchange rate for the period 1999 -2013, the prices exhibited a behavior that was skewed by the tendency to strengthen the trend for short time periods, even though H was estimated around 0.49 for the entire period. For the EUR/ALL exchange rate of the same period of time (1999 – 2013) there is some mean reverting correlation between returns. It could be

concluded that prices have a short-term memory, a persistent market in short periods of time.

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A POTENTIAL ROLE OF BACTERIAL LIPOPOLYSACCHARIDE ON THE ETIOLOGY AND PATHOGENESIS OF TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES

Burim N. AMETAJ[4]
University of Alberta, Edmonton, Canada

Transmissible spongiform encephalopathies (TSEs) known also as prion diseases are a group of neurodegenerative diseases with still unknown etiology. Although the disease has been related to misfolding of a normal cellular prion protein (PrP^{C}) into an abnormal one, known as scrapie (PrP^{Sc}), there is increasing evidence for a potential role of exogenous cofactors in the pathogenesis of the disease. In our study, we hypothesized that mouse recombinant moPrP converted *in vitro* into a beta-rich isoform ($moPrP^{res}$) through incubation with bacterial lipopolysaccharide (LPS) and LPS alone might cause prion-like disease in a murine model. LPS is a cell wall component of all Gram-negative bacteria ubiquitously found in the gastrointestinal tract, infected uterus and mammary gland, and food products. Recently we reported that incubation of LPS with PrP^{C} under normal *in vitro* conditions converted PrP^{C} to a beta rich isoform and resistant to proteinase K degradation ($moPrP^{res}$). To test our hypothesis 90 FVB/N female mice at 6 week of age were randomly assigned into 6 treatment groups (n=15 per group) including: 1) saline (negative control), 2) LPS alone, 3) $moPrP^{res}$, 4) $moPrP^{res}$+LPS, 5) RML (Rocky Mountain Laboratory scrapie strain) + LPS, and 6) RML alone (positive control). Lipopolysaccharide and saline were administered subcutaneously for a period of 6 weeks, whereas $moPrP^{res}$ and RML were injected one time subcutaneously at the start of the experiment. Mice inoculated with LPS or $moPrP^{res}$ showed prion-like disease signs with

[4] Acknowledgements: Dr. Ametaj is the principal investigator of this study. Other people involved in the study include Dagnachew W. Hailemariam (a Postdoctoral Research Fellow and Seyed Ali Goldansaz, an MSc student both in Dr. Ametaj's team. Also, many thanks go to Dr. David Wishart's lab at University of Alberta for providing prion protein used in these experiments and to Dr. David Westaway's lab for helping with the brain histology analyses and for care to the mice at the Center for Prion and Protein Folding Diseases at University of Alberta.

weight loss accompanied with 50% and 60% mortality rates, respectively, compared to the positive controls (RML). Immunohistochemical (IHC) staining of brain sections from the mice treated with LPS or moPrP^{res} showed extensive vacuolation patterns comparable to the positive control but not accumulation of PrP^{Sc} in the brain. Moreover, presence of astrogliosis and deposition of amyloid plaques were detected in some brain regions. Subcutaneous administration of LPS with RML or RML only showed high degree of vacuolation, accumulation of PrP^{Sc}, and astrogliosis. Furthermore, gene expression profiling of all treatment groups in comparison to the negative control indicated down-regulation of genes encoding the prion protein (*Prnp*) and shadow of prion protein (*Sprn*), with the exception of the LPS-only group. Down-regulation of *Prnp* and *Sprn* are considered gene expression signatures specific to prion diseases. In addition, using non-targeted metabolomics approach multiple metabolite alterations were detected in the serum and urine of RML- and RML+LPS-infected mice, which might serve in the future as predictive biomarkers of disease at early stages of prion pathogenesis.

OBITUARY

PROF. DR. BETIM MUÇO WAS AN EMINENT PERSONALITY OF SCIENCE, ARTS AND CULTURE

January 21, 1947-January 15, 2015

Born and raised in Tirana, Albania, he was resident of Rockville, Maryland where he pursued his scientific work, since 2001.

He graduated in nuclear physics from the University of Tirana in 1970, specializing in seismology. He held a PhD in Earth Sciences. Dr. Muço's scientific career spans more than four decades. He contributed enormously to the practice and development of seismology in Albania and the Balkans through his work at the Albanian Institute of Seismology (1974 – 2012). He was the head of the institution from 1993 to 1997. As director of the Seismological Network of Albania, he led several international and regional projects and published a wide array of scientific articles and books. From 1998 to 2005, Dr. Muço co-led two NATO Science for Peace projects on seismology. In 2001, Dr. Muço moved to the United States with his family. He received U.S. citizenship as an "Exceptional Ability Individual in the Sciences and Arts, who would substantially benefit prospectively the national economy, cultural or educational interests, or welfare of the United States."

It is well known the scientific contribution of Prof. Dr. Betim Muço in the following topics: Better understanding of seismicity of Albania and the confrontation between Adria microplate and Albanian orogen; different aspects of induced seismicity; the role of underground water on the seismicity

of Albania and Balkan; focal mechanism solution of Albanian earthquakes; seismic hazard of Albania.

Among the so many published scientific works performed by Prof. Muço must be mention: Evidence for Induced Seismicity in the Vicinity of Fierza Reservoir, Northern Albania (1991), Focal Mechanism Solutions for Albanian Earthquakes for the Years 1964-1988 (1994), Some features of seismicity of Albania (1995), The seasonality of Albanian earthquakes and cross-correlation with rainfall (1995), Seismic zonation of Albania using a deterministic approach (2002), Probabilistic seismic hazard assessment in Albania (2004), Seismicity of Adriatic Microplate and a triggering possibility between its edges: geodynamic implication (2006), Focal mechanism and stress field distribution in Albania (2007), Seismicity, seismotectonics and seismic hazard in Albania (2010), and Geohazards assessment and mapping of some Balkan countries (2012).

He was Science Editor and Translator at General Dynamics Information Technology and a consultant for the Incorporated Research Institutions for Seismology (IRIS) in Washington D.C. He presented scientific papers in many conferences in the U.S. and Europe. His most recent contribution is a series of papers on the correlation between rain and earthquakes in the state of Virginia (2013), which were well received by the scientific community.

It has been said that the world of arts and literature will not be the same without him

Dr. Muço's life passion was literature. He was an acclaimed writer and poet. His first poems were published when he was in high school in 1967 in Albania. He also wrote the lyrics of many songs, popular to this day. He went on to write and publish more than 25 books of poetry, short stories, novels and essays. He also wrote children's books, including three collections of fairy tales, dedicated to each of his three children. Dr. Muço has won several literary awards in Albania and overseas. Many of his poems and short stories have been translated and published in literary anthologies, journals and magazines in English, Russian, French, Dutch, Romanian and Turkish.

In addition, he was also a distinguished translator bringing into Albanian works of Graham Greene, Saul Bellow, Yukio Mishima, Reiner Maria Rilke, James Joyce, Vladimir Nabokov, and Alice Munro. In 2008, he selected and translated into Albanian an "Anthology of World Poetry of the 20th Century" which included translations of selected works by 135 world poets – making it one of the most comprehensive collections of poetry ever published in Albanian. On the evening before he passed away, he was putting the finishing touches to his latest novel, "The Stars Are Quite Close," to be published post-mortem.

Dr. Muço was honorary consul of Japan in Albania in the 1990s. During a post-doctorate program at the University of Tokyo (1990-1991), he developed

very close ties with Japan, its culture and especially its community of seismologists. Upon his return, he published a book of haiku (the first ever in Albanian) as well as a collection of essays on Japanese customs and culture.

Betim Muço's scientific success lay in his vision and an approach that did not follow bandwagons but continually challenged tradition and authority. He was a likeable and approachable and inspiring man who will be much missed.

Prof. Shyqyri Aliaj
Prof. Niko Pojani
Kiço Blushi