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PREPROCESSING TOOLS FOR THE ALBANIAN LANGUAGE: A STATE-OF-THE-ART SURVEY AND AN ANNOTATION SCHEMA PROPOSAL

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ABSTRACT

Language preprocessing tools as parser, morphological analyzer, and lemmatizer have become very popular Natural Language Processing (NLP) approaches. Firstly, in this paper, we have presented a literature review of recent researches on preprocessing tools for the Albanian language. There is a lack of works done to implement these tools for the Albanian language. To our knowledge, there is not an official published preprocessing tool for the Albanian language. Secondly, in this paper, we have proposed a part-of-speech annotation schema for the Albanian language. We mapped the annotation schema proposed by Salavaçi and Biba (2012) into a new annotation schema containing the part-of-speech tags and morphological characteristics tags. Improving the proposed annotation schema and developing a morphological tagger for the Albanian language will foster.

Keywords: POS tagger; stemming; lemmatize; Albanian language

1. INTRODUCTION

Nowadays, there are tools as part-of-speech (POS) tagger, stemmer, and lemmatizer for almost all languages. Efforts have been made to develop these tools for the Albanian Language, but to our knowledge, no one of these tools is available online and public for use. Despite the lack of NLP tools, there is a lack of high-quality public annotated corpora for NLP purposes in the Albanian Language.

This paper aims to evaluate the state-of-the-art situation of POS taggers, lemmatizers, and stemmers in the Albanian Language and to propose a POS annotated corpora for the Albanian Language. We have proposed an annotated schema based on the Universal Dependencies schema. The

proposed annotated corpus using this schema is created by mapping an existing annotated corpora into the new schema.

The Albanian language is the official language in the Republic of Albania and the Republic of Kosovo, the second official language in the Republic of North Macedonia, and the official regional language in Ulcinj, Montenegro. It is an isolated branch of the Indo-European language family. The Albanian language has a complex morphology and syntax system. There are two dialects, Tosk used by the standard Albanian, and Gheg. The Albanian alphabet is Latin and has 36 letters, 29 consonants and 7 vowels. The standard Albanian language morphology has ten different word categories: noun, pronoun, verb, adverb, numeral, particle, conjunction, preposition, and interjection. The morphological categories of the noun are the case (nominative, genitive, dative, accusative, and ablative), definiteness (defined and non-defined), gender (female, masculine and neutral), and number (singular and plural). Some masculine nouns in plural number form change the gender to female and are known as endogen nouns. The adjectives can be linked with an article or not. The adjective adapts in case, number, and gender with the word they modify. So, the adjective has the morphological categories of case, number, and gender. There are seven types of pronouns, possessive, interrogative, demonstrative, subject, relative, indefinite, and reflexive. The verbs have the most complex inflection system. The morphological categories of the verb are the person (1, 2, 3), number (singular and plural), voice (active and passive), mood (indicative, admirative subjunctive, imperative and optative), tense (present, past, and future). The adverbs have the morphological category of gradation. There are five types of adverbs of quantity, manner, time, cause, and location. The numbers, particle, conjunction, preposition, and interjection are non-changeable words and do not have morphological categories. The complex inflectional paradigms of the Albanian language make the development of NLP tools difficult. The forthcoming section discusses the efforts made to create annotated corpora and develop NLP tools for the Albanian Language.

The structure of the paper is as follows: Section 2 present a literature review of the NLP tools for English; Section 3 presents the literature review of the part-of-speech and stemmer tools of Albanian; Section 4 presents a proposal for an annotation schema for the Albanian language, and in Section 4 we conclude our work.

2. Literature review: Natural Language Processing (NLP) Tools

Natural Language Processing (NLP) tools as a part-of-speech tagger, stemmers, and lemmatizers are linguistic procedures performed in computational linguistic throw a wide range of algorithms. A part-of-speech tagger aims to assign to each word in a sentence a morphosyntactic class

based on lexical and contextual information. Stemmer and lemmatizer aim to transform the word respectively into its root (stem) and lemma. These tools can be rule-based or statistical. The rule-based algorithms are developed based on a set of language-dependent morphological rules. Language dependency is one of the most important disadvantages of these algorithms. This disadvantage has led the researchers to focus on developing statistical techniques. Statistical techniques eliminate the language dependency problem, and the developed tools can be used for different languages. These algorithms use supervised or unsupervised statistical techniques. The disadvantage of supervised techniques is the need to have annotated corpora. The creation of an annotated corpus is time-consuming and requires specialized persons to do it. The performance of these tools depends significantly on the languages they are used for. More complex languages require powerful techniques to have good performance in POS tagging, stemming, and lemmatization.

In 1992, Brill (1992) developed the first and the most widely used rule-based part-of-speech tagger for English. This tagger assigns to each word a tag based on the Penn Treebank tagset. The Pen Treebank tagset has 36 tags for morphological annotation of English. The method used by this tagger required a reduced store space, and the identification of the errors is easy, but the tagging process is slow.

The supervised technique is used to develop the TnT tagger (Brants, 2000) and the Stanford Log-linear POS Tagger (Toutanova *et al.*, 2000). The TnT tagger (Brants, 2000) is an implementation of the Viterbi algorithm for the second-order Hidden Markov Model (HMM) model. As scholastic approaches, the HMM model and Viterbi algorithm assign the most probable tag to a word when used to POS tagging. This tagger is implemented in English and German using three different corpora with an accuracy of 96.7%. The Stanford Log-linear POS Tagger (Toutanova *et al.*, 2000) is a POS tagger based on a maximum entropy algorithm. Overthrow the years it is improved and implemented in different languages. This model enhances the performance of tagging by enriching the information sources used to tag. The authors have implemented the POS tagger using the Penn Treebank with an accuracy of 96.86% and 86.91% for unseen words.

The first developed stemmer algorithm is rule-based. In 1968, Lovins (1968) developed the first context-sensitive, longest-match rule-based stemming algorithm for English. The algorithm has two phases. In the first phase, the longest matched ending of the word from a predefined list of 294 endings is removed. Then in the second phase, the stem is treated to solve some linguistic exceptions like double consonants or irregular plurals utilizing one of the 35 transformation rules. This stemmer is very fast, but too many rules needed to be implemented. As the algorithm has a limited number of predefined endings, there can be a high number of errors (Lovins, 1968).

Dawson (1974) improved and extended Lovin's stemmer by using a list of 1200 endings and using the partial matching technique to match stems that are equal within certain limits. Dawson's stemmer algorithm uses a large set of endings and requires more time and storage to be executed.

Porter's stemmer is one of the most used rule-based stemming algorithms in Information Retrieval. The first version of this algorithm was for English, and later due to its good results, it was used for languages like German, French, Russian, etc. This algorithm has five steps, uses around sixty suffixes, has two rules responsible for recording and one context-sensitive rule to decide if a suffix should be removed or not. In 2001, was created the Snowball framework (Porter, 2001) that includes an improved version of the algorithm for English, German, French, Russian, etc.

In 2013, Mayfield and McNamee (2003) proposed the first language-independent stemming algorithm. This N-gram stemming algorithm offers the possibility to develop models for different languages. The experimental results demonstrate the effectiveness of the selection of a single n-gram as a stem of a word.

Last years, researchers are using more and more neural networks or deep learning in the development of parsers, part-of-speech taggers, stemmers, or lemmatizers. The models based on these technologies offer the possibility that one proposed solution to be easily used in different languages. Different tools like these have been implemented and evaluated in the framework of a lot of shared tasks.

3. Literature review: annotated corpora for the Albanian language

In this section, we present the existing annotated corpora and morphological tagging tools for the Albanian Language. The grammar of the Albanian language is complex, and the process of the annotation is a challenge. The complicated inflectional paradigm of the Albanian language makes the development of stemming and lemmatization tools too difficult. There are some attempts to develop rule-based stemming tools that we have discussed in detail below.

Trommer and Kallulli (2004) proposed a simple rule-based morphological tagger for the standard Albanian language taking into consideration the main grammatical rules of the Albanian language. The tagger uses 340 morphological rules that indicate the relation between input lexicon and output derived forms. The tagset is a pair of sets attribute-value conform to EAGLE guidelines standard adapted to the Albanian language grammar. The tagset contains 17 labels: n, v, a, prsp, part, reflp, posp, demp, indp, intp, relp, pa, prep, adv, ptl, seq, conj. The tagger is evaluated in two small corpora, each of 500 tokens (word) so we cannot define if this is a performing tool.

The tagger proposed by Salavaçi and Biba (2012) may be considered as the first statistical part-of-speech tagger for Albanian language using the OpenNLP tool. The authors have annotated a corpus of 10.000 words using three tagsets, respectively with 100 tags, 150 tags, and 220 tags. We have discussed in detail this tagset in section 3. This annotated corpus is used to train two models, a Maxent model, and a Perceptron model. The average accuracy of the two models is nearly 60%. We will use this annotated corpus to create the annotated corpora using the Universal Dependencies (UD) schema.

The Saint-Petersburg linguists' team (Morozova and Rusakov, 2014) created The Albanian National Corpus contains 16.6 million tokens. This is the biggest annotated corpus for the Albanian language. The corpus contains text from short stories, novels, fiction and non-fiction memoirs, journals, religious official, and scientific texts. The tagset used have 62 tags which include the standard tags as a verb, adverb, preverb, adjective, adjective numeral, numeral, pronominal clitic, conjunction, preposition, prepositional article, particle, interjection, pronoun, and other tags about the case, gender, number, definiteness, mood, person, tense, verbal representation, animacy, transitivity, voice, pronominal clitics, and article position. Each word has the correspondent lemma, the English translation, and the morphological features of the lexeme and the word form. The authors report that based on four Albanian dictionaries they have manually created the files that contain a grammatical wordlist with all the information of the lexeme and the inflectional paradigm. The corpus is morphologically tagged automatically by using the morphological analyzer UniParser, which assigns separately to each word of the corpus a tag considering the information in these files (Arkhangelskiy et al., 2012). The process of tagging does not take into consideration the syntactic context of the word form when determining its grammatical features. The authors do not report the performance of the Albanian model in terms of accuracy. The tagged model is not available online to be downloaded; the annotated corpus is available online, but cannot be downloaded to be used for NLP purposes.

Kadriu (2013) developed a morphological tagger using the NLTK toolkit. To assign part-of-speech tags to new text this morphologic tagger uses a corpus annotated corpus of 32000 words and a set of regular expressions rules. In the first phase, a tokenizer is used to tokenize the sentences and the words, then only nouns and verbs are assigned the lemma by removing or replacing the suffixes. In the second phase, the word is annotated with the part-of-speech tags using the NLTK. First, the word is tagged using the tagger based on the dictionary and then is used the regular expression tagger determines the correct tag to a word by using a series of regular expressions and affixes. The tagset contains 22 tags. In case there is a word not

determined in the dictionary or cannot be generated by the regular expression pattern, it is tagged with the tag None.

The implemented model has an accuracy of up to 90% but the corpus used to train, and to test is small, and the tagger generated a considerable number of untagged words as the words non-found in the dictionary, the irregular verbs, nouns, and adjectives.

Kirov et al. (2018) have created a small morphological annotated corpus for the Albanian language. The corpus contains 589 lemmas, and 33483-word forms in total using the UniMorph schema. To each word is assigned the corresponding lemma and the morphological tags. The trained models and the annotated corpus are free downloaded.

Kabashi and Proisl (2018) have created a morphologically annotated corpus for the Albanian language, but it is not available online. The corpus contains 2020 sentences, 31584 tokens. Two native Albanian speakers have manually annotated it. They presented three tagsets, one full tagset specified by the authors, and the corresponding mapping tagset to Google UPOS and Universal Depends UPOS. The full tagset has in total 79 tags classified in 16 main tags categories: 4 tags for nouns, 14 tags for verbs, 5 tags for adjectives, 3 tags for adverbs, 14 tags for pronouns, 1 tag for preposition, 6 tags for conjunctions, 2 tags for numbers, 19 tags for particles, 1 tag for interjections, 1 tag for articles, 3 tags for pronominals, 2 tags for abbreviations, 2 tags punctuations, 1 tag for emoticon and 1 tag the non-linguistic element. Even they have used a large tagset they have not included tags for the number, gender, definiteness, and case of the name. The authors have specified a large number of tags to distinct nouns and adverbs preceded by an article. The annotated corpus is used to develop five models using SoMeWeTa tagger, HMM-based HunPos tagger, TreeTagger, Stanford POS Tagger, and OpenNLP tagger. The accuracy of the models is 85.96% to 95.10%, and SoMeWeTa is the best performant model.

Karanikolas (2009), a non-native Albanian speaker, developed the first rule-based stemming algorithm for the Albanian language.

This algorithm works on the principle of the longest-match suffix removal. The author, based on five Albanian grammatical books, has defined a list of 470 stopwords. The suffix remover is done in three steps, firstly is removed the longest suffix that matches the ending of the word, then the generated word is used to remove another matched longest suffix, and in the final step is kept only the first of a sequence of multiple ending consonants from the word generated by the second step. This algorithm is manually evaluated in a small corpus.

Sadiku and Biba (2012) presented the JStem algorithm, the rule-based stemming algorithm implemented in Java Programming for the Albanian language. For the Albanian language, this can be considered the first

stemming algorithm developed by a native speaker. The algorithm uses 134 rules that remove the suffixes and prefixes of a word to generate its root and a stopwords list. The authors have not specified rules for plural formation, feminine, masculine, and neutral gender formation. The JStem algorithm is extended by Biba and Gjati (2014) to stem the composite words in the Albanian Language. By analyzing the morphology structure of composite words in the Albanian language, the authors have added a set of rules to find the stems of the composite words. The implemented rules take into consideration the composite words formed by two words linked by a separating dash, with prefixes, with numerals, by productive nouns associated with verbs or adjectives, with international prefixes, and formed randomly by associating two different words.

4. A proposal for an annotation schema and an annotated corpus for the Albanian language

This section presents a grammar annotation schema (part of speech and morphological features) for the Albanian language using the Universal Dependencies (UD) schema. We have considered the tagset and the annotated corpus developed by Salavaçi and Biba (2012) and propose a new annotated corpus based on the rules of the UD schema.

The annotated corpus of Salavaçi and Biba (2012) does not have the lemmas of the words, so we have manually assigned to each word the corresponding lemma. To define the correct form of the lemma, we have used the Albanian National Dictionary.

The existing tagging set is mapped to the new tagging set that follows the UD schema. One of the problems we fronted in this process is that tagging in the UD schema is word-level, while Salavaçi and Biba (2012) in the annotation consider the difference between an adjective preceded or not by an article and the verbs in a composite tense are annotated as an entity and not every single part of it separately.

The corpus annotation using the new schema was done automatically by a program developed by us considering the mapping rules we defined for the old and the new annotation schema.

The tagset presented by Salavaçi and Biba (2012) has 224 tags in total. There are 39 tags for nouns, a tag for particular nouns, and 38 for nouns indicating gender, number, case, and definitiveness. They have not considered if the noun is preceded by an article or not as defined in the Albanian language grammar. We use the tag NOUN to tag a noun and PROPEN to tag a person's name. To nouns, we have assigned the morphological features of the case, definite, gender, and number. We have not assigned morphological tags to a person's names.

Salavaçi and Biba's (2012) tagset specifies 20 different tags for the article, indicating the gender, number, and case. In our schema, we have used the DET tag and no morphological feature.

Table 1. Noun annotation examples.

	Salavaçi and Biba's (2012)		Our Annotation	
	Annotation	Tag Meaning		
derën	derën_NNFNSK	Noun - Female - Singular - Definitive - Accusative	NOUN	Case=Acc Definite=Def Gender=Fem Number=Sing
Veliu	Veliu_NNP	Particular noun	PROPN	

One of the most complex word categories in the Albanian language is the verb. Salavaçi and Biba (2012) have used 75 tags for the verb. There is a tag for the compound verb tense to tag the verb kam (have) and jam (is) used in the Albanian language as an auxiliary verb to form the compound tenses. For this category, they have not specified any grammatical characteristics. They have different tags for modal verbs, negative verbs, infinitive, gerundive, and participle forms of the verb. The other 69 tags are used to tag the verbs based on the different morphological features, like mood, tense, person, number. In this tagging schema, a verb in a compound tense is tagged as the auxiliary verb with tag Vb+ and the verb with the corresponding tag that represents the mood, tense, person, and the number of this compound verb. We have adopted this schema to the UD schema using only the main tag VERB and the AUX tag for the mund (can) verb. As morphological features, we use case, number, person, and tense. For the verb in compound tense, we have used the tag VERB even for the auxiliary verb and the main verb and the same grammatical features tags for the two verbs. For the infinitive form of the verb, we have used the morphological feature VerbForm=Inf. For the gerund form of the verb, we have used the morphological feature VerbForm= Ger. For the participle form of the verb, we have used the morphological feature VerbForm= Part. Maybe our verb tagging schema will need to be revised to a better proposal. Table 2 shows some examples.

Table 2 Verb annotation examples.

	Salavaçi and Biba's (2012)		Our Annotation		
	Annotation	Tag Meaning			
kishte bërë	kishte_VB+ bërë_VBDMIIN	VB+ -> Verb - Compound verb VBDMIIN -> Verb - Indicative - Past Perfect - Person III - Singular	kishte	VERB	Mood=Ind Number=Sign Person=3 Tense=Past
			bërë	VERB	Mood=Ind Number=Sign Person=3 Tense=Past
pat kthyer	pat_VB+ kthyer_VBDKTIIS	VB+ -> Verb - Compound verb VBDKTIIS -> Verb - Indicative - Pluperfect - Person III – Plural	pat	VERB	Mood=Ind Number=Plur Person=3 Tense=Pqp
			kthyer	VERB	Mood=Ind Number=Plur Person=3 Tense=Pqp
do të ketë	do_RP të_RP ketë_VBDAIIN	PR -> Particle VBDAIIN -> Verb - Indicative - Future - Person III – Singular	do	PART	
			të	PART	
			ketë	VERB	Mood=Ind Number=Sing Person=3 Tense=Fut

Salavaçi and Biba (2012) have used 68 tags for the pronouns specifying the type and the morphological features of gender, number, and the case for the demonstrative, personal, interrogative, and indefinite pronouns. They have a different tag for subjunctive pronoun, a reflexive pronoun, short form of the personal pronoun, personal pronoun contraction short form, possessive pronoun, agglutinated pronouns, short for of personal pronoun, and indefinite form of the personal pronoun. For these pronouns' categories, they have not defined morphological features. We have tagged all the pronouns with the PRON tag. The PronType morphological feature is used to define the type of pronouns. To the demonstrative pronouns, we have assigned the morphological features of the case, gender, number, and prontype with the value Dem. The morphological features used to personal pronouns are the case, gender, number, person, and prontype with the value Prs. To the short form of the personal pronoun, personal pronoun contraction short form, and

agglutinated pronoun, we have defined the morphological features pron type with value Prs. To the pronouns tagged by Salavaçi and Biba (2012) with the tag possessive pronoun, we have assigned the morphological features Poss=Yes and PronType=Prs. We tagged the interrogative pronouns with the morphologic features case, gender, number, and prontype with the value Int. And the indefinite pronouns are tagged with the morphologic features case, gender, number, and prontype with the value Ind.

Table 3 Pronouns annotation examples.

	Salavaçi and Biba's (2012)		Our Annotation	
	Annotation	Tag Meaning		
ata	ata_PRVIIIISK	Pronoun Personal - Person III - Masculine - Plural - Accu.	PRON	Case=Acc Gender=Masc Number=Plur Person=3 PronType=Prs
kjo	kjo_PRIPFNE	Indefinite Pronoun - Female - Singular - Nominative	PRON	Case=Nom Gender=Fem Number=Sing PronType=Dem
tim	tim_PRPRN	Possessive pronoun	PRON	Poss=Yes PronType=Prs
pse	pse_PRP	Interrogative Pronoun	PRON	PronType=Int

Salavaçi and Biba (2012) have defined 6 different tags for the adverbials, one for each category in the Albanian language. We have used the ADV tag to tag an adverbial and the morphological feature AdvType to define the adverbial type.

Table 4 Adverbial annotation examples.

	Salavaçi and Biba's (2012)		Our Annotation	
	Annotation	Tag Meaning		
si	si_ABM	Adverbial - Clauses of manner	ADV	AdvType=Man
përse	përse_ABQ	Adverbial - Purpose clauses	ADV	AdvType=Cau

In the Albanian language, the conjunction is a non- inflected word category, and Salavaçi and Biba (2012) used only one tag. We have used the CCONJ and the SCONJ tag and no morphological feature.

To tag numbers, Salavaçi and Biba (2012) have used three tags, one for cardinal numbers and two for ordinal numbers specifying their gender female or masculine. We use the NUM tag for numbers and the morphological feature NumType with value Ord for ordinary numbers and with value Card for cardinal numbers.

In the Albanian language, the preposition is a non-inflected word that fits in the case with the noun or adjective that follows them. Salavaçi and Biba (2012) have used four tags for prepositions considering their case. The preposition in our schema is tagged as ADP, and we have defined the morphological feature of the case.

In the Albanian language, the adjective is inflected for the case, number, and gender. In addition, it can be either articulated or unarticulated. Moreover, the adjectives have the degrees of comparison. The tagset of Salavaçi and Biba (2012) defined 5 tags for this word-class indicating whether it is articulated or not, or gradability (affirmative, comparative, and superlative degrees). The adjective is tagged using the ADJ tag, and no morphological features are assigned. Based on Albanian grammar, we need to add the morphological features of the case, number, and gender in the future.

We have used the PUNCT tag for punctuation and INTJ tag for the interjection and onomatopoeic words.

Table 5 Annotation examples

	Salavaçi and Biba's (2012)		Our Annotation	
	Annotation	Tag Meaning		
dhe	dhe_CC	Conjunction	CCONJ	-
kur	kur_CC	Conjunction	SCONJ	-
një	një_CDRF	Ordinal numeral - Female	NUM	NumType=Card
dy	dy_CDRM	Ordinal numeral - Masculine	NUM	NumType=Card
1935	1935_CDT	Cardinal numeral	NUM	NumType=Ord
nga	nga_INE	Preposition - Nominative	ADP	Case=Nom
e thellë	e_CC	CC -> Conjunction	e	CCOJN
	thellë_JJNP	JJNP-> Adjective - With Article - Base	thellë	ADJ
shumë prekës	shumë_RP	RP -> Adjective - Without	shumë	PART
	prekës_JJPS	JJPS-> Adjective - Without Article - Superlative	prekës	ADJ
.	._.	punctuation	PUNCT	-

The Figure 1 illustrates an example of an annotated sentence by Salavaçi and Biba (2012) and Table 6 shows the annotated sentence using our proposed tag schema.

Agroni_NNP punonte_VBDPIIS për_INK ngritjes_NNFNSK e_JNFNK oxhakut_NNMSNG
bashkë_ABS me_INK brigadën_NNFNSK e_JNFNK tij_PRPRN_

Fig. 1: Salavaçi and Biba sentence annotation.

Table 6 Annotated sentence using our proposed tag schema

Word	Lemma	POS tag	Morphological features
Agroni	Agron	PROPN	
punonte	punoj	VERB	Mood=Ind Number=Plur Person=3 Tense=Past
për	për	ADP	Case=Acc
ngritjen	ngritje	NOUN	Case=Acc Definite=Def Gender=Fem Number=Sing
e	e	DET	
oxhakut	oxhak	NOUN	Case=Gen Definite=Def Gender=Masc Number=Sing
bashkë	bashkë	ADV	AdvType=Man
me	me	ADP	Case=Acc
brigadën	brigadë	NOUN	Case=Acc Definite=Def Gender=Fem Number=Sing
e	e	DET	
tij	tij	PRON	Poss=Yes PronType=Prs
.	.	PUNCT	

5. CONCLUSION

In the present a survey of the existing morphological annotation tools and annotated corpus for the Albanian language is presented. There is no official tool to be used for the Albanian Language. We have proposed a new annotation schema for the Albanian language by mapping the annotation schema of Salavaçi and Biba (2012) to a new one based on UD Schema. The annotated corpus in (Salavaçi and Biba 2012) is mapped to the new annotation schema by an automatic program. The annotation is the first step and the most important to develop modern morphological annotation tools. In the Albanian language, the annotation process is challenging due to its grammar complexity. The annotation schema in the present paper proposed can be the first step to create a stable annotation schema for the Albanian language. The annotation corpus also can be the first step towards an annotation corpus based on a multilingual annotation schema. In the future, we plan to enlarge the annotated corpus and use it to develop and implement a morphological tagger.

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DIMETHOATE ADSORPTION/REMOVAL FROM AQUEOUS SOLUTIONS BY SOME ALBANIAN CLAYS

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ABSTRACT

The present paper evaluates the adsorptive properties of some natural clays in Albania to technical dimethoate from its aqueous solutions. Consequently, these clays could be used to remove the various insecticides from aquatic environments. A new sample pretreatment method was developed for the gas chromatographic determination of dimethoate in its aqueous solutions. The representative samples of clays mineral originate from Brari, Currila, Dardha and Prrenjias regions in Albania. The initial dimethoate for the concentration are 0,200g/L and 0, 300 g/L, 0,400g/L and 0,500 g/L. Adsorption of dimethoate for the concentration of 0,200g/L was studied for a period of time from 6 to 144 hours (6 days), while the other concentrations are studied within the adsorption interval of 48-hours. The clay from Brari region exhibited better adsorptive properties compared to the others, followed by the Dardha, Currila and Prrenjasi' s clays. The results obtained show that the four clays could be used for the purification of groundwater from dimethoate residues in Agriculture.

1. INTRODUCTION

The organophosphate pesticides have been extensively used in agriculture and show considerably toxicity for humans (Colovic *et al.*, 2013; Lazarevic-Pašti *et al.*, 2019). They are persistent pollutants which cause the contamination of drinking water in the agricultural areas where are used (Ali *et al.*, 2017; Triassi *et al.*, 2017). Removal of dimethoate from aqueous solutions has been investigated, among different techniques, by means of adsorption as well (Maria *et al.*, 2010; Vagia, *et al.*, 2010)

Dimethoate is produced and marketed by many countries around the world, with many trade names and labels. The market products usually contain about 40% active ingredient (pure dimethoate), the rest consists of emulsifiers, fillers, dyes, etc. It is commercially known as Dimethoate, Rogor, Bromthion, Perfection, Phosphamide etc. In Albania it is commonly known as

Rogor and is the most widely used organophosphate insecticide for plant protection.

On the other hand, clays are found in big quantities in Albania, widely spread on the surface of plain and hilly regions. Over 90 clay deposits have been discovered in Albania (Dede 1980; IGEWE; Kola. 1986). Nowadays, clays are used for many purposes. Currently, there are 25 deposits exploited for various application purposes in Albania. Information about the use of clays in Albania as could be found in (Kola 1986; Malaj 2006; Xhaxhiu, 2013; Xhaxhiu, 2013; Tako 2015; Xhaxhiu 2020). Here, the application areas are: i) wine clearing, ii) rehabilitation of oil removal from turbines and transformers, iii) improve the physical and chemical properties of vegetable oils, iv) removal of heavy metals from waters and, iv) organophilic surface treatment.

Recent studies are focused on the use of clays for the purification of groundwater from pesticide residues from the agriculture sector.

The present investigation aims to exploit the adsorption abilities of four Albanian clays as natural adsorbents. The clays were not chemically pretreated, but were in activated form by specific treatments.

2| MATERIALS AND METHODS

2.1 Materials:

2.1.1 Chemicals

Dimethoate Pestanal-Analitical Standart-99% - MERCK (Darmstadt, Germany)

Technical Dimethoate

The technical dimethoate is provided by the Greek company “K & N-Efthyamiadis s.a” and has an active substance content of 40%. In addition, it is accompanied by the relevant certificates and approved by the Institute of Food Safety and Veterinary (IFSV), Tirana, Albania.

Dibutyl phthalate (DBP)

Analytical standard is provided by the FLUKA, Buchs, Germany, and employed as an internal standard for the laboratory analyses (99. 5%).

Methylene chloride

High pressure liquid chromatography grade (> 99, 9%), and ethyl acetate (99, 8%) is provided by the Sigma-Aldrich, Seelze, fc Germany.

The inorganic salts used: KCl (99,999%) and Na₂SO₄ anhydrous, (for synthesis grade) is provided by the MERCK, Darmstadt, Germany.

2.1.2 Clay samples

The Table 1 below summarizes the regions where sampling occurred.

Table 1. Sampling areas

Brari -Tirana:	41° 21' 14.49'' N 19° 50' 17.74 ''E
Currila- Durrës:	41° 19' 16.29 '' N 19° 25' 51.92 '' E
Dardha –Korçë:	40° 31' 16.59'' N 20° 49' 33.69'' E
Përrenjasi – Librazhd:	41° 4' 3.88'' N 20° 33' 2.33'' E

Samples were selected based on a registered database from the Institute of Geological Research, at University of Tirana. In each case a sample quantity of 25-30g of clay was drawn as a representative average amount from an initial quantity of 5 kg ground natural clay. The representative clay samples were dried in a thermostat for 4 hours at a temperature of 150°C and sieved using a 74-mesh sieve (fraction: 0-0.250 mm). The chemical composition and physico-chemical properties of the clays were determined and reported in (Kola 1986), and are in the table 2 and 3 reported. Moreover, numerous literatures are focused on their physico-chemical properties, some of which we considered in our study (Nutting 1943; Kanazawa 1989; Xhaxhiu et al., 2013; HAL 2019; USGS Pub. Rep (n.d); Wikipedia;).

A high sodium and kalium content was found in the Brari and Currila's clays, while a high CaCO_3 content was found in the Dardha's clay (22,6-26,3%), Currila's clays (19, 3-23, 9 %) and Brari's clay (21, 8-23, 2%).

Table 2. Chemical composition (%) of natural clays

Region	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	CaO	MgO	TiO ₂	Na ₂ O+K ₂ O	HK
Brari	43,4-53,9	11-15,8	5,4-7,7	7,7-11	4-7,9	-	3,03-3,93	10-14,9
Currila	42,21	12,47	5,26	13,69	5,94	-	3,35	-
Dardha	44,72	12,54	6,67	11,68	6,24	-	-	-
Prrenjasi	46,5-52,9	6,4-9,2	2,8-18	0,1-3,5	5-16	0,3-0,7	0,3-0,8	16-20

Table 3. Physico chemical parameters of natural clays

Region	pH	Density (g/cm ³)	Porosity (%)	Surface area (m ² /g)
Brari	7,5	2,77	0,490	42
Currila	9,3	2,79	0,568	45
Dardha	7,6	2,78	0,564	89
Prrenjasi	7,4	2,78	0,558	175

Traces of titan (Ti) are found in the clay of Prrenjas. It could be noted from the Table 2 that the Currila's clay has the highest pH level. Brari's clay shows the lowest density, porosity and lower surface area.

Powder XRD measurements and characterizations of the clays samples are detailed in (Xhaxhiu 2020).

2.2 Equipment

The Hewlett Packard (GC) equipped with a flame ionization detector (FID), and a nonpolar capillary column with a diameter of 0.32 mm and 30 m long, SE 30 was used for the gas-chromatographic measurements. Its working conditions are: the column working temperature was 160-280°C and rate 20°C/min. The detector temperature was 300°C. The injection temperature was 250°C. All the experiment was carried out in a splitless mode.

Centrifuge

The sample was centrifuged in an Eppendorf Centrifuge, model 5403 (Leipzig, Germany), with a rotation speed of 5000 rpm.

Sieve

The clay samples were sieved via Fritsch Analysette [Type 03502] Idar-Oberstein- Germany.

2.3 Adsorption procedure

The concentrations of the market product dimethoate with 40% active substance content are calculated after the conversion to pure substance to determine dimethoate adsorption for the four clays.

The initial concentrations of dimethoate were 0,200g/L, 0,300g/L, 0,400g/L and 0,500 g/L.

Once the work started, the solutions were prepared. The clay suspension of dimethoate aqueous solutions are prepared under the ratio: 1 gram of clay for every 5ml of solution.

Suspensions are kept under continuous stirring and, at specified time intervals samples are taken for the determination of dimethoate content in the water.

The suspension sample with a volume of 30-40 ml is centrifuged for 15 minutes at 5000 rpm, 25 ml of clear solution taken and passed through a special cork test tube. A 2,5 g of KCl is subsequently added and dissolved. Dimethoate is further extracted by adding 5-6 ml solvent (Ethyl Acetate-dichloromethane 3:1 vol/vol) and shaking on mechanical device for about 20 minutes. Once shaken, the phases are allowed to separate for 5 minutes and the organic phase gets collected. The extraction procedure is repeated three times, and the organic phases are mixed together.

The organic phase is dried over 1g anhydrous Na_2SO_4 . Once dried over, a solution of an internal standard (Dibutyl phthalate in absolute ethyl alcohol – 2,5 mg/ml) is added to obtain the concentration of the standard of 50 micrograms / ml.

Only sample volume of 1-2 ml is analyzed. The gas chromatographic analysis of the dimethoate is based on the aforementioned method with an heating rate of $20^\circ\text{C} / \text{min}$ and the splitless injection method x 1 microliter. The resulted retention time for dimethoate is 6, 30 minutes. For the internal standard DBP, it is 7, 90 minutes.

The amount of dimethoate in solution is calculated according to the corresponding factor on the basis of the chromatograms of the standard and of samples. The results are in the table 4 and 5 reported. In addition, the hydrolysis of dimethoate is determined and appears to be on average 4%. This value is used to correct all the obtained results for the adsorbed amount of dimethoate on clays.

The technical dimethoate recovery is in average 46.65%, due to the influence of emulsifiers and other accompanying substances of the technical product.

This sample preparation method for the gas chromatographic analysis of dimethoate is based on a range of experiments and (Amandola 1967; Melluso *et al.* 1994; Othmer 2000; Othmer 2000; Al Kuisi 2002; HAL. 2019; Xin *et al.*, 2021)

3. RESULTS AND DISCUSSIONS

The adsorption of technical (market) dimethoate from its aqueous solutions was investigated applying the aforementioned experimental procedure and the results on the concentration of 0.200g / L and for adsorption times from 6 hours to 144 hours (6 days) are in the Table 4 reported.

Table 4. The influence of clay type and contact time on dimethoate adsorption initial concentration $C=0.200$ g/L, $T=25$ °C)

Time Region	6	24	48	72	96	120	144
Brari	0,0875	0,1250	0,1905	0,2691	0,2812	0,3570	0,3818
Currila	0,0823	0,1155	0,1605	0,2116	0,2553	0,3205	0,3387
Dardha	0,1127	0,1494	0,1884	0,2384	0,3282	0,3554	0,3568
Prrenjas i	0,0550	0,0961	0, 1252	0,1765	0,2287	0,3065	0,3294

Brari's clay followed by Dardha's clay exhibit better the adsorptive properties to dimethoate in aqueous solutions, for the concentration C 0,200 g / L. The samples collected from Currila and Perrenjas have a mean adsorptive ability.

The results in the Table 4 reported are graphically depicted in the Figure 1. The variation of the adsorbed amount of dimethoate with the contact time for the four clay samples has similar pattern. The amount of dimethoate adsorbed increases steadily in any case for 140 hours at the initial concentration of 0.200 g/L, i.e. basically the same adsorption ability towards dimethoate, regardless the specific adsorption values of each clay. Consequently, they have the same potential to be used for the purification of water contaminated with this insecticide.

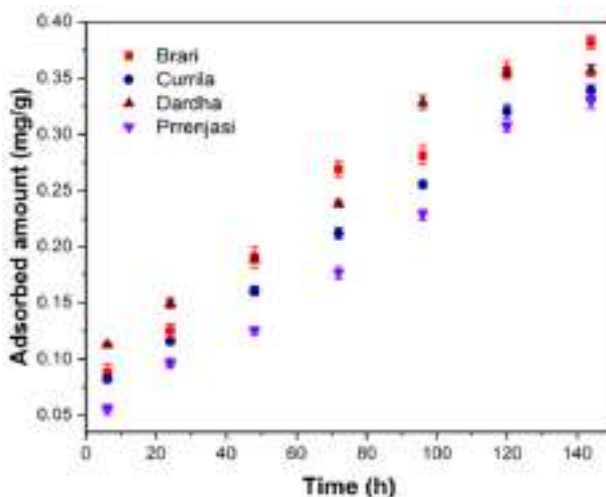


Fig. 1: Adsorbed amount of dimethoate as function of its initial concentration from its aqueous solutions at 25°C on one gram of clays of Brari, Currila, Dardha and Prrenjasi.

The investigation of dimethoate adsorption at higher concentrations and, specifically with concentrations of 0.300g / L, 0.400g / L and 0.500 g / L, was carried out in the same way.

These concentrations are not met in agricultural practices, but considered as extreme possibilities that could appear by chance, or due to indiscriminate use of insecticides by unprofessionals.

As the maximum concentration of C = 0,500g / L (or otherwise 1,250 g / L gross technical product with active substance content 40%) is inappropriate to be used due to the high content of surfactants and other accompanying substances of the product, it is not used. The concentration 0.400g /L is less problematic to the experiment process.

An average interval of 24 hours, 48 hours, and 72 hours were chosen as the clay-dimethoate aqueous solution contact time.

The contact time of 48 hours was chosen as the average time for the comparison of the adsorption results for the different clays.

In this time interval the adsorption phenomena are stabilized and comparisons could be made among the clays. No result is expected for the extension of time beyond 72 hours. As time extends, the counter-slowning down adsorption, reducing the adsorption capacity of clays, saturation and finally desorption appears.

All the results obtained experimentally for concentrations mentioned above are presented in Table 5.

The data of table 5, are presented graphically in Figures 2, 3, 4 and 5 (adsorbed amount mg/g from concentration) graphically depicted.

Table 5. The Influence of clay type, contact time and initial concentration on the adsorption of dimethoate on four different clays from its aqueous solutions at 25°C.

C, g/L	0,200			0,300			0,400			0,500		
Time, h Region	24	48	72	24	48	72	24	48	72	24	48	72
Brari	0,125	0,250	0,269	0,215	0,542	0,634	0,281	0,672	0,751	0,635	0,822	0,910
Curila	0,115	0,130	0,212	0,118	0,260	0,301	0,123	0,265	0,441	0,132	0,251	0,175
Dardha	0,149	0,188	0,238	0,099	0,207	0,255	0,190	0,250	0,330	0,191	0,193	0,204
Prrenjasi	0,096	0,125	0,176	0,103	0,289	0,384	0,122	0,233	0,318	0,154	0,389	0,376

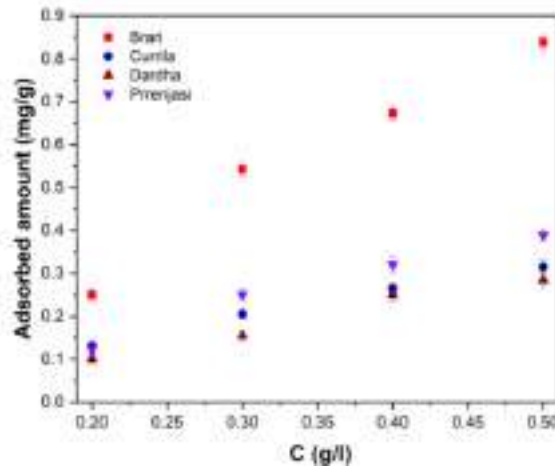


Fig. 2: Adsorbed amount of dimethoate as function of its initial concentration from its aqueous solutions at 25°C on one gram of clays of Brari, Currila, Dardha and Pprenjasi.

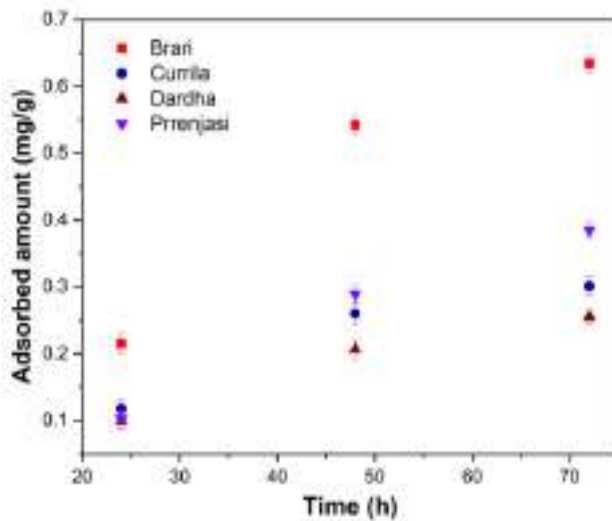


Fig. 3: Adsorbed amount of dimethoate as function of the contact time from its aqueous solution with the initial concentration of 0.300 g/L at 25°C on one gram of clays of Brari, Currila, Dardha and Pprenjasi.

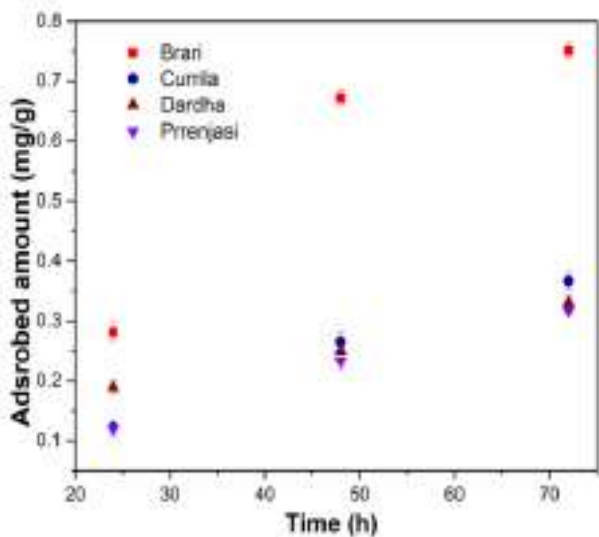


Fig. 4: Adsorbed amount of dimethoate as function of the contact time from its aqueous solution with the initial concentration of 0.400 g/L at 25°C on one gram of clays of Brari, Currila, Dardha and Pprenjasi.

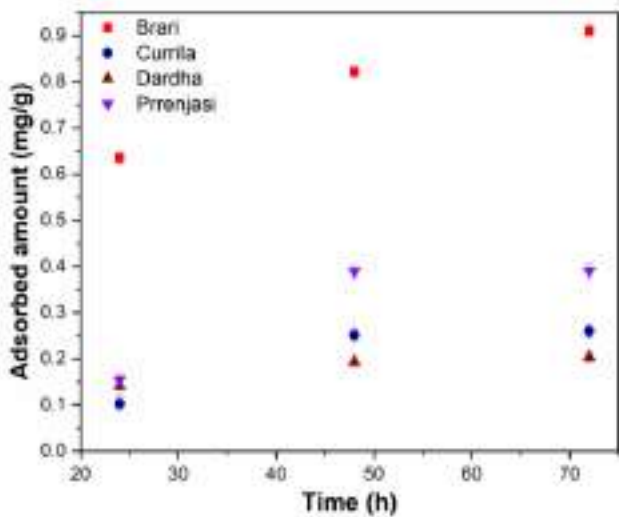


Fig. 5: Adsorbed amount of dimethoate as function of the contact time from its aqueous solution with the initial concentration of 0.500 g/L at 25°C on one gram of clays of Brari, Currila, Dardha and Pprenjasi.

4 CONCLUSIONS

The adsorption behavior of dimethoate on four different natural clay minerals of Albania from its aqueous solutions is studied for its variation with the contact time and the initial concentration of dimethoate solutions. Contact time of the clays with the dimethoate solutions ranged from 6 hours to 144 hours and the initial concentrations were 0.200 g/L, 0.300 g/L, 0.400 g/L and 0.500 g/L.

The variation of the adsorbed amount with the contact time follows a similar pattern for all the four clay samples, showing as the best adsorbent in every time interval and every initial solution's concentration the clay of Brari followed by the Prrenjas clay that displayed better properties at increased concentrations of (0.300-0,500g/ L).

Other clays have medium adsorptive properties. To make a relative comparison of clays we take Brari's clay basis, for the concentration $C = 0,200\text{g / L}$ and in time 48 hours which we estimate 100%, then the Currila's clay represent 52% of this value, Dardha 75,2 % and Prrenjas 34,2 %. As increased of concentrations, also changes the order of adsorptive properties occur, Prrenjas's sample displayed a marked increase in concentrations: $C=0,300$, $C= 0,400$, $C=0,500\text{ g/L}$ with respective values of 53, 3%, 47, 8% and 47, 3% compared with Brari's values of 100%. Dardha's and Currila's samples show average values, with stable increasing concentration almost all indicators show a downward trend. Finally, the study shows that the four selected natural clays, of our regions, can be successfully used to remove dimethoate from contaminated groundwater during the practice of using this insecticide in agriculture sector.

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ADSORPTION PROPERTIES OF ROW AND CHEMICAL ACTIVATED GRAIN-MATERIAL DERIVED FROM NATURAL SAND OF ALBANIAN COASTLINE

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ABSTRACT

The present paper investigates the adsorption properties of a natural material obtained from the sand of Albanian coastline. The heaviest fraction of the natural material is processed through mechanical and chemical activation to obtain different forms of the grain materials. Mechanical activation under wet and dry conditions was applied using 1% and 10% H_2SO_4 . Two consecutive mechanical stages followed by chemical activation were compared with simultaneous activation of the absorbent. The differently-prepared grain materials have been investigated in relation to their adsorption properties with Cu^{+2} . The experimental data were fitted into Langmuir and Freundlich isotherms theoretical model for the investigation of the adsorption procedure. Once the data were linearized, the adsorption parameters were calculated. The linearization of Freundlich equation provided the highest values of correlation coefficient, confirming that the adsorption of Cu occurs according to a heterogeneous and non-monolayer process. The differences found are related with the value of n calculated for the mechanical and chemically activated samples. Regarding the mechanical activated grain material, $n < 1$ shows the probability of chemical adsorption; while the effect of chemical activation of the grain material, is expressed in the increase of the value of n which means gradual transition from chemical adsorption to physical ones.

Keywords: adsorption, isotherm, grain material

1. INTRODUCTION

Heavy metals constitute a very heterogeneous group of elements widely varied in their chemical properties and biological functions. In addition, they are kept under environmental pollutant category due to their toxic effects on living species. Consequently, constant attention has been paid to their removal, especially from aquatic environments. Some of the techniques for removing heavy metals from aquatic environments are based on the processes of precipitation, filtration, ion exchange, etc. Meanwhile, techniques based on adsorption processes are of particular interest, especially when relatively low cost adsorbents are used. Water purification from Ni, As, and Cu, Cd and Pb has been respectively investigated in (Janusz and Skwarek 2005; Mavropoulos *et al.*, 2005; Mayo *et al.*, 2007; Vaclavikova *et al.*, 2010; Zhang *et al.*, 2020) based on the adsorption properties of some natural materials classified in the group of ilmenite-magnetite materials. Their adsorbent properties are related to the presence of TiO_2 and/or Fe_2O_3 . Also coastal sands containing oxides of silicon, iron, etc. (Vasjari *et al.*,) can be used in rapid filtration processes to remove metals from water as a result of adsorption processes. The primary objective of the present paper is the application of innovative methods employing grain materials with adsorptive properties for environmental purposes. The preparation and use of these materials which utilize industrial waste for the development of environmental cleaning technologies is an example of the "circular economy". A circular economy (also referred to as circularity and CE) is a model of production and consumption, which involves sharing, leasing, reusing, repairing, refurbishing and recycling existing materials and products as long as possible. The material here investigated was obtained from the Old Sand Enrichment Plant, Dures, and its chemical composition (Vasjari *et al.*,) makes it a likely adsorbent for metals. The natural material has been pre-treated through mechanical activation under various experimental conditions for better adsorptive properties to heavy metals. Investigation on copper ion affinity involving theoretical adsorption isotherms and linearization of the data was a means to address the present experiment.

2. MATERIALS AND METHODS

In the present investigation, reagents of analytical grade were used to prepare the required aqueous solutions. Adsorption isotherms employing different concentrations of Cu^{2+} aqueous solution, at room temperature, were used for the investigation of the adsorption process. There are two modes of adsorbent preparation: i) dry mechanical activation followed by chemical

activation (treatment in aqueous solution with different pH) and, ii) simultaneous mechanical and chemical activation.

In the first case, once grinded, the material was analyzed for its particle size distribution through a sieve system by dividing it into 6 fractions with the following size ranges: i) 0.50-0.25 mm, ii) 0.25- 0.125mm, c) 0.125-0.10mm, iii) 0.10-0.09mm, iv) 90-71 μ m and, v) 71-56 μ m. Based on preliminary studies performed in the laboratory (Broli 2013) the 90-71 μ m fraction was selected which underwent the acidic activation process.

The chemical activation was performed in plastic tube of 50 ml. 5 g from the selected fraction (71-90 μ m) were weighed and 1% or 10% H₂SO₄ solution was added. The prepared suspension was placed in a shaker for 12 hours. Once decanted, it was filtered, rinsed with distilled water several times and placed in a thermostat at 60°C overnight.

In the second case, the agate sphere mill, a device that ensures fine grinding of the materials in wet condition using different pH solution was used for the preparation procedure (Hanke 2014; Phoothinkong *et al.*, 2017). The inner part of the equipment which is in contact with the material and working solutions is a by-inert material avoiding the reaction with the materials to be treated in it. The spheres are also made of agate, a strong and inert material also.

A certain amount of natural material (table 1) was placed in the milling container together with the solution which will realize the chemical activation of the material during grinding. 1% H₂SO₄ solution and 10% H₂SO₄ solution for 4 hours were used for chemical activation purposes. Once decanted, the crushed solid material was filtered, rinsed with distilled water several times and placed in a thermostat at 60°C overnight. The prepared grain materials were fractionated using the sieve system, and each fraction was weighed to estimate losses (tab 1).

Table 1 Distribution of material by fractions (in gr.)

	Preparation procedure	Initial weight (g)	>100 μ m	100-90 μ m	90-71 μ m	71-63 μ m	\sum fractions (g)
2	Chemical activation 1% H ₂ SO ₄	12.45	4.82	0.34	0.54	6.45	12.15
3	Chemical activation 10% H ₂ SO ₄	11.53	3.53	0.33	0.63	6.86	11.35

Adsorption experiment: An amount of activated material was placed in contact with 15 ml of a standard solution of Cu⁺² (5 to 200 ppm). The systems

were kept for 12 hours at room temperature under continuous mixing conditions using Roll MIXER (80 rrot / min). The Hettich EBA 20 centrifuge for 5 min at 45,000 rpm was used to separate solution from the solid phase (adsorbent). The Flame Atomic Absorption Spectroscopy (Specter AA-10 plus) was involved for the concentration of Cu^{+2} in the solutions before and after the adsorption process. The amount of adsorbed Cu is calculated and expressed in mgCu /gr adsorbent.

Langmuir Isotherm model and Freundlich's model are used to evaluate the adsorption parameters. This isotherm model is based on the assumptions that the adsorption process occurs at particular homogeneous sites on the surface of the adsorbent. In addition, it allows to calculate the maximum adsorption in monolayer through the parameter X_{max} expressed in mg analyte per gram adsorbent. The Freundlich isotherm model predicts a heterogeneous adsorption process and provides the opportunity to judge whether adsorption is characterized by chemical or physical interactions through the value of the parameter n. Both models provide information on the affinity of analyte adsorption to the adsorbent through the constant K.

The table 2 summarizes the experimental results of the Cu+2 absorption from the mechanical activated material. The amount of absorbed Cu is calculated and expressed in mgCu/gr adsorbent is in this table reported. Logarithmic values necessary for of the data linearization are also reported. Figure 1 depicts the absorption curve, while the linearization of the data according to Langmuir and Freundlich's theoretical model are in the Figure 2 and 3 respectively depicted.

The same procedure is followed in all experiments using activated material in different modes. Figure 4(a) and (b) depict the results of data linearization when the activated material in H_2SO_4 1% used applying the first procedure (mechanical and chemical activation simultaneously). Figure 4(c) and (d) represent the case of activated material via dry mechanical activation and wet chemical activation in H_2SO_4 10%.

Table 2 Adsorption data of Cu from mechanical activated grain material

Nr	Adsorbent (g)	Initial concentration C (ppm)	Final concentration C (ppm)	ΔC (ppm)	Adsorbed (mg Cu /g)	$\log(C_{\text{ads}})$	$\log C$
1	0.0202	5	4.75	0.25	0.12	-0.90	0.70
2	0.0217	10	9.88	0.12	0.06	-1.25	1.00
3	0.02	15	14.52	0.48	0.24	-0.62	1.18
4	0.0208	25	23.12	1.88	0.90	-0.04	1.40
5	0.0213	50	44.47	5.53	2.59	0.41	1.70
6	0.0228	100	90.31	9.69	4.25	0.63	2.00
7	0.0242	150	131.99	18.01	7.44	0.87	2.18
8	0.0212	200	177.90	22.10	10.42	1.02	2.30

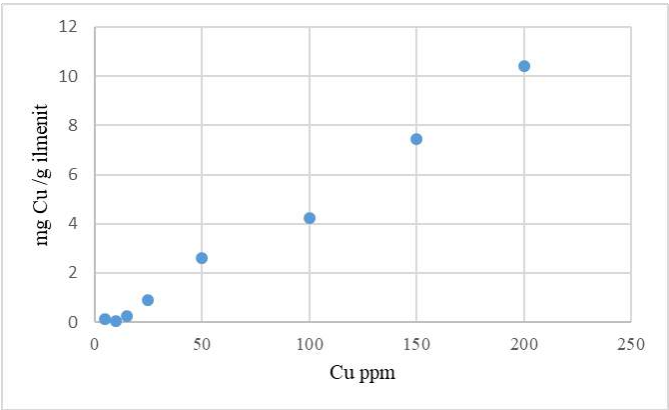


Fig 1: Adsorption isotherm using mechanical activated grain material.

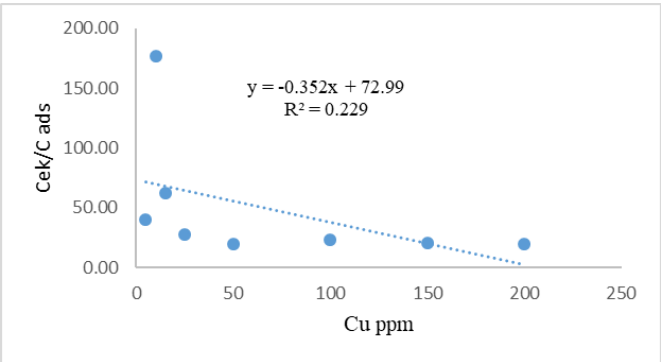


Fig. 2: Linearization of Cu adsorption data according to Langmuir isotherm model using mechanical activated grain material.

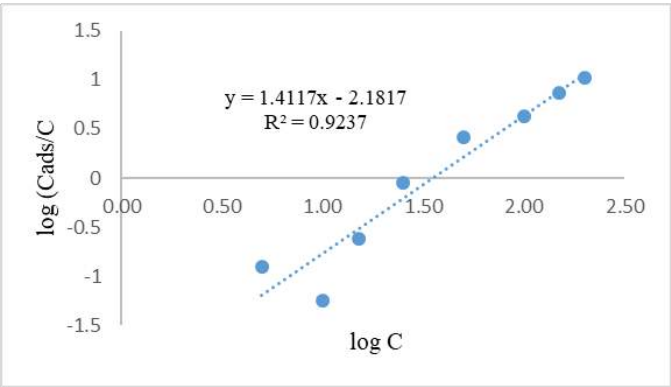


Fig 3: Linearization of Cu adsorption data based on Freundlich isotherm model using mechanical activated grain material.

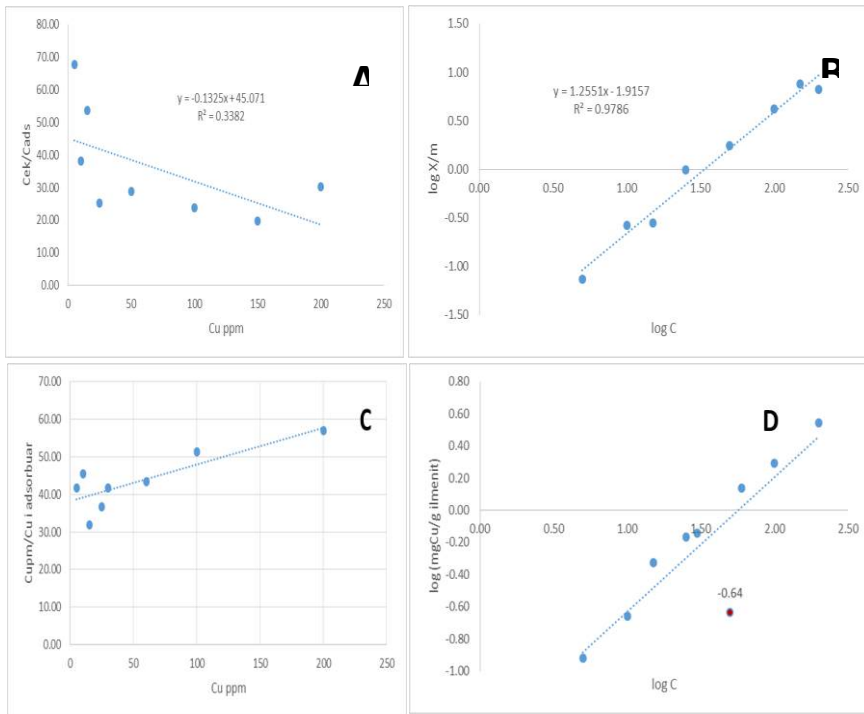


Fig. 4: Linearization of absorption isotherms: a) activated material in H₂SO₄ 1% -Langmuir model; b) activated material in H₂SO₄ 1% -Freundlich model; c) activated material in H₂SO₄ 10%- Langmuir model; d) activated material in H₂SO₄ 10%- Freundlich model.

3. DISCUSSIONS

Langmuir isotherm, in its general form is given as the degree of coverage of the surface (active centers), depending on the concentration of the solution in contact with it, in equilibrium conditions:

$$x_{ek} = \frac{X_{\max} K C_{ek}}{1 + K C_{ek}}$$

a.

where X-ek is the amount of Cu⁺² adsorbed (g Cu / g adsorbent) corresponding to C-ek of the analyte in the solution. X-max is the adsorption capacity of the material, or the maximum amount of Cu⁺² adsorbed from 1g adsorbent. K is a constant for a given system which represents the affinity of Cu⁺² for the adsorbent. Referring to the certain adsorbent system, the smaller the value of K, the greater the affinity between analyte to adsorbent. To find the constant K as well as the maximum amount that can be adsorbed by 1 g of adsorbent, the isotherm equation can be linearized as follows:

$$\frac{C_{ek}}{X_{ek}} = \frac{1}{X_{\max} K} + \frac{C_{ek}}{X_{\max}}$$

The linearization of the data following to the Langmuir theoretical model for only mechanical activated grain material (fig.2), as well as for mechanical and chemical activated grain material are given in table 3. In the case of mechanical activated grain material the negative value of the slope of the curve from which C_{\max} should be calculated as well as worst correlation coefficient, indicates that the adsorption of this grain material cannot be explained by this model. For chemical activated material in H_2SO_4 1% or 10% linearization of experimental data according to the Langmuir theoretical model results in a correlation coefficient of 0.3382 and 0.6861, respectively (Fig. 4 (a) and (c)). This means that adsorption does not approach the assumptions of this theory for monolayer and homogeneous adsorption.

Table 3 Summary of adsorption results

Preparation procedure	Theoretical model	Linearization of the data	R^2	Adsorption parameters	
				K	X_{\max} (mg/g)/n
2 steps: * Dry mechanical activation * Wet chemical activation (H_2SO_4 10%)	Langmuir	$Y = 0.098 x + 38$	0.6861	$2 \cdot 10^{-3}$	10.2
	Freindlich	$Y = 0.906 x - 1.49$	0.9844	$10^{-1.5}$	1.10
1 step *Wet mechanical and chemical activation simultaneously (H_2SO_4 1%)	Langmuir	$Y = -0.132 x + 45.07$	0.3382	-	-
	Freindlich	$Y = 1.2551 x - 1.92$	0.9786	$10^{-1.9}$	0.80
Dry mechanical activation	Langmuir	$Y = -0.352 x + 72.99$	0.2290	-	-
	Freindlich	$Y = 1.4117 x - 2.18$	0.9237	$10^{-2.2}$	0.71

To prove that adsorption can occur according to a multi-layered and heterogeneous model, the experimental data obtained are fitted to Freundlich's theoretical model. According to the Freundlich model, the relationship between adsorption and analyte concentration in solution is given by the equation:

$$\frac{C_{ads}}{C} = K * C^{\frac{1}{n}}$$

where K and n are two characteristic adsorption constants. K provides information on the adsorption capacity, the affinity between the analyte and the adsorbent. The n indicates the intensity of adsorption. The value of n indicates the deviation from the linearity of the adsorption dependence of the analyte concentration in the solution: i.e. if it results in $n = 1$, then the adsorption is linear; if $n < 1$ adsorption is a chemical process; while in $n > 1$ the adsorption process is physical (Desta 2013).

The linearization of this equation is given as a dependence:

$$\log \frac{C_{ads}}{C} = \log K + \frac{1}{n} \log C$$

The linearization of the experimental data obtained for mechanical activated grain material according to the Freundlich theoretical is shown in Fig 3. The value of the correlation coefficient is acceptable for the linear dependence $R^2 = 0.9237$. From the parameters of the straight line the affinity is evaluated presented by calculated $K = 10^{-2.18}$ while the value of $n = 0.71$ suggest the chemical adsorption ($n < 1$).

Regarding the grain materials activated in H_2SO_4 1% or 10%, linearization of experimental data has resulted in a very good correlation with $R^2 = 0.9786$ and $R^2 = 0.9844$, respectively, as based on Freundlich's theoretical model. The values of the n coefficient increase as pH (increasing the concentration of H_2SO_4 during activation) decreases, which means that the intensity of adsorption increases. Based on the results obtained, independently by the preparation procedure of the grain material, the data show the very good fitting to the Freundlich's theoretical model. Surface characterization using SEM and EDS analyzes of the chemically activated samples (Vasjari et al.,), has shown that the particles of the grain material of absorbent have irregular shape, high surface roughness and the chemical composition of the surface varies depending on the chemical activation. This explains the Cu absorption due to heterogeneous and multilayers form.

4. CONCLUSIONS

The absorption of Cu from the experimented material, derived from the sand and prepared differently using mechanical activation and/or chemical activation is studied based on Langmuir and Freundlich theoretical models of adsorption. The experimental data fit very well to theoretical model of Freundlich. Consequently, Cu^{2+} adsorption is a heterogeneous process on a heterogeneous surface.

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NUTRITIONAL STUDYING OF PRIMARY METABOLITES AND MICROELEMENTS OF HAZELNUT

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ABSTRACT

Many studies report the low incidence of cardiovascular disease associated with the consumption of nuts, particularly hazelnuts, supporting this way the beneficial role of nuts in human health. The population of the Mediterranean is prone to the Mediterranean diet, which typically includes plenty of fruits, vegetables, bread and other grains, potatoes, beans, nuts and seeds; olive oil as a primary fat source; and dairy products, eggs, fish, and poultry in low-to-moderate amounts. Besides the Mediterranean diet, the Dietary Guidelines for Americans recommend including nuts in the diet because of the fatty acids found in the n-6 / n-3 ratio as it improves the lipid profile in blood. Hazelnuts are rich in macronutrients (protein, carbohydrates, and lipids) and micronutrients (trace elements, minerals, and vitamins). The present paper investigates the fatty acid and micronutrient profiles of hazelnut marketed and consumed in Albania. Oleic acid became the major fatty acid with up to 70% of the total fatty acids. The ICP-MS was employed to analyze metals like magnesium, calcium, potassium. Based on the determined amounts in the samples referring to minerals, we calculated a recommended daily consumption amount of nuts at 42.5 g.

Keywords: hazelnut, nutritional interest, fatty acids, microelements

1. INTRODUCTION

Hazelnut (*Corylus avellana* L) is an endemic bush in many Mediterranean countries, consumed as fruit for a long time throughout the world since

prehistoric civilization. FAOSTAT (2020) stated that the world hazelnut production for 2019 was 1154496 tons. There is no information about Albania.

In modern times, hazelnuts are used as a processed ingredient in chocolates and other sweets (Platteau *et al.*, 2011). They are well known and appreciated for their organoleptic properties. In addition, they are very nutritious and healthful because of their favorable composition of nutrients and nutraceutical compounds (Kelly and Sabaté, 2006). Because of their richness in nutrients and bioactive health-promoting compounds, there are good reasons for profitably including hazelnuts as part of a nutritious and functional diet (Contini *et al.*, 2011). Hazelnuts are a rich source of fat (about 60%) and fiber (around 10%), as well as an excellent source of protein and carbohydrates; the most important minerals are potassium, phosphorus, calcium, and magnesium, while significant amounts of copper, manganese, and selenium (USDA 2009). The unsaturated FAs are main group in hazelnut's fatty acids profile, by 50—73%. Studies show that other secondary constituents are phytosterols, phenolic compounds, and other antioxidants. These compounds are supposed to intervene in the control of other cardiovascular diseases, such as blood hypertension, or control cholesterol levels in the blood (Alasalvar *et al.* 2006; Perna *et al.*, 2016). Recently, hazelnut extracts showed antimicrobial activity to gram-positive bacteria (Oliviera *et al.*, 2008). Several scientific studies have presented results on the mineral content and vitamins showed by geographical factors (Dunar and Altundag, 2004). Information about the chemical composition of hazelnuts in Albania could be found in (Osmani-Lataj *et al.*, 2013).

FAOSTAT and INSAT do not provide any information about the production and consumption of this raw food, but such information could be found (Osmani-Lataj *et al.*, 2013). In Albania, studies about foods as essential sources of fatty acids focus on olive oil (Topi *et al.*, 2013). Data on the chemical composition of *Corylus avellana* L., cultivated in Albania, are presented last decade (Osmani-Lataj *et al.*, 2013). The present study investigates the nutritional aspect of fatty acids of hazelnut as a representative of the nuts group, which are an essential part of the Mediterranean diet.

2. MATERIAL AND METHODS

Hazelnut samples were purchased in the local market of Tirana. Representative samples were prepared based on the sampling methods as recommended by the EN ISO 948 on Spices and condiments (coffee, tea, spices, beans, nuts, and dried fruits). The minimum quantity for each final sample is 100-500 g.

Analytical Methods

Total fats were extracted by Soxhlet at 60°C for 6 hours, and n-hexane was used as a solvent. Hazelnut oil samples were kept at 4°C in the dark until GC analysis. The fatty acids were analyzed as Fatty Acid Methyl Esters (FAME) via GC-FID (Thermo Quest, 2000) equipped with a capillary column (23.3m x 0.25mm x 25 µm) according to the AOCS methods (AOCS, 1990). The total ash was calculated based on (Köksal *et al.*, 2006).

The Spectrophotometer of Atomic Absorption (Varian SpectrAA – 400 Plus) was involved in investigating the mineral content. The phosphorus was analyzed as phosphomolybdate vanadium according to James (1995) by Spectrophotometer.

Statistical analysis

The investigation was carried out in September 2019. Chemical analyses were carried out in triplicate for each hazelnut sample. An amount of 15 g of hazelnut sample was processed for further analysis. Data are presented as Mean±StDev. The Minitab Statistical software (MINITAB INC. 814-238-3280) was involved in the statistical analysis. The standard error was $P \leq 0.05$.

3. RESULTS AND DISCUSSIONS

Fatty acid composition and total fat

The total fat content was analyzed in both cultivars resulted in over 60%, with a range of 60.08-61.57%. Ten fatty acids were detected above the Limit of Detection, but the most important appear to be: palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic acid (Table 1). Oleic acid was found at 80.34%, followed by linoleic acid at 14.54%. The results on analyzed samples present significant differences for five fatty acids. The palmitic acid varies from 4.53-5.69%, and the linoleic acid content resulted at the interval 11.67-14.54%.

Comparison of the results with data from the literature shows similarity in the mineral content with cultivars of the Eastern Mediterranean Sea (Alasalvar *et al.*, 2003; USDA 2007). Köksal *et al.* (2006) compared the palmitic acid values between the hazelnuts of the Balkan eastern region of the Black Sea. He reported that the hazelnuts cultivars of the Balkan Region have a higher rate than the hazelnuts of the eastern Black Sea region (4.72—5.87%).

The saturated fatty acids (SFA) resulted in 6.62-67.34%, while PUFA was higher, 11.73-14.61%. SFA group is supposed to raise total cholesterol (TC) and low-density lipoprotein (LDL), which are undesirable to human health.

However, certain SFA (as consumed in our daily diet) have beneficial effects on the ratio of LDL to high-density lipoprotein (HDL) (Ding *et al.*, 2017).

Table 1: Total fat (g/100g); fatty acids (% FAME) as Mean \pm STDEV

Fatty acid	Hazelnut cultivars	Mean	SD
Palmitic	4.53-5.69	5.11	0.82
Palmitoleic	0.36-0.41	0.39	0.04
Stearic	1.65-2.09	1.87	0.31
Oleic	76.13-80.34	78.24	2.98
Linoleic	11.67-14.54	13.11	2.03
Linolenic	0.059-0.068	0.06	0.01
SFA	6.62-7.34	6.98	0.51
PUFA	11.73-14.61	13.17	2.04
PUFA/SFA	1.60-2.21	1.90	0.43
Total fat	60.08-61.57	60.78	1.12

Analysis of minerals

Ash content was in the interval 1.91- 1.97%, with a mean value of 1.92% ($P \leq 0.02$) (Table 2). Minerals in higher amount were measured potassium by 750.25 mg /100g, phosphor by 298 mg /100g and calcium (232 mg/ 100g). The potassium varied from 112.2 mg 100g⁻¹ to 114.44 mg 100g⁻¹, Magnesium content is 8.55 mg/100g, Copper content is 1.98 mg/100g. The iron content appears to be 4.11 mg/100g. Zinc amounts resulted to 2.28 mg/100g (Table 2). The essential minerals are: potassium (58.27%), cooper (0.14%), manganese (0.30%), iron (0.26%), magnesium (12.56%), phosphor (16.66%), zinc (0.17%). These data are comparable to the publications on the literature review.

Table 2: Minerals and non-minerals (mg 100g⁻¹) expressed as Mean value \pm STDEV ($P \leq 0.05$)

Minerals	Hazelnut samples	Mineral content (% of total)	Mean \pm STDev
Calcium	140-232	11.49	180.22 \pm 65.05
Cooper	1.67-1.98	0.14	1.82 \pm 0.22
Iron	3.12-4.11	0.26	3.58 \pm 0.70
Magnesium	153-192	12.56	171.39 \pm 27.58
Manganese	3.65-8.55	0.30	5.59 \pm 3.46
Natrium	2.09-2.84	0.17	2.44 \pm 0.53
Phosphor	203-298	16.66	245.96 \pm 67.18
Potassium	710-750	58.27	735.00 \pm 28.18
Zink	2.04-2.28	0.17	2.16 \pm 0.18
Ash (g/100 g)	1.89-1.96		1.92 \pm 0.05

There are similarities between the mineral composition of the hazelnuts here reported and (Dundar and Altundag, 2004; Simsek *et al.*, 2007; Muller *et al.*, 2020).

Although there is a lack of official information about the consumption per capita, Osmani-Lataj *et al.* (2013) said that the annual consumption is estimated to be 0.07 kg/person, based on the unpublished statistics. FAOSTAT and INSTAT databases do not provide any information about the production and consumption of hazelnuts. EU countries report that hazelnut consumption is >1kg/person.

4. CONCLUSIONS

The chemical composition of hazelnuts is here investigated for a better information on the nutritional aspect. The fat content of the cultivars resulted in over 60%, similar to other publications.

Hazelnut ash content shows the high potential for nuts to introduce in the diet of the different population groups. Considering the importance of several metals, for example, Zn, as co-factors in the enzymatic reactions, it is essential to use nuts for consumers' nutritional status. As hazelnuts are rich in potassium and calcium, they are an important source of microelements. Further studies would be necessary to compare the results and the setup of a database to provide more comprehensive information about the impact of climatic conditions and anthropogenic activity factors on hazelnut cultivation.

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ON THE NEOGENE AGE OF THE EVAPORITES OF PALASA: A REVIEW¹

Petrit MAVROVI*

ABSTRACT

The present paper aims at dating the Palasa evaporites based of paleopalynological analyzes carried out in China from 1970-1971 without providing any conclusion, but complemented with additional data from current publications. Here, even a limited number of samples as there isn't any geological section would be sufficient. Evaporites are presented as tectonic blocks. Being situated at the foot of Çika Mountain (or West of Çika tectonic Belt), Palasa evaporites usually have been considered as part of Ionian tectonic zone. They have been reported to be from Permian-Triassic to Oligocene in age.

Keywords: Evaporites, Palasa, Neogene, Miocene, Pliocene, paleopalynology, microfauna

1. INTRODUCTION

The evaporitic formation could be found in the Ionian tectonic zone, the Peri-Adriatic Depression and in the Korabi tectonic Zone. The evaporites of the Ionian Zone that are exposed to the surface, especially in the Kurveleshi tectonic Belt, are Upper Triassic (Carnian) in age. The evaporites of the Peri - Adriatic Depression belong to the upper Miocene, while the evaporites in the Korabi tectonic zone are Triassic in age (Diamanti F., *et al.*, 1999).

¹ The photos of the microfossils are copies from the catalog compiled by the Chinese scholars.

* Retired from the National Hydrocarbon Center.



Fig. 1: The Llogara Pass: The fault between Ionian tectonic zone (East) and Sazani-Karaburuni tectonic zone (West). Photo credited to the author (1968).

Palasa evaporite deposits emerge in the west of the Çika tectonic belt and extend from the Dhikle spring to the vicinity of Palasa. They appear in form of lenses (Shushkov and Tkaciev 1960) and small isolated blocks often covered by slope rubbles, which make them difficult to locate. Consequently, proving their existence in surface was impossible.

Although the location of the outcrop 511 described in (Shushkov and Tkaçiev 1960) was not found, the authors offer a compelling argument by providing a detailed information about microfauna (see the list of 511 outcrop determinations, volume II, dealing with a middle Miocene age). This new fact is upholding the age previously supposed by the presence of genus *Tsuga* in the published pollen determinations (Gjikopulli and Rama 1973), sample Nr 15, see below, and a means to address palynological studies to foster the age determination of these evaporitic deposits.

The samples Nr., 15; 16; 511 (microfaune) and the sample named *Burimi i Dhikles* (spring) and Vuno-1 have been analyzed. The samples nr. 15, and 16 have unknown coordinates. They were sampled in 1969, and information about them could be found in (Gjikopulli and Rama 1973). Although the coordinates of sample 511 are known, the author has not found it (the slope rubbles might be the source). The sample named, *Burimi i Dhikles*, (Fig. 5), was collected in 1972 (Muhameti P., Pejo I., 1974), from the spring with the same name (Fig. 2). The Fig. 6 depicts the position of the sample Vuno-1 as defined by the author .

Palasa region is geologically complex. In addition, different surfaces are covered by slope robbles making it even more complex. The complexity relates to the fault between Ionian and Sazani-Karaburunu Zones (Fig.1 and 2)

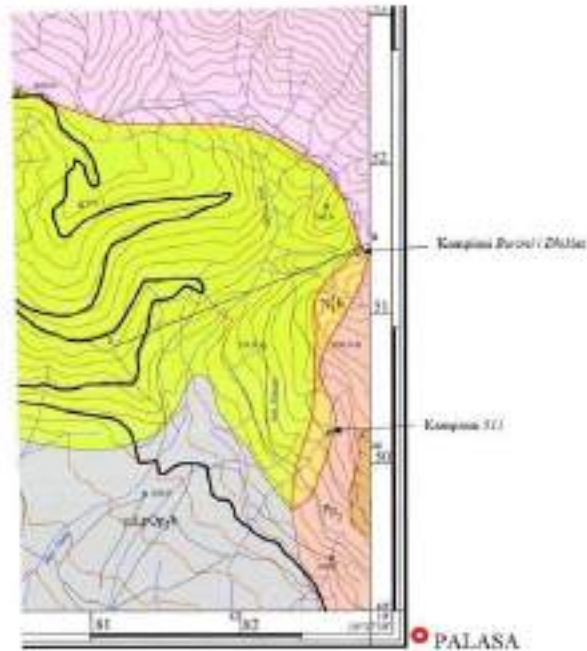


Fig. 2 Geological map NW of Palasa Village. (Albanian Geologic Service 2010), complemented by the author with the location of the sample named, *Burimi i Dhikles* (Muhameti and Pejo 1974) and the sample 511 (Shushkov and Tkaçiev, 1960).

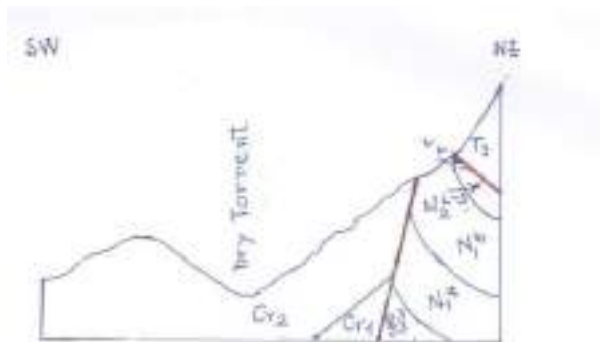


Fig. 3 B-B Cross-section, NW of Palasa Village
vvv Evaporites

History

In general, the Palasa evaporitic deposits are considered to have the same geological age with the evaporitic deposits of the Ionian zone (Mavrovi 2018), as they could be partly met near the fault between Upper Triassic dolomites and other different deposits.

Paleopalynological investigation of these evaporite deposits was first carried out in China in 1971, (samples 15, 16) without reporting their age. Information about some relevant analyses could be found in (Gjikopulli and Rama 1973) stating that the evaporitic deposits of Ionian zone (including those of Palasa) are Oligocene in age.

Due to the absence of flowering plants, our first paleopalynological study about the evaporitic deposits of the Ionian zone, defines the deposits of Palasa to be Jurassic-Lower Cretaceous in age, (but not reaching the Aptian age (Muhameti and Pejo 1974). We could explain by the time the only sample containing flowering plants as a result of contamination while collecting the sample for analysis.

Due to the lack of literature, the conclusion regarding their age remains unchanged (Muhameti *et al.*, 1982; Diamanti *et al.*, 1999).

Efforts have been made to define the age of Palasa evaporite deposits fifty years later. Table 1 is based on many papers from Italy and Greece (see below). The data in the Table 1 show that the appearance of *Dacrydium* in the region might date between the first appearance of *Tsuga* and the evaporitic thickness of Upper Miocene.

2. MATERIAL AND METHODS

The analytical investigation of samples 15 and 16 was carried out at the Palynologic Laboratory of the Institute of Mineralogy, Paywanchuang, Beijing, China. Laboratory investigation for pollen extraction involved the dark gray-dark green silts, avoiding colors resulting from oxidation processes as spores and pollen could be destroyed. The sample was collected from a depth of 20-25 cm to avoid any possible impact of water circulation and weathering processes' events. The laboratory extraction of the material from the rock required the use of hydrochloric acid (concentration rate not less than 10%) to eliminate the carbonate cement. Once rinsed by decantation to eliminate HCl, HF (concentration above 37%) was added to eliminate silica cement; rinsing by decantation; 200 microns mesh sieving. ZnCl₂ heavy liquid centrifugation with specific gravity 2.1; 10 microns mesh sieving; rinsing and slide making was carried out. In concrete terms, for the samples of Butrinti-1/s that represented salt rocks mixed with clayey silt: the author

applied the weight 800-1000gr (Shaffer 1964). Once the salt dissolved, the aforementioned procedure subsequently followed.

Detailed information about the aforementioned method could be found in (Shaffer 1964; Traverse 2007).

In addition, two other analyzes were carried out, but as the present paper aims at confirming the presence of flowering plants pollen although the negative outcome as reported in (Muhameti and Pejo 1974), these analyzes are not here reported.

Investigation on the samples collected from the wells was carried out:

Butrint-1 well, depths: 1703-1705m; 3020-3022, 1m; 3790-3791,7m; 3998-4000m. Pollen preparations were carried out at the Petroleum Geological Institute, Fier (1989) and used for the last interval. In addition, the author prepared new slides based on a greater weight for the first three intervals. Only at the interval depth 3020-3022.1m, a grain of *Corsiniipollenites* was found.

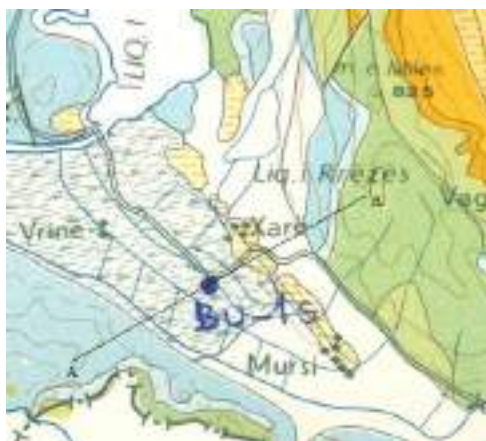


Fig. 4: Geologic map at the scale 1:200 000, with the location of the wildcat Bu-1s.

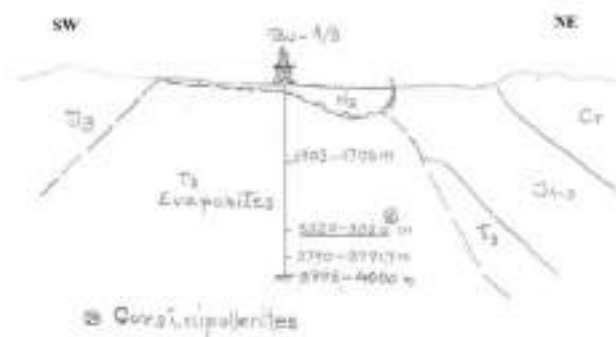


Fig.5: A-A Cross-section with evaporites penetrated by the wildcat Bu-1s (without scale). The geological profile is from the well file and sketched by the author.

No result has been reported for **Bogaz-1**: 2797-2801m.

In addition, a recrystallized globotruncanid foraminifer, typical of an age not older than Coniacian was found in a thin section resulting from a sample collected in Vuno (Vuno -1, Fig. 6) by Pëllumb Sadushi. Other sample containing pollen (Vuno-1) of genus *Callialasporites* was prepared, but this microfossil merely informs about the Mesozoic age.

Two samples investigated at the Palynologic Laboratory of the Institute of Mineralogy, Paywanchuang, Beijing, China were selected for reviewing purposes.

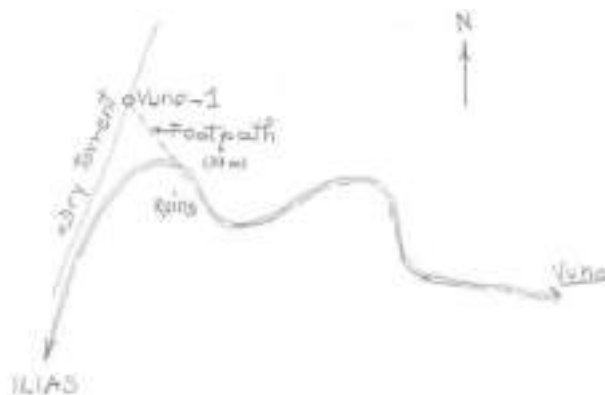


Fig. 6: The location of the sample Vuno-1, near the highway West of Vuno.

3. RESULTS

Regarding the palynological content of these two samples, the genera *Dacrydium* prevails (35%), but the flowering plants could be met in one sample only. The other sample consists mostly of unidentified tetrads. Muhameti and Pejo (1974) stated that the absence of flowering plants is a means to address the age of Palasa evaporitic deposits.

Locating the evaporitic deposits outcrops in the Palasa village for new samples was impossible. However, the age of the palynological complexes presented below was determined based on the existing literature.

1) Nr. 15- Sample containing flowering plants.

The microflora is rich and well preserved. In general, the pollen grains of gymnosperms prevail with 72%, followed by the angiosperm pollen with 21%. The following plants were particularly identified:

Pteridophyta
 Selaginella-4; Selaginella? -4; Polypodiaceae -1; Dicksonia-1;
 Unidentified spores-5.

Gymnospermae
 Ginkgo -5; Coniferae -4; Podocarpaceae -1; Dacrydium-58; Tsuga -2;
 Araucariaceae -1; Pinaceae -4; Picea -4; Pinus -8; Cedrus -15

Angyospermae
 Liliaceae -1; Betulaceae-1; Quercus -2; Ulmaceae -1; Magnolia -2;
 Ericaceae -34; Tricolporopollenites -1; Unspecified -6

Sum-165

As it could be clearly noted, the sample presents a poor variety of microflora for all three major groups. *Dacrydium* (*Podocarpaceae*) and *Cedrus* (*Pinaceae*) prevail in the group of gymnosperms, while *Ericaceae* prevails in the group of angiosperms.

The sample Nr. 16 does not contain flowering plants.

Dacrydium 35%; Pinaceae 5%; Cedrus 3%; Picea 1%; Tetrads 53%;
 Bisaccate indet. 3%

The second sample is characterized by the prevalence of the genus *Dacrydium*, in addition to unidentified tetrads.

In general, it can be said that Palasa evaporitic deposits are characterized by the predominance of *Dacrydium*, *Ericaceae* and *Cedrus*, the presence of the genus *Tsuga* and *Ginkgo* and undetermined *Tetrads*.

4. DISCUSSIONS

Mavrovi (2018) stated that the authors whose work has been a means to address the Geological map of Albania at the scale 1: 200 000 give the evaporites of the Ionian Zone (including Palasa evaporites) a general Permian-Triassic age. Later, they were given the Oligocene age (Gjikopulli and Rama, 1973). They have been considered to be Jurassic-Lower Cretaceous in age since then (Muhameti *et al.*, 1982; Diamanti *et al.*, 1999).

Information about the importance of *Tsuga* for the Neogene deposits in SE Europe is given by Traverse A. Given the fact that this microfossil is part of the palynological assemblage of Palasa evaporites, efforts have been made to find the Palasa evaporitic outcrops, but helplessly unfortunately.

An opinion has been given, however, based on the analysis of the samples 15 and 16 (Gjikopulli and Rama, 1973), partly the sample named, *Burimi i Dhikles* (Muhameti and Pejo 1974), and the sample 511, *microfaune*, (Shushkov and Tkaçiev 1960).

Since the main genera for the two analyzed samples are represented by *Dacrydium*, Ericaceae and *Cedrus*, the forthcoming paragraphs emphasize their stratigraphic importance.

Palynostratigraphically, the diagnostic form is *Tsuga* (Photo-7), which in Southeastern Europe appears during the Lower Miocene and disappears during the Pliocene (Traverse, 2007).



Fig. 7: *Tsuga*, 800 X (Gypsum).

The genus *Dacrydium* (Fig. 8) first appeared in Australia in Santonian, 86 million years ago (Hill, 1994). Flowering plants that have been met mainly appear from the Upper Cretaceous. Nevertheless, the oldest records for Ericaceae, Magnolia, *Quercus* and *Corsiniipollenites* (see below) date since the Eocene.

These palynostratigraphic data represent the other very important argument according to which, the evaporites of Palasa are not Mesozoic in age, but much younger.

Thus, *Dacrydium* begins to spread northwards, arriving in India 50 million years ago, i. e. in the Lower Eocene (Morley 2010). In addition, the author states after covering all the countries of Southeast Asia, *Dacrydium* arrived in Japan 16 million years ago, during the Middle Miocene. Taking a look at the Eurasian map and considering India as a point of reference, it is probable that *Dacrydium* has arrived in Albania more or less at the same time as in Japan.

No information has been reported about the occurrence of *Dacrydium* in Pakistan. In Eocene arrived in Georgia (Shatilova et al., 2014). Data report about Turkey emphasize Oligocene age as the time when *Dacrydium* was

distributed in Europe (<http://file.flora.cn>fastdfs>group 1 pdf>). The absence of normal geologic section makes it impossible for an accurate definition of the moment when this microfossil first appeared in the Miocene deposits in Albania. Nevertheless, it has appeared after the first appearance of *Tsuga* and before the evaporitic thickness of Upper Miocene. Therefore, this moment does not improve the situation of the age of Palasa evaporitic tectonic blocks imposed by the presence of *Tsuga* — the Neogene age.



Fig. 8: *Dacrydium*, 800X, (Gypsum).

There are many authors who reject the existence of *Dacrydium* in the Miocene deposits of Italy and Greece, (Bertolani-Marchetti 1962 a,b; Bertolani-Marchetti 1973; Benda *et al.*, 1974; Guernet *et al.*, 1970; Guernet *et al.*, 1976; Jarrige *et al.*, 1983; Jimenez-Moreno *et al.*, 2007; Kovar- Eder 2003; Orgetta *et al.*, 1976; Sauvage 1979; Mettos *et al.*, 1988;), but Lona (1973), Follieri (1977), Sauvage (1975), Sauvage 1977a), Sauvage (1979), Sauvage (1980), Guerrera *et al.*, (1985), Bertoldi *et al.*, (1994) inform about the occurrence of *Dacrydium* in Pliocene deposits. Bertoldi *et al.*, (1994) and Sauvage (1977a) stated that *Dacrydium* dates from Late Neogene to Pliocene or Pleistocene, affirming the appearance of *Dacrydium* in the Upper Miocene. Consequently, Palasa evaporites might belong to the uppermost part of Upper Miocene.

Table 1 reports on the local distribution of some palynomorphs in Italy, Greece, and Europe (for *Tsuga* and *Ginkgo*), arranged by time of appearance as considered by the author, based on the data from (Traverse 2007) about 1- SE Europe; (Lona *et al.*, 1971; Lona *et al.*, 1973; Follieri 1977; Guerrero *et al.*, 1985; Bertoldi 1988; Bertoldi *et al.*, 1994) about 2- Italy; from (Guernet *et al.*, 1970, 1976; Sauvage 1977a; 1977b; 1979; Mettos *et al.*, 1988) about 3- Greece and <https://ucmp.berkeley.edu/seedplants/ginkgoales/ginkgo.html> about 4-Europe.

Table 1. Table of local distribution of some palynomorphs in Italy, Greece, and Europe (for *Tsuga* and *Ginkgo*).

Palinoforma	Mioc 1	Mioc 2	Mioc 3	Pliocen	Pleistocen	Holocen
Ullaceae						3
				2		
Quercus					2	
				3		
Tsuga					1	
Cedrus						3
					2	
Dacrydium					2	
					3	
Ericaceae					2	
					3	
Ginkgo				4		

As there is no a clear palynological boundary of the evaporites in Palasa region, it could be accepted as Neogene in age. The author says that the evaporites are in the Peri-Adriatic Depression of Upper Miocene (Messinian) age. There are no normal sections of evaporites here, but tectonic blocks only.

Information about the extinction of *Ginkgo* in Europe could be found in (<https://ucmp.berkeley.edu/seedplants/ginkgoales/ginkgo.html>) by stating that *Ginkgo adiantoides* was particularly abundant in Europe at the beginnings of Pliocene, it was gone from that region by about 2.5 million years ago. This extinction more or less coincides with the extinction and migration of *Tsuga* from Europe. The extinction of these two microfossils would define the upper palynological boundary of the age of Palasa evaporite deposits. On the other hand, its lower boundary is determined by the appearance (in a hypothetical section) of *Dacrydium* and *Tsuga*. The complex is characterized by the presence of *Dacrydium*, *Ericaceae* (or undetermined *Tetrads*) and *Cedrus* at a considerable rate — altogether making up 65% of the complex. *Ginkgo* and *Tsuga* are typical of the complex.

The Oligocene deposits of Aranitas section have been previously studied, and this microfossil *Dacrydium* has never been met (Pejo and Muhameti 1973). It has neither been met in the sections later investigated (Jançeŕ L., Personal Communication, 2019), nor in the Tortonian-Messinian deposits. (Gjani 1989; Muhameti 1992).

Fergusson (1967) states that *Dacrydium* is little known in Europe. No information about it could be found in (Kovar- Eder 2003; Jimenez-Moreno *et al.*, 2007). Jimenez-Moreno *et al.*, (2007) provide information about the Miocene and Pliocene deposits in all Balkan countries (except Albania), including those from Crete Island, the southern coast of Turkey and northwestern Syria.

The microfauna identified from the clays beneath an evaporite lens (No. 511), (Shushkov and Tkačiev 1960), consists of these planktonic foraminifera species: *Acarinina miocenicus* Putrja; *Globigerina trilocularis* Ran.; *Orbulina universa* d'Orbigny, *Globorotalia albanensis* Mjatluk; *Cibicides helveticus* Mjatluk.

Age: Middle Miocene

The survey geologists, Shushkov and Tkačiev (1960) accepted the Lower Helvetian age. Simon Prillo, accepts the Upper Langhian-Lower Serravallian age for the aforementioned microfauna based on the current stratigraphic nomenclature (personal communication, 2021). The age of the microfauna does not automatically define the age of Palasa's evaporites, but rather its importance lies in the confirmation of the Miocene age as was initially suggested by the presence of *Tsuga*.

The author states that only two evaporite horizons exist from the Upper Triassic to Upper Miocene, within the Ionian zone and the Peri-Adriatic Depression; the Upper Triassic (Carnian) horizon and the Messinian horizon. The palynological content of Palasa evaporites is not similar to Kavaja and Ndroqi sections, which include the Serravallian (partly) -Tortonian-Messinian in the Peri-Adriatic Depression. The predominance of *Dacrydium*, *Ericaceae* (this is substituted by undetermined *Tetrads* in sample 16) and *Cedrus* is not signaled in the aforementioned sections. This evident difference may be explained by washing during the Pliocene transgression. Another less credible hypothesis may be the existence of an unknown phytogeographic province.

In the Butrinti wildcat-1s (3020-3021,5m) which is situated in the West of Çika Belt (in the South of Palasa), the microfossil *Corsiniipollenites* (Fig. 9), an herbaceous from the family *Onagraceae* which dates from Early Tertiary to Pleistocene (Song, Wang, Huang 2004), could be met. This fossil, although very rare, testifies that the age of Butrinti evaporites may be also a young one (probably Upper Miocene).



Fig. 9: *Corsinipollenites* spp. -59.76-microns, (without scale), **Butrinti-1s** (3020-3022,1m)., (black salt rock).

It could be preliminarily argued that the identification of Neogene evaporite deposits in both Palasa and Butrint might represent a regional phenomenon. In this context, it would be interesting to analyze the palynological content of evaporites from the village of Çiflik, south of Butrint.

5. CONCLUSIONS

The age evolution of Palasa evaporites is as following:

Before the application of paleopalynology: Permian-Triassic or Triassic.

1971: without any definition;

1973: Oligocene;

1974-1999: Jurassic-Lower Cretaceous.

Both *Tsuga* and *Dacrydium* are a means to address the Neogene age of the evaporites in Palasë. Consequently, the evaporites of Palasa are not part of Ionian Tectonic Zone.

The data about the microfauna prove the Miocene (Middle Miocene: Upper Langhian-Lower Serravallian) age. An evaporite lens with continental Neogene microflora overlays the microfauna clays. Between *Orbulina universa* clays and *Dacrydium-Ericaceae-Cedrus* evaporites, deposits of the Upper Serravallian-Tortonian-Messinian ages might be missing.

In this context, the scarce data about the Butrint-1s well require review of the age of the respective evaporitic deposits. Consequently, further investigation is needed to obtain additional microfossil material, all the more

so, if we recall that this diapiric structure has been attacked by deep wildcat to prepare it as a perspective structure for hydrocarbon exploration.

Dacrydium entered Albania probably during the Upper Miocene, and along with *Ericaceae*, *Cedrus* and undetermined *Tetrads* constitute a particular case in Europe.

No phytoplankton was found in the evaporites of Palasa, which proves that the environments where the evaporites were formed had a very limited connection with the sea.

Terrestrial vegetation has been represented by moist and evergreen forests both in the lowlands and in the mountains, mainly *Dacrydium* and *Cedrus*. The shrub belt is represented by *Ericaceae*, proving the existence of acidic infertile soils.

Warm-temperate climate; the region has been mainly mountainous with low coastal areas, and salty lagoons.

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PHOTOSYNTHETIC CHLOROPHYLL PIGMENTS IN *PHASEOLUS VULGARIS* TREATED WITH MUTAGENS

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ABSTRACT

Beans (*Phaseolus vulgaris*) are considered one of major leguminous plants. In addition, they are characterised by a high genetic and significant variability, appropriate for agricultural production. Unfortunately, its production has significantly reduced due to climatic changes, and the abortion of flowers is concerning. The legumes do not survive at high temperatures and droughts period. Efforts have been made to extend the flowering time and eliminate the abortion of flowers via induced mutation techniques applied on bean seeds. The Cs-137 gamma radiation, in three doses ranging from 50 to 150 Gy was applied to the seeds, in addition to the chemical mutagens dES diethylsulphate and EMS ethylmethansulphonate, in three concentrations, respectively. The IAEA protocols were followed when planting the irradiated and chemically treated seeds in the greenhouse and experimental field. The results obtained in the M1 generation showed changes compared to the plants controlled for the physical and chemical treatments. During this time, chlorophyll pigments were analyzed and the results reported the mutagenic impact. The chlorophyll meter CCM-200 was used to measure the photosynthetic pigments in leaves. Regarding the chlorophyll content in plant leaves, it was observed that the highest chlorophyll content (30.6) relates to the application of the doses 50 Gy. The lowest chlorophyll content (7.6) relates to the application of the dose 150 Gy. There were changes in the maturity period for the two gamma rays doses (100 Gy and 150 Gy). Regarding the chemical mutagens for the dES maximum concentration 0.020 M, the values of photosynthetic pigments varied between 19.6 and 39.30, whereas for the EMS in maximum concentration 0.3%, the values of photosynthetic pigments oscillated between 14.01 and 40.80. Induced mutations in *Phaseolus vulgaris* have increased the photosynthetic chlorophyll pigments at a considerable rate. Here, additional field experiments would be needed to determine mutant lines.

Keywords: chemical and physical mutagens, chlorophyll pigments, *Phaseolus Vulgaris*, mutation

1. INTRODUCTION

Mutation induction techniques are diverse, but in general they are divided into two major groups based on the type of mutagenic agents used for changes at the genetic level in chemical and physical induction techniques. Physical and chemical mutagens have been used successfully in plant breeding programs to artificially generate genetic variations for the development of new varieties with improved traits (Kodym and Afza, 2003; Ylli *et al.*, 2007; Stamo *et al.*, 2012; FAO / IAEA, 2018; Ylli *et al.*, 2018). One of the photosynthetic pigments such as green chlorophyll in plants plays the role of a biomolecule with interest that enables the process of photosynthesis. The higher chlorophyll amount, the healthier the plant is. Therefore, the chlorophyll amount provides the researchers, agronomists and farmers with valuable diagnostic information to address nutritional and irrigation management, pesticide control, stress assessment environmental etc. (Süß *et al.*, 2015).

Chlorophyll mutations and the amount of photosynthetic pigments in plants are a means to address the evolution of genetic effects as a result of mutagenic treatment (Usharani *et al.*, 2015). The frequency of chlorophyll mutations in beans was found to be linearly correlated with dose. However, at high doses the frequency of mutation is reduced due to the low fertility in M1 generations. The latter occurs due to the drastic action of mutagenic treatments on cell strands resulting in high gamete and zygote sterility, leading to M1 generation mutations with a lower probability of represented by mutants in the M2 generation. Mutants obtained by induced mutagenesis show superior traits on cultivars such as higher yields due to increased resistance to biotic and abiotic factors, improved nutrient content, and other quality traits. Mutant crop varieties are more adaptable to the environment, require less agricultural contributions, and are therefore more economical to grow and contribute to more environmentally friendly agriculture (Mba, 2013).

2. MATERIALS AND METHODS

The present investigation was carried out from 2014 to 2016 involving the bean seeds of Shijak variety obtained from the National Seed and Seedling Entity which is the Genetic Bank of Albania, of the Institute of Plant Genetic Resources, Agricultural University of Tirana. Here the method of chemical and physical mutagenesis was applied to treat the seeds. The mutagens used were EMS (ethylmethanesulfonate) and dES (diethylsulfate), in three different doses each of them. Also, three different doses of gamma-radiation were applied on the bean seeds to evaluate the radiation effect in the photosynthetic

pigments values. The experiment was carried out at the Mutagenesis Laboratory, in an experimental field in Fieri, Albania, and in the greenhouse of the Department of Biotechnology, Faculty of Natural Sciences, University of Tirana and in line with the IAEA (FAO / IAEA, 2006; FAO / IAEA, 2018) protocols. In the greenhouse experiments for mutagenesis in beans, the temperature and humidity were controlled, and plants were grown in pots with selected soil that provided the plants with necessary nutrients.

The gamma radiation with Cs-137 radiation source in three different doses; 50 Gy, 100 Gy and 150 Gy was applied for the treatment of the bean variety. The seeds that were subjected to irradiation were uniform, healthy, with high germination power and moisture content from 12-14%.

The seeds were kept for at least 3 days in a desiccator with 60% glycerol to balance the moisture content at 12-15% seed levels prior to radiation. The seeds were subsequently packaged in small containers adapted to the sample size in order to minimize air content. Some samples were packed in paper bags before being placed in the container to avoid contamination or the mixing of the seeds with each other. The Petri dishes or smaller containers were used for smaller seeds. The container filled with packaged samples was equipped with holders that were fixed in a standard position. Finally, the containers were placed in the radiation device in which the radiation dose was determined.

Once this process ended, the materials were kept in the laboratory for a week at temperatures varying between 20-22°C, and were prepared to be planted based on the relevant schemes in the laboratory greenhouse and experimental plot in field conditions (Stamo *et al.*, 2007; Jaupaj *et al.*, 2013).

The chemical mutagenic treatment of materials was in line with (FAO / IAEA, 2018), and the used doses of mutagens were in line with the manual of the IAEA atomic agency (FAO 2006). Selected seeds were introduced to be inoculated into beakers filled with distilled H₂O where each ml of H₂O corresponds to one bean. After 4 hours, the distilled water was removed from the beakers where the preparatory phase took place, and the respective solutions prepared in advance for each mutagen with the following concentrations were added:

Diethylsulfate - dES

- 0.0025M 0.140ml in 600 ml distilled H₂O
- 0.005 M 0.282 ml in 600 ml distilled H₂O
- 0.010 M 0.564ml in 600 ml of distilled H₂O

Ethyl methane sulfonate - EMS

- 0.1% 0.1510 ml in 600 ml distilled H₂O
- 0.2% 1.020ml in 600 ml of distilled H₂O
- 0.3% 1.530ml in 600 ml of distilled H₂O where they stand for one hour

During each treatment, the seeds were kept for one hour. After the respective treatments for each mutagen, the seeds were rinsed for 4 hours in tap water to confirm the absence of the chemical mutagen residues with which they were treated on plant materials. Once rinsed, the seeds were placed on filter paper for about 24 hours to dry up. Once dried up, the seeds were divided to be sown in greenhouses and plots (FAO, 2011). The seeds were planted in the plot according to a randomized block, with 2 repetitions. The seeds were watered 3 times a week to prevent them from suffering from drought stress and provide the necessary moisture conditions for growth. At the same time the materials were planted in the greenhouse in two pots with two repetitions per dose (FAO, 2017).

The Figure 1 depicts the chlorophyll meter CCM-200 used to measure the amount of chlorophyll for all doses of radiation treatment. This equipment has been designed to measure chlorophyll content in plants and cultivars, and it is particularly useful for managing the nitrogen fixation programs for scientific research.



Fig. 1: The CCM-200 chlorophyll meter and the measurement of the amount of chlorophyll in the leaves.

Measurements were made for all doses of treatment by selecting stabilized leaves, which are equally exposed to the sun. The device was first switched on and calibrated. Once switched on and calibrated, the leaves (samples) were placed in the part where the device makes the measurements and the pigment value appeared on the screen after 2-3 seconds. Pigment measurements were always made in the same direction and position of the leaf and at the same distance from its main nerve. Measurements were made to determine the effect of mutagens and compare the doses with the control.

In addition, the impact of mutagens on the mutations was also investigated for genetic analysis purposes.

The present study evaluates the effects of treatments with physical and chemical mutagens to the plant *Phaseolus vulgaris* to improve its physiological features and the possible influence in shortening the flowering time in these plants under the influence of induced mutagenesis.

3. RESULTS AND DISCUSSIONS

Figure 2 depicts experiment process with the bean plants in field and in the plots in greenhouse. During the experiment it was observed a change in the levels of photosynthetic pigments measured by the CCM-200 chlorophyll meter. Experimental work continued until the M3 generation of some plant materials.



Fig.2: Bean plants during the generation M1 / M2 in the field and in pots in the greenhouse of the generation M2.

The effect of physical mutagens

The measurements were made in several different weeks and the progress of the photosynthetic pigments in the M1 / M2 band and their effects on the doses of physical radiation by comparing it with untreated control plants was investigated (Oladosu Y., *et al.*, 2016). The results depicted in the Figure 3 show that photosynthetic pigments have increased in all doses from week to week. A difference between each treatment regarding the dose applied could also be noted.

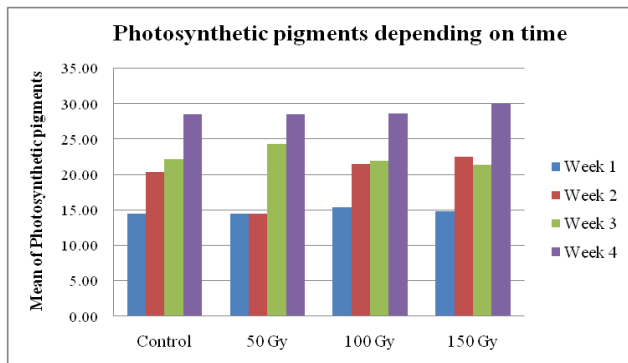


Fig. 3: The impact of physical radiation in a few weeks on the photosynthetic pigments amounts of bean plants.

After the first week the photosynthetic pigments were almost the same, and after the second week was obvious the change of photosynthetic pigments between different doses. Regarding the plants treated with 150 Gy, the photosynthetic pigments values measured in the first phases had a significant increase, something that in other phases was inhibited. The 100 Gy dose is presented with an increase in photosynthetic pigments compared to the control showing an efficacy for the effect of this dose on the amount of photosynthetic pigments. While for the 50 Gy dose, it is observed that between the two first weeks there is no difference in the values of photosynthetic pigments and then in the other two weeks a higher increase in comparison to the beginning is observed.

Photosynthetic pigments are genetically variable (Fig. 4) due to the impact of the mutagen at different doses, climatic conditions, planting location, soil type, etc. The graph plots that for both the pre-treated plants and control plants there was observed a change in the values of photosynthetic pigments. Control plants have been coming down to plants of the M2 generation. Regarding the pre-treated plants, the pigments values have increased for all three doses used.

We conclude that the impact of physical radiation doses from one generation to the next is increasing from M1 to M2 in all plants measured.

When measuring the plants pre-treated with dose 100 Gy, satisfactory results were noted during the M2 band.

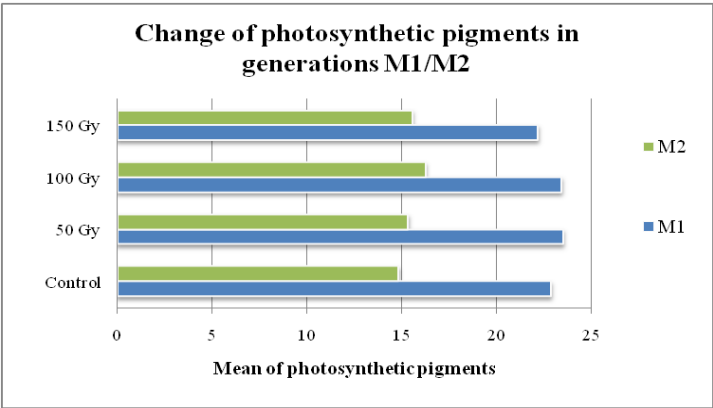


Fig.4: The impact of physical radiation on the M1 and M2 generation on the bean plant.

Investigation also involved the plants planted in two parallel plots in the greenhouse. The plants were randomly selected and photosynthetic pigments were measured via the CCM-200 chlorophyll meter. The results (Fig. 5) reported that the highest efficiency was given by the dose 100 Gy, as the stimulation of the photosynthetic apparatus of these plants was given.

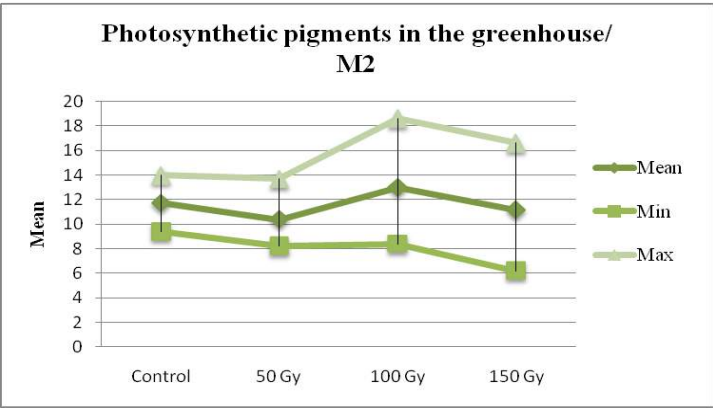


Fig. 5: The impact of physical radiation on the photosynthetic pigments on the bean plants in the greenhouse during the M2 generation.

The Table 1 reports the minimum, mean and maximum values per dose. As it could be noted, the highest values were obtained when applied the second dose showing a higher impact on the synthetic pigments of the plants planted in the greenhouse (Ibrahim R., 2010). Comparing with the control, the use with the first dose 50 Gy has led to a decrease of the mean values alike the higher dose which shows a lower value.

The effect of chemical mutagens

The M1 generation plants have been pretreated with two different chemical mutagens in three different doses such as EMS and dES. Here, the chlorophyll meter CCM-200 was also used for measurements purposes. The use of chemical mutagens in three different doses and the gamma radiation has affected the photosynthetic apparatus of plants (Table 1).

A major impact on M1 in the field compared to the control was given by the doses of EMS. The first dose appeared to be more effective than the control. The first dose of dES gave a higher variability, but an increase in photosynthetic pigments amount (Fig. 6) when applying the second dose could be slightly noted.

Table 1. The influence of chemical mutagens on photosynthetic pigments amount, maximum and minimum values

	Photosynthetic pigments amount	Max	Min
Control	29.08	38.1	22.4
dES1	27.95	41.2	16.4
dES2	28.31	36.1	19
dES3	25.26	39.3	19.6
EMS1	29.75	39.2	18.7
EMS2	26.06	36.4	14
EMS3	29.02	40.8	14.1

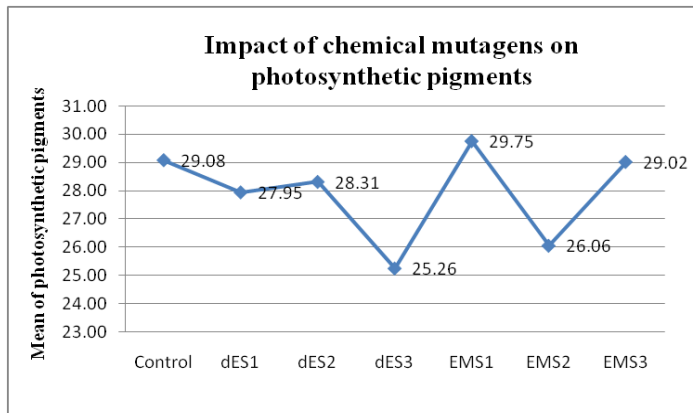


Fig. 6: The effect of chemical mutagens on photosynthetic pigments amount of bean plants during the M1 generation.

The amount of photosynthetic pigments changed from week to week, from one generation to another, and also according to the types of mutagens. The figure 7 compares the mean values of photosynthetic pigments amount during the M1 generation under the effects of chemical and physical mutagens. Here further experiments are need for accurate conclusions.

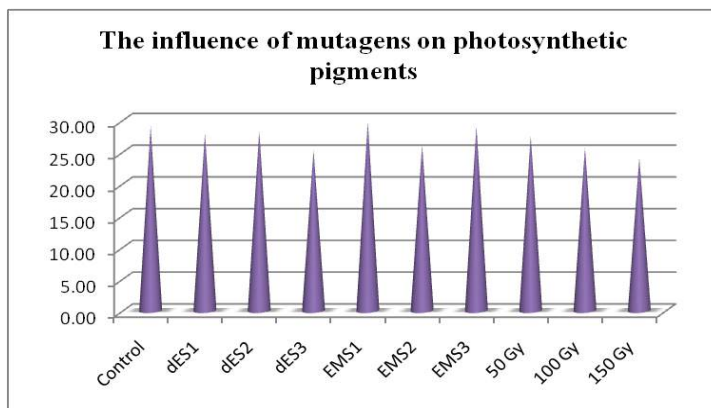


Fig. 7: Comparison of mean values of photosynthetic pigments amount during the M1 generation under the effects of chemical and physical mutagens.

Chlorophyll mutations are in themselves a phenotypic process (FAO / IAEA, 2018). The mutations appear only in the pre-treated and planted materials in generations M1, M2 and slightly in M3, and later they disappear. As chlorophyll mutations are on display for several days, they must to be constantly monitored on daily basis to be detected and evaluated. There are different types of mutations noted in the same plant regardless the treatment using the same chemical or physical mutagen. A higher number of chlorophyll mutations could be noted in the M1 band.

4. CONCLUSIONS

Bean seed-induced mutagenesis has a positive effect on the level of photosynthetic pigments, and on the occurrence of chlorophyll mutations. Since the amount of chlorophyll is an indicator of plant health, it can be said that a dose of 100 Gy radiation improves the condition of plants in the greenhouse or in the field. In addition, chemical mutagens at lower doses provided a positive influence on photosynthetic pigments. Mutations induced in *Phaseolus vulgaris* have greatly increased photosynthetic chlorophyll pigments, but further experiments to determine mutant lines are needed.

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HEAVY METALS IN *PRIMULA VERIS* L., PRIMULACEAE IN DIFFERENT REGIONS OF KOSOVO

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ABSTRACT

Primula veris L., Primulaceae is a perennial plant with oval leaves and yellow flowers. It is widespread in different regions of Kosovo, in mountainous and hilly areas and in subalpine and alpine pastures. For years it is well known as a medicinal plant for pharmacological actions. In this study we intend to evaluate the heavy metal contents and their distributions in roots, leaves and flowers of *Primula veris*. were selected as sampling locations for investigation purposes as they are environmentally clean areas. During the biennial monitoring, plant materials from the *Primula veris* population have been collected and investigated. In addition, soil samples where these plants were grown have been collected. As this plant is used of medicinal purposes, its vegetative parts such as roots, stems, stalks and leaves and yellow flowers were investigated applying the ICP-OES technique based on the EPA method 6010C: 2007 for the Cd, Cu, Fe, Ni, Pb, Zn content. The heavy metals content in soil was compared with the heavy metal content in different parts of *Primula veris* plants. During the survey of 2015 in Badovc area, Pb contamination in soil, leaves and flowers was 111.84 ppm, 2.86 ppm and 1.70 ppm, respectively, whereas in Novobërd - Artanë area the Pb content in soil, leaves and flowers was 520.34 ppm, 11.62 ppm and 0.79 ppm, respectively. So, only a small amount of lead was transferred to flowers, as the main consumable part. There was an equal distribution of heavy metals such Fe, Cu and Zn among the stalks and leaves, and flowers. The Cd concentration was found at a very low rate in both areas. As heavy metal contamination was found at a very low rate, the *Primula veris* flowers could be used for medicinal purposes.

Key words: *Primula veris*, medicinal plants, heavy metals, soil

1. INTRODUCTION

Kosovo has a great biological, ecological and landscape diversity. The flora of Kosovo is made up of 1,800 plant species. There are about 200 Balkan endemic plant species and 13 Kosovo endemic plant species that could be here found (Rexhepi 2003; Shuka *et al.*, 2010; Mustafa *et al.*, 2012;

Menczer *et al.*, 2018;). The great number of endemic and stenoendemic plants are an added value to the biodiversity. Unfortunately, anthropogenic activities pose threats to these plants. River canyons, and subalpine and alpine zones are of great importance as they are rich in endemic-relict species, and medicinal and aromatic plants (MAP) and wild berries (WB) (Millaku 1999; Menczer *et al.*, 2018). Sharri Mountains, Albanian Alps, Kamenica, Goollak regions (eastern part of Kosovo) and Mitrovica are rich in medicinal and aromatic plants as based on the surveys.

Primula veris L. are small, long-lived perennials from the family Primulaceae, growing wild in temperate Europe and western Asia (Millaku 2010). Cowslip grows on nutrient-poor grasslands, herb-rich meadows, and at the edges and in clearings of warm and bright woodlands. Cowslips produce a rosette of leaves and leafless flower stalks, up to 20–30 cm high. Cowslip flowers are fragrant, bright yellow with orange spots at the edge of each lobe. They are formed at the top of the stalks in an umbel-like inflorescence. It is in flower from April to May, and the seeds ripen from July to August (Menczer *et al.*, 2018).

Primula veris L. is one of the most important aromatic and medicinal plants grown in the territory of Kosovo. Given the increasing demand of pharmaceutical industry for the use of this plant, efforts were made to cultivate some cowslip populations with a high production and yield of flowers and roots.

The cowslip (*Primula veris* L.) is one of the most important aromatic and medicinal plants, in Kosovo and of great importance for the pharmaceutical industry. Therefore, it has an increasing demand in the domestic and foreign markets (Millaku 2010; Luma *et al.*, 2018).

Extracts from rhizomes, roots and flowers of *Primula veris* are components of many pharmaceutical products (Włodarczyk 2020). Until now, *Primula veris* L has been collected from wild habitats of SE Europe, endangering this species existence. In the future cultivation should be associated with controlled collection from wild habitats.

The characteristic constituents of flowers and roots of *Primula veris* L. are triterpene saponins, and phenolic compounds, including flavonoids (about 3% in flowers), phenolic acids, and phenolic glycosides. Saponins are responsible for the secretolytic and expectorant activity. In turn, phenolic compounds reveal antioxidant, antimicrobial, and cytostatic properties (Richards 2003; Luma *et al.*, 2018).

There is a long history in the clinical use of medicinal plants. Chemical pollutants such as heavy metals and pesticide residues as common plant pollutants, pose serious risks to human health (Shaban *et al.*, 2016). Medicinal plants can easily be contaminated with heavy metals through soil, water and air. Usually the soil is subject to pollution through atmospheric deposition of

heavy metals from various sources including industrial activities, precipitation, atmospheric dusts and products used for plant protection (Maobe *et al.*, 2012). Heavy metal pollution can occur due to factors including irrigation with polluted water, addition of metal-based fertilizers and pesticides, industrial emissions, transport, harvesting process, storage and sale (Radwan and Salama 2006; Duran *et al.* 2007; Tuzen and Soylak 2007).

The present study aims to determine the presence of heavy metals in the cowslip populations, and the way these elements were transferred from soil to roots, leaves and flowers.

Primula veris materials were collected from two different areas of Kosovo in 2015 and 2016 to investigate the heavy metals content as this plant is of great importance for the pharmaceutical industry.

2. MATERIAL AND METHODS

Figure 1 depicts the Novobërd - Artanë and Badovc - Graçanica regions, the sampling areas as they are ecologically clean. Plucking and selecting plant materials at the same period of blossoming is very important. The roots, stem, stalks and leaves and flowers were collected from the same preselected place. All plant materials and soils were collected in 2015 and 2016 in an area of 150m x 150m², for uniform materials to be used for homogeneous samples.



Fig. 1. Selected areas in Kosovo for collecting *Primula veris* plant materials

The collection of plant and soil materials for further heavy metal analysis occurred in April and May. The set of plant materials were selected at designated areas. Once selected, they were taken to laboratory to be washed with running distilled water to remove the pollution like dust, soil and other contaminants. Once cleaned, they were covered to dry up at room temperature, in the dark and with controlled ventilation.

Plant fragments (roots, stalks & leaves and yellow flowers) were isolated and oven-dried at 80 °C for 48 h, and then 0.25 g was taken and transferred into Teflon vessels. Once transferred into Teflon vessels, 6 mL of 65% (v/v) HNO₃ (Merck) and 2 mL of 30% (v/v) peroxide hydrogen (H₂O₂) were added.

Soil samples (about 500 g) were collected at a depth of about 10 cm using a stainless-steel shovel. They were oven-dried at 80 °C for 48 h and passed through a 2-mm sieve. Subsequently, 0.3 g was weighed and 9 mL 65% (v/v) HNO₃, 3 mL 37% (v/v) HCl and 2 mL 48% (v/v) HF (Merck) were added.

The extraction from plant materials is in line with the BS EN 13805: 2014. The samples were mineralized in a microwave oven (Berghof), and after cooling they were filtered via Whatman filters and the volume was made up to 50 mL with ultrapure water in volumetric flasks.



Fig. 2: Autosampler and Inductively Coupled Plasma ICP-OES, DV 2100, Perkin Elmer.

We planned to evaluate the content of metal elements like Cd, Cu, Fe, Ni, Pb and Zn in plant and soil samples. The elementary analysis was carried out using ICP-OES (Perkin Elmer, Optima 2100 DV) and the results were evaluated based on EPA method. 6010C: 2007. The samples have been analyzed at the Agrovet Laboratory in Fushe- Kosova, Prishtine, a licensed and accredited laboratory.

Materials were analysed and evaluated in line with the European

Community reference data for soil, roots, stalks, leaves and flowers: (Broome, 2000; Ceburnis *et al.*, 2000; Angioni *et al.*, 2003).

3. RESULTS AND DISCUSSIONS

Heavy metals content in the roots, stalks, leaves and flowers samples of the plant is here investigated. The Inductively Coupled Plasma – Optic Emission Spectroscopy (ICP-OES) was used to assess the concentration of Cd, Cu, Fe, Ni, Pb and Zn in soil and plants, first for the Novobërd - Artanë region, situated in the east of Prishtina. The beech forests are found at an altitude of 800 - 1000 m. In addition, this region is mentioned for its medicinal and aromatic plants. *Primula veris* or cowslip has a wide distribution in this area.

The Figure 3a depicts the heavy metals content (ppm values) in soil for the Novobërd - Artanë region. As it could be clearly noted, Fe, Pb and Zn are found at the highest levels, 20695.8 ppm (parts per million - 10^{-6} kg), 520.3 ppm 231.0 ppm, respectively, i.e., at an exceeding rate as based on (FAO/WHO 2011; Ruqia *et al.*, 2015).

The forthcoming paragraph reports about the relation between heavy metal content in soil and the roots of *Primula veris*. The heavy metal content in roots (Fig. 3b) is found as following: Fe – 525.1 ppm, 39 time less than in soil; Pd – 10.7 ppm, 48 time less than in soil; Ni 9.98 ppm, 10 time less than in soil; Zn – 25.3 ppm, 9 time less than in soil.

The mobility of heavy metal as Cd, Pb, and Ni in soils and the ability of these elements to migrate from soil to other environmental components, including medicinal plants pose a significant health risk to humans and animals (Bezlova *et al.*, 2012; Velicković *et al.*, 2020). Existing studies prove bioaccumulation of heavy metals and arsenic in different above- and underground plant parts (Kabata-Pendias, 2000). From the elementary analysis cowslip is not an accumulative plant for lead and iron, but it had more affinity for Ni, Cu and Zn, as these elements belong to three consecutive boxes in periodic table.

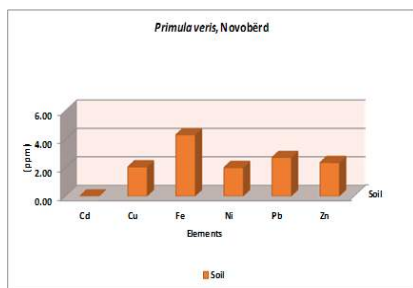
As stems connect the roots to the leaves, they help transporting even the heavy metals to the stalks, leaves and the yellow flowers of the plant. The investigation of 2015 reported that the flowers had a lower heavy metals content than the stalks and leaves. The Pb content in flowers was 0.78 ppm, while in stalks and leaves it was 11.62 pp. The Ni content was 7.72 ppm in flowers and 15.79 ppm in leaves. Fe, Cu and Zn had a roughly equal distribution.

The Ni (101.87 ppm) and Pb content in soil was above the maximum permissible values, but these heavy metals content in the roots and flowers,

the main parts used for medicinal purposes, was within permissible limits (WHO 2011; Afzal Shah *et al.*, 2013; Ruqia Nazir *et al.*, 2015).

The Zn content in roots (25.30 ppm) was ten time lower than in soil, and its transport to different parts of the plant during blooming was equal — 17.60 ppm in stalks and leaves, and 17.43 ppm in flowers. Zn is one of the most important elements that play a vital role in the physiological and metabolic processes of many organisms.

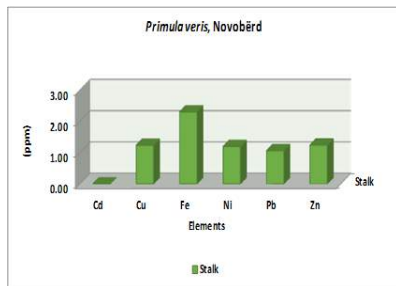
The Cd content was very low (< 0.1 ppb), probably to the ecological quality of the areas.



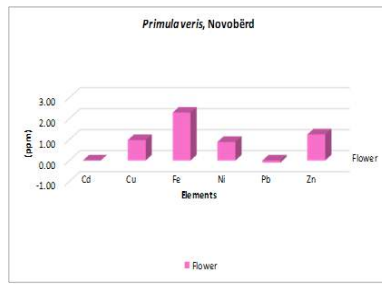
a)



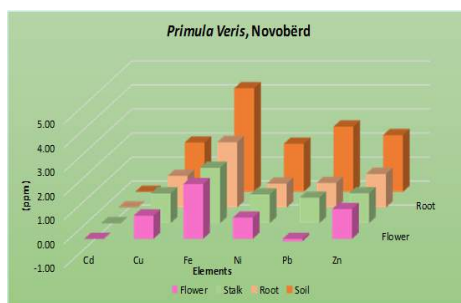
b)



c)



d)



e)

Fig.3: Heavy metals content of Cd, Cu, Fe, Ni, Pb and Zn in A - soil, B - roots, C – stalks, leaves, D – flowers and E- summary for *Primula veris*, in Novobërd - Artanë area during 2015.

Regarding the Novobërd - Artanë area, all the heavy metals, except the Cd, are found at an exceeding level, and in most cases are also found in the plant material of the *Primula veris* (Diana *et al.*, 2012).

The forthcoming paragraph reports about the heavy metals distribution in the Badovc area, near the Gračanica region, in the south-east of Prishtina. Soil samples and plant (roots, stems, stalks, leaves and flowers) were collected. The Figure 4 depicts the heavy metals content in soil, roots, stalks, leaves and flowers. Figure. 4e summarizes the heavy metals distribution.

Regarding the heavy metals content in soil, it was reported following: Fe – 19602.5 ppm, Ni – 1266.5 ppm, Pb – 111.8 ppm, Zn – 85.1 ppm and Cu – 29.7 ppm. Cd content was found within permissible limits — < 0.1 ppb (FAO/WHO 2011, Ruqia Nazir *et al.*, 2015).

This accumulation of heavy metals in roots shows the ability of these elements to migrate from soil to plant components. The Figure 4b depicts the heavy metals content in the roots: Fe – 534.2 ppm, 38 time less than in soil; Pd – 44.2 ppm, 2.5 time less than in soil; Ni 207.7 ppm, 6 time less than in soil; Zn – 42.8 ppm, 2 time less than in soil. The bioaccumulation of heavy metals is high for Pb, Ni and Zn.

The distribution of heavy elements among flowers, stalks and leaves is an important step in the mobility of heavy metals. There is a balanced distribution of Cu, Fe and Zn, alike for the *Primula veris* plant collected from the Novobërd - Artanë area. Pb and Ni showed a different situation.

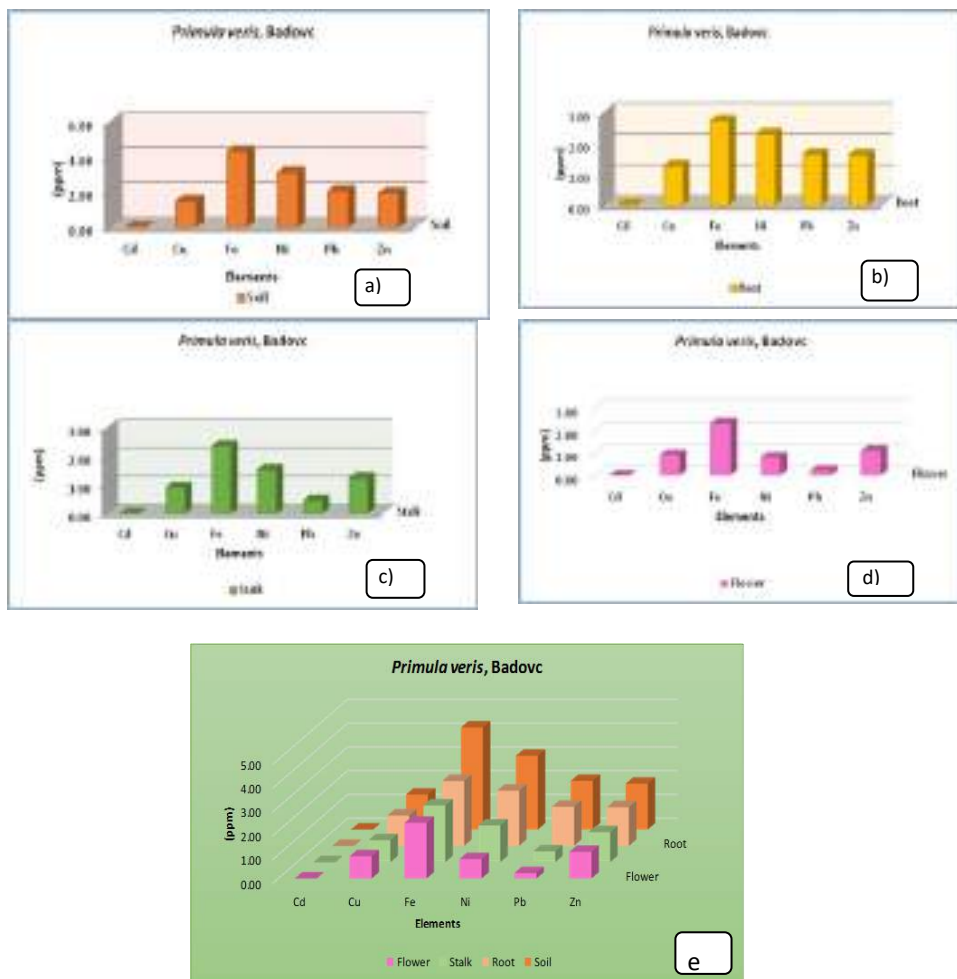


Fig. 4: Heavy metals content of Cd, Cu, Fe, Ni, Pb and Zn in a - soil, b - roots, c - stalks & leaves, d – flowers and e- summary for *Primula veris*, in Badovc area during 2015.

The Pb content in flowers was 1.70 ppm, while in the stalks and leaves it was 2.86 ppm. Ni content in flowers was 6.59 ppm, while in the leaves it was 35.21 ppm. About the same situation was reported for the Novobërd - Artanë and Badovc areas.

The roots of *Primula veris* collected from Novobërd – Artanë area reported: Fe – 525.1 ppm, Zn – 25.3 ppm and Cu – 20.85 ppm, while for Badovc area it was reported: Fe – 534.2 ppm, Ni – 207.7 ppm, Pb – 44.2 ppm and Zn – 42.8 ppm— all exceeding the permissible limits.

As Cu, Fe, Pb and Zn were found at a high rate in the soil, roots of the *Primula veris* exhibited high levels of these metals (Fig.4e). Regarding the flowers, Cu, Fe, and Zn were also found at a higher rate than the permissible (Bezlova *et al.*, 2012).

Cu content soil is below the permissible limits, while in the plant roots it is 18.74 ppm, i.e. exceeding the allowable limits. In flowers and leaves it is 8.58 ppm and 8.38 ppm, respectively, i.e. under the permissible limits (FAO/WHO 2011; Ruqia Nazir *et al.*, 2015). These plant materials could be used as pure medicinal plants (Bezlova *et al.*, 2012; Velicković *et al.*, 2020).

In the soil and roots, Ni is 1266.5 ppm and 207.7 ppm, respectively, i.e. above the permissible limits. In flowers, it is 6.59 ppm, i.e. below the permissible limits as recommended by the WHO (10mg / kg = 10 ppm). Ni found to be helpful for human and animal health (Vodyanitskii 2016).

Pb was found at high values, 111.84 ppm, but in flowers, it was 1.70 ppm. In stalks and leaves, it was 2.86 ppm, i.e. below the permissible limits (FAO/WHO 2011; Shah *et al.*, 2013; Ruqia Nazir *et al.*, 2015). The *Primula veris* flowers had low contamination from heavy metal elements.

During the surveys, it was investigated whether the heavy metals distribution in *Primula veris* remained unchangeable for both areas (Fig. 5).

The data of 2016 reported this heavy metals content was found at the highest level in roots of *Primula veris* as following: the Novobërd – Artanë area: Fe – 966.76 ppm, Zn – 59.48 ppm and Cu – 33.63 ppm, the Badovc area: Fe – 686.47 ppm, Ni – 120.12 ppm, Pb – 28.17 ppm and Zn – 66.16 ppm. Therefore, these heavy metals content remains the same.

The distribution of the metals through stalks, leaves and yellow flowers is typical of heavy metals.

In Novobërd - Artanë area, in 2016, the data reported that the flowers had a lower Pb and Ni contents than in stalks and leaves. In flowers, it was 0.64 ppm, and in stalks and leaves, it was 13.78 ppm. In flowers and leaves, Ni was 3.08 ppm and 12.18 ppm, respectively. Fe, Cu and Zn had a roughly equal distribution of heavy metals among stalks, leaves and flowers.

This difference of Pb content in flowers and leaves in the Novobërd - Artanë area remains the same throughout the investigation.

Regarding the Badovc area, the data of 2016 reported that the flowers had a lower Pb and Ni contents than the stalks and leaves. Pb content in flower was 2.69 ppm, while in stalks and leaves, it was 4.45 ppm. Ni content in flowers was 3.56 ppm, while in leaves it was 20.30. Fe, Cu and Zn had a roughly equal distribution among stalks, leaves and flowers.

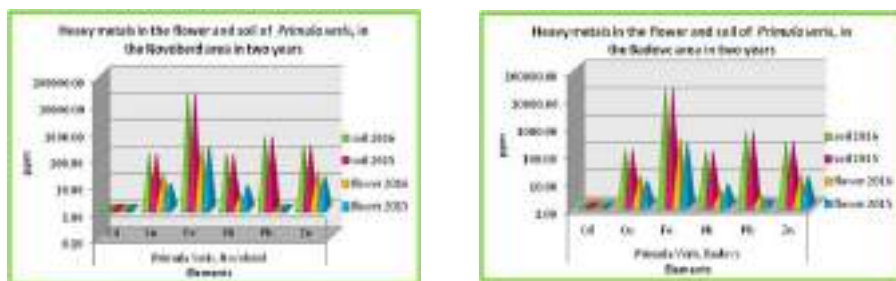


Fig. 5. Heavy metals content in flowers and soil of *Primula veris*, in the areas of Novobërd - Artanë and Badovc for 2015 and 2016.

The ratio of Ni content in leaves / flowers was 5.7 for 2016 and 5.3 for 2015, so closely the same value during two successive years for Badovc area.

In the Badovc area, the Pb content in flowers for 2015 was 1.70 ppm. In 2016 it was 2.69 ppm, i.e. above the permissible limits. In Novobërd - Artanë area, the Pb content in flowers was 0.79 ppm and 0.64 ppm, in 2015 and 2016, respectively, i.e. below the permissible limits, which means that Novobërd - Artanë area belong to a clean area.

Although *Primula veris* L. is not a heavy metal accumulator plant, some parts it reported heavy metal contamination above the permissible limits. Mainly, the roots and flowers of this plant are used in medicinal industry.

4. CONCLUSIONS

The presence Cd, Cu, Fe, Pb, Ni and Zn in soils and plant materials of *Primula veris* L (roots, leaves and flowers) is here investigated.

The results reported that the Fe content was found at a very high rate in soil. Consequently, it was found at the same rate in its roots and flowers.

There is an unequal distribution of Pb and Ni between flowers and leaves. Pb content was found at a low level in Novobërd - Artanë area. Ni content found at a low level in the Badovc - Gracanica area. Cu, Fe and Zn had approximately an equal distribution.

The main group of elements in roots, in the two selected area, remained unchanged throughout the investigation period.

Cu, Iron Fe, and Zn were found at a high rate in flowers. Pb and Ni were within the permissible limits.

Cd was found at a very low level proving that these areas are ecologically clean.

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IMPACT OF EXOGENOUS PROLINE ON VEGETATIVE GROWTH OF JOJOBA GROWN *IN VITRO* UNDER SALT AND DROUGHT STRESSES

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ABSTRACT

The present paper investigates the ability of exogenous proline to counteract salt and drought inhibitory effects in jojoba grown *in vitro*. Shootlets cultures were obtained *in vitro* by explanting nodal segments on MS medium supplemented with 1.5 mg/l BA + 1 mg/l IAA. Four concentrations (1,2,3 and 4 g/l) of proline were added to the culture medium to investigate the ability of proline to counteract the harmful effect of salt (induced by 3000 ppm of salt mixture) and drought (induced by 60 g/l mannitol) stresses. Generally, the addition of proline to saline or osmoticum medium improved the growth parameters i.e., shootlet number, shootlets height, leaf number, and fresh mass of shootlets even in the low proline application compared to control (proline-free medium). Results reported that 3 g/l of proline would be the most effective concentration for ameliorating the adverse effects of both salinity and drought. Under salt stress, the measured growth parameters were increased as proline increased till 3 g/l, and then decreased except shootlets height which took the opposite trend. On the other hand, all vegetative parameters and growth dynamics of shootlets grown under drought stress were increased with increasing the proline in the culture medium till 3 g/l. Salt or drought combating ratio took the same trend of growth dynamics. According to the obtained results, exogenous application of proline provided osmoprotection and enhanced the growth of shootlets of jojoba grown under salt and drought stresses. This can open the possibility of using exogenous applications of proline to alleviate stresses in the agronomic situation.

Keywords: jojoba, salinity, mannitol, proline, *in vitro* culture

1. INTRODUCTION

Jojoba (*Simmondsia chinensis*), a promising dioecious shrub of arid and marginal zones, is commercially precious for its seeds. Jojoba oil has various commercial uses which include cosmetics, lubricants, and adhesives, medicines, pharmaceuticals, supply of acids and alcohols, electric-powered insulators, foam control agents, and plasticizers (Jacoboni and Standarti, 1987). Besides its advanced lubricating properties, jojoba has attracted interest as a panorama and soil conservation plant due to the fact it may develop in regions of high atmospheric temperatures, low humidity, and low fertilizer requirements (Yermanos, 1982). Furthermore, jojoba plants had been discovered to be physiologically active during the whole year, indicating their capability in keeping advantageous carbon stability even under extreme drought at very low (-36 bar) water potential (Rashmi *et al.*, 2016). Although jojoba is adapted to the aridity situations, salt accumulation in irrigated soils derived from both irrigation and groundwater sources can increase salinity to such levels which can reduce growth and yield. Also, drought is one of the maximum vast abiotic stress that affects jojoba growth and development. Salinity and drought are taken into consideration to be the most serious growth-limiting elements for crop plants. Salinity is a significant abiotic stress factor that threatens agriculture in both arid and semiarid environments, affecting over 20% of the world's irrigated land (Wu *et al.*, 2017). The effects of salinity are typically summarized as water stress, salt stress, and stress because of ionic imbalance (Greenway and Munns, 1980). The primary purpose of salinity risks is enhanced ion toxicity causing impaired sequestering of sodium ions into the vacuoles. Likewise, the effects of drought or osmotic stress on plants are the reduction in vegetative growth via way of means of influencing various morphological and physiological processes. In this regard, mannitol is used as an osmotic agent in plant tissue subculture research (Bekheet *et al.*, 2016). The addition of mannitol to the plant tissue culture medium decreases the water potential of the media inducing water stress that adversely affected the growth. The components of drought and salt stress cross-talk with every different as each of those stresses, in the long run, bring about dehydration of the cell and osmotic imbalance. Therefore, there's an absolute need for salt-tolerant cultivars or species to carry the poorly utilized saline lands into the right cultivation. Plants have evolved diverse approaches to reduce the negative effects due to abiotic stressors, several of that have been associated with the metabolism of amino acids (Batista Silva *et al.*, 2019). The exogenous application of proline guarantees an additional way for enhancing growth under environmental stresses (Heuer, 2003). High levels of proline enable plants to maintain low

water potentials, permitting additional water to be taken up from the environment. In this respect, some research has confirmed that exogenous proline can mitigate the damaging effects of salt and drought stresses (Heuer 2003; Hoque *et al.*, 2007; Islam *et al.*, 2009). Despite data displaying that jojoba tolerates fairly high levels of salinity (Benzioni *et al.*, 1996) and water stress (Foster and Wright, 1980), the selections to this point have now no longer been meant for use in areas with extremely excessive levels of salinity and water stress (Botti *et al.*, 1998). Consequently, *in vitro* culture constitutes a valid system for the study of the reaction of the plant to situations of drought and salinity and the isolation and selection of tolerant lines. Otherwise, tissue culture permits the management of stress homogeneity and the characterization of cell behavior under strain situations, unbiased of the regulatory systems present at the whole plant level (Lutts *et al.*, 2004). At this point, many reviews have defined the use of nodal segment as explants as the *in vitro* propagation of jojoba (Agrawal *et al.*, 2002; Tyagi and Prakash, 2004; Singh *et al.*, 2008; Llorente and Apóstolo, 2013; Bekheet *et al.*, 2015). Moreover, the effect of various salt concentrations on jojoba seedlings has been studied, and the substantial improve of proline and total sugar contents as salt concentrations increased has been discovered. Given the promising results on different crops, the the impact of the exogenous application of proline on the vegetative growth of jojoba tissue cultures grown under salt and drought stresses would be of great importance for the present investigation.

2. MATERIALS AND METHODS

2.1. Establishment of *in vitro* cultures

Nodal segments with axillary buds taken from women flowers have been used as explants for the *in vitro* regeneration of jojoba. Nodal explants have been washed with walking faucet water for 30 minutes. Once washed, the nodal explants were dipped in ethanol 70% for 30 seconds. The explants have been then sterilized with Clorox (50 %) for 10 minutes. Once sterilized, the explants wereA after which rinsed 3 instances in sterilized distilled water to take away all strains of the disinfectants. All steps of sterilization have been completed under aseptic situations with the use of a laminar air-float cabinet. The explants have been trimmed to 0.5 cm long then and cultured into 350 ml glass jars containing (MS) medium Murashige and Skoog (1962) supplemented with 1 mg/l benzyl adenine (BA) for 5 weeks. The proliferated shootlets have been sub-cultured instances (5 weeks interval) on MS medium contained 1.5 mg/l BA + 1 mg/l IAA for shootlets multiplication (Bekheet *et al.*, 2018). When the explants began out to multiply, properly grown micro shoots have been separated with the assist of a sterile scalpel under the hood

and placed in the same media for multiplication. Contaminated and vitrified cultures have been discarded and the healthful shoot buds have been used for the subsequent experiments. Small clusters of shoot buds (approximately 0.5 g weight) taken from the third subculture have been used in the following experiments.

2.2. Effect of proline on vegetative growth of shoot buds grown under salt stress

Various concentrations (1, 2, 3 and 4 g/l) of proline were used to investigate the ability of proline to mitigate the detrimental effect of salt stress. Uniform shoot buds (about 0.5g weight and 1 cm length) were cultured on multiplication medium (MS medium contained 1.5 mg/l BA + 1 mg/l IAA). To induce salt stress, the medium was supplemented with 3000 ppm of salt mixture [3 NaCl : 1 (3 MgCl₂ : 1 CaCl₂)] as described in (Ibrahim and El-Kobbia 1986).

2.3. Effect of proline on vegetative growth of shootlets grown under drought stress

To assess the influence of proline on the proliferation and growth of jojoba shootlets grown under drought stress, proline was added to the multiplication medium at a concentration of 1, 2, 3 and 4 g/l. Drought or osmotic stress was induced by supplementation of culture medium with 60 g/l mannitol. Shoot buds (0.5 g weight and 1 cm length) were cultured on the medium with different treatments for 8 weeks.

2.4. Recorded data

- Shootlets number: the number of proliferated shootlets recorded after 8 weeks of culturing.
- Shootlet height: the length (cm) obtained by the main shootlet that arose from the explant was recorded after 8 weeks of culturing.
- Leaf number: the number of leaves that arose from all the shootlets was recorded after 8 weeks of culturing.
- Fresh mass: fresh weight (g) at the end of the experiment.

$$\text{Growth value} = \frac{\text{Final fresh mass} - \text{Initial fresh mass}}{\text{Initial fresh mass}}$$

$$\text{Salt (drought) combating ratio} = \frac{\text{Fresh mass on proline containing medium}}{\text{Fresh mass on proline - free medium}}$$

2.5. Culture conditions and statistical analysis

Culture media were solidified with 0.7 % agar, amended with 30 g/l sucrose, and adjusted to pH 5.8 before autoclaving at 126 °C and 1.5 lb/M² for 20 min. Cultures were incubated in a growth chamber at 25± 2°C under 16 h light (2000 Lux) and 8 h dark. Experiments were designed in completely

randomized design and data were statistically analyzed from 20 replicates using standard error (SE) (Snedecor and Cochran, 1967).

3. RESULTS AND DISCUSSION

3.1. Effect of proline on the vegetative increase of shootlets grown under salt pressure

3.1.1. Effect of proline on growth characteristics

Salt tolerance in plants is a complicated phenomenon that involves morphological and developmental modifications in addition to physiological and biochemical processes. The exogenous application of proline was found to be powerful in alleviating the negative results of salt stress. This experiment was performed to examine the impact of proline on the growth characteristics of jojoba shootlets grown *in vitro*. Proline in concentrations of 1, 2, 3 and 4 g/l was added to the grown medium which was salinized with 3000 ppm of a salt mixture. The shootlets characters determined in this experiment were the number of proliferated shootlets, the height of shootlets, and the number of leaves. Table 1 shows that in salt stress (proline-free medium) the shootlets and leaves numbers were reduced. Proline was found to improve the negative results of salt stress on *in vitro* multiplication of jojoba. The increase in proline level applied to culture medium triggered a significant increase in the shootlets and leaves' number. However, shootlets height values have been reduced in high levels of proline added to the saline culture medium. The maximum value (3.00 cm) of shootlets height was observed in the proline-free medium (Table 1 and Fig. 1-A). Shootlets had chlorosis. As a result, these explants appeared light green. While the highest numbers of shootlets (6.40) and leaves (12.00) have been obtained on the medium contained 3 g/l of proline (Table 1 and Fig. 1-B). The proliferated propagules in this treatment were healthy and had dark green color. It is also apparent from the coefficient of correlation obtained that the number of leaves was correlated with the proliferated shootlets. According to the results, it appeared clearly that the growth characteristics of the jojoba shootlets have been very affected by the proline added to the saline culture medium. These findings can offer perception for growing salt-tolerant lines of jojoba via *in vitro* studies.

3.1.2. Effect of proline on growth dynamic

The impact of diverse concentrations (1, 2, 3, and 4 g/l) of proline on the growth dynamic of shootlets of jojoba grown on medium salinized with 3000 ppm of the salt combination was determined. Table 2 reports the impact of the addition of proline on fresh mass, growth value, and salt combating ratio. It was found that the impact of proline on the growth of the shootlets system

was highly perceptible. Compared to the control, the proline increased the 3 growth parameters of all concentrations of proline tested. The results confirmed that the growth parameters increased as the proline increased in the saline medium. However, the addition of proline at concentrations above until 3 g/l reduced the shootlets growth dynamic, thereby signifying 3 g/l of proline to be the top-rated concentration for alleviating the damaging consequences of salt stress. At this proline level, the highest fresh mass (3.50 g), and growth values (6.00) had been registered (Table 2). Also, the salt combating ratio calculated on fresh mass values took a comparable trend since the maximum value (1.75) was found in the 3g/l proline-containing medium. Consequently, these effects indicated that exogenous proline at an optimal concentration may be utilized for mitigating the suppressive consequences of imposed salt stress and thereby enhance the mechanisms of salt tolerance in jojoba tissue cultures. Exogenous application of proline provided osmoprotection and additionally more advantages on the growth of plants grown under salt stress. Under salinity, proline has been suggested to enhance growth, physiological, biochemical, and anatomical characteristics, and also to improve the antioxidant system defense of plants (Abdelhamid *et al.*, 2013; Dawood *et al.*, 2014). Despite the useful consequences of exogenous proline application, it imparts poisonous effects if is over-accumulated or implemented at immoderate concentrations. The consequences of the prevailing research verified that the damaging consequences of salinity on jojoba grown *in vitro* might be partly offset with the aid of using the exogenous application of proline. Application of 3 g/l is the most effective concentration for combating the salt stress effects in the jojoba plants grown *in vitro*. At this concentration, the best results of growth characteristics (proliferated shootlets and leaves) and growth dynamics (fresh mass, growth value, and salt combating ratio) had been obtained. These results are in line with (Lone *et al.* 1987) who cited that the addition of exogenous proline to cultured barley (*Hordeum vulgare* L. cv. Maris Mink) embryos increased shoot proliferation under saline conditions. In addition, Athar and Ashraf (2009) confirmed that exogenously implemented proline on the germination and seedling stages alleviated the damaging consequences of salt stress on canola cultivars. In alfalfa callus (*Medicago sativa*) cultures, 1g/l of exogenous proline was very effective at lowering the consequences of salt stress and promoting traditional growth, and increased the dry weight (Ehsanpour and Fatahian, 2003). Similarly, (Khalid *et al.*, 2010) suggested that reduction in germination, growth and chlorophyll contents caused by salt stress changed into improved by exogenous software of proline in sorghum. In a similar study, the exogenous proline at concentrations starting from 200-3000 mg/L, was found to be efficient for ameliorating the damaging consequences of salt stress (100 mM sodium chloride) on *in vitro* regeneration of *Sorghum bicolor* (L. Moench) (Amali *et*

al., 2013). Likewise, Okuma *et al.*, (2000) found that exogenous proline improved the growth of salt-stressed tobacco cell cultures and the development changed into attributed to the function of proline as an osmoprotectant for enzymes and membranes against salt inhibition. Regardless of the role of proline, jojoba tissue cultures tolerate salinity up to a level of sodium chloride concentration (113 mM), without showing any stress symptoms. Above this level, the salinity pressure effect was found as succulence and chlorosis of leaves and shoots (Roussos *et al.*, 2007). Furthermore, Taha (2014) cited that growing seawater levels in tissue culture medium as much as 2000 ppm increased callus fresh weight in addition to shoots multiplication of jojoba. In this regard, Benichi *et al.*, (2010) suggested that 3 g/l of NaCl marked the beginning of negative affect at the growth of jojoba seedlings, while 5g/l of NaCl inhibited completely the emergence of plumules.

Table1. Effect of exogenous proline on growth characteristics of jojoba shootlets grown on medium contained 3000 ppm of the salt mixture

Proline concentration (g/l)	Shootlets number ± SE	Shootlets height (cm) ± SE	Leaf number ± SE
0.0	2.50 ± 0.02	3.00 ± 0.11	6.00 ± 0.11
1.0	3.50 ± 0.10	2.70 ± 0.16	8.20 ± 0.15
2.0	5.30 ± 0.12	2.50 ± 0.22	9.00 ± 0.17
3.0	6.40 ± 0.20	2.20 ± 0.20	12.00 ± 0.10
4.0	5.00 ± 0.23	2.00 ± 0.31	10.00 ± 0.09

Table 2. Effect exogenous proline on growth dynamic of jojoba shootlets grown on medium contained 3000 ppm of the salt mixture

Proline concentration (g/l)	Fresh mass (g) ± SE	Growth value	Salt combating ratio
0.0	2.00 ± 0.13	3.00	-
1.0	2.40 ± 0.20	3.80	1.20
2.0	3.00 ± 0.10	5.00	1.50
3.0	3.50 ± 0.15	6.00	1.75
4.0	2.70 ± 0.09	4.40	1.35

3.2.Effect of proline on vegetative growth of shootlets grown under drought stress

3.2.1. Effect of proline on growth characteristics

The exogenous application of amino acid proline has been employed as an osmoprotectant substance that improves the performance of plants in drought conditions. Although the mechanism of drought tolerance is poorly understood, osmotic adjustment is considered to be associated with

dehydration tolerance by allowing plants to maintain cell turgidity and physiological processes. In this experiment, the effect of proline as an osmoregulator and its role in jojoba combating drought stress was studied by the addition of proline (1, 2, 3 and 4 g/l) in the tissue culture medium that contained 60g/l mannitol. Under these conditions, the *in vitro* response of jojoba growth characteristics in terms of the number of proliferated shootlets, the height of shootlets, and the number of leaves were recorded. Data obtained revealed that all measured growth characters improved with proline treatments and improvements were recorded even in the low proline application. Obviously, the growth parameters gradually increased to 3 g/l of proline application and then decreased. The highest values of shootlet number (7.20), the height of shootlets (4.10 cm), and the number of leaves (13) were registered with 3g/l proline containing medium. However, higher proline concentration (4 g/l) doesn't prove to be of benefit. In contrast, the lowest values of the three growth parameters were observed with the control (proline-free medium) treatment. On the treatment, shootlets were vitrified and the leaves were relatively narrow and had pale green color (Table 3 and Fig. 1-C). In contrast, shootlets proliferated on medium contained 60 g/l mannitol and amended with 3 g/ l proline were healthy and had dark green color leaves (Fig 1-D). The obtained results proved that the addition of proline to the multiplication medium efficiently improved the growth characteristics of jojoba shootlets grown in the medium contained a high level of mannitol. The results could provide a platform for improving the sustainability and agricultural productivity of jojoba grown under drought conditions.

Table 3. Effect exogenous proline on growth characteristics of jojoba shootlets grown under drought stress induced by 60 g/l mannitol

Proline concentration (g/l)	Shootlets number \pm SE	Shootlet height (cm) \pm SE	Leaf number \pm SE
0.0	3.00 \pm 0.12	3.30 \pm 0.21	9.10 \pm 0.31
1.0	4.10 \pm 0.15	3.70 \pm 0.26	10.20 \pm 0.19
2.0	6.20 \pm 0.14	3.50 \pm 0.20	12.00 \pm 0.15
3.0	7.20 \pm 0.22	4.10 \pm 0.10	13.00 \pm 0.10
4.0	5.30 \pm 0.19	4.00 \pm 0.18	11.50 \pm 0.12

3.2.2. Effect of proline on growth dynamic

Growth dynamics of jojoba shootlets grown on medium contained 60 g/l mannitol (drought inducer) and various concentrations (1, 2, 3 and 4 g/l) of proline as osmoprotectant were evaluated. Generally, all growth parameters are affected positively with proline treatments under drought conditions. Results reported that proline applications provided an increase in the growth

parameters presented as fresh mass, growth value, and salt combating ratio compared to the control (proline-free medium). Although proline induced improvement in growth dynamics of jojoba shootlets at all applied levels, application of 3 g/l proved to be more effective in inducing drought stress tolerance as compared to the other level. At this treatment, the best results of fresh mass (3.90), growth value (6.80), and salt combating ratio (1.56) were obtained. A remarkable reduction of the three growth parameters was obtained with the 4 g/l proline containing medium (Table 4). These results show that exogenous applications of proline could be used to reduce the harmful effect of drought on the growth parameters of jojoba. It is important here to notice that both the number of proliferated shootlets and fresh mass values on proline-free medium (control) were higher in the mannitol-containing medium compared to the salt-containing medium. This may be explaining the differences of growth parameters reflected counteracting salt and drought stresses by application of proline.

Table 4. Effect of proline concentration on growth dynamics of jojoba shootlets grown under drought stress induced by 60 g/l mannitol

Proline concentration (g/l)	Fresh mass (g) \pm SE	Growth value	Drought combating ratio
0.0	2.50	4.00	-
1.0	2.80	4.60	1.12
2.0	3.20	5.40	1.28
3.0	3.90	6.80	1.56
4.0	3.00	5.00	1.20

Drought stress is an important restricting factor in plant increase. Of diverse abiotic stresses recognized in nature, drought stress poses the first-rate hazard to crop production because water is essential at each stage of plant growth from seed germination to plant maturation (Chaves *et al.*, 2003; Athar and Ashraf, 2005), so any degree of water imbalance may also produce deleterious results on crop growth. In this respect, numerous techniques had been proposed to reduce the harm due to drought stress and to increase the tolerance of plants towards this stress. As one of the compatible osmolytes, proline considers as a key signaling molecule capable of triggering a couple of responses and represents part of the adaptation process. At this point, preceding studies have proven that exogenous proline application effectively regulates osmotic ability and performs an important role in sustaining plant increase under osmotic stress (Ali *et al.*, 2007; Ashraf and Foolad, 2007; Hoque *et al.*, 2007). The current research found that the addition of proline accelerated both growth characteristics and growth dynamics of jojoba shootlets grown under drought stress caused by mannitol in comparison to

untreated shootlets. Application of 3 g/l of proline proved to be more effective in inducing drought stress tolerance compared to the control or other levels. Our findings reveal that exogenous applications of proline might be used to alleviate the unfavorable results of and drought in jojoba. These effects are following the ones of Ali *et al.* (2007) who suggested that exogenous application of proline at specific growth stages improved the shoot fresh and dry weights of maize and application of 30 mM proline triggered the most increase in shoot fresh and dry weights. Meanwhile, the connection among increased proline content and better biomass were observed under osmotic stress in *Arabidopsis* (Roosens *et al.*, 2002) and rice (Wu *et al.*, 2003). Because of some earlier reviews, it's far suggested that exogenously applied would possibly have triggered enhanced endogenous proline accumulation under water stress situations which not only protects enzymes, 3-D structures of proteins and organelle membranes, but, it additionally supplies energy for growth and survival thereby assisting the plant to tolerate stress (Chandrashekar and Sandhyarani, 1996; Hoque *et al.*, 2007; Ashraf and Foolad, 2007). Although obviously it's far from specific reviews that exogenous application of proline induces abiotic stress tolerance in plants, there are a few reviews that reveal that excessive concentrations of proline can be dangerous to plants, including inhibitory effects on growth or deleterious effects on cell metabolisms (Nanjo *et al.*, 2003).

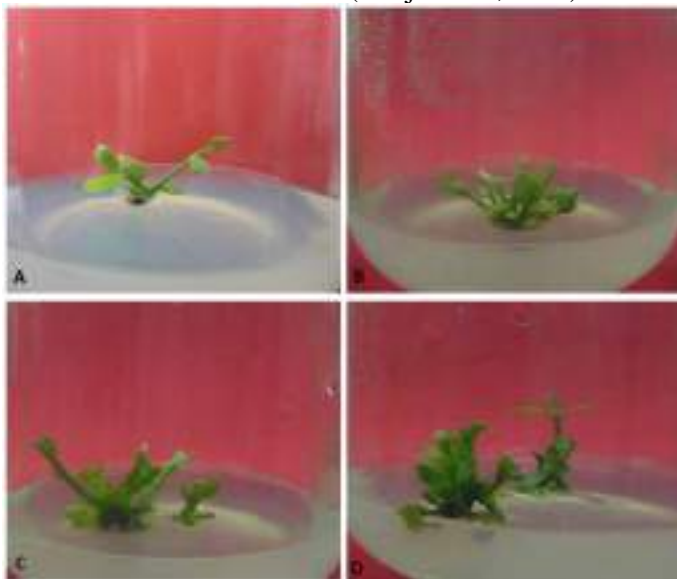


Fig. 1: a) Jojoba shootlets proliferated on: a) salt with proline-free medium, b) salt medium supplemented with 3 g/l of proline, c) drought with proline-free medium and d) medium contained 60g/l mannitol and 3 g/l proline.

4. CONCLUSION

Salinity and drought are consideration the most critical growth-restricting factors for crop plants. Although jojoba is adapted to aridity conditions, excessive salinity levels and excessive drought conditions are the most significant abiotic stresses that affect jojoba growth and production. The exogenous application of amino acid proline is thought to be effective in alleviating the damaging results of salt and drought stresses. In the present study, our findings indicated that the addition of proline to saline or osmotic medium improved the vegetative characteristics and growth dynamics of *in vitro* grown shootlets. Among numerous levels, three g/l of proline was found to be the most effective level on counteracting salt and drought inhibitory results. The acquired effects simply show that exogenous applications of proline could be used to reduce the dangerous effects of salt and drought stresses in jojoba. Also, the effects could offer a platform for enhancing the sustainability and agricultural productiveness of jojoba grown under salinity and drought conditions.

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COMPREHENSIVE UNDERSTANDING OF BIO-NANO INTERACTIONS-A CHALLENGE FOR NANOTOXICOLOGICAL RESEARCH IN ALBANIA: A REVIEW

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ABSTRACT

Nanotechnology is one of the most promising technologies of the 21st century. It is the ability to convert the nanoscience theory to useful applications by observing, measuring, manipulating, assembling, controlling and manufacturing matter at the nanometer scale. Nanotechnology is an area of science that integrates, physics, electronics, mathematics and informatics. This new science area promises scientific advancement in many sectors such as medicine, consumer products, energy, materials and manufacturing. A new research area has evolved in the last 20 years. Nanotoxicology represents a new and growing research area in toxicology. It deals with the assessment of the toxicological properties of nanoparticles with the intention of determining whether (and to what extent) they pose an environmental or societal threat. Nano particles (NPs) and nanomaterials (NMs) exhibit unique

physicochemical properties, which make them interact with cells and tissues in an unpredictable manner. Being of comparable dimensions, fabricated nanoparticles, and cellular molecular organelles and macromolecules, a possible direct interaction in the nano-bio interface could be revealed. A holistic understanding of bio-nano interactions would be necessary for a safe and intelligent design and use of nanomaterials. The present paper reviews via *in vivo*, *in vitro*, and *in silico* methods and a battery of reliable, low-cost, and specific biomarkers of nanotoxicity effects to unearth the mechanisms of bio-nano interactions. Furthermore, potential opportunities and challenges in applying these biomarkers in the study of bio-nano interfaces are also provided.

Keywords: nanoparticles; bio-nano interactions; biomarkers; nanotoxicity

1. INTRODUCTION

Research in the field of eco-physio-toxicology for the effects of toxic chemicals on biological organisms at cell to organism levels has been made in the last twenty years in Albania. Information on the adverse effects of environmental and anthropogenic-borne toxicants on biota health could be found in (Aliko et al., 2021; Qyli *et al.* 2020; Sula et al., 2020,). Different animals from different taxa such as crustaceans, amphibians, and fish, in addition to the mechanisms of homeostatic perturbation, have been studied using a multi-biomarker approach. Reactive oxygen species (ROS) response, lysosomal membrane stability, erythron profile, histopathology of liver and kidney, behavior and development, and growth parameters were evaluated as endpoints (Figure 1). Considering the complexity of biological systems, our studies clearly pointed out that even small changes that occur in the subcellular level, if persist in long-term, can alter homeostasis equilibrium determining so the fate and functions of the organism by leading it to disease.

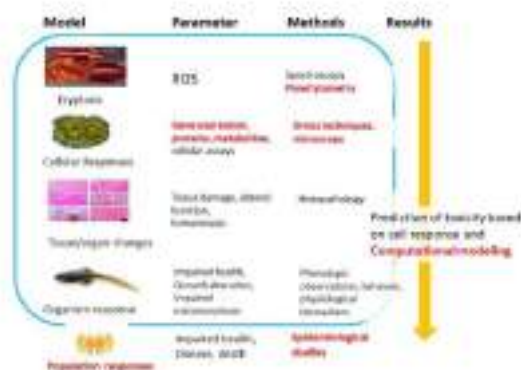


Fig. 1: General approach to evaluate toxic effects of NPs using cell and animal models, as well as a battery of reliable parameters/biomarkers.

The entire cell can be viewed as a “network of interlocking assembly of large protein machines” (Alberts, 1998), which, in turn, are often times of nanoscale dimensions (van den Heuvel and Dekker, 2007). Advancement in scientific research has made investigation on cells, tissues, and organs transformation and behavior as nanomaterials enter in contact with them unavoidable. Here, understanding the underlying mechanisms of material-biological system interaction is necessary. This could provide therapeutic gain if some features of unwanted effects of a nanomaterial in the context of disease are proved useful. This is the case when ecotoxicology and physiology/medicine can be viewed as the yin-yang of nano-bio interactions (Bondarenko *et al.*, 2021).

The present paper review summarizes the assessment approaches of toxicants’ effects on model organisms and the perspective of nanotoxicity in the development of nanomedicine. Furthermore, exploring interactions between engineered or incidental materials and biological systems at the nanoscale is a prerequisite for the safe and efficacious distribution of nanomaterials in patients (Shvedova *et al.*, 2010; Wang *et al.*, 2019).

NanoToxicology Approaches: advanced *in vitro* and *in vivo* models

Nanoparticles and nanomaterials are thought to behave differently from the bulk substance of the same chemical compound. Due to their tiny size and large surface area, the interaction of NPs with cells is far more powerful than their bulk counterparts (Tirumala *et al.*, 2021). Besides their beneficial biological effects, most often nanoparticles can cause adverse effects on biological systems, even though this is a very debatable argument. Figuring out any nanoparticles or nanomaterials toxic effects produced upon living cells or organisms is essential to understand mechanisms associated with nanotoxicity outcomes.

Generally, three approaches are used by our laboratory to evaluate the toxicity of NPs, cell-based *in vitro*, organism-based *in-vivo* assays, and *in silico* methods (Figure 2).

Cell-based *in vitro* assays

Cell-based tests, well-known as *in vitro* assays, are commonly used to assess the safety and toxicity of xenobiotics, replacing testing in animals. To reduce the increased number of animals used each year in toxicity tests, using non-mammalian red blood cells, i.e., amphibians and fish offers a great system to investigate the possible effects of toxicants in toxicology studies. Substituting experiments animals with cells go in respect with the principle of 3Rs (reduction, refinement, and replacement) proposed by Charles Hume and William Russell in 1957. According to this principle, the number of animals

sacrificed is reduced and the stress upon animals during handling and operational procedures is also minimized.

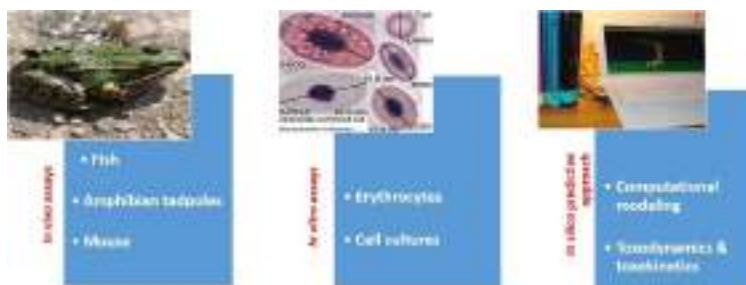


Fig. 2: Schematic presentation of scientific approaches employed for nanoparticle toxicity assessment.

Laboratory investigation about fish and frog erythrocytes was carried out to evaluate the toxic effects of different environmental toxicants such as copper nanoparticles (Aliko *et al.*, 2015), different pharmaceuticals such as fluoxetine and ibuprofen (Aliko *et al.*, 2021), and pesticides and PCBs (Sula *et al.*, 2020). In vitro toxicity is usually evaluated using cellular viability, apoptosis, oxidative stress enzymes, and genotoxicity as endpoints. Studies have shown a significant increase in the frequency of cellular and nuclear abnormalities in erythrocytes of *Bufo bufo* tadpoles exposed for seven days to two environmentally realistic concentrations of pharmaceuticals fluoxetine, ibuprofen, and their mixture, which proves the pharmaceuticals' role as erythrocyte apoptosis-inducers (Aliko *et al.*, 2021).

Non-mammalian erythrocyte cell seems to be a very good model cell in toxicity assessments, due to its morphological characteristics, long lifespan, and maturation during circulation, traits that make it susceptible to xenotoxins (Podsiedlik *et al.*, 2020). Following exposure to xenobiotics, erythrocytes undergo different morphological changes like shape changes due to disruption of membrane integrity. Usually, there is a dose-dependent shape alteration of fish erythrocytes exposed to environmental pollution, with red blood cells shifting from ellipsoidal to a circular shape, as a way to compensate for the reduction in hemoglobin concentration provoked by stress (Sula *et al.*, 2020). Thus, measuring erythrocyte rotundity, as a biomarker of cell resilience and membrane stability, can help in assessing the cytotoxic effect of xenobiotics on freshwater biota.

Red blood cells represent the main cells in circulations and they are responsible for oxygen transport towards body tissues and cells; any factor that perturbs this process could be lethal. Possessing a nucleus and being covered by a multi-component plasma membrane, the non-mammalian

erythrocyte has a characteristic ellipsoid shape, relatively high flexibility, elasticity, and deformability. Any alteration of this structure makes red blood cells suffer and compromises their function until it decides to undertake apoptosis. So, programmed cell death seems a clear sign of erythrocyte response to injury; this makes erythrocyte an excellent reliable biomarker of cell toxicity (Farang and Alagawany, 2018; Aliko *et al.*, 2021).

Erythrocytes are capable of explaining antioxidant capacity and producing reactive oxygen species (ROS) as a response to various toxicants due to the antioxidative enzymes they contain. By measuring the altered concentration of these enzymes in the blood, one can assess tissues' response to stressors. The generation of ROS in blood impairs the antioxidant defense system to eliminate oxidative stress, altering so, the normal body homeostasis. Our findings revealed that acute exposure of goldfish to manganese induced a significant increase in superoxide dismutase (SOD), catalase (CAT), and glutathion-S-transferase (GST) antioxidant enzymes in the blood, strongly suggesting that Mn^{2+} exposure caused generalized oxidative stress, which leads to activation of protective mechanisms necessary for scavenging of produced O_2 -radicals in erythrocytes (Aliko *et al.*, 2018).

Organism-based *in vivo* assays: fish and amphibians

To evaluate *in vivo* toxicity of nanomaterials and nanoparticles, model organisms must be exposed either to acute or chronic stressors and multiple biomarkers, such as biochemical, hematological, histopathological, immunological, and behavioral, must be measured. Fish and amphibians are the most investigated model organisms in our laboratory. Both species inhabit aquatic ecosystems, with fishes living entirely in water, while amphibians exhibit an amphibious life, with larval phase in water and adults sharing both areas, land, and water. The use of non-mammalian animal models such as *Carassius auratus* (Aliko *et al.*, 2018), *Carassius carassius* (Sula *et al.*, 2020), *Bufo bufo* (Aliko *et al.*, 2020), *Carcinus aestuarii* (Qyli *et al.*, 2020) represents an ideal strategy to overcome the ethical problems related to the traditional animal models used in the safety assessment of NPs.

The fishes were exposed to copper nanoparticles (CuONPs), at two environmentally relevant doses of 0.5 and 1 $\mu g/L$ for 96h for the toxicity test. In addition, ROS enzymes, tissue histopathology, biochemical and physiological parameters were also evaluated. All analyzed biomarkers showed significant differences between exposed groups and in relation to the control group (unpublished data). Copper nanoparticles at 1 $\mu g/L$ seemed to enter the cell, destabilizing the erythrocyte membrane, causing cytotoxicity and genotoxicity by leading the cell toward apoptosis. Another investigation involving the goldfish exposed to an environmentally realistic dose of manganese proves these results (Aliko *et al.*, 2018). However, the evaluation

of chronic toxicity and carcinogenic potential of nanoparticles and nanomaterial requires long-term studies of 12-24 months.

***In silico* assays: modeling tadpole growth and cytotoxicity**

In silico modeling represents a relatively new tool that combines experimental approaches, providing a powerful technique to help in understanding molecular mechanisms between NPs and biological macromolecules at the atomic level (Huang *et al.*, 2021). Even though nanomaterials are designed to be used successfully in the medical field, due to some of their properties they can create toxic effects in organisms. This is because they enter into cells, react with cellular components, and most probably interact with DNA causing breaks, altered basis, and chromosomal damage. This affects DNA function and causes a clastogenic effect producing micronuclei (Azqueta and Dusinska, 2015; Aliko *et al.*, 2021).

One of the promising approaches we are using to study the genotoxic effects of copper nanoparticles is molecular docking. This tool is based on computational simulations and evaluation of copper (II) oxide-DNA molecule interactions based on 3D structural knowledge. The molecular docking study showed that CuONPs, despite their low molecular weight, binds to the minor groove of DNA in an energetically favorable ($-2.13 \text{ kcal mol}^{-1}$) manner, suggesting a direct interaction with the genetic material, supporting the data from micronucleus assay (unpublished study).

Toad embryos and tadpoles are used for the assessment of the developmental toxicity. These life phases are very sensitive to teratogenic insults and also cover almost all morphogenic processes such as neurulation, limb bud formation, cardiac looping. Acute exposure of *Bufo viridis* tadpoles at Gosner stage 21 to different copper concentrations (0.01, 0.05, and 0.1mg/L) for 120 hours, caused poor larval development and growth (Aliko *et al.*, 2015).

Future perspectives

The present review aims at uncovering the molecular mechanisms of nano-bio interactions as the use of nanomaterials in biomedicine holds great promise, especially in diseased related with radical oxygen species-ROS. In the near future, nano-bio interface research has to be focused on: i) a full understanding of nanomaterial redox species-induction; ii) research nano-bio interactions considering the complex environment *in vivo* and, iii) applying computational simulation in order to deeply and accurately investigate the nano-bio interactions. In this case, *in silico* methods combined with experimental data offer great possibilities in evaluating the toxicological behavior of NPs and NMs. Understanding the risk produced by nanoparticles

and nanomaterials is of great concern for the safety and development of nanotechnology and nanomedicine.

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GENETICS OF SARS-COV-2 AND GENETIC DETERMINANTS OF PATHOGENICITY

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ABSTRACT

The Severe Acute Respiratory Syndrome-2 virus (SARS-CoV-2) is a SARS-like beta-coronavirus of zoonotic origin, first identified in December 2019 in Wuhan, China. The first complete sequence of the genome was deposited at NCBI Genbank on January 5, 2020. Human-to-human transmission was confirmed on January 14, 2020, at which time SARS-CoV-2 had spread in many countries around the world. RNA viruses exhibit high mutation rates, originating different genetic variants and assemblies of the viral genome which can affect the virus' infectivity, immune response, symptoms and clinical manifestations in patients. Thousands of global SARS-CoV-2 assemblies available in concentrated genetic databases are already known globally. Coronaviruses, including SARS-CoV-2, are single-positive-stranded, + ssRNA virus). Similarly to other coronaviruses, the SARS-CoV-2 genome consists of approximately 30,000 nucleotides organized into specific genes encoding structural proteins and non-structural proteins (Nsps). Structural proteins include the spike S, envelope (E), membrane (M), and nucleocapsid protein (N). Surface glycoprotein S is involved in binding to the host cell and plays an important role in rapid human-to-human transmission. Non-structural proteins enable the viral replication and transcription inside the host cell. The infection mechanism of SARS - CoV - 2 into human cells consists on the recognition and binding to ACE2 receptors of the human cell membrane, and entrance of the virus into the cell, enabled by intracellular proteases TMPRSS2. Thus, all human cells, tissues or organs that express, produce and release high levels of ACE2 are potential targets of SARS - CoV 2 infection. The target cells of SARS-CoV-2, express high levels of ACE-2 gene, determining the pathogenic effects in a variety of tissues and organs, including the most severe acute respiratory syndrome. Comparative genetic analyzes have revealed that SARS-CoV-2 is similar in 94.6% of the amino acid sequence and 80% of the nucleotide sequence to the previous SARS-CoV, a similarity to consider it a variant of SARS, hence its designation as SARS-CoV-2. Recent studies regarding mutations and rapid adaptation of the coronaviruses to the host show that genetic characteristics common to SARS-CoV-2 and the previous coronaviruses SARS-CoV and MERS-CoV affect their

virulence, high pathogenicity, the transmission capacity, the host susceptibility, the mutational ability and be zoonotically transmitted to humans. Recent studies show that the degree of severity of COVID-19 disease depends on human genetics, and specifically on the genetic variants/alleles of candidate genes such as ACE2, ADAM17 and TMPRSS2. Population genetic studies show clear correlations between the ACE2 expression level and the severity of COVID-19. On the other hand, ACE2 release from human cell is affected by other genes like ADAM17 and TMPRSS2. Genetic population studies show that their spread in different populations and ethnicities, may explain why severity and mortality rate is higher in males, why they affect individuals suffering from other concomitant diseases and why it does not show severe clinical symptoms in children. These results lead to hypotheses that the impact and risk of SARS-CoV-2, the severity of the disease and the mortality rate have a multifactorial genetic basis and that large-scale individual genetic profiling for these genes are required to identify the most genetically-vulnerable and at-risk populations.

Keywords: SARS-CoV-2, COVID-19, S protein, ACE-2 receptor, SARS etiology, ADAM-17 gene, TMPRSS-2 gene

1. INTRODUCTION

SARS-CoV-2 is a SARS-like coronavirus of potential zoonotic origin first identified in December 2019 in Wuhan, China's Hubei province. The etiological agent was characterized as a SARS-like betacoronavirus, which full genome was deposited at the NCBI Genebank on January 5, 2020. Thousands of genomes have been sequenced since that date. Transmission from animals to humans was confirmed on 14 January 2020, at which time SARS-CoV-2 had already spread rapidly to many countries around the world. Further widespread global transmission led the WHO to declare COVID-19 as a pandemic on March 11, 2020. Due to its worldwide distribution, SARS-CoV-2 exhibits a wide genetic diversity, with considerable distribution of its forms and variants in different racial/ethnic nationalities. Genetic studies in populations with geographically diverse backgrounds have reported significant genetic variation in protein coding regions, with variations in allele/genotype frequencies. This data enriches our knowledge of SARS-CoV-2 and previously known coronaviruses to understand how the virus is adapting to its new host, in order to design and produce effective drugs and vaccines especially in pandemic conditions (JHCHS 2020; Van Dorpa *et al.*, 2020).

Analysis of genetic sequence data of viral pathogens are an important tool for the epidemiological studies of infectious diseases and the molecular orientation in the production of drugs and vaccines. Data on genetic structure and sequence shed light on key epidemiological parameters such as the timing of outbreak of epidemic doubling, transmission pathogen detection and identification of potential sources and / or animal reservoirs. On the other hand, genomic data are effectively used to identify pathogenic genes that

interact with the host and characterize the variable genetic zones, in order to avoid rapid mutations that reduce the effectiveness of drugs and vaccines (Fani *et al.*, 2020).

This publication is a summary review on issues and scientific aspects related to the current state of knowledge in the fields related to SARS-CoV-2/COVID-19, pathogenicity, medical and pathological aspects and public health approaches, as reported from reliable and reputable sources and publications in scientific and informative journals until December 2020.

SARS-CoV-2: genomics

Coronaviruses, including SARS-CoV-2, are single-stranded, positive-stranded RNA viruses (+ ssRNA virus). Coronaviruses contain among the largest genomes among RNA viruses: 25-32 Kb. The SARS-CoV-2 genome consists of approximately 30,000 nucleotides organized into specific genes encoding structural proteins and non-structural proteins (Nsps). The typical CoV genome includes a 5'-cap region, 5'-untranslated region (UTR), open reading frames, a 3'-UTR and 3'-poly (A) end edge. The first two-thirds of the genome encodes unstructured proteins from 2 open reading frames; enzymatic replicase complex. The last third of the genome encodes 4 well-conserved structural proteins in CoV. Structural proteins include spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. Surface glycoprotein S is involved in interaction with the host cell angiotensin converting enzyme (ACE2) receptor 2 and plays an important role in rapid human-to-human transmission. S protein is responsible for binding to host cell receptors and viral entry into host cells. Proteins M, E and N are part of the viral particle nucleocapsid (JHCHS, 2020, Wang *et al.*, 2020).

Non-structural proteins, which are generated as cleavage products of the open reading frame 1ab (ORF1ab) viral polyproteins, are assembled and enable viral replication and transcription. RNA-dependent RNA polymerase, also known as Nsp12, is the major component that regulates viral RNA synthesis with the help of Nsp7 and Nsp8. The other five accessory proteins encoded by the genes ORF3a, ORF6, ORF7a, ORF8 and ORF10, assist in the final assembly of viral particles and their exit from host cells (Van Dorpa *et al.*, 2020; Bahrami *et al.*, 2020).

RNA viruses exhibit high mutation rates, thus creating different genetic versions of the viral genome in almost every replication cycle within the host cells. This process originates viral populations with a high diversity of genomes, known as *quasispecies*. During each viral replication cycle, the differences increase and accumulate, creating significant genetic differences between the original and the succeeding viral genome. Depending on the type and location of the mutations, genetic changes of this type can affect the infectivity, the transmission capacity of the virus, the human immune response,

symptoms and clinical manifestations in patients (Zeng *et al.*, 2020; Wang *et al.*, 2020).

There are thousands of SARS-CoV-2 sequences available in the global genetic database. The extraordinary availability of genomic data reported during the COVID-19 pandemic has been made possible by the intensive and extraordinary efforts and studies of hundreds of researchers who are constantly discovering new sequence and deposit SARS-CoV-2 ‘assemblies’ data globally (Wang *et al.*, 2020; Hu *et al.*, 2020).

Comparative sequence analysis of samples from infected individuals revealed that SARS-CoV-2 and SARS-CoV are very similar; the 2 viruses are 94.6% similar in amino acid sequence, while the nucleotide sequence similarity is 80% throughout the genome. For this reason in February 2020, the same Coronavirus Study Group classifies it as belonging to the same species and officially named the new coronavirus as SARS-CoV-2. While the clinical presentation, epidemiological patterns, and host range of SARS-CoV-2 may differ from the original SARS-CoV, it is the genetic similarity between the 2 viruses which led the group to classify them as same species. According to World Health Organisation the designation “SARS-CoV-2” is associated with the name of the virus, while “COVID-19” refers to the name of the disease caused by infection by SARS-CoV-2 (Hu *et al.*, 2020; Rabaan *et al.*, 2020).

SARS-Cov-2: infection mechanism

CoV binding to the host cell surface receptors and membrane fusion is mediated by the Spike (S) protein, composed of two subunits, S1 and S2. In the case of SARS - CoV - 2, the cleavage and activation of S proteins are controlled by the TMPRSS2 intracellular protease. This promotes the initial entry of the virus inside the host cell. Besides the changes in the amino acid sequences of the SARS-CoV-2 and SARS-CoV S proteins, both use the same cellular receptor for entering into the cell: the angiotensin-converting enzyme-2 (ACE2) receptor. SARS protein CoV- 2 has high affinity for the ACE2; the RBD-S complex with ACE2 further promotes the binding of S2. S2 is highly expressed in SARS-CoV-2, compared to SARS, which may explain the higher infectivity of SARS-CoV-2. In particular, two specific repetitions of S2 subunit play the key role for fusion between the virus and the human cell membrane (Lotfi *et al.*, 2020; Sironi *et al.*, 2020).

All cells, tissues and organs with high ACE2 expression are potential susceptible targets of SARS - CoV 2 infection. Liver damage, intestinal inflammation, renal and testicular insufficiency and pancreatitis in patients with COVID-19, correlate closely with high expression of ACE2 in cholangiocytes, in the gastrointestinal tract (both small intestine and duodenum), urinary organs (kidneys), testicles and pancreas. ACE2 represents

a high level of expression in the heart, which can contribute to acute myocardial damage and chronic damage to the cardiovascular system. Although ACE2 is primarily expressed in human type II (AT2) alveolar cells, the ratio of type I and type II alveolar cell surface area in alveoli is about 9:1. Type II alveolar cells expressing ACE2 occupy only 1.4% of all AT2 cells, which hypothesizes existence and inclusion of other receptor-assisted or unknown molecular interactions in regulating of SARS-CoV-2 infection and explaining its high infectivity. Other potential receptors, such as DC - SIGN (CD209 genotypes), L - SIGN (CLEC4M) and CD147 may also allow the virus to either capture and anchor in host cells or bind to the S protein. However, the role of these receptors and other influencing factors are still under investigation (Lotfi *et al.*, 2020; Nakagawa and Miyazawa 2020).

SARS-CoV-2; human pathogenicity

Coronaviruses (CoV) are a large family of viruses that are spread from animal hosts to humans, causing life-threatening respiratory illnesses such as Middle East Respiratory Syndrome (MERS) and severe acute respiratory syndrome (SARS). The SARS-CoV-2 virus is genetically closely linked to the SARS-CoV coronavirus, the first pandemic threat of a new and deadly coronavirus that appeared in late 2002 and caused an outbreak of severe acute respiratory syndrome (SARS). SARS-CoV was extremely deadly but was extinguished after major public health mitigation measures. SARS-CoV-2 has spread rapidly worldwide compared to 2002 SARS-CoV and 2012 Middle East Respiratory Syndrome (MERS-CoV) coronavirus, although the estimated fatality rate in confirmed cases is 6.6% in SARS-CoV-2 (as of August 2020), which is lower than those of SARS-CoV and MERS-CoV, (9.6% and 34.3% respectively) (Petersen *et al.*, 2020; Harrison *et al.*, 2020).

In contrast, the SARS-CoV-2 that appeared in December, 2019, rapidly triggered a global pandemic. The spread of SARS 2003 ended in June 2003, with 8098 reported cases and 774 deaths, and a fatality rate of 9.7%, with the majority of cases received in hospital. By comparison, the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) - another deadly coronavirus appeared in 2012 and caused 2494 reported cases and 858 deaths in 27 countries, with a fatality rate of 34%. The new SARS-CoV-2 coronavirus is less lethal but more contagious than MERS-CoV or SARS-CoV. The virus was detected in December, 2019 and in 6 months in the first pandemic wave, counting about 10 million infected and 500,000 deaths. Due to the wide clinical spectrum and high transmissibility, the eradication of SARS-CoV-2 with the 2003 SARS-CoV policies and measures does not seem to be a viable goal and equally effective, at least in the short terms (Zhu *et al.*, 2020; Harrison *et al.*, 2020).

Comparative genomics studies show that the genetic characteristics of SARS-CoV-2 and two other coronaviruses with High Fatality Rate (HFR), SARS-CoV and MERS-CoV affect both virulence and pathogenicity, as well as their host-transmitting ability from animals to humans. The evolutive changes of high Case Fatality Rate (CFR) viruses did not happen immediately, but as gradual events and trends. These characteristics are common to high-CFR coronaviruses and their host animals (bats in particular) that infect relatives in the same genus, proving that the emergence of SARS-CoV-2 is a natural process of continuous evolution of the coronavirus and is consistent with the possibility of future zoonotic transmission of other highly pathogenic strains to humans (Abdelrahman *et al.*, 2020; Bahrami *et al.*, 2020).

From the comparison of these viruses in terms of population-level mortality, transmission and severity pandemic evidence, fatality rate (mortality in individuals with the disease) and population-level mortality it turns out that: a) R_0 - (basic reproductive rate) of SARS-CoV-2 is similar to or higher than R_0 of SARS-CoV and pandemic influenza; b) Mortality due to SARS-CoV-2 and SARS-CoV is prone to people older than 70 years, unlike the influenza pandemics of 1918 and 2009; c) The percentage of symptomatic people seeking hospital treatment is higher for SARS-CoV-2 infections than for the 2009 influenza pandemic; and d) The mortality rate (case fatality rate) is probably about 1%, if asymptomatic individuals / mild symptoms are considered; under investigation and monitoring (SARS-CoV-2 data as of September 2020) (Abdelrahman *et al.*, 2020; Toyoshima *et al.*, 2020).

SARS-CoV-2: Genetics of human pathogenicity

Current research shows that genetics of specific human (host) genes such as ACE-2, ADAM17 and TMPRSS2, play an important role in susceptibility, pathogenicity and clinical manifestations caused by SARS-CoV-2 in humans. Population-level studies reveal clear negative correlation between the degree of ACE2 expression and the severity of COVID-19 onset. Recent data show that ACE2 is effectively released from human cell membranes through a process that is regulated at different levels and engages two types of membrane proteases: ADAM17 disintegrants and metalloproteinases and TMPRSS2 transmembrane protease. ADAM17 protein acts directly on ACE2 by directing its release into the extracellular space, while TMPRSS2 degrades both ACE2 and SARS-CoV-2 protein S, leading to the fusion of the virus with the membrane and its introduction into the cell. When the proteolytic activity of ADAM17 and TMPRSS2 increases, it triggers releasing rather than cell entry, creating a biochemical barrier to virus infection. This may be because the ACE2-virus interaction occurs away from virus-sensitive tissues. Severity of COVID 19 disease manifestations correlates with low levels of

ACE2 expression, and genetic polymorphisms of ADAM17 expression regulation that affect the intensity of ACE2 extraction from cells (Burgess, 2020; Gussow *et al.*, 2020).

Clinical studies have also revealed that incidence and mortality levels from SARS-CoV-2 are significantly different between male and female COVID-19 patients and the disease is associated with pre-existing concomitant conditions, such as cancer and cardiovascular disorders, and in particular individuals with hypertension taking anti-hypertensive medication. The ACE-2 gene is located on the X chromosome. It catalyzes the conversion of angiotensin II to angiotensin- (1-7), which acts as a vasodilator and exerts important modulating effects on the cardiovascular system. TMPRSS2 is an important influencing gene in prostate cancer, identified as an oncogene of the ETS family and detected in several types of tumors (Afewerky 2020; LoPresti *et al.*, 2020).

Comparative genomic studies of genetic variants encoding several regions of the ACE2 gene suggest that genomic variants of ACE2 may play an important role in susceptibility to COVID-19 and its associated cardiovascular conditions. In addition to differential polymorphisms which may explain the sensitivity and even outcome in different ethnic populations, the fact that ACE2 is an X-linked gene, might explain the increased / higher risk in males. As such, even in the absence of variations in this gene, the allelic presence of this gene can affect the natural history and prognosis of COVID-19 in males, regardless of ethnicity (Burgess 2020; Di Maria *et al.*, 2020).

Comparative genomic studies of coding genetic variants for some regions of the TMPRSS2 gene show that all populations carry p.Val160Met variants, with the highest allele frequency (~ 25%), in Eurasian population with a 40% allele frequency. The p.Asp435Tyr variant is carried only by the European population. These unique widespread polymorphisms in TMPRSS2 provide possible explanations for differential genetic susceptibility to COVID-19 as well as for risk factors, including those with cancer and the high-risk group of patients. Because TMPRSS2 is located at 21q22.3, it can be assumed that individuals with Down syndrome would be at higher risk for COVID-19 infection. Furthermore, the role and oncogenic involvement of TMPRSS2 variants may be related to the results also demonstrated for patients with COVID-19. From RNA analysis it is observed that the expression of TMPRSS2 is higher in ciliated cells and type I alveolar epithelial cells and an increase with aging, which suggests that regulation of TMPRSS2 expression may explain the higher protection of infants and children from COVID-19 (LoPresti *et al.*, 2020; Russo *et al.*, 2020).

2. CONCLUSIONS

Due to its genetic properties and structure, its very high mutation capacity and the rapid and effective mechanism of infection, SARS-CoV-2 presents a very high degree of pathogenicity expressed in terms of infectivity, transmissibility of the virus, the human immune response, symptoms and clinical manifestations in patients.

The binding of sars S-protein to ACE2 receptors in various human tissues is the basis of its serious pathological consequences in humans. This also explains the distinctive features of infection of this virus, compared to others of the coronavirus family in terms of infectivity, hospitalization and mortality.

The genetics of SARS-CoV-2 pathogenicity, in addition to the virus genetics, is influenced by a set of human genes involved in the mechanism of infection, severity and response.

Human genes such as ACE-2, ADAM17 and TMPRSS2, play an important role in susceptibility, pathogenicity and clinical manifestations caused by SARS-CoV-2 in humans. ACE2 degree of expression relates to the severity of COVID-19, ADAM17 gene protein acts on ACE2 release from the cell, while TMPRSS2 degrades both ACE2 and SARS-CoV-2 protein S. ACE2 also affects the susceptibility to COVID-19 and the increased incidence in males. The expression of TMPRSS2 in some human cells and its increase with aging, may explain the protection of infants and children from COVID-19.

These results show that the risk and severity of the disease is genetically multifactorial. Further genetic analysis and systematic investigations of functional polymorphisms of potential candidate genes would enable assessment of the most risked and genetically-vulnerable populations and in-depth studies for designing of effective medical treatments and protection for COVID-19.

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ANTIBIOTIC USE IN PATIENTS WITH COVID-19: PRESENT AND FUTURE PERSPECTIVES

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ABSTRACT

The COVID-19 pandemic, caused by the SARS-CoV-2 virus has become a public health emergency with more than 120 million confirmed cases and 2 million deaths worldwide, as of 18 March 2021. Several studies report that the pandemic has led to a further increase and inappropriate use of antibiotics, which might have a long-term impact on public health due to antibiotic bacterial resistance (AMR). The present study aims to: i) provide updated information on antibiotic prescriptions in patients with COVID-19, ii) discuss issues related to irrational use of antibiotics and, iii) provide guidelines to face the growing threat of antibiotic-resistant bacteria. This is a summary review on issues and scientific aspects related to the current state of knowledge in the field, as a result of the selection of publications until March 2021. The rationale for antibiotic treatment in COVID-19 patients seems to be related to the previous experience on bacterial superinfection in influenza, where the rate of co-infection/secondary bacterial pneumonia was 11-35% in hospitalized patients. On the other hand, the currently limited evidence suggests that in COVID-19 this incidence is much lower. Several studies report a high prevalence (more than 70%) of broad-spectrum antibiotic use in patients with COVID-19. In fact, the most prescribed antibiotics were fluoroquinolones, third-generation cephalosporins, piperacillin/tazobactam and carbapenems. Moreover, empirical antibiotic prescriptions seem to be based on clinical symptoms, rather than on laboratory markers of inflammation or radiology findings. High rates of empiric prescriptions of broad-spectrum antibiotics were reported during the COVID-19 pandemic, which suggests that more guidance on the use of antibiotics is needed. Guidelines need to be repurposed and promoted even in these difficult times where there is a massive pressure on all healthcare workers. A close and continuous surveillance of the

antibiotic use, along with national recommendations and continuous education, might contribute to the improvement of the appropriate use of antibiotics and mitigate the consequences due to AMR. Further investigations through larger and prospective studies are required to help and guide stewardship practices.

Keywords: COVID-19; antibiotics; AMR; SARS-CoV-2; antimicrobial stewardship

1. INTRODUCTION

COVID-19 is an infectious disease caused by the recently discovered virus, known as SARS-CoV-2 (Severe Acute Respiratory Syndrome-Related Coronavirus 2) (WHO, 2020).

The COVID-19 pandemic has become a public health emergency with more than 120 million confirmed cases and 2 million deaths worldwide, as of March 18th, 2021 (WHO, 2021). However, as many people are most likely asymptomatic and tests and/or surveillance is limited, the real number of infected or dead persons may be underestimated.

Data indicate that this pandemic is the most severe of the three coronavirus-induced pandemics in the past two decades after SARS-CoV-induced respiratory viral disease in 2002–2003 and MERS-CoV respiratory syndrome in 2012 (da Costa *et al.*, 2020).

A substantial portion of SARS-CoV-2–infected individuals report a few, if any, symptoms and recover completely (80%). However, approximately 20% of the patients develop a severe disease requiring hospitalization and oxygen support, 5% of whom are admitted to Intensive Care Units (ICU) (Matheson *et al.*, 2020).

The deadly nature of SARS-CoV-2 might be partially related to bacterial co-infections. In fact, many colonizing bacteria, such as *Streptococcus pneumoniae* or *Staphylococcus aureus*, might take advantage of the compromised immunological status of patients with underlying viral infection, to cause secondary bacterial pneumonias (Ginsburg *et al.*, 2020). For instance, during the pandemic there has been an increase in the number of hospitalized patients fulfilling the criteria for community-acquired pneumonia (CAP). The diagnosis of bacterial co-infection in patients positive for SARS-CoV-2 remains a major challenge because fever, cough and lung infiltrates are common symptoms of both diseases (Sieswerda *et al.*, 2021).

Despite the viral nature of the disease, several studies report that the pandemic has led to a further increase and inappropriate use of antibiotics mainly due to suspected bacterial co-infection or to prevent secondary infection during hospitalization such as hospital-acquired pneumonia (HAP), ventilator-associated pneumonia (VAP) and so on (Rawson *et al.*, 2020; Lansbury *et al.*, 2020; Langford *et al.*, 2020). These trends could have a long-

term impact on public health due to the emerging antimicrobial resistance (AMR).

The increased and irrational use of antibiotics is not new since antibiotics are among the most widely used drugs worldwide. Between the years 2000-2015, the consumption of antibiotics increased by 39% (CDDEP, 2019).

Furthermore, the evolution of AMR represents nowadays a serious threat to the public health worldwide as well as a catastrophic economic impact. Thus, AMR is considered another global pandemic. Millions of infections and approximately 700,000 deaths occur each year due to antibiotic-resistant infections. At this rate, it is estimated that by 2050 mortalities will reach 10 million per year (O'Neill, 2014). For these reasons there is a great worldwide concern that the increase in antibiotic prescriptions during the COVID-19 pandemic could further exacerbate the existing problem of AMR.

The present study aims to: i) provide updated information on antibiotic prescriptions in patients with COVID-19, ii) discuss issues related to the irrational use of antibiotics and, iii) provide guidelines regarding the growing threat of antibiotic-resistant bacteria.

2. MATERIALS AND METHODS

This study is a literature review on issues and scientific aspects related to the current knowledge in the field regarding antibiotic prescription practices during the COVID-19 pandemic. Consequently, databases such as Pubmed and Hinari for published eligible and reliable studies on human subjects in English up to March 2021 were used. Studies were included if they evaluated patients with confirmed COVID-19 and/or reported the prevalence of bacterial co-infections.

3. RESULTS

Several studies in different countries have indicated high rates of antibiotic prescriptions in COVID-19 patients, raising concerns about the irrational use of antibiotics and the pandemic's potential impact on AMR.

These trends occur both in the community and in the hospital sector. The results of a behavioral research conducted by the World Health Organization (WHO), in nine countries and areas of Europe, showed that 79-96% of the community that was using antibiotics reported no infection with COVID-19, but that antibiotics were used to "prevent infection" (Di Guardo, 2021). This problem was even more complicated in the hospital sector where the anxiety/uncertainty during the pandemic, the difficulty in excluding the risk of co-infection upon admission, the possibility of secondary infections during

hospitalization and the increased workload of the healthcare workers could contribute to a further increase in the use of antibiotics (Lucien *et al.*, 2021).

Rate of co-infection

The rationale for antibiotic treatment in COVID-19 patients seems to be related to the experience with bacterial superinfection in influenza, where the rate of co-infection/secondary bacterial pneumonia was 11-35% in hospitalized patients (Chien *et al.*, 2009; Sheng *et al.*, 2011). In fact, due to the increase in mortality linked to bacterial superinfection, several guidelines advocated the initiation of empirical antibiotic use in patients with severe COVID-19 (Alhazzani *et al.*, 2020; WHO 2020). On the other hand, the current although limited evidence suggests that this incidence is much lower for COVID-19. One rapid systematic review, considering 3506 patients, estimated that bacterial co-infection was present in 3.5% of patients and bacterial secondary infection was identified in 14.3% of patients. When stratified by patient population (an estimate of COVID-19 illness severity), bacterial infection ranged from 5.9% in all hospitalized patients to 8.1% in critically ill patients (Langford *et al.*, 2020). In another systematic review and meta-analysis, 7% of hospitalized COVID-19 patients had a bacterial co-infection, with the highest prevalence observed in ICU wards (14%) (Lansbury *et al.*, 2020). Moreover, other studies conducted in the United Kingdom showed that bacterial co-infections seem to be uncommon among Covid-19 patients. For example, a review study conducted in the United Kingdom showed that 8% patients (62/806) experienced bacterial/fungal co-infection during hospitalization (Rawson *et al.*, 2020). Another study in the Whiston hospital (Prescot, UK) estimated that only 4% of the patients had pneumococcal co-infection (Adler *et al.*, 2020). These results are consistent with other studies, such as those conducted by the Dutch Working Party on Antibiotic Policy (SWAB), where bacterial co-infection upon admission was reported in 3.5% of COVID-19 patients, while bacterial secondary infections during hospitalization occurred in around 15% of the patients (Sieswerda *et al.*, 2021). Even more lower rates are reported in another study that evaluated the data of patients admitted in 5 hospitals in the Johns Hopkins Health System, where the prevalence of bacterial co-infection was 1.2% (Karaba *et al.*, 2020). Same rates are also reported from hospitals in Netherland (Karami *et al.*, 2021).

The data on pathogens causing bacterial superinfections in COVID-19 seem to be scarce and yet unknown. The systematic review and meta-analysis of Langford *et al.*, (2020) concluded that the most frequent microorganisms reported were *Mycoplasma* species, *Haemophilus influenzae* and *Pseudomonas aeruginosa*. However, bacterial co-pathogens were reported only in 45.8 % of the cases, representing less than 14% of the patients with

reported infections. In other studies, the most frequent pathogens were *Staphylococcus aureus*, *Haemophilus influenzae* and *Streptococcus pneumoniae* (Sieswerda *et al.*, 2021). When physicians were consulted, most of them declared that the need to start empirical therapy was due to the coverage of atypical pathogens, followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. On the other hand, Methicillin-Resistant *Staphylococcus Aureus* (MRSA) was recognised as non-critical (Beović *et al.*, 2020).

Rate of antibiotic use

Although data on antibiotic prescriptions given to patients with SARS-CoV-2 infection are scarce, current studies report a high prevalence (more than 70%) of antibiotic use, especially broad-spectrum antibiotics. A large systematic review conducted in Canada showed that the prevalence of antibiotic prescriptions was 74.6%. The most common antibiotic classes prescribed were fluoroquinolones (20.0%), macrolides (18.9%), β -lactam/ β -lactamase inhibitors (15.0%) and cephalosporins (15.0%) (Langford *et al.*, 2020).

Moreover, the results of an international web-based survey, addressed to physicians from 23 countries, estimated that 71.8% of the patients were receiving antibiotics. Although the piperacillin/tazobactam was the most used antibiotic, different trends were seen in different countries. In Italy, the most prescribed antibiotics were carbapenems in combination with fluoroquinolones, whereas in North America, anti-MRSA antibiotics were mainly used and in Turkey, fluoroquinolones. The decision on antibiotic use is based on clinical symptoms and on laboratory markers or radiology. An interesting result of this study is that, although 61.8% of the participants followed the local guidelines for the use of antibiotics in patients with COVID-19, 82.9% of these cases were following local CAP guidelines. The duration of the antibiotic therapy also differs by country, ranging from 5 days in the UK and North America, to 7 or even 8 days in Italy (Beović *et al.*, 2020).

During the New York City pandemic, out of 5,853 COVID-19 patients admitted to the hospital in a 7-week period, 71% were receiving at least one antibiotic such as doxycycline, azithromycin, levofloxacin, ciprofloxacin, ceftriaxone, cefepime, intravenous vancomycin, and piperacillin/tazobactam. Furthermore, 79% of the patients were exposed to antibiotic therapy in the 30 days that preceded positive bacterial diagnosis. The duration of the therapy rather empirical or targeted was high (approximately 8.5 days) (Nori *et al.*, 2020). High rates of antibiotic use are also reported from other studies conducted in the United States. For example, a single-center retrospective analysis conducted in a medical center in Philadelphia, estimated that 67% of

patients received antibiotic therapy, yet 72% did not have an obvious source of bacterial infection. The most common antibiotics used were cefepime (45%), ceftriaxone (54%), vancomycin (48%), and azithromycin (47%) (Goncalves Mendes Neto *et al.*, 2021).

These data are also consistent with several studies reported from China. Chen *et al.* reported that 71% of the patients received antibiotic treatment, 25% of whom were treated with a single antibiotic and 45% with a combined therapy. The most prescribed antibiotics were cephalosporins, quinolones, carbapenems, tigecycline (against MRSA) and linezolid. Antibacterial therapy by moxifloxacin (64%), ceftriaxone (25%) and azithromycin (18%) is very common in Chinese hospitals (Chen *et al.* 2020; Wang *et al.*, 2020).

On the other hand, surprisingly very lower rates are seen only in a Point Prevalence Survey study conducted in Singapore, with a prevalence of antibiotic use approximately 6.2%. Although authors stated that “Despite low prevalence of antibiotic use among confirmed and suspected COVID-19 patients, there was significant proportion of inappropriate antibiotics use where bacterial infections were unlikely (Tan *et al.*, 2021).

Incidence of antibiotic resistance

Studies on the possible impact of the pandemic on antibiotic resistance are very scarce and fragmentary. However, available data show an increase in multidrug resistant bacteria (MDR). A retrospective study in the Wuhan Union Hospital concluded that among 159 strains isolated from COVID-19 patients with bacterial secondary infections, 85.5% were Gram-negative, mainly *A.baumannii*, *K. pneumoniae* and *S. maltophilia*. At the same time, the carbapenem resistance rate of *A. baumannii* and *K. pneumoniae* was 91.2% and 75.5%, respectively (Li *et al.*, 2020).

Another retrospective study in Italy estimated that there was an increase in the incidence of Carbapenem-Resistant *Enterobacteriaceae* (CRE) from 6.7% in 2019 to 50% in March–April 2020 (Tiri *et al.*, 2020).

One study in the New York City hospital showed that there was a significant decline (>10%) in the susceptibility of multiple species (*K. pneumoniae*, *P. aeruginosa*, and *E. cloacae*) to one antibiotic at least (Nori *et al.*, 2020).

4. DISCUSSION

The COVID-19 pandemic has become a public health emergency worldwide. Our review shows that antibiotic prescriptions for COVID-19 patients, especially broad-spectrum antibiotics, are very common compared to other therapeutic agents (more than 70%). Although the amount and type of prescriptions may vary in the different countries, there is a consistently high

prevalence of antibiotic consumption in the healthcare settings. Therefore, it becomes very important to highlight the major long-term impact that these trends could have on the emergence of antimicrobial resistance.

The use of antibiotics in medical practice began in the 1940s and their role has been revolutionary in the treatment of severe infections in patients who have undergone interventions, suffering from neoplasms, immunosuppressed and for the prevention or treatment of diseases in the veterinary field (Aminov, 2010). However, the discovery and development of new antibiotic classes were accompanied by the onset of resistance and nowadays all antimicrobials used in humans are affected by this phenomenon (Ukuhor, 2021). The onset of resistance is a natural evolutionary phenomenon of microorganisms, that is accelerated by the selective pressure due to irrational and overuse of antibacterial drugs (Prestinaci *et al.*, 2015). Hospital conditions, where the consumption of antibiotics is high and where the spread of resistance is facilitated, constitute a particularly favorable environment for the emergence and spread of resistance against multiple antibiotics (Almagor *et al.*, 2018).

On the other hand, the widespread empirical use of antibiotics is currently not supported by the evidence, as the overall rate of co-infections/secondary bacterial infections in SARS-CoV-2 positive patients is low. Higher rates are reported only in critically ill patients in the ICU wards. As such, it seems there is a huge gap between the prevalence of antibiotic prescriptions and the rate of co-infections, that suggests a potential irrational use of these therapeutic agents.

There are several reasons that lead physicians to this practice of prescriptions. First, this tendency seems to be related to the experience with previous epidemic and pandemic outbreaks, such as the H1N1 influenza A in 1918, where the incidence of reported bacterial superinfections, that complicated viral infections, was relatively high (approximately 23%) (Ginsburg *et al.*, 2020). Second, guidelines regarding the use of antibiotics in patients with suspected or confirmed COVID-19 vary by country and, in some cases, may be confusing, since they do not differentiate between CAP and COVID-19 (Beović *et al.*, 2020; Huttner *et al.*, 2020). Third, healthcare professionals involved in the treatment of COVID-19 patients have a high workload, high levels of pressure and are continuously aware of the high risk of infection and mortality surrounding the pandemic every day. All the above reasons may impact on their inability to strictly follow the local clinical practice guidelines. In fact, as mentioned in the results of our study, the decision on antibiotic use is based on clinical symptoms rather than laboratory data.

In this background, Antibiotic Stewardships (AS) are an essential key to guide the rational use of antibiotics. Several guidelines, such as those

published by WHO and the National Institute of Health (NIH), advocate the empirical use of antibiotics in critically ill patients (WHO, 2020; NIH, 2021; Alhazzani *et al.*, 2020). Furthermore, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) has published some more detailed guidelines, encouraging microbiological tests before prescription, and therapy re-evaluation/de-escalation. These guidelines also advise not to exceed 5-day treatment in most cases (Huttner *et al.*, 2020). As described in the Results section, many of the routine prescription practices used by physicians during the pandemic differ from these guidelines.

These findings suggest that ASs need to be re-proposed during the COVID-19 pandemic. Guideline design should be evidence-based, but at the same time easy and accessible, to facilitate and promote a more proper use of antibiotics.

The impact that antibiotic prescription practices during the pandemic will have on future AMR still remains unclear. Given the excessive use of broad-spectrum antibiotics worldwide, there are several reasons for concern, and emerging studies are already showing an increase in the incidence of MDR bacteria.

According to some studies “the impact on AMR will be uneven, varying between epicenters and non-epicenters, geographic regions, hospital-to-hospital within regions and within specific hospital units”. The increase is estimated to be higher in countries that were simultaneously epicenters for COVID-19 and AMR, such as USA, Brazil, Russia, UK, Spain and Italy.

Moreover, the pressure against AMR is expected to be significant in hospitals with the largest number of COVID patients and even in facilities with specific wards that were converted in COVID units, even if the rest of the hospital experienced an overall reduction in antibiotic consumption (Cornelius *et al.*, 2020).

Conversely, other authors found that the chances for an increase in AMR are poor, due to the improved good health practices, the greater prevention/disinfection, both in healthcare facilities and in the community, and due to the national/international travel restrictions. However, the same authors state that this decrease is likely to happen especially in developed and wealthier countries, whereas in areas with poor infrastructure and infection control measures, AMR will still remain a major concern (Collignon *et al.*, 2020). Unfortunately, it can take many years for these questions to be answered.

The current situation highlights a major need for further research to better comprehend several critical points that could drive irrational antibiotic use and help in the design of guidelines during the pandemic. These include the exact incidence of co-infection/secondary infections in COVID-19 patients,

the identification of the main pathogens causing those infections and the role of biomarkers in diagnosis.

In conclusion, a close and continuous surveillance of the antibiotic use, along with international/national recommendations and continuous education, could contribute to improve the appropriate use of antibiotics and mitigate the consequences due to AMR.

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ON BETA THALASSEMIA MUTATIONS, LABORATORY FINDINGS AND TREATMENT OF ALBANIAN PATIENTS

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ABSTRACT

The β -thalassemia syndromes (major and intermedia) are caused by mutations that result in the reduced or non-production of beta- globin chains. More than 300 disease-causing mutations have been identified so far. Here, the chronic anemia prevails among all the of thalassemia syndromes. It is estimated that 1,5 % of the world's population has β -thalassemia, while in Albania this prevalence goes to approximately 7 %, (ranging from 7-11% in particular areas). The treatment protocol of patients has significantly improved in the last decades. In the present study there are 65 patients involved to investigate their molecular diagnosis of beta -thalassemia and to correlate these data with their follow up. The IVS-I-110 mutation is found in 37% of the patients. The same data are reported for the East Mediterranean region. Cod 39, a severe mutation responsible for severe clinical outcomes in patients, especially with homozygote manifestation, is found in 18% of the patients. So, patients with this mutation-s type have mean hb level 8,1 compare to mean hemoglobin level in total group which is 8,5 g/dl. This is statistically significant. ($t= 1,954$, $p=0,05$). The mean number of PRBC-s / month is 3,8 units of PRBC compared to the total of patients' needs which is 3,1 units of PRBC. A meticulous follow up of these patients, taking into account their molecular presentation at the beginning, is an important predictor for the future outcome.

Keywords: β - thalassemia syndromes, allele, mutation, pure red blood cells lactate dehydrogenases, Ca 15-3, indirect bilirubin

1. INTRODUCTION

Haemoglobinopathies are very serious clinical conditions caused by genetic mutations. They represent the commonest monogenic diseases in the world.

The recombinant DNA techniques have been used for the for a better understanding of thalassemia syndromes (including beta thalassemia and sickle cell disease), the first diagnosed diseases from the intrauterine life. Consequently, the treatment protocol of patients has significantly improved.

There 530 patients with haemoglobinopathies registered in Albania.

Continuous monitoring of biochemical indexes is important for the follow up of the patients with haemoglobinopathies.

Haemoglobinopathies (β thalassemia and sickle cell disease) refer to a heterogeneous inherited group of hemoglobin, characterized by a quality or quantity deficit of hemoglobin synthesis.

Haemoglobinopathies are part of autosomal recessive diseases, the most prevalent genetic blood diseases in the world (Ther *et al.*, 2006). As people affected from these types of diseases suffer from a chronic hemolytic anemia throughout their life, permanent regular blood transfusion therapy is a crucial to them.

The Thalassemia International Federation stated that there are approximately 500 000 patients with beta thalassemia registered in treatment and follow-up. There are more than 100 000 patients suffering from sickle cell disease worldwide (data are mainly collected from USA database) (Kountouris *et al.*, 2014).

It is estimated that nearly 7,1 % of the population is a carrier of any type of haemoglobinopathies in Albania. Currently, there are at least 530 patients (children and adults) diagnosed and in treatment in the country. The majority of our patients are diagnosed major and intermediate with beta thalassemia (84,5%), and the reminder (15,5%) diagnosed with the sickle cell disease. It is not a gender-based disease. There are approximately 300 genetic mutations of β thalassemia. There are 20 most mutations manifested among the Mediterranean population. 12 out of 20 mutations appear to decrease the beta globin chain synthesis, while 8 mutations seem to be responsible for the absence of beta globin chain (Antoniou and Grosveld 1999).

Wonke (2001) said that there are a lot of clinical manifestations which vary from mild to severe anemia, and could be fatal if systematically untreated. Patients with anemia during their treatment with blood intake, manifest high ferritin levels in blood, developing iron overload in major organs not only due to frequent transfusions, but also to the increased

intestinal absorption. Iron overload causes cardiac, hepatic, endocrine dysfunction. In addition, inappropriate treatment might be fatal.

Kountouris *et al.*, (2014) stated that appropriate treatment improves significantly peoples living conditions. Today, their longevity goes to 60 years. Consequently, frequent monitoring and assessment of laboratory data are important.

Hemolysis, the destruction of red blood cells and the release of their contents (cytoplasm) into surrounding fluid (e.g. blood plasma), is a typical phenomenon of the disease. Here, indirect bilirubin, LDH and tumor marker Ca 15-3 are of crucial importance (Taher *et al.*, 2010).

The present study aims to give some insights on the molecular diagnosis of the beta thalassemia mutations, laboratory findings and treatment of 65 randomly selected Albanian patients aged between 2 - 18 years, and to correlate these data with their follow up. The consent for data publication has been given.

2. MATERIALS AND METHODS

In the present study 65 patients with haemoglobinopathies are randomly selected. They are followed up only at our clinic and their consent for data publication has been given. Other pathologies are excluded in this group of patients who are aged between 2 - 18 years.

Data on beta thalassemia mutations are here reported, and blood samples for Ca15-3. LDH, Indirect Bilirubin and pre transfusion level of haemoglobine collected. Laboratory investigation is routinely carried out at the laboratory of the University Hospital Center 'Mother Teresa' and Laboratory of National Center of Blood Transfusion in Tirana, Albania.

3. RESULTS AND DISCUSSIONS

The chart 1 plots demographic distribution of the patients showing that the majority of the patients comes from western Albania probably due to plasmodium falciparum here present in the past which confirms the hypothesis of this co-existence.

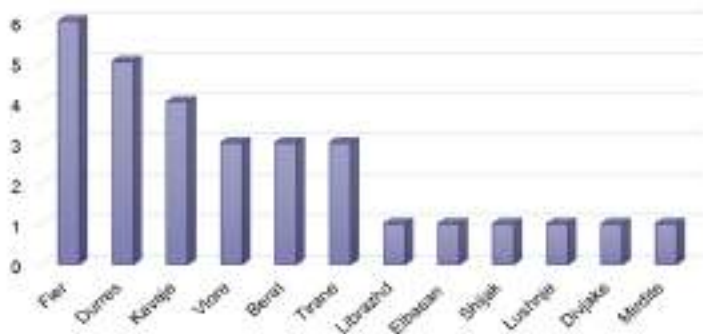


Chart 1. Demographic distribution of the patients.

Table 1. Types of beta-thalassemia mutations among the patients

MILD MUTATIONS β^{++}	MODERATE MUTATIONS β^{+}	SEVERE MUTATIONS β°
-101 -87 -88 -28 -IVSI-106	IVS I-110 (36,4%) IVS I-6 (18,3%)	IVS I-1 (9,1%) Cod 39 (18,3%) Cod 44 (13,6%) IVS I-116 (4,5)

Table 1 informs about the types of beta-thalassemia mutations among the patients. It could be noted that the IVS-I-110 mutation is the most frequent mutation among the patients by counting 37% of patients.

Table 2. The mean value of pre -transfusion hemoglobin level

	Gender	Number of patients	Mean value	S.D.
Hb	Male	37	7,64	1,00
	Female	28	7,65	1,07

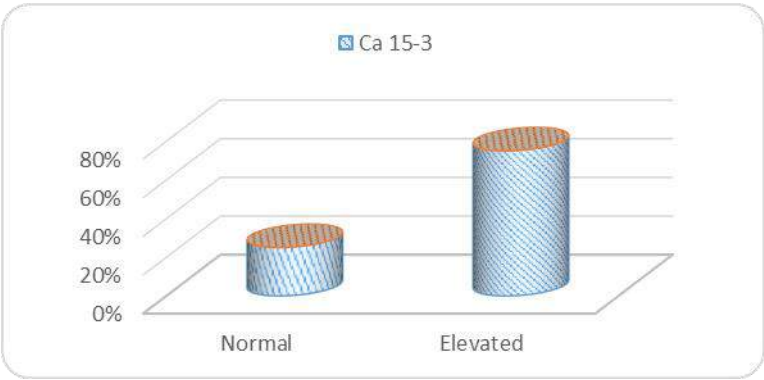
Table 2 reports the mean value of pre -transfusion hemoglobin level. As it could be noted both males and females patients manifest very low pre-transfusion hemoglobin levels due to severe hemolysis caused by the mutation, lack of blood donation, especially in summer, and rare antigenic structures of rhesus blood groups.

Table 3. Codon 39 mutation, hemoglobin level and quantity of blood needed

	Patients with Cd 39 mutation	Number of patients	t	p
Hb (g/dl)	8.1 (0.9)	8.5(0.7)	1.9	0.03
PRBC (n/month)	3.8 (1.1)	3.1(1.1)	2.3	0.02

**PRBC- Pure Red Blood Cells*

Patients with genotype Cd39, have low hemoglobin level compared to total number of patients which is statistically significant (independent sample t test statistic =1.954 p= 0.03). Therefore, they need more blood units (PRBC) in order to cope with a qualitative life (independent sample t test statistic = 2.33 p= 0.02).



Graph.2 Ca 15-3 indicator in patients with haemoglobinopathies.

Ca 15-3 is present in 75% of the patients, compared to 25% of patients who have normal values.

Table 4. The mean value of Ca 15-3 in patients with haemoglobinopathies.

	Gender	Number of patients	Mean	S.D.
Ca 15-3	Male	37	49,08	17,6
	Female	28	48,20	17,2

Males exhibit slightly higher mean value of Ca 15-3 than to females (49,08 vs. 48,20).

Table 5. The mean value of LDH in patients with haemoglobinopathies

	Gender	Number of patients	Mean	S.D.
LDH	Male	37	696,7	414,4
	Female	28	879,6	494,4

Table 5 shows a higher LDH value in females compared to males.

The majority of patients manifest double and triple LDH level. The number of patients with normal values is 15 patients, there are 12 patients with double LDH value and there are 16 patients with triple LDH value.

Table 6. The mean indirect bilirubin level in patients with haemoglobinopathies

	Gender	Number of patients	Mean value	S.D.
Indirect Bilirubin	Male	37	1,95	0,87
	Female	28	2,96	2,32

A student test has been made for two separated variables. It is estimated that there is no significant statistical difference between patients (male/female) for all variables studied in the confidence interval 95%.

Table 7. Student test used to compare hemolytic indicators

	t value	p
Ca 15-3	0,127	0,99
LDH	-1,428	0,19
Indirect Bilirubin	-1,988	0,05
Hemoglobin	-0,034	0,99

There is a significant correlation between high levels of Ca 15-3 and high levels of LDH, indirect bilirubin and the low levels of pre-transfusion hemoglobin ($p < 0,05$).

Patients who do not undergo transfusion based on their needs have high levels of tumor marker Ca 15-3.

There is a good correlation between the high values of Ca 15-3 and the high values of LDH, indirect bilirubin and low hemoglobin level ($p < 0,05$).

Patients with haemoglobinopathies have high levels of Ca 15-3, probably related to ineffective erythropoiesis at bone marrow level due to the accelerated turnover of erythroblasts. The latter is closely related to the high levels of other hemolytic biological parameters like: LDH and mostly indirect

bilirubin. Ca 15-3 is found at higher levels in 75% of the patients, while 25% of the patients have normal levels of the Ca 15-3.

Albania reports a high prevalence of haemoglobinopathies alike in other Mediterranean countries. The guidelines provided by the Thalassemia International Organization are followed for the prevention and treatment of blood disorders. So, the most common treatments for this disease are blood transfusion plus iron chelation (BTIC) therapy and bone marrow transplantation (BMT). Consequently, patients using these procedures experience different health-related quality of life (HRQoL).

4. CONCLUSIONS

The haemoglobinopathic data here reported reflect our clinical and laboratory practices.

The hemolytic data of the 65 patients are here evaluated and compared to other neighboring Balkan and Mediterranean countries. Here we could mention the frequencies of the most common β -thalassemia alleles (IVS-I-110, Codon 39, IVS-I-6) (Kohler et al., 2021). Babameto-Laku *et al.*, (2011) and Aessopos *et al.*, (2014) stated that the IVS-I-110 mutation has its highest frequency in the east Mediterranean region (Cyprus, Lebanon, Greece, Republic of Macedonia, Turkey), whereas the codon 39 mutation is found mainly in the west (Sardinia, Sicily, Spain).

The correlation between type of genetic mutation and some laboratory data are a means to address appropriate management of beta thalassemia syndromes. The genotype Codon 39 is a severe mutation (the third as per percentage among our patients) People with this genotype (Codon 39) manifest lower pre transfusion hemoglobin level compare to total group of patients, and this is statistically significant ($t=1,954$, $p=0,05$)

These patients need more blood compared to total of patients and this is statistically significant: $t=2,33$ and $p=0,02$.

The mean number of pure red blood cells per month is 3,8 units compare of the total of patients' need. All these results are similar with those of literature, especially data reported from Mediterranean countries (Cappellini *et al.*, 2019).

The Ca 15-3, LDH, indirect bilirubin and level of pre transfusion hemoglobin are in the present study evaluated and compared with (Antoniou and Grosveld 1999; Wonke 2001; Taher *et al.*, 2010) where it is stated that hemolysis is always present in our patients.

The tumor marker Ca 15-3 is found at higher levels in 75% of the patients, while 25% have normal levels of the tumor marker Ca 15-3.

The majority of patients showed higher LDH level (70%).

The mean hemoglobin values was 7,64 in males and 7,65 in females.

There is a good correlation between the high values of Ca 15-3 and the high values of LDH, indirect bilirubin and low hemoglobin level ($p < 0,05$).

Kreka (2017) stated that patients with hemoglobinopathies manifest higher levels of Ca 15-3, which might be due to ineffective erythropoiesis of bone marrow. This marker is induced by an increased erythroblasts apoptosis. Cappellini *et al.*, (2019) stated that hemolysis always present is supported scientifically by the high values of LDH, indirect bilirubin, and low levels of hematocrit and hemoglobin.

The treatment protocols and guidelines followed have been updated across the years. Consequently, their longevity goes to 60 years.

5. RECCOMENDATIONS

Blood supply remains the most efficient therapeutic treatment. Blood donation is a continuous need.

Iron chelation therapy has significantly improved their quality of life and patients longevity (removing iron overload from patient's organs).

On the other hand, the improvement of personalized treatment using genetic modifiers still in trials and other specific drugs seem to be of a great benefit for all patients with haemoglobinopathies (Stephanou *et al.*, 2019).

Collaboration among specialists is of irreplaceable importance

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HISTONE DEACETYLASE INHIBITORS; AN OVERVIEW OF THE CLINICAL STUDIES IN HEMATOLOGICAL MALIGNANCIES AND SOLID TUMORS

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ABSTRACT

Histone deacetylase inhibitors (HDACi) are considered novel potential drugs in cancer treatment and so far, they represent an important area of research. Four HDACi, Romidepsin, Belinostat, Vorinostat and Panobinostat have been currently approved by the U.S FDA as anticancer agents,³ and many others are in ongoing clinical trials. Over the last decades, histone deacetylase enzymes (HDACs) have been recognized as therapeutic targets, due to the correlation between irregular covalent modifications catalyzed by them and tumor development. The rationale for developing HDACi is based on their ability to induce differentiation, apoptosis and cell cycle arrest in cancer cells. Objectives: This review aims to summarize the recent progress toward HDACi evaluating their efficacy as single agents, or in combination with other chemotherapeutic agents for the treatment of different types of tumors. A literature research was conducted in MedLine, PubMed, Caplus, SciFinder Scholar databases from 2015 to 2021. The overall future for the use of HDACi in combinatory regimens in a variety of malignancies, looks promising, although more information regarding life quality changes associated with the administration of these agents, should be forthcoming. So far, poor outcomes have been reported in the treatment of solid tumors by using HDACi as single agents, meanwhile, they have shown distinguished effectiveness in the treatment of hematological malignancies. The use of HDACi in combinatory anticancer regimens is considered a successful strategy in

terms of lower toxicity and better clinical outcomes. Although, a better comprehension of HDACi effectiveness is required in order to optimize their efficacy, toxicity and overcome therapy resistance.

Keywords: epigenetic factors, HDACs, HDACi, clinical trials, novel anti-cancer therapies, FDA, efficacy, combination therapy.

1. INTRODUCTION

Cancer is considered an essentially genetic disease, in which genomic expression alterations alter the cellular control pathways causing the cell to proliferate in an uncontrolled way (Baylin and Jones, 2016). However, in recent decades, numerous clues have led to the recognition that epigenetic factors may also be critical in the evolution of human cancer (Porta-Pardo *et al.*, 2020).

Among the post-translational modifications of chromatin, which results in the creation of various gene expression patterns, histone acetylation is one of the most studied processes and appears to play a fundamental role in the regulation of nucleosomes (Tolsma and Hansen, 2019). In fact, the activity of histone acetyltransferases (HATs) and histone deacetylases (HDACs) were identified among the transcriptional coactivators and corepressors, respectively, providing strong evidence of the functional connection between histone acetylation and transcription (Sanei and Kavoosi, 2019; Milazzo *et al.*, 2020).

A critical event contributing to tumor formation seems to be the imbalance between HATs and HDACs, resulting in an abnormal HDAC activity leading to transcriptional repression of specific genes that promote tumor suppression (Fraga and Esteller, 2005). Despite the HDACs catalytic activity in removing the acetyl groups from the ϵ -amino lysine residues on histones, they also deacetylate several non-histone proteins (Li and Seto, 2016; Porter and Christianson, 2019). Thus, the idea emerging from this scenario was that the use of HDAC inhibitors (HDACi) might be a useful approach to cancer therapy (Singh *et al.*, 2018).

Currently, in humans, there have been identified 18 HDAC family members which are classified into four classes based on sequence homology to yeast HDACs (Mottamal *et al.*, 2015; Lee *et al.*, 2021). Class I includes HDAC1, -2, -3, and -8, which are primarily situated in the nucleus of the cells (Gregorette *et al.*, 2004; Brosch *et al.*, 2008; Li *et al.*, 2020). Class II HDACs are further subdivided into: class IIa (HDAC enzymes 4, 5, 7, 9) and class IIb (HDAC 6, 10). Class III Sir 2-like enzymes (human sirtuins 1–7), and class IV (HDAC11) (Trapp and Jung, 2006; Wang *et al.*, 2011; Tang *et al.*, 2013; Zhang *et al.*, 2017; Zhang *et al.*, 2019). Classes I, II and IV are zinc-dependent metalloproteins and they are present in the nucleus or cytosol,

while the class III HDAC family requires NAD⁺ as a co-factor and they appear in the cytoplasm, mitochondria or nucleus of the cell (Marks and Xu, 2009; Alexander *et al.*, 2019).

The HDAC inhibitors are generally classified into benzamides [compounds such as Entinostat, Mocetinostat, Ci-994, Tacedinaline, 4SC202], hydroxamates [such as Vorinostat, Panobinostat, Belinostat, Givinostat, Trichostatin, Resminostat, Quisinostat, Abexinostat, Practinostat, CHR-3996], depsipeptides [Romidepsin], short chain fatty acids [such as Valproate, Pivanex, Sodium phenyl butyrate] and sirtuins inhibitors [Nicoitinamide, sirtinol, Cambinol, EX-527] (Miller *et al.*, 2003; Ceccacci and Minucci, 2016; Eckschlager *et al.*, 2017). The majority of HDACi, present a common recognition domain (called “cap”), linked to a hydrophobic chain which bears a zinc-binding group (ZBG) of different nature (Ni *et al.*, 2015). Based on their isoform specificity, they are classified into; selective HDAC inhibitors and pan-inhibitors, which act on all HDAC classes (Ceccacci and Minucci, 2016). Following the discovery of dose-limiting toxicity of pan HDAC inhibitors, there has been a trend toward the development of class-specific HDAC inhibitors to improve the outcomes and limit the toxic effect (Eckschlager *et al.*, 2017).

Yadav *et al.*, (2019) stated that clinical studies report that class III and IV HDAC inhibitors appear to have some adverse effects such as thrombocytopenia, neutropenia, anemia, fatigue, and diarrhea. Meanwhile, class II HDAC inhibitors are considered promising agents, but due to several side effects associated with their use, such as cardiac toxicity, therefore more data is needed in order to progress with their application in cancer therapy (Manal *et al.*, 2016; Zhang *et al.*, 2017).

To date, selective Class I HDAC inhibitors are the most investigated isoforms, since they are highly expressed in several cancers as well due to their limited toxic effects.

The ongoing intense research conducted in this field has shown that HDACi have a complex anticancer activity exerted through multiple pathways from their interaction with histone deacetylases to numerous other molecular targets (Hull *et al.*, 2016; Karagiannis and El-Osta, 2006).

Several clinical trials performed during the last years proved that HDACi compounds are potent inducers of growth arrest, differentiation, cell death by autophagy/apoptosis, accumulation of ROS and inhibition of angiogenesis in transformed cells (Bolden, 2006; Mehnert and Kelly, 2007; Jazirehi, 2010; Cheng *et al.*, 2017). Furthermore, the selective toxicity of HDACi on mutated cells and their ability to synergistically promote the efficacy of many conventional chemotherapeutic agents has aroused further interest in this new category of drugs (Suraweera *et al.*, 2003; Diyabalanage, 2013).

To date, there are four HDACi agents approved by the USA Food and Drug Administration for the treatment of different type of tumors and many others are being evaluated in preclinical or clinical studies (Vander Molen *et al.*, 2011; Autin *et al.*, 2019). Vorinostat (SAHA) was approved by the USA FDA in 2006 for the treatment of CTCL followed by Romidepsin (FK228), approved for the treatment of Cutaneous T-cell Lymphoma, (CTCL) and Peripheral T-cell lymphoma (PTCL) three years later. Belinostat (PXD 101) and Panobinostat (LBH-589) have been recently approved (2014 and 2015 respectively) for the treatment of PTCL and Multiple Myeloma (MM) (Chun, 2015; Guha, 2015; Yoon and Eom, 2016; Li and Sun, 2019).

The present paper aims to provide a comprehensive and updated overview of the current state-of-the-art of different U.S. FDA-approved HDACi, as well as the recent progress toward combination with other chemotherapeutic agents in the treatment of a variety of tumors.

2. METHODS AND MATERIALS

This manuscript is a descriptive review based on previous research papers. In order to provide an overall picture of recent advances on the topic, a broad literature review of only published studies over the period 2015-2021, was performed. The selected databases were as follows: PubMed, MEDLINE, CAPLUS and SciFinder Scholar. In addition, the keywords and phrases used to generate this research were: epigenetic factors, HDACs, HDACi, FDA, clinical trials, novel anti-cancer therapies.

3. RESULTS

As it has been previously reported in the literature, despite the approval by the FDA, HDACi have been shown to have limited therapeutic efficacy against solid tumors as single therapeutic agents. Thus, combining HDACi with other conventional drugs is being considered an important approach toward their full therapeutic potential benefits (Vancurova *et al.*, 2018).

- **Vorinostat (SAHA)** belongs to the hydroxamate derivatives class and causes inhibition of both class I and II HDACs (Park *et al.*, 2017). It has been approved for the treatment of CTCL with a daily recommended dose of 400 mg.

Numerous clinical trials have been performed and others are planning to evaluate the use of this compound in monotherapy or in combination for the treatment of hematological and solid tumors (Chun, 2016; Wang *et al.*, 2020).

Other investigations were conducted considering the possible combination of HDACi with other agents based on promising results which indicate the ability of these compounds to reduce the metastatic potential of tumor cells (Damaskos *et al.*, 2017).

A promising strategy, against breast cancer cells in vitro and in mouse models, includes the combination therapy using Vorinostat and other agents to improve the treatment efficacy (Schech *et al.*, 2015, Chiu *et al.*, 2016).

In the phase I clinical study, a total of 60 patients (26 with Diffuse large B-cell lymphoma (DLBCL), 21 with Hodgkin lymphoma, 8 with T-cell lymphoma and 5 with B-cell lymphoma) were treated with Vorinostat-Gemcitabine- Busulfam- Melphalan in combination with Azacitidine (Nieto *et al.*, 2016). The overall survival rate resulted to be respectively 77% among the patients with DLBCL and 95% among those with Hodgkin lymphoma (Apuri and Sokol, 2016).

A phase IIb trial study evaluated the efficacy of Vorinostat in combination with lenalidomide and dexamethasone in 25 lenalidomide-refractory patients. The response rate was 24% and the clinical positive feedback rate was 80%. The most common reported adverse events of Vorinostat were nausea, fatigue, diarrhea and only 6% of the patients experience more severe side effects (Sanchez *et al.*, 2017).

Vorinostat has demonstrated promising effects against advanced leukemias, solid tumors, glioblastoma multiforme and further investigation of its profile is underway (Golabek *et al.*, 2015; How *et al.*, 2015; Singh *et al.*, 2015; Stahl *et al.*, 2016, Montalban-Bravo *et al.*, 2017; Reddy *et al.*, 2020).

In a phase II clinical trial, Vorinostat in combination with bortezomib and Dexamethasone was found to be effective in the treatment of patients with RRMM. (ClinicalTrials.gov Identifier: NCT00773838).

- **Belinostat (PXD-101)** is a hydroxamic acid-type HDACi with inhibitory activity towards class I, II and IV HDAC isoforms. It has been approved by FDA for the treatment of PTCL (Garmpis *et al.*, 2019). The recommended dosage of belinostat for the treatment of PTCL is 1,000 mg/m² administered over 30 minutes by intravenous infusion once daily on days 1 to 5 of a 21-day cycle.

To date, phase I and II clinical trials have been conducted in patients with solid tumor and RRMM therefore, Belinostat results being well tolerated with minimal side effects such as diarrhea, nausea, fatigue and anorexia (Tandon *et al.*, 2016; ClinicalTrials.gov Identifier: NCT01273155). Meanwhile, the combination of various proteasome inhibitors, and HDAC inhibitors for the treatment of lymphoma, and PTCL in particular, is currently under evaluation (Sawas *et al.*, 2015).

A phase II clinical trial is also testing the clinical activity of Belinostat as a single agent applied in patients with advanced-stage thymus tumors (ClinicalTrials.gov Identifier: NCT00589290).

Phase I clinical trials, trying to prove the synergistic interactions of Belinostat with Volasertiv (for B and T-cell lymphomas) and Zidovudine (for ATL), are in recruiting participants phase (ClinicalTrials.gov Identifier: NCT02737046; ClinicalTrials.gov Identifier: NCT02875002).

The efficacy of Belinostat and Erlotinib combination is underway phase II clinical trials in patients with non-small cell lung cancer (ClinicalTrials.gov Identifier: NCT0118870).

Another phase I study demonstrated the synergic effect of the combination of Belinostat and CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) in a group of 23 patients with PTCL. The observed overall response rate was 89%. The most adverse events were reported when CHOP were administrated alone (Johnston *et al.*, 2021).

The safety and efficacy of Belinostat combination with Bortezomib, was also tested in patients with RRMM, but considering the observed dose-limiting toxicity, the study was terminated in the Phase II trial (ClinicalTrials.gov Identifier: NCT00431340).

- **Panobinostat (LBH-589)** is a class I and II HDACi agent approved by the FDA for the treatment of CTCL and PTCL and in combination therapy with other agents in RRMM (Prebet and Vevy, 2011). Panobinostat is a hydroxamic acid HDAC inhibitor with high potency.

The antitumor activity of Panobinostat is associated with the induction of angiogenesis, induction of apoptosis and autophagy (Ganai, 2016).

Phase I clinical trials are investigating the combination of Panobinostat and Letrozole for the treatment of metastatic breast cancer. The recommended and tolerable dose for this co-treatment is; Panobinostat at 20 mg (oral route) three times a week administration and Letrozole at 2,5 mg daily. Researchers found that Panobinostat was able to prevent the growth of aromatase inhibitor-resistant cells establishing the innovative role of this compound in the treatment of metastatic breast cancer (Tann *et al.*, 2016).

The efficacy of Panobinostat in inhibiting breast cancer growth and metastasis in mouse models via up-regulating APLC expression, was recently demonstrated (Qin *et al.*, 2019).

A study conducted by Lee *et al.*, reported that the combination therapy Bortezomib/Panobinostat and Docetaxel showed better inhibition efficacy of tumor growth in cell line xenograft models in comparison with single treatment (Lee *et al.*, 2018).

The combination therapy of Panobinostat and proteasome inhibitors for the treatment of relapsed or/and refractory MM appear to be effective and well tolerated (Liu *et al.*, 2016).

A phase I clinical trial aiming to prove the possible treatment benefits in patients with RRMM, by combining Panobinostat with daratumumab, bortezomib and dexamethasone, is in recruiting participants phase (ClinicalTrials.gov Identifier: NCT04956302).

Encouraging results in terms of efficacy were observed, in phase III clinical trial, in which the combination therapy of Panobinostat with bortezomib and dexamethasone, was applied to RRMM patients. Researchers found that the co-treatment was able to reduce cancer size in 59% of the patients (ClinicalTrials.gov Identifier: NCT01023308). The most common side effects include; stomach pain, confusion, headache, loss of appetite, nausea and vomiting.

For the time being, clinical studies are in progress to evaluate the efficacy of Panobinostat in combination with other agents for the treatment of different type of cancer.

- **Romidepsin (FK228)** chemically belongs to the class of depsipeptides. It is a highly selective class I HDAC agent, approved by the FDA in 2009 for the treatment of CTCL and in 2011 for the treatment of PTCL (Valdez *et al.*, 2015). The recommended dose of romidepsin is 14 mg/m² administered intravenously over a 4-hour period on days 1, 8, and 15 of a 28-day cycle.

At present time, Romidepsin is being considered a potential strategy mainly for the treatment of T-cell lymphomas, but also for hematologic and solid malignancies (Chun, 2015; Cacabelos and Tejjido, 2018).

The combination Romidepsin- Alisertib is in phase I clinical trial for the treatment of relapsed/refractory aggressive B and T lymphomas (Strati *et al.*, 2020).

Wuet *al.* (2016) evaluated the combinatory effect of Romidepsin with Temozolomide (TMZ) in glioma cell lines, observing a significant level of apoptosis in glioma cells. Furthermore, in mice models, it was confirmed that the co-treatment, FK228 – TMZ is much more effective compared to each drug alone.

The potential anti-cancer activity of this agent in endometrial carcinoma, has been investigated by Li *et al.* in a study in which, the ability of Romidepsin in inducing tumor cell proliferation arrest at G0/G1 phase and apoptosis, was confirmed (Li *et al.*, 2016).

Falchi *et al.* in a phase I trial, found that the combination of oral 5-azacytidine and romidepsin, among 25 patients with relapsed/refractory PTCL, resulted to be safe and effective, with an overall response rate of 61% (Falchi *et al.*, 2021).

Promising efficacy results for CTCL patients were demonstrated in a phase I trial study, conducted by Vu *et al.* A combined therapy of romidepsin and liposomal doxorubicin was administered to 11 patients with CTCL and 12 patients with PCTL and the overall response rate was 60% and 27% respectively (Vu *et al.*, 2020).

Many clinical trials are evaluating the therapeutic combination benefits of Romidepsin- Azacitidine, Oxaliplatin, Gemcitabine, Dexamethasone in various lymphoid malignancies (ClinicalTrials.gov Identifier: NCT01846390; Yamaski *et al.*, 2019, Reiman *et al.*, 2019).

Romidepsin is also being investigated in phase I/II trials as a novel drug in the cure of human immunodeficiency virus infection (ClinicalTrials.gov Identifier: NCT02092116; Mc Mahen *et al.*, 2020; ClinicalTrials.gov Identifier: NCT02616874; ClinicalTrials.gov Identifier: NCT02850016). The most common side effects observed were; stomach pain, confusion, headache, loss of appetite, nausea and vomiting.

Additional clinical studies using HDACi in combination therapy for pancreatic, breast and non/small cell lung cancer are in progress (Yao *et al.*, 2015).

Considering all the data we reviewed for the above-approved HDACi, the current clinical results are promising, and they support the potential use of these compounds (as single agents and in combination regimens) for the treatment of a variety of malignancies.

4. DISCUSSION

The goal of this study was to investigate the recent progress toward the combination of approved HDACi with other chemotherapeutic agents in the treatment of a variety of tumors. In this review, we summarize the results of different clinical trials which could potentially lead the way to discover other HDAC inhibitors with greater therapeutic relevance.

Our findings suggest that the use of HDACi in combinatory anticancer regimens is considered a successful strategy in terms of lower toxicity and better clinical outcomes. Nevertheless, gathering more information relative to the higher safety doses, children's response toward these novel therapies and long-term toxicity remains still a challenge (ClinicalTrials.gov Identifier: NCT04308330).

Despite evidence suggesting that combination therapy regimens are more effective, the clear demonstration of the molecular events that define the cumulative benefit remains moderately understood.

Following the promising outcomes of HDACi used in CTCL, PTCL, RRMM, and in a small number of hematological lymphomas, further investigation must be carried out to evaluate their safety and efficacy profile against solid tumors. Regarding this issue, previous studies conducted have shown that HDACi are more effective in hematological malignancies than in solid tumors, and the reason why is probably related to stability matters. Nevertheless, we found that HDACi combined with other anticancer drugs, are commonly being tested in several phase I/II clinical trials for solid tumors malignancies, including ovarian cancer (Yadav *et al.*, 2019; Janyst *et al.*, 2018).

We also found that a promising strategy against breast cancer includes the combination therapy using Vorinostat and Tamoxifene in patients with advanced ER-positive breast cancer who had been treated with hormonal therapy and chemotherapy before. Furthermore, combining Panobinostat with Letrozole, was found to prevent the growth of aromatase inhibitor-resistant cells in metastatic breast cancer (Tan *et al.*, 2016). As well, the efficacy of Belinostat and Erlotinib combination is underway clinical evaluation in patients with non-small cell lung cancer (ClinicalTrials.gov Identifier: NCT0118870).

In our analysis, we also noticed that despite the interesting advances in cancer therapy, HDACi are achieving success in many other pre-clinical and clinical trials for non-neoplastic diseases, such as Alzheimer's disease, metabolic disease, HIV infection, and multiple sclerosis. In particular, Romidepsin is under investigation in phase I/II trials as a novel drug in the cure of human immunodeficiency virus infection (McMahan *et al.*, 2020). A

deeper investigation of this approach although, requires larger-scale clinical trials in order to prove the full potential therapeutic benefits of these agents.

In addition, Ricolinostat (ACY-1215) and Citarinostat (ACY-241) are two experimental HDACi, which are being tested in Phase I/II clinical trials in patients with relapsed or relapsed/refractory multiple myeloma (ClinicalTrials.gov Identifier: NCT01583283; ClinicalTrials.gov Identifier: NCT01997840).

The development of dual inhibitors is another interesting approach that could be further evaluated, as they will lead to compounds with simpler pharmacokinetic, lower toxicity and more efficient clinical efficacy.

The overall future perspective for the use of HDACi in a variety of malignancies, looks promising. However, more information regarding life quality changes associated with the administration of these agents, should be forthcoming. Finally, the development of suitable predictive biomarkers is necessary in order to provide information on the probability of response to the therapy in future clinical trials.

5. CONCLUSION AND FUTURE PERSPECTIVES

The findings reported in this paper emphasize the role of HDACi, as innovative pharmacological treatments for anticancer therapies. They induce numerous anticancer effects such as growth arrest, differentiation and in some cases cell death by apoptosis. Especially their significant synergistic effect with other conventional chemotherapeutic agents, has shown very promising results in clinical trials, ensuring high therapeutic efficacy, improvement of the clinical conditions and a reduction in side effects. However, a better comprehension of HDACi effectiveness and further evaluation of these anticancer agents is needed to optimize their efficacy, toxicity and overcome therapy resistance. Moreover, as most of these agents are broad-spectrum and nonselective, further research is required for the development of selective and more tolerable HDACi. Therapeutic strategies that include epigenetic drugs can therefore revolutionize the ongoing fight against cancer.

ABBREVIATIONS

CHOP - Cyclophosphamide, doxorubicin, vincristine and prednisone

CTCL -Cutaneous T-cell Lymphoma

DLBCL -Diffuse large B-cell lymphoma

ER+ -Estrogen Receptor-positive

FDA- Food and Drug Administration

HAT- Histone Acetyl Transferase

HDACi- Histone Deacetylase Inhibitors

HDACs- Histone Deacetylase enzymes

MM- Multiple Myeloma

PTCL -Peripheral T-cell lymphoma

RRMM- Relapsed/ Refractory Multiple Myeloma

SCFA – Short chain fatty acids

CONFLICT OF INTEREST

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STATUS OF PLANT GENETIC RESOURCES IN THE ALBANIAN NATIONAL INVENTORY

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ABSTRACT

Biological characteristics of crops, their collecting source, germplasm storage type, acquisition data, donor code and geographic distribution of plant species are here used for the assessment of qualitative and quantitative traits of the Plant Genetic Resources found in the Albanian National Inventory. Modelling distribution of species (interaction species x climatic data) and spatial analysis (geographic, taxonomic, ecological and conservation data) of all 1050 geo-observations helped assess the impact of bioclimatic factors that delimitate the potential distribution of fruit trees species. Comparison analysis showed an increase of quantity of landraces and wild species in ex situ collections of fruit trees, vegetables, and medicinal crops. Representativeness analysis found a high presence of wild and low domesticated genes per species. The reserve of high-quality genes is very important for breeders and field users. Spatial-modelling analysis found fruit tree species sites situated into excellent suitable areas (from 20 to 37%), very high suitable areas (from 10 to 20%), and high suitable areas (from 5 to 10%). Bioclimatic predictors capture information about annual conditions (annual mean temperature, annual precipitation, annual range in temperature and precipitation), as well as seasonal mean climate conditions and intra-year seasonality (temperature of the coldest and warmest months, precipitation of the wettest and driest quarters). So, temperature seasonality, annual precipitation of driest month appears to be the most limiting factors for the distribution of fruit tree species in the northern Albania. The annual mean temperature, and maximum temperature of warmest month for central Albania; and seasonality and maximum temperature of warmest month, precipitation seasonality and precipitation of driest month seems to be the most limiting factors for the southern Albania.

Keywords: bioclimatic factors, genetic resources, tree species distribution

1. INTRODUCTION

Plant Genetic Resources (PGR) refers to germplasm or genetic diversity (GD) of actual or potential value that exists among individuals or group of individuals belonging to a species, and are vital for crop improvement

ensuring food security. They comprise the diversity of genetic material contained in landraces populations and crop wild relatives (LPCWR), modern varieties and other economically important plant species contributing to nutritional, medicinal, energy, shelter, fuel, amenity, and cultural uses. Genetic diversity, providing raw materials for crop improvement (Alina *et al.*, 2017) and abiotic-biotic stresses resistance, adaptation to climate change (Endresen *et al.*, 2011; Khazaei, *et al.*, 2013; Gixhari *et al.*, 2016), allows crops to evolve and adapt, and is a major resource for plant breeders to use and meet the challenges of food security and environmental stability. The full spectrum of PGR consists of diverse type of collections such as those derived from the centres of diversity, centres of domestication, breeding institutions and represent the biological foundation for maintaining and improving crop productivity having played a central role in crop development from antiquity.

In general, geneticists are interested in introducing monogenic or polygenic traits into modern cultivars or in germplasm adapted to specific biotic or abiotic conditions, meanwhile plant breeders seek in genebank for the best genotypes to be used as parents (source of genes) at various stages of crop breeding programme. Production factors such as the reduction of land availability suitable for agriculture, environmental degradation and global climate change make breeders identify the adapted genotypes (AG) that can grow on poor soils or soils with an excess of harmful ions or plants adapted to drought, waterlogging, extreme temperatures or very steep terrain, and other delimited plant growing factors (Witcombe *et al.*, 2008; Gixhari *et al.*, 2012; Butler *et al.*, 2013; Al-maskri *et al.*, 2014; Challinor *et al.*, 2014;).

Plant genetic resources are a means to address the sustainable development of agriculture by helping to increase agricultural food productions. Today, preservation of genetic resources is regarded as an important need for human society. The gene banks are used to store and conserve the GD of plants, and they protect genetic materials, providing the raw material for the improvement of crops. Nowadays the objective and collecting strategies of genebanks for existing germplasm collections aim to fill gaps of the representativeness in genebank shifting the goal for PGR from quantitative perspective to the quality of collections, and to maximize the genetic representativeness (GR) of collection while minimizing the economic cost of collecting missions (Parra-Quijano *et al.*, 2011a; Tyack *et al.*, 2015). Doing this ex-situ collections of genebank are expected to be more representative of the existing genetic variation and the range of environmental adaptation, present in nature or in the field across the distribution of the target taxa or species.

To identify the best adapted genotypes (AG) breeders' need, a high genetic representativeness (GR) in genebank of GD present in nature or in the field, and any information that may help to select parents with the desired traits. The morphological phenotypic characterization and evaluation (C&E) of

PGR, very useful to determine the GR in genebank, have great importance in discovering the genes of interest for a successful breeding crop (Thormann *et al.*, 2016).

Greene and Hart (1999) said that eco-geographical representativeness (ER), (combining climatic, ecological, and geographical data) reflect indirectly the GR, due to the relationships among the environmental components (EC) of a site and the genotypes of the populations occurring at that site through natural selection and local adaptation. Thus, ER, based on the G x E interaction (where g – genotype; e – environment), can be useful in estimating the GR of collections using simple and cost-effective methods.

Albania is mentioned for its rich in plant biodiversity, including wild relatives of cultivated crops. Albanian flora identified about 3 250 species of plants in the country, from 11 000 types of plants that are in Europe (or 29.5%) (Flore de L'Albanie, 1996), but only 162 species (5%) of them is included in the Albanian National Inventory (ANI). Currently, the Albanian National Genebank maintains more than 4500 accessions of 162 plant species. Most of the germplasm, included in the EURISCO (a web-based catalogue that provides information about ex situ plant collections maintained in Europe), is represented by landraces, cultivated and wild plants as wheat, barley, crop wild relatives (CWR), wild vegetable plants (beet, carrot, onion, etc.), leafy vegetables and grain legumes (lentil, vetch, pea, clover, alfalfa, etc.).

Good database on genetic materials conserved in the gene-bank leads to enhanced utilization of germplasm by farmers and ensure that "tomorrow's plant breeders will have today's genetic resources for use in their plant breeding programs". To increase usage value and effective ex situ conservation of plant genetic resources, it is necessary to have all information in a well-organized documentation system (database) and the database must be available for everyone interested (Endresen, 2011; Green *et al.*, 2012).

The present paper aims to: i) evaluate the genetic diversity of plant genetic resources stored in the Albanian National Genebank, and ii) analyse the quality of genes stored in ex situ collections, diversity and amount of data information recorded in the Albanian National Inventory (ANI) database.

2. MATERIALS AND METHODS

Assessment of genetic diversity of Albanian National Inventory (ANI), is carried out via unique accession number, genus and species, common crop name and group of crops (cereals and maize, vegetables, legumes, medicinals, fruit trees, forages and industrials), biological status of accession (wilds, weedy, landraces, breeding materials, advanced cultivars, others); collecting source (wild habitat, farm or cultivated habitat, experimental stations,

research organization or gene-bank); by location of collecting site and geographic coordinates (latitude, longitude and elevation of collecting site), storage status (seed, field, genebank, experimental stations); acquisition data of sample (1996-2000, 2001-2005, 2006-2010, 2011-2015, 2016-2020); collecting institute and donor institute code, total stored accessions and species, using EURISCO Descriptors for uploading passport data to EURISCO (2017); FAO/Biodiversity multi-crop passport descriptors (MCPD), version 2.1, (2015). The statistical data of PGR from the Albanian National Genebank database and EURISCO catalogue database were used, and comparison analysis, synthesis and interpretation of statistical data made.

3. RESULTS AND DISCUSSION

Genetic diversity represented in the National Genebank in 2000

The Albanian National Genebank was set up in 1998, and plant germplasm stored as seeds under long-term storage conditions, until 2000 consisted of 2686 accessions (acc.) of 46 different plant species. Plant germplasm was represented by the most important group of cultivated crops as follow: cereals 28%; maize 26%; legumes 10%; forages 9%; vegetables 7%; industrials 14% and others 6%. Plant germplasm (seeds) was maintained under long-term storage conditions, in cold vertical freezers. Analysis by crops found “no” adequate representativeness of species & cultivated crops in genebank, so only 2 genera (*Triticum*, *Zea*) were more than 50%.

Comparison analysis by source and biological status showed that the 46% of total germplasm (1235 acc.), provided by ex-Research Agricultural Institutes to the genebank, was presented by narrow genetic base materials (breed materials, breeder lines, advanced cultivars). Collected genetic materials, with a potential large genetic base, were only 14% (440 acc.) of the total germplasm stored in genebank, but these materials were without EC information. Kresovich *et al.*, (2006) said that collecting germplasm without ECCS information or geographical data would not be very useful to the breeders. The most useful information on AG and bioclimatic factors that delimitates the distribution of one species, is related to the ECCS, which reveals the most important EC for adaptation and its impact on the heredity process. The results reported that all seed germplasm stored in the National Genebank (1998-2000) was little useful to be used directly by plant breeders and by farmers.

Genetic diversity represented in genebank in 2021: Albanian National Inventory database of PGR was organized in 2008, including for the first time 504 acc., in the NordGen bank database system (SESTO), and at the end of the same year, the ANI database (2111 acc., of 62 species) was included in the EURISCO catalogue database (Gixhari *et al.*, 2013).

Albanian National Inventory by acquisition data: The analysis of ANI by acquisition data of samples; found the most part of plant germplasm (46%) was accepted in the Genebank from 1998-2005 and from 2011-2015 with 23%. Three important collecting activities supported by Agricultural Service Project (ASP) 2003-2005; South Eastern Europe Development Network (SEEDNet) Project (2008-2010) and FAO projects (2014-2015) were carried out during these periods. The most important contributors were SEEDNet Project (630 acc., of 36 species) collecting 17 new species (alleles), and FAO Project (551 acc., of 52 species), collecting 25 new species (alleles) not collected before by other collecting missions.

ANI by donor code: Currently, ANI of PGR in genebank and EURISCO database includes 4570 accessions of 162 plant species. The institutional contributors to the ANI by donor code are as follow: Albanian Genebank (FAO code - ALB026) 40%; Agriculture Technology Transfer Centre of Lushnja (ALB019) 16%; Agriculture Technology Transfer Centre of Shkodra (ALB022) 16%; Agriculture Technology Transfer Centre of Vlora (ALB020) 6%; Agriculture Technology Transfer Centre of Korca (ALB021) 5%; Agriculture Technology Transfer Centre of Fushe-Kruja (ALB023) 5%; Tobacco Station (ALB027) 4%; Repatriated especially from Germany (DEU538) 4%.

ANI by storage type: Conservation of ANI is maintained in two storage forms: as seeds in genebank under long term storage conditions (76%) and under field collection conditions (24%). The principal contributors of plant germplasm conserved as field collections are: Albanian genebank 67%; Agriculture Technology Transfer Centre of Vlora (ALB020) 25%, and Agriculture Technology Transfer Centre of Korca (ALB021) 9%.

Quantitative and qualitative analysis of ANI:

ANI by group of crops: From 2000-2021 composition of ANI is changed in favour of plant species none represented in genebank. There was an increase of legumes and vegetables and especially of fruit trees species not presented before in genebank database. The principal cultivated crops that represent Albanian NI in 2021 were: cereals and maize 32%; vegetables 10%; legumes 7%; Medicinal and aromatic plants 12%; fruit-trees 25%; forages 5% and industrials 9%. Legumes, vegetables and especially the fruit trees species presence which were previously absent in the Genebank database has increased.

ANI by collecting source: Results by collecting source showed the increase of collected materials in wild and farm habitats as follow: genetic materials collected on the wild habitats were 15%; materials collected on the farm habitats were 34% and materials received by experimental stations were only

7%. Wild habitats sites are considered as source of CWR and respective wild genes (highly adapted genotypes), farm habitats are source of primitive and landraces genes (well adapted genotypes), and experimental stations are source of experimental genes (genotypes adapted for the specific areas) where they can be used directly on the farm production. Analysis by collecting source, highly related to the adaptation of genotypes represented in genebank has lead the priority of acquisition in genebank as follow: CWR shaped by the environment and highly adapted to the disfavour environment conditions, primitive and landraces shaped by climate and humans adapted to the disfavour climate conditions and resistance/or tolerance sources especially against parasites, rare and threatened species, modern cultivated crops mostly shaped by humans (plant breeders) adapted to the specific local conditions.

ANI by biological status: Comparison analysis by biological status, highly related to the quality of genotypes (genes) represented in genebank, found that: 16% of all germplasm was composed by CWR and wild food plant species (or wild genes with a very large genetic base); 45% was represented by primitive and landraces (or genes with large genetic base); 18% was represented by breed or experimental materials (or genes with narrow genetic base) and only 2% was represented by advanced cultivars (or genes with very narrow genetic base). The analysis demonstrates the high presence of qualitative genes (wild and landraces) with a very large genetic base and highly adapted to the disfavour environment conditions, which are very useful to fulfil the quality and quantity needs of plant breeders and farmers.

The aim of genebank is to conserve and use PGR providing basic materials to the crop experts to use genetic variability for the development of high yielding cultivars with a broad genetic base. However, the utilization of these genetic resources depends upon their efficient and adequate information related to ecogeographic data, characterization and evaluation data, molecular data (Gixhari *et al.*, 2014), etc., which requires efficient standards and appropriate strategies.

For effective utilization of PGR in practice geographic data of ECCS are very important for the plant breeders. They help the breeders to verify how is the GR in genebank of GD in nature or in the field, and to identify the AG for the specific areas. Witcombe *et al.*, (2008), Mackay, (2011), Bari *et al.*, (2012), Khazaei *et al.*, (2013), Gixhari *et al.*, (2016) stated that germplasm strategy including geographic data of ECCS is highly related to identification of AG that can be grow on delimited growing factors such as poor soils, salt soils, drought, waterlogging, extreme temperatures, etc. as stated in (Fiedler *et al.*, 2016; Cowan *et al.*, 2020). The genetic materials (or GD) of Albanian NI with ECCS information represent 56% of total germplasm stored in genebank.

The environment has empirically been considered as a key factor by farmers and conventional plant breeding (PB) since agriculture began. The phenotype (P) equation $P = G + E + G \times E$ explained the relationship between adaptation and GD and the effect of certain EC in the distribution of species. The most useful information on AG and bioclimatic factors that delimitates the distribution of one species, is related to the ECCS (Hijmans *et al.*, 2005a; Scheldeman *et al.*, 2010; Parra-Quijano *et al.*, 2012 a). The ECCS reveals the most important (EC) for adaptation and its impact on the heredity process. All the data provide accurate information about the EC and bioclimatic factors that either support or delimitate the distribution of one species (Parra-Quijano *et al.*, 2011a; Gixhari 2021).

Application of geographic information systems (GIS), useful to the management of georeferenced data of collecting sites, make the study of the EC under which LPCWR have acquired their adaptive traits possible. The GIS tools were also identified as prerequisites for effective utilization of PGR and as new future tools to improve the conservation and use of PGR (Pacicco *et al.*, 2018). Genebank uses GIS tools to analyse the in-situ status of endangered wild plant species in protected areas (Rodrigues *et al.*, 2004), and to identify the appropriate sites of conservation of CWR (Shehadeh *et al.*, 2013). Analysing the spatial aspect of collecting sites and geographical distances using international genebank descriptors FAO (FAO, 2015; EURISCO, 2017) and distribution of a particular group of species (Gixhari *et al.*, 2012; Parra-Quijano *et al.*, 2015), GIS methodologies improve the GR of ex situ collections, the efficiency of conservation and use of PGR, the field explorations, identification of EC and geographic areas which are likely to contain specific desired traits of interest for breeders (Parra-Quijano *et al.*, 2011b; Rosa *et al.*, 2017; Jostelle *et al.*, 2019).

Using ecogeographical representativeness (ER), and methodology that manage information of ECCS under which LPCWR have acquired their adaptive traits, genebank has identified EC and genetic representativeness (GR) of natural GD presented in NI collections.

An analysis combining trees species occurrence data (genotype) with climatic data (environment), realized by genebank in 10 counties of Albania (Shkodra, Dibra, Kukes and Lezha in the north; Korca, Gjirokastra, Berat and Vlora in the south; Tirana and Elbasan in the centre), to assess the influence of bioclimatic factors that delimitates the potential distribution of each species, was carried out. Interaction analysis of fruits tree species genotypes x 19 bioclimatic factors (G x E) that delimitates the potential distribution of each fruit tree species at the district level demonstrate that: annual precipitations, precipitation of driest month and temperature seasonality, seems to be the most limiting factors for the distribution of fruit tree species in northern part of Albania; and temperature annual range, maximum

temperature of driest month, and annual precipitations for central and central east part of Albania; and precipitation seasonality, temperature seasonality, annual precipitations seems to be the most limiting factors for south and south-western part of Albania (Figure 1).

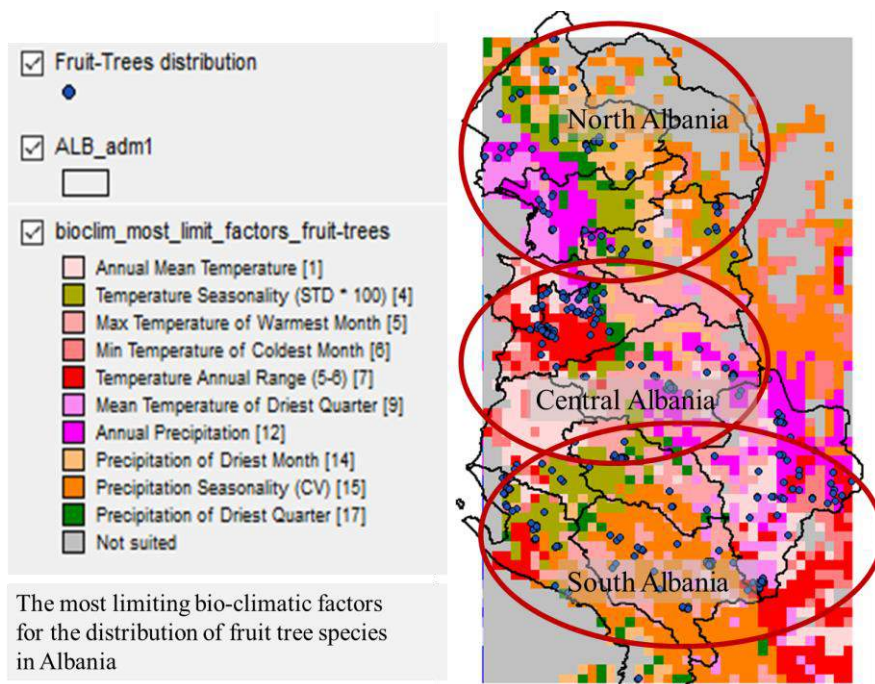


Fig. 1. The most limiting bio-climatic factors for the distribution of fruit tree species in Albania.

4. CONCLUSIONS / PROSPECTS

Ecogeographic studies provide critical information on plant genetic resources (PGR) to assess their current conservation status and prioritize areas for conservation. They have also proven useful for effective genebank management, such as the definition of core collections and identification of collection gaps. Geographic information systems (GIS) are useful tools for mapping ecogeographic distributions of biodiversity. GIS allow complex analyses to be performed, as well as clearly visualizing results in maps, which facilitates decision making and implementation of conservation policies by authorities.

So, the link between genotypes and the environment components, helping plant breeders to select sets of germplasm containing specified interested traits is provided.

Each collecting source can be individually profiled for the most important environment components as precipitation, driest, seasonality, humidity, temperature, agro-climatic zoning, and soil characteristics. Ecogeographic and GIS tools can identify the most suitable sites for conservation and regeneration, as a key factor in reducing genetic erosion.

An increase of ecogeographic and GIS tools in conservation and use of PGR is expected in the present decades, particularly as regards the challenges implied by global climate change for agriculture. Furthermore, studies on adaptation of LPCWR should continue to increase the importance of the environment components in explaining the phenotype, the abiotic-biotic aspect of adaptation, and the influence of farmers on the ecogeographical patterns of cultivated plants.

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GENETIC RESOURCES DIVERSITY OF ALBANIAN DRIED LEGUMES' SEED

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ABSTRACT

Seed dried legumes are nutritional powerhouses that mankind has used since the ancient times. Over the centuries, farmers have made gradual selections of the best varieties of legumes with regard to the taste, production, and appearance. Albania is rich in natural genetic diversity of legume plants, which are cosmopolitan in distribution, representing important ecological constituents in almost all biomes across the globe and occur in even the most extreme habitats. Legume plants particularly require a warm or temperate climate. However, the great diversity of varieties and cultivars has allowed them to adapt to various climatic conditions. So, they could be found planted in the fields, hills, and mild mountains. There is a long range of local landraces and advanced varieties of legume plants playing a significant role to the growth of agricultural products and diversity of daily diets for both humans and animals. The national collection of legume plants is represented by 550 accessions of 22 species, but only 10 legume species are used as seed dried legumes for human consumption purposes. Comparison analysis found that 95% of seed dried legumes used for human consumption purposes is represented by common beans landraces (*Phaseolus* spp.). Other seed dried legumes such as cowpeas (*Phaseolus unguiculatus*) and (*Vigna unguiculata*), runner bean (*Phaseolus coccineus*), chickpea (*Cicer arietinum*), and lentil (*Lens culinaris*) represent only 5% of dried legumes used as food. Spatial analysis reported that seed dried legume species are grown successfully on the low hills and mild height mountains areas of a North-West to South-East extension. Here, where excellent suitable areas range from 20 to 40%, and high suitable areas from range 10 to 20%.

Keywords: Legume landraces, dried seeds, genetic diversity.

1. INTRODUCTION

Seed dried legumes are nutritional powerhouses that mankind has used since the ancient times. Over the centuries, farmers have made gradual selections of the best varieties of legumes with regard to the taste, production, and appearance. Pulses are the delicious, protein-packed, affordable and earth-friendly foods one knows as beans, chickpeas, lentils and dry peas.

Legumes are also important to the functioning of pasture ecosystems. Through a symbiotic relationship with rhizobium bacteria, legumes fix atmospheric nitrogen (N). Nitrogen fixation enables legumes to be self-sufficient for N and increases the availability of soil N (Heichel, 1985). Legume species diversity was also found to be greater on back-slope landscape positions than summit or toe-slope positions, and as legume species diversity in the pastures increased, legumes as a percentage of dry matter (DM) also increased (Harmony *et al.*, 2001).

The word "legume" is an agricultural and botanical term. In agriculture under this name are included the cultivated annual herbaceous plants which have legume fruit with seeds in different shapes, sizes, and colors. From the botanical point of view, this group includes annual and self-pollinating plants of the botanical family of legumes (*Leguminosae*). Paparisto *et. al.*, (1988) stated that the geographic position and favorable climatic conditions— from coastal subtropical to inland continental climate—make Albania rich in legumes' natural genetic diversity. There are also many other grain legume species cultivated around the world, and also many wild ones that are harvested in times of need by local people. Some of these are being investigated for their potential cultivation (Hymowitz 1990).

Seed dried beans, of different colors, are the most important pulse crops of the world, whereas chick-pea, pigeon pea, and lentils occupy the third, fourth, and sixth positions, respectively. Notwithstanding their high production potential, their productivity is generally low as these are cultivated on poor lands, with no or little inputs, and are susceptible to several abiotic and biotic stresses. Legumes are grown for since ages in different parts of the world. They are well suited to diverse environments and fit in various cropping systems owing to their wide adaptability, low input requirements, fast growth, nitrogen-fixing, and weed smothering ability. Their short growing period and photoperiod sensitivity make them suitable for crop intensification and diversification. Legumes plants are adapted to a wide range of ecological habitats in the country, in wild habitats, in forest habitats, on the hills and mountains habitats (Papadhopulli 1976; Salillari *et.al.*, 2007). Legumes plants have a major contribution to the growth of agricultural products in all Albanian regions. There are many reasons lying in the inclusion of legumes in

the diet: i) being inexpensive, ii) having low cholesterol content and high fibre content (both soluble and insoluble), high in plant protein, are packed with micronutrients, and maintain a low-fat level. Legumes are over 90% soluble and can help manage body weight, blood sugar levels, and lower cholesterol. There are many studies proving the link between legumes consumption and reduced risk of coronary heart disease in men and women, iii) having high protein content. Protein is an essential building block for the human body. Without it, we would lose muscle mass, our metabolism would slow down, and we would feel weak and fatigued, iv) fuel of the immune system, v) being rich in fiber. Fiber acts as a cleaning and cleansing agent in our gut and intestinal tracts, vi) being environmentally friendly as meat industry is one source of greenhouse gas emissions, vii) being versatile, viii) being regulator of blood sugar as they are low on glycemic index, and ix) reducing the risk of cancer as they are rich in antioxidants. In many countries, they are part of the cultural heritage and are consumed regularly or even every day. However, these tiny legume seeds, in many shapes, colors, and sizes, have been one of the products used for food since ancient times (<https://www.fao.org/fao-stories/article/en/c/1176990/>). The combination in the cooking of legumes with cereals (e.g., lentils with rice) increases the protein quality of legumes. Extraction of legume extracts is used commercially to increase the value of cereal products.

Grain legumes have an impact on life on our planet. Seed dried legumes are a type of miracle plant that grows in all conditions and climates since ancient times. This group includes hearty plant species that have existed for millions of years. Legumes grow in regions with extremely hot and cold climates and are found in all four corners of the world, with the exception of poles and barren deserts. They include groups of herbaceous plants with similarities in nutritional, agronomic values, and biological characteristics but also with distinctive features that originate from the place of origin and the environment where they grow.

The National Genebank collection of legume plants is represented by 550 accessions of 22 species, but only 10 legume species, including 313 accessions of many well-known local populations, are used as seed dried legumes for human food. Comparison analysis found that about 95% of seed dried legumes used as the food was represented by common beans landraces (*Phaseolus vulgaris*). Cowpeas (*Phaseolus unguiculatus*) and (*Vigna unguiculata*), runner bean (*Phaseolus coccineus*), chickpea (*Cicer arietinum*), and lentil (*Lens culinaris*) represent only 5% of dried legumes used for human consumption purposes. Spatial analysis showed seed dried legume species are grown successfully on the low hills and mild height mountains areas of a North-West to South-East extension. Here, excellent suitable areas range from 20 to 40%, and high suitable areas from range 10 to 20%.

The present paper assesses the genetic diversity of the current legumes' plants, part of the Albanian National Genebank collection.

2. MATERIALS AND METHODS

Data sampling. Information on the total occurrence of legumes plant species in Albania obtained from ex-situ data collection of legumes plants stored in the Albanian genebank's database was used for the data sampling. Additional data were obtained from the EURISCO database, a web-based catalog that provides information about *ex situ* plant collections of Europe (<http://eurisco.ecpgr.org>), and the Global Biodiversity Information Facility (GBIF) database (Gixhari *et. al.*, 2013).

Geographic distribution. The present investigation was carried out in all-natural growing areas of legumes plants in eight districts of Albania. Each population (group of individuals) represents a geo-referenced observation point. Each observation point presupposes the presence of legumes plants-or population. All geo-referenced observations (ex-situ data) chosen to carry out spatial analysis, were entered into the GIS analysis, as presence points, (Hijmans *et. al.*, 2005; Hyso *et.al.*, 2005) and were spatially represented as point maps using DIVA-GIS tools (Hijmans *et.al.*, 2001; Hyso *et.al.*, (2005).

3. RESULTS AND DISCUSSION

Analysis by geographic distribution

Significant information about each legume species has been collected and recorded. All data were checked for inconsistencies, and data points without coordinates were removed from the legumes ex-situ data. Sites with incorrect coordinates were assigned coordinates where possible, while duplicate or doubtful data were removed (Scheldeman *et al.*, 2010). In addition, all the legume species were carefully screened to resolve any scientific name conflicts (Chapman, 2005). The accessions not present physically as genetic material stored in the National Genebank were also removed.

Once the presence or absence of accessions of the data included in the legumes plants database with partial or complete information for a total of 280 presence points in total, only 210 presence points including of legumes plants were compiled and used to evaluate the geographic distribution of seed dried legumes currently observed in Albania (Figure 1).

Spatial analysis showed that seed dried legume species are grown successfully on the low hills and mild height mountains areas of a North-West to South-East extension. Here, excellent suitable areas range from 20 to 40%, and high suitable areas from range 10 to 20%.

Detailed analysis of ex situ data of seed dried legumes stored in the Albanian National Genebank show that *Phaseolus vulgaris* was represented by a higher number of local landraces distributed across the country, followed by *Cicer arietinum*, *Lens culinaris*, *Phaseolus coccineus*, *Phaseolus unguiculatus*, and *Vigna unguolata*.

Higher number of traditional varieties (landraces) of dried legumes and especially of *Phaseolus vulgaris* populations were found in the Shkodra region, (63 populations), Elbasani region (16 populations), in Korça region (54 populations), Berati region (31 populations), and in Gjirokastra region (19 populations). Lezha, Dibra, Durrësi, and Tirana region (Figure 1) have a low number of legume populations.

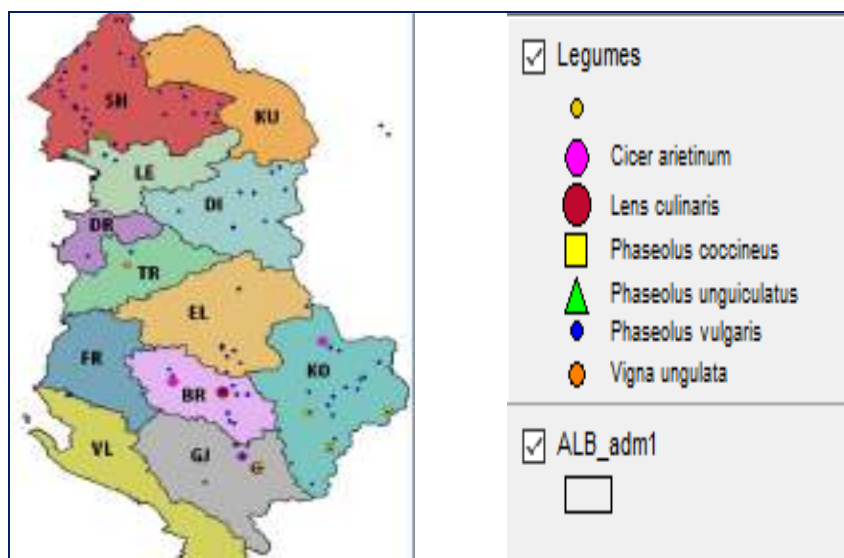


Fig. 2: Geographic distribution of seed dried legumes in 8 districts in Albania.

Analysis of seed dried legumes by species

The National Genebank stores many different samples of current and potential legumes used by breeders, farmers, seed producers, researchers, etc. All dried legume populations were collected across the country including a wide range of economically important food crops such as phaseolus, lentil, cicer, peas, peanuts etc.

Comparison analysis reported that 95% of seed dried legumes used as food is represented by common beans landraces (*Phaseolus* ssp.). Cowpeas (*Phaseolus unguiculatus*) and (*Vigna unguolata*), runner bean (*Phaseolus coccineus*), chickpea (*Cicer arietinum*), and lentil (*Lens culinaris*) represent only 5% of dried legumes used for human consumption purposes (Figure 2).



Fig. 3: Legumes stored in the Albanian National Genebank.

Collection results and acquisition of seed legumes in the Albanian National Genebank

Activities of the collection and acquisition of plant genetic resources were made from 2009 to 2016. A total of 313 accessions of 6 legume species was collected. The collected dried seeds of *Phaseolus vulgaris* were represented by more than 28 local varieties or landraces, due to climatic diversity of a great number of micro-climatic conditions of the areas. The environment has an influence on the phenotype of legumes and shape their genotypes through adaptation. *Phaseolus unguiculatus* 1 acc., *Phaseolus coccineus* 4 acc., *Lens culinaris* 2 acc., *Cicer arietinum* 4 acc, *Vigna unguiculata* 1 acc etc, were also collected (Table 1, Figure 3).

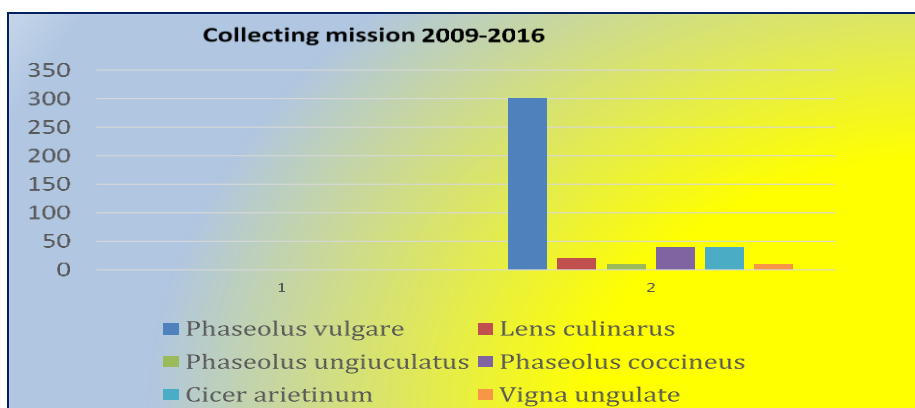


Fig. 4: Collecting results and acquisition of seed legumes in the National Genebank 2009-2016.

4. CONCLUSIONS

The following conclusions could be drawn:

i) Spatial analysis showed that seed dried legume species are grown successfully on the low hills and mild height mountains areas of a North-West to South-East extension.

ii) Albania is rich in natural genetic diversity of legume plants, which are grown over a wide range of ecological habitats in the country, on the fields, on the hills and mild height mountains habitats.

iii) Legume collections inherit a high number of local landraces and advanced varieties of legume plants (550 accessions of 22 species).

iv) Data analysis report that 95% of seed dried legumes used for human consumption food is represented by common beans landraces (*Phaseolus* spp.).

v) Cowpeas (*Phaseolus unguiculatus*) and (*Vigna unguolata*), runner bean (*Phaseolus coccineus*), chickpea (*Cicer arietinum*), and lentil (*Lens culinaris*) represent only 5% of dried legumes used as food.

vi) The principal collecting activities related to enrichment of legume germplasm were carried from 2009 to 2016. Albania is very rich in legume populations, so more collection activities must further.

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Scientific information

NEW FRONTIERS OF SCIENCE DEVELOPMENT IN ALBANIA: NANOTECHNOLOGY, THE ACCEPTED WORKHORSE IN HEALTH, AGRICULTURE, SECURITY AND DEFENCE

Nanotechnology is an area of science that integrates, physics, electronics, mathematics and informatics. This new science area promises scientific advancement in many sectors such as medicine, consumer products, energy, materials and manufacturing. This is why Albanian scientists involved in the area came together under the "Albanian Nanoscience and Nanotechnology Unit" of the Albanian Academy of Sciences to discuss, share ideas and learn from one another on current technologies, as innovation is a means to address sustainable development.



The NANOALB Unit of the Albanian Academy of Sciences, intellectual powerhouse where the most eminent Albanian scientists from Kosovo, Montenegro, the Republic of North Macedonia come together to express their determination to foster the intellectual pursuits determined by experiment with the support of the European Community and other organizations, scientific centres involved in the area, organized the *21st International Conference on Trends in Nanotechnology* in close partnership with Phantom Foundation and Catalan Institute of Nanoscience and Nanotechnology from October 4-8, 2021.

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A symbol of the importance of NANOALB is its contribution to science areas of vital importance for the member countries such as early diagnosis of diseases (including epidemics), analysis of emergency pollutants (heavy metals such as mercury, lead, cadmium, pesticides used in agriculture, and other chemical materials hazardous to human health used in various

industries). The backbone of NANOALB is active participation and sincere and concrete collaboration among stakeholders for the benefit of society by developing the modern techniques such as biosensors and cost-effective equipment.

The topics covered but not limited to were: i) Atoms and molecular computing, ii) High spatial resolution spectroscopies under SPM probe, iii) Graphene and 2DM / Carbon nanotubes, iv) Low dimensional materials (nanowires, clusters, quantum dots, etc.), v) Nanobiotechnologies and nanomedicine, vi) NanoChemistry, vii) Nanofabrication tools and nanoscale integration, viii) Nanomagnetism and Spintronics, ix) Nanomaterials for energy, x) NanoOptics / NanoPhotonics / Plasmonics / Nanophononics, xi) Nanosensors, xii) Nanostructured and nanoparticle based materials, xiii) Nanotechnologies for Security & Defense, xiv) Risks-toxicity-regulations, xv) Theory and modelling at the nanoscale, and xvi) Topological insulators.

As the participation of the young generation is of crucial importance, supporting them is fundamental. Only students received poster awards. The awarded students were Leyla Karadurmus from the University of Ankara, Turkey, Shanil Gandhi from the Bluesense Diagnostic APS, Denmark and Massimo Urban from UCN2, Spain.



The 22nd International Conference on Trends in Nanotechnology will take place in Tirana, Albania for the second time. The TNT 2022 is a good opportunity for socializing and Tirana is worth visiting.

Further information could be found: <https://www.eventseye.com/fairs/f-tnt-trends-in-nanotechnology-26375-1.html>

Blerina Shkreta

THE POLYTECHNIC UNIVERSITY OF TIRANA CELEBRATED ITS 70TH ANNIVERSARY OF FOUNDATION

The Polytechnic University of Tirana (PUT) is a public university located in Tirana, the capital of Albania and offers degrees in engineering and related fields.

The university includes the Faculty of Civil Engineering, Faculty of Information Technology, Faculty of Mechanical Engineering, Faculty of Geology and Mining, Faculty of Electrical Engineering, Faculty of Mathematics and Physics, Faculty of Architecture and Urbanism and Institute of Geosciences (IGEO), the institute of the Polytechnic University of Tirana.

The mission of the PUT is to: i) recognise, promote, and support excellence in science and to encourage the development and use of science for the benefit of humanity, ii) invest in future scientific leaders and in innovation, ii) invigorate science and education, iii) increase access to the best science internationally, and iv) inspire an interest in the joy, wonder and excitement of scientific discovery.

Its priorities emphasise the impact that young scientists have on science for the benefit of society.

As 2021 marked its 70th anniversary, a range of activities run on the occasion.

So, the Faculty of Information Technology organized *The 1st International Conference on Information Technologies and Educational Engineering (ICITEE21)*. The conference aimed to enhance collaboration between leading academic scientists, researchers and research scholars in the field of Engineering and Information Technology. At the same time, it provided an



opportunity for researchers and educators to present, discuss and share their experiences and research results, trends, and concerns as well as practical challenges and solutions in all areas of computer systems and applications, communications software, telecommunications and educational engineering.

The conference was organised by the Faculty of Information Technology, Polytechnic University of Tirana with the support of the Academy of Sciences of Albania and included but was not limited to the following topics: i) advanced technology in education and learning, ii) antenna design and simulations, iii) artificial intelligence, iv) communication theory, v) cyber security - challenges and techniques in the business sector, vi) database technology and design, vii) distributed and cloud computing, viii) education simulation and methodology, ix) embedded software and digital systems, x) grid, cluster, cloud and P2P computing systems, xi) information and knowledge management, xii) intelligent computing and networking, xiii) interfaces and communication protocols, xiv) IoT technologies and applications, xv) machine learning and deep learning, xvi) multimedia systems and services, xvii) natural language processing, xviii) network operations and management, xix) optical and photonic communications, xx) parallel computing and multicore systems, xxi) radio-frequency engineering and technologies, xxiii) research/education methodology in ICT, xxiv) resource management in multi-core, GPU and FPGA parallel architectures, xxv) satellite communication, xxvi) semantic Web and internet applications, xxvii) software engineering and software ecosystems, xxviii) WSN Technologies and Systems.

The Faculty of Architecture and Urban Planning (FAU) organised on April 19, 2021 the webinar, *Restoration of cultural sites damaged by the earthquake*. This event was run in close partnership with the CHWB, IKTK, Ministry of Culture, U.S. Ambassadors Fund for **Cultural** Preservation Project and the US Embassy in Tirana, Albania.

A Honouring Ceremony was held at the Faculty of Electrical Engineering on 15 July 15, 2021 to honour all the professors who have made outstanding contributions to progress of the Faculty.

The workshop, Looking for Tratturo Celano Foggia, was organized by the FAU, in collaboration with the University of Chieti Chieti Pescara and La Salle University, Bogota, in October 2021, and was run online.

The National Agency of Territory Planning (NATP) presented on October 22, 2021 its library building at the premises of FAU as a co-organizer of the event.

FAU and Epoka University co-organized on November 2021, the *International Conference on Architecture and Urban Planning* (4ICAUD).



A thematic exhibition on Albanian architecture, constructions with pre-prepared panels was made on December 2021 at the premises of FAU.

The Department of Textile and Fashion of the Faculty of Mechanical Engineering organized, *The 9th International Textile Conference and the 3rd International Conference on Engineering and Entrepreneurship 2021*. This event was run from 18-19 November 2021, and the technical

program consisted of oral and poster presentations on a wide range of topics, subsequently followed by a fashion show at the premises of the university.

IGEO ran the symposium *Geosciences, achievements and future challenges*. The symposium was held on 25 November 2021. This one-day event consisted of a large symposium providing information on earth science related areas such as geology, geohazards, meteorology, hydrology and natural hazards.

Blerina Shkreta

In memoriam

ASSOCIATED ACADEMICIAN ARTAN BORIÇI (1965-2021)

Artan was born on April 19, 1965, in Tirana, to parents Hamid Boriçi and Myzejen Boriçi (née Bushati) originating from Shkodra and descendant of families Boriçi and Bushati, both very well-known Shkodra based Albanian families.

He graduated from the 5-years physics degree of the University of Tirana (UT) Albania in 1988, receiving the title “Physicist”. His academic



career begins immediately after the graduation as a research and teaching assistant at the theoretical physics chair of UT, before going to Switzerland where he started graduate studies in computational and theoretical physics at the Swiss Federal Institute of Technology in Zurich (ETH).

Artan Boriçi’s scientific legacy is so abundant that it cannot be framed in his lifetime. He left this life as a realized man, with his many quality works in modern physics, numerical analysis and computational finance, however, his continuous scientific work and results were ceased.

His work on surface segregation of binary metallic alloys gave him the opportunity to choosing between different projects for his graduate studies, i.e. either computational quantum chemistry or computational elementary particle physics.

Artan got his PhD at the CSCS/SCSC (Centro Svizzero di Calcolo Scientifico/Swiss Center for Scientific Computing) in 1996 under the guidance of Prof. Dr. Martin Gutknecht, Prof. Dr. Philippe de Forcrand and Prof. Dr. Jürg Fröhlich. His thesis is Diss. ETH No. 11689 for the

degree of Doctor of Mathematics. He investigated the usefulness of various Krylov space methods for the numerical approximate solution of QCD problems. He was probably also among the first to apply “BiCGStab2” algorithm to QCD problems, for which it became quite popular.

In one of his recommendation letters for Artan, Prof. Dr. Martin Gutknecht writes:

“Artan Boriçi was a very ingenious Ph.D. student, who was able to thoroughly understand both the physics and the numerical methods that are applied to simulate it, and who was also able to turn new ideas quickly into computer code.

His thesis was a remarkable contribution to computational QCD (Quantum Chromo Dynamics) and received a lot of attention. It was a truly interdisciplinary work involving physics, numerical analysis, and computer science.

What might also be of importance to you is that Artan Boriçi has not only lots of interesting ideas but is also a very good person to collaborate and to get along with.”

In the following three years Boriçi held a postdoc position at the Paul-Scherrer Institute (PSI), the largest Swiss research facility, which is mainly concerned with all kinds of energy research, including medical applications of particle physics. There, he continued working in Lattice QCD, and in particular he devised a special form of the nonsymmetric Lanczos algorithm making use of the so-called γ_5 -hermiticity for solving the Wilson fermions problem. Boriçi kept an interest in sparse matrix problems relevant for QCD, such as the computation of the inverse square root of such a matrix. For this and other matrix functions, the Lanczos algorithm also provides efficiently approximations. He also presented a nice new algorithm for shifted unitary matrices.

In the meantime, Artan was also focused on lattice QCD theories, he has worked on the “Formulation of Chiral fermion theories/ Truncated Overlap Fermions” and exploring “The Link between Overlap and Domain wall fermions”.

In November 1999, for his next temporary project funded position, Boriçi chose to switch to computational finance, where methods related to some used in physics have been developed and widely applied recently. Boriçi's ability to quickly understand new concepts and

theories have made him a most valuable collaborator of the then newly founded RiskLab at ETH Zurich. His excellent computer skills were another asset. During this period Boriçi developed the linearly scaling algorithms for American option pricing, *the world state of the art algorithm in the field since 2001*.

After his experience at RiskLab, Artan made a comeback to particle physics and accepted a temporary position at the School of Physics and Astronomy, the University of Edinburgh, Scotland, where he worked on “Development of formulations and algorithms for chiral fermions” part of UKQCD research program. UKQCD has adopted a variant of Domain Wall Fermion formulation.

As member of the Department of Physics, University of Tirana since October 2003, Artan founded and led the lattice QCD group. His scientific work was focused on the Formulation of local chiral fermions theories, i.e. Creutz fermions on an orthogonal lattice and Minimally Doubled Fermion Revival. He continued his work on the field of chiral fermion algorithms and local chiral fermion formulation of Borici-Creutz type. He also developed QCDCAL software, a high level Octave functions for teaching as well as algorithmic prototyping in lattice QCD.

Recently, Artan got interested on Yang-Mills problem. He published a paper on development and analytical solution of Yang-Mills theory: “Disordered fermions, extra dimensions and a solvable Yang-Mills theory, Phys. Rev. D 100 (2019) 3, 034502”. Until the end of his life, Artan was working on the solution of Yang-Mills theory, he was writing another paper on this subject, “Resolving the scales of the Yang-Mills theory by means of an extra dimension”.

Artan's experience in management duties includes the position of the vice-dean (2012) and Council member of the Faculty of Natural Sciences at UT (2008-2012). During 2012-2018, he led Computational Physics group at UT and, recently (November 2018-March 2021), High Energy and Astrophysics group. Two PhD students completed in the field of chiral fermion algorithms and local chiral fermion formulation of Borici-Creutz type.

Artan was member of several professional organizations, i.e. Albanian Physics Society, Former member of UKQCD (UK), SPEEDUP (Switzerland). He was also paper reviewer in leading journals and research bodies: *Physical Review*, *Physics Letters*, *Journal*

of Computational Physics, Computational Physics Communications, Journal of Global Optimization, U.S. NSF (U.S National Science Foundation). He was one of the evaluators and pre-evaluators of the annual Nobel Prize in Physics.

Artan was a fellow of the Albanian Academy of Sciences. He was elected thanks to his high level of scientific work and qualities, as a prominent scholar of his time. He worked closely with other prestigious scientific institutions such as CERN (European Nuclear Research Center), Supercomputing Centre of the Institute for Advanced Simulations (Research Center Juelich, Germany). He was member of organizing committees of international conferences at home and abroad. His theses adviser, Prof. Dr. Philippe de Forcrand (ETH Zurich & CERN/Theoretical Physics) writes:

“With his international conferences and collaborations, Artan was doing outstanding work to connect Albania with the rest of the world. I hope some of his colleagues will carry the torch and continue his very successful efforts.”

He will surely be dearly missed by his colleagues and friends.

Mimoza Hafizi
Rexhep Mejdani
Floran Vila

A TRIBUTE TO THE LIFE OF ACADEMICIAN PETRAQ PETRO

DISTINGUISHED SCHOLAR AND EDUCATOR

One year has passed since Professor P. Petro (1944–2021), a mathematician and member of the Academy of Sciences of Albania, left us unexpectedly and prematurely. We, the authors of this article, former students and colleagues of Academician P. Petro, like many others, feel deeply the loss of our professor who is remembered as a passionate and energetic man until the last moments of his life. In this article we attempt to give a modest outline of his large contribution to science and education. Since our specialty is not in the field of algebra, for technical research details we consulted some of Petraq's algebraist colleagues, and their valuable input is highly acknowledged.

Petraq Petro, was born in the village of Prodan in Cologne, Albania. After completing secondary education at the "Raqi Qirinxhi" high school in Korça, he studied mathematics at the Faculty of Natural Sciences, University of Tirana where he graduated with distinction in 1965. After graduation he started working as a lecturer in the Department of Mathematics at the Faculty of Natural Sciences where he remained an active member until his retirement. In the following we mentioned some important markers of Petraq's distinguished career as a mathematician. He received his degree of Candidate of Sciences in 1981 followed by the title of Docent in 1984 and culminating with the degree of Doctor of Science in 1986. He was awarded the title of Full Professor in 1991. In 2008 he was elected an associate member of the Academy of Sciences of Albania, and from 2011 he was a full member of its Assembly. From 2019, with the establishment of the Permanent Commission of Mathematics and Informatics at the Academy of Sciences, Prof. Petro headed it with energy and passion.

Teaching Activity

Immediately after graduating from the university in 1965, Petraq Petro began working as an assistant to the eminent Professor Aleko Minga in algebra, initially giving exercises in several subjects of algebra. Later, he became a lecturer in algebra and established himself as an authority in

algebraic subjects remaining a fierce promoter of their modernization until his retirement from the university in 2013.

In the 70s, the mathematical education in Albanian schools and universities was in need to substantial changes following the world-wide modernization of curricula and teaching methods in mathematics. Prof. Petraq Petro together with other prominent professors in the Department of Mathematics pioneered this modernization process, in which the French school of mathematics had a significant influence thanks to traditional links. In this spirit, P. Petro together with A. Minga, M. Fundo, A. Karçanaj, S. Gjinushi, B. Pepo wrote the textbook *Introduction to Basic Mathematical Concepts*, otherwise known as *Chapter Zero*, which contained a modern treatment of fundamental concepts of mathematics. Chapter zero also provided the basis for other mathematical subjects. Prof. Petro is a published author of around 25 textbooks and special topics lecture-notes, counting here new editions. Among the most prominent are: *Basics of Modern Algebra* (1984), *Exercises of Modern Algebra* (1985), *Algebra I, II* (1987, with co-author) and later, *Exercises of Linear Algebra* (2000), *Basic Mathematical Concepts* (2005), and *Algebraic Structures* (2007). During 2007-2013, P. Petro has played a leading role in the establishment of the Doctoral School in Algebra and Geometry in the Department of Mathematics at the Faculty of Natural Sciences. During his teaching career which spans almost half a century, P. Petro has educated several generations of teachers and mathematicians. He designed and taught a variety of new algebra courses at the level of Bachelor and Masters and beyond, so influencing several generations of mathematicians. The graduate school in mathematics at the Faculty of Natural Sciences became the main source of qualification in algebra for the whole country of many algebraists who received their doctorate there, and continued their academic activities in several universities in Albania and abroad. Prof. Petro was scientific advisor of 8 Ph.D students, has mentored many successful algebraists who have worked or are working at the University of Tirana, and has been a great inspiring supporter and inspiration of young researchers in his field.

Scientific Activity

Prof. Petro's main research activity was focused on the field of algebraic structures of semigroups, rings and their generalizations, more specifically in the study of Green's relationships in these structures. The investigation of these relations within the structure of rings is a novelty aspect of his research in this direction. His first idea to develop Green's relationships in rings, as often used to be mentioned by him, stemmed from reading the celebrated monograph by one of the authorities in the theory of semigroups, Ottó Steinfield, *Quasi Ideals in Rings and Semigroups*, 1978. Independently,

despite limited literature available to him due to isolation of the political system at that time, Petraq Petro succeeded to solve some of the open problems highlighted in Steinfeld's monograph, more specifically he provided the first solution of two open problems in the theory of quasi-ideals. Part of this contribution is subject of his doctor's dissertation entitled *Quasi-ideals in some algebraic structures and the impact of these structures on the modernization of algebra* (1986), and part were further developed and later published in international journals after the opening of Albania to the World in 90s. To provide some perspective of his work, it is worthwhile mentioning that quasi-ideals are important algebraic structures which play an essential role in the characterization of regular and semi-inverse semigroups and other algebraic objects. In this work, Petraq Petro introduced for the first time a new method, which he called the "ring animation", that translates some important properties from semigroups to rings obtained as a result of this "animation" process. Rings that have Steinfeld's property, used by Petro, have similarities with semicircles related to more general rings, hence his approach can be extended to the study of such algebraic structures. Furthermore, Green's type theorems for rings, algebras, and semigroups developed in his work, simplify the proof of similar relations in quasi-ideals. Generally speaking, a trademark of Petro's work is the introduction of appropriate transformations which enable to establish relations on one structure by using the corresponding ones from a different structure. As an example, his method "turning off" the ring operation, enables to turn the proof of a statement in the theory of rings into the proof of the analogous statement in the theory of semi-groups. His work has contributed to the unification of concepts and methods in various algebraic structures. This conceptually new apparatus provides an elegant and compact approach to algebraic studies. It also simplifies teaching important algebraic concepts, and give a framework of using semi-groups and rings of type $\mathbb{Z}_2(\mathcal{S})$ as a rich source of counterexamples.

Prof. Petro has presented his work at numerous national and international conferences in algebra, and his results were well received by the international community. He has published over 25 scientific articles many of which have appeared in highly regarded international peer-reviewed journals with impact factor.

In the following we describe in some details two of his most noticeable papers co-authored with E. Pasku. The article entitled *The Green-Kehayopulu relation \mathcal{H} in le-semigroups*, published in *Semigroup Forum* (2002) deals with some interesting properties of the relation \mathcal{H} in ordered semigroups, which generalizes the well-known Green's relation \mathcal{S} in standard semigroups. More specifically, in this paper it is proven that certain properties of \mathcal{S} -classes essentially differ from those of \mathcal{H} -classes in that, as oppose to \mathcal{H} , non-trivial

\mathcal{S} -classes are never groups. However, sufficient conditions were found in this paper under which they form sub-semigroups.

The other noteworthy paper is titled *The relation \mathcal{B} in le -semigroups*, and has appeared in *Semigroup Forum* (2007). The approach in this paper is in the same spirit as in the aforementioned article, but it considers a different type of relation in ordered semigroups known as *relation \mathcal{B}* .

This relation in general is finer than the Green-Kehayopulu relation \mathcal{H} , and it turns out to have better properties. In particular, in this framework the regularity (intra-regularity) of an element induces the regularity (respectively intra-regularity) of the whole \mathcal{B} -class containing that element. Various conditions under which \mathcal{B} -classes are sub-semigroups are also provided here.

The history of semigroups was born and developed based on the ideas induced by the ring theory. In Christopher Hollings's famous book on the history of semigroups, *"Mathematics across the Iron Curtain: A History of the Algebraic Theory of Semigroups"* 2014, Petraq Petro is mentioned as a mathematician to walk in the opposite direction of mainstream investigations, by trying to study the theory of rings with tools and ideas similar to those of semi-group theory.

Concluding, it is certain that Petraq Petro was a leader in the algebraic research in Albania, and has earned recognition from the international research community in his field.

Contributions to Professional Service

During his half a century academic career, Petraq Petro was engaged not only in teaching and research, but was also an active scholar in various higher education institutions. He has held several leadership positions at department and university level, as well as has served on numerous scientific and educational committees of national interest. Since 1987 for about 35 years Petraq Petro was the leader of the algebra section in the Department of Mathematics of the Faculty of Natural Sciences at the University of Tirana. In 1991 he became the head of the Department of Mathematics and continued on this position until 1995. He was a member of the Scientific Council of the Faculty of Natural Sciences (1990–2007) and later of the Council of Professors of the Faculty of Natural Sciences (2008–2013). During 1999–2012, he served as a member of the Senate of the University of Tirana. In addition to this outstanding service in academic institutions, during 1992–1994 he was a member of the Science and Technology Committee affiliated with the Albanian Ministry of Sciences, and in the time period of 1993–1997 he served on the Scientific Qualification Commission at national level. During the years 2007–2013 he was a member of the Council of Higher Education and Science. Prof. Petro's outstanding professional service includes outreach and scholarly activities at large. He has co-organized several national and

international scientific conferences in mathematics. He has been on the editorial board of scientific journals, including *Mathematica Balkanica* a quarterly publication of the Bulgarian Academy of Sciences, and most recently *Journal of Natural and Technical Sciences* affiliated with the Academy of Sciences of Albania.

Academician Petraq Petro's scholarly accomplishments and his service to mathematical community at large remain part of the long-lasting Albanian tradition in sciences and education.

Prof. Fioralba Cakoni
Prof. Fatmir Hoxha