



# **PROCEEDING BOOK**

**5<sup>TH</sup> WORLD CONFERENCE ON SUSTAINABLE LIFE SCIENCES**

**WOCOLS 2023**

**07-10 DECEMBER 2023**

**CAPPADOCIA, TURKEY**

**Web: <http://wocols.com>**





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## **Preface**

**Dear Colleagues,**

**We pleased and honored that “World Conference on Sustainable Life Science (WOCOLS 2023 CAPPADOCIA)” was been held in Cappadocia, Nevsehir, Turkey on 07-10 December 2023.**

**WOCOLS 2023 CAPPADOCIA promoted and disseminated the knowledge concerning several topics and technologies related to life sciences.**

**The main goal of WOCOLS 2023 CAPPADOCIA was to bring together researchers, scientists and experts in universities, companies, institutions, communities, agencies, associations and societies to provide them a unique platform for sharing worldwide ideas well as the recent developments on life sciences.**

**Thank you all for your great contributions to the conference.**

**Sincerely yours,**

**On Behalf of the Conference Organizing Committee**

**Asst. Prof. Dr. Aziz Satana, Chair**

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# **KEYNOTE SPEAKERS**

## **ABSTRACT**

## **Application of Biotechnology in Medicine**

**Munis Dundar**

Department of Medical Genetics, Faculty of Medicine, Erciyes University, Kayseri, Turkey

### **ABSTRACT**

Various applications of biotechnology have changed the field of medicine and reshaped lives. In relation to human health, biotechnology has done a lot in terms of improving diagnosis, treatment and prevention of diseases. Mostly, an early detection of diseases is critical for diagnosis by biotechnological methods. Genetic tests and use of biomarkers helps in diagnosing many genetic disorders, cancers, as well as infectious diseases at early stages. This early recognition helps to manage treatment more effectively and tailor it to individual needs. There has also been tremendous development in treatment methodologies due to biotechnology. Among them include biologics, which are produced through recombinant DNA technology and used to cure a number of diseases including several types of cancers, diabetes and rheumatoid arthritis. Furthermore, gene therapy which is an advanced technology is going towards treating genetic defects. Future holds promise for more research and development in this area with a view to creating healthcare solutions that are more effective, safer and accessible.

**Keywords:** Biotechnology, Therapy, Medicine, Genetics.



## **Highlights of Current Research Projects- Sustainable Agriculture and Precision Nutrition**

**Sevim Erhan<sup>1\*</sup>**

<sup>1\*</sup>United States Department of Agriculture (USDA), USA

### **ABSTRACT**

## **One Health in Turkey: A Roadmap Study**

**Hakan Yardımcı<sup>1\*</sup>**

<sup>1</sup>Ankara University, Veterinary Faculty, Microbiology Department, 06110, Altındag-Ankara, Turkey

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### **ABSTRACT**

The world has entered the era of epidemics, especially since the second millennium. When we look around us, many of the issues we see and criticize are actually the reasons that prepared this period. Population growth, urbanization, destruction of the ecosystem, climate change due to global warming, food shortages, gradual decrease in clean water resources and drought, pollution of the environment with irreversible wastes, easier global transportation, increased resistance to antimicrobial agents are the main ones. These issues have caused and are likely to continue to cause the re-emergence of old diseases as well as new ones. These problems have turned our world into a patient with clinical symptoms. The solution to this disease is a One Health System in which professions such as agricultural engineers, food engineers, biologists, zoologists, ecosystem specialists and sociologists will work in cooperation, with veterinarians and human physicians at the center. Because the patient is now our world. The era of controlling both human and animal diseases with clinical interventions in hospitals is over. Public health and preventive medicine have come to the fore. Control of diseases must now be in the field and predisposing causes must be eliminated. One health is recommended by organizations such as WHO, FAO, WOA and UNEP. It is inevitable that a One Health platform and/or legislation should be prepared in Turkey as soon as possible, where relevant professions work together to overcome this challenge.

**Keywords:** Onehealth, Zoonoses, Antimicrobial Resistance, Türkiye.

## Desalination of Salt Water Via Polyelectrolyte Hydrogels and Osmotic Motors

**J. Höpfner, L. Arens, K. Schlag, C. Pfeifer, H. Zhang, Ch. Fengler, L. Arens, A. Jangizehi, M. Wilhelm\***

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### ABSTRACT

Charged hydrogels are a class of materials with a high affinity for water. On a microscopic scale, they are composed of cross-linked polyelectrolyte polymer chains that incorporate a large amount of water or aqueous solution. Due to the large water uptake of such networks, often 1000 times more than their own weight, the term “superabsorber” is used.

Superabsorber have different applications, whereby the largest market is personal hygiene products (like diapers) with a production of more than  $8 \times 10^6$  tons of absorbent material in 2011 worldwide.

We investigated and described a novel approach for water desalination using such charged hydrogels under externally applied mechanical forces. The desalination mechanism is based on the unequal distribution of an added salt between gel and surrounding solution phase. We synthesized acrylic acid-based hydrogels of various compositions and investigated their desalination properties with a specially designed experimental press setup that allowed us to control online the force exerted on the gel and to measure the water elution from the gel bed as well as the salt concentration of the eluate.

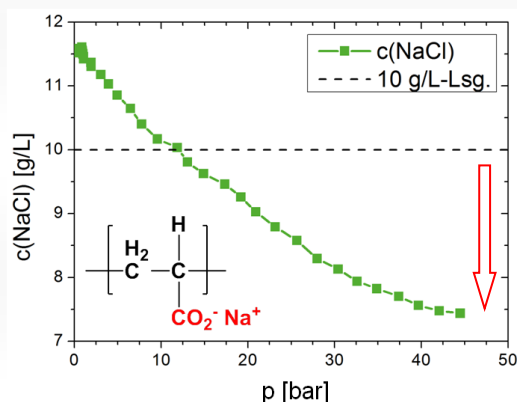


Figure 1: Effective desalination of a brine solution (10 g/L NaCl) by applying pressure on swollen hydrogel beads (left), self-constructed experimental setup to measure the desalination performance online (right).

Furthermore, we were looking at an inverse approach – using a salt gradient in water solution to obtain mechanical force: The electrostatic interaction of polymers in solution depends on the ionic strength, hence additional salt in the medium will reduce interactions between the polymer chains. By adding additional charges to the water, the electrostatic repulsion of the crosslinked polymer chains will decrease, resulting in a less swollen hydrogel. As a consequence of the screened electrostatic interaction and the rubber elasticity, a polymeric hydrogel and the concentration gradient between seawater and fresh water can be used to propel a mechanical motor.

A prototype osmotic motor was built to evaluate this effect on hydrogels of various compositions (Fig. 2): First, the hydrogel in salt water expands only to a small extend. In a second step, the hydrogel is washed by fresh water. Consequently, it swells to its maximum extend. This volume expansion can be used to create mechanical energy, via  $W = \int p \, dV = \int F \, dx$ .

For our first setup a weight of 1 kg could be lifted.

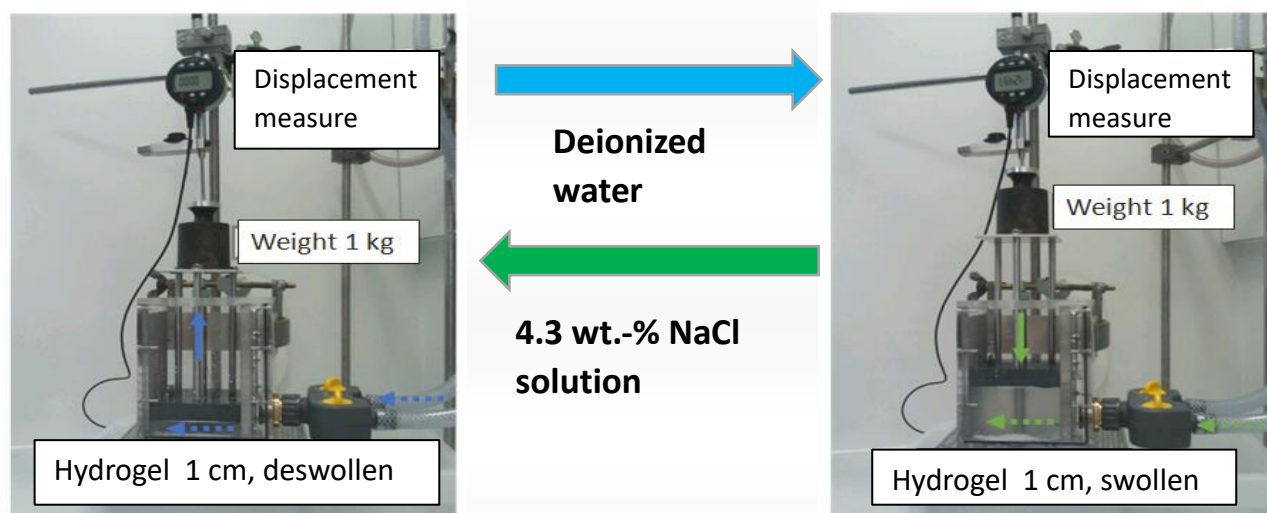


Figure 2: Prototype of an osmotic motor based on crosslinked polyacrylic acid. The hydrogel is able to lift 1 kg by changing the salt concentration of a feed solution between seawater and fresh water concentration.

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## **Rural Development: Policies, Strategies and Implications**

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### **ABSTRACT**

The rural areas mainly composed of land intended for agriculture, forests and other natural areas occupy most of the European Union. Rural development is a complex process focused on improving the quality of life in rural areas, rural activities, infrastructure and socio-economic indicators. Farm to Fork Strategy is core strategy of European Union (EU) for a greener rural area and a better quality of life of rural communities.

Strategic Plans approved in 27 EU countries are the main instruments of the EU Common Agricultural Policy (CAP) 2023-27 for rural development. Strategic Plans of EU member states contribute to sustainable use of natural resources and enhancement of rural economy.

Role of rural policies and strategies must be focused on improving the attractiveness of rural areas for young generations, enhancement of rural infrastructure development and create jobs.

**Keywords:** EU Farm to Fork Strategy, Common Agricultural Policy (CAP), EU Strategic Plans, rural areas.

## Fermented Fireweed (*Chamerion Angustifolium* (L.) Holub) Leaves - A New, High-Quality Food for Human Health

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### ABSTRACT

More and more people around the world are interested in food and medicinal plants. One such plant that has a wide range of uses is the fireweed (*Chamerion angustifolium* (L.) Holub). This study aimed to investigate the influence of technological parameters of solid-phase fermentation on the change of flavonoids, phenolic acids, tannins, carotenoids, chlorophylls, and antioxidant activity. The raw material for the experiment was collected from a naturally growing fireweeds habitat, located in Giedres Nacevicienes ecological farm (no. SER-T-19-00910), which is in Safarka village (55°00'22" N; 24°12'22" E), Jonava district (Lithuania) in 2019–2021. Polyphenolic compounds, carotenoids, and chlorophylls were quantitatively and qualitatively evaluated by high-performance liquid chromatography (HPLC). The ABTS radical-cation coupling method was used to evaluate the tendency of the antioxidant activity of the biologically active substances. The results of the study showed that solid-phase fermentation has a significant effect on the quantitative composition of biologically active substances in the leaves of the fireweeds. Thus, after modeling the technological parameters of solid-phase fermentation, it is possible to propose to the food and pharmaceutical industry the production of health products from the leaves of the fireweeds.

**Keywords:** fireweed, fermentation, health, polyphenols, antioxidant activity.

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**Biological invasion and urbanization: the spread of alien *Ambrosia psilostachya* DC. and the regression of native *A. maritima* L. (Asteraceae) in Italy.**

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**ABSTRACT**

Biological invasions are an important component of global environmental change and one of the leading causes of native biodiversity and ecosystem function disruption. Urbanization and habitat degradation are promoting factors of biological invasions. Therefore, understanding and predicting large-scale invasion patterns is pivotal; the first step may be the identification of the environmental variables (climatic, geographic, or ecological) influencing species distribution along the invasion timeline. Predictions can be made through Ecological Niche Models (ENMs), correlative models which estimate the ecological requirements of species by relating their known geographic distributions to a set of environmental variables. In the present study, based on herbarium and field data, we reconstructed through ENMs the change of the niche of *Ambrosia psilostachya* DC. (Asteraceae) along its invasion history in Italy from the first record, dated back to the beginning of the 20<sup>th</sup> century, until today; we focused on three temporal windows (1910 - 1930, 1940 - 1960, 1990 - 2010) that better represent the period of early introduction, late introduction and first spread, naturalization and spread of the species in the country. *A. psilostachya* is a North American species arrived to Europe at the end of 19<sup>th</sup> century. In Italy, it has spread especially along the coastline likely taking advantage of the massive urbanization of the coasts. While the alien plant gained ground, a severe regression of *A. maritima* L., congener native to the Mediterranean basin, occurred. So, we compared the dynamisms of the niches of the two species to understand differences and possible elements influencing the resilience of the two species during the profound changes of coastal landscape during the last century.

**Keywords:** Ragweed, Niche Dynamism, Urbanization, Alien Plants

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# **KEYNOTE SPEAKERS**

## **FULL-TEXT**

## **New Protein Sources and Biomolecules in the Developing World**

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### **ABSTRACT**

Increasing global population, together with changing sociodemographic factors, increases the pressure on world resources. Even though animal protein sources have negative environmental impacts, cause greenhouse gas emissions, and require more land and water, animal-based protein is a part of sustainable nutrition. Amino acid deficiency is an important problem in plant-based proteins. However, the discovery of new animal protein sources is among the current topics of recent years. It is expected that alternative protein sources will be a part of sustainable nutrition and contribute to food security. Bee products such as honey, pollen, bee bread, propolis, royal jelly and apilarnil are used for both their nutritional properties, protection against diseases and support for treatment. Among these products, bee bread (perga) and drone larvae (apilarnil), which have become popular in recent years, are remarkable products with their high protein content and rich amino acid content. In this study, these two bee products of animal and plant origin are mentioned.

**Keywords:** Protein Sources, Herbal, Animal, Bee Bread, Apilarnil

### **1. INTRODUCTION**

According to the United Nations, global population growth is expected to increase by approximately 50% since 2000, reaching 9.5 billion in 2050. Increased food demand as a result of more population to feed, other changes such as economy and urbanization will cause changes in consumption activities. Thus, not only the amount of food consumed will change, but also the type of this food (1-3).

Today, plant protein sources meet 57% of the world's protein needs, while meat meets 18%, dairy products 10%, fish and shellfish (6%) and other animal products 9% (4).

Cereal proteins meet the majority of protein needs globally and are important for animals as well as humans. While grains are gaining particular importance in the diets of developing countries, wheat is the most important of this group (5). Wheat, used as bread, is an important component of protein distribution in Europe. A typical loaf of bread has 8g of protein per 100g. Protein content in grains varies (10-15%), with the largest protein content being storage protein (prolamins, globulins and germens) (6). Corn, rice and wheat are the main basic plant resources. However, it should be known that plant-based protein sources lack one or more amino acids that will meet human nutritional needs (7).

The expectation that the demand for animal-based protein will double by 2050 is worrying. These concerns include the fact that animal foods produce higher levels of greenhouse gases than plant

foods and that this causes climate change. As a matter of fact, the need for land will increase as the increase in demand for animal-based protein creates the need for more animal feed production. The increase in the conversion of forests, wetlands and natural grasslands to agricultural areas will have negative effects on greenhouse gas emissions, biodiversity and other ecosystem activities (7).

It is expected that alternative protein sources will be a part of sustainable nutrition and contribute to food security. Among these products, bee bread (perga) and drone larvae (apilarnil), which have become popular in recent years, are remarkable products with their high protein content and rich amino acid content.

Nectar and pollen are essential components of honeybee, *Apis mellifera* L., diet. Nectar provides carbohydrates for honeybee, while pollen supplies protein, lipid, and vitamins. Pollen collected by foraging worker bees is combined with honeybee secretions. After loading to the pollen basket (corbicula) pollen is covered with a thin layer of honey and wax to avoid deterioration. By the time, this mixture undergoes a set of biochemical changes caused by different enzymes, microorganisms, moisture and temperature. This chemically modified pollen is called as beebread (8).

Chemical composition of beebread is different from collected pollen. Beebread has higher contents of vitamin K, reduced sugar and digesting enzymes of microorganisms than the corresponding plant pollen. It comprises about 24-35% carbohydrates, 20% protein, 3% lipids and 3% vitamins and minerals. Beebread, also, contains vitamins, all essential amino acids that cannot be biosynthesized by the human body and a variety of biologically active compounds such as pigments, enzymes, flavonoids, carotenoids and hormones. The high biological activity of beebread inhibits mold and mildew development that ensure better protection of this apiculture product (9).

There have been many studies published revealing the chemical composition of pollen from different botanical origins (10,11). Yet there are limited studies available on various properties of beebread. Antioxidant effect of the beebread was studied by Nagai (2005) and antibacterial effect of Morocco's beebread were reported by Abouda et al. (2011) (9, 12). Herbert and Shimanuki (1978) studied the chemical composition and nutritional value of bee collected and stored bee pollen samples (13). Fatty acid composition of beebread was studied by several authors. Human and Nicolson (2006) investigated the amino acid and fatty acid composition of fresh, bee collected and stored pollen (14). In another study, Ceksteryte et al. (2008) identified twenty-four fatty acids in the beebread collected in spring and summer time. Oleic and arachidonic were the most abundant unsaturated fatty acids in beebread constituting around 15% of total fatty acids. Moreover, Ceksteryte and Jansen (2012) studied fatty acid composition in beebreads collected from various floral origins (15). Linolenic acid (n-3) was reported to be the highest (27-43.8%) within twenty-two fatty acids identified in spring beebread. Unsaturated fatty acids have many beneficial health effects such as reducing the level of serum triglycerides possessing cardio protective properties through reducing blood cholesterol, triglyceride level and exerting an anti-arrhythmic, anti-thrombotic, anti-inflammatory impact (16).

Apilarnil is a bee product with biologically active properties. It is obtained by filtration and pulverization of drone larvae homogenate harvested at the 7-day larval stage, before the

honeycomb eyes close. Apilarnil is a homogeneous and milky substance, yellowish gray in color and sour in taste. It is easily ripened and stored in its raw form in the deep freezer. Its structure contains water (65-75%), proteins (9-12%), carbohydrates (6-12%), fatty acids and lipids (3.5-8%), K, Na, Ca, Mg minerals (1-1.5%). amino acids (threonine, leucine, isoleucine, methionine) as well as sex hormones such as testosterone, prolactin, progesterone and estradiol. Although its physicochemical properties are similar to royal jelly, it has some differences. It is also reported that it has male-specific strengthening effects because it contains male-specific hormones. For example, it is recommended as a natural anabolism stimulator in men because it increases muscular body weight. Its basic components are amino acids, and it is among the unique products that contain all the essential amino acids that cannot be synthesized by human or animal organisms, vitamins (A, B1, B6, PP, choline vitamins, beta carotene, etc.) minerals (calcium, phosphorus, sodium, zinc, manganese, iron, copper and potassium). Silici et al. (2019) research, protein content of apilarnil (7-day-old larva) is 48.75 g/100g, carbohydrate content is 21.62 g/100g, fat content is 21.13 g/100g, ash content is 4.07 g/100g, moisture content is 4.43 g/100g and energy content is 472 g/100g determined as kcal/100 g (17). According to the results of analysis with HPLC-UV, 16 amino acids were determined in apilarnil. Among the essential amino acids, only tryptophan could not be detected. The highest amino acid amount is lysine amino acid, which is 7198 mg/100g, while the lowest is methionine amino acid, whose value is determined as 500 mg/100g. In another study, Hu and Li (2001) determined that the moisture content of pupae and larvae was 81.69-73.48%, the protein in the dry matter of pupae and larvae was 42.36-48.47%, the fat level was 15.75-20.63% and the ash content was 9.99-18.47%. They determined that the dry matter contains 17 types of amino acids and is especially high in glutamate, aspartate, lysine and leucine, and the content varies between 26.35-44.72%. They also determined that there are high levels of vitamins such as vitamin C and vitamin D. Pharmacological activities of Italian bee (*Apis mellifera ligustica*) worker bee larvae and pupae were determined in three ways; antitumoral, antiaging, and regulation of immune function. The results determined that worker bee larva and pupa S180 showed a significant inhibition effect on tumor development, tumor inhibition rates were 60.92% and 64.37%, respectively, and life extension rates were 80.65% and 81.74%. They found that worker bee larvae and pupae could significantly increase SOD activity, while reducing the MDA content in the brain, liver and blood tissues, and significantly increasing the RNA and protein content in the brain tissue of mice. While it increased the T lymphocyte transformation function of worker bee larva and pupa, it also significantly increased the charcoal clearance index and thymus index, and stated that it had a clear effect on cell-mediated immunity and non-specific immunity. The results indicated that the nutritional components of worker bee larvae and pupae were close to those of queen and drone larvae and pupae. They reported that there was no significant difference between the pharmacological activities of worker bee larvae and pupae and queen bee larvae. They reported that worker bee pupae and larvae are promising as food and medicine in the future. Since apilarnil contains important nutritional elements and is a natural environment, it is a suitable environment for the development of microorganisms and fungi. Therefore, it may deteriorate under inappropriate storage conditions. The most suitable way to stabilize unstable moist environments is lyophilization. As a matter of fact, lyophilized royal jelly obtained by lyophilization, also known as the freeze-drying method, is widely used in pharmaceutical production. The adsorbed homogenate method can be preferred for drug

production. In adsorbent selection; Minimum homogenate according to the adsorbent ratio, taste of the resulting product, stability in storage, optimized physical characteristics of the final product are important parameters.

## CONCLUSION

Bee products discovered in recent years have been the subject of many studies due to both their nutritional content and positive effects on health. Among these products, bee bread of plant origin and apilarnil of animal origin attract attention. It is thought that it will take an important place among the global population growth and the alternative protein sources caused by this increase.

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## **Common Misconceptions in the History of Medicine: Some Examples of Information That is Known to be Correct but Wrong in the History of Medicine**

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### **ABSTRACT**

Some aphorisms, oaths, or booklets attributed to Hippocrates, who became an important figure in the history of medicine, long after his death, are attempted to be presented as belonging to him, although they do not actually belong to him. This article tries to correct this kind of misinformation and illuminate it within the resource framework.

**Keywords:** Primum non nocere, harm, ethics, Hippocrates, Medical oath

### **PRIMUM NON NOCERE (FIRST, DO NO HARM) DOES NOT BELONG TO HIPPOCRATES**

The exact Latin phrase itself, Primum non nocere, of course, does not date from the era of ancient Greece or even from the later Galenic promotion of the principle. Research by the New York Medical pharmacologist, Dr Cedric Smith, showed that the phrase, Primum non nocere, was first used by one of the founders of modern medicine, the English physician Dr. Thomas Sydenham (1624-1689). This attribution was made by an anonymous reviewer, "H.H.," [probably Henry Hartshorn] of a book, Foundations of a New Theory and Practice of Medicine, written by Dr Thomas Inman and published in 1860 in London.<sup>1</sup>

Contrary to popular belief the phrase does not appear in the Hippocratic Oath or the Hippocratic Corpus (If Hippocrates wrote, he wrote in Greek, not Latin). Rather the saying is attributed to Thomas Inman, as recently as 1860.<sup>1</sup>

That same year Oliver Wendell Holmes Senior famously remarked in a lecture to the Massachusetts Medical Society, "*If the whole materia medica, as now used, could be sunk to the bottom of the sea, it would be all the better for mankind—and all the worse for the fishes.*"<sup>2</sup>

### **What does "Primum non nocere" mean first?**

According to Inman, overmedication-related injuries were frequently concealed by the illness. The maxim "First do no harm" is still a crucial guideline against excessive therapy. However, akin to numerous axioms or aphorisms, this counsel is rudimentary. Healthcare professionals frequently cause harm to patients through procedures like tracheotomies, cannula insertions, chemotherapy, abdominal openings, and skull drilling. The majority of efforts aimed at helping a patient entail risk of harm or necessitate doing harm. The doctor hopes that the advantages will exceed the disadvantages. If "first do no harm" were to be taken literally, the clinician would take no action.<sup>3</sup>



The ancient ethical precept of not exacerbating an unfavorable circumstance is known as pragmatic ethics.<sup>4</sup> It originates from the Hindu Rig Veda's verse, "*Do no harm to any living creature*".<sup>5</sup> The requirement implied avoiding intentional harm. This idea, which has its roots in the third millennium B.C.E., was institutionalized in the eighth century B.C.E. in the shape of the code of conduct known as "ahimsa." Ahimsa, or the duty to inflict no damage, whether deliberate or not, later developed into one of Hinduism's core ethical precepts. Later, in the latter half of the sixth century B.C.E., it was also absorbed into Buddhism, particularly Jainism. The Sanskrit term for "*non-injury*" (ahimsa) is the virtue.<sup>6</sup>

The "Hippocratic injunction" to do no harm has been a fundamental principle in clinical pharmacology and medical and graduate student education. This study and review aimed to identify the source of this distinctive Latin phrase in light of the recent reexamination of the type and severity of adverse medication responses. According to reports, the author was neither Galen nor Hippocrates.

The axiom first appeared in literature dating back to the Middle Ages around 1860. It was attributed to the English physician Thomas Sydenham and was expressed in both English and Latin. It was mainly conveyed orally and was widely used from the late 1800s to the early 1900s; in the early 1900s, it was hardly ever published in print. Its suitability and limits as a manual for the moral conduct of pharmaceutical research and medicine are examined. It is nevertheless a powerful reminder that every medical and pharmaceutical choice has the potential to be harmful, even in the face of insufficiencies.<sup>7</sup>

### **Oath of Graduates of Medicine School**

Evidence is a conceptual justification for a viewpoint or course of action. Thus yet, nothing has been said on the caliber of the evidence and, thus, the warrant's strength. Being ignorant is defined as holding incorrect beliefs or not having any beliefs at all about how the world functions. If I don't think that germs cause disease, and if I do think that they do, then I don't know that germs cause disease. This would be a serious flaw in a doctor practicing in the twenty-first century, but not in a doctor practicing, say, in the fourth century BC. This is due to the fact that whereas my ignorance from the fourth century was collective, it was individual in the twenty-first century. No one understood, or should have known, that germs caused disease 25 centuries ago; today, everyone knows this, or at least everyone who does medicine. In any case, the requirement that medical professionals have a basic understanding of their profession is not new and has always been justified as a moral duty.<sup>8</sup>

It is possible to interpret the Hippocratic Oath—which was most likely not penned by Hippocrates—as much a celebration of educators and learning as it is a list of obligations and virtues. The oath-taker is celebrating knowledge transmission when she promises to “regard him who has taught me this technique as equal to my parents” and to “not cut, and certainly not those suffering from stone, but I will cede [this] to men [who are] practitioners of this activity”; she is also promising not to practice beyond her knowledge or capacity.<sup>9</sup>

"Grant me the strength, time, and opportunity always to correct what I have acquired, always to extend its domain; for knowledge is immense and the spirit of man can extend indefinitely to enrich

itself daily with new requirements" is the Oath of Maimonides, named for the twelfth-century physician, rabbi, and philosopher Moses Maimonides.<sup>10</sup>

This should undoubtedly be interpreted as an appeal to keep up with the rapidly evolving body of medical knowledge, a medieval foreshadowing of the significance -the ethical significance- of ongoing medical education. Nothing could be different. The relationship between health and knowledge suggests a moral obligation since specialized ignorance either causes or permits harm to others. The fact that a patient might have fared better under the care of someone else whose expertise (or lack thereof) would have saved the day makes a clinician's ignorance somewhat culpable. We shall address these intriguing questions regarding how far behind one might go without being held accountable later. We now seek to distinguish between community and idiosyncratic ignorance, which makes the idea crucial.

## CONCLUSION

Writing history with the assumption and/or dream that all kinds of knowledge, development, and culture belong only to Europe, with the motivation of Europe-centered historiography, will eventually collapse with the emergence of the truth.

With the support of conscientious and honest writers, mostly Western, who are taken as references, it has been revealed that most of the things attributed to Hippocrates do not belong to him and do not reflect the truth.

*Primum non nocere* is said by Thomas Sydenham and was introduced by Thomas Inman. The oaths for the graduates of medical faculties in the ancient Indian civilization and the physicians working in the palace in the Babylonian Empire were established in ancient times.

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# **ORAL PRESENTATIONS**

## **ABSTRACT**

## Assessment of Depositional Environments of Sebkha Sediment: Application of Geochemical and Mineralogical Tracers

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### ABSTRACT

In order to better comprehend the depositional environments and to define the minerals origin of sebkha Mhabeul sediments, a 91.5 cm core (Mh 2) was collected from this sebkha. Based on the age model of Marquer et al. (2008), the core spans the last 2400 years. This work aimed to identify the geochemical and mineralogical sedimentary composition and the factors intervening on sediment distributions and mineralogical composition. This core was analyzed by X-ray fluorescence (XRF), Bernard calcimetry, AFNOR sieves, X-ray diffraction (XRD) and scanning electronic microscope (SEM). The sedimentary investigations show the dominance of the detrital minerals (especially the quartz). Moreover, the mineralogical investigations prove the existence of evaporitic (bassanite and halite) and carbonate (dolomite and calcite) minerals. The previous study by Fourier transforms infrared (FTIR) absorption spectra (Gammoudi et al. 2021) confirms the existence of OM. The principal component analysis (PCA) of the obtained results proves the good correlation between major and minor chemical elements and different minerals.

**Keywords:** Geochemistry, Mineralogy, Depositional environment, PCA, South-Eastern Tunisia

**Acknowledgment:** All authors thank the Sfax Preparatory Engineering Institute which provided logistical support and authorization for coring. Also, they would like to thank Mrs. S. Chtourou and Mr. H. Gargouri for their help during the laboratory measurements. Also, the authors thank Mr. M. S. Ben Reguiga, the laboratory member of ETAP (Tunisia), for his advice and for his help in the SEM analysis.

## **Study on Preparation and Characterization of the Chitosan/Grape Seed/ZnO-Based Bio-nanocomposites**

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### **ABSTRACT**

The increase in the world population and the limited resources of required raw materials have increased the importance of renewable, environmentally friendly, and sustainable materials from natural resources. Chitosan (CH) is an abundant biomaterial that attracts much attention due to its superior properties, such as biocompatibility, sustainability, functionality, and biodegradability. Grape seed (GS), the by-product of the grapes, is rich in bioactive compounds, such as phenolic acids, stilbenes, and flavonoids, including flavan-3-ols, flavonols, a stilbene and anthocyanins which enables for potential use in many applications. This study aimed at the production of CH/GS-based biopolymer composites that are reinforced with ZnO nanoparticles. CH/GS/ZnO-based composite materials with variable properties have been prepared using solution mixing and casting techniques, characterized, and compared. The intra and intermolecular interactions between CH, GS, and ZnO were studied by Fourier transform infrared spectroscopy and X-ray diffraction analysis. Scanning electron microscopy and equilibrium swelling corroborate the change of morphologies of composites. The swelling behaviors of prepared composites depend on the solution medium pH and the interactions between materials. The prepared composites are potential candidates for various applications, including food packaging, wound dressing, and tissue engineering.

**Keywords:** Chitosan, Grape Seed, ZnO.

## **Analyses of *Crocus sativus* Genome Using Cytogenetic Approach**

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### **ABSTRACT**

*Crocus sativus* (Saffron) belongs to genus *Crocus* and family Iridaceae. *C. sativus* is an economically important aromatic and medicinal plant. Saffron is known the world's highest priced spice which is derived from its dry stigmas. This species cultivated mostly in Europe, Asia, America but major saffron growing regions include Türkiye, Iran, Azerbaijan, Spain, Italy, India and Greece. *C. sativus* is a triploid male-sterile plant ( $2n = 3x = 24$ ) which vegetatively propagated by corms. Although the economic, medicinal importance and common cultivation in Türkiye, there is limited research on Saffron genome. The aim of the project was to analyse of chromosome number and the genome size of *C. sativus* which cultivate in Safranbolu, Türkiye. Genome size of all analysed plants was estimated by flow cytometry and somatic chromosome counts were performed based on slides stained with DAPI. The analyses showed that studied samples had similar genome size (approximately 9 pg/2C) with  $2n = 24$  chromosome number. The data obtained in the project is planned to be used in the induction and analyses of the polyploidy.

**Keywords:** *Crocus sativus*, Saffron, Genome size, Chromosome number

**Acknowledgment:** This study was supported by Ondokuz Mayıs University The Scientific Research Projects Coordination Unit (Project number: PYO.ZRT.1902.23.001).

## **Fabrication and Characterization of Cellulose-Based Composite Scaffolds Incorporated with Psyllium Seed for Potential Healthcare Applications**

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### **ABSTRACT**

Cellulose (CL) is the most abundant natural raw, biodegradable, nontoxic, semi-crystalline biopolymer material used in the paper industry, obtained from various biomass containing poly (1,4- $\beta$ -D-anhydroglycopyranose) that is the main component of the plant fibers consisting of –OH groups and strong hydrogen bonds between them. Biopolymer materials, such as cellulose, can be naturally degraded after usage and are promising to transform into healthcare applications. Psyllium is a plant with a high water-soluble fiber content, which exerts several positive effects on health and possesses pharmaceutical properties and desirable nutritional effects. Psyllium seeds consist of the acidic arabinoxylan and xylan backbones that are strongly branched. They are widely studied due to their potential health benefits and applications in various clinical and diagnostic applications, food, and pharmaceutical industries. To evaluate organic wastes and to bring them into the economy, cellulose was obtained from wasted plane tree leaves along with the pulping, alkali treatment, and bleaching processes, subsequently under the motto "Zero Waste." This paper presents CL/ Psyllium Seed (PS) biopolymer composites prepared with a simple solvent casting and evaporating method following ultrasonication. Cellulose and its three composites, including a 5, 10, and 20 weight percent of PS gel, were fabricated as non-crosslinked and crosslinked. The structural and morphological properties of biocomposites were studied using Fourier transform infrared spectroscopy, X-ray diffractions, and field effect scanning electron microscopy techniques. The composite that was produced is a promising candidate for various healthcare applications.

**Keywords:** Cellulose, Psyllium, Biopolymer, Composite.

**Acknowledgment:** This study is a part of the project TEKF.23.57 " Extraction of the Cellulose and Cellulose-based Biopolymer Composites: Fabrication and Properties " funded by Fırat University Scientific Research Projects Unit.

## **Treatment of Defense Industry Wastewater with Electrocoagulation (EC)**

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### **ABSTRACT**

Industrial wastewater is the major source of hazardous bio-accumulative and highly soluble toxic heavy metals like arsenic, copper, chromium, cadmium, nickel, zinc, lead, and mercury in the aquatic environments. There has, therefore, always been a need for the removal and/or recovery of these toxic, non-biodegradable, and persistent heavy metals from the defense industry wastewater. The defense industry wastewater was treated with electrocoagulation (EC) using a sacrificial Fe anode in a batch reactor. In EC process, TOC, toxicity, and heavy metal contents of the wastewater were decreased during 30 min retention time as a result of response surface methodology (RSM) optimization with following parameters; current density (10.0, 15.0 and 20.0 mA/cm<sup>2</sup>) supporting sodium sulfate electrolyte amount (0.0, 5.0 and 10.0 mM Na<sub>2</sub>SO<sub>4</sub>) and initial water pH (3, 6 and 9). It was observed that the main metal and heavy metal contents were Cr, As, Sb, Pb, Hg, Cd, Al, Fe, Zn, Ni, Cu, Ag, Sn and Pb when their concentrations were listed in descending order at the level of µg/L, and these heavy metals were removed more efficiently in the EC process. The FTIR-ATR spectrum of the sludge observed the strong peaks around 875 cm<sup>-1</sup> and 884 cm<sup>-1</sup> related to vibrations of the Fe–O bonds in iron oxide after the EC. This study showed that defense wastewater was successfully treated with EC first time in the literature even if it had high TOC, toxicity, and heavy metal contents.

**Keywords:** defense industry, wastewater treatment, electrocoagulation, heavy metals, toxicity.

## **Easy4digit - European Agriculture System for Digital-Based Technologies**

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### **ABSTRACT**

Digital agriculture, also known as precision agriculture, is the use of advanced technologies and data analysis techniques to optimize and improve agricultural production. This includes the use of sensors, drones, GPS mapping, satellite imagery, and other technologies to collect data on soil moisture, nutrient levels, weather patterns, crop growth, and other factors that impact agriculture. Digital agriculture enables farmers to make more informed decisions about how and when to plant, irrigate, fertilize, and harvest crops. By analyzing data in real-time, farmers can identify problems early on and take corrective action before crops are affected. This can lead to higher yields, better crop quality, and reduced waste. Digital farming has the potential to revolutionize agriculture and bring significant benefits for farmers and the society overall, as we need new ways to grow more food more sustainably, but there are bottlenecks limiting in terms of time, investments and effectiveness the take-off of the new digital technologies in agriculture. Concretely, it means applying new technologies and innovative solutions, such as data science, advanced sensors in the field and flying drones, digital communication channels, and on field automation starting from an in-depth and well-developed decision system supporting right, practical and sustainable planning and management. The mission of the EASY4DIGIT VET Erasmus+ KA220 is mainly focused on reducing the existing gap of specific competences in digital education supporting Decision Support Systems and tackling lack of knowledge and information dedicated to young as well as adult farmers as final users and trainers as intermediary category. EASY4DIGIT intends to address the challenges by developing awareness and competences of farmers towards an advanced digital knowledge focused on smart and resilient farming. EASY4DIGIT will raise the skills capacity and in-house functional knowledge and best practices in order to improve the overall competitiveness of the agriculture industry and succeed in an increasingly digital, green and knowledge driven economy.

**Keywords:** Smart agriculture, Digitalization in agriculture, Training, E-learning

## **Coal Clinker Ash Assessment for Nitrogen, Potassium, and C/N Transformations in Sandy Soil**

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### **ABSTRACT**

Statement of the problem: The disposal of coal clinker ash remains a challenge globally, especially for developing countries which has resulted in it becoming an environmental menace. Its utilization remains subdued and is mostly hindered by its heterogeneous particle size. However, its characterization reveals that it has the capability to improve poor agricultural soils. Purpose of the study: to assess the potential of CA to improve nitrogen and potassium retention as well as its influence on the C/N ratio in sandy soils. Materials and method: Nutrient leaching experiments were conducted using cylindrical PVC pipes, 50 cm in length and a diameter of 10.5 cm, one end was closed with a water-porous material to allow for drainage. The treatments included control (sandy only), CA1 10 %, CA1 20 %, CA2 10 %, CA2 20 %, and rice biochar (positive control) at 5 % w/w, replicated three times. Results: The concentration of total  $\text{NO}_3^-$ -N leached was significantly affected by the application of clinker ash, with CA at 10 % for both types leaching at par with the positive control, biochar. Solution K and  $\text{NH}_4^+$ -N were leached highest in CA1 at 20 %, on the other hand, CA2 treatments retained the highest amount of these nutrients. C: N ratio of sandy soil was equally affected and improved by the application of clinker ash. After the leaching period, pH and EC tested were significantly affected by the application of clinker ash. Conclusion: Clinker ash is embedded with characteristics that are critical and can be utilized to improve the nutrient and chemical properties of sandy soil.

**Keywords:** Nitrate, Ammonium, Potassium, Leaching, Incubation, Retention.

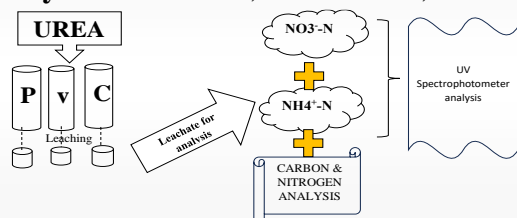


Fig 1: Experiment layout and analysis



## **Characterization of the Risks Induced By Domestic Discharges on the Physicochemical and Microbiological Quality of the Waters of the Urban Foggaras of the Beni-Abbes Oasis**

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### **ABSTRACT**

This work focuses on studying the physicochemical and microbiological quality of the water coming from the urban foggaras of Beni-Abbes (1250 km south of Algiers, the capital of Algeria). The foggaras are of great economic and social importance. They are an important historical heritage of the Beni-Abbes oasis and a source of drinking water and irrigation. The results show that 84% of the foggaras have a good physico-chemical quality ( $T^{\circ}=24^{\circ}\text{C}$ , conductivity= 4974  $\mu\text{S}/\text{cm}$ , pH= 8.03,  $\text{Cl}^{-}= 802.3 \text{ mg/l}$ ,  $\text{Na}^{+}= 238 \text{ mg/l}$ ,  $\text{K}^{+}= 26 \text{ mg/l}$ ), on the other hand, 100% of the bacteriological test results are unsatisfactory and (total germs  $21.7 \times 10^4 \text{ CFU}$ , total coliforms  $1.4 \times 10^3 \text{ CFU}$ , faecal coliforms  $1.4 \times 10^3 \text{ CFU}$ , coliforms  $1.4 \times 10^3 \text{ CFU}$ , faecal streptococci 240 CFU, Clostridium sulphito-reducing, Clostridium spore-forming forms, Salmonella). This is an alarming state of affairs due to neglect and lack of sanitation, which could turn it from an important source of life into an unsafe source of contamination, given the frequency of bacterial contamination indicators (total coliform, faecal coliform, faecal streptococcus, salmonella, etc.).

**Keywords:** Water, Foggara, Beni-Abbes, Physico-chemical quality, Microbiological quality.

## **Calf Behaviour: Investigation of Milk Feeding Behaviour in Calves**

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### **ABSTRACT**

Feeding of calves with milk or milk replacer during the period between birth and weaning can have significant effects on their behavior, performance and welfare. The amount of milk given to calves during this period, the duration of milk feeding, the number of meals per day, the characteristics of the milk given, and the method of feeding may vary from farm to farm. In studying the milk drinking behavior of calves, data on how often they come to drink milk in a day (12 hours), how long they spend drinking milk and the amount of milk they consume are used. In this review study prepared within the scope of the study supported by Erciyes University BAP commission (FUI-2023-12867), the practices performed during the milk feeding period in calves were discussed and examined.

**Keywords:** *Holstein*, calf, sucking-drinking, behaviour, milk feeding management.

**Acknowledgment:** This study was prepared within the scope of the 'Investigation of sleep behavior in calves' [FUI-2023-12867] project supported by Erciyes University Scientific Research Projects Department.

## **Determination of Fatty Acid Contents of Some Wild Sunflower Species (*Helianthus Spp.*)**

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### **ABSTRACT**

Wild sunflower species have many sources of genes resistant to biotic and abiotic stresses, broomrape parasite as well as having higher quality traits. The transferring of these useful genes to the cultivated sunflower is so important issue to obtain a continuous resistance and then develop better quality and yielding cultivars in sunflower production. The use of molecular methods in the breeding studies shortens the breeding cycles by providing an effective and accurate selection as well as saving time. The study was conducted in wild sunflower garden which set up via a TUBITAK project in previous years. Fatty acid compositions of all wild species materials were determined largely in Trakya University laboratory for the first time in the world via Gas Chromatography (GC). The molecular analysis was performed in the laboratory of Trakya University to identify individuals with high oleic acid trait containing the homozygous oleic gene with 4 molecular markers (3 INDEL markers F4-R1, F4-R2 and F4-R3) and an SSR marker N1-3F) / (N2-1R HO)). Based on GC analysis; no species with a high oleic rate (80% or more oleic acid) was found. However, among the examined wild species, three of them were found to have mid oleic acid content (between 60 - 80% oleic acid). Among these species, *H. annuus* species ranked first with the highest oleic acid content of 77.46%, followed by *H. hirsutus* with 69.71% and *H. floridanus* with 67.19%. In conclusion; Based molecular marker results conducted with 3 INDEL markers, *Helianthus californicus*, *Helianthus exilis*, *Helianthus giganteus*, *Helianthus gracilentus*, *Helianthus grosseserratus*, *Helianthus laciniatus*, *Helianthus mollis*, *Helianthus neglectus*, *Helianthus petiolaris*, *Helianthus petiolaris subsp. petiolaris* were determined to be high oleic. However, these were not determined higher oleic acid content based on GC analysis because there was no genotype containing high oleic acid among wild species, the selectivity of the markers may not be accurate.

**Keywords:** Wild sunflower, Fatty Acid, Oleic Acid, Molecular markers

## **Vegetative Propagule Pressure as a Driver of Plant Invasion Success: The Strange Case of *Caulerpa taxifolia* and *Caulerpa prolifera***

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### **ABSTRACT**

Propagule pressure is considered to be a major driver of plant invasion success in a wide range of ecosystems including coastal marine ones. Nevertheless, the information on the importance of propagule pressure for the diffusion of marine macroalgae is still scarce. The aim of this study was to better understand the role of propagule pressure in the invasion dynamics of the green macroalga *Caulerpa taxifolia* (Vahl) C. Agardh, currently among the most successful invasive clonal seaweeds in the Mediterranean. A mesocosm experiment was conducted to evaluate the establishment and growth ability of its fragments. Two factors were considered, the composition of the fragments (5 treatments: blades, stolons, rhizoids, blades+stolons, stolons+rhizoids, blades+stolons+rhizoids) and their density (2 treatments: low and high pressure). The number of established and grown fragments of *C. taxifolia* were considered as response variables and compared with those of the native congener *Caulerpa prolifera* Forssk J.V. Lamouroux. Results show that fragment establishment and growth seemed to be more relevant for complete portions (blades+stolons+rhizoids) for both the species. Nevertheless, also the other types of fragments appeared to be able to establish and grow, proportionally to their density, regenerating the lacking parts. Anyway, some significant differences between the two species were highlighted, such as a surprisingly higher number of fragments established and grown for *C. prolifera*. These results suggest that, despite propagule pressure appears to be important for the spread of both the species, other factors are key to the successful invasion of *C. taxifolia*.

**Keywords:** Invasive macroalgae, propagule pressure, invasion success, mesocosm experiment.

**Acknowledgment:** Project funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4 - Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU. Award Number: Project code CN\_00000033, Concession Decree No. 1034 of 17 June 2022 adopted by the Italian Ministry of University and Research, CUP, H43C22000530001 Project title “National Biodiversity Future Center - NBFC”.

## Determination of Heavy Metals in Some Fish and Molluscs from the Black Sea

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### ABSTRACT

This research aims to determine the content of heavy metals in the edible part of the three most consumed species of fish (Mediterranean horse mackerel (*trachurus mediterraneus ponticus*), black sea gobies (*neogobius melanostromus*), and lefer (*pomatomus saltatrix*), and black sea mollusks (mussels and shrimps) in Bulgaria, collected from the southern coast of the Black Sea. Samples were prepared for analysis by nitric acid digestion in a microwave system and analyzed by ICP-OES. The mercury content of the fish and molluscs is determined with a mercury analyzer. A significantly higher lead, cadmium, mercury, arsenic, zinc, and copper content was found in the Black Sea mollusks (mussels and shrimp) than in Black Sea fish. Of the Black Sea fish, the highest content of As was found in *Pomatomus saltatrix* (0.84 mg/kg), Hg in *Neogobius melanostromus* (0.02 mg/kg), Zn in *Trachurus mediterraneus ponticus* (10.1 mg/kg) and Cu in *Neogobius melanostromus* (0.54 mg/kg). According to the concentration of Pb and Cd, no significant difference was observed in the studied fish. The content of heavy metals in fish, mussels, and shrimps is at the maximum permissible standards established for Bulgaria and the EU. The results from this study were compared with data from previous research, showing an insignificant decrease in heavy metal levels. Consuming Black Sea fish and mollusks poses no risk to human health.

**Keywords:** toxic metals, Black Sea, Bulgaria, human health

**Acknowledgment:** This study is a part of the project 04/22 "Determination of the content of toxic metals and As in the water-biota-sediment system" funded by Center for scientific research, transfer of technologies and protection of intellectual property at Agricultural University-Plovdiv.

## **Determination of *In Vitro* Antioxidant Potential of Iced Tea Enriched with Linden Extracts**

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### **ABSTRACT**

Iced tea is a form of cold tea that may or may not be sweetened and is also a popular packaged drink. Teas prepared from herbs have been popular because of their health benefits. This research aimed to produce iced tea enriched with *Tilia cordata* flower (harvested from the City of Ohrid) extracts and to determine its antioxidant potential. European Pharmacopoeia indicates the necessity to evaluate the metal content of vegetal samples used as medicinal herbs. According to the results of this research, in the fresh *Tilia cordata* flowers low presence of the analysed metals was determined (Mn, Zn, Cu and Fe), while the presence of Pb, Cd and Ni was not determined. Furthermore, iced teas enriched with both aqueous and ethanolic extracts showed higher ( $p < 0.05$ ) antioxidant activity compared to the control variant: the ability to capture DPPH radicals > conjugated diene method > ability to chelate iron ions. On the other hand, iced teas enriched with ethanolic extracts were characterized by higher values for all three tests compared to teas enriched with aqueous extracts. Therefore, this product can be classified as a functional food in terms of its antioxidant potential, while it has a beneficial impact on the consumer's health. Industrial production of this product does not require special conditions, due to which there may be great potential and market advantage over conventionally available linden teas.

**Keywords:** iced tea, *Tilia cordata*, antioxidant potential.

## **The Amount of Acrylamide Formed in Different Types of Potatoes: Does It Change with Traditional Baking, Fryer and Airfry Fring Cooking Methods?**

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### **ABSTRACT**

**Objective:** Advancements in culinary technologies continually introduce new cooking methods, adapting to the evolving lifestyles of modern society. Notably, air frying has gained popularity in recent years due to the widespread use of home-based airfryer devices. This method of food preparation presents itself as a novel and environmentally favorable cooking instrument, as it mitigates the generation of oil waste and, in comparison, reduces both cooking durations and energy consumption, particularly in terms of electricity and fossil fuels while due to its cooking nature, it may also carry the risk of creating various carcinogens in foods. This research study involved the assessment of acrylamide -a chemical compound, forms under specific culinary conditions- levels in different potato varieties subjected to three distinct cooking methods: conventional oven baking, deep-frying, and air frying.

**Methods:** In this study, measurements were made on two forms of normal potatoes (*Solanum tuberosum*) (peeled, cut and prepared for research and ready-frozen), as well as purple potatoes (*Dioscorea alata*) and sweet potatoes (*Ipomoea batatas*), for the purpose of making fried fries. Acrylamide levels were determined using a fixed cooking protocol across all three cooking methods for all potato samples. The analytical method for acrylamide measurement involved adapting the technique developed by İnce K.O et al. in 2009 to contemporary laboratory conditions and subsequent analysis using UV-Vis spectroscopy, as advanced by Chong et al. in 2019. Each potato variety underwent three measurements, with results expressed as mean and standard deviation. Using the calibration measurements drawn from the acrylamide standard, the acrylamide contents of the samples were calculated as mcg/kg by making the necessary conversions based on the absorbance readings at 420 nm.

**Results:** The findings of this study revealed that While deep-frying produced the highest acrylamide levels for sweet potatoes and purple potatoes ( $p<0.05$ ), baking in the oven resulted in the lowest levels. There was no significant difference in acrylamide levels among potato samples baked in the oven ( $p<0.05$ ). However, particularly in the case of deep-frying and air frying, sweet potatoes and purple potatoes exhibited higher acrylamide content compared to conventional potatoes and also the air frying method generally appears to produce more acrylamide than the oven-baking method for these two potato samples. ( $p<0.05$ ).



**Conclusion:** As a result of this research, it was determined that among different potato varieties, the frying method resulted in the highest amount of acrylamide formation, specifically with air frying, while the most favorable amount was found in baking. Additionally, it was revealed that sweet potatoes and purple potatoes, which have become popular in the market, are riskier in terms of acrylamide formation compared to regular potatoes.

**Keywords:** Acrylamide, Air Fryer Frying, Potato, Sweet Potato, Purple Potato.

## **A Comparative Study: Effects of Commercial CMC and CMC Produced from Sugar Beet Pulp on Ice Cream Model**

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### **ABSTRACT**

Lignocellulosic agricultural residues are by-products of the agricultural industry and there are problems with their disposal. In terms of sustainability and waste management, it is critical to convert agricultural waste into high-value-added products. The use of agricultural residues as a raw material in the production of cellulose has many benefits such as the abundance, low cost and easy accessibility of these agricultural wastes. Sugar beet pulp, which is the by-product of sugar production process, is accepted as a good source of cellulose due to its cellulose content. CMC is most value using cellulose derivatives. In this study, the effects of CMC (SCMC) produced from sugar beet pulp and commercial CMC (CCMC) on the ice cream model were compared. Total dry matter, ash content and protein value produced SCMC ice cream and the produced CCMC ice cream samples was no statistical difference ( $p>0.05$ ). The pH value and color properties of both ice cream samples statistically difference ( $p<0.05$ ). When the rheological properties of ice cream mixes were compared, it was determined that ice cream produced with CCMC had better rheological properties than ice cream produced with SCMC. When the melting properties of ice creams were compared the end of the 30 minutes while SCMC ice cream completely was melted but 12.52% CCMC ice cream still wasn't melted. Sensory analysis result gumminess, aftertaste and overall acceptability was statistically significant ( $p<0.05$ ) but colour, appearance, hardness, iciness properties was statistically no difference ( $p>0.05$ ).

**Keywords:** sugar beet pulp, cellulose, carboxymethyl cellulose (CMC), ice cream

**Acknowledgment:** This study is a part of the project TEYDEB/3180003 “Cellulose and Carboxymethyl Cellulose (CMC) Gum Production from Sugar Beet Pulp” funded by TUBITAK.

## **Development of Some HPLC Analysis Methods to Prevent Silo Losses in Sugar Beet**

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### **ABSTRACT**

In the sugar production process, the quality parameters of the sugar beet entering the factory are expected to be at the highest level. Unfavorable weather conditions, beet head cutting, damaged and unclean beets, and fermentation of beets during storage all cause losses that reduce sugar output and quality. The aim of this study is to minimize sugar production losses and produce higher-quality sugar with fewer losses. In collaboration with Erciyes University and Kayseri Sugar R&D Center, a sugar analysis method in the HPLC-RID system was developed, and sugar beet intermediate products were examined. The results obtained from the study aim to improve the process, increase the quality and efficiency of the sugar produced, and contribute to the development of the sustainability of the sugar sector. In order to minimize silo losses, sucrose, invert sugar (glucose and fructose), raffinose, and kestose analyses in siloed beet, press water, raw syrup, watery-dark syrup, and molasses samples were analyzed.

According to the analysis results, an increase was observed in the invert sugar values of the intermediate products during the production period. No significant change was observed in the amounts of raffinose and kestose, while the amount of sucrose was constant throughout the period. In the molasses sample, which is a residue in the sugar production process, the biggest loss is in sucrose, which is 50%. In addition, raffinose and kestose in molasses were determined to be 1% on average. This prevents sucrose from precipitating and reduces the amount of sugar entering the bag.

**Keywords:** HPLC, Sucrose, Invert Sugar, Raffinose and Kestose.

**Acknowledgments:** This paper is a study of the project named "Development of Some HPLC Analysis Methods to Prevent Silo Losses in Beet" supported by ERÜ-BAP.

## **To Making of a Productive Green Campus: AGU-ORCHARD as an Eco-Cultural Practice**

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### **ABSTRACT**

Abdullah Gül University (AGU) is developing a new university model with an education and research approach that focuses on producing "social impact". AGU has adopted the principle of structuring itself as an "open campus" where classrooms, workshops, laboratories, libraries and social spaces are redefined in communication with each other. This is not only for students and employees, but also for the people living in Kayseri and its surroundings, who will meet with science and art, and will be shaped by the principle of a living laboratory environment. Together with the local government, AGU is developing a series of urban ecology-oriented projects that will contribute positively to the city and its citizens within the framework of the Green Campus Green District principle. In this context, by utilizing the principles of sustainability, AGU has established the AGU Orchard as the first step of this approach in designing the campus as a sustainable way of life and method. AGU Orchard is intended to create an eco-cultural space by bringing together university staff, students, local neighborhood residents, NGOs, and institutions. This space is expected to function as a socio-cultural interaction platform with a focus on education, research, and societal contribution. The goal is to remind the city's residents of gardening culture and integrate the orchard into the daily life of the city. By incorporating it into educational activities, this initiative, designed with an educational focus, will facilitate the gathering of local residents, students, civil society organizations, local institutions, and AGU members in the orchard.

**Keywords:** Productive Campus, Edible Campus, Eco-cultural Hub, Kayseri, AGU Orchard, Sustainable Cities and Communities.

## **A Course Proposal Aiming to Increase Students' Environmental Awareness “Organic Design; Back to Nature”**

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### **ABSTRACT**

This research reveals a course proposal developed to increase environmental awareness of university students by learning from nature and thus contribute to the design process. The main purpose of this course is to prevent students from being disconnected from their environment as a result of the current low environmental awareness levels reaching serious levels with the Covid-19 epidemic and to raise awareness for a sustainable environment. In short, understanding nature, which is the starting point of all our living spaces, experiencing it visually, tactilely and semantically, and redesigning it are the basic points of this course.

The course consists of three modules; The first module is soil. In the soil module, in addition to classroom activities on producing products from soil and mud, discussions are held on the importance of soil for our world and humans. The second module involves the use of planting elements to produce different textures and colors. Within the scope of this module, studies are carried out to create different texture and color varieties from natural materials collected through trips to regions with different plant diversity. The subject of the third module, which is the last module, is to design artificial miniature habitat with collected natural materials, based on different city definitions.

At the end of these three modules, it is aimed to provide input to the designs that students will make by observing their environment better and being inspired by the formation and combination of colors and textures in nature at the point where their environmental awareness increases.

**Keywords:** Environmental Perception/ Awareness, Design Process, Artificial Habitat.

## **Oil Palm Smallholders in Management-Assistance Programs Vary in Socio-Demographics, Attitudes, and Management Practices**

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### **ABSTRACT**

Smallholder farmers produce over 40% of global palm oil, the world's most traded and controversial vegetable oil. Awareness of the effects of palm oil production on ecosystems and human communities has increased drastically in recent years, with ever louder calls for the private and public sector to develop programs to support sustainable cultivation by smallholder farmers. To effectively influence smallholder practices and ensure positive social outcomes, such schemes must consider the variety in perspectives of farmers and align with their priorities. We conducted social surveys on smallholder farmers in Indonesia and Malaysia with varying degrees of cooperation with management-assistance programs (an industrial palm oil producer and a conservation focused NGO, respectively) as examples of the patterns in, and heterogeneity of, three main factors ("survey sections") associated with farmers: demographics, attitudes, and management decisions. These examples act as case studies to investigate the associations between smallholder palm oil producers involved in different schemes, allowing us to determine the alignment between the intentions of partnership programs and the current realities of smallholder plantations. The relationship between heterogeneity of response and degree of program

involvement differed by survey section and region: Indonesian smallholders most closely partnered with the private sector were the most varied in socio-demographics and attitudes but showed little variation in management inputs, while Malaysian smallholders most closely partnered with an NGO were the most heterogeneous across all survey sections. We found that income, geographic proximity, herbicide usage, and vegetation management were consistently among the most distinct factors in both contexts. Our findings demonstrate the wide variety of smallholder farmers in both regions, directly opposing a ‘one-size-fits-all’ approach to sustainability. The wide variety of existing management practices also provides a potentially valuable natural experiment to identify high-yield, environmentally-friendly management approaches. When taken in context, our findings may inform the interventions of management-assistance programs, ensuring they are approaching the most relevant farmer groups in the most effective way.

**Keywords:** Sustainable agriculture, palm oil, management-assistance, sustainability certification, smallholder farmers

**Acknowledgements:** This study is funded by the Biotechnology and Biological Sciences Research Council (BB/T012366/1) and the Gates Cambridge Trust.



# **POSTER PRESENTATIONS**

## **ABSTRACT**

## **Valorization of the Whole Plant of *Clematis Cirrhosa* L. (Ranunculaceae) As A Source of Bioactive Compounds Within a Circular and Sustainable Bioeconomy**

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### **ABSTRACT**

*Clematis cirrhosa* L. is an Algerian medicinal specie used to treat burns, joint aches, rheumatism pain and sexual dysfunction and as a diuretic agent [1,2,3,4], yet there is little information available concerning its bioactive composition and its potential economic value has not been explored. The aim of this study was to quantify bioactive compounds and to investigate antioxidant and acetylcholinesterase inhibitory activities of extracts obtained from the whole plant of *C. cirrhosa* in order to prove its possible use as potential natural source for human health. Phenolic compounds were distinctively profiled in the different extracts using TLC and standard phenolics. The antioxidant activity was evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), N,N-dimethyl-p-phenylenediamine (DMPD), nitric, hydroxyl and superoxide radicals,  $\beta$ -carotene bleaching, cupric reducing, ferric reducing, and metal chelating activity methods. Maceration in ethyl acetate and methanol allowed recovering the highest cumulative phenolic (96.13 and 99.98 mg GAE/g DE, respectively), flavonoid (44.90 and 24.62 mg CE/g DE, respectively), flavonol (16.05 and 22.13 mg QE/g DE, respectively), and hydrolyzable (585.21 and 85.54 mg TAE/g DE, respectively) and condensed tannin (3.15 and 6.23 mg CE/g DE, respectively) contents. The phytochemical analysis led to the identification of several phenolic compounds that were dominated by chrysophanol, emodin, caffeic acid, chlorogenic acid and kaempferol-3,7-O-dirhamnoside. The ethyl acetate and methanol extracts showed potential antioxidant activity in the different assays and this could be attributed to their polyphenol, tannin, and flavonoid contents. The ethyl acetate and methanol extracts exhibited also anti-acetylcholinesterase activity (IC<sub>50</sub> values of 1.12 and 0.71 mg/mL, respectively). This study provided a fundamental reference for the research of polyphenols in *C. cirrhosa* and found that this plant has the promising prospects of additives used in food.

**Keywords:** natural antioxidants, phenolic compounds, neuroprotection, sustainable life

## **DNA And HSA Binding Study of Naturally Occurring Monoterpene Ketone, Carvone**

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### **ABSTRACT**

Carvone has been characterized as the main component in the essential oils of many species of Lamiaceae and Asteraceae families. Our previous work showed that this monoterpene ketone is the main compound of *Tanacetum balsamita* essential oils isolated from the flowers, leaves, and stems by employing Clevenger-type apparatus. It has been known that this compound has promising pharmacological properties, such as neuroprotective, anti-diabetic, antifungal, antibacterial, antibiofilm, and anticancer effects, which qualified him as a key candidate in drug development. Bearing in mind carvone's beneficial effects, the aim of this study was to evaluate its interaction modes with biomacromolecules human serum albumin (HAS) and deoxyribonucleic acid (CT-DNA) using experimental and theoretical techniques. Considering that HSA plays the main role in the bioavailability, distribution, and elimination of numerous biologically active moieties from the body and that DNA is the major cellular target for many therapeutic agents, including plant secondary metabolites, understanding these interactions may provide valuable information on carvones therapeutic abilities.

**Keywords:** Carvone, DNA, HSA, Interactions.

**Acknowledgment:** This work has been supported by the grant of the Serbian Ministry of Education, Science, and Technological Development (Agreement No. 451-03-68/2023-01/200122) and the grant APVV-20-0058, "The potential of the essential oils from aromatic plants for medical use and food preservation".

## **A Comparative Study: Sustainable Soil Management and Rehabilitation of Degraded Soils**

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### **ABSTRACT**

The physical and chemical characteristics of the soil environment in which a plant grows have an impact on how well it develops during agricultural production activities. The addition of organic materials to the soil is one way used to enhance the physical characteristics of the soil and assure its continuity. The effects of leonardite and zeolite on a few soil parameters were investigated in this study after they were administered to the trial area where alfalfa was grown under irrigable conditions.

The investigation was carried out using a randomized block trial design with three replications in the trial area of the Erciyes University Faculty of Agriculture Research Application Farm (ERUTAM) (plot dimensions: 1000 m<sup>2</sup> and distance between plots: 0.6 m). Plant height was statistically significant for the applied organic soil conditioner at the 0.05 level. In the LSD test, Zeolite and Leonardite were placed together while the control group was kept apart.

Zeolite and Leonardite don't differ statistically, according to observations. The effect of soil amendments on the number of main stems was significant at the 0.05 level. It has been determined that the effects of Zeolite and Leonardite on pH and EC are significant at the 0.05 level, and the application that reduces pH the most is Zeolite, and the application that reduces EC is Leonardite. It has been observed that the effect of soil conditioners on yield is significant at the 0.05 level and the application that increases yield is Zeolite.

**Keywords:** Alfalfa, Soil, Sustainability, Soil Conditioner.

**Acknowledgment:** This study is a part of the I would like to thank Erciyes University Agricultural Research Center (ERUTAM).

## **Inhibition of Kv1.3 Channel by Scorpion Neurotoxin: Alleviating LPS-Induced Lung Injury Through Anti-Inflammatory Mechanisms**

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### **ABSTRACT**

The voltage-gated potassium (Kv) 1.3 channel plays a important role in the immune responsiveness of T-lymphocytes. In this study, we evaluated the effect of kaliotoxin (KTx), a hight specific Kv1.3 potassium channel blocker, purified from *Androctonus australis* hector venom, on induced pulmonary inflammation by lipopolysaccharide (LPS). Kaliotoxin was systemic delivery (0,8 ug/25 g) by intraperitoneal (i.p) route, after the injection of LPS (5 mg/kg) to induce pulmonary inflammation in murine model.

Obtained results showed that the administration of KTx after LPS injection induced a significant decrease in lung wet weight/dry weight in inflammatory markers characterized by a myeloperoxidase activity decrease, a marker of neutrophiles cells activation. KTx seems to be able to reverse the observed oxidative damage induced in LPS model characterized by a decrease of NO and lipid peroxidation (MDA) accompanied by an increase of anti-oxidant markers (catalase and GSH) in the lung. Histological examination of lung tissues confirmed a reduction in tissue lesions and inflammatory cell infiltration in the presence of Kaliotoxin compared to the LPS-only model.

The study highlights the remarkable pulmonary anti-inflammatory effects of Kaliotoxin, achieved through the inhibition of Kv1.3 potassium channel. These findings underscore the potential of Kaliotoxin as a targeted therapeutic strategy for pulmonary inflammatory diseases.

**Keywords:** Inflammation, Kv.13 Channel Blocker, Lung Injury, Oxidative Stress.

## **The Release of IL-6 and IL-8 In Response to Cup S1 Allergen**

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### **ABSTRACT**

Allergic diseases have a central place in chronic pathologies. For over 20 years, their frequency has been increasing. Allergies to pollen, at present, are a major public health problem because of pollen diversity. However, all the pollens are not allergenic, their nature and quantity vary significantly depending on the region and climatic conditions. *Cupressus sempervirens* is one of the most widespread species in Algeria with very high allergenic capacity. The aim of this work is to evaluate the stimulatory effect of the major allergen of this species

The present study was carried out on human bronchial epithelial cells (BEAS-2B) transformed by an adenovirus 12 SV40 hybrid. In this context we are interested in the stimulation, in vitro, of those cells by different doses of the major allergen Cup s1 to test the viability and the release of IL-8 and IL-6. After a series of culture, the cells were exposed for 24 hours at a concentration of 0.02µg/µl, 0.06µg/µl, 0.1 µg/µl, 0.3 µg/µl and 0.9µg/µl of allergen Cup s1. The viability was assessed by the MTS assay and the assay of cytokine was carried out in the supernatant using the technology Luminex100. The MTS test showed that cells exposed to different doses were all viable. The release of IL-8 by the cells exposed to different concentrations of Cup s1 showed a highly significant increase with cells exposed to 0.1 µg/µl, 0.3µg/µl and 0.9µg/µl of the major allergen. However, cell culture with 0.1 µg/µl, 0.3 µg/µl and 0.9µg/µl stimulated significant release of IL-6. Our experiments showed that the allergen Cup s1 represents no risk vitality of the cells and had the potential to stimulate the release of IL-8 and IL-6 in a dose-dependent manner.

**Keywords :** Cupressus Sempervirens, Allergy, BEAS-2B, IL-8, IL-6.

## **Performance Comparison of Sustainable Bio Surfactants and Petrochemical Derived Surface Actives in Stain Removal Products**

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### **ABSTRACT**

Surfactants are the active ingredients in detergent formulations as they are responsible for the main cleaning power. It is known that petroleum-derived surfactants cause various ecological damages. Biosurfactants are surfactants of microbial or plant origin. In cleaning products, it is essential to produce a detergent product that is efficient and will meet the needs of the consumer and ensure their satisfaction. There are some desirable quality factors in the detergent product. Rapid and complete dissolution in water, stability, high cleaning power, high biodegradability, safe discharge into the environment. Although it is claimed that petroleum-derived surfactants have higher stain removal power, new generation bio surfactants have been tested to be as effective as petroleum-derived surfactants.

In the study, stain remover products that can be used in laundry containing biosurfactants and petroleum-derived surfactants were created. The stain removal performance of these products was tested using 14 different EMPA standard stains and the results were examined with a D65 light source with UV cut-off filter, Konica Minolta Spectrophotometer, at 420 nm, reflection through a spectrophotometer using the Y value of Y, x, y color coordinates measurement. The results obtained from the sum of 14 spots were calculated as delta L value. As a result of multiple washing performance tests, the  $\Delta L$  value of the stain remover containing biosurfactant was calculated as 402, and the  $\Delta L$  value of the stain remover containing petroleum-derived surfactant was calculated as 400. Although similar results were obtained in total performance, a separate performance comparison was made for each stain.

**Keywords:** Biosurfactant, Stain Remover Performance, Cleaning Product.



## **Trace Elements Analysis in Nutraceuticals with FAAS**

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### **ABSTRACT**

Over the last 30 years, the use of nutraceuticals has received great attention in many countries, not only due to increased consumer awareness but also due to public economic and health crises caused by pandemics, earthquakes, and increasing populations (Djaoudene et al. 2023). Nutraceuticals are used for disease prevention, medical support, control of symptoms, and maintaining health (Vishvakarma et al. 2023; Helal et al. 2019; Başaran 2008). Nutraceuticals can be used as functional foods and beverages or dietary ingredients and are available in the market in powder, capsule, tablet, liquid, and gummy forms and sold in pharmaceutical dosage forms. Nutrients are certain substances, such as vitamins, minerals, amino acids, and fatty acids, that serve important nutritional functions. By law, manufacturers and distributors are responsible for ensuring that nutraceutical dietary supplements contain only labeled ingredients and do not contain harmful substances such as pesticides and heavy metals (Mindak et al. 2008; Avula et al. 2011; Thakkar et al. 2020). Unfortunately, studies have shown that these products cause adverse effects such as hepatotoxicity, renal failure, palpitations, chest pain, tachycardia, and carcinogenicity (Thakkar et al. 2020; Zhu et al., 2019; Avigan et al., 2016; Geller et al., 2015). In addition, various studies have reported that nutraceuticals may contain certain elements, especially arsenic, cadmium, and mercury which can cause harmful effects on humans due to their toxicity (Dolan et al. 2003; Amster et al. 2007; Mindak et al. 2008; Avula et al. 2011). Heavy metal toxicity can lead to various health problems, as well as extreme damage due to oxidative stress caused by free radical formation. Some metals accumulate in the body and the food chain and exhibit a chronic nature.

The purpose of this study is to analyze the inorganic element compositions of nutraceutical products and calculate the deviation of daily intake values from regulatory guidelines and claims. Therefore, 17 different nutraceutical products - including tablets, capsules, powders, and liquids - were selected and purchased online. Their elemental compositions were determined using flame atomic absorption spectroscopy (FAAS) to measure the presence of Fe, Mn, and Zn. Gooseberry supplements are found to be the richest in Fe, Mn, and Zn. To ensure the accuracy and precision of the method, three certified reference materials will be analyzed.

**Keywords:** Nutraceuticals, Heavy Metal, Flame Atomic Absorption Spectroscopy.

## **Chemical Profile, Antimicrobial Potential and Combinatory Antimicrobial Effect of Tea Tree Essential Oil and Propolis**

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### **ABSTRACT**

The antimicrobial resistance is one of the most serious global health threats, heightening the risk of a therapeutic deadlock that is becoming increasingly tangible. Therefore, the development of alternative treatment strategies has become an urgent necessity. Essential oils and natural products such as propolis are potential alternatives worth exploring due to their interesting biological properties. In this line, our study aims to determine the chemical composition of tea tree essential oil (TTO) and propolis (PP), to investigate their in-vitro antimicrobial activities and their combinatory antimicrobial effect to provide data that will enable their use in the development of cost-effective antimicrobial products. The gas chromatography coupled with mass spectrometry (GC–MS) analysis revealed that Terpinen-4-ol (42.89%) was the major components of TTO, whereas pinocembrine (21.22%) was the main constituent of PP. The results of the antimicrobial susceptibility tests demonstrated that the two tested products exhibited an inhibitory effect against all tested bacterial and fungal strains, with MIC values ranging between 0.048 and 0.097 v/v% for TTO and 0.097-0.39 v/v% for PP. The most prominent inhibitory effect of TTO was exerted against *S. aureus* ATCC25923, while PP was more active against *C. albicans* ATCC 26790. Terpinen-4-ol and pinocembrine are mainly responsible for the antimicrobial activity of the two tested products. The checkerboard method indicated an indifferent effect of the TTO/PP combination against all tested microorganisms except for *C. albicans* ATCC 26790, where a synergistic effect was observed. We presume that the indifferent effect is due to the similarity of the sites of action of the constituents of the two tested products.

**Keywords:** Tea Tree Oil, Propolis, Antimicrobial Activity, Checkerboard Test.

## **The Impact of Vacuum Drying on the Quality of Powdered Khalas Dates**

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### **ABSTRACT**

The purpose of this study is to find out how vacuum drying affects the various quality characteristics of Khalas date powder. Three levels of maltodextrin ratio (0, 20, and 40% (w/w)), drying temperature (50, 60, and 70 °C), drying pressure (10, 25, and 40 k Pa), and grinding duration (10, 20, 30, and 40 s) were all examined in relation to the quality of Khalas date powder. Total phenolic content (TPC), antioxidant activity (DPPH), total color difference ( $\Delta E$ ), and basic color parameters (L, a, and b) were used to analyze the samples' quality. Increasing the drying temperature led to an increase in the total phenolic content (TPC). At 70 °C, and 10 kPa, TPC dropped to 5,72 mg GAE/g dry weight as the maltodextrin ratio increased to 40% (w/w). As the maltodextrin ratio and pressure increased, the antioxidant activity (DPPH) decreased but raised with increasing the drying temperature.

**Keywords:** Date, Drying, Maltodextrin Ratio, Phenolic Content

**Acknowledgment:** The authors would like to thank King Abdulazizi City for Science and Technology (KACST) for financially supporting this research through project number 2-17-04-001-0021. The authors also extend their thanks to the Deanship of Scientific Research, King Saud University, for support via the Vice Deanship of Scientific Research Chairs.

## **The Impact of Vacuum Drying Conditions on The Mechanical Attributes of Sukkari Date Powder**

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### **ABSTRACT**

The aim of the current research was to find out the effect of drying conditions on the mechanical properties of Sukkari date powder. The study was done by vacuum drying (VD) the date paste to check the effect of drying conditions on the mean cake strength, bulk density, cohesion index, compressibility, and flow stability of the date powder. The conditions of drying consisted of three levels of drying temperature (50, 60, and 70°C), three levels of drying pressure (10, 25, and 40 kPa), three levels of maltodextrin ratio (0, 20, and 40% (w/w)), and four levels of grinding time (10, 20, 30, and 40 s). The mean cake strength and cohesion decreased with increasing maltodextrin ratio, drying temperature, and drying pressure. The cohesion index increased with increasing grinding time, while mean cake strength decreased to its lowest value of 0.334 N m<sup>-2</sup> with increasing grinding time to 28 s. Mean cake strength increased with increasing grinding time to reach 38.6 N m<sup>-2</sup> at grinding time 40 s. Bulk density and flow stability decreased with increasing maltodextrin ratio, drying pressure, and grinding time, while they increased with increasing drying temperature. Compressibility increased with increasing maltodextrin ratio, drying temperature, and drying pressure, while decreasing with increasing grinding time. The compressibility was in the range of 1.24 to 20.29, and the bulk density was in the range of 0.5872 to 0.7312 g/cm<sup>3</sup>.

**Keywords:** Cake Strength, Maltodextrin Ratio, Mechanical Properties, Sukkari Dates Powder, Vacuum Drying.

## **Content of Heavy Metals, Micro and Macro Elements in Bulgarian Honeydew Honey and Risk Assessment**

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### **ABSTRACT**

This study aimed to determine the content of heavy metals and micro and macroelements in honeydew honey collected from different regions in Bulgaria. A honey samples purchased from apiaries and hypermarkets were tested. Honey samples were prepared for analysis by digestion with nitric acid in a microwave system and analyzed with ICP-OES. The mercury content of the honey was determined without sample digestion with a mercury analyzer. The health risk assessment in the consumers due to ingestion of toxic metals via consumption of honey was estimated by calculating the hazard quotient (HQ), total hazard quotient (THQ), and cancer risk (CR).

The content of elements varies among the honey samples. The range of K predominant in all types of honey (209.9-1911.9 mg/kg), followed by Ca (69.1-192.5 mg/kg), P (31.5-149.8 mg/kg), Mg (28.1-146.7 mg/kg), Na (23.1-130.8 mg/kg) and Al (0.5- 2.99 mg/kg). In terms of microelements, the content of Mn (0.54-28.1 mg/kg) and Fe (3.1-13.0 mg/kg) was the highest, followed by B (1.6 - 9.0 mg/kg), Zn (0.53-2.88 mg/kg) and Cu (0.34-1.15 mg/kg). The content of the toxic metal's ranges from 0.02 to 0.32 mg/kg for Pb, 0.01 to 0.10 mg/kg for Cd, 0.21 to 0.78 mg/kg for As, and 0.10 to 10.9 ng/g for Hg. The composition of the studied honey samples depends mainly on the geographical and geochemical origin. The heavy metal content tested in honey was found to be low, except for the Pb content in 3 honey samples, due to anthropogenic contamination sources around the hives.

The health risk assessment showed that consumers of honeydew honey in most regions of Bulgaria are at a safe range.

**Keywords:** Honey, Trace Elements, Mineral Content, Health Risk Assessment

**Acknowledgment:** This study is a part of the project KP-06-H54/7 "Opportunities to limit mercury exposure to the environment and human health" funded by the Bulgarian National Science Fund.

## **Effect of Mulberry Leaf Powder Additive on Chemical Content and Sensory Properties of Butter Cookies**

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### **ABSTRACT**

Cookies can be enriched with various plant raw materials, which improves their nutritional value and sensory properties. A mulberry leaf powder additive was chosen to enrich the cookies. The aim of this research was to investigate the effect of freeze-dried mulberry leaf powder additives on the chemical and sensory properties of wheat flour butter cookies.

The ingredients of the butter cookies: white wheat flour (550 C type), butter (82% fat), sugar, eggs, and baking powder were purchased from the local market in Kaunas. Mulberry leaf powder amounts were used to replace the percentage (0, 4, 8, and 12%) of wheat flour, improving the nutritional value of the butter cookies. By standard methods, the amounts of protein, fiber, ash, and pH were determined. All sensory properties (inside color, color, taste, flavor, texture, overall assessment) of cookies were evaluated using a 5-point scale.

The use of 4, 8, and 12% mulberry leaf powder additives in butter cookies significantly increased the amounts of protein, ash, fiber, and pH compared to the cookies without additives. Assessing the overall sensory evaluation of butter cookies, cookies with an 8% mulberry leaf additive were rated at 4.55 points.

The current study showed that the freeze-dried mulberry leaf powder additive significantly improved the chemical composition, such as protein, ash, and fiber, of the butter cookies. Cookies with an 8% mulberry leaf additive were most liked by panelists.

**Keywords:** Cookies, Mulberry Leaf, Additive, Fiber, Ash.

## **Effects of Sweet Potato Puree Additive on the Spelt Wheat Bread Quality**

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### **ABSTRACT**

The purpose of this study was to establish the effect of a sweet potato tuber puree additive on the contents of fiber, sugar, ash, some mineral elements, and the antioxidant activity of spelt wheat bread. The bread was made with whole spelt wheat flour, salt, sugar, dry yeast, water, and sweet potato puree. Sweet potato puree substituted a particular amount (0, 20, 35 and 50%) of the spelt wheat flour in the bread. Experiment treatments: 1) control (spelt wheat bread without sweet potato puree); 2) spelt wheat bread with 20% sweet potato puree; 3) spelt wheat bread with 35% sweet potato puree; 4) spelt wheat bread with 50% sweet potato puree. The contents of fiber, ash, and sugar in the bread were determined by standard methods. The DPPH assay was used to measure antioxidant activity, inductively coupled plasma mass spectrometry - to quantify calcium, potassium, magnesium, and phosphorus. The results have shown that the spelt wheat bread with 50% sweet potato puree had the highest amounts of ash, sugar, calcium, and potassium, as well as the highest antioxidant activity. Bread samples enriched with 35% and 50% sweet potato puree contained a significantly higher amount of fiber compared to bread without puree. In conclusion, the enrichment of spelt wheat bread with sweet potato tuber puree might increase its nutritional value and antioxidant activity.

**Keywords:** antioxidant activity, potassium, sweet potatoes, spelt wheat bread, sugar



## **Quantitative Composition of Biologically Active Compounds in the Leaves of the Fireweeds**

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### **ABSTRACT**

The demand for organic production is increasing worldwide. The fireweeds, grown in an organic way, contribute greatly to the idea of a healthier society and clean land. Fireweeds are widespread in the world and have high polyphenols, carotenoids, and antioxidant properties. They can be used for cancer treatment. At present, foods and food supplements rich in antioxidants are very important, but more important is that these products are made from organic raw materials. More and more people tend to consume organic products, because they are associated with higher quality, compared to conventionally grown products. The fireweed (*Chamerion angustifolium* (L.) Holub) (also known as willowherb) is a plant that can solve health problems and is a very popular medicinal plant in Lithuania. One of the current ways to improve extraction and modify bioactive compounds in fireweeds fermented leaves and their bioavailability is solid-phase fermentation (SPF). The purpose of this experiment was to determine the influence of SPF under various conditions on the quantities of polyphenols (phenolic acids, tannin oenothien B, flavonoids) and carotenoids (lutein, zeaxanthin, beta-carotene) in organic fireweeds leaves. The solid-phase fermentation method can be used to purposefully change the quantitative and qualitative composition of biologically active substances in the leaves of the fireweeds. 24 hours aerobic solid-phase fermentation significantly increased the amounts of oenothien B and ellagic acid, which have anticancer properties, in the leaves of fireweeds.

**Keywords:** Fireweed, Organic, Carotenoids, Antioxidant Activity.

**Acknowledgments:** The study was funded by the Ekhagastiftelsen for application “Studies of the variability of biologically active and anticancer compounds in organically and biodynamically grown and fermented fireweed leaves” (no. 2021-67).

**Expression of Neurofilament Medium Chain (*NEFM*) and DEAD-Box Helicase 5 (*DDX5*) genes in ovine mammary epithelial cells after stimulation with *Staphylococcus aureus* *in vitro***

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**ABSTRACT**

*Staphylococcus aureus* is gram-positive bacteria that cause mastitis disease in dairy animals. Mastitis is a condition where the udder of lactating animals gets inflamed due to various pathogens, mainly bacteria. This ailment affects the health of dairy animals and reduces milk production, significantly negatively impacting the dairy industry's economy. In this study, the mammary tissue from a local Turkish breed (Akkaraman) was collected from the slaughterhouse and was isolated according to the used protocol. First the mammary cells were taken from the mammary epithelial cells, then the cells were cultured, stimulated with *S. aureus*, then total RNA was extracted, and cDNA was synthesized. Finally, by using the quantitative real-time PCR, mRNA expression levels of Neurofilament Medium Chain (*NEFM*) and DEAD-Box Helicase 5 (*DDX5*) were investigated as they are involved in the pathways related to the innate immunity, and *GAPDH* gene as the reference gene. Our results showed that the differential mRNA expression of *NEFM* and *DDX5* are higher in stimulated group as compared to control which is involve in the resistance mechanism of ovine mastitis. In conclusion our study revealed for the first time that the exposure to *S. aureus* stimulates the immune response in the sheep mammary gland.

**Keywords:** Sheep, mRNA expression, mastitis, cell culture

**Acknowledgements** This research was funded by Erciyes University Scientific Research Projects Coordination Unit under grant number FYL-2023-12822. The authors also thank the Betül Ziya Eren Genome and Stem Cell Centre (GENKÖK) at Erciyes University for providing laboratory facilities.

## **Application of Bioremediation in Reducing the Content of Hydrocarbons in Soil Polluted from Fuel Oil**

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### **ABSTRACT**

A big percentage of soil and water pollution comes from accidental spillage during exploitation, transport, processing, storage, and extensive use of petroleum. One of the technologies that are increasingly used in the world for the remediation of polluted environments, primarily soil, is bioremediation (use of non-pathogenic microorganisms that use organic pollutants as a source of nutrients). The main objective of this investigation was to answer whether it is possible in the applied conditions of bioremediation to successfully purify the soil contaminated with hydrocarbons from fuel oil? In this study, *ex-situ* bioremediation was performed on the soil from different areas in Serbia contaminated with waste oils from petroleum products. The *ex-situ* bioremediation process was performed at the BREM plant in Dobanovci (N 44°48'52.42" E 20°13'13.08"). Bio-pile for bioremediation was made on a waterproof asphalt surface. Sampling was performed from the bio-piles before/and after bioremediation and from control bio-piles (without added microorganisms) and total petroleum hydrocarbons (TPH) were gravimetrically determined according to Beškoski et al. (2011). For the determination of petroleum hydrocarbons, gas chromatography (GC) was used. The content of TPH, obtained by the gravimetric method, for the sample before bioremediation and the control sample was around 22%, while TPH content for the soil sample after bioremediation was 1.62%. The TPH content decreased by 92.9 % in the bioremediation process, while in the control sample it decreased by only 3.02%. GC-analysis shows a significant decrease, the content of hydrocarbons with 17C and 18C atoms (pristane and phytane). The applied bioremediation procedure has significantly reduced the TPH content, which indicates that the soil contaminated with fuel oil was successfully cleaned.

This research has been financially supported by the Ministry of Science, Technological Development and Innovation of Republic of Serbia (Contract No: 451-03-47/2023-01/200026).

**Keywords:** Bioaugmentation, *Ex-Situ* Bioremediation, Fuel Oil, Pristane, Phytane

## **Okara-Enriched Gluten-Free Bread: The Changes in Antioxidant Properties**

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### **ABSTRACT**

The aim of this study is to determine the changes in antioxidant capacity of soluble and insoluble fractions of gluten-free bread enriched with okara during *in vitro* gastrointestinal digestion (GID). Changes in antioxidant capacity were monitored after the oral, gastric, and intestinal phases using two antioxidant assays: total antioxidant capacity (ABTS) and total antioxidant reducing capacity (TPC). Results of the ABTS and TPC assays show that the antioxidant capacity of the soluble fractions increased during *in vitro* digestion ( $97 \pm 14,23$  to  $306,7 \pm 13,10$  mg Trolox/100 g and  $91,83 \pm 1,08$  to  $263,17 \pm 9,03$  mg GAE /100 g, respectively). Antioxidant capacity of the soluble fraction measured by ABTS assay remained the same after oral and gastric phases and then increased after intestinal phase, while measured by TPC assay results showed that the antioxidant capacity of the soluble fraction increased after each stage of digestion. For the insoluble fraction, results showed that antioxidant capacity measured by ABTS assay did not change during or after *in vitro* GI digestion compared to the control, while TPC results showed that the antioxidant capacity of the insoluble fraction decreased after the gastric phase compared to the end of oral phase and reached the highest value after the intestinal phase. Antioxidants from okara and gluten-free flours contributed significantly to the antioxidant capacity of the bread studied, regardless of the assays applied. The overall effect of *in vitro* GI digestion on the antioxidant capacity of the digest, considered as the sum of the antioxidant capacity of the insoluble and soluble fractions after the intestinal phase, was three to four times higher than the antioxidant capacity of whole undigested bread, depending on the antioxidant assay applied (TPC or ABTS).

**Keywords:** gluten-free bread, okara, antioxidant capacity, *in vitro* gastrointestinal digestion (GID)

**Acknowledgment:** This work was supported by the Ministry of Science, Technological Development and Innovation of Republic of Serbia Grant No. 451-03-47/2023-01/200116

## **The Effects of Rhizobacteria on Sugar Beet**

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### **ABSTRACT**

This research, was carried out to observe the effects of nitrogen-fixing rhizobacteria applications at different zinc doses on yield and quality of sugar beet (*Beta vulgaris* L.) in Kayseri climate conditions. Planting, it was carried out in the application area of Erciyes University Faculty of Agriculture in May. In the experiment, 4 different zinc doses (0,100, 200, 300 ppm; Ç0, Ç1, Ç2, Ç3, respectively) according to field capacity, 2/3 as base fertilizer DAP, 1/3 after first hoeing, top fertilizer urea were applied. Two different symbiotic bacteria B1 (*Bacillus atrophaeus*) and B2 (*Bacillus taylori*) were in the research. In the experiment, the effects of rhizobacteria on yield and quality of sugar beet were observed in different doses of zinc applications. According to the results the highest sugar beet root yield Ç2B2 (3023.00 kg/da), technological beet yield BB (17.15%), sugar yield Ç2B2 (560.46 kg/da), the highest sugar presence rate BB (19.31%), alpha amino nitrogen control (4.63%), ash value Ç0 (5.24%), S value BB (28.26%), sodium rate Ç2B1 (0.87%) and potassium Ç2B1 (6.37%) applications. According to the determined results, it was determined that zinc x rhizobacteria applications were effective on agricultural properties of sugar beet. In this regard, it is recommended for sugar beet farmers and producers to apply both rhizobacteria, but the use of zinc at the Ç3 level is not recommended. It is recommended to use a Ç0 level zinc dose against salt stress.

**Keywords:** Sugar beet, Rhizobacteria, Zinc, Yield, Quality

## **Determination of Antioxidant Activity and Total Phenolic and Flavonoid Content of Walnut (*Juglans Regia*) Leaves Collected from Kayseri Region**

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### **ABSTRACT**

Since ancient times, medical plants have been used to treat various diseases<sup>1</sup>. The green shell, seed, kernel and leafs of walnut (*Juglans regia*) plant are used in traditional medicine for their hypoglycemic, antidiarrheal, antifungal, hypotensive, sedative, vascular strengthening, hemostatic, and anthelmintic properties<sup>2</sup>. As it is known, oxidative stress is the cause of many important diseases, and for this reason, the antioxidant effect of walnut plant has been examined pharmacologically in some studies<sup>3</sup>.

In this study, walnut leaves were collected from some walnut (*Juglans regia*) trees growing in the Kayseri region (Büyük Büzüngüz area in the Koramaz Valley, Türkiye) at different periods of the year. Then, the antioxidant activity and total phenolic content of these collected walnut leaves were determined. For this purpose, firstly, walnut leaves were collected periodically at 3-month intervals from June 2022 to November 2022. Then the leaves were dried properly. After that, dried walnut leaves were extracted successively with methanol: water (3:4) by using Soxhlet technique. The phenolic and flavonoid content of obtained extracts were determined by spectrophotometrically. The antioxidative activity was also tested by spectrophotometrically. Then, the antioxidant activity, total phenolic and flavonoid contents of the extracts obtained from periodically collected leaves were investigated and compared with those collected in other periods.

**Keywords:** Medical plants, *Juglans regia*, antioxidant activity,

**Acknowledgments:** This study is supported by the Scientific Research Projects Committee of Erciyes University (Project No: FYL-2022-12239).

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# **ORAL PRESENTATIONS**

## **FULL-TEXT**



## **Determination Genetic Diversity of Some Confectionery Sunflower (*Helianthus annuus* L.) Cultivars Cultivated using SRAP Markers**

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### **ABSTRACT**

Sunflower is generally produced and consumed as oil around the world. However, in addition to this broad use, confectionery consumption is also common lots of countries such Turkey. Turkey is one of the countries in the world where confectionery foods are consumed extensively. For high yield and adaptation in sunflower, there are new breeding programs in Türkiye like around the world. For breeding programs, it needs genetic diversity. Determination of genetic diversity in plants, there are different methods. One of them is molecular markers. A lot of molecular marker system have used molecular characterisations. Different kinds of molecular markers were used for sunflower. Sequence-related amplified polymorphism (SRAP) markers have used for genetic characterisation in plants such as sunflower. In present study, it was aimed that determination genetic diversity of some confectionery sunflower cultivars cultivated using SRAP Markers. 11 confectionery sunflower genotypes and 5 ornamental sunflower genotypes as control were evaluated 10 SRAP primer combinations. Results showed that there are broad variation among confectionery sunflower genotypes and SRAP marker system is suitable for determination in sunflower genotypes. Obtained results from present study can be use for sunflower breeding programs.

**Keywords:** Confectionery Sunflower, SRAP, Genetic Diversity.

### **INTRODUCTION**

Sunflower is the fourth most important oil plant worldwide and the second most important oil crop Europe, along with rapeseed. Sunflower is a very important oil plant that is widely planted in our country and around the world. It is extremely important to increase the yield of sunflower, which is the most planted oil and snack plant in our country. There are three types of sunflower oilseed, confectionary and bird food. The confectionary and bird food sunflower contain high protein contents (>40%) and low oil contents (≤30%). The confectionary and bird food sunflower are large seeded and stripped with 100-seed weight greater than 10g(Fig 1). Oil contents types are small seeded and black color. Oil-containing species have small seeds and are black in color. Confectionery sunflowers have white, striped colors. Ornamental sunflowers also come in many different colors (Figure 2).

In order to increase grain yield and therefore production and to obtain higher quality sunflowers, it is absolutely necessary to transfer genes that are resistant to adverse environmental conditions and new yield-enhancing features to currently planted varieties. It is possible to increase the genetic productivity capacity to the upper limit by using biotechnological methods as well as varieties obtained using traditional breeding methods. Biotechnological methods generally include plant tissue culture and a combination of molecular biology and cytochemical methods. This study will focus more on plant molecular biology methods.

Several different methods are used to develop inbred lines depending on the source populations available and specific program objectives. The most common procedure involves selection of individual plants within open pollinated cultivars or segregating generation of planned crosses and after development inbred lines must evaluate its.

Identification of sunflower cultivars, lines and hybrids is mainly based on morphological traits which were among the earliest markers used in germplasm management (Stanton, et al., 1994) despite a number of limitations, including low polymorphism, low heritability, late expression, and vulnerability to environmental influences (Smith, and Smith, 1992; Konarev, 2000 and Muthusamy et al., 2008), which, in turn may affect the estimation of genetic relationships. Molecular genetic markers have many advantages over morphological traits including higher information content, which is of primary importance for systematization and registration of collection specimens. The random amplified polymorphism DNA (RAPD) (Isaacs et al., 2003 and Wangsomnuk et al., 2011) and inter simple sequence repeats (ISSR) (Preeya et al., 2011) were among the earliest developed molecular tools used to assess plant genetic diversity due to technical simplicity and practical feasibility. For example, the RAPD and ISSR markers require no prior sequence information for the survey of plant genomes, but generally suffer from low resolution due to various issues associated with reproducibility; dominance and non homologous DNA fragment (Nybom, 2004). The sequence-related amplified polymorphism (SRAP) (Li and Quiros 2001) represents another simple and reliable PCR-based marker tool for genetic diversity analysis (Ferriol et al., 2003 and Budak et al., 2004).

The objectives of this study were: For breeding programs, 11 confectionery sunflower genotypes and 5 ornamental sunflower genotypes as control were evaluated 10 SRAP primer combinations., it needs genetic diversity Different kinds of molecular markers are available for sunflower, Sequence-related amplified polymorphism (SRAP) markers have used for genetic characterisation in plants such as sunflower.

## **MATERIAL AND METHODS**

DNA isolation was performed by taking leaf samples from sunflowers grown in the greenhouse of Erciyes University. . 11 confectionery sunflower genotypes and 5 ornamental sunflower genotypes as control were evaluated 10 SRAP primer combinations.

DNA concentration was measured with a microplate spectrophotometer (BioTek Instruments, Inc. Vinoski, USA), and DNA was diluted to 10 ng/mL using TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). A total of 6 SRAP primer combinations were used in this study (Table. 1).



Figure 1. The confectionary and bird food sunflower are large seeded and stripped



Figure 2. Confectionery sunflowers

PCR reaction components and PCR cycling parameters for SRAP analysis was performed as described by UZUN et al. (2009). Each of the 15  $\mu$ L reactions consisted of 1.33 mM of primers, 200  $\mu$ M of each dNTP, 1.5  $\mu$ L of 10X PCR Buffer (Biorun, Nantes, France), 2 mM of  $MgCl_2$ , 0.8  $\mu$ g/ $\mu$ L Bovine serum albumine, 5.8  $\mu$ L ddH<sub>2</sub>O, 1 unit of Taq polymerase (Biorun, Nantes, France) and 20 ng of template. DNA Thermal Cycler (Sensoquest Progen Scientific Ltd. Mexborough, South Yorkshire, UK) was used and cycling parameters included 2 min of denaturing at 94 °C, five cycles of three steps: 1 min of denaturing at 94 °C, 1 min of annealing at 35 °C and 1 min of elongation at 72 °C. In the following 35 cycles, the annealing temperature was increased to 50 °C, and the extension per cycle was set to 5 min at 72 °C. PCR products were separated on a 2% agarose gel in 1X TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA) at 115 volt for 2.5-3 h. The fragment patterns were photographed under UV light for further analysis. A 100 bp standard DNA ladder was used as the molecular standard to confirm the appropriate markers for SRAP analysis.

Table. 1 Total number of bands (number), number of polymorphic bands (number), number of monomorphic bands and polymorphism ratios obtained as a result of amplification of SRAP primer combinations (%)

Marker combination	Base	Total	Polymorphic	Monomorphic	% Polymorphism
Em1Me2	50-550	6	4	2	67
Em5Me3	100-900	7	3	5	43
Em5Me4	100-800	7	6	1	86
EM6Me7	100-900	8	2	6	25
Em3Me7	100-1000	6	4	2	67
Em2Me2	100-900	8	4	4	50
	<b>Total</b>	<b>42</b>	<b>23</b>	<b>20</b>	<b>55</b>
	<b>Max.</b>	<b>8</b>	<b>6</b>	<b>6</b>	
	<b>Min</b>	<b>6</b>	<b>2</b>	<b>1</b>	

## DATA ANALYSIS

Following the imaging processes, band existence was scored as (1), inexistence was scored as (0) and non-amplification was scored as (9). Data obtained with the use of NTSYSpc 2.1 software were analyzed, similarity matrix was generated with the use of DICE, (1945) method and a dendrogram was generated for wild pear genotypes in accordance with UPGMA method. For each marker used in present study, total number of bands, number of polymorphic bands and

polymorphism ratios were determined. The formula of (number of polymorphic bands x 100 / Total number of bands) was used while calculating polymorphism ratio.

## RESULTS AND DISCUSSION

In this study, 16 sunflower genotypes were used as material and 6 SRAP marker combinations were used. Molecular characterization was performed using a combination of markers. A total of 42 scoreable bands were obtained from 6 SRAP marker combinations, and 20 of these bands were determined as polymorphic. The mean polymorphism was 55%. The highest number of bands was obtained from primer combinations EM6Me7 and EM6Me7 (8 bands), while the lowest number of bands was obtained from primer combination Em1Me2 and Em3Me7. The highest polymorphism was obtained with Em5Me4 (86%) primer combination, while the lowest polymorphism was obtained with EM6Me7 (25%).

The dendrogram obtained as a result of the characterization study performed on 16 sunflower genotypes with 6 SRAP marker combinations is given in Figure 3.

According to the dendrogram obtained, 2 main groups were formed with sunflower genotypes. OR1, OR2 and OR3 were in the 1st main group, while the Confectionary genotypes were in the 2nd main group with OR5 and OR4 ornamental genotypes. On the other hand, while genetic similarity was determined between 0.54-1.00 in dendrogram, genotypes CON1 and CON2 were determined to be similar to each other.

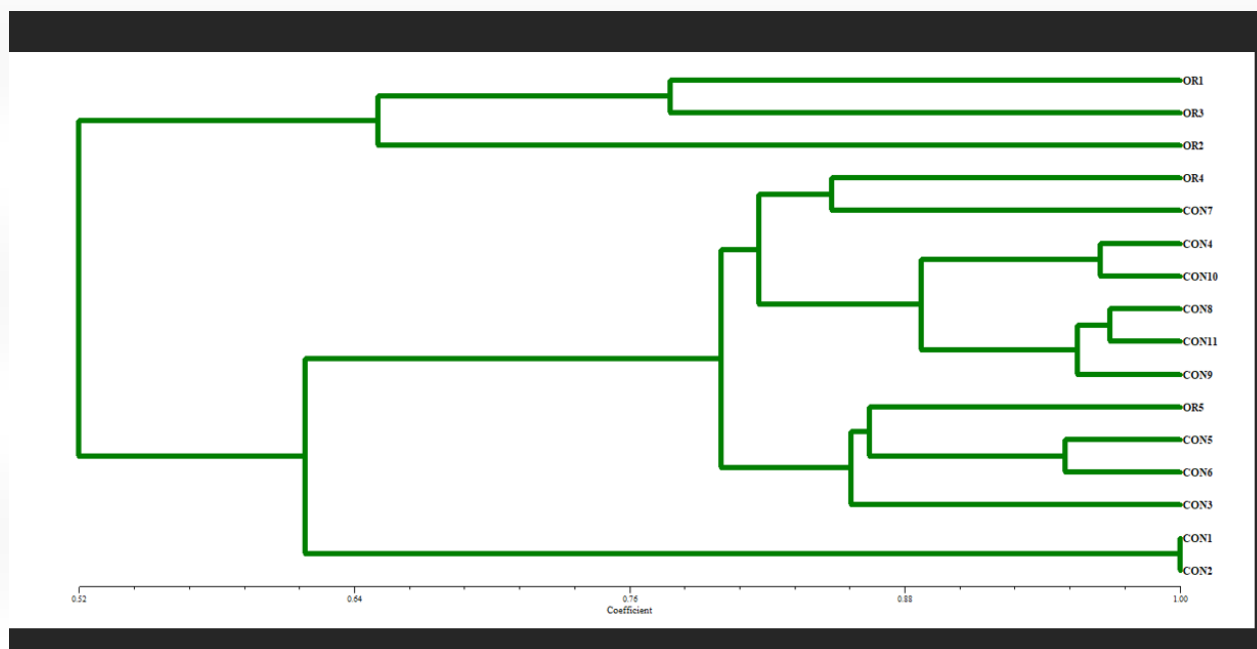


Figure 3. Dendrogram obtained from POX molecular markers characterization of sunflower genotypes



## **DISCUSSION**

Molecular markers are crucial for understanding genome organization and provide important advantages in the means of development of new lines and determination of differentiation between initial germplasm (Balalic, 2009 and Ahmed et al., 2022). The development of molecular markers in sunflower is at an advanced level and different types of markers have been developed for marker-assisted selection (MAS) over the years (Zia et al., 2014). There are numerous different molecular markers available which can be used in sunflower breeding (Şahin et al., 2018). The alternative approach is the application of molecular markers for varietal identification, hybrid confirmation and parental selection for hybridization programs. Genetic impurity of hybrids is a widely spread issue in cross-pollinated crops like sunflower while morphological description and discrimination based merely upon phenotype, are clumsy and less reliable (Zeinalzadeh-Tabrizi et al., 2018).

The development of sunflower hybrids set up sunflower as a major viable crop worldwide and encouraged the founding of numerous public and private breeding centers (Bohra et al., 2016 and Seiler et al., 2017). In recent years, public and private sector contributed to assemble huge plant genetic resources in sunflower, to identify markers for marker assisted selection (MAS) and to establish the use of new high-throughput technologies in sunflower (Yadav et al., 2018 and Dimitrijevic 2018).

The Rf gene from the confection line RHA 280 was assigned to LG 7 of the SSR map. The PET1/Rf1 system is extensively used for commercial sunflower hybrid seed production worldwide (Jan and Vick 2007). New CMS sources and the corresponding Rf genes will provide more diversity and reduce the risk for sunflower production. A previous study reported that Rf3 in the confection line RHA 280 is a unique fertility restoration gene, different from Rf1 and Rf2, which can restore CMS HA 89 with PET1 cytoplasm or the 22 induced CMS mutants from HA 89 (Jan and Rutger 1988).

So far, 72 new CMS sources have been described for sunflower (Serieys, 2005). However, only for very few of these CMS sources markers have been detected linked to the corresponding restorer genes (Horn et al., 2016). Feng and Jan (2008) tagged an additional restorer gene Rf4 with molecular markers and assigned it to LG3 of the sunflower general reference map (Tang et al., 2003). Rf4 is restoring male fertility to a newly identified CMS cytoplasm GIG2. Schnabel et al. (2008) identified AFLP markers that mapped in close vicinity of the restorer gene Rf\_PEF1, which represent a major restorer gene for the PEF1 CMS cytoplasm, another potentially interesting CMS source for commercial sunflower hybrid breeding.

## **CONCLUSIONS**

It is seen that wide variation is obtained in the molecular characterization studies of some sunflower genotypes in Turkey. Results showed that there are broad variation among confectionery sunflower genotypes and SRAP marker system is suitable for determination in sunflower genotypes. Obtained results from present study can be use for sunflower breeding programs.

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## **Possibilities to Increase the Production of Medicinal and Aromatic Plants in Türkiye**

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### **ABSTRACT**

Due to social concern about healthy living and the changes that will occur now, and, in the future, more and more people are turning to try and use medicinal and aromatic herbal products. In today's modern society, consumers are becoming increasingly knowledgeable about healthy nutrition and clean ingredients. For this reason, consumers are turning to organic food, natural agricultural products and alternative treatments, and the demand for medicinal and aromatic plants in the global market is expected to continue increasing. To meet the increasing demand for medicinal and aromatic plants, rapid cultivation of these plants is of vital importance in protecting biodiversity and preventing smuggling. In this context, many different types of field agriculture are produced in different countries and medicinal plants are produced. Turkey already has the necessary infrastructure and control mechanisms for dosage, indication and standardization of herbal products licensed under the status of 'Traditional Herbal Medicinal Product'. There is no control mechanism regarding the marketing of herbal medicines or preparations that are available in the market as food supplements or sold openly by herbalists. In the use of these products, necessary sensitivity must be shown in terms of standardization, drug interaction information and side Activities that should be implemented to increase the production of medicinal and aromatic plants: 1)Problems Related to Collection from Nature, 2)Cultivation, 3)Sustainable Raw Material Production and Planning, 4)Production Planning, 5)Support Policies, 6)Seed Supply, 7)Determination of Agronomic Breeding Packages, 8)Production of Value-Added Products, 9)Harvest, Drying and Preservation Techniques, 10)Combating Adulteration, 11)Productivity-Quality-Innovation and 12)Education and Social Awareness.

**Keywords:** *Medicinal and aromatic plants, production, essential oil*

### **1. INTRODUCTION**

Medicinal and aromatic plants are often used for a variety of purposes, including medicinal, cosmetic, aromatherapy and culinary. These plants have been used by people for centuries because they often have special properties (Alston ve Pardey, 2014). Medicinal plants are used to treat or provide relief from health problems. Various parts of these plants, especially their leaves, roots,

flowers, or fruits, are used in traditional medicine and in the modern pharmaceutical industry. Its natural ingredients often contain a range of bioactive substances and can therefore have a variety of health benefits. Aromatic herbs generally provide pleasant odor and flavor. The essential oils of these plants are used in the perfumery, cosmetics, food, and beverage industries. Additionally, it can be used in aromatherapy applications to provide sensory relaxation and well-being. The scents, flavors, and essential oils of these plants often make them valuable to many industries. These various uses of medicinal and aromatic plants play an important role in meeting people's needs regarding health, beauty, and pleasure. Additionally, commercial production of these plants is an important source of agricultural income for many countries Arslan et al., 2015).

Due to demographic characteristics such as age, culture, and income level in society, as well as social concern about healthy living and changes that will occur now and, in the future, more and more people are turning to try and use medicinal and aromatic herbal products (Bayram et al., 2010).

For this reason, the demand for medicinal and aromatic plants in the global market is expected to continue to increase. In today's modern society, consumers are becoming increasingly knowledgeable about healthy nutrition and clean ingredients. In addition, discussions and concerns about issues such as traditional medical treatments, genetics and pesticides in agricultural products are increasing. For this reason, consumers are turning to organic food, natural agricultural products, and alternative treatments. In the definition made by the World Health Organization (WHO) for traditional medicine; In addition to the treatment, diagnosis, healing, and treatment of mental and physical diseases, it is a set of practices based on the experiences and beliefs of different cultures for sustainable health (WHO, 2016). It is also known that approximately 20,000 plants are used for medicinal purposes and approximately 4 billion people (50% of the world's population) try to solve their health problems with herbal drugs in the first place. In developed countries, approximately 25% of prescription drugs consist of drugs whose active ingredients are obtained from plants (such as vinblastin, reserpine, quinine, aspirin).

According to the International Union for Conservation of Nature (IUCN), some species are in danger of extinction in regions where medicinal plants are used extensively today, such as Asia and Africa. According to the latest data, 15,000 medicinal plant species in the world are endangered to varying degrees for many reasons. Therefore, to meet the increasing demand for medicinal and aromatic plants, rapid cultivation of these plants is of vital importance in protecting biodiversity and preventing smuggling. Cultivation efforts have been continuing for many years in countries that trade medicinal plants around the world. In this context, many different types of field agriculture are produced in different countries and medicinal plants are produced (Muluneh, 2021).

### **The Importance of Türkiye in Terms of Medicinal and Aromatic Plants**

Turkey is a country with a high potential for natural growth of medicinal and aromatic plants due to its rich biodiversity, climate diversity and soil structure. Türkiye is a small continent in terms of biodiversity. The fact that it hosts three phytogeographical regions: Europe-Siberia, Mediterranean and Iran-Turan has brought a wide variety of ecosystems to our country (Figure 1). This diversity allows the growth of many medicinal and aromatic plants. Additionally, the fact

that Türkiye has hosted many civilizations throughout history indicates a rich plant knowledge in traditional medicine practices. Many medicinal plants are known and used among the public (Acıbuca ve Bostan Budak, 2018). Many farmers earn income from growing these plants. In addition, Türkiye is an important country where medicinal and aromatic plants are exported. Especially medicinal plants and spices are in demand in international markets.



Figure 1. Map of Phytogeographical regions in Türkiye

Agricultural and scientific institutions in Turkey conduct research for the sustainable production and trade of medicinal and aromatic plants. Studies on the conservation and breeding of plant genetic resources are important. However, it is important to increase studies on the status and potential of medicinal and aromatic plants in Turkey, collect more information and data in this field, promote sustainable agricultural practices and make strategic plans to increase trade (Karık and Tunçtürk, 2019). These efforts can help Turkey evaluate its potential in these plants more effectively and use them sustainably.

It is estimated that 70% of the medicinal plants used today are collected from nature and 30% are cultivated. These products are mostly offered to the public and purchased by herbalists and spice sellers in our country. Selling these products freely or without adequate supervision in places other than pharmacies may pose a danger to public health. With the local pharmaceutical industry taking ownership of the issue, expensive imported herbal products can be made cheaper with domestic production, and it may be possible to provide real treatment by increasing the use of standardized herbal products.

The number of plant species collected from nature for commercial purposes in Türkiye and sold in the domestic and foreign markets is approximately 350. The number of endemic species among them is 35. In other words, approximately 10% of traded species are endemic. Around 200 natural plant species are sold in herbalists in the country. The number of natural plant species collected from nature and sold abroad is approximately 100. On average, 10-12% of natural plant species

are used for various purposes. When all these are evaluated together; It is estimated that at least 1000 of the species in our country are used in various ways and recently about 500 of them have been traded.

Thanks to its geographical location, climate and plant diversity, agricultural potential and large surface area, Türkiye has an important place in the production (including production by collecting from nature) and trade of medicinal and aromatic plants. To maintain and increase the market share, standardization must be ensured in the products offered to the market and the quality must be at the desired specifications. To obtain quality and standard products that can meet consumer and industrialist demands; It is necessary to produce breeding information on the species and varieties needed by the domestic and foreign markets, under farmer conditions, to disseminate this information, and to develop post-harvest operations and processing technology. When the issue is approached from a public health perspective; In our country, there are currently necessary infrastructure and control mechanisms for dosage, indication and standardization in human medicinal products and herbal products licensed with the status of 'Traditional Herbal Medicinal Product'. However, since there is no control mechanism or obligation to market the herbal drugs or preparations that are available in the market as food supplements or sold openly through herbalists, the necessary sensitivity must be shown regarding the use of these products in terms of standardization, drug interaction information and side effects (Bayram et al., 2010).

## Activities to Increase the Production of Medicinal and Aromatic Plants

### 1. Problems and solution suggestions regarding collecting from nature

Procuring raw materials from natural flora is mostly achieved by collecting non-wood forest products. When collecting from nature, plants that "true to their name" should be used within the balance of conservation and use, and the principle of "sustainable use" should be taken into consideration. Plants growing in our country's flora are in danger of extinction due to reasons such as indiscriminate collection, industrialization, urbanization, field clearing, overgrazing, tourism, reclamation of barren-saline areas, pesticide residues and weed control and afforestation. Due to these mentioned factors, there is a decrease in biological diversity, the extinction of the collected species and the disappearance of treatment opportunities for the people treated with these plants. Species in danger of extinction (*Gentiana lutea* L., *Galanthus elwesii* Hooker fil., *Orchidaceae* ssp., *Rubia* ssp., *Isatis* ssp., *Gypsophila arsusianum*, *Glycyrrhiza* ssp., *Sideritis* ssp.) should be protected in the natural flora (Akalan et al., 2020).

To reduce the harms of collecting from nature; I. Regulation and control of collection (Alternate collection, Rest collection, Regular collection, Restriction of exports), ii. Training (Collector training, technical personnel training on the subject), iii. Administrative and Legal Measures and iv. National Parks and nature conservation areas need to be established (Karagoz et al., 2016).

### 2. Cultivation

Failure to ensure standardization in collections from natural flora and collection of unintended materials reduces the chance of competition in international trade and negatively affects continuity in marketing. After the international "CITES Convention", which was made for the purpose of protecting plant diversity and preventing its destruction, the phrase "obtained from culture" is



required in the import and sale of these plants to prevent plant collection. The species to be cultivated should be determined in line with inventory studies and market research, and value chain studies on this subject. should be done. It should be ensured that materials that are in demand in the product market and that can be grown under our country's conditions are grown in appropriate climate and soil conditions, in line with the active ingredient contents appropriate to the pharmacopoeias.

The benefits of culturing medicinal plants can be listed as follows: 1. Due to collection, there is a decrease in nature over time, these plants cannot be used, therefore they need to be cultured., 2. Cultivation is more advantageous, especially for those that are rare in the flora., 3. More product is obtained from unit area., 4. Changing and mixing processes are minimal., 5. Quality control is easier in cultivated species., 6. New varieties can be developed through breeding in cultivated plants., 7. Loss of rare and endangered plants is prevented (Alamgir, 2017).

### **3. Sustainable raw material production and planning**

To provide raw materials to the sector without interruption, importance should be given to both environmental sustainability, which will protect biological diversity, and economic sustainability, which will ensure the continuity of the parties involved in the production phase. Carrying out collection activities from nature in accordance with the criteria of "good collection" and cultivation in accordance with the criteria of "good agricultural practices" will make significant contributions to environmental sustainability. For economic sustainability, contract production and organization of small-scale producers are of great importance. Medicinal and aromatic plants, grains, vegetables, and fruits etc. It does not have a widespread marketing network and usage area like the products. In this case, there is a marketing problem for farmers who grow or collect these products from nature, and there is a problem of access to raw materials for manufacturers who use these products as raw materials (Mathe et al., 2015). In addition, the same quality and quantity of products cannot be obtained every year by simply collecting them from nature. For this reason, a contract production model is needed for the overall sustainability of the sector. Contract production should be encouraged through regulations. Cooperative etc. of the contract production model. If carried out together with organizational models, the integration of small-scale production in the form of family farming with the market will be easier, and economic sustainability will be established.

### **4. Production planning**

There is a need to determine the production amounts of the species and varieties that are and will be cultivated. A production plan should be planned, giving priority to products with high added value and that can be procured from our country. For this purpose, in addition to sectoral reports, product-based reports should also be prepared and presented to the manufacturer and relevant stakeholders. Medicinal plant production zones should be established in our country by ensuring coordination between the relevant faculties of universities, relevant units of ministries and local governments and relevant commercial organizations operating in the field of medicinal and aromatic plants (Inoue et al., 2019). The species to be grown on the lands to be allocated for medicinal and aromatic plant cultivation should be determined by experts. Allocating more arid and marginal lands rather than wetland and bottom lands will allow these areas to be utilized and

brought to our country's agriculture with higher added values.

### **5. Support policies**

To increase the production of medicinal and aromatic plants, increase efficiency and quality, ensure sustainability, and develop environmentally friendly alternative agricultural techniques, the General Directorate of Plant Production of the Ministry of Agriculture and Forestry has a special support program for producers. However, these supports need to be increased.

### **6. Seed supply**

It stands out as one of the most important problems encountered in the cultivation and cultivation of medicinal and aromatic plants. Supply of sustainable and quality raw materials; This makes it necessary for these plants to be cultivated. No product planning or quality standardization is possible with material collected from natural flora. Considering the richness of our country's flora and its importance in terms of medicinal plants, the number of existing varieties (119) is low (TTSM, 2023). A significant portion of the herbal drugs still produced are produced from seeds collected from the population, local ecotype, or nature. Precautions should be taken to protect variety purity during both variety development and seed production stages. It is very important to develop and commercialize the certified seed, seedling and sapling sector in the seed growing of medicinal and aromatic plants. In this context, seed producers, breeders and seed processors who want to operate in medicinal and aromatic plants should be encouraged.

### **7. Determination of agronomic breeding packages**

Depending on the characteristics of medicinal and aromatic plants, their generative and/or vegetative parts can be used as seeds. In the agronomic package to be determined in production.

- fertilization biology (specific to self and foreign pollinated plants),
- Amount and number of seeds to be sown/planted per decare,
- cultural procedures to be applied (e.g. weed control, irrigation, hoeing, thinning, tip breaking, etc.), mechanization status,
- harvesting-threshing methods (manual or machine) and
- should be determined specifically for each plant, considering the characteristics of the targeted product.

When the seedlings and cuttings needed in perennial plantation plants (e.g. lavender, sage, thyme, mint, etc.) are grown by the producers themselves in hot and cold pads or seedlings in a part of the field, the initial plant cost will be quite low. Although mulch application in perennial species increases the facility cost slightly, it will facilitate weed control, reduce labor costs, and will also prevent weed confusion during harvest. The use of agronomic methods suitable for farming will reduce labor costs (especially the establishment of row distances suitable for mechanization for weed control). Production by clone or stolon can sometimes be advantageous compared to production by seed. Although obtaining 8-10 cm seedlings from seeds can take two months, rooting of clones taken with upper cuttings can be achieved in three weeks when conditions are suitable. Medicinal and aromatic plants are species that have many by-products (e.g. leaves,



flowers, essential oils and hydrosols, etc.), and correct evaluation and marketing of by-products can reduce the production cost. The most important factors behind reducing production costs are conscious production practices as well as following the developments on the subject.

### **8. Production of value-added products**

It is known that our country's biggest problem regarding medicinal and aromatic plants is its inability to produce value-added products. Obtaining sustainable, high-quality, and standardized products is very important in the production of value-added products. Plants used medicinally must have properties that comply with the standards in pharmacopoeias. In products produced according to pharmacopoeias, pesticides, aflatoxins, microbiological contamination, and heavy metal analyzes must be tested both in the plant and in the finished product according to pharmacopoeia requirements. Contents of the products; Since it may vary depending on geographical conditions, collection conditions, time of production, and product production conditions, the content amounts must be analyzed and standardized to be used as a nutritional supplement and medicine. Before the content analysis of the products, the type and type of the product must be determined correctly, and after the determination, toxic substance analyzes must be determined after it is extracted with appropriate extraction methods (Rao et al., 2022). “Turkish Standards Institute” has studies on some medicinal and aromatic plants. However, these are not sufficient and only cover certain plants. These standards should be expanded and adapted to current conditions.

### **9. Harvest drying and preservation techniques**

In the cultivation of medicinal and aromatic plants, harvesting is generally done by human power. The need for labor in the production process of medicinal and aromatic plants is higher than in the production stages of other plants. The need for labor increases in the harvesting and drying stages, as well as in the production process where chemical use is limited. The excess of labor-related costs weakens the competitiveness of our country compared to other countries that cultivate medicinal plants. To increase our competitiveness, medicinal plant producers, university mechanical engineering, agricultural equipment and machinery departments and agricultural machinery manufacturers should be brought together. There is a need for mechanization of medicinal plant harvest and subsequent medicinal plant processing (sifting; washing; leaves, stems, foreign matter separation machines, etc.). Drying and preservation are important quality parameters in the medicinal plant trade. A well dried plant; humidity, light, heat, etc. When stored in an environment where conditions are unfavorable, the effort and time spent on drying is wasted. There is a lack of knowledge of producers and collectors regarding good and correct drying techniques. Training activities on these issues should be carried out by relevant ministries in regions where cultivation or gathering is prevalent. In our country, there are no incentives or guidance regarding drying methods and drying systems for businesses operating in agricultural machinery production. Bringing together industry stakeholders is important in terms of harmonizing supply and demand in this field. It would be appropriate to create incentive policies for the establishment of drying and processing facilities in regions where collection and cultivation are high. In appropriate packaging, there should be criteria on the packaging regarding the Latin drug name of the plants, harvest date, drying, sterilization method and how they will be stored

until the end consumer. Standardization studies to be carried out with all stakeholders on this subject; It will ensure that quality is maintained in the process from the producer to the trader and the end consumer (Oztekin and Martino, 2014).

### **10. Efficiency-quality-innovation**

Developing production in accordance with domestic and foreign demand, Producing for the market at international standards, -Ensuring the protection and sustainability of natural and biological resources, -Establishing contract production models that will ensure the supply of products in the quantity and quality desired by the buyer, -Strengthening producer, processor and exporter ties , - Obtaining geographical indications of commercially prominent products under current conditions in the target region, - Growing the right product in the right region and supporting this production planning, - Establishing quality, effectiveness and reliability criteria for the use of herbal products prepared from medicinal plants used in the pharmaceutical, cosmetic and food industries. and raising consumer awareness through public service announcements are important.

### **11. Education and social awareness activities**

Lack of awareness about the usage areas and forms of medicinal and aromatic plants, especially products that pose health risks, can cause serious health problems. On the other hand, according to the results of the survey conducted by the Ministry of Health; It has been determined that 60% of people in our country use traditional and complementary medicine methods for treatment at some point in their lives. It was recorded that 59% of them used herbal or herbal mixtures. On the one hand, the existence of masses who want to be treated with plants, and on the other hand, the fact that these masses do not have sufficient information, reveals the importance of education and social awareness activities in this field. There is insufficient information on which part of the plants to harvest/collect and when. To collect the right plant species, precautions must be taken before collection. Collectors should be trained in basic botanical knowledge and the importance of species maintenance. However, it would be appropriate to subject plant collecting to certification.

## **CONCLUSION**

When the current situation of medicinal and aromatic plants in our country, their strengths and weaknesses, opportunities and threats are evaluated together, all stages of these plants, from conservation to production, from processing to marketing, should be addressed with a holistic approach. In addition, there is a need to manage production with the principle of process improvement. As a result of the "production planning of medicinal and aromatic plants" that will be created by taking these principles into consideration, their contribution to the economy will also increase.

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## **Nitrogen Consumption Effect on the Correlation Between Grain Yield and Nutrient Uptake in Wheat Under Irrigation Cessation Conditions**

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### **ABSTRACT**

To investigate the impact of nitrogen application timing on nutrient uptake and grain yield in bread wheat cultivars under water deficit conditions, two field experiments were carried out at the Agricultural Research Station of Miandoab in West Azerbaijan during the 2013-2014 and 2014-2015 growing seasons. The experimental design involved treatments organized in a split-plot factorial layout based on a randomized complete block (RCB) design with three replications. Irrigation treatments were implemented at three levels: full irrigation (I1), irrigation interruption from flowering to maturity (I2), and irrigation interruption from booting to maturity (I3), serving as the main plot factors. Concurrently, 120 kg/ha of pure nitrogen was applied across four timing levels, with N1 involving 20 kg/ha at the planting stage and 100 kg/ha at the tillering stage, N2 comprising 20 kg/ha at the planting stage and 100 kg/ha at the stem elongation stage, N3 including 20 kg/ha at the planting stage, 50 kg/ha at the tillering stage, and 50 kg/ha at the stem elongation stage, and N4 consisting of 20 kg/ha at the planting stage, 50 kg/ha at the tillering stage, and 50 kg/ha at the ear emergence stage. These nitrogen treatments were randomized in subplots. Additionally, five distinct bread wheat cultivars (G1=Zarrin, G2=Peshgam, G3=Orum, G4=Zareh, and G5=Mihan) were assigned to sub-subplots, completing the experimental design. The findings indicated that under full irrigation conditions (I1), there was a positive and statistically significant correlation between grain yield and nitrogen concentration ( $r = 0.619^{**}$ ) as well as grain iron ( $r = 0.606^{**}$ ). The observed increase in grain yield was closely linked to the elevated concentrations of these two elements in the grain. Conversely, under conditions of irrigation interruption at the spike emergence stage, a positive and statistically significant correlation was observed between grain yield and grain nitrogen concentration ( $r = 0.662^{**}$ ), grain phosphorus ( $r = 0.465$ ), and grain iron ( $r = 0.499$ ). Furthermore, these correlations were found to be meaningful. Cultivars, particularly Pishgam and Mihan, experiencing water shortage stress exhibited a higher accumulation of nutrients in the grains. This was attributed to the re-transfer of a greater amount of photosynthetic materials to the grain, resulting in elevated grain yield under irrigation interruption conditions. Additionally, a positive and statistically significant correlation was observed between grain nitrogen concentration and grain phosphorus ( $r = -0.462$ ), grain potassium ( $r = -0.682$ ), and grain iron ( $r = 0.574$ ).

**Keywords:** Nitrogen consumption, grain yield, correlation between traits.

## **INTRODUCTION**

Drought, a major limitation in agriculture, not only substantially diminishes crop yield but also impedes crucial processes such as plant respiration, photosynthesis, and stomatal movement, thereby adversely affecting overall plant growth and physiological metabolism (Yang, Lu et al. 2021). One of the primary objectives of modern agriculture is the optimal use of water and nitrogen resources through the implementation of appropriate management practices (Tavakoli, 2012). The quality of wheat grains and the uptake of micronutrients can be influenced by interactions with other mineral elements. In this regard, Shi et al. (2010) conducted a long-term study to investigate the effects of different nitrogen levels (0, 130, and 300 kg. ha<sup>-1</sup>) on the concentration and uptake of micronutrients (iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn)) and their distribution in various parts of wheat grains. The results revealed that the application of nitrogen fertilizer increased the concentrations of Fe, Zn, and Cu in wheat grains, but had no significant effect on the Mn concentration in the grains.

In a study evaluating the effects of different levels of nitrogen and potassium fertilizers on the content of Fe, Zn, Mn, and Cu in corn, it was found that the application of nitrogen, along with potassium sulfate and potassium chloride, resulted in an increase in the concentrations of Zn, Mn, Fe, and Cu in corn kernels (Manasek et al., 2013). Khamadi et al. (2015), in their investigation of the impact of crop residues and various levels of nitrogen fertilizer on the nutrient uptake of wheat, observed that an increase in nitrogen fertilizer led to an increase in the uptake of Fe, Zn, and Mn in wheat grains, while Cu uptake decreased.

In another study assessing the effects of different levels of nitrogen fertilizer on the yield and growth-related traits of wheat genotypes under post-flowering heat stress conditions, it was reported that under both favorable and post-flowering heat stress conditions, a reduction in nitrogen consumption resulted in a significant decrease in grain yield. However, the effect of nitrogen fertilizer quantity on thousand grain weight, grain filling rate, and filling duration was not statistically significant (Lak and Modhej, 2011). Mousavi et al. (2012) conducted an experiment to investigate the impact of nitrogen application methods on agronomic traits of dry land winter wheat. The results revealed that the surface broadcasting of fertilizer during the tillering stage and surface broadcasting covering two-thirds of the tillering stage, along with foliar spraying at the wheat heading stage, resulted in lower grain yield compared to other nitrogen treatment methods. Among the surface-applied nitrogen treatments, the highest grain yield was associated with the application of fertilizer during tillering and before the heading stage. Shahrabi et al. (2016), in their investigation on the impact of drought stress and nitrogen fertilizer on grain yield and nitrogen use efficiency in the Sirvan wheat variety, observed that irrigation cessation during the heading and grain-filling stages was associated with a decrease in grain yield and an increase in grain protein content in both years. Nitrogen application up to 150 kg. ha<sup>-1</sup> in both years had a positive effect on grain yield, yield components, and water use efficiency. However, the magnitude of this effect varied under different irrigation conditions, with the positive impact of nitrogen diminishing with increased drought intensity. This research aimed to explore the genetic diversity of wheat varieties in terms of nutrient uptake and grain yield potential. The goal was to identify and introduce superior varieties based on these traits under drought stress



conditions and different nitrogen application timings, specifically for recommendations in cold and water-deficient regions.

## **MATERIALS AND METHODS**

This research was conducted during the agricultural years 2013-2014 and 2014-2015 at the Agricultural Research Station of Miandoab, situated at an altitude of 1142 meters above sea level in the southeast of West Azerbaijan Province, Iran. Meteorological information for the research location is provided in Table 1. The experiment was carried out using a split-plot factorial design based on a randomized complete block layout with three replications. Irrigation treatments were implemented at three levels: full irrigation (I1), irrigation interruption from flowering to maturity (I2), and irrigation interruption from booting to maturity (I3), serving as the main plot factors. Concurrently, 120 kg. ha<sup>-1</sup> of pure nitrogen was applied across four timing levels, with N1 involving 20 kg. ha<sup>-1</sup> at the planting stage and 100 kg. ha<sup>-1</sup> at the tillering stage, N2 comprising 20 kg. ha<sup>-1</sup> at the planting stage and 100 kg. ha<sup>-1</sup> at the stem elongation stage, N3 including 20 kg. ha<sup>-1</sup> at the planting stage, 50 kg. ha<sup>-1</sup> at the tillering stage, and 50 kg. ha<sup>-1</sup> at the stem elongation stage, and N4 consisting of 20 kg. ha<sup>-1</sup> at the planting stage, 50 kg. ha<sup>-1</sup> at the tillering stage, and 50 kg. ha<sup>-1</sup> at the ear emergence stage. These nitrogen treatments were randomized in subplots. Additionally, five distinct bread wheat cultivars (G1=Zarrin, G2=Peshgam, G3=Orum, G4=Zareh, and G5=Mihan) were assigned to sub-subplots, completing the experimental design.

In both years of the experiment, planting was carried out in mid-October with disinfected wheat seeds, and a planting density of 450 seeds. m<sup>-2</sup>. After planting, one irrigation session was performed in the fall to promote the emergence and establishment of seedlings. In mid-March, following the improvement of environmental conditions, chemical weed control was implemented using the herbicide 2, 4-D. To ensure uniform irrigation across plots, water meters and pressurized irrigation pipes were used, maintaining a consistent water volume for each plot throughout the growing season. This approach aimed to standardize the amount of water consumed in each plot.

The irrigation amount for each irrigation cycle, based on the reference evapotranspiration, was set at 0.3 m<sup>3</sup> for each experimental plot. Grain yield was determined at the final harvest stage by removing the half-meter section from the beginning and end of each plot using a combine harvester. The grain yield was then converted to kg. ha<sup>-1</sup>. After the final harvest, samples were selected from the grain yield of each plot to determine the nutrient content, including nitrogen (N), phosphorus (P), potassium (K), iron (Fe), magnesium (Mg), and zinc (Zn), using the samples (Gupta, 2000). To calculate the grain protein content, the following equation, derived from Voltas et al. (1997), was used:

$$7.5 \times \text{Grain Nitrogen (\%)} = \text{Grain Protein Content (\%)}$$

This equation provides an estimate of the grain protein content based on the nitrogen content in the grain. After conducting the necessary tests and ensuring the normal distribution of the data, the compound variance analysis was performed in the form of a split-plot factorial design within the framework of a randomized complete block design. For statistical analyses and graph plotting, SPSS and Excel software were utilized. Mean comparisons were carried out using the Duncan's multiple range test at a significant level of five percent.

Table 1. The weather statistics in the experimental site

	Growing seasons 2013-2014			Growing seasons 2014-2015		
Months	The mean of Temperature (°C)	Total Evaporation (mm)	Total Precipitation (mm)	The mean of Temperature (°C)	Total Evaporation (mm)	Total Precipitation (mm)
October	15.2	119.5	4.9	15.2	83.2	4.4
November	5.4	38.9	47.5	7.4	19.6	32.5
December	-0.2	0	1.4	4.6	0	52.7
January	2.5	0	13.5	2.7	0	4.2
February	1	0	8.4	4.9	0	34.5
March	2.1	0	20	0.6	0	13.7
April	10.9	65.9	41.2	10.9	77	25.4
May	16.6	155.9	23.3	15.7	156.7	28.5
June	21.1	28.2	2.7	21.8	246.8	0.4
August	23.9	276.9	4.6	26.3	307.1	1
September	26	316.5	0	26.9	379.1	0
November	23	301.2	2.1	24.1	321.3	0

Table 2. Nitrogen timing in two growing seasons

	Growing seasons 2013-2014					Growing seasons 2014-2015				
The growth stages of wheat	Nitrogen timing date	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>	Nitrogen timing date	N <sub>1</sub>	N <sub>2</sub>	N <sub>3</sub>	N <sub>4</sub>
Planting	October 12	20*	20	20	20	October 11	20*	20	20	20
tillering	April 4	100	0	50	50	April 6	100	0	50	50
stem elongation	April 29	0	100	50	0	May 1	0	100	50	0
Ear emergence	May 10	0	0	0	50	May 13	0	0	0	50

## RESULTS AND DISCUSSION

### Grain Nutrient Concentration

The variance analysis of the data indicated that the effects of year, irrigation, nitrogen fertilizer, cultivar, irrigation × nitrogen fertilizer, cultivar × nitrogen fertilizer, and irrigation × nitrogen fertilizer × cultivar on the concentration of essential elements in the grain were statistically significant (Table 3).



This suggests that factors such as the specific year, irrigation levels, nitrogen fertilizer application, and the interaction of these factors with cultivar choice have a meaningful impact on both high and low-consumption elements in the grains. Further exploration and discussion of these results will provide insights into the nuanced relationships between these variables and their influence on grain composition.

### **Correlation between Yield and Grain Nutrient Concentration under Full Irrigation Conditions (I1)**

The correlation between grain yield and nutrient concentration under full irrigation conditions is presented in Table 4. The correlation between grain yield and nitrogen concentration ( $r = 0.619^{**}$ ) and iron concentration ( $r = 0.606^{**}$ ) under full irrigation condition was positive and significant. An increase in grain yield was associated with an increase in the concentration of these two nutrients in the grain. McDonald (2007) reported a strong correlation between zinc concentration and grain weight in wheat. The correlation between nitrogen and iron concentration in the grain was positive and significant ( $r = 0.555^{**}$ ), indicating that an increase in nitrogen concentration in the grain was accompanied by an increase in iron concentration. The correlation of nutrient concentration in wheat grain has been previously investigated, and Garvin et al. (2006) reported a positive association between zinc and iron concentrations.

Table 3. Analysis of variance (mean square) of yield and grain nutrients concentration of wheat cultivars.

Source of Variance	Degrees of Freedom	Means Square							
		Grain yield	Grain Protein content	N	P	K	Fe	Mg	Zn
Y	1	2320849 <sup>**</sup>	70.9 <sup>**</sup>	2.19 <sup>**</sup>	71.04 <sup>ns</sup>	47.38 <sup>**</sup>	711.2 <sup>**</sup>	4.68 <sup>**</sup>	460.40 <sup>**</sup>
R(Y)	4	4411888 <sup>*</sup>	537.1 <sup>**</sup>	16.52 <sup>**</sup>	30.05 <sup>ns</sup>	12.23 <sup>ns</sup>	1158.2 <sup>**</sup>	3.74 <sup>**</sup>	656.60 <sup>**</sup>
I	2	87930956 <sup>**</sup>	713.2 <sup>**</sup>	21.99 <sup>**</sup>	64.24 <sup>*</sup>	12.92 <sup>ns</sup>	188.8 <sup>ns</sup>	58.94 <sup>**</sup>	911.50 <sup>**</sup>
I×Y	2	53611798 <sup>**</sup>	24.1 <sup>ns</sup>	0.73 <sup>ns</sup>	28.46 <sup>ns</sup>	0.43 <sup>ns</sup>	107.7 <sup>ns</sup>	1.04 <sup>ns</sup>	9.23 <sup>ns</sup>
Error	8	663746	5.7	0.18	22.98	3.25	51.4	0.40	16.34
N	3	7967036 <sup>**</sup>	24.6 <sup>**</sup>	0.76 <sup>**</sup>	14.47 <sup>**</sup>	7.05 <sup>**</sup>	75.1 <sup>*</sup>	19.69 <sup>**</sup>	20.60 <sup>ns</sup>
N×Y	3	289566 <sup>ns</sup>	0.30 <sup>ns</sup>	0.01 <sup>ns</sup>	0.15 <sup>**</sup>	0.38 <sup>ns</sup>	50.8 <sup>ns</sup>	0.19 <sup>ns</sup>	5.20 <sup>ns</sup>
N×I	6	602685 <sup>ns</sup>	1.45 <sup>**</sup>	0.04 <sup>**</sup>	6.16 <sup>**</sup>	1.61 <sup>**</sup>	105.1 <sup>**</sup>	17.88 <sup>**</sup>	19.88 <sup>ns</sup>

N×I×Y	6	502918 <sup>ns</sup>	0.20 <sup>ns</sup>	0.01 <sup>ns</sup>	0.13 <sup>**</sup>	0.63 <sup>ns</sup>	66.2 <sup>*</sup>	0.14 <sup>ns</sup>	49.76 <sup>**</sup>
G	4	45650052 <sup>**</sup>	161.7 <sup>**</sup>	4.98 <sup>**</sup>	2.83 <sup>**</sup>	28.59 <sup>**</sup>	1297.9 <sup>**</sup>	12.35 <sup>**</sup>	179.20 <sup>**</sup>
G×Y	4	3673785 <sup>**</sup>	0.35 <sup>ns</sup>	0.01 <sup>ns</sup>	0.06 <sup>*</sup>	2.75 <sup>**</sup>	52.2 <sup>ns</sup>	0.07 <sup>ns</sup>	35.64 <sup>ns</sup>
G×I	8	3867231 <sup>**</sup>	4.59 <sup>**</sup>	0.14 <sup>**</sup>	1.66 <sup>**</sup>	5.12 <sup>**</sup>	94.1 <sup>**</sup>	9.70 <sup>**</sup>	115.41 <sup>**</sup>
G×I×Y	8	4463608 <sup>**</sup>	0.24 <sup>ns</sup>	0.01 <sup>ns</sup>	0.05 <sup>*</sup>	3.72 <sup>**</sup>	41.1 <sup>ns</sup>	0.07 <sup>ns</sup>	96.05 <sup>**</sup>
G×N	12	667523 <sup>ns</sup>	1.83 <sup>**</sup>	0.06 <sup>**</sup>	1.25 <sup>**</sup>	0.75 <sup>ns</sup>	199.9 <sup>**</sup>	6.88 <sup>**</sup>	56.40 <sup>**</sup>
G×N×Y	12	344337 <sup>ns</sup>	0.48 <sup>*</sup>	0.02 <sup>*</sup>	0.04 <sup>ns</sup>	0.48 <sup>ns</sup>	27.6 <sup>ns</sup>	0.06 <sup>ns</sup>	10.67 <sup>ns</sup>
G×N×I	24	5056675 <sup>ns</sup>	0.48 <sup>**</sup>	0.05 <sup>**</sup>	1.60 <sup>**</sup>	0.72 <sup>ns</sup>	97.1 <sup>**</sup>	10.58 <sup>**</sup>	21.80 <sup>ns</sup>
G×N×I×Y	24	527257 <sup>ns</sup>	0.15 <sup>ns</sup>	0.004 <sup>ns</sup>	0.04 <sup>ns</sup>	0.45 <sup>ns</sup>	39.1 <sup>ns</sup>	0.06 <sup>ns</sup>	27.60 <sup>ns</sup>
Error	228	440917	0.03	0.007	0.02	0.51	26.9	0.08	20.33
CV		9.87	3.24	3.24	3.21	12.39	12.21	11.54	14.27

\* and \*\* are significant at the level of probability of 1 and 5%, ns is nonsignificant  
Year(Y), Replication (R), Irrigation(I), Nitrogen(N), wheat cultivars(G)

**Table 4. Correlations between grain yield and grain nutrient concentration of wheat cultivars under full irrigation conditions (I1)**

	Grain yield	N	P	K	Mg	Fe	Zn
Grain yield	1						
N	.619 <sup>**</sup>	1					
P	.301	.376	1				
K	.366	.271	-.043	1			
Mg	.124	.224	.047	-.338	1		
Fe	.606 <sup>**</sup>	.555 <sup>*</sup>	.138	.292	.169	1	
Zn	-.536 <sup>*</sup>	-.003	-.042	.027	.057	-.171	1
Grain Protein content	.619 <sup>**</sup>	1	.376	.271	.224	.555 <sup>*</sup>	-.003

\* and \*\* are significant at the level of probability of 1 and 5%

### **Correlation between Yield and Nutrient Concentration in Grain under Irrigation Cessation from Flowering to Maturity (I2)**

The correlation between grain yield and nutrient concentration in the grain under irrigation cessation at the flowering stage is presented in Table 5. The correlation between grain yield and

nitrogen concentration was positive and significant ( $r = 0.480^{**}$ ), indicating that an increase in grain yield was associated with an increase in nitrogen concentration. Moreover, positive, and significant correlations were observed between nitrogen concentration and iron in the grain ( $r = 0.582^{**}$ ), phosphorus concentration and potassium in the grain ( $r = 0.596^{**}$ ), as well as iron and potassium concentration in the grain ( $r = 0.476^{**}$ ). The increase in nitrogen concentration in the grain was accompanied by an increase in iron concentration. The positive correlation between phosphorus and potassium concentration in wheat grain has been previously reported by Rezaei et al. (2010).

Table 5. Correlations between grain yield and grain nutrient concentration of wheat cultivars under water deficit condition from flowering to maturity (I2).

	Grain yield	N	P	K	Mg	Fe	Zn
Grain yield	1						
N	.480*	1					
P	.240	.101	1				
K	.450	.536	.596**	1			
Mg	-.271	-.376	-.115	-.056	1		
Fe	.443	.582**	-.049	.476*	.220	1	
Zn	.191	.237	-.112	.210	.089	.208	1
Grain Protein content	.480*	1	.240	.450	-.271	.443	.237

\* and \*\* are significant at the level of probability of 1 and 5%

### **Correlation between Yield and Nutrient Concentration in Grain under Irrigation Cessation from Booting to Maturity (I3)**

The correlation coefficients between grain yield and nutrient concentration in grain under irrigation cessation at the heading stage are presented in Table 6. In the conditions of irrigation cessation at the heading stage, positive and significant correlations were observed between grain yield and nitrogen concentration in the grain ( $r = 0.662^{**}$ ), phosphorus concentration in the grain ( $r = 0.465^{**}$ ), and iron concentration in the grain ( $r = 0.499^{**}$ ). Genotypes tolerant to water deficit, such as Pishgam and Miham, exhibited higher accumulation of nutrients in the grains, indicating a retranslocation of more photosynthetic materials to the seeds and higher grain yield under irrigation cessation. Moreover, positive, and significant correlations were found between nitrogen concentration in the grain and phosphorus concentration in the grain ( $r = 0.462^{**}$ ), potassium concentration in the grain ( $r = 0.682^{**}$ ), and iron concentration in the grain ( $r = 0.574^{**}$ ). Under irrigation cessation conditions at the heading stage, the distribution of nitrogen fertilizer resulted in an increase in the concentrations of phosphorus, potassium, and iron in the grain. This could be a reason for the positive correlation among them. Considering the positive and significant correlation between phosphorus concentration in the grain and magnesium ( $r = 0.512^{**}$ ), it is noteworthy that Graham et al. (1999) reported that phosphorus and magnesium in the grain are primarily in the form of phytic acid. Their correlation with zinc indicates that the high

concentration of zinc in the grain may be correlated with the high phytate of the wheat grain. This condition was also present in rice.

Table 6. Correlations between grain yield and grain nutrient concentration of wheat cultivars under water deficit condition from booting to maturity (I3)

	Grain yield	N	P	K	Mg	Fe	Zn
Grain yield	1						
N	.662**	1					
P	.465*	.462*	1				
K	.431	.682**	.213	1			
Mg	.260	.223	.512*	-.121	1		
Fe	.499*	.574**	.181	.599**	-.141	1	
Zn	-.146	.261	.113	.190	.159	.033	1
Grain Protein content	.662**	1	.462*	.682**	.223	.574**	.261

\* and \*\* are significant at the level of probability of 1 and 5%

## CONCLUSION

In cultivars such as "Pishgam" and "Mihan" with higher grain yields under non-water stress conditions, the concentrations of nitrogen and iron were also high. The significant negative correlation between grain yield and the concentration of zinc under non-water stress conditions suggests that zinc does not have a significant impact on grain yield in the absence of water stress. It was also revealed that under water stress conditions, plants tend to absorb more of these elements to mitigate the effects of stress and tolerate unfavorable conditions. This leads to the accumulation of these elements in the grain during the retranslocation of photosynthetic materials.

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## **Changes In Granulocytes Number in Peripheral Blood During Infection Observed by Flow Cytometry: Preliminary Results**

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### **ABSTRACT**

Neutrophils are listed among the first type of cells that reach the site of infection. Generally, Neutrophils moves through chemotaxis due to their response toward chemical signals produced at sites of infection. Neutrophils circulate in the peripheral blood representing the major percentage of white blood cells ranging from 50 to 80% of all populations. This percentage can be different in different species. Flow cytometry is a relatively new technique in veterinary medicine that has many functions, especially in identifying different cell populations using specific antibodies for each population found in the peripheral blood. Moreover, flow cytometry is often used to identify and stage leukemia, lymphoma and mast cell tumors in dogs and cats. We aimed to observe the movement of Neutrophils in the peripheral blood when determined quantity of Lipopolysaccharides of E. Coli was administered to a specific site in rabbit species. To achieve this goal two individuals' peripheral blood, infected artificially, was collected at different moments: prior to the infection and during the post infection period to observe the presence of Neutrophils in the peripheral blood. The infective dose was not lethal and the rabbits involved in this experiment was subject to another experiment allowing us to perform these analyzes. Maximum efforts were made to minimize animal distress and to use only the number of animals necessary to produce reliable results. Results showed that number of Neutrophils decreases significantly during the first thirty minutes considered as T1 (3.11%), compared to T0 (31.2%). Their number increases significantly after the first two hours and remains almost the same till the end of the experiment. Based on these results we hypothesized that Neutrophils reached the site of infection and after a general sensitization occurs due to the infective status. However further studies with a higher caseload are warranted to better define the association between differences in Neutrophils percentage in the peripheral blood and the infectious dose.

**Keywords:** Neutrophils, Flow Cytometry, Rabbit, E. Coli

## **INTRODUCTION**

Neutrophils count in peripheral blood circulation is considered as a highly important indicator of new bacterial infection in the organism. Usually, neutrophils move to the site of infection through chemotaxis due to their response to various chemicals produced at the site of infection (1). Moreover, neutrophilic movement from peripheral blood toward the infection site is one of the mechanisms used by the immunitary system when an infection, usually bacterial is present. This movement can be observed by different techniques in human and veterinary medicine to observe the neutrophils behavior on different pathogens. One of the techniques is Flow Cytometry which is considered to be relatively new in veterinary medicine. Flow Cytometry is actually used for many purposes in veterinary medicine including cell populations identification and immunophenotyping, identification and staging of lymphoproliferative and myeloproliferative neoplastic disorders such as lymphoma and leukemia, detection of minimal residual disease (2,3,4) and the detection of various prognostic indexes such as Ki67. The objective of this study was to observe the changes in percentages of neutrophils in the peripheral blood of rabbits when a local infection is present.

## **MATERIAL AND METHODS**

Two healthy rabbit individuals were selected for the experiment. Both rabbit were New Zealand breed aging six months and having no history of previous diseases. Moreover vaccine against Myxomatosis and Rabbit Hemorrhagic disease were administered at the right moment. Rabbits were infected locally using Lypopolysaharides of E.coli which was administered to a specific site, same for both rabbits. The infective dose was not lethal while maximum efforts were made to minimize animal stress. Peripheral blood was collected in different moments: T0 – time when infection was iduced, T1 – thirty minutes after infection, T2 – sixty minutes after infection T3 – ninety minutes and T4 – 120 minutes after infection was induced. Flow cytometric analyses took place immediately after all blood collections were performed. Briefly fifty micro liters of each sample was placed in cytometry tubes. An NxT Attune flow cytometer (Thermo Fisher Scientific) was used to perform all analyzes. A lysis step of five minutes was performed to exclude erythrocytes from the analysis and then cells were centrifuged at 1200 rpm for five minutes, supernatant was discarded and another wash using saline buffer was made. At the end cell were acquired to the cytometer in order to produce results. Appropriate gates were designed to analyze only events that are considered as cells excluding debris from the gate of analyzes. Neutrophils percentages were detected each time of acquisition and data were recorded.

## **RESULTS**

Results showed that number of Neutrophils in peripheral blood changes depending on time after infection was made. In T0 no movement of neutrophils is observed, while controversially in T1 the number decreases significantly (3,11%), compared to T0 (31.2%). Neutrophils number significantly increases after two hours (T4), showing a general sensitization of these cells in the peripheral blood. During T2 and T3 number of neutrophils remains low showing that a specific time is needed for these cells in the rabbit species to increase their number in the peripheral blood after induced infection. Results can be viewed in figure 1 represented by dot plots produced by flow cytometry.



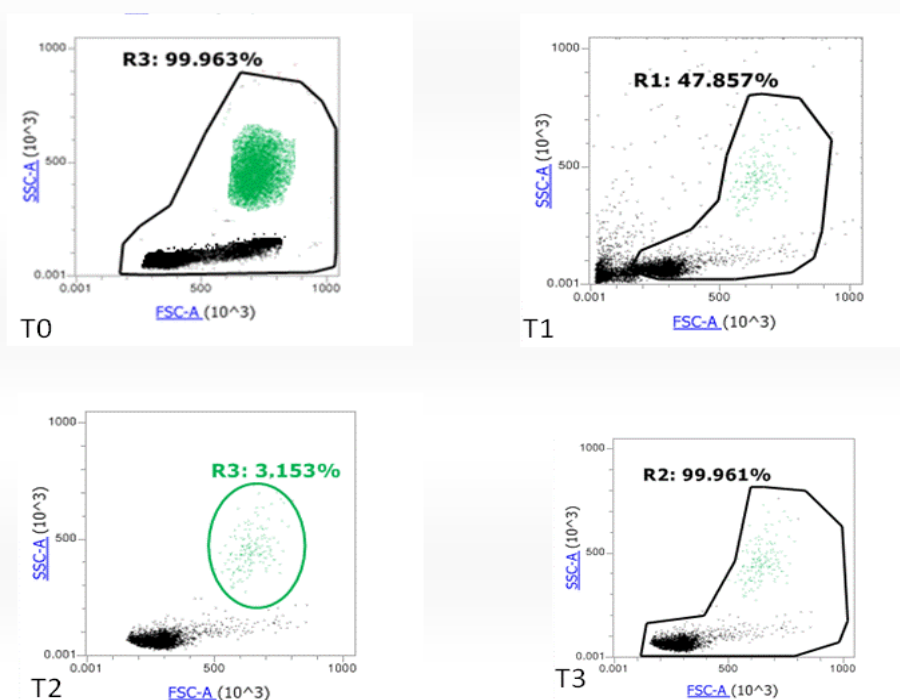


Figure 1. Flow Cytometry dot plots representing percentages of Neutrophils in different moments after induced infection.

## CONCLUSIONS

Based on these results we hypothesized that Neutrophils reached the site of infection and after a general sensitization occurs due to the infective status. However further studies with a higher caseload are warranted to better define the association between differences in Neutrophils percentage in the peripheral blood and the infectious dose.

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## **Toward Regional Waste Management, Developing and analyzing waste management scenarios in Fier Waste Functional Area in Albania**

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### **ABSTRACT**

In the Fier waste area (a region with six municipalities Fier, Lushnjë, Divjakë, Mallakaster, Patos and Roskovec), an area in the west part of Albania with around 300.000 inhabitants and a production of about 230 tons of municipal solid waste per day, waste management practices are environmentally unsustainable and underdeveloped, with poor waste collection systems especially in rural areas, minimal waste recycling activities and frequent illegal dumps or informal landfill.

To support decisions regarding future sustainable waste management solutions for this situation, five waste management scenarios have been outlined and quantitatively assessed.

All waste management scenarios include the existing SWM facilities, the Fier waste to energy plant and accompanying regional sanitary landfill, which will be the final waste disposal route for all three scenarios. Scenario 1 follows the recommendations of the approved Waste Management Sector Study 2018-2036, under which 95% of all collected mixed waste is incinerated with energy production; Scenarios 2 which include management of collected mixed waste, plus source separation of dry recyclables in the main urban areas, and limited home composting of organic waste in rural areas; and Scenario 3 that follows the requirements of the national legislation on ISWM and the related waste Acquis, under which only a progressively decreasing residual proportion of the collected waste is incinerated, after all efforts for separate collection at source, reduce, reuse, recycling and composting have been made. Scenarios 2 and 3 also include two sub-scenarios.

A multi-criteria environmental based waste management analysis (MCA) has been developed to evaluate and select the most appropriate scenario as the preferred option for the ISWM system in the Fier Waste Area. From the comparative results of the evaluation of the five scenarios, the highest score of preference is shown to Scenario 3b. A second preference shows Scenario 2b. The results demonstrate that a future regional waste management system that is based on a combination of more recycling, thermal treatment, composting, and improved landfilling reaches the objectives of sustainable waste management much more closely than the present, inadequate system which is in place.

**Keywords:** Integrated waste management, waste management scenarios, multi-criteria analysis, Fier waste area.

**Acknowledgment:** This study is a part of the project Infrastructure Project Facility Technical Assistance 9 (IPF9) Fier functional waste area feasibility study, WB21-Alb-env-02

## INTRODUCTION

The Fier waste area is one of the 10 waste areas defined in the Sectoral Plan, which coincides with Fier County, with an area of 1,887 km<sup>2</sup>. In this waste area there are 6 Municipalities of Fier District (Fier, Lushnje, Divjakë, Patos, Roskovec and Mallakastër). [1]

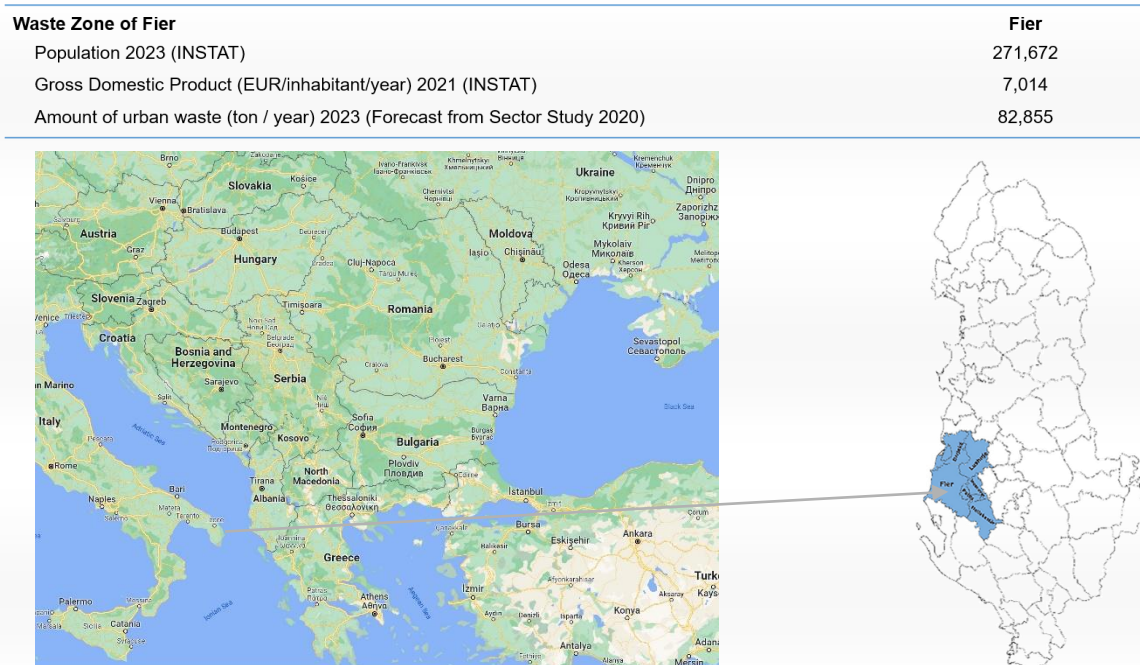


Figure 1. Waste Zone Area of Fier, Albania [Source: Developed by author]

The waste collection service is provided throughout the waste area, where the urban area is fully covered, while the rural areas have lesser coverage. The service is mainly subcontracted by the municipalities, but there are also municipalities that provide the service themselves, mainly in rural areas. Most municipalities use a network of metal containers (2,410 pieces) with a capacity of 1.1 m<sup>3</sup>, and some other types that include underground, stationary containers with a capacity of 80 m<sup>3</sup>, open bins, and 80- and 260-liter containers. In all municipalities of the waste area, 29 vehicles operate for their collection and transport with capacities ranging from 5 to 16 tons. Separate collection of waste is not applied in any municipality, and it is collected mixed and sent to the final point, which is the newly Waste Treatment Plant in Fier. [2]

As part of the feasibility study for the waste area of Fier, during the analysis of service organization scenarios, international data were taken as a basis, for the projection of the amount of waste and data for the forecast of the Gross Domestic Product, for the population projections and expected economic factors. Based on these references, the following data were evaluated, which then served in the evaluation of the scenarios for the organization of the service in the Fier Waste Zone:

- Waste generation is projected to decrease from 83,898 tons in 2020 to 77,342 tons in 2040.

- Waste collection rates will increase throughout the waste area, with rates varying from 33% (rural areas) to 80% in urban areas. 57,201 tons of waste will be collected in 2020, increasing to 71,992 tons in 2032. The overall collection rate is 68%.
- Recycling potential is high, with approximately 60% of MSW (municipal solid waste) potentially recyclable, and approximately 50% potentially compostable.
- Waste generation is low (~235 kg municipal waste per person per year) compared to Europe (~500 kg per person per year).

### **OBJECTIVES OF THE DESIGN OF THE INTEGRATED SOLID WASTE MANAGEMENT SYSTEM**

The drafting and analysis of the scenarios for the organization of the integrated waste management system was based on the principles for the protection and improvement of the quality of the environment, in accordance with the criteria of the national legislation for waste management and the relevant regulations and directives of the EU and an appropriate institutional framework. Regarding this fact, the proposal of the scenarios and then their analysis to select the most appropriate scenario, aimed to meet the objectives below, starting from the most basic level, up to the most optimal level of providing a service of waste management as sustainable as possible: [3]

- Achieving legal compliance (environmental protection and resource efficiency):
  - Application of hierarchy principles for integrated waste management, including waste prevention, reduction and recovery.
  - Improvement (100%) of municipal waste collection in all accessible areas, including source separation of recyclable materials and organic fractions of waste.
  - Reduction of landfills or uncontrolled landfills and the exercise of operational control until their closure is achieved.
  - Stimulating recovery and recycling of valuable materials (paper, plastic, metal) in accordance with national objectives.
  - Reducing the amount of waste deposited in landfills (especially biodegradable waste).
- Creation of a long-distance transfer and transport system, consisting of a reasonable network of transfer stations, connected to a system of long-distance transport vehicles between each other and the new place of disposal.
- Establishing a sustainable waste management subsystem targeting mixed waste, recyclable materials and biodegradable/organic waste.
- Assessment of the potential for intermediate waste disposal in the existing landfills with immediate remedial measures for these collection sites.

## **INSTITUTIONAL FRAMEWORK AND URBAN WASTE SECTOR IN ALBANIA**

Level of transposition of main waste directives (WFD, Landfill, Packaging, Batteries and Accumulators, WEEE, and ELV) in Albania is estimated at 80%, starting from 2011, and their implementation is at initial stage.

Urban solid waste management sector is organized at two levels: At the central level it is the **Ministry of Tourism and Environment** that has the responsibility to draft policies and strategies, ensure implementation of national legislation and legal acts, and recently responsible for development of infrastructure projects, mainly regional investments in waste treatment infrastructures. At the local level there are **municipalities** that are responsible for organizing the service of collection, transport to final treatment and disposal of urban solid waste.

Waste collection per se is organized with bring in points of 1.1m<sup>3</sup> containers for mix waste collection. The service is currently covering approximately **75%** of the population in the country, mainly in the urban areas. No separation at source is established and the recycling industry is weak. Main treatment methods are dumpsites (199 illegal dumpsites identified from a study in 2018). Only 5 sanitary landfills operate at regional level (Bushat, Maliq, Bakaj, Sharra and recently Sherishtë in Vlora to be opened by the end of 2023) and 2 waste to energy plants in Elbasan and in Fier (still not operating) are constructed. The other waste to energy plant in Tirana is under construction phase.

Albania has approved the new national waste strategy (NWS) for 2020 – 2035<sup>1</sup>, which defines the main priorities on waste management. The NWS sets as an initial priority for the waste service being provided by the municipality:

- **First:** the extension of the service to **100%** in the urban territories and the disposal of waste in a controlled sanitary plant.
- **Second:** the extension of the service to **90%** in the rural territories.
- **Third:** separation of waste for further recycling.

Based on the study the country is divided into 10 waste functional zones. In each waste functional zone are set priorities for infrastructure investments at to short-term, medium-term, and long-term phases covering waste collection and transportation, reduction, and recycling, as well as plants for primary or even secondary waste treatment. Meanwhile, the following figure show the forecast for waste collection at the county level, referring to the same years as for the population and waste generation forecast that at the end will reflect the type and capacity of waste infrastructure for final treatment should be needed for all 10 waste zones in Albania.

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<sup>1</sup> It is highly recommended to increase the collection coverage of urban territories to 100% in the short term and in the short to medium term to extend the waste collection service of rural areas. In parallel, it is highly recommended to introduce separate collection of more streams i.e., recyclables (through recycling yards/ green corners), bio-degradable waste (home composting, programs oriented to business).



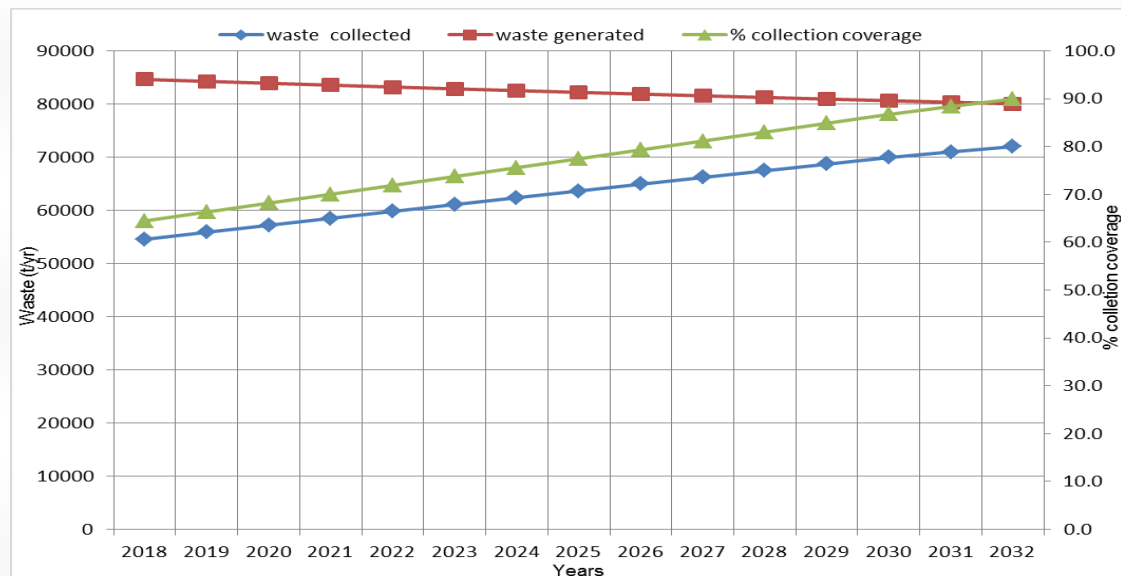


Figure 2. Waste forecast in Albania [Source: Adapted by author]

As showed in the graph during 2020 it was estimated an amount of 26,697 tons of MSW that remains uncollected and to resolve this critical issue it was needed to take measures to increase the waste collection such as investments in mobile equipment (bins and vehicles) and awareness campaigns for the users of the integrated waste management system.

### ANALYSIS OF SCENARIOS FOR THE ORGANIZATION OF THE SERVICE OF COLLECTION, TRANSPORT AND FINAL TREATMENT OF WASTE

According to the National Strategy on Waste, ISWM systems developed over a **20-year planning period (up to 2040)** will focus on management of **three key waste streams**:

- **Dry recyclables** – to be separated, sorted, and sold into the market.
- **Bio-waste** – to be separated, composted and the compost output (sold into the market or used for site restoration purposes).
- **Residual waste** – to be landfilled.

Key waste **collection and recycling targets** to be met in line with Albanian DCMs:

- Self-Local Governance Units (municipalities) should:
  - Collect waste streams, such as paper, metals, plastics, and glass separately.
  - Reduce *the waste generation rate in 2025 to 50%* of the amount of waste in 2014 by recycling paper, metal, plastic, and glass streams.
- **90%** of households in **2025** should receive adequate waste collection service.
- Landfill waste treatment rate to reach **50% in 2025** and the rest will be recovered from recycling, composting, and other technologies with energy recovery.

Based on the objectives outlined above, as well as the fact that an integrated waste management system must take into consideration elements such as the waste management hierarchy: i) Prevention and reuse of waste; ii) Waste collection, including recycling; iii) Waste transfer and transport; iv) Waste treatment; v) Final disposal in landfill (sanitary landfill and for construction and demolition waste), it was recognized that it is not possible for a single integrated management system option to simultaneously fulfill all European and national waste legislation, policies and criteria. For this reason, 3 possible scenarios were proposed, where scenarios 2 and 3 also have their own sub-scenarios, based on the proposal of the following elements: i) Different mixed waste collection systems, separate collection of biodegradable and recyclable waste; ii) Transport to waste transfer stations or directly to the waste treatment plant in Fier; iii) Reducing the amount of waste, applying recycling and composting processes; iv) Final treatment of waste at the waste treatment plant in Fier.

**Scenario 1 (business as usual – 1 container collection system)** – it will evaluate the provision of the basic service - business as usual with 1 container for the collection of mixed waste, while scenarios 2 & 3 will also evaluate the additional services of separate collection of biodegradable waste and recyclable packaging waste by proposing: **i)** Organization of the service using the collection system with 1 common container in the neighborhoods of the municipalities; **ii)** Establishing a network of waste transfer stations; **iii)** Potential use of rehabilitated landfills as transfer stations and for construction and demolition waste; **iv)** Reducing the amount of waste going to landfill; **v)** Incineration of 95% of mixed waste in the Waste to Energy treatment plant in Fier

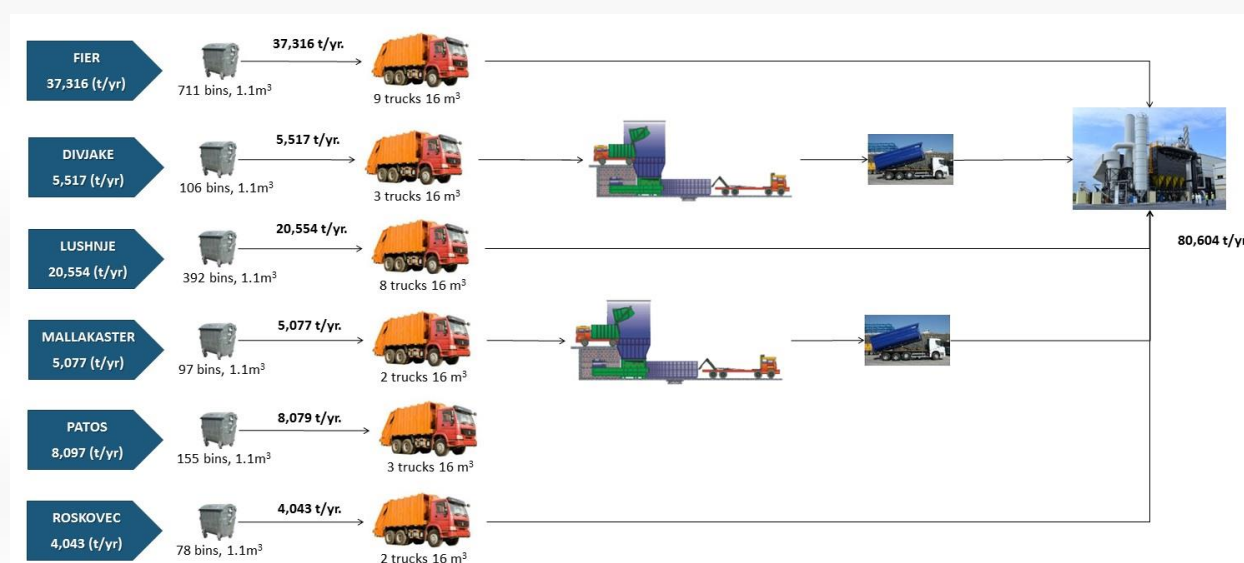


Figure 3. Scenario – One-container collection system – business as usual, in the Fier waste area  
[Source: Developed by author]

This scenario does not envisage infrastructure for waste separation at source. Community awareness remains at low levels and the only possible solution for waste treatment is to use the available infrastructure, the waste to energy treatment plant in Fier. Under this scenario, there is



only one option: collection and incineration of mixed waste, with a limited amount of ash being deposited in a sanitary landfill, or perhaps in the cement industry. It is worth noting that valuable materials lost due to burning are high, e.g. a ton of plastic collected with mixed waste has zero value. Its value increases when it is collected and processed separately by recycling companies (it is usually sold at 600-900 euros/ton and after conversion into a product can be sold at 1500-2000 euros/ton). This means that by burning a ton of plastic waste, a potential value of 2000 euros is lost.

**Scenario 2 (Transition to waste hierarchy – 2-container system).** This scenario includes the above proposal for mixed waste, including source separation of dry recyclables in major urban areas and limited home composting of organic waste in rural areas [6]. This scenario approaches the improvement of resource management. In this scenario, it is proposed to apply the collection system with two containers for dry and wet waste. The final treatment of the waste, which will not manage to be separated for recycling or composting, will go to the end point, in the waste to energy treatment plant in Fier.

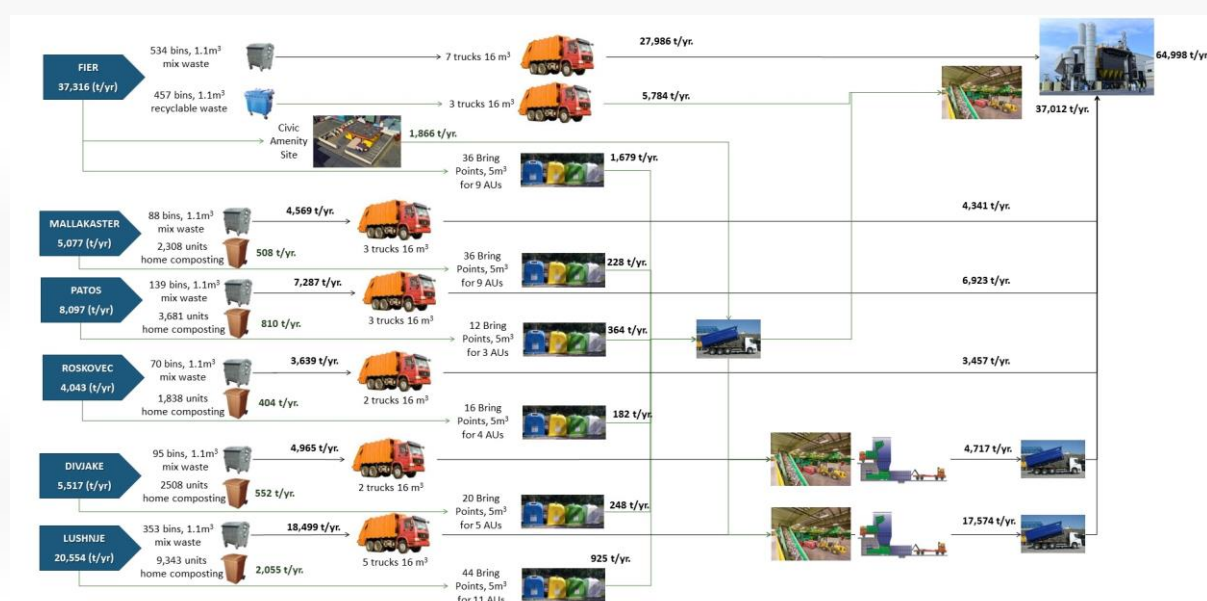


Figure 4. Scenario – Two-container collection system – transition to the waste hierarchy, in the Fier waste area [Source: Developed by author]

**Scenario 3 (Waste hierarchy scenario – 3 container system).** This scenario considers not only waste, but also resources, where waste is seen as a resource. The scenario aims to take measures and make greater investments to keep materials and resources in use for as long as possible in economic cycles, preventing them from turning into waste. The scenario is based on the application of the waste hierarchy, starting with the waste prevention process, as the most appropriate way to improve resource efficiency and reduce the environmental impact of waste. After exhausting all efforts to prevent the generation of waste as much as possible, it is passed to the next stage of the waste pyramid, such as its reuse. Recycling, composting and anaerobic digestion remain the last option for keeping materials in use. Material recovery and chemical treatment are considered more

favorable than simple energy recovery through incineration, after which the material ceases to exist and cannot be used in other cycles.

The last option that is proposed is disposal and it can only be applied to the remaining waste, after all the valuable materials have been returned to the economic cycles. Under this scenario, investments focus on waste separation at source and to a much greater extent than recommended in the Sectoral Study, including a 3-container system for urban areas, dry, wet and other waste. The scenario stipulates the construction of sorting centers for dry recyclables to further separate them into their specific fractions. This should be accompanied by the creation of Extended Producer Organizations that will collect different waste streams such as packaging waste, WEEE (waste from electrical and electronic equipment), batteries and accumulators, etc. These measures will reduce the costs of waste management for municipalities and ensure progress towards the goal of complete separation at source. Also, measures for home composting and investments for municipal composting are included in this scenario.

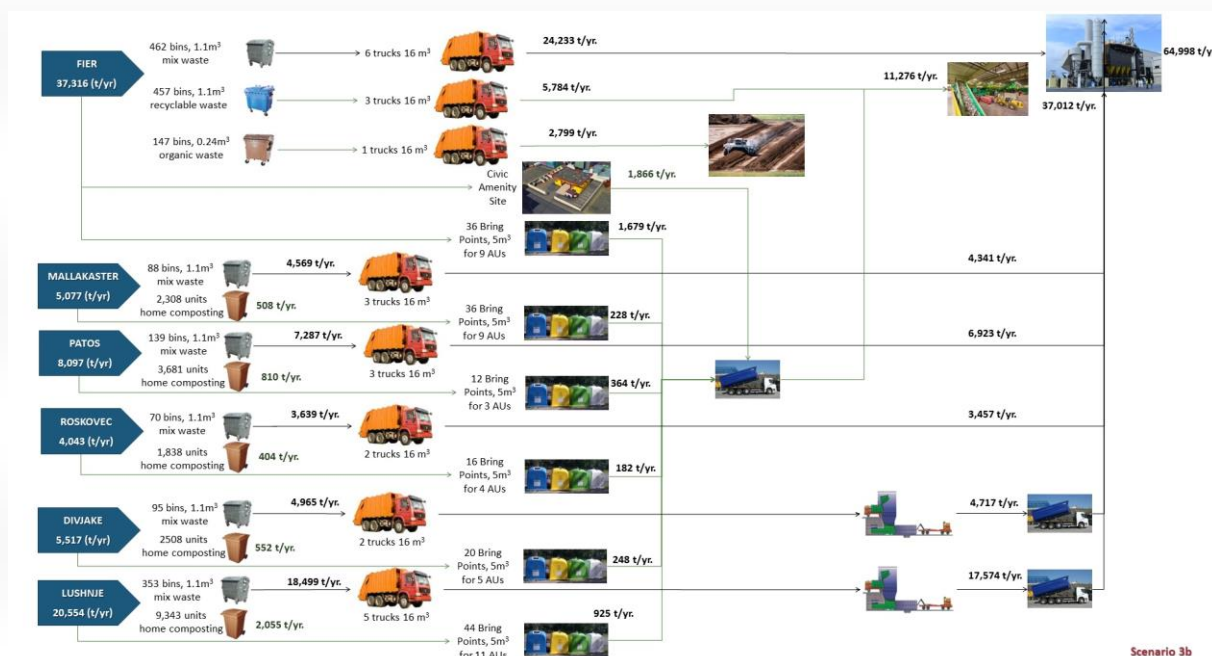


Figure 5. Scenario – Three-container collection system – implementation of the waste hierarchy, in the Fier waste area [Source: Developed by author]

At the end of the proposal and analysis of the scenarios, the 3 detailed scenarios are presented in the table below, where the collection/transfer system and the waste treatment system are analyzed for each scenario. Scenario 2 & 3 have two sub-scenarios each, where two different ways are proposed regarding the final treatment of mixed waste.

Table 1. Forecast detailed scenarios for collection/transfer and treatment in Waste zone of Fier  
[Source: Developed by author]

Designation		Scenario 1	Scenario 2		Scenario 3	
			2a	2b	3a	3b
Collection/Transfer	<b>USW collection</b>	<b>1-Container System</b>	<b>2-container system</b>		<b>3-Container System</b>	
		Mixed waste (urban & rural areas)	1 mixed waste (urban & rural areas) 1 recyclable (only the city of Fier) – 25% of mixed waste		1 mixed waste (urban & rural areas) 1 recyclable waste (only the city of Fier) – 25% of mixed waste 1 organic waste (only the city of Fier – 20% of organic waste (50% of mixed waste)	
	<b>Household waste collection centers</b>	-o	The city of Fier		The city of Fier	
	<b>Home composting</b>	-	Rural areas - 20% of organic waste (50% of mixed waste)		Rural areas – 20% of organic waste (50% of mixed waste)	
	<b>Collection points</b>	-	Rural areas		Rural areas	
	<b>Waste transfer stations</b>	2: Divjakë + Mallakstër	2: Divjakë & Lushnjë, including mobile devices		2: Divjakë & Lushnjë, including equipment	
Treatment	<b>Mixed waste treatment - separation (MRC-materials recovery center)</b>	In the Waste to Energy Treatment Plant in Fier	Local – in two Waste Transfer Stations	Central – Waste to Energy treatment plant + Collection points in the city of Fier	Local – in two Waste Transfer Stations	Central – Waste to Energy treatment plant + Collection points in the city of Fier
	<b>Treatment of organic waste</b>	-	-		-	Central – Waste to Energy Treatment Plant only for the city of Fier
	<b>Waste to Energy treatment plant</b>	Existing Waste-to-Energy Treatment Plant	Existing Waste-to-Energy Treatment Plant		Existing Waste-to-Energy Treatment Plant	
	<b>Regional sanitary landfill</b>	Existing landfill	Existing landfill + expansion		Existing landfill + expansion	
	<b>Construction and demolition waste Plants</b>	Local plants (1 per municipality)	Local plants (1 per municipality)		Local plants (1 per municipality)	
	<b>Rehabilitation of the dumpsites</b>	Waste disposal sites planned for Transfer Stations and plant for construction and demolition waste	Waste disposal sites planned for Transfer Stations and plant for construction and demolition waste		Waste disposal sites planned for Transfer Stations and plant for construction and demolition waste	

## CRITERIA AND METHODOLOGY FOR EVALUATING THE SCENARIOS

A Multi-criteria environmental based waste management analysis (MCA) has been developed to evaluate and select the most appropriate scenario as the preferred option for the ISWM system in the Fier Waste Area. Four criteria were evaluated and analysed: i) *Legal and policy criteria*; ii) *Environmental and social criteria*; iii) *Technological Criteria*; iv) *Financial and economic criteria*. In the tables below are listed the sub evaluation criteria per each four evaluation criteria and the criteria analysis used per each sub evaluation criteria.

The MCA criteria are used to score and rank the proposed ISWM scenarios. The importance of the sub-criteria is weighted on the perceived importance in achieving the ISWM system objectives or decision making. The relative importance of the criteria was selected according to an analysis matrix and was applied as a percentage of importance during the ranking process. Each criterion is considered to be of equal importance and is scored between 1 - 10. The importance factor (weighting) is then subsequently applied to the total scores to each of the four criterion, and overall totals combined to provide the overall scores for each scenario. The table below shows the evaluation criteria and importance factor (weighting) assigned to each criterion.

The raw scores for each of the four criteria were then weighted and adjusted for the final comparative evaluation. For the comparative evaluation, weighting was used as follows: i) Legal and policy: **0.3 (30%)**; ii) Environmental and social: **0.3 (30%)**; iii) Technological: **0.2 (20%)**; iv) Financial/economic: **0.2 (20%)**.

The evaluation shows the following results (scenarios ranked from highest to lowest score):

1. **Scenario 3b: 8.5**
2. **Scenario 2b: 7.9**
3. **Scenario 3a: 7.8**
4. **Scenario 2a: 7.1**
5. **Scenario 1: 6.0**

From the comparative results of the evaluation of the five scenarios, the highest score of preference is shown to **Scenario 3b**. A second preference shows **Scenario 2b**.

Table 2. Multi Criteria analysis [Source: Developed by author]

1.	Legal and Policy Criteria	
1.1	Compliance with European legislation and objectives for integrated SWM	Assess the compliance of each scenario with the objectives and requirements of EU legislation regarding integrated SWM – Waste Framework Directive, Landfill Directive, Circular Economy Package, etc. in context of achieving objectives for recycling and recovery of materials, reducing the amount of biodegradable waste disposed of to landfill, etc.
1.2	Compliance with national laws, regulations, and policies/strategies	Assess each scenario against meeting national laws on SWM, i.e., Law on integrated waste management, Strategic Policy Document and National Integrated Waste Management Plan 2020-2035 and the Sector Study for Investment Demand for Integrated Solid Waste Management in Albania 2018 – 2032 with regards to integrated SWM
2.	Environmental and Social Criteria	

2.1	Air pollution & GHG emissions. Discharge of gaseous pollutants, within permitted limits	Potential emission of gaseous pollutants, dust, and the impact on the atmosphere (contribution to greenhouse gases) from the application of the proposed measures
2.2	Pollution of soil, groundwater, and surface water. Discharges within permissible limits	Assess the impacts on land, groundwater and surface water from the construction and operation of the proposed measures
2.3	Ability to identify suitable locations for the location of SWM measures and their environmental impacts	Assess the need and ease of finding locations for the proposed measures and their environmental impact on receiving environment and receptors
2.4	Social impacts from proposed measures	Assess the likelihood of positive and negative social impacts from the proposed measures on receiving environment and receptors
2.5	Environmental mitigation measures	Assess the likelihood of being able to mitigate negative environmental and social impacts within the design and operation of the proposed measures
<b>3.</b>	<b>Technological Criteria</b>	
3.1	Appropriateness of the measures (processes) towards future change of waste volume and quality	Evaluates the possibility of adapting the process to future changes and changes in the quantity and quality of waste
3.2	Appropriate technology to guarantee efficient operation for the presented SWM measures	Evaluates the existence of appropriate technology on an operational scale, considering any operational problems proven during operation and maintenance
3.3	The need for qualified personnel for the operation and maintenance of the selected technology	Assess whether there is a need and presence of qualified personnel for the proper functioning of the proposed measures
<b>4.</b>	<b>Financial and Economic Criteria</b>	
4.1	Investment costs	Estimating the cost of capital investments such as investment costs for the main measures, including physical assets and services over the lifetime of the ISWM system. Estimates the operating and maintenance cost of investments and services over the lifetime of the ISWM system
4.2	Annual Operating and maintenance costs	Estimates of revenues, operating and maintenance costs and residual value of the investments over the lifetime of the ISWM system
4.3	Existence of a market for the sale of the final product	Assess whether the main end products (compost, recyclable materials, etc.) from the operation of the proposed measures are usable and available in the existing market. Furthermore, it evaluates whether these products meet, from the qualitative and quantitative point of view, the current required standards, to be considered usable. Finally, it assesses the possibility of alternative markets in the event of a change in the existing legal framework or market needs, to ensure the sustainability of technology
4.4	Employment generation potential	Assess employment opportunities for staff, especially in relation to the population of the neighboring area for the placement of facilities. It is an important factor especially as a compensatory benefit to one who undertakes to accept waste produced by others

Table 3. Weighing of Evaluation criteria [Source: Developed by author]

No	Evaluation Criteria	Unit	Importance Factor (100%)
1.	<b>Legal and Policy Criteria</b>		100
1.1	Compliance with European legislation and objectives for integrated SWM	0-10	40
1.2	Compliance with national laws, regulations, and policies/strategies	0-10	60
2.	<b>Environmental and Social Criteria</b>		100
2.1	Air pollution. Discharge of gaseous pollutants, within permitted limits	0-10	20
2.2	Pollution of soil, groundwater, and surface water. Discharges within permissible limits.	0-10	20
2.3	Ability to identify suitable locations for the location of SWM measures and their environmental impacts	0-10	30
2.4	Social impacts from proposed measures	0-10	10
2.5	Environmental mitigation measures	0-10	20
3.	<b>Technological Criteria</b>		100
3.1	Appropriateness of the measures (processes) towards future change of waste volume and quality, recycling, and diversion	0-10	40
3.2	Appropriate technology to guarantee efficient operation for the presented of SWM measures	0-10	50
3.3	The need for qualified personnel for the operation and maintenance of the selected technology	0-10	10
4.	<b>Financial and Economic Criteria</b>		100
4.1	Investment costs + operating and maintenance costs	0-10	30
4.2	Discounted Net Revenues	0-10	40
4.3	Existence of a market for the sale of the final product	0-10	20
4.4	Local community employment	0-10	10



Table 4. Evaluation through multi criteria evaluation of scenarios proposed in Waste zone of Fier  
[Source: Developed by author]

No.	Evaluation Criteria	Unit	Scenario 1	Scenario 2a	Scenario 2b	Scenario 3a	Scenario 3b
1.	<b>Legal and Policy Criteria</b>						
1.1	Compliance with European legislation and objectives for integrated SWM	0-10	4	7	7	8	9
1.2	Compliance with national laws, regulations, and policies/strategies	0-10	5	6	7	8	9
Sub-total 1			<b>9</b>	<b>13</b>	<b>14</b>	<b>16</b>	18
2.	<b>Environmental and Social Criteria</b>						
2.1	Air pollution & GHG emissions. Discharge of gaseous pollutants, within permitted limits	0-10	6	8	8	9	9
2.2	Pollution of soil, groundwater, and surfacewater. Discharges within permissible limits	0-10	8	7	7	7	7
2.3	Ability to identify suitable locations for the location of SWM measures and their environmental impacts	0-10	7	8	9	8	9
2.4	Social impacts from proposed measures	0-10	5	8	8	9	9
2.5	Environmental mitigation measures	0-10	6	8	9	8	9
Sub-total 2			<b>32</b>	<b>39</b>	<b>41</b>	<b>41</b>	43
3.	<b>Technological Criteria</b>						
3.1	Appropriateness of the measures (processes) towards future change of waste volume and quality, recycling, and diversion	0-10	5	8	8	9	9
3.2	Appropriate technology to guarantee efficient operation for the presented of SWM measures	0-10	7	6	9	6	8
3.3	The need for qualified personnel for the operation and maintenance of the selected technology	0-10	8	7	7	6	6
Sub-total 3			<b>20</b>	<b>21</b>	<b>24</b>	<b>21</b>	23
4.	<b>Financial and Economic Criteria</b>						
4.1	Investment costs + operating and maintenance costs	0-10	9	8	7	8	7
4.2	Discounted Net Revenues	0-10	7	6	9	6	8
4.3	Existence of a market for the sale of the final product	0-10	5	8	8	9	9
4.4	Local community employment	0-10	6	8	8	9	9
Sub-total 4			<b>27</b>	<b>30</b>	<b>32</b>	<b>32</b>	33
Scenario totals			88	103	111	110	117



## CONCLUSIONS

In order to meet the objectives set in the national legislation, all municipalities must follow the steps of the pyramid, which means that they will all have a quantity of waste that will remain after passing through all the stages of the waste hierarchy (prevention, reuse, recycling, recovery) from which more valuable materials can be recovered. Based on this assessment, the scenario which best meets the national objectives and those of the EU Directives, but which at the same time is quite ambitious and requires the commitment of all local and central institutions is Scenario 3b. In the table below, this scenario is given in more detail, where it is specifically highlighted:

- The system with 3 containers is proposed to be applied in the city of Fier
- Household waste collection center only in the city of Fier
- Composting at home only in rural areas, while the composting center (as a facility) only in the city of Fier
- 2 Transfer Stations for the Municipality of Divjakë and Lushnjë, as a transit point for the intermediate transfer of waste and to be sent afterwards to the Waste to Energy Treatment Plant in Fier
- The other municipalities of the Waste Zone in Fier will send the waste directly to the end point – the Waste to Energy Treatment Plant in Fier.

For the implementation of the **3-bin system to be applied in Waste Zone of Fier, it is recommended to keep in mind these activities for collection and transfer:**

Table 5. Recommendation for implementation of 3-bin system in Waste zone of Fier [Source: Developed by author]

COLLECTION	TRANSFER
<ul style="list-style-type: none"> <li>• Mixed waste is collected in 1 bin – this is proposed for all the municipalities of Fier Waste Area</li> <li>• Recyclables are collected: <ul style="list-style-type: none"> <li>• Fier City: <ul style="list-style-type: none"> <li>• 1 extra bin (the second bin)</li> <li>• Civic amenity centre</li> </ul> </li> <li>• For the municipalities of Divjakë, Lushnjë, Mallakastër, Patos and Roskovec in bring points (closed type skips 5m<sup>3</sup> capacity)</li> <li>• Bio-waste is collected in 1 other bin (the third bin) – this is proposed for selected business producers in Fier Municipality – the aim is that approximately 20% of the bio-waste generated will be treated at a composting facility.</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• The collected mixed waste is transferred to Fier WtEP as follows: <ul style="list-style-type: none"> <li>• Direct – municipalities of Mallakastër, Patos, Roskovec and Fier; and</li> <li>• Indirect via 2 transfer stations serving the municipalities of Divjakë and Lushnjë.</li> </ul> </li> <li>• The collected recyclables (via bins and civic amenity centre for Fier City and bring point for the rest of the municipalities in Fier Waste Area) are: <ul style="list-style-type: none"> <li>• Either sorted locally in the 2 proposed transfer station; or</li> <li>• Transferred to Fier WtEP for further sorting.</li> </ul> </li> <li>• The collected bio-waste from selected business producers of Fier City is treated at a composting plant (open windrows) at Fier WtEP</li> </ul>

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## **Evaluation of the Eutrophication State of The Butrinti Lake (Albania) During the Years 2012-2021**

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### **ABSTRACT**

Eutrophication is one of the most common phenomena affecting water bodies worldwide. This phenomenon is caused by an excess input of nutrients, especially phosphorus and nitrogen, to naturally oligotrophic or mesotrophic ecosystems. In this study we aimed to evaluate the eutrophication state of the Butrinti Lake during the 2012 – 2021 period. Butrinti is a wetland complex located in the central area of the National Park of Butrinti, Albania, (UNESCO List of World Heritage 1992), belongs to the wetland complex of Butrinti proclaimed Ramsar Zone (1997–2005), famous for its archaeological monuments, historical significance and natural richness. The eutrophication was evaluated based on the data from the State of the Environmental Reports, published annually from the National Environmental Agency in Albania. The main parameters taken into consideration for the eutrophication state were water transparency, water turbidity, COD (chemical oxygen demand), BOD (biological oxygen demand), nutrients (total phosphorus, total nitrogen), TSI (trophic state index), measured at one monitoring station. Results showed that Butrinti Lake has a mesotrophic state. Based on these results we hypothesize that the main reasons of this trophic state could be urban wastewater, solid waste and farming. Thus, it is necessary to undertake urgent measures in order to prevent the eutrophication of the studied area.

**Keywords:** Eutrophication state, Butrinti Lake, Nutrient enrichment, wastewater treatment

### **INTRODUCTION**

Eutrophication is characterized by excessive plant and algal growth due to the increased availability of one or more limiting growth factors needed for photosynthesis [1], such as sunlight, carbon dioxide, and nutrient fertilizers. Eutrophication occurs naturally over centuries as lakes age and are filled in with sediments [2].

Lakes are an important inland water ecosystem with irreplaceable ecological functions, social benefits and economic value. However, in recent decades, increased human activities have seriously affected the ecosystem of lakes, leading to a rapid deterioration in their water quality. Some lakes have such serious water pollution that it exceeds the carrying capacity of the lake itself [3].

Eutrophication is one of the most important and concerning water quality problems in freshwater ecosystems. The occurrence of water eutrophication is mainly caused by the increase of nitrogen, phosphorus and other nutrients in the water [3].

In this study we aimed to evaluate the eutrophication state of the Butrinti Lake during the 2012 – 2021 period.

## **MATERIALS AND METHODS**

### **Study Area**

Butrinti Lake located in the central area of the National Park of Butrint, Albania, (UNESCO List of World Heritage 1992), belongs to the wetland complex of Butrinti proclaimed Ramsar Zone (1997–2005). Having a surface of 1600 ha, it has a tectonic origin, and a water regime typical for coastal lagoons [4]. In contrast to most Albanian coastal lagoons, Butrint is unique due to its depth, with an average depth of 11m and a maximum of 22m, largely enclosed by steep rocky slopes and its stable physical environment (Figure 1). Butrinti Lake is part of a hydrographic system together with the Kalasa River, Bistrica and Pavllo River, Butrinti wetland (known as Butrinti Lake) and Bufi Lake (Figure 2). Butrinti Lake communicates with the Ionian Sea through the Vivar natural channel (Figure 1) (3600 m long, 60-100 m wide and up to 5-6 m deep). Butrinti lagoon is also exceptional because its natural fish populations are complemented by aquaculture in the form of mussel farming [5].

Butrint supports a large number of plants and animals considered as having an unfavorable conservation status either nationally or internationally, such as *Numenius tenuirostris*, *Caretta caretta*, *Dermochelys coriacea*, and *Monachus monachus*. The area is also an important spawning ground, food source and migration path for fishes [6].



Figure 1. Vivari Channel

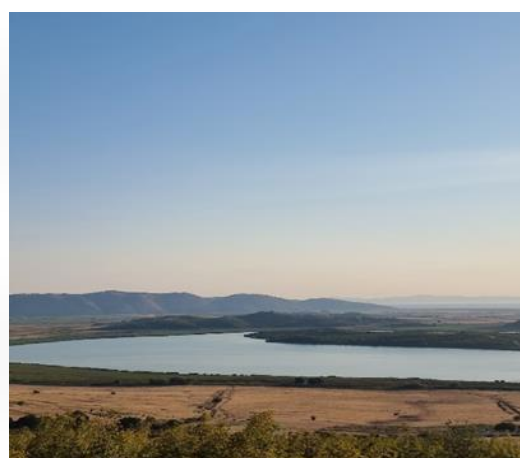


Figure 2. Bufi Lake

Information about the eutrophication state of Butrinti Lake was obtained according to the site visit, collection of data and activities inside the catchment area. The catchment area of Butrinti Lake is

in the risk of overpopulation because of the development projects (increased demand for tourist facilities and accommodation structures, for recreation and agricultural purposes). It is paradoxical, but it is worth saying that these threats to the National Park will increase the importance of the site as a protected site in the future.

The trophic state of Butrint Lake was evaluated at one monitoring stations, located in its strategic area, in two different depth, respectively 0 m and 5 m. At this monitoring station was gathered information related to the water quality, water level and meteorological data to assess the trophic state of the lake and the impact of various factors on its environment.

Sampling site in this aquatic ecosystem was selected with reference to criteria such as the degree of pollution, fresh water supply of lagoon ecosystems, etc.

The eutrophication was evaluated based on the data from the Reports of the State of the Environment prepared by National Environmental Agency (NEA) of Albania. The Report is prepared and published every year, together with the National Program of Environmental Monitoring.

We collected the result of NEA monitoring from the State of the Environment Reports for the period 2012 – 2021.

The NEA laboratory is accredited for the following parameter and measurement techniques:

Nitrate: .01-0.2 mg/l, measured with spectrophotometry, Standard IRSA-CNR (2015)

Nitrate: > 0.001 mg/l, measured with spectrophotometry, Standard APHA 4500- NO<sub>3</sub>-B 2017 (2021)

P-total: 0.005 – 0.4 mg/l, measured with spectrophotometry, Standard ISO 6878: 2004 (2015)

P-total: > 0.005 mg/l, measured with spectrophotometry, Standard S SH EN ISO 6878: 2004 (2021)

COD: 2-1500 mg/l (without dilution), measured with photometry/ titration, Standard S SH ISO 15705:2002 (2015), (2021)

BOD<sub>5</sub>: 1-6000 mg/l, measured with oxi-top system, Standard S SH ISO 5815-1:2003 (2015).

BOD<sub>5</sub>:1-4000 mg/l, measured with respirometric method with oxi-top system, Standard APHA 5210-D 2017(2021) [7].

Transparency was measured with Secchi Disk.

Turbidity was measured with FTU (Formalin Turbidity Units) multiparametric probe (HaNNA HI 93703-11).

Chlorophyll – a, was measured using a spectrophotometer.

A numerical index of trophic status has been calculated on a scale from 0 to 100. Each major division (10, 20, 30, etc.) represents a doubling of algal biomass. The index number can be calculated from any of several parameters, including Secchi disk transparency, turbidity, chlorophyll a and total phosphorus.



The Trophic Status Index (TSI) is a classification system designed to "rate" individual lakes, ponds, and reservoirs based on the amount of biological productivity occurring in the water. Using the index, one can get a quick idea of how productive the lake is. The classifications range from 1 to 100, tab 2 [9], [10].

Lake Butrinti monitoring station has the corresponding GBS coordinates:

Station 1-→ N-39.93908, E-20.027069 WS (2020) [11].



Figure 3. Monitoring station (yellow is for mesotrophic state) [8]

## RESULTS AND DISCUSSIONS

The monitoring station is located in the Butrint canal at the entrance of the lake (Figure 3). On the bank located in the southern area of the channel near the station, there is a considerable number of wastes. At this station the water is relatively calm and clear in July. The transparency of the water at this station has increased greatly in August and its color is almost blue. In the following month, the transparency of the water drops to the minimum transparency value that was observed in the second half of September.

In this period the water is very cloudy and its color is expected to be light green. In October the water is noticeably greener compared to other months [10].

Turbidity and Transparency.

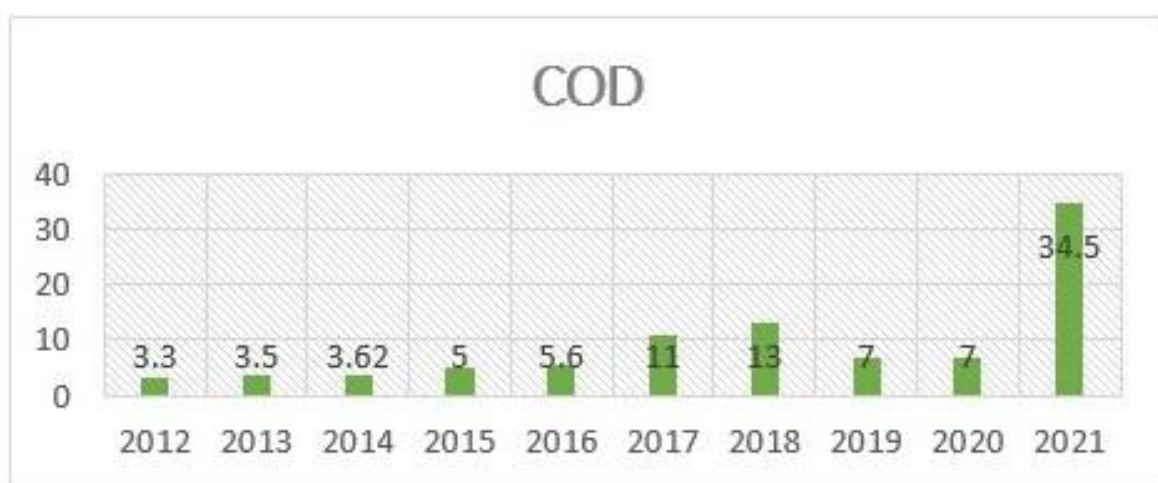
Turbidity in Butrinti Lake varied from 0, 41 to 7, 15 FTU.

Graph 1 shows the transparency levels in Butrinti Lake, from 2012 to 2021. The lowest value is in the 2017, 1 m and the highest value is 3.8 during 2021. According to the transparency results Lake Butrinti is in the oligotrophic state (Tab.1).



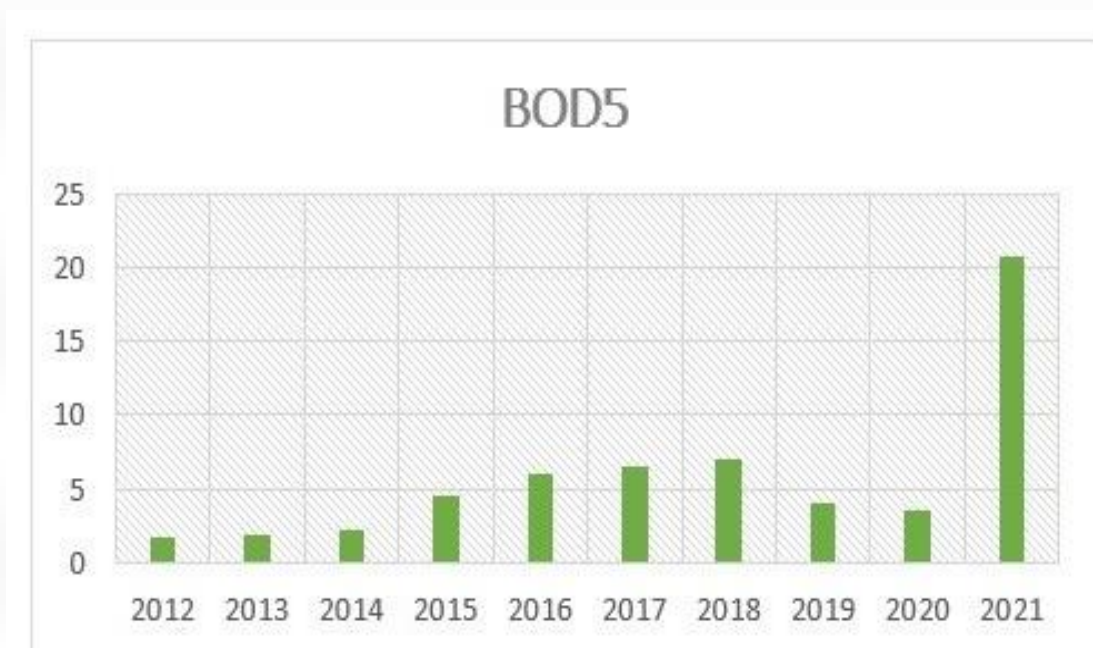
Graph 1. Transparency levels in Butrinti Lake, from 2012 to 2021

Graph 2 and 3 shows the CODs and BOD<sub>5</sub> levels in Butrinti Lake from the year 2012 to 2021. The lowest value of COD is in 2012, 3.3 mg/l. The same situation is also for BOD<sub>5</sub> level. The lowest value is in 2012, 1.8 mg/l. During the year 2021, Butrinti Lake has the highest COD level, 34.5 mg/l, and the highest BOD<sub>5</sub> level, 20.8 mg/l. According to COD and BOD<sub>5</sub> values Lake Butrinti is in the eutrophic state (Tab.1).



Graph 2. CODs values in Butrinti Lake during 2012-2021





Graph 3. BOD5 values in Butrinti Lake during 2012-2021

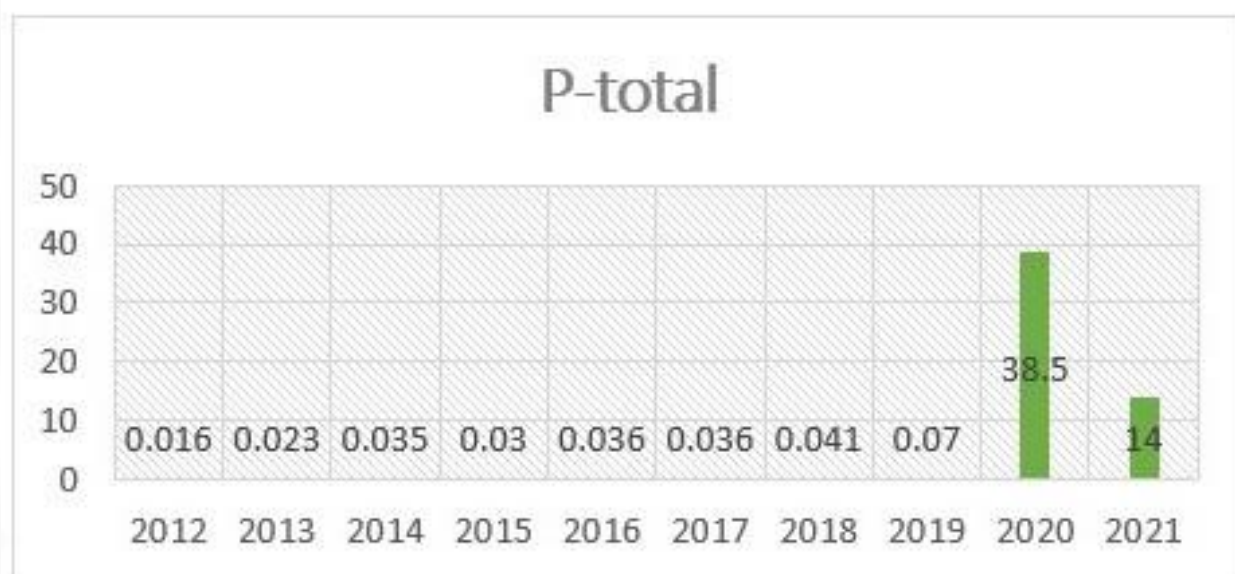
### Nutrients

Nitrates and Phosphates are the main factors enhancing the eutrophication phenomena.

Graph 4 shows the nitrate levels in Butrinti Lake from the year 2012 to 2021. Nitrate had the highest value in 2014, and the lowest one in 2017, 0.072 mg/l, while in 2021 Nitrate was 0.4 mg/l. According to the nitrate value Butrinti Lake is in the oligotrophic state (Tab.1). Graph 5 shows the P-total levels in Butrinti Lake from the year 2012 to 2021. P-total had the highest value (38.5 mg/l) in 2020, and the lowest one in 2012, 0.016 mg/l while in 2021 P-total was 14 mg/l. According to the phosphate value Butrinti Lake is in the eutrophic state (Tab.1).

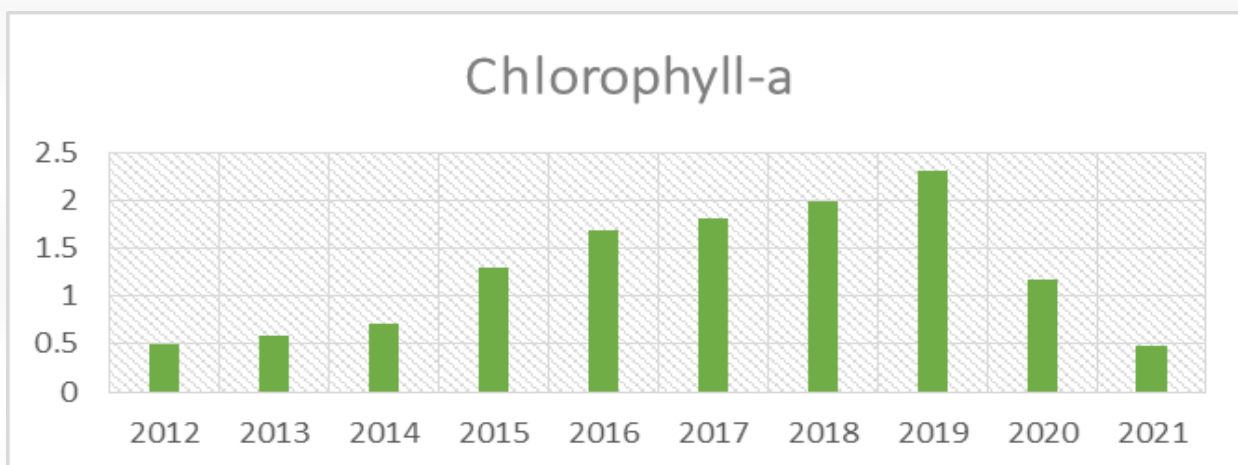


Graph 4. Nitrate values in Butrinti Lake during 2012-2021



Graph 5. P-total values in Butrinti Lake during 2012-2021

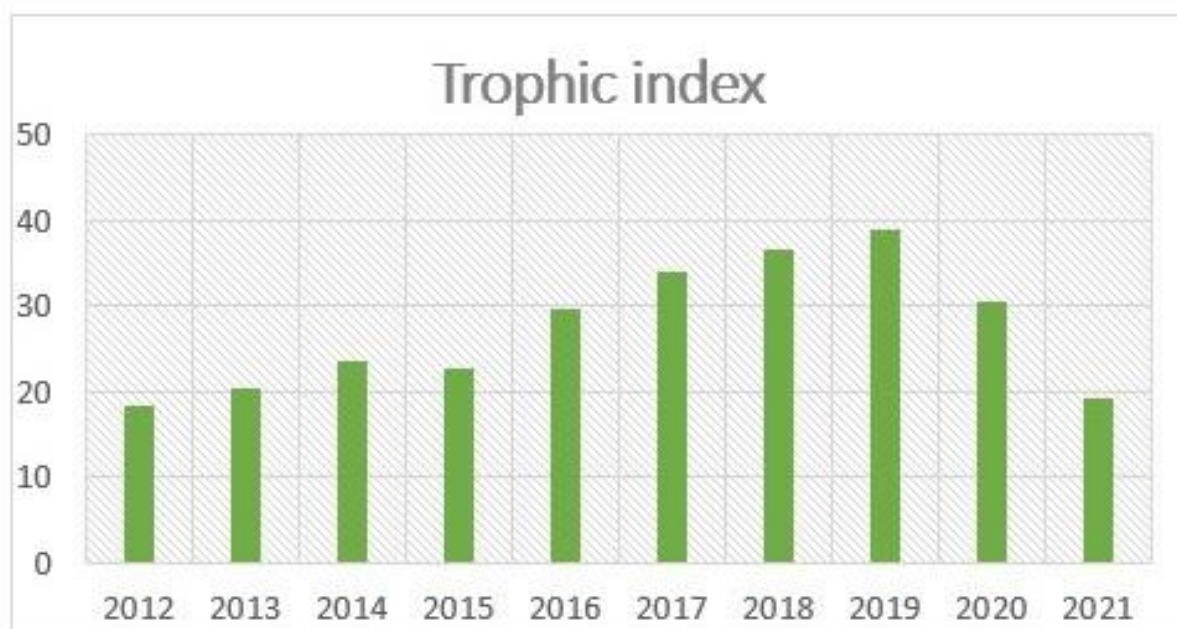
Graph 6 shows the chlorophyll-a levels in Butrinti Lake from the year 2012 to 2021. Chlorophyll-a had the highest value (2.4) in 2019, while in 2021 chlorophyll-a was 0.5 mg/l, the lowest value. According to the chlorophyll-a values Butrinti Lake is in the oligotrophic state (Fig. 4).



Graph 6. Chlorophyll –a values in Butrinti Lake during 2012-2021

We can compare the annual average values of chlorophyll to see if the amount of algae in the lake is increasing, decreasing or staying the same.

The amount of algal growth in a lake depends on many factors, including water transparency, water temperature, predation by zooplankton, and the availability of nutrients (especially phosphorus and nitrogen). There are natural seasonal changes in algae concentrations.



Graph 7. TSI index in Butrinti Lake during 2012-2021

Graph 7 shows the TSI levels in Butrinti Lake from the year 2012 to 2021. TSI had the highest value in 2019, and the lowest in 2012, respectively 38.8 and 18.3. TSI in 2021 was 19.15. According to the TSI index Butrinti Lake is in the oligotrophic state (Tab. 2).

The assessment of the quality of the lakes is determined through water quality limit values of lakes according to the Water Framework Directive and Carlson trophic state index (TSI) as below:

Table 1. Limit values of Lake water quality according to Water Framework Directive [9]

Parameters	Unit	Limit values for determining trophic state		
		Oligotrophic	Mesotrophic	Eutrophic
Transparency	m	5-10 (max 15-20)	1-2 (max.5-10)	<1 (max 2-3)
COD	mg O <sub>2</sub> /l	1-2	8-9	20-65
BOD <sub>5</sub>	mg O <sub>2</sub> /l	< 3	3 – 5.5	5.5 - 14
Nitrate (NO <sub>3</sub> -N)	mg N/l	<1	< 1	>2
Total Phosphor (P- total)	µg P/l	4 – 10	10 – 35	35 - 100

Table 2. Carlson trophic state index (TSI) [9]

TSI Index	Description
<b>TSI &lt;30</b>	Classical oligotrophic; clear waters, oxygen throughout the year in the lipolimnion layer, fish in depth
<b>TSI 30-40</b>	Deep lakes appear classically oligotrophic, but shallow waters become anoxic in summer.
<b>TS 40-50</b>	Still clear waters, but the probability of anoxia in the hypolimnion layer increases during the summer
<b>TS 50-60</b>	Low level of classical eutrophic: reduced transparency, anoxia in the hypolimnion during summer, macrophytes present, and warm water fish.
<b>TSI 60-70</b>	Blue-green algae predominate, possible algal foam, appreciable presence of macrophytes.
<b>TSI 70-80</b>	"Bloom" of algae during the summer, a dense layer of macrophytes, but with a limited extent due to of light penetration. Often the condition can be classified as hypertrophic.
<b>TSI &gt; 80</b>	Algal foam, dead fish in summer, macrophytes, and resistant fish dominate.



Figure 4. Chlorophyll – a related to trophic state  
(source:<https://www.rmbel.info/primer/chlorophyll-a/>)

These differences in the transparency values of can be explained by sea-lagoon communication, fresh water supply as well as pollution caused by human activity near the station.

On the other hand, human impact related turbidity in Lake Butrint, can be water pollution from activities such as illegal constructions in the surroundings of the lake and illegal fishing, which can lead to the destruction of the lake's ecosystem and water salinization.

As can be seen from all te results of the measured parameters there are changes during the studied period. These changes can be attributed to the following contributors:

### **1. Water supply: The irrigation and drainage network is highly amortized.**

The drainage system needs significant improvement, including the cleaning of the channels and the rehabilitation of the pumping systems. A good part of the agricultural land is flooded for a relatively long period (autumn-winter-early spring).

**2. Sewage of urban waste water:** The main problem in the sewerage network remains the pipeline of the urban wastewater treatment plant, where according to the Saranda water supply, this main pipeline is out of standard and often breaks. The last cracks of this pipeline have caused the polluted urban waters to flow into the lake of Butrint, endangering the pollution of the water of the lake as well as the cultivation of mussels, which according to the latest analyzes of the AKU, the level of salmonella bacteria is alarming.

In the area around the lake, there are various sources of water pollution, including discharge of polluted water from industry, illegal sewage and general water pollution from human activities. The wastewater treatment plant also has a significant impact. It was built near the existing hydropower station, Cukë village. The Saranda plant treats the polluted sewage water of the 30,000 inhabitants of the Saranda municipality. It is designed to handle an average flow of about 1,920 m<sup>3</sup>/day. The wastewater treatment plant faces different problems, such as power outage, lack of investment and maintenance, impact of the tourist season which can increase the load on wastewater treatment plants and challenge their capacity to supply sufficient amounts of clean water.

The discharge of polluted water from the waste water treatment plant into Lake Butrint in Saranda is a serious and problematic issue. The discharge of polluted water in an attractive touristic and natural area such as Lake Butrint can have harmful consequences for the ecosystem and human health. According to experts, in 2020, a considerable increase of these discharges into the lake has been calculated. This was the main reason why this year there was a big increase in P-total in the waters of Lake Butrint (Graph 5).

**3. Communicating Channels:** Vivar Channel Through this canal, Lake Butrint communicates with the Ionian Sea.

The Vivar canal creates a unique situation in the Butrint Lake, which is fed with fresh water and partly with salt water, thus creating ideal conditions for the cultivation of mollusks.

Vivar Channel is important for many reasons. First, it supplies Lake Butrint with fresh water, keeping the water ecosystem stable and clean.

Çuka Channel:

The Cukes Canal is an artificial channel, created by the people of the area during the reclamation of the Vurgu field. The waters of the Bistrice River and the Kalasa River flow together in this channel. This channel is distinguished by a great pollution.

This channel, constructed time ago, can not support the pressure of rainfall, causing flooding of the agricultural lands located along this canal.



**4. Chemical pesticides and fertilizers:** Unsafe agricultural practices, such as the use of excessive pesticides and improperly managed agricultural waste, can lead to lake water pollution. These chemical substances can flow into the percola waters and affect the biodiversity and water quality of the lake.

In some cases, the cultivation of agricultural lands around the lake can have negative consequences for the environment and the health of the lake.

**5. Soil Erosion:** Stagnant or undisturbed farming practices can lead to soil erosion around the lake. Soil erosion creates major problems related to the loss of agricultural land, the change in the shape of the lake and the further risk of water pollution.

## CONCLUSIONS AND RECOMMENDATIONS

The Butrinti Lake trophic state based on the transparency, Nitrate, chlorophyll-a, and TSI, in 2021 is oligotrophic. Classical oligotrophic; clear waters, oxygen throughout the year in the lipolimnion layer, fish in depth (Tab.2).

Based on the values of transparency, COD, BOD<sub>5</sub>, P-Total, during 2012 where we had the lowest values, the situation has progressed from the lake to oligotrophic state towards mesotrophic state.

Trophic state of the Lake based on COD, BOD<sub>5</sub>, and P-total is eutrophic for 2021.

The biggest pollution of the lake from which eutrophication is affected is the discharge of polluted water. Related to this main contributor, the authors provides the following recommendations:

- *Monitoring and implementation of pollution control*

It is important that the waste water treatment plant in Saranda, one of the main causes, is monitored and strict rules for the control of treated water pollution are implemented. Butrint Lake meets the standards set for environmental protection. Reducing the external load of nutrients is the most vital step in lake management. Through monitoring, an active water management is ensured, the main function of which is to reduce the concentration of phosphorus in the lake, which comes in significant quantities from the effluents of this plant in the lake waters.

- *Advanced technologies for the treatment of polluted waters*

The use of advanced wastewater treatment technologies can help increase the efficiency of the plant and reduce the pollution levels of water discharged into the lake. This may include the use of advanced filters and the construction of appropriate infrastructure for the retention and control of treated water. For the technology mission, it is also related to the provision of treated water discharge infrastructure that must be secure and equipped with equipment for monitoring and controlling the pollution of water discharged into the lake.

- *Chemical treatment*

One method of biological treatment of lake waters to protect against eutrophication is the application of algacides, chemicals designed to kill algae. There are concerns that algacides may also pose a threat to humans and have harmful effects on lake ecosystems and the "queen of the

lake" mussels. I would suggest this treatment in very small quantities and in a non-fertile period, because the purpose of the chemical treatment is to protect the ecosystem from its aging by eliminating old algae and enabling the creation and development of new algae and organisms.

Copper sulfate is a common algaecide and controls algae by freeing the phytoplankton cell. This can cause the rapid release of toxins contained within the algae such as cyanotoxins. There is also evidence that algae are becoming resistant to the application of algaecides such as copper sulfate (method not recommended for the current eutrophication values of Lake Butrint).

- *Physical treatment*

Destratification, physical removal, or harvesting and dredging of sediments are possible control methods, although they have the significant drawback of being very expensive. Destratification can be done by pumping nutrient and oxygen rich waters from the surface down to the surface using an axial flow propeller pump. However, this would not be feasible on a large scale. Destratification by pumping air across the lake can be very expensive and energy intensive, there is a proposed method that is even more economically and environmentally friendly.

Harvesting algae when blooms begin to develop is another management technique. This is also a relatively expensive method due to equipment, fuel and labor costs and is unlikely to be feasible for larger lakes and reservoirs. A benefit of this method is that many algae have commercial value. Similarly, for sediment cleanup, the economic costs would be substantial and there would also be negative ecosystem effects, particularly for benthic organisms that could lead to effects throughout the entire trophic web. Studies also suggest that sediment cleaning can improve water quality in the short term, but is not a sustainable long-term solution.

- *Awareness of people*

Awareness of people is one of the main links of the chain which, if it works properly, enables a smaller problem. Human activity in eutrophication affects the spilling of fuel, fuels from vessels such as boats, throwing waste during tourist trips.

Another major connection can be seen in the field of agriculture, where awareness should influence the use of lower doses of pesticides, chemicals and not illegally taking water from the lake in the peak summer period through various pumps.

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## ***Staphylococcus pettenkoferi*: An Emerging Resistant Bacteria in Veterinary Medicine?**

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### **ABSTRACT**

*Staphylococcus pettenkoferi* is a coagulase-negative bacteria that has been linked to several drug resistant bacterial infections in humans and animals in recent years. *S. pettenkoferi*'s drug resistant nature has led to increasing interest in the pathogen within the medical and veterinary communities. Given these findings, it was hypothesized that *S. pettenkoferi* is a potential animal and zoonotic pathogen that can contribute to multi-resistant infections with the ability to form multi-resistant biofilms. To investigate the bacteria's zoonotic and drug resistant potential, methods including disk diffusion tests and polymerase chain reaction (PCR) tests were used to assess the drug resistant potential of *S. pettenkoferi* strains of human and animal origin. The study was conducted using 14 bacterial strains collected from human patients at the Wrocław Clinical Hospital in Wrocław, Poland and 7 strains collected from healthy dogs and cats from Wrocław University of Environmental and Life Sciences. The study found that over half of the tested human strains were resistant to penicillins, as well as certain lincomycins, macrolides, fluoroquinolones, and monocarboxylic acids, while similar levels of drug resistance were not present in the tested animal strains. These results suggest the potential for *S. pettenkoferi* infections in animal patients to translate to drug resistant infections in certain at-risk human populations, especially those in frequent contact with animals.

**Keywords:** *S. pettenkoferi*, drug resistance, One Health, biofilms

## **INTRODUCTION**

*Staphylococcus pettenkoferi* (*S. pettenkoferi*) is a coagulase negative human commensal bacterium first discovered in 2002 [1, 12]. More recently, the bacteria have also been isolated from animals in several studies [2]. While the bacteria's clinical significance was not fully understood at the time of discovery, *S. pettenkoferi* has since been identified as the causative agent in a number of opportunistic infections in humans including in patients with catheters, prostheses, and diabetic foot infections [9]. The bacteria has also been identified in bloodstream infections [9, 13, 14] and has been isolated across a vast number of regions including North America, Western Europe, Japan, Brazil, South Korea, Russia, Poland, and Kenya [3]. The bacteria has proven to be of veterinary importance given its propensity to develop multi-drug resistance in companion animals, i.e., cats and dogs [4, 5].

The aim of this study was to determine the epidemiological characteristics of *S. pettenkoferi* strains of human and animal origin. This study also sought to determine whether *S. pettenkoferi* exhibits antibiotic resistance on the phenotypic or genotypic level.

## **MATERIAL AND METHODS**

### **Sample Collection**

Bacterial isolates were collected from archived strains of *S. pettenkoferi* housed in the Wroclaw University of Environmental and Life Sciences Department of Epizootiology and Clinic of Birds and Exotic Animals. A total of 12 human and 10 animal strains were used in this study. The human strains were collected from patients undergoing treatment at Wroclaw Clinical Hospital and the animal strains were collected from clinically healthy cats and dogs at Wroclaw University of Environmental and Life Sciences' veterinary hospital. Four of the swab samples from animal subjects were collected from animals' conjunctiva, one was collected from the groin, and one was collected from the pinna region of the ear. All human strains were obtained from blood samples. Identification of the cultured species was performed previous to the study using matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry.

### **Phenotypic Resistance**

Phenotypic testing was achieved via the disk diffusion method performed according to EUCAST recommendations (<https://www.eucast.org/>). Samples from liquid cultures were streaked across Mueller-Hinton Agar (MHA) and tested using disks loaded with various antibiotics (µg/disc) including penicillin G (10), oxacillin (1), amoxicillin-clavulanate (30), erythromycin (15), clindamycin (2), gentamicin (10), tobramycin (10), ampicillin (10), rifampin (5), tetracycline (30), marbofloxacin (5), ciprofloxacin (5), chloramphenicol (30), fusidic acid (10), tigecycline (15), trimethoprim/sulfamethoxazole (1.25/23.75), and linezolid (30) (Antimicrobial Susceptibility Disks, Oxoid Ltd., Wade Road Basingstoke, United Kingdom). The plates were then incubated at 37°C for 24 hours and interpreted according to EUCAST guidelines.

### **Genotypic Resistance**

Bacterial DNA was purified using A&A Biotechnology's Genomic Mini AX *Staphylococcus* Spin (A&A Biotechnology, Gdansk, Poland) kit in accordance with the manufacturer's instructions.

The presence of genes involved in resistance to the tested antibiotics was determined using the PCR method as described in previous literature (Bierowiec et al. 2016). The genes detected indicated potential resistance to penicillin (*blaZ*), aminoglycosides (*aac(6')Ie-aph(2'')Ia*),  $\beta$ -lactamase (*mecA*, *mecC*), glycopeptides (*vanA* and *vanB*), macrolide-lincosamide-streptogramin (*ermA*, *ermB*, and *ermC*), tetracyclines (*tet(K)*, *tet(L)*, *tet(M)* and *tet(O)*), fusidic acid (*fusB*), and mupirocin (*mupA*).

## RESULTS

### Phenotypic Resistance

The preliminary results for both the phenotypic and genotypic antibiotic resistance tests showed significant levels of drug resistance in human strains of *S. pettenkoferi* and mild resistance in animal strains. The table below illustrates the study's phenotypic antibiotic resistance results.

Table 1. Phenotypic Resistance with Disk Diffusion - Human Strains

Antibiotic	Percent of Resistant Strains
Penicillin	63%
Ampicillin	63%
Amoxicillin	54%
Oxacillin	36%
Clindamycin	63%
Erythromycin	63%
Ciprofloxacin	63%
Marbofloxacin	45%
Mupirocin	36%

Human strains of *S. pettenkoferi* showed marked phenotypic resistance to a variety of antibiotic classes. Of the 10 human strains tested, 63% were shown to be resistant to penicillin and ampicillin along with a further 54% and 36% resistant to amoxicillin and oxacillin respectively.

With regard to the other antibiotics tested, 63% of human strains were found to be resistant to clindamycin, erythromycin, and ciprofloxacin, with a further 45% resistant to marbofloxacin and 36% resistant to mupirocin.

Table 2. Phenotypic Resistance with Disk Diffusion - Animal Strains

Antibiotic	Percent of Resistant Strains
Clindamycin	33%
Erythromycin	33%

Animal strains of *S. pettenkoferi* were shown to be much less phenotypically resistant than their human counterparts. Of the 12 animal strains tested, only 33% were found to be resistant to clindamycin and erythromycin respectively.

#### Genotypic Resistance

The results of the study's genotypic resistance tests were consistent with the phenotypic resistance results in that they showed significantly higher levels of resistance in human strains than in animal strains.

81% of human strains were found to be harboring the *ermA* and *ermB* genes, with only 9% of strains found to be positive for *ermC*. These genes have been shown to be responsible for resistance to macrolides.

Of the strains analyzed, 63% were found to be positive for the *mecA* gene responsible for resistance to penicillin and methicillin, while an additional 54% tested positive for *blaZ*, another gene known to code for penicillin resistance.

Interestingly, 100% of the tested human strains were found to harbor the *fusB* gene which is responsible for resistance to fusidic acid.

Table 3. Genotypic Antibiotic Resistance in Human Strains

Gene	Antibiotic Resistance	Percentage of Strains Harboring Gene
<i>ermA</i>	Macrolide-Lincosamide-Streptogramin	81%
<i>ermB</i>	Macrolide-Lincosamide-Streptogramin	81%
<i>ermC</i>	Macrolide-Lincosamide-Streptogramin	9%
<i>blaZ</i>	Penicillin	63%
<i>mecA</i>	$\beta$ -lactam Antibiotics	54%
<i>fusB</i>	Fusidic Acid	100%

Table 4. Genotypic Antibiotic Resistance in Animal Strains

Gene	Antibiotic Resistance	Percentage of Strains Harboring Gene
<i>ermA</i>	Macrolide-Lincosamide-Streptogramin	33%
<i>ermB</i>	Macrolide-Lincosamide-Streptogramin	33%
<i>fusB</i>	Fusidic Acid	100%

As stated above, the strains of *S. pettenkoferi* tested in animals demonstrated comparatively lower levels of genotypic resistance than strains found in humans. Notably, the presence of the *ermA* and *ermB* genes was confirmed in only 33% of animal strains.

The highest level of genotypic resistance in animal strains was seen for the *fusB* gene with 100% of strains being found to be harboring this gene.

## DISCUSSION

The study's preliminary trials found that levels of both phenotypic and genotypic antibiotic resistance were significantly higher in human strains of *S. pettenkoferi* compared to levels in animal strains. Given the increasing global threat of antibiotic resistance within a One Health framework, further testing is needed to better understand whether or not *S. pettenkoferi* could pose a risk as a zoonotic pathogen. Several studies have shown the potential for *S. pettenkoferi* infections to be transmitted from companion animals to their caregivers [4, 5]. These previous studies coupled with the high levels of antibiotic resistance in human strains found in our study suggest the potential for *S. pettenkoferi* to pose a public health risk for those who are frequently in close contact with animals, whether or not the animals they encounter exhibit clinical signs of an *S. pettenkoferi* infection. More data is needed to fully support this claim and subsequent trials will focus on the risk for multiresistant zoonotic infections.

The preliminary results of this study offer important insight into the state of *S. pettenkoferi*'s antibiotic resistance and more broadly, the antibiotic resistance of *Staphylococcus* species as a whole. While *S. aureus* is known to be the most resistant staphylococcal species [6, 7], several studies have suggested *S. pettenkoferi*'s virulence to be comparable to that of *S. aureus* [2, 8, 9,10]. While more data is needed to fully understand *S. pettenkoferi*'s virulence potential, these findings are important as they allow veterinary practitioners to more accurately assess infection risks and mitigate the risk of interspecies disease transmission.



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## **Effects of Adding Black Cumin Seed Meal to Laying Hen Diets on Performance and Egg Quality**

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### **ABSTRACT**

Feeding expenses constitute approximately 70% of the expenses in animal breeding. In order to reduce feeding costs, it is necessary to include cheap feed sources in feed. At the same time, the use of various industrial residues as long as their nutritional values enough is important in terms of reducing environmental pollution and utilizing resources appropriately. This study was conducted to determine the effects of black cumin (*Nigella sativa* L.) seed meal, which is produced as a residual material after the oil is extracted, on performance and egg quality in laying hens. A total of 48 commercial white laying hens of Lohman breed, 58 weeks old, were used in the study. Black cumin seed meal (BCSM) was added to the laying hen diets at 0 (control), 100 and 200 g/kg. Chickens were placed in individual cages in 16 replicates for each treatment. Live weight measurements were determined at the beginning and on the last day of the experiment. Feed consumption and egg internal and external quality measurements were made on days 21, 35 and 49. The addition of BCSM did not significant effect on live weight and live weight change values of hens. Feed consumption values were significantly lower in the group with 20% BCSM added group than the control and 10% BCSM group ( $P<0.05$ ). Feed efficiency of 20% BCSM group was significantly lower in 49 days and the overall period ( $P<0.01$ ). Adding of 20% BCSM to the diets significantly reduced egg production and mass ( $P<0.01$ ). As a result, adding 20% BCSM to laying hen diets negatively affects feed consumption and egg production parameters, so it is recommended to use it at 10% or lower levels in the laying hen diets

**Keywords:** Black Cumin Seed Meal, Economic Production, Sustainability, Egg,

### **INTRODUCTION**

In order for people to survive, alternative feed sources must be found in animal husbandry and animal feeding must be made more economical. Soybean meal, a protein source widely used in poultry nutrition, creates a great economic burden because it is imported due to produce in sufficient quantities in some countries. Some industrial residues, including black cumin seed meal

(BCSM), which is obtained after the extraction of black cumin oil, can be used as a feed raw material that can be fed to animals in certain amounts economically. Producers' ability to find alternative feed raw materials easily may reduce the need for protein sources.

In some studies conducted so far, black cumin (*Nigella sativa* L.) seeds and oil have been used in various animal nutrition studies in low concentrations (Bölükbaşı et al., 2009; Demirci et al., 2019; Seidavi et al., 2020; Fathi et al., 2023). We aware only a small number experiment BCSM percentage was high (El-Tohamy et al., 2010) However, there are no sufficient studies on the use of black cumin seed meal remaining after the oil is extracted. The cake remaining after the extraction of black cumin seed oil contains high levels of crude protein (approximately 33%). Black cumin meal is often not considering by oil producers because it is not produced sufficiently and its nutritional value is not well understood. On the other hand, since the usage levels and effects on animals are not well known, it is not known exactly what negative effects it will cause when given to animals in large amounts. Therefore, in this study, the effects of adding 0, 10 and 20% black cumin seed meal to laying hen diets on performance and some egg quality characteristics were investigated.

## **MATERIAL AND METHODS**

For this study the local experimental animal ethics committee report was approved with decision number 17/022, dated 15.03.2017.

In the study, a total of 48 commercial Lohman white laying hens 58-weeks olds were used as animal material. Before the research, the body weights (BW) of the animals were measured and grouped according to their body weight to minimize differences BW. Also egg yields of hens were recorded for 20 days and considered for grouping.

The black cumin meal (BCSM) were bought from a commercial oil store which processing cumin seed for oil. Chemical composition of BCSM was analyzed and found that contents of dry matter (DM) 91.72%, crude protein (CP) 36.23%, crude oil (CO) 17.16% and crude ash (CA) 6.30%. Black cumin seed meal was added to the concentrated feed at the levels of 0, 100 and 200 g/kg. In the study, corn, wheat, soybean meal and sunflower meal based feed was used. The experimental diets were prepared according to meet laying hen's nutrient requirement. The diets contained corn, wheat, soybean meal, sunflower meal, plant oil, salt, lysine, vitamin and mineral premix, black cumin seed meal, di-calcium phosphate, limestone and phytase enzyme. Black cumin seed meal included the formulas and used as a feed material 0, 10 and 20% in diets. Diets chemical composition was 2750 metabolizable energy (ME), 17.50% crude protein, 3.45 % calcium, 0.28 % available phosphorus, 0.30 % methionine, 0.73 % lysine and 0.15% sodium.

Body weight of laying hens measured initial and last day of experiment and BW differences calculated. The total feed consumption was measured for 21, 35 and 49 days and calculated by weighing the remaining feed at the end of the each period. Individual feed consumption was calculated for the animals was measured for aforementioned days. Feed conversion ratio (FCR) was calculated by dividing periodic feed consumption: egg mass ratio. Egg production were recorded daily and the number of eggs laid by each chicken in period days and calculated. Egg yields were calculated as percentage of total "egg number: (hen\*period days) x 100".

To determine egg traits, the eggs were collected for consecutive two days and analyzed every at 21, 35 and 49 days. Eggs were numbered and two eggs analyzed for each chicken. When the analysis started, the eggs were weighed with a scale with a precision of 0.1 g and recorded. Egg mass was calculated by multiplying the average % egg yield and egg weight in the same period.

The data were statistically analyzed by using the SPSS 9.05 statistical program. Duncan test was applied to detect differences in means. For significance level,  $P < 0.05$  level was taken into account in the test.

## RESULTS AND DISCUSSION

The effects of addition of BCSM to ration on live weight, feed consumption and feed conversion values are given in table 1. No significant difference were detected between the groups in terms of body weight (BW), and BW change values between the groups ( $P > 0.05$ ). It was observed that the addition of 20% BCSM meal to the ration decreased feed intake in all periods of experiment compared to control and 10 BCSM groups. There were no significant differences among the control and 10% BCSM meal supplemented groups. Feed conversion ratio (FCR) of groups were not significantly affect by the treatments at 14 and 28 days. However, 20% BCM addition caused an increase in FCR values and it was higher than those of control and 10 % BCSM added groups at 42 days and overall period ( $P < 0.01$ ).

Table 1. Effect of trials on live weight and change, feed intake and feed conversion ratio

	Groups			
Item	Control	%10 BCSM	%20 BCSM	P
BW-initial	1480.94+21.65	1455.31+29.72	1422.50+12.71	0.194
BW-end	1498.13+22.68	1462.00+29.58	1452.00+11.48	0.305
Body weight change	17.19+13.80	8.00+10.19	29.50+9.64	0.419
Feed intake, g/day				
14	102.38+3.26 <sup>a</sup>	97.41+2.40 <sup>a</sup>	71.40+4.17 <sup>b</sup>	0.001**
28	101.96+3.28 <sup>a</sup>	96.65+2.44 <sup>a</sup>	71.61+4.18 <sup>b</sup>	0.0001**
42	102.86+4.56 <sup>a</sup>	104.59+5.22 <sup>a</sup>	60.62+5.84 <sup>b</sup>	0.001**
Overall	102.40+2.48 <sup>a</sup>	99.55+1.81 <sup>a</sup>	67.88+2.91 <sup>b</sup>	0.0001**
Feed conversion ratio, g feed/g egg mass				
14	1.98+0.07	1.86+0.11	2.14+0.19	0.327
28	1.83+0.05	1.64+0.07	1.93+0.13	0.081
42	1.96+0.13 <sup>b</sup>	1.93+0.17 <sup>b</sup>	4.21+0.75 <sup>a</sup>	0.001**
Overall	1.93+0.04 <sup>b</sup>	1.77+0.07 <sup>b</sup>	2.81+0.33 <sup>a</sup>	0.0001**

<sup>a, b, c</sup>: Differences between averages with different letters in the same column are statistically significant. BW: Body weight, P: level of significance, \*\* $P < 0.01$ : BCSM: Black cumin seed meal

The effects of adding BCSM to ratio on egg yield (number and %), egg weight and mass are given in table 2. The addition of 20% BCSM to the diet significantly reduced egg production, yield, weight (without 0-21 d) and mass compared to the 10% BCSM and control group in all periods and average values ( $P < 0.01$ ). The addition of 10% BCSM meal to the diet did not significantly affect egg yields, weight and mass values compared to the control group.

Table 3. Effects on egg production (number) and % egg yield, egg weight and mass of chickens fed with feed added to BCSM

Item	Measurement days			
	0-21	21-35	35-49	Overall
Egg production, number				
Control	11.56+0.56 <sup>a</sup>	11.88+0.66 <sup>a</sup>	11.63+0.70 <sup>a</sup>	11.69+0.51 <sup>a</sup>
BCSM 10%	11.69+0.44 <sup>a</sup>	12.94+0.32 <sup>a</sup>	11.75+0.70 <sup>a</sup>	12.13+0.30 <sup>a</sup>
BCSM 20 %	7.50+0.52 <sup>b</sup>	8.94+0.64 <sup>b</sup>	5.44+0.90 <sup>b</sup>	7.38+0.52 <sup>b</sup>
P	0.0001**	0.0001**	0.0001**	0.0001**
Egg yield, %				
Control	82.74+3.96 <sup>a</sup>	84.82+4.70 <sup>a</sup>	83.04+5.00 <sup>a</sup>	83.53+3.58 <sup>a</sup>
BCSM 10%	83.04+3.22 <sup>a</sup>	92.41+2.30 <sup>a</sup>	83.93+4.99 <sup>a</sup>	86.46+2.12 <sup>a</sup>
BCSM 20 %	53.57+3.57 <sup>b</sup>	63.84+4.59 <sup>b</sup>	38.84+6.42 <sup>b</sup>	52.08+3.93 <sup>b</sup>
P	0.0001**	0.0001**	0.0001**	0.0001**
Egg weight, g				
Control	62.56+1.33	62.16+0.79 <sup>a</sup>	61.72+1.27 <sup>a</sup>	62.19+0.86 <sup>a</sup>
BCSM 10%	65.07+1.06	64.50+0.90 <sup>a</sup>	64.05+1.10 <sup>a</sup>	64.54+0.78 <sup>a</sup>
BCSM 20 %	61.27+1.07	57.05+0.96 <sup>b</sup>	56.94+0.99 <sup>b</sup>	58.42+0.63 <sup>b</sup>
P	0.071	0.0001**	0.0001**	0.0001**
Egg mass				
Control	54.19+2.58 <sup>a</sup>	56.44+1.76 <sup>a</sup>	54.01+1.58 <sup>a</sup>	54.40+1.44 <sup>a</sup>
BCSM 10%	54.47+2.31 <sup>a</sup>	59.70+1.90 <sup>a</sup>	56.83+1.80 <sup>a</sup>	57.16+1.19 <sup>a</sup>
BCSM 20 %	34.89+2.14 <sup>b</sup>	39.35+2.17 <sup>b</sup>	22.51+3.86 <sup>b</sup>	31.51+2.40 <sup>b</sup>
P	0.0001**	0.0001**	0.0001**	0.0001**

a, b, c: Differences between averages with different letters in the same column are statistically significant.

P: level of significance, \*\* $P < 0.01$ . BCSM: Black cumin seed meal

Effects on egg width and length of chickens fed with feed added to BCSM meal in the experimental groups are given in table 3. Egg width and length of 20% BCSM added group were lower than control and 10% BCSM added groups in all periods ( $P < 0.01$ ), without egg length in 0-21 d period.

Also, the eggs of the control group and 10% BCSM meal added group, egg widths were slightly larger in than those of the hens fed with 20% BCSM group.

Table 4. Effects on egg width and length of chickens fed with feed added to BCSM meal in the experimental groups.

Item	Measurement days			
Egg width, mm	0-21	21-35	35-49	Overall
Control	43.75±0.21 <sup>ab</sup>	43.55±0.16 <sup>b</sup>	43.74±0.16 <sup>a</sup>	43.68±0.20 <sup>a</sup>
BCSM 10%	44.13±0.24 <sup>a</sup>	44.17±0.13 <sup>a</sup>	44.01±0.20 <sup>a</sup>	44.10±0.16 <sup>a</sup>
BCSM 20 %	43.09±0.31 <sup>b</sup>	42.22±0.21 <sup>c</sup>	42.34±0.24 <sup>b</sup>	42.58±0.18 <sup>b</sup>
P	0.0001**	0.0001**	0.0001**	0.0001**
Egg length, mm				
Control	58.49±0.58	58.48±0.36 <sup>a</sup>	58.44±0.47 <sup>ab</sup>	58.44±0.55 <sup>a</sup>
BCSM 10%	59.43±0.33	59.51±0.37 <sup>a</sup>	60.25±1.02 <sup>a</sup>	60.25±0.88 <sup>a</sup>
BCSM 20 %	59.32±0.48	57.20±0.40 <sup>b</sup>	57.31±0.38 <sup>b</sup>	57.31±0.44 <sup>b</sup>
P	0.290	0.0001**	0.0001**	0.0001**

a,b,c: Differences between averages with different letters in the same column are statistically significant. P: level of significance, \*:P<0.05, \*\*P<0.01. BCSM: Black cumin seed meal

## DISCUSSION

While the addition of high percentage (20%) BCSM to the diet did not significantly affect live weight values, it decreased feed consumption, feed conversion ratio, egg production, mass, and egg width and length values compared to the control and 10% BCSM groups. The addition of 10% BCSM to the diet significantly affected feed consumption, FCR and egg production characteristics compared to the control group. It is thought that the addition of 20% BCSM in the diet primarily reduces feed consumption in animals and, accordingly, causes a decrease in productivity parameters and egg characteristics. Black cumin seeds and meal have a distinctive bitter, metallic, peppery, and pungent taste (Singletary, 2023). Due to this unpleasant bitter taste, 20% consumption in high amounts caused a decrease in appetite and feed consumption in hens. In a study conducted with broiler chickens (Fathi et al., 2023), BCSM supplementation used in low amounts (20, 40 and 60 g/kg) in the diet increased live weight and feed consumption, improved feed utilization, increased antioxidant and immunity, and increased the amount of pathogenic microorganisms. It was determined that it decreased. In another study, using black seed at a similar rate (10 to 20g/kg) improved FCR of broilers and improved plasma lipid profile and antibody-mediated immunity (Ghasemi et al., 2014). Saleh et al. (2020) reported that replace 50% and 100% of BCSM with wheat bran (20 and 40 g/kg in diets) in laying hens, did not affect body weight,



however, hen-day egg production, egg weight and egg mass significantly increased, while feed conversion ratio significantly decreased. Similarly, in another study, it was reported that replacing bran (2.5 and 5.0%) in the diet and also adding enzymes and polyethylene glycol to the feed increased performance, egg quality and egg yolk color values (Mansoori et al., 2006). The BSCM rate used in laying hen rations so far (max 5%) is lower than the rate used in the current study (10 and 20%), therefore, no negative results were reported in studies conducted by other authors. As a matter of fact, in the current study, giving 10% BCSM did not negatively affect the results compared to the control group, while only a high rate (20%) gave negative results. As a result, while the use of the highest 10% BCSM in laying hen diets seemed safe, the use of 20% BCSM caused a decline in performance and egg quality values.

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## **Nevşehir Province Angstrom-Prescott Coefficients and Reference Evapotranspiration Comparisons**

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### **ABSTRACT**

Atmospheric evaporation demand on crop water consumption have been presented by reference evapotranspiration. Crop water consumption was estimated by multiplying reference evapotranspiration with crop coefficients depending on crop growth stage. Recently, FAO Irrigation and Drainage Paper 56 Penman-Monteith method was offered as a sole standard method to estimate reference evapotranspiration. Local Angstrom-Prescott (AP) coefficients are required to convert sunshine duration to short wave solar radiation ( $R_s$ ), which is driving force for crop evapotranspiration. Sunshine duration data has been recorded since 1965 but  $R_s$  data since 2016 in Nevşehir province. Using limited daily  $R_s$  data, local AP coefficients of Nevşehir provinces in The Central Anatolian Region of Türkiye were determined as yearly and monthly. Then, reference evapotranspiration ( $ET_o$ ) series were computed by these local AP coefficients and AP coefficients ( $a_s= 0.25$  and  $b_s= 0.50$ ) offered by FAO 56 for the regions without any AP coefficients. Control  $ET_o$  series were computed monthly by  $R_s$  series for their recording period between 2016 and 2019. Mean absolute error (MAE) and root mean square error (RMSE) were used to compare the  $ET_o$  series to the control  $ET_o$  series.  $ET_o$  series computed with yearly local AP coefficients ( $a_s= 0.2361$  and  $b_s= 0.5368$ ) resulted in reasonable error (MAE= 35.9 mm/year and RMSE= 41.8 mm/year) that were not considerably different from  $ET_o$  series computed by AP coefficients ( $a_s= 0.25$  and  $b_s= 0.50$ ) with negligible error differences (MAE= 38.2 mm/year and RMSE= 46.2 mm/year). Monthly local AP coefficients resulted in the least error (MAE= 7.8 mm/year and RMSE= 10.7 mm/year) and improved  $ET_o$  estimations. Annual  $ET_o$  values were 1065 mm for FAO 56 AP coefficients, 1085 mm for yearly local AP coefficients and 1088 mm for monthly local AP coefficients in Nevşehir. Therefore, FAO 56 AP coefficients work as good as yearly local AP coefficients, but monthly local AP coefficients work better for  $ET_o$  estimations for Nevşehir province.

**Keywords:** Reference evapotranspiration, Local Angstrom-Prescott coefficients, and frequency analysis

## **INTRODUCTION**

Crop evapotranspiration ( $ET_c$ ) could be estimated by multiplying atmospheric evaporative demand ( $ET_o$ ) and plant coefficients ( $K_c$ ) (Doorenbos and Kassam 1986; Doorenbos and Pruitt 1975; Allen et al. 2005). A hypothetical grass surface, which has 70 s/m air flow resistance, 0.23 albedo, and 0.12 m height, was accepted a standard surface to compute  $ET_o$ . Different regions can be compared to each other by using  $ET_o$  and the periods during the plant growth season can also be compared (Allen et al. 1998).  $ET_o$  has been also using for hydrological models. Some drought indexes, which include  $ET_o$  as a factor, are used to evaluate drought situation of different provinces in Türkiye (Yürekli ve ark. 2010; Yürekli ve Ünlükara, 2013a; Yürekli ve ark. 2013b, Sekendur, 2022).

Accurately estimating crop evapotranspiration primarily depends on accurate  $ET_o$  calculation and using plant coefficients that suit the region. One of the major parameters to estimate  $ET_o$  is solar radiation or sunshine duration data recorded by meteorological stations. If solar radiation or other data used to compute  $ET_o$ , some alternative calculations were offered by Allen et al. (1998). Solar radiation can be estimated with local AP coefficients or with accepted AP coefficients ( $a_s = 0.25$  and  $b_s = 0.50$ ). Then sunshine duration can be converted to solar radiation. Solar radiation data is not required only for  $ET_o$  estimations but also required primarily for energy sectors and others. Solar energy applications such as photovoltaics, solar thermal systems, and passive solar designs need solar radiation data as a basic factor. In designing solar technologies, and optimizing and evaluating performance, solar radiation data in anywhere should be modern, reliable, and usable (Bulut and Büyükalaca, 2007). Solar radiation cannot be measured easily because of requires expensive equipment and technology in many developing countries.

Although meteorological records have been found since 1959 in Nevşehir Province, sunshine duration records were started in 8<sup>th</sup> month of 1965 and solar radiation records in 5<sup>th</sup> month of 2016. In this study, it was aimed to determine Angstrom-Prescott coefficients by using daily solar radiation and sunshine duration data between 2016-2019 and to calculate solar radiation from the beginning of meteorological records by using Angstrom-Prescott relationship, and to estimate reference evaporations. Furthermore, it was aimed to compare reference evaporations and evaluate  $ET_o$  variations in Nevşehir Province.

## **MATERIALS AND METHODS**

Nevşehir Province is in the Middle Anatolian Region of Türkiye and is located on 38°37'N latitude and 34°43'E longitude. Height above sea level of Nevşehir is 1260 m. The primary economic sectors are agriculture and tourism. Continental semi-arid climate dominates the province. Some climatic features are listed in Table 1 (MGM, 2023). Total precipitation is 420.6 mm, mean annual temperature is 10.8°C, and mean annual sunshine is 7.1 hours per day.

54-year meteorological data (1965-2019) were used to compute the  $ET_o$  of Nevşehir. Monthly mean data for  $ET_o$  computing: Minimum and maximum temperatures ( $T_{min}$  and  $T_{max}$ ), minimum and maximum relative humidities ( $RH_{min}$  and  $RH_{max}$ ), wind speed, short wave solar radiation ( $R_s$ ), and sunshine duration ( $n$ ). All meteorological data was derived from the Meteorology General Directorate of Turkish State (MGM, 2020).

Table 1. Some climatic features of Nevşehir Province in Türkiye (1959-2022)

	Months											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
T <sub>mak</sub> °C	3.8	5.5	10.2	15.9	20.6	24.9	28.5	28.5	24.5	18.3	11.7	6.1
T <sub>min</sub> °C	-3.7	-2.6	0.5	5.1	8.8	11.6	13.6	13.5	10.4	6.7	2.3	-1.4
T <sub>ort</sub> °C	-0.2	0.9	4.9	10.1	14.5	18.3	21.3	21.2	17.3	12.1	6.6	2.0
n (h/day)	3.1	4.1	5.3	6.7	8.4	10.5	11.9	11.3	9.4	6.5	4.6	3.1
Precip. (mm)	46.2	40.9	47.4	49.5	56.9	36.6	9.7	8.0	13.7	28.4	34.3	49.0

n: Sunshine duration.

Although meteorological data has been recorded since 1959 in the province, sunshine duration since 1965. Solar radiation measurements have been recorded since 5<sup>th</sup> month of 2016. ET<sub>o</sub>, which represents the evaporative demand of the atmosphere, was computed the methods represented by Allen et al. (1998):

$$ET_o = \frac{0.408 \Delta (R_n - G) + \gamma \frac{900}{T + 273} u_2 (e_s - e_a)}{\Delta + \gamma (1 + 0.34 u_2)} \quad (1)$$

In where, ET<sub>o</sub> is reference evapotranspiration (mm/day),  $\Delta$  is slope of the saturated vapor pressure curve (kPa/°C),  $R_n$  is solar energy at the reference surface (MJ/m<sup>2</sup>.day),  $G$  is soil heat flux (MJ/m<sup>2</sup>.day),  $\gamma$  is psychrometric constant (kPa/°C),  $T$  is mean temperature (°C),  $u_2$  is wind speed at 2 m height from the soil surface (m/s),  $e_s$  and  $e_a$  are saturated and actual vapor pressure (kPa), respectively. Extraterrestrial radiation ( $R_a$ ), clear sky solar radiation ( $R_{so}$ ), net solar radiation ( $R_{ns}$ ), net longwave radiation ( $R_{nl}$ ) and net radiation ( $R_n$ ) were computed by following equations (Allen et al., 1998):

$$R_a = \frac{24 \cdot (60)}{\pi} G_{sc} d_r [\omega_s \sin(\varphi) \sin(\delta) + \cos(\varphi) \cos(\delta) \sin(\omega_s)] \quad (2)$$

$$R_s = \left( a_s + b_s \frac{n}{N} \right) R_a \quad (3)$$

$$R_{so} = (a_s + b_s) R_a \quad (4)$$

$$R_{ns} = (1 - \alpha) R_s \quad (5)$$

$$R_{nl} = \sigma \left[ \frac{T_{mak, K}^4 + T_{min, K}^4}{2} \right] (0.34 - 0.14 \sqrt{e_a}) \left( 1.35 \frac{R_s}{R_{so}} - 0.35 \right) \quad (6)$$

$$R_n = R_{ns} - R_{nl} \quad (7)$$

In where,  $G_{sc}$  is solar constant (0.082 MJ/m<sup>2</sup>.min),  $d_r$  is inverse relative distance Earth-Sun,  $\omega_s$  the sunset hour angle (rad),  $\varphi$  is degree of the latitude (rad),  $\delta$  is solar declination angle (rad),  $n$  is sunshine duration (h/day),  $N$  is day light duration (h/day),  $a_s$  and  $b_s$  are Angstrom-Preseott (AP) coefficients,  $\alpha$  is albedo (0.23),  $\sigma$  is Stefan-Boltzmann constant (4.903×10<sup>-9</sup> MJ/K<sup>4</sup>m<sup>2</sup>),  $T_{mak}$  and  $T_{min}$  are monthly mean minimum and maximum temperatures (K) (Allen et al. 1998).

Relative solar radiations ( $R_s/R_a$ ) were installed against to relative sunshine durations ( $n/N$ ) at coordinate axes. Regression and correlation analyses were performed by excel program and

obtained regression equation and determination coefficient. Daily  $R_s$  and  $n$  data were used to obtain AP coefficients since there were last few years  $R_s$  data. Some data was eliminated depending on those rules:  $R_a > R_{so} \geq R_s$ ,  $n \leq N$  and  $R_s > 0$  (Allen et al. 1998, Ünlükara 2014). Furthermore, data considerably diverged from the regression line was also eliminated (Ünlükara 2014, Kırtan 2019, Kaymaz 2020, Baran 2021, Polu 2021, Kılıç 2022). The coefficient of  $a_s$  is the value that the regression line crossed at Y axis and coefficient of  $b_s$  is the slope of the regression line. The AP relationship is given just below, and Figure 1 shows the relationship (Angstrom 1924, Prescott 1940).

$$\frac{R_s}{R_a} = a_s + b_s \frac{n}{N} \quad (8)$$

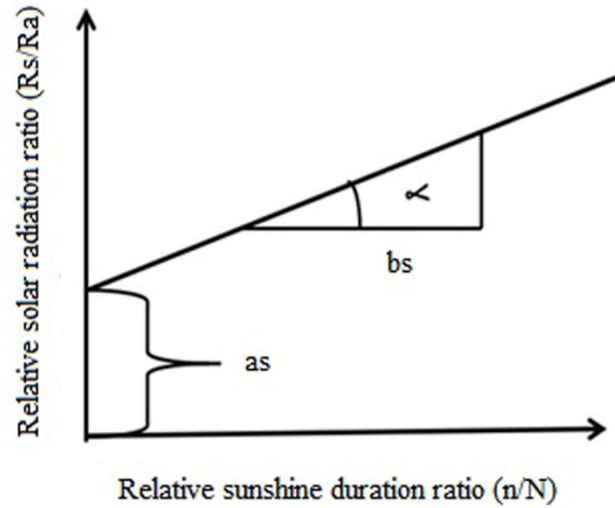


Figure 1. Angstrom-Prescott relationship between relative sunshine duration and relative solar radiation ratios.

Using  $R_s$  data between 2016 and 2019, and local AP coefficients were used to compute control reference evapotranspiration series ( $ET_{oc}$ ).  $ET_{o1}$  series were calculated by using  $a_s = 0.25$  ve  $b_s = 0.50$  coefficients when the region has no local coefficients.  $ET_{o2}$  and  $ET_{o3}$  series were calculated by local monthly and yearly AP coefficients obtained in this study. Sunshine duration data was also used to compute the last three  $ET_o$  series. Mean Absolute Error (MAE) and Root Mean Square Error (RMSE) were used to evaluate these  $ET_o$  series according to the  $ET_{oc}$  series:

$$MAE = \frac{\sum |ET_{oc} - ET_{oi}|}{n} \quad (9)$$

$$RMSE = \sqrt{\frac{\sum (ET_{oc} - ET_{oi})^2}{n}} \quad (10)$$

The series that has the least error was accepted as the best estimating series. This series was also used for frequency analysis.

Frequency analysis was performed to obtain  $ET_o$  values at 95, 75, 50, 25, 10 and 5% probabilities (James 1988):

$$RP = \frac{100}{P} \quad (11)$$

In where, RP is the repetition period and P is the probability, which is equal to or higher than considered  $ET_o$  value. After computing the monthly  $ET_o$  series and ordering from the least one to biggest one, occurring probability of each  $ET_o$  value were found as below:

$$P = \left(1 - \frac{R}{M+1}\right) \cdot 100 \quad (12)$$

Where P is the probability (%) of considering the  $ET_o$  values which is equal to itself or higher than itself, R is the rank of the ordered  $ET_o$  and M is number of total  $ET_o$  values. After performing the Weibull transform for P values by Equation 10,  $ET_o$  values were marked against their W values and were determined frequency equations by regression for monthly and annual  $ET_o$  values.

$$W = \log \left[ -\log \left( \frac{P}{100} \right) \right] \quad (13)$$

$ET_o$  values at 95, 75, 50, 25, 10, and 5% probabilities were determined by using these frequency equations.

## **RESULTS AND DISCUSSIONS**

### **The relationship between solar radiation ratio and sunshine duration ratio, and Angstrom-Prescott coefficients**

The yearly relationship found by dividing the daily sunshine duration ratio ( $n/N$ ) to the solar radiation ratio ( $R_s/R_a$ ) was shown in Figure 2 for Nevşehir. Mean 23.61% of  $R_a$  ( $a_s = 0.2361$ ) reaches to earth's surface on a fully cloudy day in Nevşehir while 77.29 of  $R_a$  ( $a_s + b_s = 0.7729$ ) reaches the earth's surface on a clear sky day. Yearly local  $a_s$  was found a bit lower than  $a_s = 0.25$  and the sum of local AP coefficient was a bit higher than 0.75. The yearly local  $b_s$  coefficient on a relatively cloudy day was found a bit higher than  $b_s = 0.50$  which was offered Allen et al. (1998). Using linear regression Bakirci (2009) reported coefficients of  $a_s$  between 0.0790 and 0.5286, and coefficients of  $b_s$  between 0.0528 and 0.6481 for 18 provinces in Türkiye. On a clear sky day, 70.73% of  $R_a$  reaches to the earth's surface in Balıkesir, 65.28% in Bilecik, 65.09% in Bursa, 64.59% in Çanakkale, 54.49% in Edirne, 59.22% in İstanbul, 79.59% in Kırklareli, 59.64% in Kocaeli, 72.45% in Sakarya, 67.6% in Tekirdağ and 74.17% in Yalova (Baran 2021). Local AP coefficients may vary depending on climatic conditions, altitude, geographic position, and air pollution.



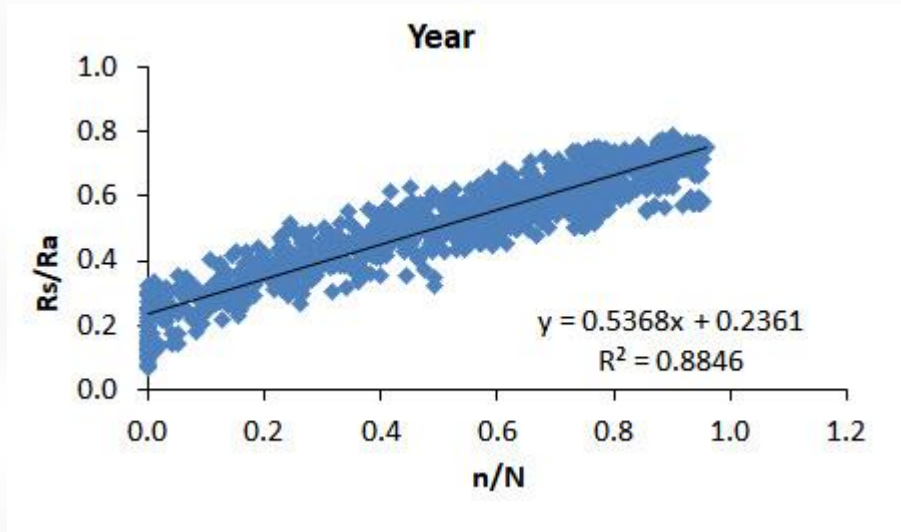


Figure 2. Yearly Angstrom-Prescott relationship of Nevşehir Province in Türkiye

Monthly Angstrom-Prescott relationships for Nevşehir Province were shown in Figure 3 and all local AP coefficients were listed in Table 2.

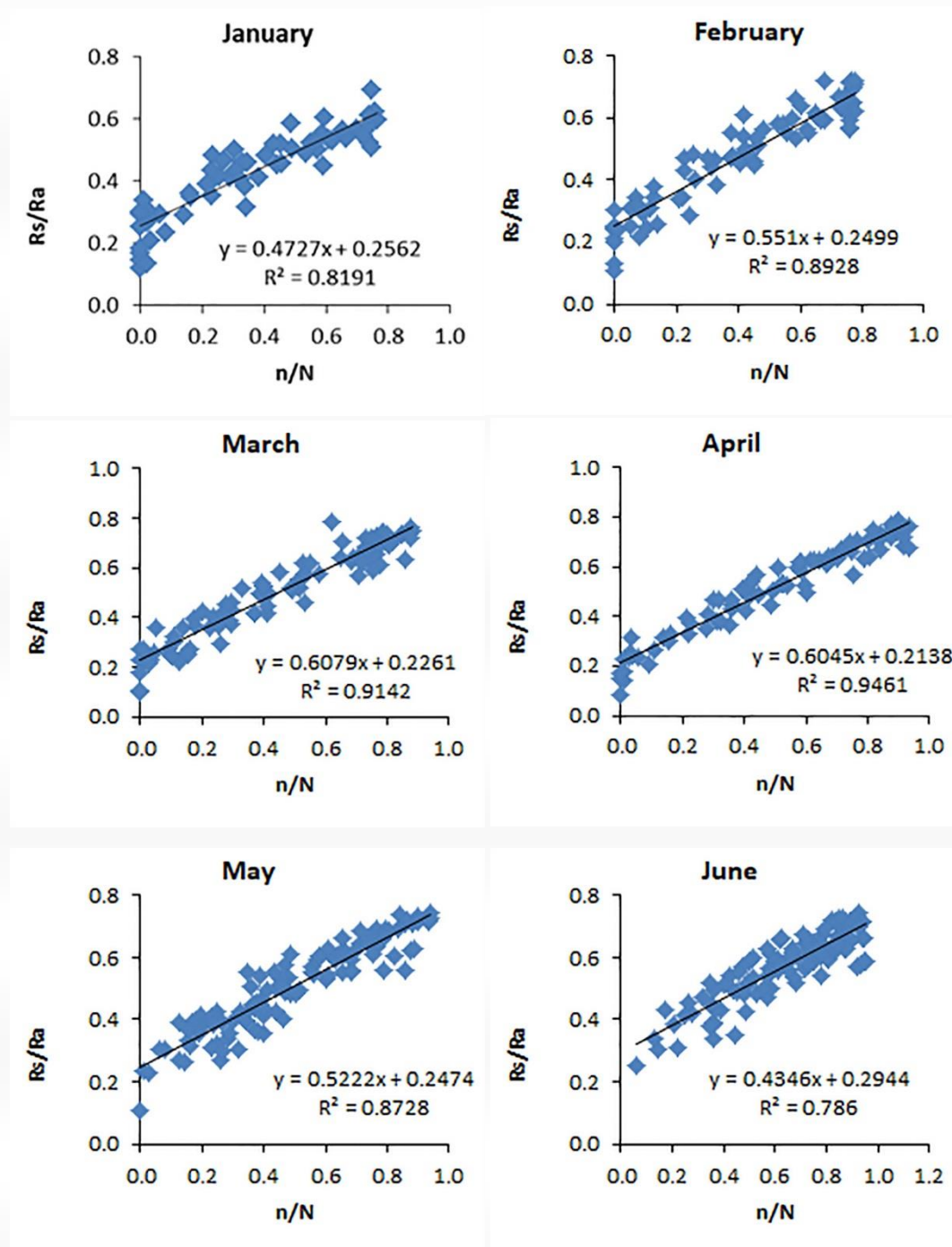
Table 2 Local monthly Angstrom-Prescott coefficients for Nevşehir Province in Türkiye

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
$a_s$	0.2562	0.2499	0.2261	0.2138	0.2474	0.2944	0.3015	0.3335	0.2243	0.2496	0.2226	0.2089
$b_s$	0.4727	0.5510	0.6079	0.6045	0.5222	0.4346	0.4397	0.4359	0.5737	0.5567	0.4803	0.5156
$a_s + b_s$	0.7289	0.8009	0.8340	0.8183	0.7696	0.7290	0.7412	0.7694	0.7980	0.8063	0.7029	0.7245

Sum of  $a_s$  and  $b_s$  represents the shortwave solar radiation that reached the earth's surface on a clear sky day. Therefore, the highest sum (0.8340) was found in March, and the lowest sum (0.7029) in November for Nevşehir. The coefficient of  $a_s$  represents shortwave solar radiation that reaches the earth's surface on a fully cloudy day. This ratio varied from 0.2089 in December to 0.3335 in August. The coefficient of  $b_s$  represents the part of  $R_a$  that reaches to the earth's surface depending on cloudiness and cloud types. The coefficient  $b_s$  for Nevşehir varied from 0.4346 in June to 0.6079 in March. If any region has no local AP coefficients, Allen et al. (1998) offered to continue  $ET_o$  calculations by using  $a_s = 0.25$  and  $b_s = 0.50$ . These AP coefficients are in local AP limits for Nevşehir. Kılıç (2022) reported local AP coefficients varying between 0.0624-0.2670 for  $a_s$ , and between 0.3128-0.5061 for  $b_s$  in Adıyaman, Kaymaz (2020) reported varying between 0.0204-0.2474 for  $a_s$ , and between 0.5088-0.7732 for  $b_s$  in Kahramanmaraş, and Polu (2021) reported varying between 0.0799-0.5180 for  $a_s$ , and between 0.1772-0.6759 for  $b_s$  in Erzurum.

Ahrens (2009) stated that 19% of the solar radiation that reaches the atmosphere are absorbing by the atmosphere and clouds, 6% of the solar radiation is scattering, and 20% and 4% of the solar radiation is reflecting by clouds and the earth's surface. Consequently, 51% of  $R_a$  is absorbing by the earth. Albedo, reflecting ratio of incidence radiation, is varying 60-90% for thick clouds and 30-50% for thin clouds. The sky can be defined depending on cloudiness ratios as no clouds, few cloudy, partly cloudy, mostly cloudy, and overcast (Ahrens 2009). Atmospheric conditions are

dynamic and can be change instantaneously and seasonally. Therefore, local AP coefficients may represent this dynamism and can be used to transform sunshine duration ratio to solar radiation data.



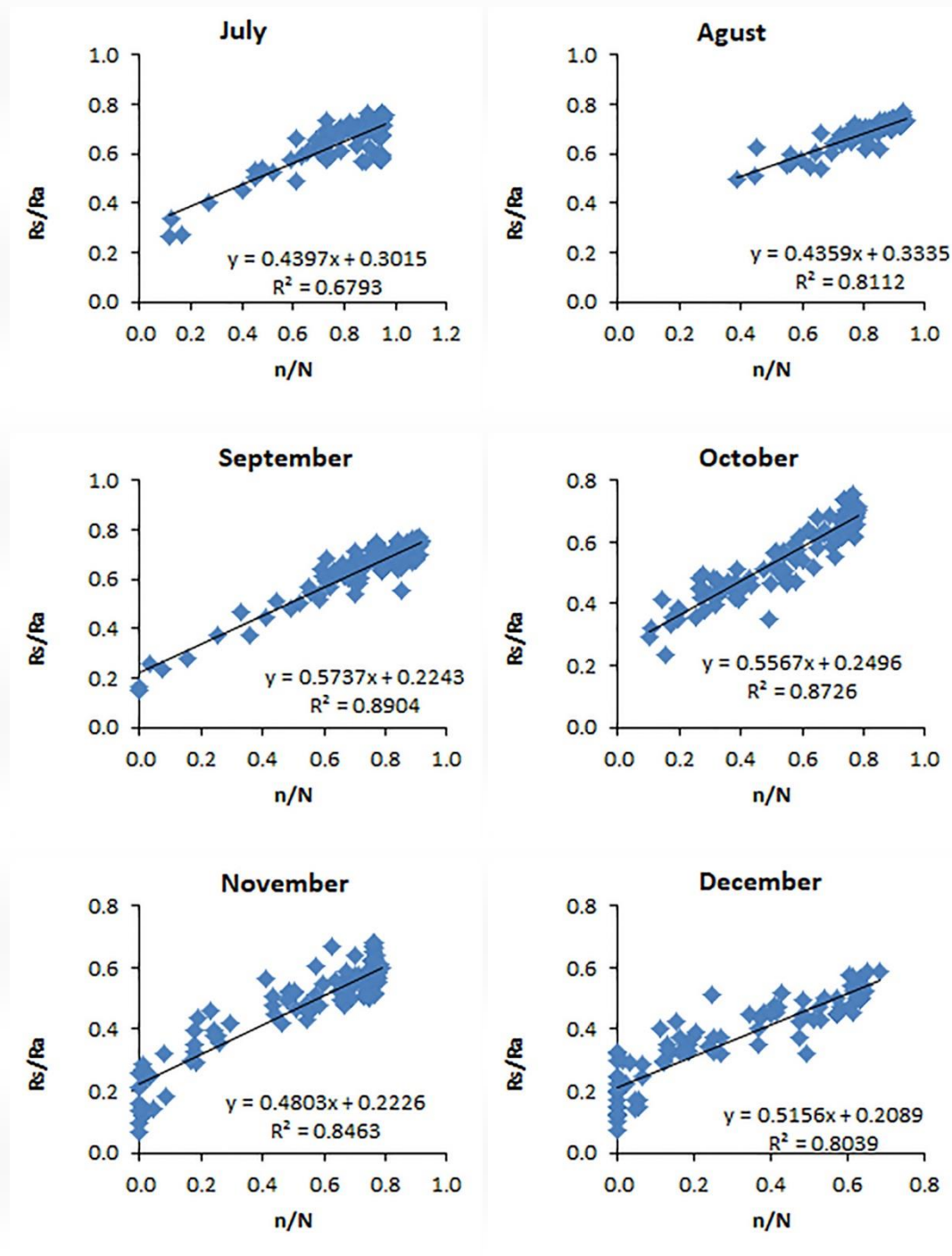


Figure 3. Monthly Angstrom-Prescott relationships of Nevşehir Province in Türkiye

### Different reference evapotranspiration series and comparisons

Control  $ET_{oc}$  series, which were estimated by  $R_s$  data (1916-1919), were compared to  $ET_{o1}$ ,  $ET_{o2}$ , and  $ET_{o3}$  series.  $ET_{o1}$  series were estimated by AP coefficients of  $a_s = 0.25$  and  $b_s = 0.50$  while  $ET_{o2}$  series were estimated by monthly local AP coefficients. Yearly local AP coefficients were used to obtain  $ET_{o3}$  series. Annual total  $ET_o$  values were found as 1065 mm for  $ET_{o1}$ , 1088 mm for  $ET_{o2}$ , and 1085 mm for  $ET_{o3}$ . MAE and RMSE were 38.2 and 46.2 mm/year respectively for  $ET_{o1}$ . Nearly similar MAE and RMSE results were found for  $ET_{o3}$  as 35.9 and 41.8 mm/year respectively. The lowest MAE and RMSE results were 7.8 and 10.73 mm/year respectively for  $ET_{o2}$  series obtained by monthly local AP coefficients. Therefore, using monthly local AP coefficients improved the precisions of  $ET_o$  estimations. Monthly or seasonal changes in cloudiness and cloud type may have been well reflected in estimations of  $R_s$  directly and  $ET_o$  indirectly. Therefore,  $ET_o$  frequency analysis was performed by using the  $ET_{o2}$  series. The mean annual total  $ET_{o2}$  was found as 1092 mm between 1965 and 2019.

The relationships between different  $ET_o$  series were shown in Figure 4 by regression and correlation analysis. There are very strong linear relationships between these  $ET_o$  series. Therefore, any  $ET_o$  series can be determined from another  $ET_o$  series by these relationships.

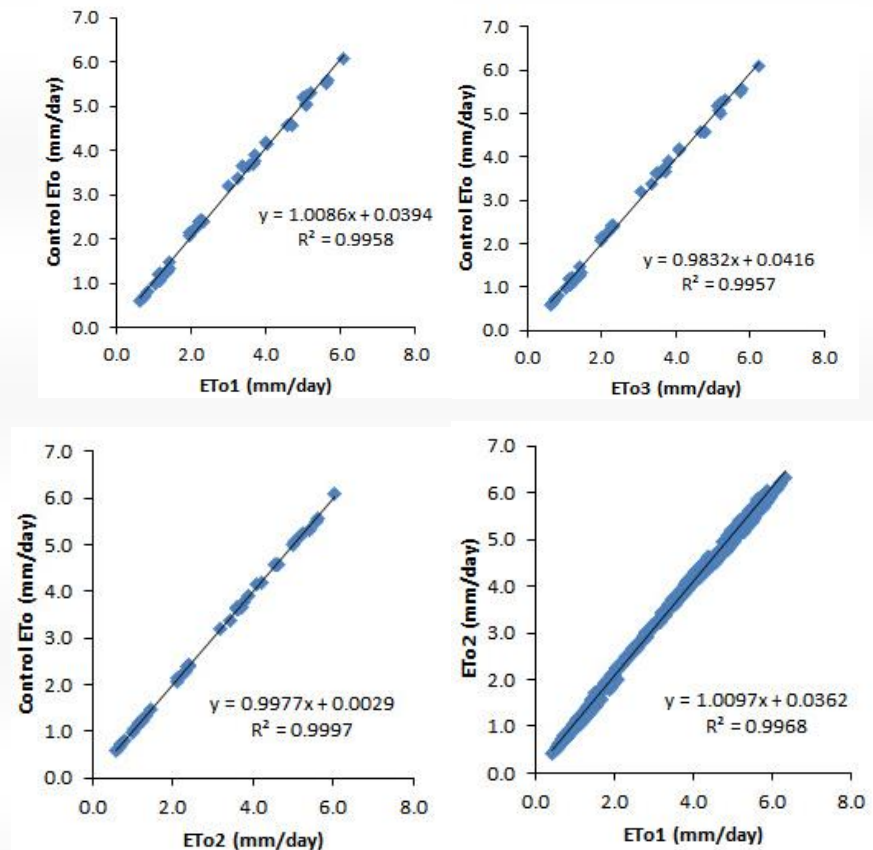


Figure 4. The relationships between different reference evapotranspiration series for Nevşehir Province in Türkiye.

## REFERENCE EVAPOTRANSPIRATION FREQUENCY ANALYSIS

Annual total evapotranspiration varied between 999 and 1261 mm for 54 years (1966-2019) in Nevşehir and the mean annual  $ET_o$  was found as 1092 mm. Annual total  $ET_o$  values that equal or bigger than themselves at 5, 10, 25, 50, 75 and 95% probabilities are determined as 1160, 1149, 1128, 1099, 1063 and 992 mm. When it is eliminated at both ends of the probability curve, annual total  $ET_o$  in Nevşehir varied between 992 and 1160 mm and  $ET_o$  difference year to year may be 168 mm.  $ET_o$  at 5% probability may be 6.2% greater the mean  $ET_o$  (1092 mm) or  $ET_o$  at 95% probability may be 9.2% lower. TAGEM-DSİ (2017) reported 1048 mm for Nevşehir based on the ASCE standardized Penman-Monteith method. The mean annual total  $ET_o$  is found as 1113 mm based on Climwat 2.0 and Cropwat 8.0. Dadaser-Celik et al. (2016) reported that most meteorological stations in Türkiye had annual reference evapotranspiration between 750 and 1200 mm for the last 32 years and  $ET_o$  increased gradually from North to South.

Table 3 and Table 4 show daily and monthly total  $ET_o$  values equal or higher than themselves at 5, 10, 25, 50, 75 and 95% probabilities for each month, respectively.  $ET_o$  values varied between 0.40 and 1.08 mm/day in January when the lowest  $ET_o$  values were observed while  $ET_o$  values varied between 4.94 and 6.11 mm/day in June when the highest  $ET_o$  values were observed. Mean monthly  $ET_o$  values are 0.81, 1.23, 2.03, 3.10, 4.03, 4.90, 5.64, 5.39, 4.02, 2.46, 1.30, and 0.87 mm/day for I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and XII. Mean monthly  $ET_o$  values are as like  $ET_o$  values at 50% probability in Table 3. Therefore, any  $ET_o$  value would be lower or higher than the  $ET_o$  value at 50% probability for a given period. July and August are the months in which many plants consume at peak rates. For example, sugar beet, potato, maize, and sunflower have respective plant coefficient values ( $K_c$ ) of 1.20, 1.15, 1.20, and 1.15 in these months. Higher  $K_c$  values mean that those plants would consume more water than  $ET_o$  in these periods. Mean  $ET_o$  in July is 5.69 mm/day and may pass over 6.1 mm/day at a 5% probability that corresponds 7.4% higher than normal. This increment also rises 8.0-9.0% crop evapotranspiration for some plants. It may be important to design irrigation systems considering these increments to supply enough water to the plant depending on the investment and management economy.

Table 3. Daily evapotranspiration values (mm/day) according to months at different probabilities in Nevşehir province.

P	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
%95	0.40	0.61	1.35	2.24	3.20	4.16	4.94	4.82	3.44	1.83	0.85	0.48
%75	0.69	1.05	1.82	2.85	3.79	4.69	5.44	5.22	3.85	2.28	1.17	0.76
%50	0.84	1.27	2.06	3.17	4.09	4.96	5.69	5.43	4.06	2.51	1.33	0.90
%25	0.95	1.45	2.25	3.42	4.33	5.17	5.89	5.59	4.23	2.69	1.46	1.01
%10	1.04	1.58	2.39	3.60	4.50	5.33	6.04	5.71	4.35	2.83	1.55	1.10
%5	1.08	1.64	2.46	3.69	4.59	5.41	6.11	5.77	4.41	2.90	1.60	1.14

P: Probability



Table 4. Monthly total evapotranspiration values (mm) at different probabilities in Nevşehir province.

P	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
%95	12.5	17.2	41.7	67.1	99.1	124.9	153.3	149.4	103.3	56.7	25.6	14.8
%75	21.4	29.4	56.4	85.6	117.4	140.7	168.6	161.9	115.6	70.7	35.1	23.5
%50	25.9	35.6	63.9	95.0	126.8	148.8	176.5	168.3	121.8	77.9	39.9	27.9
%25	29.5	40.5	69.8	102.5	134.1	155.2	182.6	173.3	126.8	83.5	43.7	31.4
%10	32.2	44.1	74.1	107.9	139.5	159.9	187.2	177.0	130.4	87.7	46.5	34.0
%5	33.5	46.0	76.4	110.7	142.3	162.3	189.5	178.9	132.3	89.8	47.9	35.3

P: Probability

Monthly total  $ET_o$  varied from 12.5 mm to 33.5 mm in January and from 153.3 mm to 189.5 mm in July. Total monthly  $ET_o$  values at 95, 50, and 5% probability were depicted in Figure 5. Normal distributed  $ET_o$  values peak in July. Lower  $ET_o$  values are observed in both December and January. The mean annual  $ET_o$  in Nevşehir was found 1092 mm. The annual  $ET_o$  varies between 992 and 1160 mm at 95 and 5% probabilities based on the frequency analysis for the period (1965-2019). Therefore, annual  $ET_o$  in some years may be 6.2% greater than 1092 mm or may be lower as 9.2 %. Negligible minor differences occurred between the  $ET_o$  series computed by solar radiation data and the  $ET_o$  series by monthly local AP coefficients. Consequently, using monthly local AP coefficients in Nevşehir would improve the precision of  $ET_o$ .

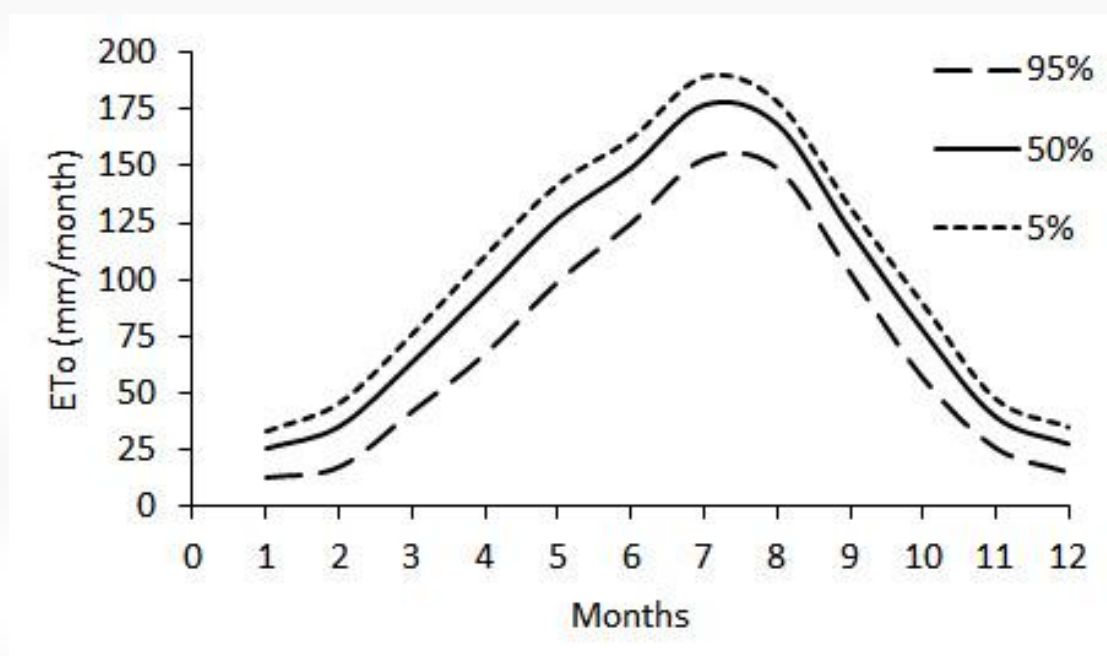


Figure 5.  $ET_o$  variations in Nevşehir province at 95, 50, and 5% probabilities.



ET<sub>o</sub> variations in the period of 54 year (1965-2019) are shown in Figure 6. Lower ET<sub>o</sub> values are observed around 1995 while greater values are observed along the beginning and end of the period. Annual ET<sub>o</sub> values may be 5.5% greater than the annual mean ET<sub>o</sub> value (1092 mm) or may be 9.8% lower.

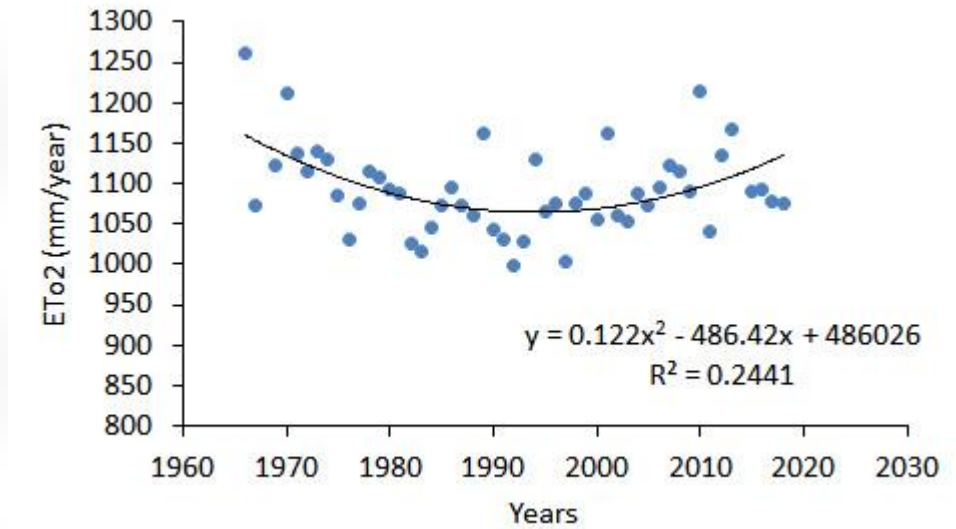


Figure 6. Annual total ET<sub>o</sub> variations in Nevşehir province from 1966 to 2019.

## CONCLUSION

Angstrom-Prescott coefficient ( $a_s$ ), which represents solar radiation reaching the earth's surface on an overcast day, varied between 0.2089 and 0.3335 in Nevşehir Province. Angstrom-Prescott coefficient ( $b_s$ ), which represents the cloudiness effect on a relatively cloudy day, varied between 0.4346 and 0.6079. Summation of these coefficients, which represent solar radiation reaching on a clear sky day, were found between 0.7029 and 0.8063.

The mean annual ET<sub>o</sub> in Nevşehir was found 1092 mm. The annual ET<sub>o</sub> varies between 992 and 1160 mm at 95 and 5% probabilities based on the frequency analysis for the period (1965-2019).

Reference evapotranspiration (ET<sub>o3</sub>) values found by yearly local Angstrom-Prescott coefficients ( $a_s = 0.2361$  and  $b_s = 0.5368$ ) and ET<sub>o1</sub> values computed by standard Angstrom-Prescott coefficients ( $a_s = 0.25$  and  $b_s = 0.50$ ) resulted in greater errors than the ET<sub>o2</sub> values found by monthly local Angstrom-Prescott coefficients. The ET<sub>o1</sub> and ET<sub>o3</sub> series produced nearly similar errors that were not considerably high. It can be concluded that using monthly local Angstrom-Prescott coefficients would improve ET<sub>o</sub> estimations because of variations in cloudiness and cloud type over a year.

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## **Investigation of the Use of *Lentilactobacillus Diolivorans* as A Starter Culture in Olive Fermentation**

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### **ABSTRACT**

In the present study, the use of olive-derived *Lentilactobacillus diolivorans* Z141B isolate as a starter culture in Gemlik olive variety was investigated. Physicochemical and microbiological analyses were carried out during 35 days of storage in olive trials. As a control, analyses were performed on samples exposed to spontaneous fermentation. The beta glucosidase activity of *Lentilactobacillus diolivorans* Z141B isolate was determined as 5.61 micromol/min/mL in the enzyme activity tests which is important in the degradation of oleuropein. On the 4th day of fermentation, pH values were determined as 5.06-4.78 for *Lb. diolivorans* and control samples, respectively, while on the 35th day, these values were determined as 4.24-4.55, respectively. Lactic acid bacteria count was 5.23-6.53 log/ml for *Lb. diolivorans* and control samples on the 4th day of fermentation and 6.35-5.99 log/ml on the 35th day. The results showed that the use of *Lentilactobacillus diolivorans* Z141B isolate as a starter in natural olive processing is promising.

**Keywords:** Lactic acid bacteria, starter culture, oleuropein, olive

### **1. INTRODUCTION**

Olive is an important fruit that is consumed by people and is also very rich in nutritional value. Dating back to ancient times, olives are one of the first cultivated plants on earth. Olives, which are the only fruit that is not consumed after harvesting, undergo a number of processes in order to become ready for consumption. In its natural state, olives cannot be consumed after harvesting due to the phenolic compound called as oleuropein, which gives a bitter taste. The most basic step of table olive production in the olive industry is to remove the bitter taste by hydrolyzing the oleuropein naturally presents in the olive fruit.

For this purpose, although it varies according to the type of olive processing, the most common method involves the hydrolysis of oleuropein by soaking the olives in alkaline solutions and then removing the alkali from the environment by washing. Following the removal of bitterness, the alkaline substances present in the environment is removed by washing (Anonymous 2011).

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NaOH solution is known to produce high amounts of heavily polluted wastewater. This refers not only to alkaline solutions but also to other washing waters needed to remove excess alkalis from the olive flesh (Habibi et al. 2016). Furthermore, in many countries, the use of NaOH for flavoring organic table olives is prohibited (Habibi et al. 2016; García et al. 2008). Studies have revealed that bitterness in olives can be removed without the use of alkali, and it has been stated oleuropein can be broken down by some enzymes and its bitter taste can be eliminated. In this context, it has been reported that it is possible to remove the bitter taste by degrading oleuropein with microbial  $\beta$ -glucosidase enzyme (Abriouel et al. 2011; Cocolin et al. 2013; Garcia et al. 2008). Fermenting olives with lactic acid bacteria, which have the capacity to synthesize  $\beta$ -glucosidase enzyme capable of hydrolyzing oleuropein, also has a positive effect on the quality of the final product. The aim of the present study is to assess the use of *Lentilactobacillus diolivorans* strain in table olive fermentation. This type of study is essential to assess the efficiency of using starter cultures for the production of table olives with a higher value added.

## **2. MATERIALS AND METHODS**

Gemlik type olives were used for fermentation process. The previously identified *Lactobacillus diolivorans* Z141B isolate was used as a starter culture.

### **2.1. Olive processing**

Fermented olive processing consists of control (spontaneous fermentation) and GD encoded sample (*Lentilactobacillus diolivorans* added). After adding 8% rock salt to 250 g of washed olives, they were put into 500 ml jars after they were thoroughly blended. *Lentilactobacillus diolivorans* was activated by incubation for 22 hours at 30 °C in MRS broth medium. The supernatant was removed by centrifuging at 10000 rpm for 5 minutes at 4 °C. The remaining bacteria were washed with buffered saline solution. One mL of the culture of approximately 8 log CFU/mL was inoculated. Olives were stored at 20–25 °C for 35 days and during the fermentation pH, colour and microbiological analyzes (total LAB), bioactive properties and oleuropein content were determined for the olive samples at the different storage days.

### **2.3. Determination of pH values**

A 15 mL of brine was taken from the samples and pH was measured with a calibrated pH meter using standard buffers of pH 4.1, 7.0 and 10.1.

### **2.4. Microbiological analyzes**

Dilutions of previously prepared olive homogenates were prepared using PBS. These dilutions were inoculated on De Man, Rogosa and Elisabeth Sharpe (MRS) media for LAB count. Colony counts were performed by incubating the samples at 30 °C for 24–48 h and determined by calculating log CFU/mL.

### **2.5. Color values**

Internal and outer colour of olive were assessed with a Chromameter for 10 olives taken from each treatment at random, as L, a, and b values.

## 2.6. Bioactive properties

Total phenolic content of the samples was determined using the method suggested by Singleton and Rossi (1965). For this purpose, 200 µl of the brine was mixed with 1800 µl of distilled water. Then 1 mL of diluted (1/10) Folin Ciocalteu reagent and after 1 min later, 2 mL of sodium carbonate (2% w/v) were added into all tubes. The samples were incubated for 2 hours at room temperature and dark conditions. At the end of the incubation, the absorbance values of the samples were recorded at 765 nm using a UV-vis spectrophotometer. Total phenolic content of the samples was calculated as mg oleuropein equivalent /L sample using a calibration curve.

DPPH radical scavenging activity of the samples was determined as described by He et al. (2016). A 100 µL of brine sample was mixed 3900 µL of DPPH radical solution in methanol (2mM) and mixed well using vortex. After the incubation of the samples at room conditions in a dark place for 30 min, the absorbance values were recorded at 517 nm by a UV-vis spectrophotometer. DPPH radical scavenging capacity was calculated as % inhibition using the following equation):

$$\% \text{ Inh} = [(Ab_{\text{control}} - Ab_{\text{sample}}) / Ab_{\text{control}}] \times 100$$

## 2.7. Oleuropein content

Brine samples were filtered through a 0.45 mm filter and then directly injected to HPLC equipped with a photodiode array detector. The wavelength used for quantification of oleuropein was 280 nm. Separation was achieved by using a C18 column (250 4.6 mm) with the temperature of the column set to 30 °C. A isocratic program was utilized with a mobile phase consisting of acetonitrile (21%) and 1% (v/v) phosphoric acid in water (79%). The flow rate during the mobile phase was 1.0 ml/min, and the injection volume was 20 µl. Oleuropein was quantified using calibration curves of authentic oleuropein standard.

## RESULTS AND DISCUSSION

The pH changes of the brine samples of olives during the fermentation of Gemlik type black olives were illustrated in Figure 1. Values of brine pH decreased rapidly within the first 14 days of fermentation, further decreased until the day 35 and stabilized thereafter.

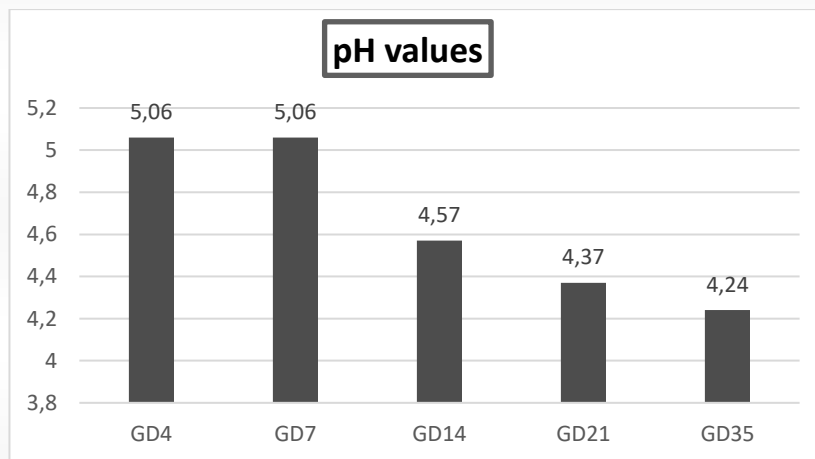


Figure 1. Changes in pH values in Gemlik type black olive fermentation



The LAB count of the brine samples of olives during the fermentation of Gemlik type black olives was illustrated in Figure 2. The amount of LAB in olive samples varied between 5.60 and 5.99 log CFU /mL for control samples while it was 7.25-6.39 log/mL for *Lb. diolivorans*. In the spontaneous process, LAB growth started on the 7th day after brining the olives and showed a marked lag phase. In the treatments, an initial slight decrease (1–2 decimal logarithm) in the inoculum populations was noticed but after approximately 7 days, the population increased rapidly. Total LAB count values obtained in this study were similar to some studies while some of them were different to our results. At the end of various olive fermentation, the number of LAB in the brine was determined as in the range of 3.3–6.8 log CFU/mL (Cortes-Delgado et al., 2016), 6.25–7.84 log CFU/mL (Tofalo et al., 2012).

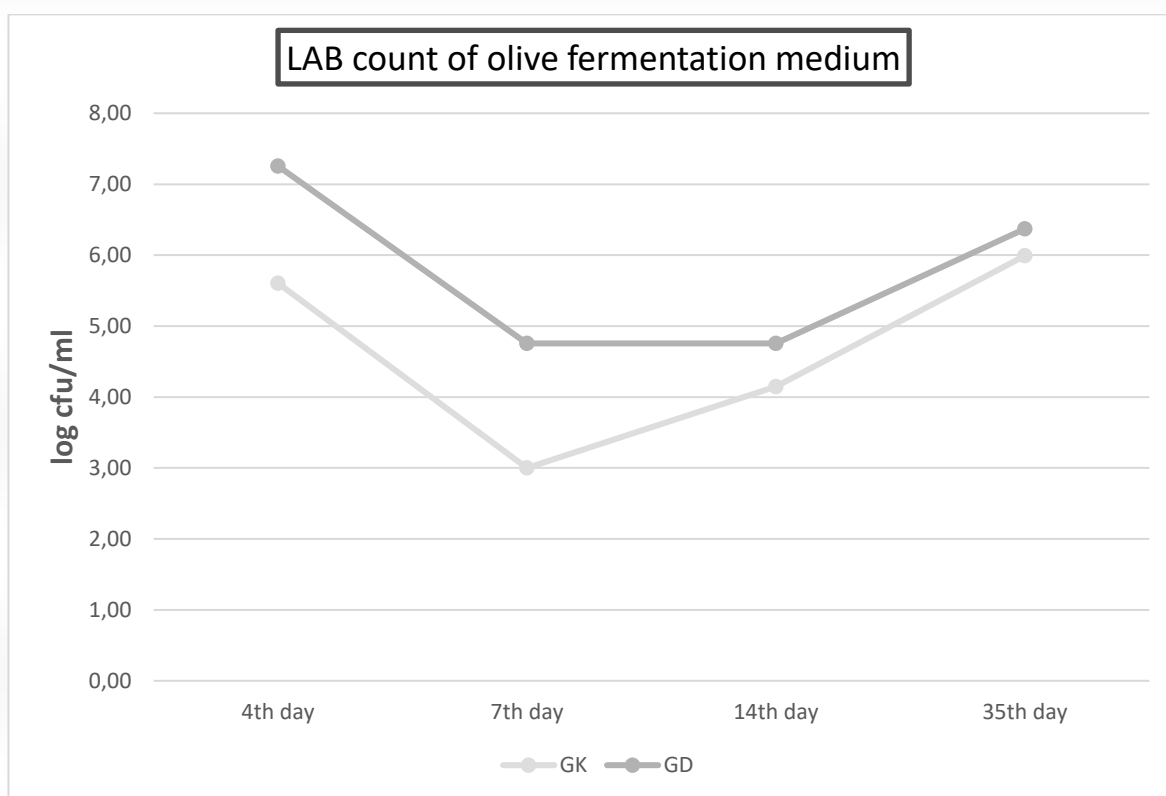


Figure 2. Changes in the number of lactic acid bacteria (LAB) in Gemlik type black olive fermentation. (GK: spontaneous fermentation, GD: *Lentilactobacillus diolivorans* fermentation)

The L, a\* and b\* values of shell colour of olive samples during the fermentation of Gemlik type black olives were illustrated in Figure 3. The L, a and b values of internal fruit colour of olive samples during the fermentation of Gemlik type black olives were illustrated in Figure 4. Fruit colour is also an important quality attribute of table olives for the consumer. From the 4th to the 35th day of fermentation, no significant L value variations for outer and internal part of olives were recorded. For b\* values of outer shell, an increase was observed. LAB strain used in this study did not affect the olive colour negatively.

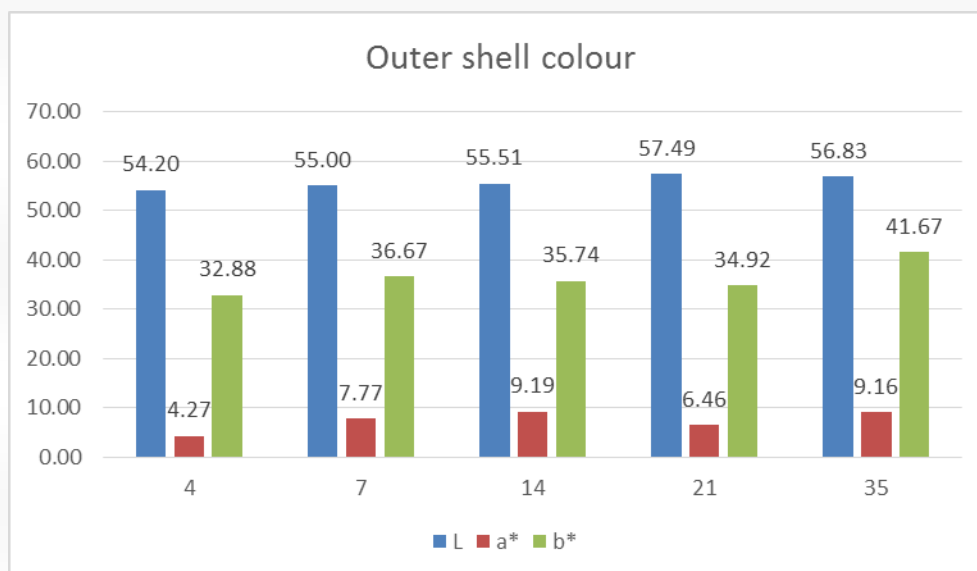


Figure 3. Changes of outer shell colour values of olive during fermentation period

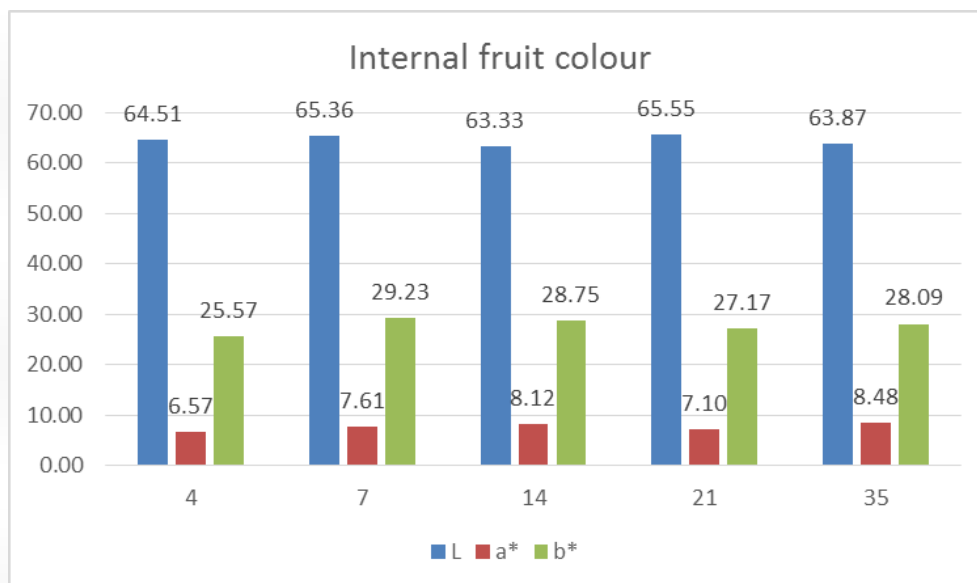


Figure 4. Changes of internal colour values of olive during fermentation period

The total phenolic content values of brine samples during the fermentation of Gemlik type black olives were illustrated in Figure 5. And the antiradical activity values of brine samples during the fermentation of Gemlik type black olives were illustrated in Figure 6. The level of phenolic compounds and antiradical capacity in brines of olives increased significantly during the fermentation process. There was an important loss of phenolic compounds from the olive to the brine during fermentation. The increase of total phenolic compounds in brines was due to the

diffusion of phenolics from the olives during processing and fermentation (Chammem et al., 2005; Chranioti et al. 2018).

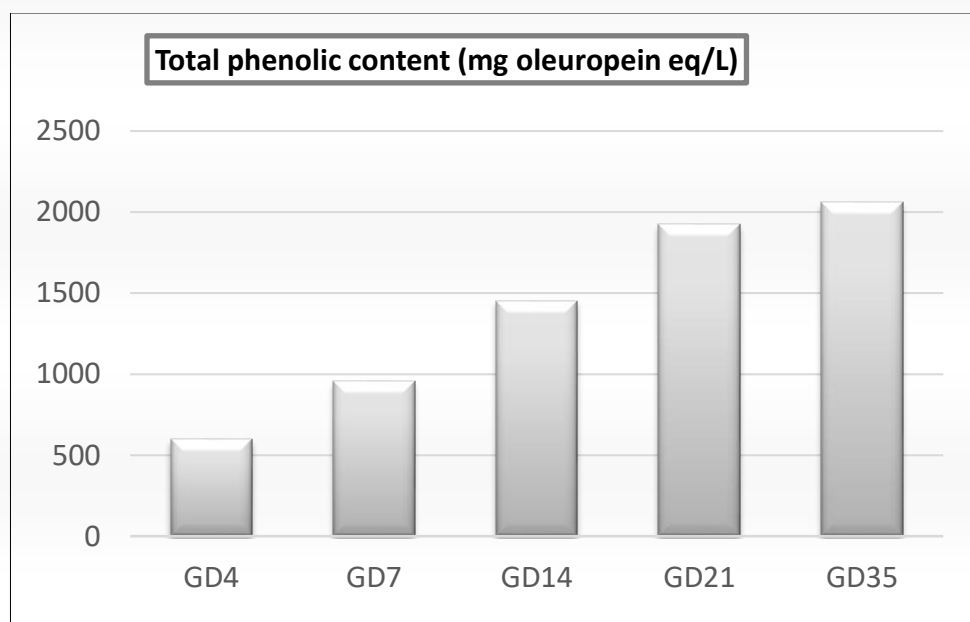


Figure 5. Changes of total phenolic content values of brine during fermentation period

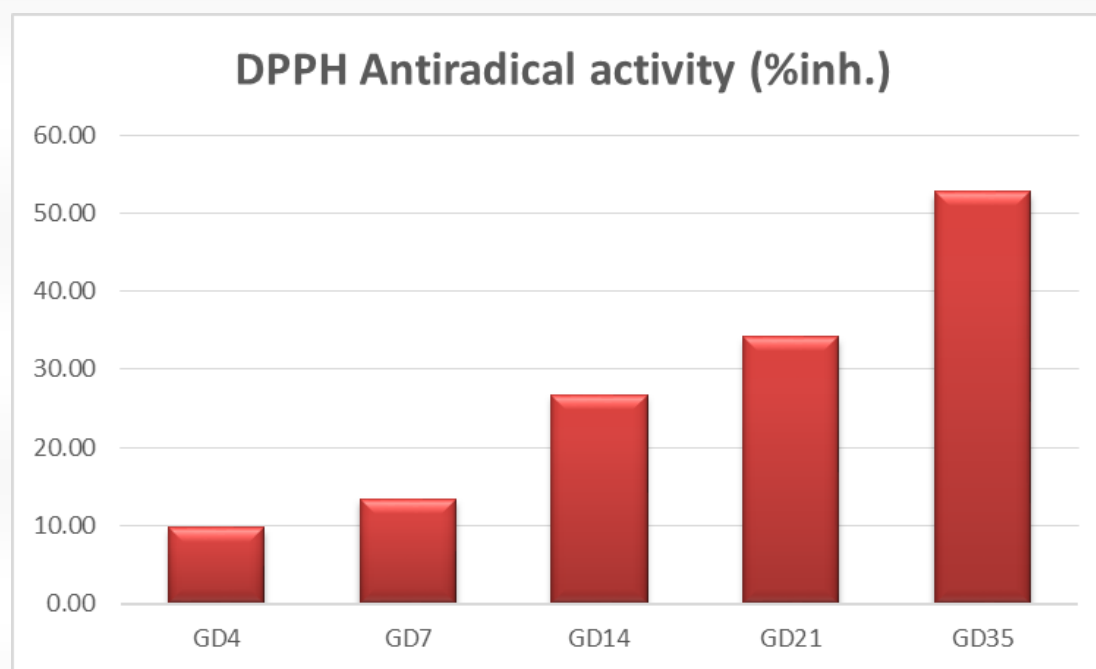


Figure 6. Changes of antiradical activity values of brine during fermentation period

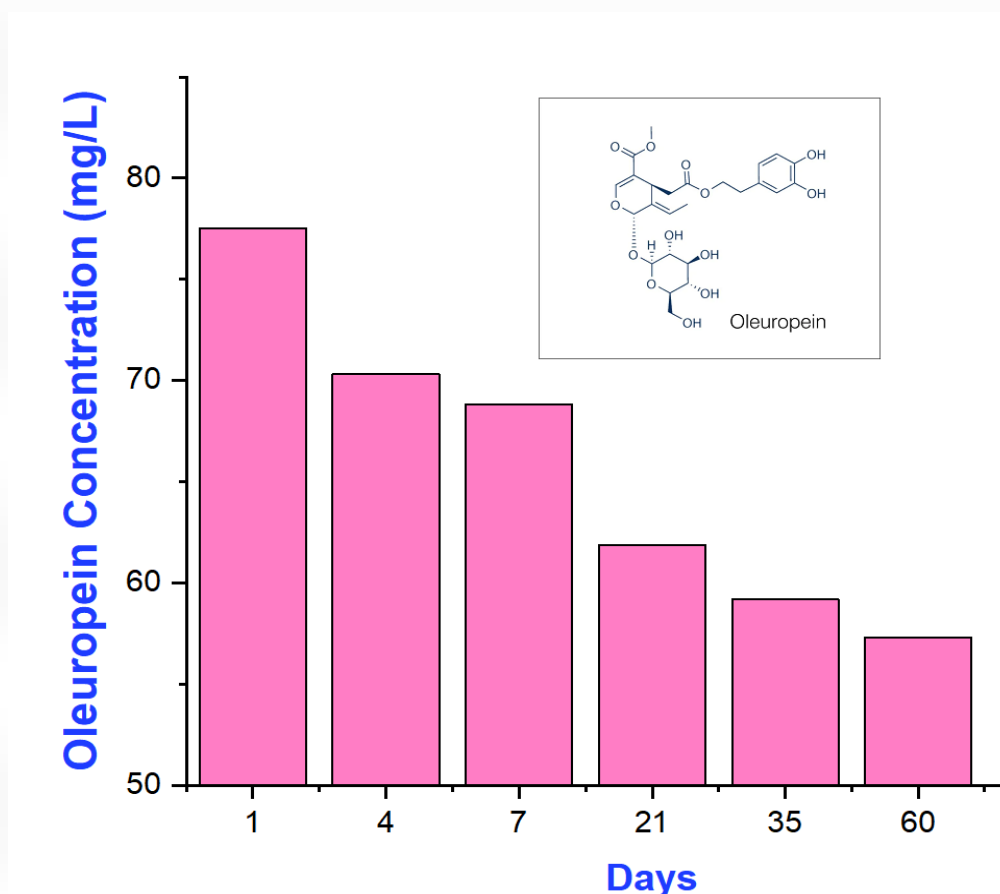


Figure 7. Changes oleuropein content of brines during fermentation period

The oleuropein content of brines during the fermentation of Gemlik type black olives was illustrated in Figure 7. Oleuropein amount was about 77.53 mg/L at first day of fermentation, then it became about 57.31 mg/L at the end of 60 day.

The use of starter culture positively affected fermentation, acidification rate and bitterness loss of the final product. The results show that the selected oleuropeinolytic strains belonging to *Lb. diolivorans* contribute to a significant decrease of the oleuropein content. The main advantage of the fermentation by using oleuropeinolytic strains may be concluded as biological debittering without the need of chemical treatments which are known to reduce the nutrient content of the processed olives and to create excess amount of washing water. This study reports, for the first time, a study on the ability of *Lentilactobacillus diolivorans* in olive fermentation.

The present study showed that olives added with *Lentilactobacillus diolivorans* starter cultures will contribute to olive production by exhibiting a good fermentation profile compared to self-fermented olives.

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## **Effect Of Seed Color on Phenolic and Antioxidant Activity in Grains and Sprouts in Lentils**

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### **ABSTRACT**

Legumes, known as important macro and micronutrient sources, are known as functional foods that support health and have therapeutic properties in addition to their nutritional values. Lentils have a great importance among legumes in terms of the nutritional values they contain. The aim of the study was to compare the phenolic substance and antioxidant activity contents of lentil seeds and sprouts in three different colors (red, green and black). Extraction was performed on red, black and green lentil seeds and sprouts, and the findings obtained showed that the total phenolic substance content (TPC) in the seeds and sprouts was between 732.163 mg GAE/g and 115.243 mg GAE/g, and the total flavonoid substance content (TFC) was 27.300 mg CE/g and 2.710 mg CE/g, DPPH scavenging activity content between 20.871 % and 10.567 %, iron chelating (ICA) content between 92.395 % and 30.757 %, Ferric reducing antioxidant activity (FRAA) content between 1.860 mg AAE/g and 0.0767 mg AAE/g, total hydrolysable tannin content varies between 3102.5 mg/kg and 720 mg/kg, and condensed tannin content varies between 1959.2 mg/kg and 158.66 mg/kg. The results differed according to seed color, and the highest phenolic substance and antioxidant substance values were obtained from black lentil seeds, and the lowest value was obtained from black lentil sprouts. Lentil seed extracts gave superior results compared to sprouts.

**Keywords** lentils, sprouts, different color, antioxidant, phenolic

### **INTRODUCTION**

Lentils, which are known to have developed with wheat and barley, which have become the basic products of agriculture, and have been cultivated for more than 7.000 years, are one of the five important legumes produced in the world (Muehlbauer, F. J., & McPhee, K. E., 2005). Lentils (*Lens culinaris Medik*), an important food product among legumes, are known to grow in various soil structures as well as to have the ability to grow in less favorable environments. (Kaale et al., 2023). Lentils, which are rich in protein, have been stated to have 18 out of 20 amino acids, including all 8 essential amino acids (Anoma et al., 2014). It is one of the best and cheapest sources of vegetable protein (Vidal-Valverde et al., 1994). It is sometimes called “poor man's meat” due to the high amount of protein it contains. (Samaranayaka, A., 2017).



Legume seeds are generally among the phytonutrients that are rich in phenolic compounds and have high antioxidant capacity, which may be beneficial in preventing many health-related conditions such as coronary and cardiovascular diseases. It is known that lentil seeds contain approximately 25% protein, 56% carbohydrates and 1% fat. Lentils are considered a type of legume whose seeds contain mainly condensed type of tannin components and is known as a nutritious food rich in protein. The high antioxidant potential of tannins has been reported in numerous studies (Amarowicz et al., 2010). It is known that regular consumption of lentils can prevent and reduce the development of chronic diseases such as cancers, obesity and diabetes.

It is known that the antioxidant activity of legumes increases during germination due to the increase in polyphenols and vitamin C content. Lentils have been noted as an important plant material for obtaining sprouts because they have a high protein concentration and a higher polyphenol content than other legumes (Peñas et al., 2015). Germination is known to have the ability to improve levels of GABA, a compound involved in the regulation of blood pressure, and to promote the release of bioactive peptides in legumes. It is known that the inclusion of lentil sprouts in staple foods such as bread has the potential to provide functional, nutritional and nutraceutical benefits in human nutrition (Kahve and Bayrak., 2023).

## **MATERIAL AND METHODS**

### **Extraction of the seeds and sprouts samples**

For lentil sprouts, 1 g of sample was weighed and 15 ml of acetone-water-acetic acid (80-18.5-1.5) solvent was added to the powder sample. It was shaken at room temperature for 40 minutes. At the end of the process, the samples were centrifuged at 7500 g for 5 minutes and the resulting supernatant was filtered and used in analyses (Mokrani, 2023).

For lentil seeds, 1 g of sample was weighed, 10 ml of solvent was added and shaken at room temperature for 3 hours. Extraction was performed using 70% acetone for red lentil seeds, 70% ethanol for green lentil seeds and 50% acetone for black lentil seeds. After the samples were kept for 1 night, they were centrifuged at 7500 g for 5 minutes and the resulting supernatant was filtered and used in analyses (Xu & Chang, 2007).

### **Bioactive analysis of seed and sprouts extracts**

#### **Total phenolic content (TPC)**

TPC of the samples was determined using the method suggested by Singleton and Rossi (1965). For this purpose, 200 µl of the extract was mixed with 1800 µl of distilled water. Then 1 mL of diluted (1/10) Folin Ciocalteu reagent and after 1 min later, 2 mL of sodium carbonate (2% w/v) was added into all tubes. The samples were incubated for 2 hours at room temperature and dark conditions. At the end of the incubation, the absorbance values of the samples were recorded at 765 nm using a UV-vis spectrophotometer (Shimadzu, Japan). Total phenolic content of the samples was calculated as mg GAE/g sample using a calibration curve.

#### **Total flavonoid content (TFC)**

TFC of the samples was measured according to method of Zhishen et al. (1999). For this aim, 0.5 ml of sample was mixed with 2 mL of distilled water and then 150 µl of sodium nitrite (5% w/v)

was added into the tubes and the samples were waited for 5 min. Then 150  $\mu$ L of  $\text{AlCl}_3$  (5% w/v) was incorporated into samples and after 6 min waiting, 1 ml of NaOH (1 M) and 1.2 ml of distilled water was added and the final mixture was vortexed and the absorbance of these mixtures was recorded at 510 nm by a UV-vis spectrophotometer (Shimadzu, Japan) and the total flavonoid content of the samples was calculated as mg catechin equivalent (CE)/g sample.

### **DPPH radical scavenging activity**

DPPH radical scavenging activity of the samples was determined as described by He et al. (2016). A 100  $\mu$ L of extract sample was mixed 3900  $\mu$ L of DPPH radical solution in methanol (2mM) and mixed well using vortex. After the incubation of the samples at room conditions in a dark place for 30 min, the absorbance values were recorded at 517 nm by a UV-VIS spectrophotometer (Shimadzu, Japan). DPPH radical scavenging capacity was calculated as % inhibition using the following equation):

$$\% \text{ Inh. (Remaining)} = 100 - [(A_{\text{Scontrol}} - A_{\text{Ssample}}) / A_{\text{Scontrol}}] \times 100$$

### **Ferric reducing antioxidant activity (FRAA)**

Reducing power, which gives an idea about the antioxidant capacity of the samples, was determined based on the method applied by Malomo et al. (2011). One ml of the sample extracts of various concentrations and standard (ascorbic acid) were mixed with 2.5 ml of 0.2 M phosphate buffer solution (pH = 6.6) and then 2.5 ml of 1% w/v potassium ferricyanide [ $\text{K}_3\text{Fe}(\text{CN})_6$ ] was added. The samples were incubated for 20 min at 50 °C. After this step, 2.5 ml of trichloroacetic acid (10% w/v) was added to the reaction mixture and centrifuged at 1000 g for 10 minutes and 2.5 ml was taken from the top of the solution. 2.5 ml of distilled water and 0.5 ml of 0.1%  $\text{FeCl}_3$  were added to the separated part of the solution (2.5 mL) and the samples were mixed by vortex. Then the absorbance values of the samples were measured by UV-vis spectrophotometer (Shimadzu, Japan). The results are given in mg ascorbic acid equivalent (mg AAE / kg).

### **Analysis of condensed tannin and total hydrolyzable tannin**

The contents of the condensed tannins of the samples were determined by applying the method proposed by Sun et al. (1998). For this purpose, the sample extracts were diluted as 1:10 and then 1 mL of extract was mixed with 2.5 mL vanillin (prepared with 1% in methanol) and final sample was mixed with 2.5 mL of  $\text{H}_2\text{SO}_4$  (prepared with 25% in methanol). Subsequently, the samples were incubated in a water bath at 30 °C for 15 min and the absorbance values were recorded at 510 nm by a UV-vis spectrophotometer (Shimadzu, 1800; Japan). The results were expressed as mg catechin equivalent (CE)/g sample using a calibration curve. For the determination of total hydrolyzable tannins of the samples, 1 mL of diluted extract was mixed with 5 mL of potassium iodate (2.5% w/v) and after 4 min of the incubation in the dark conditions, the absorbance of the samples was measured at 550 nm by a UV-vis spectrophotometer (Shimadzu, 1800; Japan). Using the calibration curve prepared with tannic acid, the results were given as mg tannic acid/100 g dry sample. All experiments were replicated with four repetitions.

### **Ferrous ion chelating activity (ICA)**

Iron chelating activities of the samples were determined according to the method suggested by Rival et al. (2001). For this purpose, 1 mL of the sample extract diluted as 1:10 was taken and 3.7 mL of ethanol (95% v/v) was added. Then, 100 µL of FeCl<sub>2</sub> was incorporated into the samples and immediately after vortexing the samples, 200 µL of ferrozine (5 mM) was placed into the tubes. The homogeneously mixed samples were allowed to incubate for 10 min at room temperature in the dark and the absorbance values of the samples were measured by UV–vis spectrophotometer (Shimadzu, 1800; Japan) at 562 nm.

Iron chelating activity values of the samples were calculated as % inhibition using the following equation.

$$\% \text{ Chelating activity} = [(Abs_{\text{control}} - Abs_{\text{sample}}) / Abs_{\text{control}}] \times 100$$

where Abs<sub>sample</sub> is the absorbance of sample; Abs<sub>control</sub> is the absorbance of control. All experiments were replicated with four repetitions.

### **Statistical Analysis**

Data were subjected to variance analysis using SAS (SAS Inst., 1999) statistical software. The LSD multiple range test was employed to compare the treatment means as a complement of the ANOVA procedure.

## **RESULTS AND DISCUSSION**

Lentil seeds and sprouts of different colors (black, green, red) contained total phenolic substance (TPC), total flavonoid substance (TFC), DPPH scavenging activity, iron chelation (ICA), Ferric reducing antioxidant activity (FRAA), total hydrolysable tannin and condensed tannin content is presented in Table 1.

When the results were examined, the seeds included total phenolic substance (732.162 mg GAE/g), DPPH (20.871 %), condensed tannin (1959.2 mg/kg), total hydrolyzable tannin (2547.5 mg/kg) and Ferric reducing antioxidant activity (1.8596 mg AAE /g) in terms of black lentil seed, the lowest values are TPC (234.054 mg GAE/ g), TFC (11.459 mg CE/g), CT (292.768 mg/kg), THT (720 mg/kg), FRAA. It was obtained from green lentil seeds in terms of (0.7527 mg AAE/ g) values. The highest value in TFC content was obtained from red lentil seeds, 27,300 mg CE/g. The lowest value in DPPH content was determined to be 12.070% in red and green lentil seeds. In a study conducted by Pal et.al., (2016) on 5 types of lentil sprouts, it was reported that gallic acid decreased by 13.67-40.41%, catechin dihydrate decreased by 38.99-58.32% and quercetin decreased by 3.17-51.76%. The highest values among sprouts are green in TPC, TFC, DPPH, CT, THT, FRAA as 163.108 mg GAE/ g, 5.343 mg CE/g, 14.978 %, 392.500 mg/kg, 3102.5 mg/kg, 0.8643 mg AAE/ g, respectively. It was detected in lentil sprouts the highest ICA value was determined in red lentil sprouts as 48.631%. In a study, it was stated that in different lentil varieties, processing conditions such as optimal germination temperature and time should be provided in order to maximize the nutritional, bioactive and quality properties of germination on the sprout, and that germination conditions are also effective on phenolic and antioxidant substances (Kahve and Bayrak, 2023). The lowest values of TPC, DPPH, ICA, THT contents were 115.243 mg GAE/

g, 10.568 %, 30.755 %, 1433.8 mg/kg, respectively, in black lentil sprouts and the lowest values in TPC, CT and FRAA contents were obtained from red lentil sprouts as 2.709 mg CE/g, 158.661 mg/kg, 0.0776 mg AAE/g, respectively. When black lentil seeds and sprouts were compared, it was determined that the TPC, TFC, DPPH, ICA, CT, FRAA contents of the seed were higher than the sprouts, and only the THT value of black lentil sprouts was higher than the seed. When the green lentil seed and sprout results were examined, it was determined that TPC, TFC, DPPH, ICA contents were higher in the seed, while CT, THT, FRAA values were higher in the sprout. In red lentil sprouts and seeds, TPC, TFC, DPPH, ICA, CT and FRAA contents were higher in the seed, while only THT content was found to be higher in the sprouts (Table 2).

**Table 1: Bioactive properties of lentil sprouts and seeds**

Genotypes	TPC	TFC	DPPH	ICA	CT	THT	FRAA
Red Lentil Seeds	451.622 <sup>b</sup>	27.300 <sup>a</sup>	12.070 <sup>a</sup>	55.614 <sup>b</sup>	313.661 <sup>b</sup>	1210 <sup>b</sup>	1.8293 <sup>a</sup>
Black Lentil Seeds	732.162 <sup>a</sup>	18.071 <sup>b</sup>	20.871 <sup>a</sup>	87.157 <sup>a</sup>	1959.2 <sup>a</sup>	2547.5 <sup>a</sup>	1.8596 <sup>a</sup>
Green Lentil Seeds	234.054 <sup>c</sup>	11.459 <sup>c</sup>	12.070 <sup>a</sup>	92.395 <sup>a</sup>	292.768 <sup>b</sup>	720 <sup>b</sup>	0.7527 <sup>b</sup>
Sg Df.	**	**	*	**	**	*	**
LSD	24.45	3.856	13.711	7.203	25.54	1182.1	0.087
Red Lentil Sprouts	155.405 <sup>b</sup>	2.709 <sup>c</sup>	11.978 <sup>b</sup>	48.631 <sup>a</sup>	158.661 <sup>c</sup>	1911.3 <sup>a</sup>	0.0776 <sup>c</sup>
Black Lentil Sprouts	115.243 <sup>c</sup>	3.629 <sup>b</sup>	10.568 <sup>b</sup>	30.755 <sup>c</sup>	205.804 <sup>b</sup>	1433.8 <sup>a</sup>	0.6795 <sup>b</sup>
Green Lentil Sprouts	163.108 <sup>a</sup>	5.343 <sup>a</sup>	14.978 <sup>a</sup>	43.768 <sup>b</sup>	392.500 <sup>a</sup>	3102.5 <sup>a</sup>	0.8637 <sup>a</sup>
Sg Df.	**	**	*	**	**	*	**
LSD	6.226	0.633	2.745	3.675	21.013	2543	0.0843

When the results were compared, it was determined that the samples with the best phenolic substance and antioxidant content among the seed and sprout samples were seeds, while the values of sprouts were lower.

Table 2: Comparison of sprouts and seeds according to their colors

Genotypes	TPC	TFC	DPPH	ICA	CT	THT	FRAA
Red Lentil Seeds	451.623 <sup>a</sup>	27.300 <sup>a</sup>	12.070 <sup>a</sup>	55.613 <sup>a</sup>	313.663 <sup>a</sup>	1210 <sup>b</sup>	1.8267 <sup>a</sup>
Red Lentil Sprouts	155.407 <sup>b</sup>	2.710 <sup>b</sup>	11.977 <sup>a</sup>	48.630 <sup>a</sup>	158.66 <sup>b</sup>	1911.3 <sup>a</sup>	0.0767 <sup>b</sup>
SD	*	*	*	*	*	*	*
LSD	24.617	3.2201	16.783	8.105	26.032	3432.2	0.2484
Black Lentil Seeds	732.163 <sup>a</sup>	18.070 <sup>a</sup>	20.870 <sup>a</sup>	87.153 <sup>a</sup>	1959.2 <sup>a</sup>	2547.5 <sup>a</sup>	1.860 <sup>a</sup>
Black Lentil Sprouts	115.243 <sup>b</sup>	3.630 <sup>b</sup>	10.567 <sup>b</sup>	30.757 <sup>b</sup>	205.803 <sup>b</sup>	1433.8 <sup>a</sup>	0.680 <sup>b</sup>
SD	*	*	*	*	*	*	*
LSD	13.827	4.541	8.346	4.471	33.97	1512.9	0.163
Green Lentil Seeds	234.057 <sup>a</sup>	11.457 <sup>a</sup>	12.313 <sup>a</sup>	92.397 <sup>a</sup>	292.77 <sup>b</sup>	720 <sup>a</sup>	0.750 <sup>a</sup>
Green Lentil Sprouts	163.11 <sup>b</sup>	5.343 <sup>b</sup>	14.98 <sup>a</sup>	43.77 <sup>b</sup>	392.503 <sup>a</sup>	3102.5 <sup>a</sup>	0.863 <sup>a</sup>
SD	*	*	*	*	*	*	*
LSD	39.933	2.880	10.706	9.4703	33.744	2615.3	0.1497

TPC: Total phenolic content (mg GAE/g), TFC: Total flavonoid content (mg CE/g), DPPH: DPPH scavenging activity (%), ICA: Iron chelating activity (%), CT: Condensed tannin (mg/kg), THT: Total hydrolyzable tannin (mg/kg), FRAA: Ferric reducing antioxidant activity (mg AAE/g),

## CONCLUSION

Phenolic substance and antioxidant substance contents of red, black and green lentil seeds and sprouts vary according to seed color, and when Tables 1 and 2 are examined, it has been determined that seed color affects the phenolic substance and antioxidant content and the highest values are obtained from black lentil seeds.

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## **Sustainability in Disaster Management: A Meta-Thematic Analysis on Disaster Studies**

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### **ABSTRACT**

This study, focusing on the sustainable crisis communication literature in disaster management, aims to meta-thematically analyze academic articles on disaster management published in the world and especially in countries such as America and Japan, which frequently struggle with disasters. United Nations (UN) definition of disaster; It is defined as "all kinds of serious events that cause loss of life, physical, economic and social losses for people, affect societies by stopping or interrupting normal life, and cannot be coped with by local means." The definition of crisis is explained in social sciences as "a state of tension that occurs as a result of a significant disruption of normal relations in the face of an unexpected situation or any development, and the ways found to solve the problem encountered are not sufficient" (Demir and Acar, 2015: 65). The study was carried out through international databases and directories. In this context, databases such as Scopus, Web of Science and Science Direct constitute the sample of the research. Within the scope of the study created in a qualitative pattern, academic articles obtained from the databases in question were examined with the meta-thematic analysis method. The research findings are interpreted according to certain themes and the details of the findings are reflected in the conclusion of the study, ending with the interpretation of the findings on what kind of studies have been done on sustainable crisis communication in disaster management in the international arena and suggestions for future studies to be conducted in Turkey.

**Keywords:** Disaster Management, Sustainable Crisis Communication, Meta-thematic Analysis

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## **INTRODUCTION**

The definition of crisis is explained in social sciences as "a state of tension that occurs as a result of a significant disruption of normal relations in the face of an unexpected situation or any development, and the ways found to solve the problem encountered are not sufficient" (Demir and Acar, 2015: 65).

The crisis process is a complex process with difficulties. Every process consists of certain stages and time periods. Crisis stages may last long or short depending on the type of crisis, its cause, and its severity. It requires analysis of each emerging crisis and its stages in order to detect crises, prevent their progression and end the crisis after it occurs, and then take precautions. The stages of the crisis process are as follows (Tutar, 2000: 51), (Tüz, 2001: 16-18), (Filiz, 2007: 11):

- The period before the crisis,
- Moment of Crisis,
- The period after the crisis.

### **Pre-Crisis Process**

The pre-crisis period is the period before the crisis occurs, when the symptoms of the crisis gradually manifest themselves, something goes wrong, and negativities begin to be perceived. Hints that the crisis will break out become evident during this period.

This period, where the first intervention is made in the event of a crisis, consists of two stages: the escalation phase and the regression phase, where the escalation phase is stopped and the regression phase is started. The escalation phase is the moment when the situation is not yet understood, the first shock is experienced and panic prevails. This phase ends with the arrival of the first aid and the escalation stops. When measures are taken to solve the crisis, interventions are made and the necessary measures are taken, the escalation stops and the regression phase begins (Filiz, 2007: 13).

This phase, which we call post-crisis, refers to the period in which the severity of the crisis decreases, but its effects still continue due to its consequences. The crisis process is now under control, and the next period, the post-crisis, is the period in which improvement, correction and destruction are reduced, and re-improvement efforts begin. This process and the measures taken set an example for future periods and determine whether similar crises will occur again (Tutar, 2000: 64).

The concept of sustainability is a basic principle and a perspective that has three different dimensions: economic, social and environmental, based on an institution's understanding of social responsibility. This phenomenon, which is more commonly heard and discussed within the scope of the concept of responsibility, needs to be adapted to the management systems, business styles and cultures of institutions and managed with different dimensions within this period (Bıçakçı, 2012: 48). It is seen that institutions are becoming more sensitive to the society and environment they live in day by day in terms of their structure, style, understanding and activities (Güllüpunar, 2010: 33).

Crisis management means overcoming problems in times of chaos and always being prepared to reduce the effects of the crisis (Sapriel, 2003:350). With effective management skills, it is possible to overcome crises with very little damage. An effective management approach is very important in increasing or decreasing the level of crises (Akdağ and Taşdemir, 2006: 143). The main purpose of crisis management is to create institutions that can predict crises, distinguish their types, take some precautions against crises and recover as quickly as possible (Pearson and Mitroff, 1993:49).

The word disaster is derived from the Latin word "astrum" meaning star. While in ancient times, disasters were considered as natural events that could not be overcome and events involving the negative judgment of God, in the modern age they began to be examined from a broader perspective and turned into a dynamic structure (Eryılmaz, 2007, pp. 8-9).

### **Natural Disasters in Turkey and the World**

Due to Turkey's geopolitical location, 95% of the land area is considered a disaster area. Because of this reason; According to the Global Risk Index, Turkey ranks 45th among 191 countries and is in the "high risk" group with an index score of 5.0 (AFAD, 2018). When we look at Turkey's disaster profile, it is seen that natural disasters seen in other parts of the world, except tropical storms and active volcanoes, are also present in Turkey (Dölek, 2016, p. 322).

In 2014, TAMP (Turkey Disaster Response Plan) was created by AFAD in order to intervene effectively in disasters. The purpose of TAMP is; To define the roles and responsibilities of the working groups and coordination teams that will take part in response efforts for disasters and emergencies, and to determine the basic functioning of response planning before, during and after the disaster. This response plan covers ministries, institutions and organizations, private sector, non-governmental organizations and real persons who will work in responding to disasters and emergencies of all types and severity that may occur in Turkey. With its integrated planning approach and modular structure, TAMP is designed as a system that will minimize operational risks during disasters (AFAD, 2023).

Japan is among the most effective countries in disaster management because it is located in the earthquake zone and has experienced many devastating earthquakes to date. Early warning systems and observation networks have been established in Japan. The observation network was built by the Japan Meteorological Agency (JMA). The Disaster Relief Law came into force in Japan in 1947, and the Basic Law on Disaster Measures was enacted in 1961 to reduce disaster risks (Ishiwatari, 2021).

The United States of America (USA) established the Federal Emergency Agency (FEMA) as a result of the disasters they experienced in 1972 and 1979. Disaster management is gathered under one roof with FEMA. With some legal regulations, federal institutions with capacity and responsibility for disaster response were given the task of cooperating with FEMA, and FEMA was given the task of coordinating disaster preparedness and assistance (Yılmaz, Balun & Erbay, 2019, pp. 382-383).

## METHOD OF THE RESEARCH

The study was designed according to the qualitative research method. The universe of the research consists of academic databases operating internationally. In this context, research; It was carried out in databases such as Web of Science, Science Direct and Scopus. Since they are international and academic in nature, the representative potential of these databases is considered to be strong. Research data were analyzed using the meta-thematic analysis method. Meta-thematic analysis; It can be defined as expressing and giving meaning to the data of research studies that have been studied nationally and/or internationally with a qualitative method on a particular research subject and collected by document review, by considering them on a common level and creating new themes and codes (Batdı, 2019, p. 11).

Meta-thematic analysis, which includes a verbal/textual analysis process based on document analysis, aims to combine the qualitative findings of the studies by creating themes and codes. In other words, meta-thematic analysis involves examining qualitative studies on any subject from the perspective of the researcher and obtaining comprehensive and general findings (Batdı, 2017).

## DATA COLLECTION PROCESS AND ANALYSIS

The research covers studies between January 2018 and January 2023. The process followed in collecting and analyzing data is shown in Fig. It is explained in detail in 1. In this context, first of all, the international database was scanned according to the keywords "natural disaster management" and "sustainable crisis communication". Detected publications; It is categorized as "research article, year, title, journal, journal type, article type, language, number of pages, subject". Articles were selected from these publications because they constitute the focus of the research. Another starting point regarding research data is the exclusion of duplicate studies in databases and directories.

Table.1 Picos Diagram of Selected Studies

Describing	Number of studies identified in the database: 1592
Division	Number of studies after removal of repeats: 1439
Selection	Number of studies allocated in title and abstract: 1318
Inclusion	number of full-text articles to choose from: 112
	number of studies included in the analysis: 76
Records excluded for reasons:	
<ul style="list-style-type: none"> <li>• Natural disasters that do not occur in Turkey</li> <li>• Multi-global natural disasters</li> <li>• Technical studies with very local solutions</li> </ul>	

As a result of the screening, 76 studies with the theme of 'natural disaster management' were identified in international databases (Web of Science, Science Direct and Scopus) databases and directories. In this context, as a result of this research conducted in the web of science database, 162 articles were reached, and by re-evaluating these articles in terms of their keywords, 27 articles were reached.

In the first search made by entering keywords in the Science Direct database, 1300 articles were reached. After the titles and abstract, 137 articles were reached, and when certain restrictions were made, such as research articles in social sciences and studies in English between January 2018 and January 2023, 24 articles were reached.

In the study conducted by entering keywords in the Scopus database, 130 articles were reached. As a result of the study, taking into account the necessary constraints of the article, 25 articles were selected for review.

When the data collection procedures and process of the research are summarized, it is possible to state that 76 publications with the theme of 'natural disaster management' were detected in international databases between January 2018 and January 2023. In other words, the findings of the research were obtained and interpreted through 76 articles.

## EVALUATION OF RESEARCH FINDINGS

In the scope of the research; The words "natural disaster management" and "sustainable crisis communication" were scanned in databases such as Web of Science, Science Direct, and Scopus and were included in the findings. The articles in question were compiled in chronological order and tabulated according to categories such as 'author', 'title', 'year', 'journal', 'journal type', 'article type', 'language', 'number of pages', 'subject'. Categories were created by the researchers themselves.

Table. 2. Articles and Thematic Features on Natural Disaster Management Published in Journals in International Databases

<u>Author</u>	<u>Title</u>	<u>Ye ar</u>	<u>Journal</u>	<u>Journal Type</u>	<u>Article Type</u>	<u>Langu age</u>	<u>Page Num ber</u>
Dharmasena, MKGI; Toledano, Margalit), Weaver, C. Kay	The Role of Public Relations in Building Community Resilience to Naturaldisasters: Perspectives From Sri Lanka and New Zealand	20 20	Journal of Communicati on Management	Internat ional Referee d Journal	Researc h	Englis h	17
Chan, Chung-Shing; Nozu, Kazuo; Cheung, Ting On Lewis	Tourism and Natural Disastermanagem ent Process: Perception of Tourism Stakeholders in the Case of Kumamoto Earthquake in Japan	20 20	Current Issues in Tourism	Internat ional Referee d Journal	Researc h	Englis h	21
Mukherjee, Shubhadeep; Kumar, Rahul; Bala, Pradip Kumar	Managing a Natural Disaster: Actionable insights from Microblog Data	20 21	Journal of Decision Systems	Internat ional Referee d Journal	Researc h	Englis h	16
Moerschell, Linda; Novak, Susan S.	Managing Crisis in a University Setting: The Challenge of Alignment	20 20	Journal of Contingencies and Crisis Management	Internat ional Referee d Journal	Researc h	Englis h	11

Elbedour, Salman; Alsubie, Futiem; Al'Uqdah, Shareefah N.; Bawalsah, Joseph A.	School Crisis Management Planning	20 20	Children & Schools	International Refereed Journal	Research	English	8
Houston, J. Brian; Schraedley, Megan K.; Worley, Mary E.; Reed, Katherine; Saidi, Janet	Disaster Journalism: Fostering Citizen and Community Disaster Mitigation, Preparedness, Response, Recovery, and Resilience Across The Disastercycle	20 19	Disasters	International Refereed Journal	Research	English	12
Dao, Minh Tuan; Lim, Seunghoo	Fear of Disasters Within The Risk Communication Network of Foreign Students in Japan Amid The COVID-19 Pandemic Crisis: A Cohort Design	20 22	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	12
Mirbabaie, Milad; Stieglitz, Stefan; Brünker, Felix	Dynamics of Convergence Behaviour in Social Media Crisis Communication- A Complexity Perspective	20 22	Information Technology & People	International Refereed Journal	Research	English	27
Kinsky, Emily S.; Chen, Li; Drumheller, Kristina	Crisis and Emergency Risk Communication: FEMA's Twitter Use During the 2017 Hurricane Season	20 21	Public Relations Review	International Refereed Journal	Research	English	10
Xu, Zhan	How Emergency Managers Engage Twitter Users During Disasters	20 20	Online Information Review	International Refereed Journal	Research	English	18
Park, Hyejeong; Nam, Kihun; Lee, Junsik	Survival Communication Under Uncertainty And Complexity: A Review	20 22	International Journal of Advanced And Applied Sciences	International Refereed Journal	Compilation	English	9
Kouskouna, Vasiliki; Sakkas, Georgios; Cecic, Ina; Tsimpidaros, Vasileios-Ioannis; Sakkas, Stylianos;	Earthquake induced Crises: Game Tree Approached Risk Communication and Lessons Learnt	20 21	Annals of Geophysics	International Refereed Journal	Research	English	24



Kaviris, George; Tertulliani, Andrea							
Christensen, Tom; Ma, Liang	Coordination Structures and Mechanisms for Crisis Management in China: Challenges of Complexity	20 20	Public Organization Review	Internat ional Referee d Journal	Researc h	Englis h	18
Liu, Yihong; Christensen, Tom	The Long-Term Development Of Crisismangement in China-Continuity, Institutional Punctuations And Reforms(Sic)(Sic)(Sic)Palabra s Clave	20 22	Review of Policy Research	Internat ional Referee d Journal	Researc h	Englis h	21
Lu, Xiaoli	Online Communication Behav ior at The Onset of A Catastrophe: An Exploratory Study of The 2008 Wenchuan Earthquake in China	20 18	Natural Hazards	Internat ional Referee d Journal	Researc h	Englis h	18
Doyle, Emma E. H.; Johnston, David M.; Smith, Richard; Paton, Douglas	Communicating Model Uncertainty for Natural Hazards: A Qualitative Systematic Thematic Review	20 19	International Journal of Disaster Risk Reduction	Internat ional Referee d Journal	Compil ation	Englis h	28
Chon, Myoung,Gi	Government Public Relations When Trouble Hits: Exploring Political Dispositions, Situational Variables, and Government-Public Relationships to Predict Communicative Action of Publics	20 19	Asian Journal of Communicati on	Internat ional Referee d Journal	Researc h	Englis h	17
W. Seeger, Matthew; Islam, Khairul; S.Seeger, Henry	Emergency Preparedness, Response, and Strategic Communication for Natural Disasters	20 21	HANDBOOK OF STRATEGIC COMMUNIC ATION		Book Chapter	Englis h	14
Liu, Wenlin; Xu, Weiai Wayne	Tweeting To (Selectively) Engage: How Government Agencies Target Stakeholders on Twitter During Hurricane Harvey	20 19	International Journal of Communicati on	Internat ional Referee d Journal	Researc h	Englis h	23
Seeger, Matthew W.; Pechta, Laura E.; Price, Simani M.; Lubell, Keri M.; Rose, Dale A.; Sapru, Saloni ; Chansk	A Conceptual Model for Evaluating Emergency Risk Communication in Public Health	20 18	Health Security	Internat ional Referee d Journal	Researc h	Englis h	11

y, Melanie C.; Smith, Belinda							
Hu, Xi; Zhang, Xiujuan; Wei, Jiuchang	Public Attention to Natural Hazard Warnings on Social Media in China	20 19	Weather Climate And Society	Internat ional Referee d Journal	Researc h	Englis h	15
Wehde, Wesley; Pudlo, Jason M.; Robinson, Scott E.	"Is There Anybody out There?": Communication of N atural Hazard Warnings at Home and Away	20 19	Social Science Quarterly	Internat ional Referee d Journal	Researc h	Englis h	18
Amiresmaili, Mohammadreza ; Zolala, Farzaneh; Neko ei-Moghadam, Mahmood; Sala vatian, Siavash; Chash myazdan, Mohammadreza ; Soltani, Ahmad; Savabi, Jaber	Role of Social Media in Earthquake: A Systematic Review	20 21	Iranian Red Crescent Medical Journal	Internat ional Referee d Journal	Compil ation	Englis h	8
Goswick, Jeri; Macgregor, Cynthia J.; Hurst, Beth; Wall, Patricia J.; White, Renee	Lessons Identified by the Joplin School Leadership After Responding To a Catastrophic Tornado	20 18	Journal of Contingencies and Crisis Management	Internat ional Referee d Journal	Researc h	Englis h	10
Kitagawa, Kaori	Living With an Active Volcano: Informal and Community Learning for Preparedness in South of Japan	20 18	Observing The Volcano World: Volcano Crisis Communicait on	Internat ional Referee d Journal	Researc h	Englis h	13
Rodgers, Janise; Su, Guiwu; Qi, Wenhua; Milled ge, David; Densmor e, Alexander; Davi s, Craig; England, Philip; Young, John; Cao, Yue; Chakos,	Creating an Earthquake Scenario in China: A Case Study in Weinan City, Shaanxi Province	20 20	International Journal of Disaster Risk Reduction	Internat ional Referee d Journal	Researc h	Englis h	14

Arrietta; Li, Xiaoli; Sim, Timothy; So, Emily; Parsons, Barry; Sun, Lei; Yu, Junlei; Guo, Chunlan							
Eddie Lucero, Jessica Trounstone, Jennifer M. Connolly & Casey Klofstad	A Matter Of Life Or Death: How Racial Representation Shapes Compliance With City Disaster Preparedness Orders	20 22	Journal of Urban Affairs	International Refereed Journal	Research	English	19
Wei Zhai	A Multi-Level Analytic Framework For Disaster Situational Awareness Using Twitter Data	20 22	Computational Urban Science	International Refereed Journal	Research	English	15
Somya D. Mohanty a , Brown Biggers a , Saed Sayedahmed a , Nastaran Pourebrahim b , Evan B. Goldstein b , Rick Bunch b , Guangqing Chi c , Fereidoon Sadri a , Tom P. McCoy d , Arthur Cosby	A Multi-Modal Approach Towards Mining Social Media Data During Natural Disasters - A Case Study Of Hurricane Irma	20 21	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	14
Tisha Joseph Holmes a, Patrice C. Williams b , Sandy Wong c , Kathryn Smith d , John T. Bandzuh c , Christopher K. Uejio	Assessment Of An Evacuation Shelter Program For People With Access And Functional Needs In Monroe County, Florida During Hurricane Irma	20 22	Social Science & Medicine	International Refereed Journal	Research	English	9
Seungyoon Lee Bailey C. Benedict Yue 'Gurt' Ge Pamela Murray-Tuite Satish V. Ukkusuri	An Application Of Media And Network Multiplexity Theory To The Structure And Perceptions Of Information Environments In Hurricane Evacuation	20 20	Research Article	International Refereed Journal	Research	English	16

Chung-Shing Chan, Kazuo Nozu & Qinrou Zhou	Building Destination Resilience In The Tourism Disaster Management Process From The Past Experiences: The Case Of The 2018 Hokkaido Eastern Iburi Earthquake In Japan	20 22	Tourism Recreation Research	International Refereed Journal	Research	English	18
Shefali Juneja Lakhina, Elaina J. Sutley Jay Wilson	“How Do We Actually Do Convergence” For Disaster Resilience? Cases From Australia And The United States	20 21	Int J Disaster Risk Sci	International Refereed Journal	Research	English	13
Meredith Jacobson, Hollie Smith, Heidi R. Huber-Stearns, Emily Jane Davis, Antony S. Cheng & Alison Deak	Comparing Social Constructions Of Wildfire Risk Across Media, Government, And Participatory Discourse In A Colorado Fireshed	20 21	Journal of Risk Research	International Refereed Journal	Research	English	19
"Jennifer M. First, Mansoo Yu, J. Brian Houston	Coordinating Non-Established Disaster Relief Groups: A Case Study Of Hurricane Irma In Florida, United States	20 21	Disasters Journal	International Refereed Journal	Research	English	29
Kati Orru, Sten Hansson, Friedrich Gabel, Piia Tammppuu, Marco Krüger, Lucia Savadori, Sunniva Frislid Meyer, Sten Torpan, Pirjo Jukarainen, Abriel Schieffellers, Gabriella Lovasz, and Mark Rhinard	Approaches To ‘Vulnerability’ In Eight European Disaster Management Systems	20 21	Disasters Journal	International Refereed Journal	Research	English	26
Tomoki Tanaka , Yuki Matsuda , Manato Fujimoto , Hirohiko Suwa , and Keiichi Yasumoto	Evacuation Shelter Decision Method Considering Non-Cooperative Evacuee Behavior To Support The Disaster Weak	20 21	Sustainability	International Refereed Journal	Research	English	21
Celine Rendon, Khalid K. Osman, Kasey M. Faust	Path Towards Community Resilience: Examining Stakeholders’ Coordination At The Intersection Of The Built, Natural, And Social Systems	20 21	Sustainable Cities and Society	International Refereed Journal	Research	English	12

Mohammadsepehr Karimizariani , Keighobad Jafarzadegan , Peyman Abbaszadeh , Wanyun Shao , Hamid Moradkhani	Hazard Risk Awareness And Disaster Management: Extracting The Information Content Of Twitter Data	20 21	Sustainable Cities and Society	International Refereed Journal	Research	English	11
Tara Powell, Jennifer Scott, Paula Yuma	Surviving The Storm: A Pragmatic Non-Randomised Examination Of A Brief Intervention For Disaster-Affected Health And Social Care Providers	20 22	Health Soc Care Community	International Refereed Journal	Research	English	11
Amer Hamad Issa Abukhalaf , Jason von Meding a , Jake R. Dooling b , Deyaaldean M. Abusal	Assessing International Students' Vulnerability To Hurricanes: University Of Florida Case Study	20 22	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	14
Louis Ngamassi , Hesam Shahriari, Thiagarajan Ramakrishnan, Shahedur Rahman	Text Mining Hurricane Harvey Tweet Data: Lessons Learned And Policy Recommendations	20 22	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	11
Rejina Manandhar a, Laura K. Siebeneck	Information Management And The Return-Entry Process: Examining Information Needs, Sources, And Strategies After Superstorm Sandy	20 20	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	11
Hamilton Bean, Ana Maria Cruz, Mika Shimizu Keri K. Stephens Matthew McGlone, Sharon Strover	Mobile Alert And Warning In The United States And Japan: Confronting The Challenges Of International Harmonization	20 21	Int J Disaster Risk Sci	International Refereed Journal	Research	English	7
Kamol Chandra Roy, Samiul Hasan	Modeling The Dynamics Of Hurricane Evacuation Decisions From Twitter Data: An Input Output Hidden Markov Modeling Approach	20 21	Transportation Research Part C	International Refereed Journal	Research	English	16
Dimitrios Tzioutzios , Jeong-Nam	Appetite For Natech Risk Information In Japan: Understanding Citizens' Communicative Behavior	20 22	Int J Disaster Risk Sci	International Referee	Research	English	19

Kim,Ana Maria Cruz	Towards Risk Information Disclosure Around Osaka Bay			d Journal			
Jim P Mann & Brian D Williams	Policing In The Eye Of The Storm	2020	Journal of Police and Criminal Psychology	International Refereed Journal	Research	English	10
Hiroko Oe a, Sachiyo Kawakami	A Disaster Prevention Programme Using Virtual Schemes: Recommendation Of Tradition Populaire Integrated With Tendenko As An Approach To Immersive Training	2021	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	6
Oliver Nahkur , Kati Orru a , Sten Hansson a , Pirjo Jukarainen , Miia Myllyl , Marco Krüger , Matthias Max , Lucia Savadori , Tor-Olav Nævestad , Sunniva Frislid Meyerf , Abriel Schieffellers , Alexandra Olson, Gabriella Lovasz , Mark Rhinard	The Engagement Of Informal Volunteers In Disaster Management In Europe	2022	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	14
Patricia A. Champ a, Hannah Brenkert-Smithb, Jonathan P. Riley c , James R. Meldrum d, Christopher M. Barthe , Colleen Donovan f , Carolyn J. Wagner	Actionable Social Science Can Guide Community Level Wildfire Solutions. An Illustration From North Central Washington, US☆	2022	International Journal of Disaster Risk Reductio	International Refereed Journal	Research	English	11
Naim Kapucu, Qian Hu, Mitchel Harmon, and Parker Toro	Coordinating Non-Established Disaster Relief Groups: A Case Study Of Hurricane Irma In Florida, United States	2021	Disasters Journal	International Refereed Journal	Research	English	21
Timothy Schemppa,*, Haoran	A framework to integrate social media and authoritative data for disaster	2019	International Journal of	International Referee	Research	English	10



Zhangb,**, Alexander Schmidt, Minsung Hongd, Rajendra Akerkard	relief detection and distribution optimization		Disaster Risk Reduction	d Journal			
Maziar Yazdani a,*, Mohammad Mojtahedi a, Martin Loosemore b, David Sanderson	A modelling framework to design an evacuation support system for healthcare infrastructures in response to major flood events	20 22	Progress in Disaster Science	Internat ional Referee d Journal	Researc h	Englis h	12
Juan C. Marcillo- Delgado a,*, A. Alvarez-Garcia b, Agueda García-Carrillo	Analysis of risk and disaster reduction strategies in South American countries	20 21	International Journal of Disaster Risk Reduction	Internat ional Referee d Journal	Researc h	Englis h	17
Amer Hamad Issa Abukhalaf a,*, Jason von Meding a, Jake R. Dooling b, Deyaaldeen M. Abusal c	Assessing international students' vulnerability to hurricanes: University of Florida case study	20 22	International Journal of Disaster Risk Reduction	Internat ional Referee d Journal	Researc h	Englis h	14
David N. Nguyena,*, Fumihiko Imamurab, Kanao Iuchic	Barriers towards hotel disaster preparedness: Case studies of post 2011 Tsunami, Japan	20 18	International Journal of Disaster Risk Reduction	Internat ional Referee d Journal	Researc h	Englis h	9
Juan C. Marcillo- Delgado a,*, A. Alvarez-Garcia b, Agueda García-Carrillo	Communication strategies on risk and disaster management in South American countries	20 22	International Journal of Disaster Risk Reduction	Internat ional Referee d Journal	Researc h	Englis h	12
Sten Hansson a,*, Kati Orru a, Andra Siibak a, Asta Bäck b, Marco Krüger c, Friedrich Gabel c, Claudia Morsut d	Communication-related vulnerability to disasters: A heuristic framework	20 20	International Journal of Disaster Risk Reduction	Internat ional Referee d Journal	Researc h	Englis h	9
Sifan Xu	Crisis communication within a community: Bonding, coping, and making sense together	20 18	Public Relations Review	Uluslar arası Hakeml i Dergi	Researc h	Englis h	13

John L. Renne	Emergency evacuation planning policy for carless and vulnerable populations in the United States and United Kingdom	2018	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	7
Milan Stute a,*, Max Maass a, Tom Schons a, Marc-Andre Kaufhold b, Christian Reuter b, Matthias Hollick	Empirical insights for designing Information and Communication Technology for International Disaster Response	2020	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	10
Juhani Latvakoski b,*, Risto "O"orni a, Toni Lusikka b, Jaana Keränen	Evaluation of emerging technological opportunities for improving risk awareness and resilience of vulnerable people in disasters	2022	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	22
Hitomi Nakanishia,*, John Blackb	Implicit and explicit knowledge in flood evacuations with a case study of Takamatsu, Japan	2018	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	9
Mohammed Ali Berawi a,c,*, Pekka Leviakangas b, Sutan Akbar Onggar Siahaan c, Alya Hafidza c, Mustika Sari c, Perdana Miraj c, Ruki Harwahyu d, Gunawan Saroji c	Increasing disaster victim survival rate: SaveMyLife Mobile Application development	2021	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	14
Brooke Fisher Liua,*, Brooke M. Fowlera, Holly A. Robertsb, Emina Herovicc	Keeping hospitals operating during disasters through crisis communication preparedness	2018	Public Relations Review	International Refereed Journal	Research	English	12
Vanessa A. Coopera,*, Giuseppe Forinob, Sittimont Kanjanabootrab, Jason von Medingc	Leveraging the community of inquiry framework to support web-based simulations in disaster studies	2020	The Internet and Higher Education	International Refereed Journal	Research	English	15

Maila D.H. Rahiem a,*, Robin Ersing b, Steven Eric Krauss c, Husni Rahim	Narrative inquiry in disaster research: An examination of the use of personal stories from the child survivors of the 2004 Aceh tsunami	20 21	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	10
Scott Curtis a,*, Anuradha Mukherji b, Jamie Kruse b, Jennifer Helgeson c, Ausmita Ghosh b, Nelson Adeniji b	Perceptions of risk to compound coastal water events: A case study in eastern North Carolina, USA	20 22	Progress in Disaster Science	International Refereed Journal	Research	English	8
Katsushige Kitazawa a,b,*, Scott A. Hale a,c	Social media and early warning systems for natural disasters: A case study of Typhoon Etau in Japan	20 21	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	10
Leila Daddoust a, Ali Asgary a,b,*, Kenneth J. McBey b,c, Steve Elliott d, Alain Normand e	Spontaneous volunteer coordination during disasters and emergencies: Opportunities, challenges, and risks	20 21	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	11
Angus Naylor a,*, Joanna Faure Walkera, Anawat Suppasrib	Suitability of the early warning systems and temporary housing for the elderly population in the immediacy and transitional recovery phase of the 2011 Great East Japan Earthquake and Tsunami	20 18	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	8
Oliver Nahkur a,*, Kati Orru a, Sten Hansson a, Pirjo Jukarainen b, Miia Myllylä b, Marco Krüger c, Matthias Max d, Lucia Savadori e, Tor-Olav Nævestad f, Sunniva Frislid Meyer f, Abriel Schieffellers g, Alexandra Olson g,	The engagement of informal volunteers in disaster management in Europe	20 22	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	14

Gabriella Lovasz h, Mark Rhinard i							
Eun-Seon Park, D.K. Yoon	The value of NGOs in disaster management and governance in South Korea and Japan	20 22	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	15
V. Nespeca *, T. Comes, K. Meesters, F. Brazier	Towards coordinated self-organization: An actor-centered framework for the design of disaster management information systems	20 20	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	12
Marion Lara Tan a,*, Raj Prasannaa, Kristin Stock b, Emma E.H. Doylea, Graham Leonard c, David Johnston	Understanding end-users' perspectives: Towards developing usability guidelines for disaster apps	20 20	Progress in Disaster Science	International Refereed Journal	Research	English	13
Philippe Weyrich a, Isabelle Ruin b, Galateia Terti b, Anna Scolobig	Using serious games to evaluate the potential of social media information in early warning disaster management	20 21	International Journal of Disaster Risk Reduction	International Refereed Journal	Research	English	17

Dharmasena, MKGI; The title of his article published in Toledano, Margalit), Weaver, C. Kay (2020) is "The Role of Public Relations in Building Community Resilience to Natural Disasters: Perspectives from Sri Lanka and New Zealand". The article was published in the Journal of Communication Management. The article, published as a research article in the International Refereed Journal, is in English and consists of 17 pages. The article defines the role of public relations in disaster management by analyzing disaster and communication managers' understanding of community resilience and their use of communication in the context of two different cultural environments.

Chan, Chung-Shing; Nozu, Kazuo; The title of the article Cheung, Ting on Lewis (2020) is "Tourism and Natural Disaster management Process: Perception of Tourism Stakeholders in The Case of Kumamoto Earthquake in Japan". The journal published in Current Issues in Tourism is an International Refereed Journal. It is a research article in English. The research article consists of 21 pages. It is about the role of tourism in disaster management based on disaster management frameworks in the case of the earthquake that occurred in Kumamoto Prefecture, Japan in April 2016.

Mukherjee, Shubhadeep; Kumar, Rahul; The title of the article Bala, Pradip Kumar (2021) is "Managing A Natural Disaster: Actionable insights From Microblog Data". It was published as an English research article in the Journal of Decision Systems, an international refereed journal. It is an article consisting of 16 pages. The research aims to measure whether the popularity of Tweet,

which mobilizes people in the event of a disaster, is affected by the style of communication on social media.

Moerschell, Linda; The title of the article published in Novak, Susan S. (2020) is “Managing Crisis in A University Setting: The Challenge of Alignment”. It was published in English in the international refereed journal *Journal of Contingencies and Crisis*. It is an article consisting of 11 pages. This article explores the unique challenges university administrators face when planning and organizing communications and activities before, during, and after a crisis.

Elbedour, Salman; Alsobie, Futiem; Al'Uqdah, Shareefah N.; The title of the article written by Bawalsah, Joseph A. (2020) is "School Crisis Management Planning". It was also published in English in the International Refereed Journal called *Children & Schools*. The article consists of 8 pages. A study was carried out on what the Crisis Management Plan should be like in a school.

Houston, J. Brian; Schraedley, Megan K.; Worley, Mary E.; Reed, Katherine; The title of Saidi, Janet (2019)'s article is “Disaster Journalism: Fostering Citizen and Community Disaster Mitigation, Preparedness, Response, Recovery, and Resilience across The Disastercycle”. It was published in the peer-reviewed journal *Disasters*. It consists of 12 pages. This article considers these possible functions of disaster journalism and is conducted in the form of semi-structured interviews with 24 journalists in the United States to better understand their approaches to this discipline.

Dao, Minh Tuan; In the article he wrote in Lim, Seunghoo (2022), the title is “Fear of Disasters Within the Risk Communication Network of Foreign Students in Japan Amid The COVID-19 Pandemic Crisis: A Cohort Design”. It was published in the international peer-reviewed journal called *International Journal of Disaster Risk Reduction*. It consists of 12 pages. The main purpose of this study is to examine the role of crisis communication during the COVID-19 process.

Mirbabaie, Milad; Stieglitz, Stefan; The title of the article by Brünker, Felix (2022) is “Dynamics of Convergence Behavior in Social Media Crisis Communication- A Complexity Perspective”. It is a research article published in the International Refereed Journal of *Information Technology & People*. It is in English and consists of 27 pages. The purpose of this study is to investigate communication on Twitter during two unforeseen crises (Manchester bombings and Munich attacks) and a natural disaster (Hurricane Harvey). The study aims to contribute to the understanding of the dynamics of convergent behavioral archetypes during crises.

Kinsky, Emily S.; Chen, Li; The title of the article by Drumheller, Kristina (2021) is “Crisis and Emergency Risk Communication: FEMA's Twitter Use During the 2017 Hurricane Season”. It was published in English and 10 pages in the International Refereed Journal called *Public Relations Review*. This study was created by examining FEMA's national and regional Twitter account posts throughout the historic 2017 hurricane season and explored expanded use of the Crisis and Emergency Risk Communication (CERC) model in researching risk and crisis communication during the hurricane season.

The title of the article by Xu, Zhan (2020) is “How Emergency Managers Engage Twitter Users During Disasters”. Research article published in the international journal *Online Information Review* and published in English. This 18-page study examined social media engagement of

official emergency accounts at each disaster stage, based on Fink's four-stage crisis and disaster model.

Park, Hyejeong; Nam, Kihun; In the article Lee, Junsik (2022), the title is “Survival Communication Under Uncertainty and Complexity: A Review”. It was written as an English review in the International Journal of Advanced and Applied Sciences, an International Refereed Journal. It consists of 9 pages. In this study, which made an extensive content review and synthesis based on the literature on the concepts of risk and crisis communication published before 2015, the literature was reviewed and a concept of survival and communication was tried to be framed and discussed through the lessons learned from two successive disasters.

Kouskouna, Vasiliki; Sakkas, Georgios; Cecic, Ina; Tsimpidaros, Vasileios-Ioannis; Sakkas, Stylianos; Kaviris, George; In his article by Tertulliani, Andrea (2021), the title is “Earthquake induced Crises: Game Tree Approached Risk Communication and Lessons Learnt”. The article, published as an international refereed article in the journal Annals of Geophysics, is 24 pages long and in English. In this study, policy selection is approached through the application and construction of a risk game tree.

Christensen, Tom; The title of the article by Ma, Liang (2020) is “Coordination Structures and Mechanisms for Crisis Management in China: Challenges of Complexity”. It was published in English and 18 pages in the international peer-reviewed journal Public Organization Review. This article aims to combine vertical and horizontal dimensions to develop a theoretical framework to explain the patterns and performance of interorganizational coordination during crisis periods.

Liu, Yihong; In the article of Christensen, Tom (2022), the title is “The Long-Term Development of Crisismanagement In China-Continuity, Institutional Punctuations and Reforms (Sic)(Sic)(Sic)Palabras Clave”. The journal of the article is Review of Policy Research, an international refereed journal. The article is a research article in English and consists of 21 pages. This study focuses on the long-term development of crisis management in China.

The article Lu, Xiaoli (2018) wrote is titled “Online Communication Behavior at the Onset of a Catastrophe: an Exploratory Study of the 2008 Wenchuan Earthquake in China”. It was published in English and 18 pages in the international peer-reviewed journal Natural Hazards. In this article, to describe the information communication behavior of internet users at the beginning of the 2008 catastrophic Sichuan earthquake in China, we first examined how China's crisis communication environment changed and identified the challenges faced by crisis communication managers. Secondly, the behavior of Chinese citizens during the 2008 Wenchuan earthquake was examined.

Doyle, Emma E. H.; Johnston, David M.; Smith, Richard; The title of Paton, Douglas (2019) article is “Communicating model uncertainty for natural hazards: A qualitative systematic thematic review”. It was published as a review in the International Refereed Journal called International Journal of Disaster Risk Reduction. It is in English and 28 pages. Developing a shared uncertainty management scheme with users facilitates the management of different epistemological perspectives, accommodates the different values that underpin model assumptions and the judgments they prompt, and increases uncertainty tolerance.



The title of Chon, Myoung, Gi (2019) article is “Government public relations when trouble hits: exploring political dispositions, situational variables, and government-public relationships to predict communicative action of publics”. It has also been published in the International Refereed Journal called Asian Journal of Communication. It is a research article in English. The subject explains that government institutions may face crises due to inappropriate responses to natural or human-made disasters. However, crisis communication in government public relations has not been extensively researched. This study aimed to do this research.

W. Seeger, Matthew; Islam, Khairul; The title of the article by S. Seeger, Henry (2021) is “Emergency Preparedness, Response, and Strategic Communication for Natural Disasters”. It was published as a book chapter in the book titled Handbook of Strategic Communication. It is in English and has 14 pages. In the book section; Firstly, disasters and disaster management are defined. Communication functions in crisis are discussed both as tactics to support emergency management and as strategic components. It is stated that emergency preparedness and response require a set of specific forms of strategic communication (SC) throughout the disaster lifecycle.

Liu, Wenlin; The title of the article by Xu, Weiai Wayne (2019) is “Tweeting to (Selectively) Engage: How Government Agencies Target Stakeholders on Twitter during Hurricane Harvey 2019.” It was published in the international peer-reviewed journal International Journal of Communication. It is a research article and published in English. It consists of 23 pages. Using Hurricane Harvey as a case study, this study examined the stakeholder engagement practices on Twitter of 42 government and emergency management (EM) organizations across three phases of this natural disaster.

Seeger, Matthew W.; Pechta, Laura E.; Price, Simani M.; Lubell, Keri M.; Rose, Dale A.; Sapru, Saloni; Chansky, Melanie C.; The title of the article by Smith, Belinda (2018) is “A Conceptual Model For Evaluating Emergency Risk Communication In Public Health”. It was published as a research article in English in the international peer-reviewed journal Health Security. It consists of 11 pages. This article applies a conceptual model of public health developed in collaboration with the Centers for Disease Control and Prevention (CDC). The model was created through a comprehensive review of the emergency risk communication literature, interviews with researchers, and discussions with CDC stakeholders. This model is adaptable to a wide range of emergency events and includes basic structures for evaluating internal processes as well as the outcomes of emergency risk communication to the target audience. This can help public health communicators learn how their various activities contribute to emergency risk communication outcomes.

Hu, Xi; Zhang, Xiujuan; Wei, Jiuchang (2019) article title is “Public Attention to Natural Hazard Warnings on Social Media in China”. It was published in the International Refereed Journal of Weather Climate and Society. It is a research article and is in English. It consists of 15 pages. This study investigates factors affecting public attention to natural disaster warning information on social media.

Wehde, Wesley; Pudlo, Jason M.; Robinson, Scott E. The title of his article is “Is There Anybody Out There?”: Communication of Natural Hazard Warnings at Home and Away.” Published in Social Science Quarterly International Refereed Journal. It is a research article. It is in English and

has 18 pages. This article examines various determinants of communication behaviors related to natural disasters and how these determinants vary for those at home or away from home.

Amiresmaili, Mohammadreza; Zolala, Farzaneh; Nekoei-Moghadam, Mahmood; Salavatian, Siavash; Chashmyazdan, Mohammadreza; Soltani, Ahmad; The title of the article by Savabi, Jaber (2021) is “Role of Social Media in Earthquake: A Systematic Review”. It was published in the Iranian Red Crescent Medical Journal International Refereed Journal. This compilation article is in English and consists of 8 pages. Social networks can play a special role in people's communication in situations of crisis and disaster. These media are interactive, digital or mobile-based tools. In this regard, this study aimed to evaluate the role of social media in earthquakes.

Goswick, Jeri; Macgregor, Cynthia J.; Hurst, Beth; Wall, Patricia J.; The title of the article by White, Renee (2018) is “Lessons identified by the Joplin School Leadership after responding to a Catastrophic Tornado.” It was published in the Journal of Contingencies and Crisis Management International Refereed Journal. This research article is in English and has 10 pages. This case study describes the lessons learned by the school board following a devastating tornado that destroyed a third of the city, injured more than 1,000 people, and claimed the lives of 158 people, including members of the school community. Data was collected through one-on-one interviews with 10 district administrators and educators directly affected by the crisis. The findings presented identify a number of lessons learned that may guide other educational institutions to be better prepared for a crisis situation, including the importance of seeking expert help, creating a plan for obtaining resources, and establishing communication practices that support communities in crisis situations.

The title of Kitagawa, Kaori (2018) article is “Living with an Active Volcano: Informal and Community Learning for Preparedness in South of Japan. Observing The Volcano World: Volcano”. It was published in the International Refereed Journal of Crisis Communication in English and 13 pages. The study suggests that two specific local beliefs – 'distrusting authorities' and 'effectively intimidating' – are principles underlying volcanic preparedness in the region. The study also suggests that the concept of 'kyojo (collaborative partnerships)' plays a central role in the planning and implementation of preparatory programs such as the Sakurajima Taisho Explosion Centennial Project, which offers a wide range of informal teaching and learning opportunities.

Rodgers, Janise; Su, Guiwu; Qi, Wenhua; Milledge, David; Densmore, Alexander; Davis, Craig; England, Philip; Young, John; Cao, Yue; Chakos, Arrietta; Li, Xiaoli; Sim, Timothy; So, Emily; Parsons, Barry; Sun, Lei; Yu, Junlei; The title of the article by Guo, Chunlan (2020) is “Creating an earthquake scenario in China: A case study in Weinan City, Shaanxi province”. It was published as a research article in the International Journal of Disaster Risk Reduction International Refereed Journal. It is in English and has 14 pages. In the study, an earthquake scenario was created, and after the earthquake scenario, they used the storytelling approach to create two science-based narratives in Chinese and English. One of them is a short comic book that provides earthquake prevention and preparedness tips to the public; The other is a narrative story with technical content and recommendations for relevant local institutions. Narratives can help people visualize projected losses and impacts and offer mitigation and preparedness recommendations that, if implemented, will help reduce earthquake damages and consequences.

The title of the article by Eddie Lucero, Jessica Trounstein, Jennifer M. Connolly & Casey Klofstad (2022) is “A matter of life or death: How racial representation shapes compliance with city disaster preparedness orders”. It is a research article in the Journal of Urban Affairs International Refereed Journal. It was published in English and 19 pages. The article has shown that as political institutions and the public cooperate, inform and raise awareness during a disaster, trust increases & they accelerate evacuation by promoting good will and more.

The title of Wei Zhai (2022) article is “A Multi-Level Analytic Framework For Disaster Situational Awareness Using Twitter Data”. It was published as a research article in the International Refereed Journal of Computational Urban Science and in English. The article, consisting of 15 pages, covers the situational awareness of the public during a natural disaster, their perceptions, infrastructure activities, volunteering, support, necessary information, etc. developed a Twitter-based analytical framework to measure human-level situational awareness.

Somya D. Mohanty a , Brown Biggers a , Saed Sayedahmed a , Nastaran Pourebrahim b , Evan B. Goldstein b , Rick Bunch b , Guangqing Chi c , Fereidoon Sadri a , Tom P. McCoy d , Arthur Cosby (2021) The title of the article is “ A Multi-Modal Approach Towards Mining Social Media Data During Natural Disasters - A Case Study Of Hurricane Irma”. It was published as a research article in the International Journal of Disaster Risk Reduction. The research is in English and consists of 15 pages. In America, a model is being developed for the different stages of disaster (preparation, response and recovery), the infrastructure offers detailed research.

Tisha Joseph Holmes a, Patrice C. Williams b, Sandy Wong c , Kathryn Smith d , John T. Bandzuh c , Christopher K. Uejio (2022) article is titled “Assessment of an Evacuation Shelter Program for People with Access and Functional Needs in Monroe County, Florida During Hurricane Irma” was published as a research article in the International Refereed Journal of Social Science & Medicine. It is in English and consists of 9 pages. The article provides communication, evacuation and transportation, shelter, and interagency coordination in disasters in the United States, and provides management lessons to inform adaptation, preparedness, and response plans to produce public health and emergency services.

The title of the article by Seungyoon Lee Bailey C. Benedict Yue ‘Gurt’ Ge Pamela Murray-Tuite Satish V. Ukkusuri (2020) is “An Application of Media and Network Multiplexity Theory to The Structure and Perceptions of information Environments in Hurricane Evacuation”. Research Article This article published in the International Refereed Journal is a research article and is published in English as 16 pages. This article provides practical inferences regarding the problems and lack of information in evacuation decisions during disasters, contributing to uncertainties, accessing correct information in disaster situations, difficulties in decision-making, ensuring coordination and needs.

The title of the article by Chung-Shing Chan, Kazuo Nozu & Qinrou Zhou (2022) is “Building Destination Resilience In The Tourism Disaster Management Process From The Past Experiences: The case of the 2018 Hokkaido Eastern Iburi earthquake in Japan”. This article, which is a research article in the International Refereed Journal of Tourism Recreation Research, was written in English. It is 18 pages. The study reveals that post-disaster recovery can lead to new product

creation, image improvement, local knowledge enrichment, people-to-people and people-to-place connections that facilitate sustainable tourism development with long-term vision and strategy.

Shefali Juneja Lakhina, Elaina J. Sutley Jay Wilson (2021) article is titled “How Do We Actually Do Convergence” for Disaster Resilience? Cases from Australia and the United States”. It was published as a research article in the International Refereed Journal of Int J Disaster Risk Science. It is in English and consists of 13 pages. The aim of the research is to produce a web-based application to model and evaluate resilience at the community level (community, environmental and urban resilience).

The title of the article by Meredith Jacobson, Hollie Smith, Heidi R. Huber-Stearns, Emily Jane Davis, Antony S. Cheng & Alison Deak (2021) is “Comparing Social Constructions Of Wildfire Risk Across Media, Government, And Participatory Discourse in A Colorado Fireshed” Journal The research article was published in the International Refereed Journal of Risk Research and in English. It consists of 19 pages. It describes the risk and difficulties of uncontrollable fire. Community Bushfire Protection Plan prepared (for support and coordination)

The title of the article by Jennifer M. First, Mansoo Yu, J. Brian Houston (2021) is “Coordinating non-established disaster relief groups: a case study of Hurricane Irma in Florida, United States”. The research article was published in Disasters Journal International Refereed Journal in English and 29 pages. The Disaster Adaptation and Resilience Scale was developed to ensure the development and validation of a protection measure at the individual level.

Kati Orru, Sten Hansson, Friedrich Gabel, Piia Tammpuu, Marco Krüger, Lucia Savadori, Sunniva Frislid Meyer, Sten Torpan, Pirjo Jukarainen, Abriel Schieffellers, Gabriella Lovasz, and Mark Rhinard (2021) article is titled “Approaches to 'Vulnerability' in Eight European Disaster Management Systems”. It is a research article in Disasters Journal International Refereed Journal. It was published in English and 26 pages. A heuristic model is put forward to facilitate different understandings of vulnerability along the dimensions of reducing social vulnerability to disasters, private relationships, and social support through state actors.

The title of the article by Tomoki Tanaka, Yuki Matsuda, Manato Fujimoto, Hirohiko Suwa, and Keiichi Yasumoto (2021) is “Evacuation Shelter Decision Method Considering Non-Cooperative Evacuee Behavior to Support the Disaster Weak.” It is a research article in the International Refereed Journal of Sustainability. It is in English and has 21 pages. This study proposes an evacuation decision method to draw attention to the scarcity of shelters, limited capacity, and difficulties experienced during evacuation in disaster situations.

The title of the article Celine Rendon, Khalid K. Osman, Kasey M. Faust (2021) is “Path towards community resilience: Examining stakeholders’ coordination at the intersection of the built, natural, and social systems”. A research article was published in the International Refereed Journal of Sustainable Cities and Society in English. It consists of 12 pages.

The title of the article by Mohammadsepehr Karmiziarani, Keighobad Jafarzadegan, Peyman Abbaszadeh , Wanyun Shao , Hamid Moradkhani (2021) is “Hazard risk awareness and disaster management: Extracting the information content of twitter data”. Research article published in the International Refereed Journal of Sustainable Cities and Society in English. It consists of 11 pages.

It provides valuable information and recommendations to county-level disaster managers and response teams before, during and after Harvey that can significantly assist in minimizing the consequences of the event and increasing residents' preparedness.

The title of the article by Tara Powell, Jennifer Scott, Paula Yuma (2022) is “Surviving the Storm: A Pragmatic Non-Randomised Examination of A Brief intervention for Disaster-Affected Health and Social Care Providers”. It was published as a research article in the International Refereed Journal of Health Social Care Community in English. It consists of 11 pages. This study examines the psychological distress of health and social care providers using a brief group intervention, Resilience and Coping for a Health Community, and then assesses pre-disaster and post-traumatic stress, anxiety, burnout and secondary traumatic stress, and prepares an action, well-being plan to reduce this distress is acquiring.

The title of the article by Amer Hamad Issa Abukhalaf, Jason von Meding a, Jake R. Dooling b , Deyaaldeen M. Abusal (2022) is “Assessing International Students’ Vulnerability to Hurricanes: University of Florida Case Study International.” The research article was published in the Journal of Disaster Risk Reduction International Refereed Journal and was published as 14 pages in English. The purpose of the study is to develop new knowledge about the behavior of international students at U.S. institutions of higher education in relation to hurricanes with the goal of improving overall campus crisis management.

The title of the article by Louis Ngamassi, Hesam Shahriari, Thiagarajan Ramakrishnan, Shahedur Rahman (2022) is “Text mining hurricane harvey tweet data: Lessons learned and policy recommendations”. It was published as a research article in the International Journal of Disaster Risk Reduction. It is in English and has 11 pages. People's needs and expectations during disasters can help reduce the gap between basic needs and expectations for emergency response, based on themes such as concern about a particular city, event or travel cancellations, the threat to the oil and gas (energy) industry, and the threat of climate change. The disaster plan for disaster management institutions, disaster victims and citizens offers recommendations to help them be better prepared.

The title of the article by Rejina Manandhar a, Laura K. Siebeneck (2020) is “Information Management and The Return-Entry Process: Examining Information Needs, Sources, and Strategies After Superstorm Sandy.” It is a research article in the International Journal of Disaster Risk Reduction. It is in English and has 11 pages. It provides information management activities for both organizations and the public, seeking information from a variety of sources to reduce disaster-related uncertainties during and after a disaster. This research focuses on information needs and source trusting behaviors.

Hamilton Bean, Ana Maria Cruz, Mika Shimizu Keri K. Stephens Matthew McGlone, Sharon Strover (2021) article is titled “Mobile Alert and Warning in the United States and Japan: Confronting the Challenges of International Harmonization.” It was published as a research article in the International Refereed Journal of Int J Disaster Risk Sci. It is in English and 7 pages. In the USA, a project plan agenda for the Disaster Risk Reduction Framework 2015-2030 has been prepared.



The title of Kamol Chandra Roy, Samiul Hasan (2021) article is “Modeling The Dynamics of Hurricane Evacuation Decisions From Twitter Data: An input Output Hidden Markov Modeling Approach” is a research article in the International Refereed Journal of Transportation Research Part C. It is in English and 16 pages. This study presents a method for inferring individual evacuation behaviors (evacuation decision, timing, destination, etc.) from social media.

Dimitrios Tzioutzios, Jeong-Nam Kim, Ana Maria Cruz (2022) article is titled “Appetite for Natech Risk Information in Japan: Understanding Citizens’ Communicative Behavior Towards Risk Information Disclosure Around Osaka Bay”. He published a research article in English and 19 pages in the International Refereed Journal of Int J Disaster Risk Sci. This study aims to assess whether residents around Osaka Bay, Japan, have this demand for disclosure of risk information and to understand and resolve their communicative behaviors and perceived challenges through the prism of Situational Problem Theory.

The title of the Jim P Mann & Brian D Williams (2020) article is “Policing in the Eye of the Storm.” It was published in a research article in the Journal of Police and Criminal Psychology International Refereed Journal and in English. It consists of 10 pages. After Hurricane Harvey, the issue is to prepare an emergency plan, its implementation and to reveal the difficulties encountered.

The title of the article by Hiroko Oe a, Sachiyo Kawakami (2021) is “A Disaster Prevention Program Using Virtual Schemes: Recommendation of Tradition Populaire Integrated With Tendenko as an Approach to Immersive Training”. It was included as a research article in the International Journal of Disaster Risk Reduction International Refereed Journal. It is in English and has 6 pages. The purpose of this study is to discuss and propose a community action plan to increase citizens' preparedness for natural disasters.

Oliver Nahkur, Kati Orru a , Sten Hansson a , Pirjo Jukarainen , Miia Myllyl , Marco Krüger , Matthias Max , Lucia Savadori , Tor-Olav Nævestad , Sunniva Frislid Meyerf , Abriel Schieffellers , Alexandra Olson, Gabriella Lovasz , Mark Rhinard (2022) article Its title is “The engagement of informal volunteers in disaster management in Europe”. It is a research article in the International Journal of Disaster Risk Reduction. It is published in English and has 14 pages. This study attempts to fill this gap by focusing on policies and institutional arrangements for the integration of informal volunteers (Finland, Germany, etc.) and the methods and tools used for the integration of volunteers.

The title of the article Patricia A. Champ a, Hannah Brenkert-Smithb, Jonathan P. Riley c , James R. Meldrum d, Christopher M. Barthe , Colleen Donovan f, Carolyn J. Wagner (2022) “Actionable Social Science Can Guide Community Level Wildfire Solutions. An Illustration From North Central Washington, USA. It was published as a research article in the International Journal of Disaster Risk Reduction. It is in English and has 11 pages. The focus is on developing wildfire preparedness and mitigation programs that address risk reduction behaviors, fire mitigation, and communication preferences among communities.

The title of the article by Naim Kapucu, Qian Hu, Mitchel Harmon, and Parker Toro (2021) is “Coordinating Non-Established Disaster Relief Groups: A Case Study Of Hurricane Irma in Florida United States”. Disasters Journal is a research article in an International Refereed Journal. It was published in English and 21 pages. Examines the role of non-resident relief groups (NERGs)



and their involvement in the response to Hurricane Irma. The main aim of the study is to discover more about the involvement of NERGs. It is aimed to ensure disaster response, motivation and coordination with other emergency management institutions.

The title of the article by Timothy Schemppa\*, Haoran Zhangb, Alexander Schmidtc, Minsung Hongd, Rajendra Akerkard (2019) is “A framework to integrate social media and authoritative data for disaster relief detection and distribution optimization”. It was published as a research article in the International Journal of Disaster Risk Reduction. It is in English and has 10 pages. The output/result of the research manages to iteratively optimize disaster aid distribution with social media data using the temporal dimension with the established system.

The title of the article by Maziar Yazdani a, Mohammad Mojtahedi a, Martin Loosemore b, David Sanderson (2022) is “A Modeling Framework to Design an Evacuation Support System for Healthcare infrastructures in Response to Major Flood Events”. It is a research article in the International Refereed Journal of Progress in Disaster Science. It is in English and has 12 pages. As outputs, it addresses the limitations of current hospital evacuation models and suggests factors that should be included in future models to increase community resilience to increased flood risks due to climate change.

The title of the article Juan C. Marcillo-Delgado a,\*, A. Alvarez-Garcia b, Agueda García-Carrillo (2021) is “Analysis of Risk and Disaster Reduction Strategies in South American Countries”. It was published as a research article in the International Journal of Disaster Risk Reduction. It is in English and has 17 pages. As the output of the research, 87 disaster research report strategies were found in the selected countries. The categories of these strategies are divided into four separate management plans. Studies such as social engagement planning and stakeholder management, response to disaster feedback and post-disaster continuity management planning, and planning for effective sector continuity have been carried out.

The title of the article by Amer Hamad Issa Abukhalaf a, Jason von Meding a, Jake R. Dooling b, Deyaaldeen M. Abusal c (2022) is “Assessing International Students’ Vulnerability to Hurricanes: University of Florida Case Study”. Research article published in the International Journal of Disaster Risk Reduction International Refereed Journal and in English. It consists of 14 pages. Research shows that higher education students have limited knowledge about hurricane preparedness. The research aimed to develop new knowledge for international students when they encounter hurricanes or similar weather conditions.

The title of the article by David N. Nguyena\*, Fumihiko Imamurab, Kanako Iuchic (2018) is “Barriers towards hotel disaster preparedness: Case studies of post 2011 Tsunami, Japan”. It is a research article in the International Journal of Disaster Risk Reduction. It is in English and has 9 pages. As a result of the research, the 2019 Rugby World Cup and the 2020 Tokyo Olympic Cup were perceived as a danger and an uncertain risk. For the Japanese municipal government of the region, transportation and tourism were a priority emergency for the local government in terms of their DMO management. Although DMOs and CVBs were common to the Americas and Europe, few were common to Japan. The predicted increase in tourism when new DMOs emerge. This study shows how DMOs can be used more effectively in the light of disaster risk management.

Juan C. Marcillo-Delgado a,\*, A. Alvarez-Garcia b, Agueda García-Carrillo (2022) “Communication strategies on risk and disaster management in South American countries” International Journal of Disaster Risk Reduction, International Refereed Journal Research, English, 12 pages.

The title of the article by Sten Hansson a,\*Kati Orru a, Andra Siibak a, Asta Bäck b, Marco Krüger c, Friedrich Gabel c, Claudia Morsut d (2020) is “Communication-related vulnerability to disasters: A heuristic framework”. It is a research article in the International Journal of Disaster Risk Reduction and is written in English. It consists of 9 pages. The outcome of the research is to improve our understanding of how communication ecosystems – new media environments can transform the various forms and effects of false or harmful information characteristic of the modern era, the ways in which people learn about dangers and cope with disasters, and the design of the research is presented as a heuristic framework.

The title of Sifan Xu (2018) article is “Crisis communication within a community: Bonding, coping, and making sense together”. It is a research article in the Public Relations Review International Refereed Journal. It is in English and consists of 13 pages. The method of coping with crises constitutes the output of the research.

The title of the article by John L. Renne (2018) is “Emergency evacuation planning policy for carless and vulnerable populations in the United States and United Kingdom”. It is a research article in the International Journal of Disaster Risk Reduction International Refereed Journal and written in English. It consists of 7 pages. The outcomes are local, regional and national resilience forums to coordinate emergency planning activities. In the design of the research, comparative analysis was made using the focus group interview technique.

The title of the article by Milan Stute a, Max Maass a, Tom Schons a, Marc-Andre Kaufhold b, Christian Reuter b, Matthias Hollick (2020) is “Empirical insights for designing Information and Communication Technology for International Disaster Response”. International Journal of Disaster Risk Reduction is a research article in an International Refereed Journal. It is in English and consists of 10 pages. Output of the research ADI Operating principles 1 Uniqueness of the disaster 2 Interfaces 3 Self-sufficiency 5 Local resources 9 Centralization 11 Regular synchronization 12 Well-known processes System requirements 4 Flexibility 6 Simplicity 8 Scalability 10 Timeliness, integrity and accuracy Support systems 7 Robust communication 13 Automated reporting 14 Cyber -are physical systems.

The title of the article by Juhani Latvakoski b, Risto O’orni a, Toni Lusikka b, Jaana Keränen (2022) is “Evaluation of Emerging Technological Opportunities for improving Risk Awareness and Resilience of Vulnerable People in Disasters”. International Journal of Disaster Risk Reduction is a research article in an International Peer-Reviewed Journal. It is in English and has 22 pages. It had neutral or positive opinions about the perceived effectiveness and efficiency of the tools evaluated. This also applies to other statements regarding users' acceptance, such as perceived ease of use and willingness to use tools again, as well as perceived suitability for civil protection, crisis management and disaster risk management.

The title of the article by Hitomi Nakanishia, John Blackb (2018) is “Implicit And Explicit Knowledge In Flood Evacuations With A Case Study Of Takamatsu, Japan”. International Journal

of Disaster Risk Reduction is a research article in an International Refereed Journal. It is in English and has 9 pages. Outcomes of the research: Japan is a relatively monocultural society; Here there is not much variation in knowledge/residents' experiences of disasters. But Australia's multicultural society will need increased understanding of differences in experience. A social survey research is useful in synthesizing tacit knowledge for cross-cultural practice.

The title of the article by Mohammed Ali Berawi a,c,\*, Pekka Levi`akangas b, Sutan Akbar Onggar Siahaan c, Alya Hafidza c, Mustika Sari c, Perdana Miraj c, Ruki Harwahyu d, Gunawan Saroji (2021) is "Increasing disaster victim survival rate : SaveMyLife Mobile Application development". It is a research article in the International Journal of Disaster Risk Reduction. It is in English and consists of 14 pages. The outcome of the research aims to improve the response time of the search and rescue team and increase the victim survival rate by taking into account victim prioritization and technology use through mobile disaster applications.

Brooke Fisher Liua,\*, Brooke M. Fowlera, Holly A. Robertsb, Emina Herovicc (2018) "Keeping hospitals operating during disasters through crisis communication preparedness" which is written in Public Relations Review and International Refereed Journal Research written in English. There are 12 pages in journal.

The title of the article by Vanessa A. Coopera,\*, Giuseppe Forinob, Sittimont Kanjanabootrab, Jason von Medingc (2020) is "Leveraging The Community Of Inquiry Framework To Support Web-Based Simulations in Disaster Studies". It is a research article in The Internet and Higher Education International Refereed Journal. It is in English and consists of 15 pages. Web-based simulations (WBS) as output are increasingly used by educators to deliver higher education curricula. As the outcome of the research, the paper reports qualitative semi-structured interviews.

Maila D.H. The title of the article Rahiem a,\*, Robin Ersing b, Steven Eric Krauss c, Husni Rahim (2021) is "Narrative inquiry in Disaster Research: An Examination of The Use of Personal Stories From the Child Survivors of the 2004 Aceh Tsunami". It is included as a research article in the International Journal of Disaster Risk Reduction. It is in English and has 10 pages. Research outcome 1) narratives describe complex situations and explain why. 2) narratives are one of the best possible methods for studying children's development. 3) narratives provide interdisciplinary connections that enrich the analysis. The design of the research; qualitative research and narrative inquiry.

Scott Curtis a, Anuradha Mukherji b, Jamie Kruse b, Jennifer Helgeson c, Ausmita Ghosh b, Nelson Adeniji b (2022) article is titled "Perceptions of Risk to Compound Coastal Water Events: A Case Study in Eastern North Carolina, USA". It is a research article in the International Refereed Journal of Progress in Disaster Science. It is in English and has 8 pages. Case study participants felt they understood the CCWE better than their state and federal counterparts and wanted to be more involved in response and recovery decision-making. The outcome of the research is CCWE risk and perceived barriers to communicating this risk to their constituencies.

The title of the article by Katsushige Kitazawa a,b,\*, Scott A. Hale a,c (2021) is "Social Media And Early Warning Systems For Natural Disasters: A case study of Typhoon Eta in Japan". It is a research article in the International Journal of Disaster Risk Reduction. It is in English and consists of 10 pages. The outcome of the study is 1) emergency warnings are likely to make people

pay more attention to warnings, but this does not mean that discussions about actions such as evacuation are increasing; 2) The expected shift in public attention (from awareness to preparedness to action) appears to be occurring on social media.

The title of the article by Leila Daddoust a, Ali Asgary a,b,\*, Kenneth J. McBey b,c, Steve Elliott d, Alain Normand e (2021) is “Spontaneous Volunteer Coordination During Disasters and Emergencies: Opportunities, Challenges, and Risks”. It is a research article in the International Journal of Disaster Risk Reduction. It is in English and consists of 11 pages. The results of the research show that SV use is common, but they perceive the SV relationship. Predominantly from a 'managerial' perspective or perspective, recruitment, utilization etc., focusing on control, predictability and potential. related liability issues and obstacles.

The title of the article by Angus Naylor a,\*, Joanna Faure Walkera, Anawat Suppasrib (2018) is “Suitability of The Early Warning Systems And Temporary Housing for the Elderly Population in The immediacy and Transitional Recovery Phase Of The 2011 Great East Japan Earthquake and Tsunami”. It is a research article in the International Journal of Disaster Risk Reduction. It is in English and consists of 8 pages. Its subject includes adults affected by Japan and the Tsunami during the emergency and temporary recovery phase of the 2011 Great East Japan Earthquake.

Oliver Nahkur a, Kati Orru a, Sten Hansson a, Pirjo Jukarainen b, Miia Myllyl a b, Marco Krüger c, Matthias Max d, Lucia Savadori e, Tor-Olav Nævestad f, Sunniva Frislid Meyer f, Abriel Schieffellers g, Alexandra Olson g, Gabriella Lovasz h, Mark Rhinard (2022) article is titled “The engagement of informal volunteers in disaster management in Europe”. It is a research article in the International Journal of Disaster Risk Reduction. It is in English and consists of 14 pages. Policies and institutional arrangements for the integration of informal volunteers, (2) methods and tools used for the integration of volunteers were used in the intervention of the research.

Eun-Seon Park, D.K. The title of Yoon (2022) article is “The value of NGOs in disaster management and governance in South Korea and Japan”. International Journal of Disaster Risk Reduction International Refereed Journal

## **CONCLUSION AND RECOMMENDATIONS**

Natural disaster, in the simplest terms, is an event caused by nature that is sometimes impossible to predict and therefore beyond human control, causing great destruction from time to time. In the modern world, as a result of the destruction caused by humans to nature, large-scale natural disasters are becoming more common as the ecosystem is disrupted and the balance of nature is disrupted. Sustainability of disaster management, which is the most interesting and discussed topic today and where sustainable solutions are tried to be found, has been examined through academic articles in the international literature. The data of the research were collected from academic articles published in international databases. In this context, the limitation of the research consists of other academic publications on sustainable disaster management - books, symposium texts, theses, etc. - and non-academic publications - newspaper and magazine articles, social media posts, etc.

The findings obtained in the research are parallel to the literature review. In this context, literature information and research findings are similar based on issues such as sustainable disaster

management studies, scope and interdisciplinary nature. The most striking data in the general evaluation of the research findings are the phone applications that are written and used in a practical way to manage the disaster or to give notifications about whether the person is safe or not. Apart from this, another noteworthy point is that in studies carried out especially in developed countries such as Japan or America, they provide information through disaster management information kit distribution and special training to foreign nationals who come to the country for various reasons such as Erasmus or the Olympics, and who do not know the language and culture of the country.

Another striking factor is that in case of a possible disaster, an agreement is made with the municipalities and the large number of foreign nationals make agreements with reliable and durable hotels where they can take shelter.

Within the scope of the research findings, when we look at the distribution of scientific articles on sustainable disaster management published in the international arena by years, 10 articles belong to 2018, 7 articles belong to 2019, 15 articles belong to 2020, 23 articles belong to 2021. It is seen that 21 articles belong to 2022. From this point of view, it can be said that sustainable disaster management has been given more importance and has been researched and studied in recent years.

In accordance with the research findings, scientific articles on sustainable disaster management published internationally in the world; They have been published in academic and international peer-reviewed journals. The maximum number is research articles, only 1 of which is a compilation and 1 is a book chapter.

When the journals in which the articles are published are examined in terms of their scope, it can be seen that they are largely international, peer-reviewed and field-indexed. When the articles included in the research are examined in terms of the number of authors, it can be seen that there are single-authored articles and co-authored articles. However, to a large extent, articles written by 3 or more people are encountered. In terms of the number of pages, the work was carried out mainly between 14 and 23 pages, with a minimum of 7-8 pages. Most of the journals are on disaster, disaster management, crisis communication, communication management. Many articles have been published, especially in the International Journal of Disaster Risk Reduction and Public Relation Review.

Another research finding is related to the topics of the articles. The topics of the article are mainly the role of public relations in sustainable disaster management, the role of tourism, the effect of crisis communication on risk reduction, the operability of pre-made and trained disaster plans and their transfer to students, and collecting information from people who have survived major disasters to prevent larger disasters. In line with all these findings and conclusions, the following recommendations are presented for future studies and research:

- Research data was obtained through international databases. Studies conducted in developed countries on sustainable disaster management have been compiled.
- The data of the research was compiled based on academic articles. Different research can be carried out by selecting academic publications such as books, symposium texts and theses, or non-



academic publications such as newspaper and magazine articles and social media posts as samples of the study.

- Intercultural comparisons can be made by investigating the concepts of sustainable disaster management and crisis communication in Turkish and different languages.

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## **Determination of Rainwater Harvesting Potential of Tokat Gaziosmanpaşa University Rectorate Building**

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### **ABSTRACT**

Increases in temperature and instability in precipitation due to Global Climate Change bring up the issue of drought and therefore water scarcity. Sustainable water management systems are needed to address the imbalance between increasing water demand and limited water resources. As a renewable resource, rainwater offers great potential for achieving this goal. Sustainable methods for water resources, such as reducing water consumption by using innovative technologies, purifying and reusing domestic wastewater, obtaining fresh water from seawater in regions where water shortage is intense, and rainwater harvesting in buildings, are becoming increasingly common all over the world. This study aims to evaluate the capacity to meet outdoor irrigation demand at the university scale with the rainwater harvesting system (RWHS), an important sustainable water management strategy. In this context, the irrigation usage capacity of the RWHS system to be applied to the Tokat Gaziosmanpaşa University Rectorate building with a roof area of 1734 m<sup>2</sup> was calculated. Annually 610 m<sup>3</sup> of rainwater can be provided from the roof of the rectorate building, 3233 m<sup>3</sup> of annual water needs can be met from this rainwater, and according to this calculation, 18.9% of the annual water need for the landscape areas around the rectorate building can be provided from rainwater and 21.423,20 TL can be saved annually.

**Keywords:** Sustainable water management, water conservation, rainwater harvesting.

### **1. INTRODUCTION**

The concept of sustainability is an approach that creates a balance between nature and humans and ensures that existing resources are transferred to future generations without being destroyed. Approaches that deal with economic values and environmental values in a balance system are known as sustainable management systems (Yılmaz FH, 2020).

Water is one of the greatest needs for the sustainability of biological life and thus for the continuity of green areas. Despite the rapidly increasing demand for water, the gradual decrease in usable water resources due to water misuse and global warming puts water at the top of the international agenda (Aküzüm et al., 2010; Kalipci et al., 2020).



In water-rich and developed countries, rainwater is used to feed groundwater in order to manage water resources sustainably, and in water-poor countries with water shortages, it saves tap water for domestic and non-domestic uses. Rainwater harvesting system (RWHS) is used to save water due to the limited amount of water resources; It is defined as the collection of water from collection areas such as roofs in urban buildings to meet demands for domestic, industrial, agricultural and environmental purposes (Aladenola and Adeboye, 2009; Worm and Hattum, 2006; Üstün et al. 2020). RWHS is considered a simple and effective approach to alleviate the worsening of urban water stress (Chiu et al., 2020). RWHS generally consists of an impermeable roof surface, a storage tank, and a conveying system between the roof and the tank (Figure 1). Recent studies indicate that a significant part of the drinking water demand in cities, between 25-60%, can be reduced by using harvested rainwater (Wallace et al., 2015; Kalipci et al., 2020).

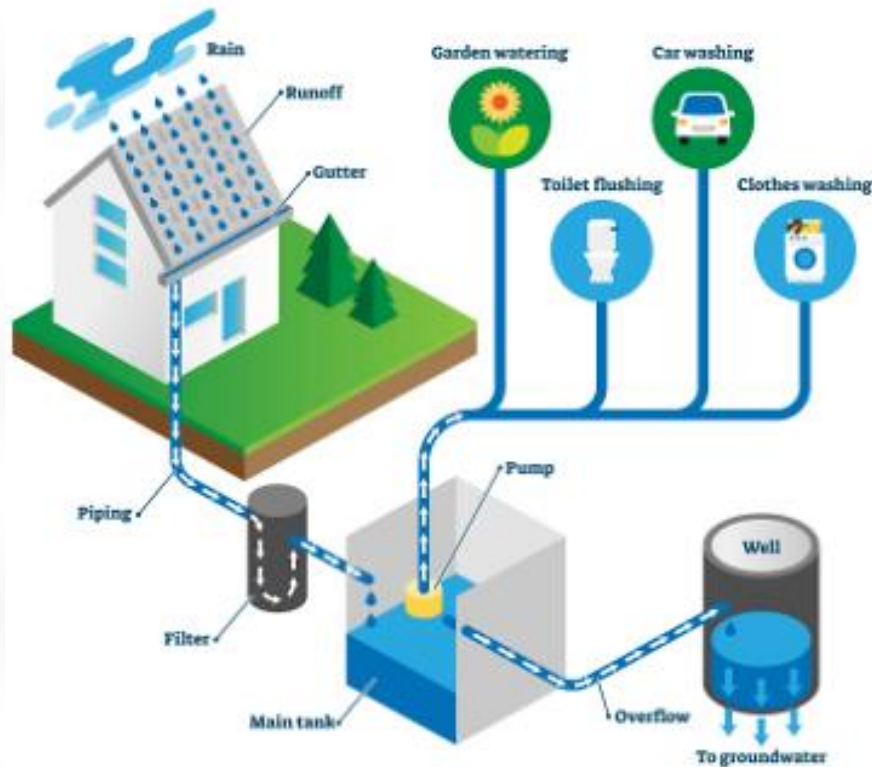


Figure 1. Rainwater Harvesting (RWHS) System (URL-1)

Within the framework of sustainable water use, it is of great importance to collect and utilize rainwater in order to ensure effective use of water in a way that is compatible with the environment and without wasting even a single drop of it. In this context, since there are areas around Tokat Gaziosmanpaşa University Rectorate Building with a roof area of 1734 m<sup>2</sup> and areas where rainwater can be used as irrigation water, the water saving potential of the rainwater harvesting system was investigated and the required amount of water was calculated and the potential of the rainwater to be collected with RWHS to meet the need was determined.

## 2. MATERIAL-METHOD

Tokat Gaziosmanpaşa University is located on the Tokat-Turhal highway, 9 km from Tokat city center. It continues its activities in Taşlıçiftlik Campus located in the distance, Ali Şevki Erek Campus in the city center and 8 districts. The Rectorate Building is located on Taşlıçiftlik campus (40°19'51"N 36°28'47"E) and there are landscape areas around it. The roof area for the Rainwater Harvesting System, which is planned to be implemented on the roof of the Rectorate of our University, is calculated as approximately 1734 m<sup>2</sup>. Figure 2 shows the visual of the roof area where rainwater harvesting will be done.



Figure 2. Rectorate building roof area

### 2.1 Rainwater efficiency calculation

The following "rainwater efficiency" formula was used to calculate the amount of rainwater to be collected from the roof surface of the Rectorate building (Sutema, 2015; Dadhich and Mathur, 2016; Tema 2017). Economic analysis of rainwater harvesting; It was determined based on the total roof area, annual average rainfall, flow coefficient and filter efficiency coefficient, which vary depending on the roof material.

$$\Sigma V_y = A_y \times Y \times K_a \times \beta$$

$V_y$ : Rainwater yield

$A_y$ : Rain catchment area represents the total roof area.

$Y$ : Annual rainfall amount is the total annual rainfall determined by the General Directorate of Meteorology (GDM).

$K_a$ : Roof flow coefficient

$\beta$  : Filter efficiency coefficient

Roof material is an important factor in determining how much rainwater will be collected on the roof. The runoff coefficient is a dimensionless factor that shows the effect of water losses and depends on the nature of the surface slope and rainfall intensity (Singh et al., 2011). Therefore, there is a need to take the runoff coefficient into account as not all the water falling on a roof area can be collected. Table 1 gives the flow coefficient of different roof materials. The roof type of this study is metal roof and the flow coefficient is 0.9.

Table 1. Flow coefficient for different roof types (Dadhich and Mathur, 2016; Taşçı,2021)

Roof type	Flow coefficient
metal roof	0,9
asbestos roof	0,8
tiled roof	0,75
concrete roof	0,70

The filter efficiency coefficient is the coefficient (0.9) specified by German standards in DIN1989. It is the efficiency coefficient of the first filter passed to separate the rainwater obtained from the roof from visible solids. It is a coefficient given by calculating that some amount of water cannot pass through here.

The roof surface area of the Rectorate is approximately 1734 m<sup>2</sup>. Rainfall amounts vary across provinces. The average amount of precipitation per square meter in Tokat province between 1929 and 2022 was measured as 434.3 mm (434.3 L/m<sup>2</sup>) (Table 2).

Table 2. General Directorate of Meteorology, general statistical data of Tokat Province (GDM, 2023)

Tokat	January	February	March	April	May	June	July	August	September	October	November	December	Annual
<b>Measurement Period (1929-1922)</b>													
Average Number of Rainy Days	10.82	10.06	11.82	11.90	13.11	8.73	2.73	2.35	4.72	7.63	8.83	10.94	103.6
Average Total Monthly Precipitation (mm)	43.2	34.1	43.1	52.6	58.7	41.0	11.5	8.7	19.5	35.9	41.5	44.5	<b>434.3</b>



### 3. RESULTS AND DISCUSSION

#### 3.1 Rainwater efficiency

For the rectorate building with a roof area of 1734 m<sup>2</sup>; The average annual rainfall for Tokat, given in Table 2, is calculated as 434.3 mm = 434.3 L/m<sup>2</sup>. According to this;

$$V_y = 1734\text{m}^2 \times 0.9 \times 0.9 \times 434.3 \text{ L/m}^2 = 609991 \text{ L} = 610 \text{ m}^3 / \text{year}$$

#### 3.2 Total annual water need

To maximize the potential benefits of a rainwater harvesting system and derive maximum benefit from it, it is important to have large areas on rooftops to serve as gathering areas. For this reason, it is a very common practice to simply collect rainwater and use it for garden irrigation. There is an irrigable area of 15404 m<sup>2</sup> behind the Rectorate building (Figure 3).

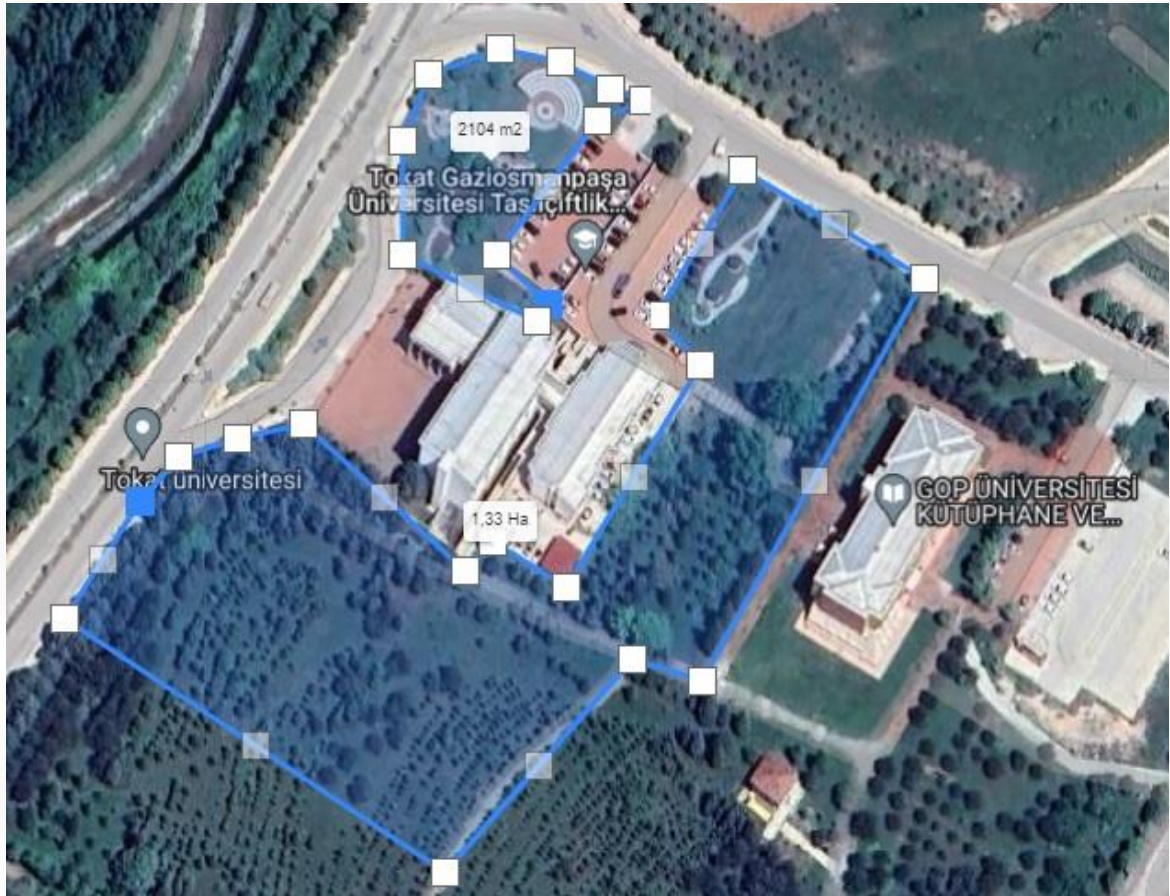


Figure 3. Irrigable area where rainwater can be used

In calculating the water need of green areas, the amount of water for each irrigation was accepted as 4 L / m<sup>2</sup>.

For irrigation of an area of 15404 m<sup>2</sup>;

$$15404 \text{ m}^2 \times 4 \text{ L /m}^2 = 61616 \text{ L} = 62 \text{ m}^3 / \text{ day}$$

If watering is done once a week;  $62 \text{ m}^3 / \text{ day} \times 365/(7) \text{ days} = 3233 \text{ m}^3 / \text{ year}$

Total Annual Water Need =  $3233 \text{ m}^3 / \text{ year}$ .

### **3.3 Tank volume calculation**

The storage volume calculation of the system was made considering May, which is the month with maximum precipitation (Table 2).

May: 58.7 mm

Tank volume = rainfall amount x roof square meters x 0.9 x 0.9

$$\text{Tank volume} = 58.7 \text{ L/m}^2 \times 1734 \text{ m}^2 \times 0.9 \times 0.9 = 82446.5 \text{ L}$$

= 83 m<sup>3</sup> tank volume is required.

### **3.4 Financial amount saved annually**

610 m<sup>3</sup> of water consumption of the region, which is planned to have a total annual water consumption of 3233 m<sup>3</sup> (including arable land), can be met from rainwater. The m<sup>3</sup> cost of water billed by Tokat Municipality in May for public and special areas is 35.12 TL/m<sup>3</sup> (URL-2).

$$\text{Annual saved amount} = 610 \text{ m}^3 \times 35.12 \text{ TL/m}^3 = 21.423,20 \text{ TL}$$

18.9% of the total consumption can be provided from rainwater and 21,423.20 TL can be saved annually.

## **4. CONCLUSION**

When the roof area of Tokat Gaziosmanpaşa University Rectorate building, the flow coefficients of roof types and the amount of collectable water determined by the average rainfall amount were examined, the total water need was found to be 3233 m<sup>3</sup> / year. When the rainwater collection potential of the building was calculated, it was determined that 610 m<sup>3</sup> / year of this water could be met from rainwater and this amount would meet 18.9% of the total consumption. Accordingly, if RWHS is established in the rectorate building of the university, an annual savings of 21,423.20 TL will be achieved. At the same time, it will contribute to the United Nations Sustainable Development Goals by ensuring sustainable use of water. Considering Turkey's usable water potential, there is a need to create alternative water resources. In this context, it is recommended to popularize RWHS and encourage water harvesting in all commercial and official buildings, especially buildings with large roofs, through government institutions, tax deductions and financial state incentives, and to allocate resources for this system within the state's environmental management plans.

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# **POSTER PRESENTATIONS**

## **FULL-TEXT**

## **Synthesis and Characterization of Novel 3,4-Dihydro-1,4-Benzoxazin-2-One: HSA Binding Investigation**

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### **ABSTRACT**

Heterocycles such as benzoxazines are structural motifs of a wide spectrum of biologically active compounds. In order to investigate new potential therapeutically active agents, we synthesized 3,4-dihydro-1,4-benzoxazin-2-one by heterocyclization of enol-esters with o-aminophenol in toluene with reflux. Considering the simple work-up, broad substrate scope, requires no chromatographic purification, with good yields in relatively short-reaction times used synthetic procedure can be very suitable for one-pot conversion of similar substrates into the corresponding products. Obtained compound is characterized using IR and NMR spectroscopy. Bearing in mind that the efficiency of drugs strongly depends on their ability to bind to protein, we decided to investigate the affinity of selected compound to bind to human serum albumin (HSA). The binding parameters values for selected compound HSA complex showed that compound bound reversibly to protein which means that can be stored and carried by HSA.

**Keywords:** 3,4-dihydro-1,4-benzoxazin-2-one, heterocyclization, HSA binding, site markers.

**Acknowledgment:** The authors are grateful to the Ministry of Education, Science and Technological Development of the Republic of Serbia for financial support (Agreement No. 451-03-68/2022-14/200122).

### **INTRODUCTION**

Cancer is one of today's most pressing health issues, as well as one of the key goals in medicinal chemistry. Metal complexes have emerged as a result of cisplatin's use as a chemotherapeutic drug that drew the interest of a huge number of scientists. [1,2] Cisplatin's use in clinical therapy is limited by various side effects, including neurotoxicity, nephrotoxicity, [3-5] limited application, and others. The primary focus of medicinal chemistry is the development and production of novel nonplatinum drugs with fewer side effects. Besides this, there has been a lot of interest in quinoxaline and benzoxazine-based compounds as an important class of nitrogen containing heterocyclic compounds. [6,7] These types of compounds displays a broad spectrum of

pharmacological activity such as antimicrobial [8], anti-inflammatory [9], antidiabetic [10], antiviral [11] and anticancer [12]. Bearing in mind these facts we synthesized novel 3,4-dihydro-1,4-benzoxazin-2-one (**3**) by heterocyclization of enol-ester (**1**) with *o*-aminophenol (**2**) in toluene with reflux (**Figure 1**). This newly synthesized compound was evaluated for HSA (human serum albumin) binding and also competitive experiments with site markers were performed to determine the position of binding of compound to HSA (site I and/or II). Warfarin is a marker for site I (subdomain IIA), whereas ibuprofen is a marker for site II (subdomain IIIA). [13,14]

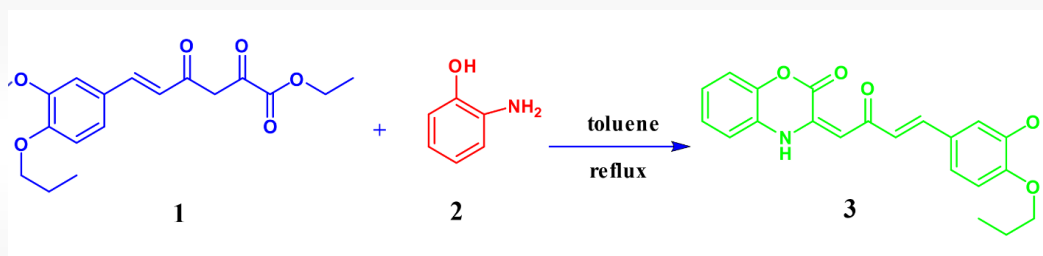


Figure 1. Synthesis of 3,4-dihydro-1,4-benzoxazin-2-one

## MATERIALS AND METHODS

### Synthesis and characterization

Appropriate dehydrozingerone ester (**1**) (1 mmol) was dissolved in toluene (15 ml), then *o*-aminophenol (**2**) (1.2 mmol) was added. After 5-6 hours of reflux, the precipitate of 3,4-dihydro-1,4-benzoxazin-2-ones appeared. The obtained compound was filtered, washed with toluene and dried at room temperature. The obtained yield was 68%.

The NMR spectra of newly synthesized compound were performed in DMSO-*d*<sub>6</sub> with TMS as the internal standard on a Varian Gemini 200 MHz NMR spectrometer. The IR spectra was recorded by using a Perkin-Elmer Spectrum One FT-IR spectrometer on a KBr pellet.

Spectral data of (*E*)-3-((*E*)-4-(3-methoxy-4-propoxyphenyl)-2-oxobut-3-en-1-ylidene)-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazin-2-one (**3**)

Dark orange powder; yield: 68%; IR (KBr):  $\nu$  3376, 3016, 2970, 2877, 1757, 1604, 1592, 1511, 1466, 1351, 1259 cm<sup>-1</sup>; <sup>1</sup>H NMR (200 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 11.9 (s, 1H, NH), 7.46-7.40 (m, 1H, CHAr), 7.20 (d, 1H, CH=), 7.25-7.18 (m, 1H, CHAr), 7.15-7.08 (m, 5H, CHAr), 7.00 (d, 1H, CH=), 6.34 (s, 1H, CH=), 3.90 (t, 2H, OCH<sub>2</sub>), 3.83 (s, 3H, OCH<sub>3</sub>), 1.84-1.62 (m, 2H, CH<sub>2</sub>), 0.96 (t, 3H, CH<sub>3</sub>) ppm; <sup>13</sup>C NMR (50 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  = 10.46, 22.13, 55.90, 69.82, 97.76, 114.49, 116.41, 116.75, 119.50, 123.12, 124.38, 125.33, 125.65, 139.08, 140.51, 141.16, 149.25, 150.41, 155.91, 188.13 ppm. (See SI)

### Protein binding experiments

20  $\mu$ M HSA solution was prepared in 10 mM PBS buffer (pH = 7.4) and stored in the darkroom at 5 °C for 6 h. The **3**-HSA complex was prepared by mixing an even amount of HSA and increasing amounts of compound **3**. The HSA: **3** molar ratios followed the order: 1: 0 (control), 1: 0.1, 1: 0.2, 1: 0.3, 1: 0.4, 1: 0.5, and 1: 0.6, in a total volume of 2.5 mL, pH 7.4, at 25 °C with an incubation

time of 3 h. The fluorescence emission spectra were obtained upon excitation at 290 nm and recorded immediately after the incubation. The emission intensity was recorded at the wavelength range of 300–500 nm. Competitive experiments with site markers (warfarin or ibuprofen) were performed in an identical way, while concentration of site marker was the same as concentration of HSA.

## RESULTS AND DISCUSSION

### Interactions of compound 3 with human serum albumin and markers

It is well known that the efficiency of drugs depends on their ability to bind to transport protein. Based on this fact, we evaluated the binding affinity of compound 3 to human serum albumin (HSA). To investigate the binding capabilities of HSA, a fluorescence emission titration with selected compound 3 was performed in the wavelength range of 300–500 nm. The obtained results revealed that the intensities of HSA decreased gradually as compound 3 concentrations increased (Figure 2). The fluorescence quenching data were analyzed by using Equation 1 [15]:

$$\log(I_0 - I/I) = \log K_a + n \log [Q] \quad (1)$$

where  $I_0$  and  $I$  are the emission intensities in the absence and presence of the quencher,  $K$  is the binding constant for complex 3-HSA,  $n$  is the number of binding sites to HSA molecule, and  $[Q]$  is the concentration of the quencher. The plots of  $\log [(I_0 - I)/I]$  versus  $\log [Q]$  are depicted in Figure 2. The values of  $K_a$  and  $n$  are determined from the segment and slope of the straight line and values are given in Table 1. The value of  $K_a$  implied the presence of strong interactions of tested compound with HSA, while  $n$  showed that compound 3 binds to HSA in a 1.7: 1 molar ratio.

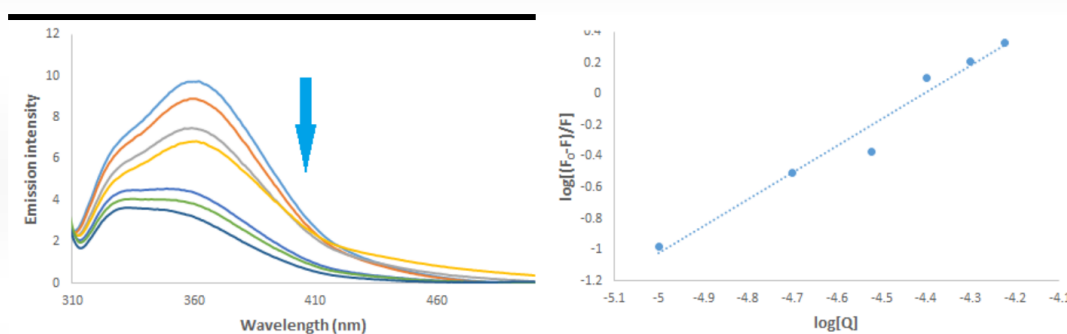


Figure 2. Left: Emissions of HSA spectrum solution in the presence of compound 3. The arrows show the change in the emission intensity after adding the increasing concentration of 3. Right:  $F_0/F$  independence of  $[Q]$  for HSA

The fluorescence of HSA is caused by tryptophan, tyrosine, and phenylalanine residues. Because phenylalanine has a very low quantum yield and tyrosine's fluorescence is completely quenched if it is ionized or near an amino group, a tryptophan residue, or a carboxyl group contributes mainly to the intrinsic fluorescence of many proteins. [16] Competitive experiments were carried out utilizing different site markers, namely warfarin and ibuprofen for sites I and II, while maintaining the concentrations of HSA and the site constant. Compound 3 was added to the warfarin-HSA or

ibuprofen-HSA systems. Fluorescence spectra were recorded with an excitation wavelength of 280 nm in the range of 300–500 nm. According to the results shown in **Figure 3**, a decrease in HSA fluorescence intensity after the addition of site markers suggests the binding of mentioned markers to HSA molecule. The fluorescence competitive studies indicate that if the compound can bind to the same site as the corresponding marker it will lead to a significant change in the value of binding constants. In this experiment, binding constants decrease in both cases, with warfarin from  $4.01 \times 10^7$  to  $4.1 \times 10^1$  and with ibuprofen from  $4.01 \times 10^7$  to  $6.06 \times 10^4$  (**Table 1**). This means that compound **3** binds to site I in HSA, but there is a possibility that some part of the molecule also binds to site II according to the value of the constant. This could be clarified by further investigations such as molecular docking.

Table 1. Binding parameters ( $K_a$  and  $n$ ) for compound **3** with HSA (without and with site markers).

Compound	$K_a$ [M <sup>-1</sup> ]	n	R
<b>3</b>	$(4.01 \pm 0.2) \times 10^7$	1.73	0.985
$K_a(\text{warfarin})$ [M <sup>-1</sup> ] $(4.1 \pm 0.2) \times 10^1$	0.36		0.937

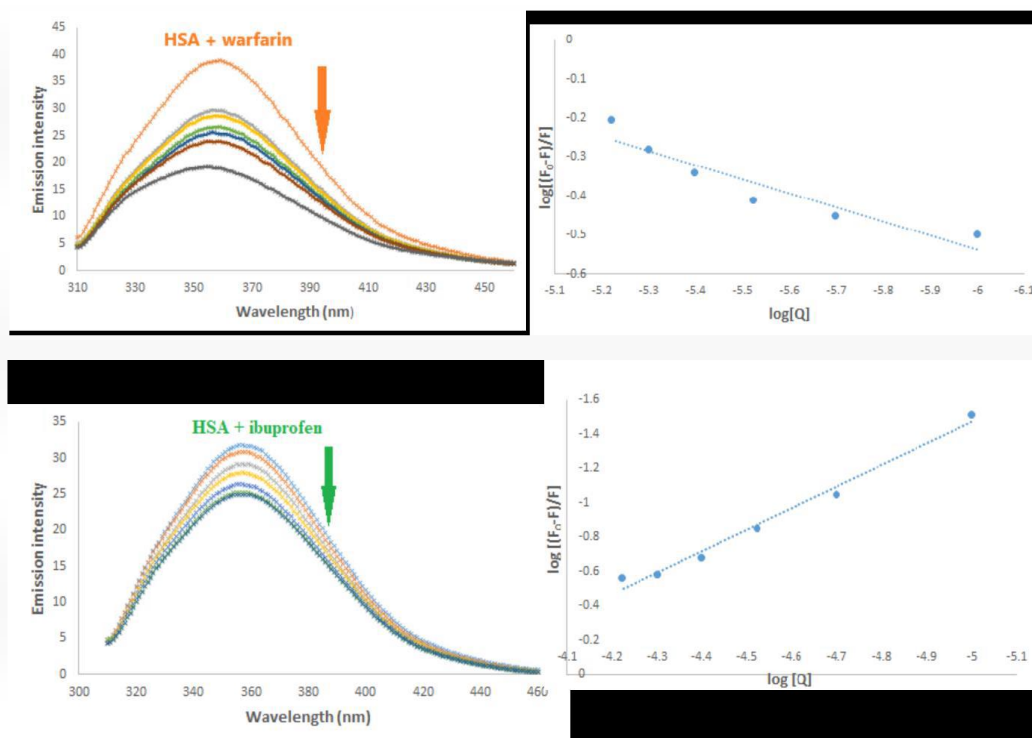


Figure 3. Left: Emission spectra of HSA in the presence of site markers warfarin (top) or ibuprofen (bottom) and the examined compound **3**. Right:  $F_0/F$  independence of  $[Q]$  for HSA for warfarin or ibuprofen.

## CONCLUSION

The newly 3,4-dihydro-1,4-benzoxazin-2-one (**3**) was synthesized by heterocyclization of ester with o-aminophenol (**2**) in toluene with reflux. This compound was characterized by IR and NMR spectroscopy. In order to investigate the possibility of the selected compound to be transported as a potential drug by HSA, certain experiments were performed. To investigate the binding capabilities of HSA, a fluorescence emission titration with selected compound **3** was performed. Obtained results indicate the presence of strong interactions of tested compound with HSA. Also, competitive experiments were carried out utilizing different site markers, namely warfarin and ibuprofen for sites I and II in order to determine the binding site. Results show that compound **3** binds to site I in HSA, but there is a possibility that some part of the molecule also binds to site II according to the value of the constant. This could be clarified by further investigations such as molecular docking.

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### Supplementary Information

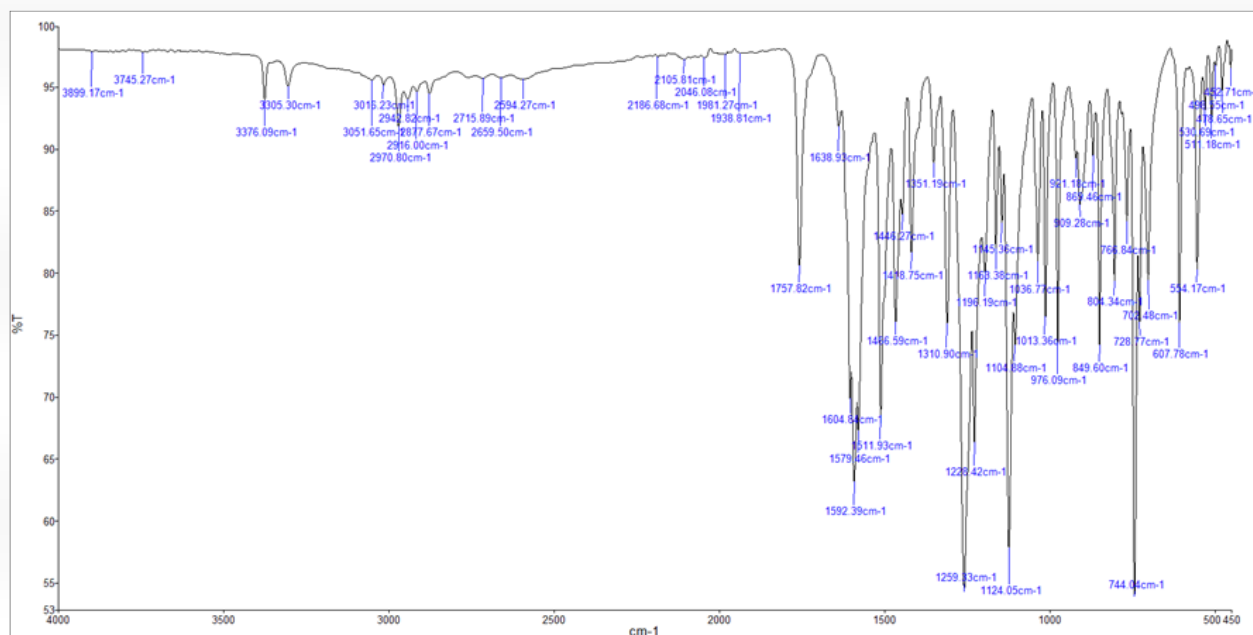


Figure SI 1. IR spectrum of compound 3

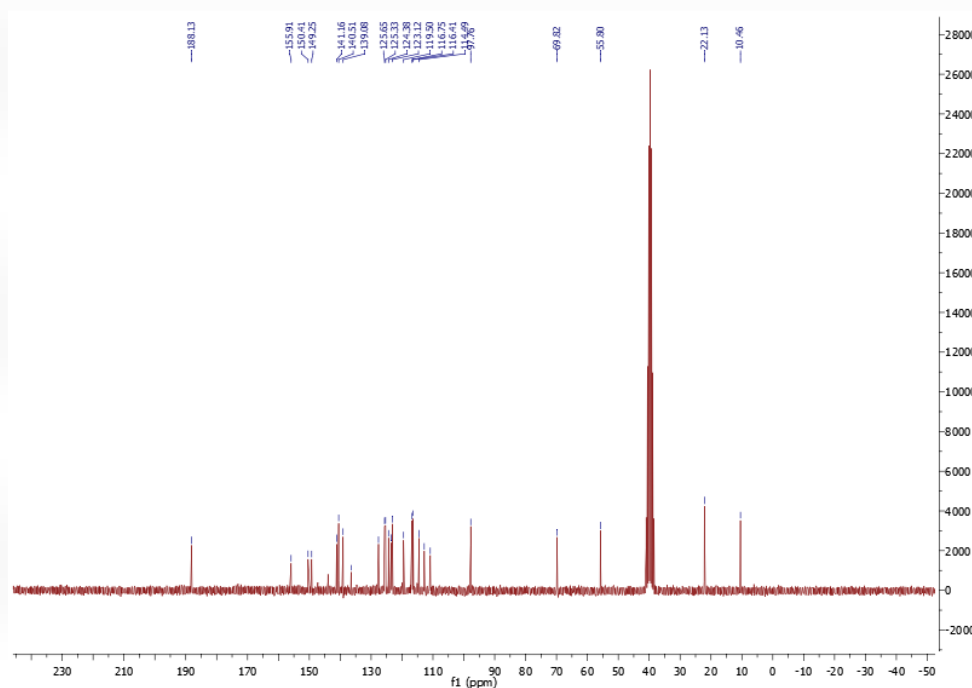


Figure SI 2. <sup>13</sup>C spectrum of compound 3

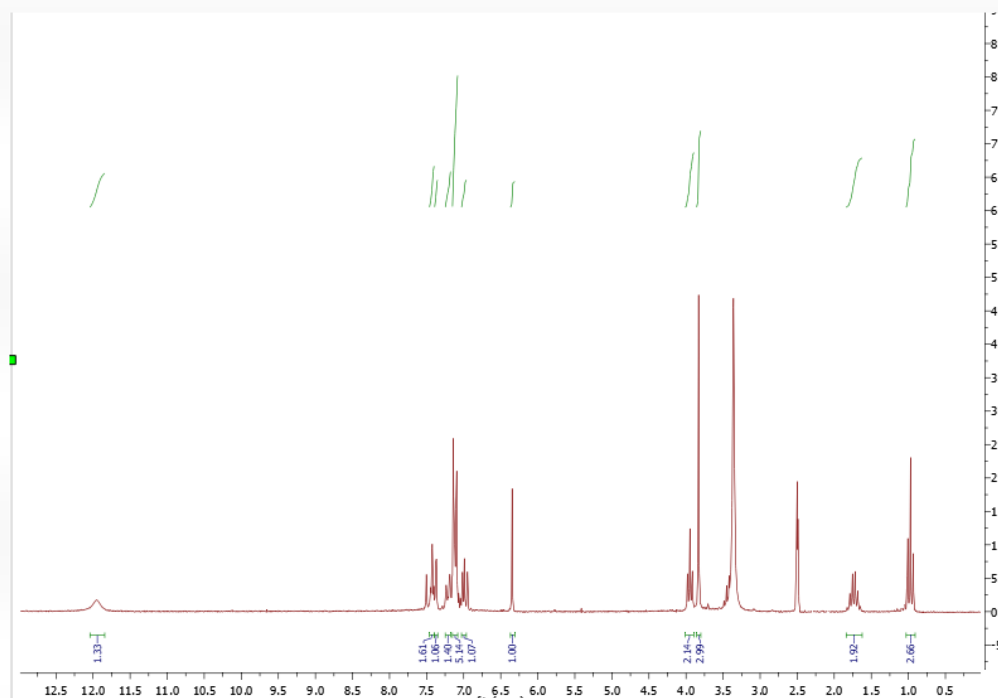


Figure SI 3. <sup>1</sup>H spectrum of compound 3

## **Environmental Assessment of the Wastewater Treatment Plants in Albania, Using the Biological Treatment as The Secondary Treatment During Operational Phase Case Study: Pogradeci WWTP**

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### **ABSTRACT**

Wastewater treatment plants (WWTP) are built in almost all the major cities of Albania, as an urgent need for public health safety and a clean environment. Nowadays, Albania has 16 WWTP in function, 5 WWTP in construction phase, 2 WWTP in reconstruction phase, and 11 WWTP in the phase of design and feasibility study. The WWTPs in operation phase use as the main treatment method, the biological one. The biological treatment method has its advantages and disadvantages. Although it treats the wastewater in less time, produces energy, fertilizers, reduces water pollution and public health risk, they have their negative impacts on the environment. In this case study, WWTP of Pogradeci, uses the method of trickling filter as secondary treatment. The results of the chemical analyses show a high efficiency of the treatment and control of the phosphorus and nitrogen content. However, the large space for the setup, installation and power supply costs, pungent smell can be considered as a lack for this treatment method, and a negative environmental impact. This paper proposes maintenance of the WWTP as the key measurement to avoid negative impacts to the environment.

**Keywords:** urban WWTP, environmental impact assessment, pollution control, biological treatment.

### **1. INTRODUCTION**

#### **1.1. Overview of the current situation of the Albanian WWTPs**

Wastewater treatment plants (WWTP) are built in almost all the major cities of Albania, as an urgent need for public health safety and clean environment. Nowadays, Albania has 16 WWTP in function, 5 WWTP in construction phase, 2 WWTP in reconstruction phase, and 11 WWTP in the phase of design and feasibility study [1]. The WWTPs in operation in Albania use as main treatment method, the biological one.

Biological wastewater treatment method, also known as the conventional method, is a common and widely used method of treatment. It takes into account biodegradation bleaching by taking aid of several micro-organisms, fungi, bacteria, yeasts, and algae. This is a cheap and easy process that goes through a combination of aerobic and anaerobic processes. In biological wastewater treatment organic material is oxidized by microbial communities maintained in either a suspended growth or an attached growth reactor. Both types of reactors make use of mixed cultures, that is, cultures including a number of microbial species.

## **1.2. Legal framework**

The environmental assessment is based on the Law No. 11/2012, “On Integrated Water Resources Management”, Law No. 10431, 09.06.2011 “On Environmental Protection” and the Law No. 1044, 07.07.2011 “Environmental Impact assessment”, DoCM no 177, 31.03.2005 “On the allowed norms of discharges of liquids and zoning criteria for the receiving water environments”.

## **2. MATERIALS AND METHODS**

### **2.1. Case study: Pogradeci Wastewater treatment plant**

For this study was chosen the Waste Water Treatment Plant (WWTP) of Pogradeci, which is a touristic city, lying near Ohrid Lake, one of the Europe’s deepest and oldest lakes, declared a World Heritage site by UNESCO in 1979. This WWTP is serving with waste water treatment an urban population of 50,000 residents, which is going to cover with his service also the rural area with a number of 75,000 inhabitants approximately in the III phase [2]. (Fig. 1).

The treatment capacity of WWTP is 1, 68 million m<sup>3</sup>/year. The waste water treatment process is based on biological treatment using technology of trickling filter, polyethylene construction, resistant of UV, with a diameter of 23 m and depth of 4 m (Fig. 2). Flow at the inlet of WWTP is about 38 l/s, which enters in the treatment plant through a pumping station in a distance of 2 km from this plant. The current surface of WWTP is approximately 13.5 ha [2].



Figure 1. Trickling filter of Pogradeci WWTP (photo credit by author)



Figure 2. Ponds used for sedimentation and treatments of N and P (photo credit by author)

Pogradeci WWTP is using for N removal a step-by-step process with ponds which occupy a total area of 8.5 ha, which before the construction of the waste water treatment plant was used for fish cultivation (Fig. 3).

## 2.2. Environmental Assessment of the Wastewater Treatment Plants and sample analysis

Environmental Assessment is based on:

- the efficiency of the wastewater treatment plant
- water quality of Ohrid lake
- environmental impact of the nearby population.

Water quality is assessed based on the WWTP laboratory analyses. The WWTP laboratory is measuring these parameters: BOD, COD, SS, PO<sub>4</sub>-P, NH<sub>4</sub>-N, NO<sub>3</sub>-N, P total. BOD, COD and SS are measured once a week, while the other parameters every day. Samples are collected in the same point, at the same depth, and in the same way. Samples collection:

- *simple analyses*: only one sample in short time.
- *qualify analyses*: 5 samples every 2 hours and with a time distance 2 min from each other.
- *complex analyses*: some or many samples, mixed with each other in time from 2 to 24 hours.

Ohrid Lake because of its depth is under the stratification phenomenon. The measured parameters are from two monitoring points, the first one is at a reference station 150 m depth and the samples are taken in 8 different depth from the surface to the end of the lake, respectively 0, 20, 40, 60, 80, 100, 120, 150 m, and the second station (littoral) is 200 m from the lake side, above 5 m depth [3].





### **3. RESULTS AND DISCUSSIONS**

#### **3.1. Efficiency of the wastewater treatment plant**

Pogradeci WWTP is constructed to treat the urban before discharging to the Ohrid Lake. This waste water treatment plant is using as the secondary treatment the method of trickling filter, the biological treatment. Biological wastewater treatment is an effective technology to remove oxygen demanding compounds (COD) and nutrients (N and P) from wastewater. However, the role of wastewater treatment plants (WWTPs) is no longer merely constraint to protecting the aquatic environment (e.g. eutrophication) or solely evaluated based on the effluent quality [4].

The advantage of this method is that the treatment efficiency is 90% compare to other methods. Table 1, presents the result of the laboratory analyses for water quality during the year 2021. The results show a very high efficiency of the treatment. BOD is reduced in 92.135 %, COD is reduced in 87.50%. Table 2, presents the result of the laboratory analyses for water quality during august 2022. The results show also a very high efficiency of the treatment. BOD is reduced in 93.57 %, COD is reduced in 93.46 %. The values of water quality after treatment are below the standards (Tab. 3).

#### **3.2. Operational nitrification/denitrification units will be built in the III phase**

The nitrification process will be carried out by a filter filled with plastic material. The filter inlet will be connected to the bottom of the 10 sedimentation/maturation tanks. The treated water will then pass to the other 10 sedimentation tanks. To enable denitrification, the water from this filter will recirculate in the anaerobic tub at a rate of 300%. In the anaerobic tank, the oxygenated nitrates will be converted into simple nitrates under the action of bacteria.

As part of the II phase of the works for the expansion of the urban wastewater treatment plant, an additional unit for the elimination of phosphorus is built. This consists in adding salts to the distribution channel before water goes to the sedimentation tanks.  $\text{PO}_4^{3-}$ , precipitation chemicals make it possible to reduce the  $\text{PO}_4^{3-}$  concentration to 2 mg/l. The  $\text{PO}_4^{3-}$  precipitating agent are added to the untreated water in the distribution chamber that distributes the water from the trickling filter to the sedimentation tanks.  $\text{FeClSO}_4 (\text{Fe}^{3+})$  is used as a precipitating agent, as it can be used as a ready solution and does not need pre-treatment in the plant area. The sludge composed of  $\text{FePO}_4$  will precipitate together with the sludge composed of the trickling filter biofilm in the secondary sedimentation basins. Part of the phosphorus will be eliminated by biological treatment from the anaerobic pond.

Additional units of the existing WWTP to reduce N, P are fundamentals. Table 3 shows that we are in the conditions of a sensitive area and we should have low levels N, P.

In the NEA Quality of surface water Report in our country 2021, regarding the environmental condition of Lake Ohrid [3], [4], since it is part of the protected lakes, the growth of phytoplankton in the lake was reported, which indicated a lake in the process of eutrophication. For this reason, the designer of the wastewater treatment plant of Pogradec decided that the discharge rates of P, N should be at the allowed rates of the EU, in case the water was poured into the lake and not used for agricultural purposes.

Table 1. Laboratory analyses of water quality, Year 2021. (Source: Laboratory of Pogradeci WWTP)

Average Value	Influent Flow	Influent Load		Concentration of WWTP Influent						Concentration of WWTP Outlet						Efficiency	
	Pump 1,2	COD	BOD	COD	BOD	COD/BOD	NH <sub>4</sub> -N	PO <sub>4</sub> -P	P <sub>total</sub>	COD	BOD	NH <sub>4</sub> -N	NO <sub>3</sub> -N	PO <sub>4</sub> -P	P <sub>total</sub>	COD	BOD
	m <sup>3</sup> /d	kg/d	kg/d	mg/l	mg/l		mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	mg/l	%	%
January	4,262.00	1,542.48	851,65	285	156.5	1.83	17.5	2.92	3.82	28.1	15.53	4.075	8.195	3.39	3.58	86.255	89.584
February	5,563.50	1,540.11	916,189	277	162.65	1.7	13.5	3.64	3.86	21.45	10.58	3.9	7.98	3.35	2.66	90.43	93.156
March	6,371.50	1,849.25	896,257	290	140.67	2.06	15.25	3.19	5.58	19.98	7.93	4.05	9.54	3.83	2.53	87.05	94.15
April	5,730.90	1,264.45	815,220	220.6	142.25	1.55	12.7	4.46	3.63	22.73	9.875	3.82	8.15	3.93	3.05	88.73	92.994
May	5,858.80	1,277.115	589,768	218	153.50	1.42	9.55	4.64	4.06	25.06	14.60	4.55	8.56	3.17	3.87	87.48	89.84
June	5,098.00	1,301.74	717,543	255	140.75	1.81	12.45	4.14	3.88	28.41	12.4	4.05	7.88	4.16	3.46	88.03	90.964
July	2,994.00	1,301.97	886,774	285	194	1.46	17.75	3.64	4.59	28.1	11.27	5.215	11.65	3.02	3.97	87.17	93.728
August	5,455.00	1,358.01	810,5411	287	171	1.67	17.15	3.44	4.51	34.68	14.63	4.60	12.75	2.87	4.77	83.70	90.053
September	4,133.00	1,612.18	742,017	391	179	2.17	18.66	2.74	5.25	35.53	13.43	5.25	9.60	3.55	4.27	89.12	90.193
October	4,137.00	1,204.33	887,386	291	214.5	1.35	15.55	2.66	4.47	31.15	12.1	4.07	11.25	3.02	4.09	86.98	93.995
November	4,283.00	1,542.86	109,430	360	255.50	1.4	15.85	3.36	7.08	32.329	10.70	6	11.40	2.67	3.61	89.38	95.547
December	3,746.00	930.881	930	383	248.5	1.54	14.5	0.96	6.97	55.6	22.5	8.65	8.765	1.82	4.05	85.75	91.41
Average value /year	4.802.79	1,316.53	843,211	295	179.86	1.64	15.08	3.31	4.73	30.26	12.96	4.853	9.643	3.23	3.6658	87.50	92.135

Table 2. Laboratory analyses of water quality, August 2022. (Source: Laboratory of WWTP Pogradeci).

August 2022	Influent Flow	Influent Load		Concentration of WWTP Influent						Concentration of WWTP Outlet						Efficiency	
	Pump 1,2 m <sup>3</sup> /d	COD kg/d	BOD kg/d	COD mg/l	BOD mg/l	COD/BOD	NH <sub>4</sub> -N mg/l	PO <sub>4</sub> -P mg/l	P <sub>total</sub> mg/l	COD mg/l	BOD mg/l	NH <sub>4</sub> -N mg/l	NO <sub>3</sub> -N mg/l	PO <sub>4</sub> -P mg/l	P <sub>total</sub> mg/l	COD %	BOD %
Average Value	4,610	2,245.98	1,306	492	286	1.7	19.8	3.76	5.81	32.2	18.4	6.7	9.243	3.06	4.37	93.46	93.57

Table 3. Norms of discharges liquids to Albania (source DoCM no 177, dated 31.03.2005 “On the allowed norms of discharges of liquids and zoning criteria for the receiving water environments”)

Parameters	Concentration	Efficiency
a) Parameters for water discharges to normal areas		
BOD <sub>5</sub>	25 mg/l	70-90 %
COD	125 mg/l	75%
TSS	35 mg/l (area with >10.000 inhabitants) 60 mg/l (2.000-10.000 inhabitants)	90 % (WWTP for 10.000 inhabitants) 70 (WWTP for 2.000-10.000 inhabitants)
b) Parameters for water discharges to sensitive areas		
P <sub>tot</sub>	2 mg/l (10.000-100.000 inhabitants) 1 mg/l (>100.000 inhabitants)	80 %
N <sub>tot</sub>	15 mg/l (10.000-100.000 inhabitants) 10 mg/l (>100.000 inhabitants)	70-80 %

### 3.3. Lake Ohrid water quality

Water of Ohrid Lake is saturated with oxygen, the values varied between 10 – 13 mg O<sub>2</sub>/l [3], during year 2021 and 7.58 –13 mg O<sub>2</sub>/l during 2022 [6]. The water is alkaline with pH values 7.2- 8.9 and with the high transparency from 5 to 11 m. Water temperature varies from 16 -26 °C [3].

Table 4. Water quality of Lake Ohrid (source: NEA, 2021)

Parameters	Station one (reference)	Station two (littoral)	Unit
Transparency	11	5	m
COD	4	5.3	mg O <sub>2</sub> /l
BOD <sub>5</sub>	2.6	3	mg O <sub>2</sub> /l
Nitrate	<1	<1	mg N/l
P total	11	11	µg P/l
Chlorophyll a	4.75	2.69	mg/m <sup>3</sup>
Trophic index	45.88	40.29	TSI (Carlson Index)

Based on the monitoring parametrers from the National Environmental Agency of Albania, waters of Ohrid Lake, are classified as mesotrophic [3] and in Class 1 – Good condition [6].

### **3.4. Environmental Impact of the WWTP**

Pogradeci WWTP has occupied a large surface as we mentioned above, 13.5 ha. The sludge from the WWTP is treated with ponds and there are no qualitative analyses. The large space for the setup, installation and power supply costs, pungent smell can be considered as a lack for this treatment method as well as negative environmental impact. The air pollution is minimal, but odours can be smelly because of the biological treatment, especially anaerobic processes.

## **4. CONCLUSION**

WWTP are necessary because they treat wastewater before the discharge to the environment. However if the wastewater treatment is inadequate, it can be a source of environmental pollution. The biological treatment has its advantages, mainly the low cost, and high efficiency of organic load removal. The results of water quality of Pogradeci WWTP show a very high efficiency of the treatment. BOD is reduced in 92.135 %, COD is reduced in 87.50%. The sensitive area as consider the Lake Ohrid should treat also the nutrients N, P. The environmental impact of WWTP to the air, soil, landscape, are minimal. The main problem is the sludge disposal. Maintenance of the WWTP is proposed as a key measurement to avoid negative impacts to the environment.

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## **The Contribution of The Wind Source in The Production of Electrical Energy - Case Study of The Milot Kurbin Field Area in Albania**

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### **ABSTRACT**

Wind power is crucial for combating climate change. The power sector accounts for around 40% of global CO<sub>2</sub> emissions. In Albania electricity sector the contribution of renewable energy sources is very important to strengthen national energy security, diversifying energy sources and reducing dependence on imports.

The purpose of this paper is to assess the theoretical, technical, and economic potential of the wind source in the region Fushë Milot - Kurbin for electricity production. Calculations of all technical - economic and environmental parameters are performed through the RETScreen Expert program. This analysis is sufficient for pre-implementation studies that do not require very detailed calculations and at the same time facilitates decision-making on project implementation.

The study area has considerable technical potential for the use of wind energy. In this area, the analyzed project envisages the installation of 8 turbines of the Siemens SWT 113 type, each with a power of 3 MW. The height of the turbines is 92.5 m. The total amount of electricity produced by this plant is estimated at 50 GWh / year, at a cost of 0.047 € / kWh. The total emission reduction for all proposed projects is 965,000 tCO<sub>2</sub> per year. The project has a positive net present value and a benefit-cost ratio greater than 1. The payback time is 4.7 years.

**Keywords:** Renewable energy source, wind energy.

### **1. THE CONTRIBUTION OF THE WIND RESOURCE TO EU AND NATIONAL ENERGY POLICIES**

The continuous increase in energy demand and consumption of fossil fuels is accompanied by the emission of large and increasing amounts of CO<sub>2</sub> into the atmosphere, which is the main contributor to global warming. So because of these emissions, it is estimated that the temperature on earth will increase by 1.5 °C between 2030 and 2050 [1].

Freeing the energy sector from carbon dependence and reducing carbon emissions to reduce the effects of climate change are at the heart of world policies for the gradual shift from the fossil fuel-based energy system to a sustainable renewable energy-based system. (RE).

After the Rio conference, climate change and the use of RES were at the centre of debate and policymaking worldwide. In Europe in 1997, RE made a modest contribution, with only 6% of the total energy consumption, while the dependence of EU countries on energy import was 50% [2].

Following these efforts, in 1997 Europe adopted the first RES strategy, Energy for the Future, which determined for the EU countries the doubling of the existing contribution of RE to the total energy consumption in the EU from 6% to 12% by 2010 [3]. The strategy estimated that to achieve this doubling of the role of RE, an increase in the general investment of the energy sector by nearly 30% was required, which would be accompanied by the opening of 500,000 - 900,000 new jobs and the reduction of CO<sub>2</sub> emissions by 402 million tons/year.

In 2001, the target for the contribution of RE to electricity production was 21% by 2010 with the adoption of the Directive on the promotion of electricity from RES (2001/77/EC) [4]. Although the share of electricity produced by RES has grown significantly over that time, reaching 13.6% of total EU energy consumption in 2005 and 19.5% in 2010 [5], the EU still failed to meet the target established by 21%. However, of the 641 TWh of electricity produced by RS in 2010, wind is the main contributor with 155 TWh after hydropower.

Many steps forward have been made since then and in 2009 the Council of Europe adopted for the first time a binding target for all member countries, where the energy produced by RES should occupy 20% of the total energy consumption by the year 2020 [6]. The member countries already have a legal obligation to define the national objectives for RES as well as to prepare every two years the National Action Plan for RES accompanied by the measures taken to achieve the objectives and the development of the energy infrastructure.

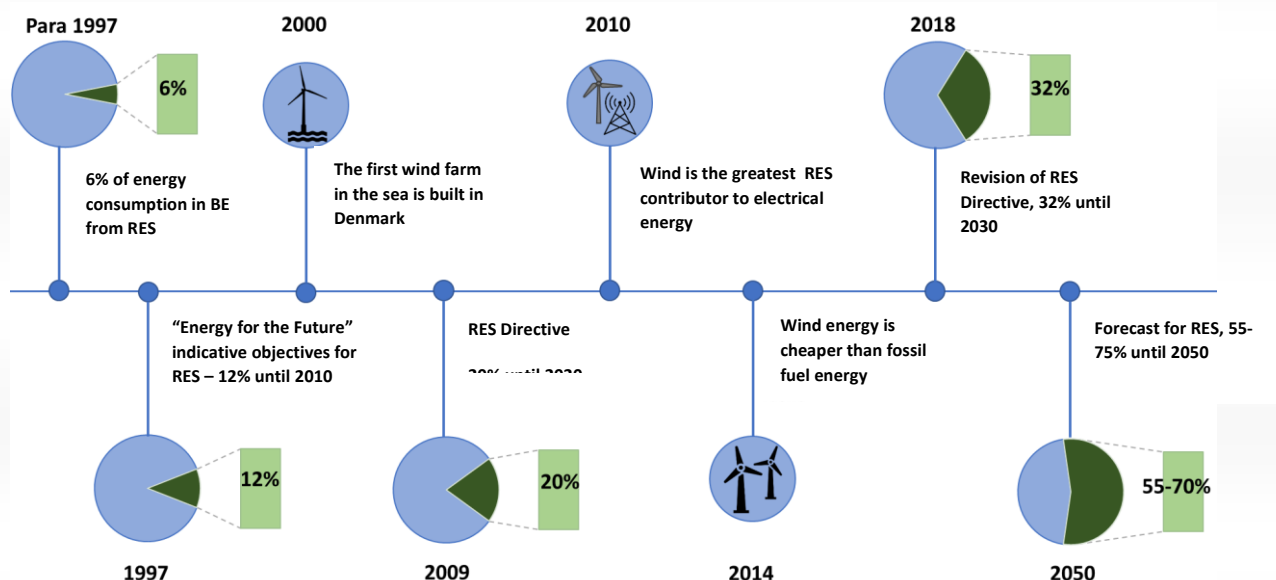


Figure 1. Development of EU RES targets and the role of wind energy [Source: EC 2020, adapted by author]

For the year 2030, the EU has adopted new objectives that are a 40% reduction in greenhouse gas emissions compared to 1990, the mandatory coverage of 32% of total energy consumption from RES [7] and the achievement of an improvement with a minimum of 32.5% in energy efficiency [8].



In the longer term, the EU has set the ambitious goal of building a competitive low-carbon economy, which will be able to reduce greenhouse gas emissions by 80% - 95% by 2050 [9]. Thus, the energy produced by RES can increase significantly in the European Union and can occupy from 55% to 75% of the total energy consumption in 2050 [10].

From the years 2000 - 2010, electricity produced by wind has had the fastest growth among all RES (with 127 TWh) [11]. While the contribution of electricity produced by the wind in relation to the electricity produced by all RES, there has been a positive increasing trend from the years 2004 - 2016 as shown in figure 2. [12].

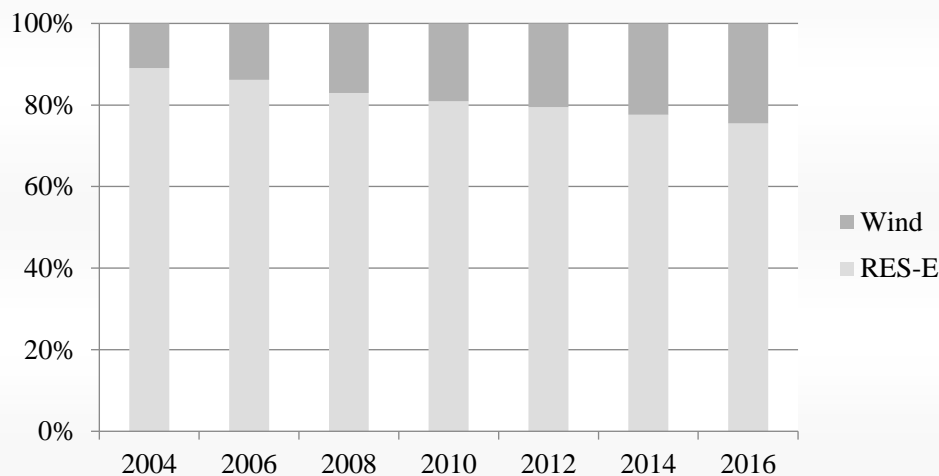


Figure 2. Wind's contribution to RES electricity production [Source: EC 2020, adapted by author]

The contribution of the use of renewable energy sources in general and of the wind source in particular in the Albanian electro-energy sector is very important to strengthen national energy security, diversifying energy sources and reducing import dependence. It also directly affects economic growth, employment and reduces the discharge of pollutants into the atmosphere, which are contributing to the warming of the planet and the greenhouse effect.

The main policy document in the energy sector is the National Energy Strategy 2018-2030, where one of its main objectives is the diversification of energy sources, aiming for the contribution of RES to total energy consumption to be 42%, while gas emissions greenhouse against the total to decrease by 11% until 2030 [13].

The National Action Plan for RES, which defines the supporting measures for achieving the national objectives, has been revised twice since its creation. What is observed is a growing trend in the planning of energy produced by wind and PV plants [14].

The interest of local and foreign investors in the use of RES has always been on the rise in the last 10 years. About 88 applications for the construction of PV with a capacity of up to 2MW have been submitted to MIE, of which 12 have been authorized with a total installed capacity of 24 MW. The largest plant is under construction in Akërnë Vlorë, with an installed capacity of 100 MW. As for wind energy, there were about 70 applications, of which 3 were authorized for the construction of wind parks up to 3 MW with an installed capacity of 41 MW [15].

The most important entry point to drive investment is to create incentives and secure financing for wind projects that produce electricity at above-market prices. These incentives must be supported by a legal and institutional framework that guarantees RE producers both the ability to connect to the grid and the ability to sell energy.

The new law on RES approved in 2017 provides energy producers for installations above 3 MW for wind parks with the sale of energy for 15 years to OSHE. Also, the law promotes new support schemes, such as the tariffs regulated today (feed in tariff) and those where the "Contract for Difference" concept (feed premium) is applied in the future. These schemes are expected to support the producer when the price of electricity produced by wind farms is above the market price. Furthermore, the law also states that for small plants up to 500 kW, excess energy can be fed back into the distribution network.

This law is a good start. However, it needs to be completed with the drafting of by-laws so that these incentive measures become applicable. The price to produce 1 MWh from wind plants with an installed capacity of over 3 MW is €76 (feed in tariff) [16].

Table 1. Forecast for the expansion of installed capacities for the generation of electricity from RES [Source: Energy Community 2020, adapted by author]

Estimated capacity (MW) for energy production from RES	2015-2020	2018 - 2020	2019 - 2020
Hydro	750	600	57
PV	50	120	490
Wind	30	70	150
<i>Table 1. Forecast for the expansion of installed capacities for the generation of electricity from RES</i>	0	8	41
<b>TOTAL</b>	830	798	738

Table 2. Price for electricity production from RES plants. [Source: ERE 2020, NREP 2018-2020]

The RES technologies that are applied	Tariffs (feed in) €/MWh
PV modules with installed capacity of electricity up to 2 MW	100
Wind turbines with installed capacity of electricity up to 3 MW	76
Small HPPs with installed capacity up to 15 MW	69

## 2. THE AREA UNDER STUDY

In the Fushë - Milot Kurbin area, it is planned to build a wind park with a capacity of 24 MW. The project is planned to be built in the district of Kurbin, located between the villages of Gurës, Fushë Kuqe and Gorre. The project will be spread over an area of 10 km<sup>2</sup> at a height of 10 m above sea level. It is planned to install 8 Siemens SWT 113 turbines, each with a power of 3 MW. The height of the turbines is 92.5 m.

Figure 3 shows the map of the area with the distribution of the average wind speed at a height of 100 m, and Table 3 provides the main technical data of the project.

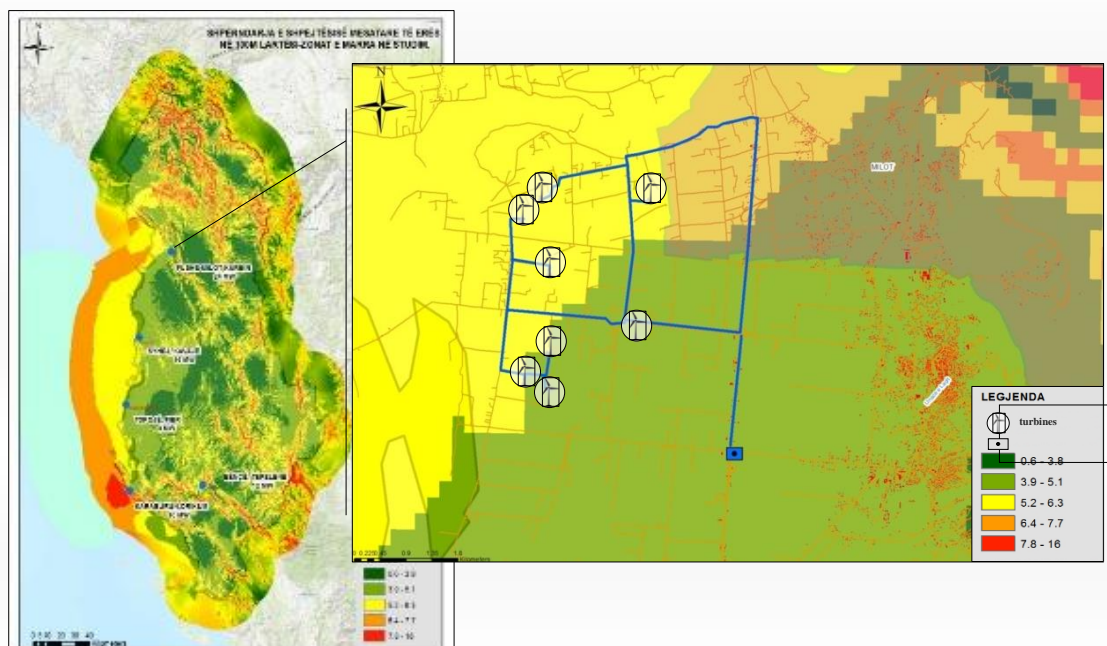


Figure 3. Distribution of average wind speed at 100 m height in the area of Fushë Milot, Kurbin

Table 3. Technical data for the proposed project in the Fushë Milot area, Kurbin

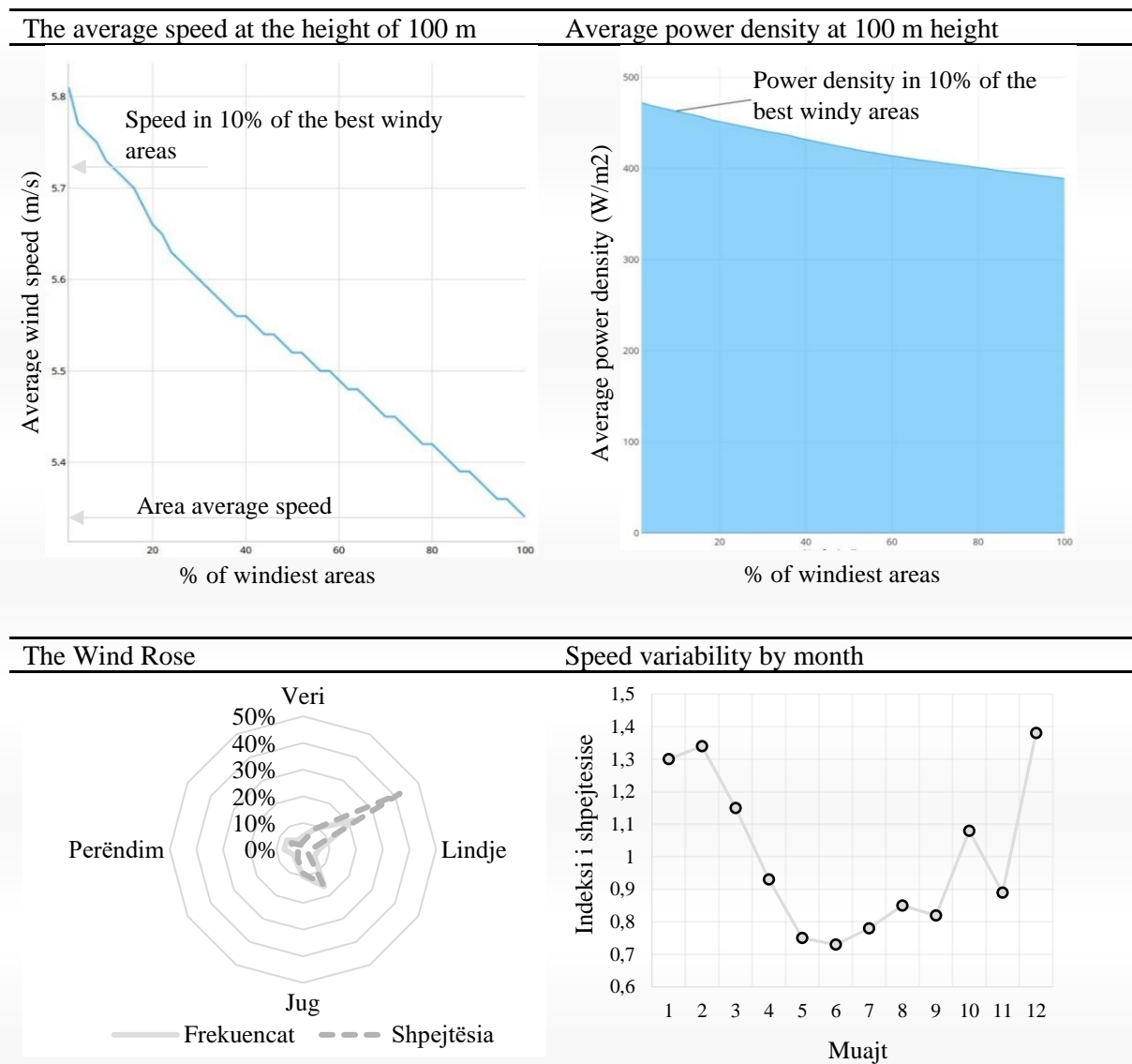
Zone	Fushë-Milot
Installed capacity	24 MW
Number of turbines and their capacity	8 x 3MW
Type of turbines	Siemens SWT 113
The height of the turbines	92.5 m
Rotor diameter	113 m
Average speed in 100 m	5.34 m/s
Average speed in the best areas	5.73 m/s
No. of wind hours	3000
Dominant direction	N – E – S – E
Connection point and distance from the network	110 kV Lac – Fushë Kuqe

Table 4 summarizes the technical information for the Fushë Milot - Kurbin area. The area presents an average speed of about 4.9 m/s. However, the wind speed is distributed in the area in different values, which vary from 4.9 - 5.4 m/s. While we are interested in the best areas, i.e. the areas with the most wind. This means that we gradually focus on smaller and smaller areas to identify areas with higher speeds. For example, in the graph if we look at 10% of the best windy areas in this region we will identify the average speed 5.28 m/s.

The power density in the 10% of the best areas, in the region we are studying, at a height of 100 m is 450 W/m<sup>2</sup>. The dominant wind direction is North-East and South-East. The wind speed index, which represents a statistically normal period of annual wind energy content, is highest during the months of January, February and December. These are the best months for using wind energy in this region. The

following tables summarize the results of the energy - environmental - financial analysis for the proposed project in Fushë Milot, Kurbin [17] [18].

Table 4. Average speed, frequencies, wind density at a height of 100 m and their variability in the area of Fushë Milot, Kurbin



### 3. METHODOLOGY

The calculations of all technical, economic and environmental parameters were carried out with the RETScreen Expert program. The analysis carried out using this program is sufficient for first studies - the applicability in which very detailed calculations are not required [19]. For the area studied through this program, the following parameters were analyzed:

**Energy Analysis:** The net energy (MWh) produced by the wind is calculated with the following expression:

$$E_{net} = E_{gross} \times C_{losses}(1)$$

The gross energy (MWh) that a turbine produces is calculated by multiplying the instantaneous power of a turbine, according to its power curve, by the probability distribution of the speed.

$$E_{gross} = (24 \times 365) \sum_{v=0}^{25} P_v f(v) \quad (2)$$

The loss coefficient takes into account losses as a result of mutual influence (1.5%), air losses (1%), technical losses (3%) and losses due to the absence or inadequacy of the network (0.03%).

$$C_{losses} = (1 - 0.15) \times (1 - 0.01) \times (1 - 0.03)(1 - 0.0003) = 0.9(3)$$

The capacity factor of the plant is calculated as the ratio of the net energy produced with the production of the capacity of the plant with the number of hours per year.

$$CF = \frac{E_{net}}{(24 \times 365) \times Cap.Plant} \quad (4)$$

The specific output of the plant (kWh/m<sup>2</sup>) is used to determine the performance of the turbine according to the wind regime, and is defined as the ratio of the net energy to the output of the number of turbines and the surface of the rotor.

$$Y = \frac{E_{net}}{(No.turbins \times Area_{rotor})} \quad (5)$$

To realize the energy performance in each area studied, regional climate data according to AEE were used, which were corrected where possible with field measurements and technical data of the turbines proposed by the project developers.

**Economic analysis:** The program analyzes in detail initial costs, annual costs, annual savings and periodic costs. To orient the user to the initial costs that are also the largest costs, the program suggests the percentages that each of these costs occupies in the total cost of a project for a large or small capacity wind farm.

Table 5. Analysis of initial costs for small and large wind farms. [Source: RETScreen]

Type of cost	Small plants	Large plants
Feasibility study	1 - 7%	< 2%
Development	4 - 10%	< 1 - 8 %
Engineering	1-5%	1 - 8%
The electrical system	47 - 71%	67 - 80%
System balancing	13 - 22%	17-26%
Others	2 - 15%	1 - 4%

**Environmental analysis:** This analysis is based on two scenarios. The baseline scenario calculates the net emission of greenhouse gases (GHGs) that would be emitted into the atmosphere if the same amount of energy were produced in an alternative way from a conventional plant. In this scenario, it is assumed that the entire amount of energy produced in

the area comes from an imaginary plant that works with fuel in the ratio of 30% natural gas and 70% diesel.

This scenario is taken as a reference and compared with the proposed scenario, the wind one and calculate the reduced GHG emissions produced by the proposed project throughout its life cycle. The analysis takes into consideration to calculate the GHG reduction, CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O emission factors of 234.5 kg/GJ, 0.0083 kg/GJ and 0.057 kg/GJ respectively.

***Financial analysis:*** This analysis through financial indicators provides information on the viability of a project. It uses these parameters as input data; inflation, discount rate, project life, grants or incentives, loan level, loan, capital, loan interest rate, loan duration and its payment.

In all cases considered, we have assumed that the life of the project is 25 years, the inflation rate is 3%, the discount rate is 9%, the loan level is 50% of the initial investment, which is taken for a period of 15 annual with 6% interest.

The program also calculates the annual income that the project realizes, both from the sale of electricity and from the reduction of GHG in case the project is supported by any incentive policy, for example that takes into account carbon credits. In all cases we have taken into consideration that the price of electricity produced by wind plants is €76/MWh. Also, the annual income takes into account only the income obtained from the sale of electricity.

The results of the financial analysis are; internal rate of return (IRR), return on capital, net present value, life cycle savings, cost benefit ratio, debt coverage and energy production cost.

#### **4. ANALYZING THE RESULTS**

For the case taken in the study, it results that the areas have a considerable technical potential for the use of wind energy. At the end of the energy - environmental - financial analysis, we can say that the proposed project is financially viable. It has a positive net present value and a benefit-cost ratio greater than 1. Investment payback time 4.7 years. The total amount of electricity produced by this plant is calculated at 50 GWh/year, with a cost of 0.047 €/kWh. The total emissions reduction for all proposed projects is 965,000 tCO<sub>2</sub> per year. The following tables and figures summarize the energy, environmental and financial results.

Table 6. Results of the energy analysis for the Fushë Milot project, Kurbin

Energy production results for the proposed plant in Fushë Milot - Kurbin	
Net energy per turbine (MWh)	6,823
Loss ratio	0.9
Capacity factor (%)	23.5
Turbine efficiency kWh/m <sup>2</sup>	617
Electricity produced annually by the plant (MWh/year)	49,326

Table 7. Results of the environmental analysis for the Fushë Milot project, Kurbin

GHG reduction results for the proposed plant in Fushë Milot - Kurbin	
Amount of emissions for the base case	41,945.9
Amount of emissions for the proposed case (tCO <sub>2</sub> )	3,357.7
Reduction of annual emissions (tCO <sub>2</sub> )	38,588.2
Reduction of emissions throughout the life cycle (tCO <sub>2</sub> )	964,705



Table 6. Results of the financial analysis for the Fushë Milot project, Kurbin

Financial results for the proposed plant in Fushë Milot - Kurbin	
Total investment cost (€)	22,565,547
Internal rate of return on capital IRR (%)	21.6
Internal rate of return on IRR assets (%)	10.4
Payback period of initial investment (years)	6.3
Years of capital return (year of positive flows in years)	4.7
Net present value (€)	14,152,588
Annual Lifetime Savings (€)	1,440,822
Benefit-cost ratio	2.3
Debt coverage (years)	3.1
Electricity production cost (€/kWh)	0.047

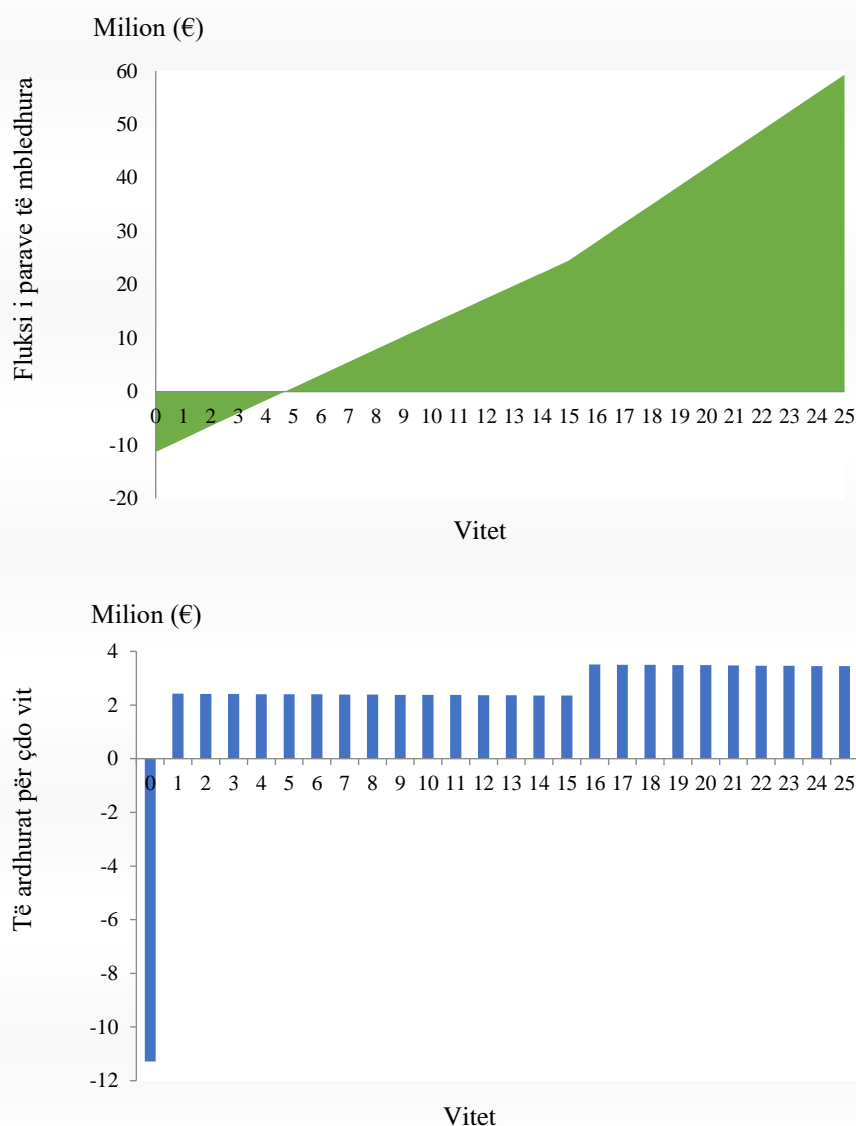


Figure 4. Income flows for the project in Fushë Milot, Kurbin

## **5. CONCLUSIONS AND RECOMMENDATIONS**

Wind energy, among all renewable energy sources, occupies a significant place in the energy market, experiencing during the last decade the greatest growth in the whole world. Among the main benefits of wind-generated energy are the environmental advantages it presents compared to energy generated from traditional fuels. Moreover, the cost of producing electricity from wind is among the cheapest compared to other energy sources, and it is more and more comparable to the costs of producing electricity from fossil fuels.

The use of the energy potential of the wind, as a renewable source of energy, for the production of electricity in Albania has not yet begun. Despite the increased interest presented to invest in this direction, it seems that the main barriers faced by investors are:

- (i) lack of long-term data and measurements in the areas of interest for the use of this energy,
- (ii) high investment costs and uncertainty about the income and expenses of these projects
- (iii) lack of effective support schemes as long as the cost of wind power production is higher than the market price of electricity.

Based on the results of this case study for further research in this field I recommend:

- (i) The design and implementation of concrete projects aimed at a detailed assessment of the wind source in the area studied as the most interesting for the use of this energy.
- (ii) More detailed assessment of the wind source, based on monitoring with field measurements of wind speed. This is the main element to determine the financial viability of a wind energy project;
- (iii) Drafting favorable policies for the use of BR in general and the wind source in particular, to create more security for investors;
- (iv) The construction of effective financial support schemes and the creation of successful business models per exploitsresource source teeres

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## **Utilisation of Non-Specific Antibodies to Detect Goat Leukocyte Populations by Flow Cytometry**

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### **ABSTRACT**

Routinely, manual and automatic techniques are used to identify granulocytes and monocytes in goat species, with good results for both techniques. However, there are other techniques such as Flow cytometry that can be used for this purpose. To achieve this, use of species-specific antibodies that are mandatory to identify each white blood population. However other species-specific antibodies can be used in other species if the antibodies reactivity is proven. Ten peripheral blood samples from healthy goats were collected and processed with an Attune NxT Cytometer (Thermo Fisher Scientific) to test their granulocytes and monocytes reactivity to the following antibodies: CD11b (Clone:M1/70) conjugated PeCy5, CD5 (Clone: YKIX322.3) conjugated FITC, cocktail CD4/CD8 (YKIX302.9/ YCATE55.9) conjugated FITC/PE. All analyses were performed at the Faculty of Veterinary Medicine at the Agricultural University of Tirana within 24 hours from peripheral blood collection. Results showed a satisfactory reactivity only to CD11b. Stain index of this antibody was considered good in all cases included in this preliminary study. Low number of cases is considered as a lack of this study, thus a higher number of peripheral blood samples from healthy goats should be included in future investigations.

**Keywords:** Leukocytes, Flow Cytometry, Goat, Antibodies

### **INTRODUCTION**

Identification of different leukocyte populations is considered a very important step since it can serve as a first approach toward a correct diagnosis in veterinary hematology. The classical way to identify each leukocyte population is the microscopic one that involves a peripheral blood smear being analyzed. Automatic techniques are also available for this purpose showing a great accuracy in leukocyte differentiation. Usually goat leukocytes are identified as in other mammals due to their membrane and nucleus characteristics. Flow cytometry represents a relatively new technique used in veterinary medicine for different purposes including leukocyte differentiation with and without use of antibodies (1), identification and staging of neoplastic disorders mainly in dogs and cats (2), Ki67 index (3),

DNA content (4), allergies and other medical conditions. In order to correctly identify each leukocyte population, use of species specific antibodies may be necessary. However if a different species specific antibody is used in another species, in some cases, a positive signal can be detected. To make this the antibody specificity and reactivity of one species should be proven to another species. The aim of this study was the evaluation of different species specific reactivity in goat leukocytes.

## **MATERIAL AND METHODS**

Peripheral blood samples from healthy goats were collected in 2.5 ml Ethilendiamintetraacetat tubes. Samples were then sent to the Laboratory of Public Safety at the Faculty of Veterinary Medicine/Agricultural University of Tirana. All samples were processed using an Attune NxT flow cytometer within 24h from sampling. Briefly four non species specific antibodies were used: CD11b (Clone M1/70) conjugated PeCy5, CD5 (Clone: YKIX322.3) conjugated FITC, cocktail CD4/CD8 (YKIX302.9/ YCATE55.9) conjugated FITC/PE (tab 1). The adequate quantity of peripheral blood ( $10^6$ ) was placed in flow cytometry tubes with the adequate quantity of the antibody. Incubation took place for thirty minutes at dark in  $4^0\text{C}$ . A RBC lysis step was performed in order to exclude red blood cells from the analysis. Centrifugation of samples was done, the supernatant discarded and finally the remaining cells were resuspended in 150  $\mu\text{l}$  of phosphate buffered saline. In order to compare the performances of antibodies the Stain Index was calculated in all cases using the following formula:  $\text{MFI of Positive} - \text{MFI of Negative} / 2 * \text{SD of Negative}$  where **MFI** = median fluorescence intensity and **SD** =  $\text{CV} * \text{Mean Negative} / 100$ ) (5,6).

Table 1. List of antibodies used to identify goat leukocytes

Antibody	Clone	Conjugation	Target Cells	Species Reactivity
<b>CD5</b>	YKIX 322.3	FITC	Mature T-Cells	Dog
<b>CD4</b>	YKIX 302.9	FITC	T-Helper Cells	Dog
<b>CD8</b>	YCATE 55.9	PE	T-Cytotoxic Cells	Dog
<b>CD11b</b>	M1/70	PECy5	Myeloid Cells	Mouse

## **RESULTS**

In total ten cases were collected in this preliminary study. A positive signal was detected only for CD11b, while no positive signal was detected for CD5,CD4/CD8. In the last case no signal was detected meaning that no link between antibodies and antigens were present. Regarding CD11b it showed a positive signal in all cases for Granulocytes. In figure 1 results are showed in flow cytometry dot plots.

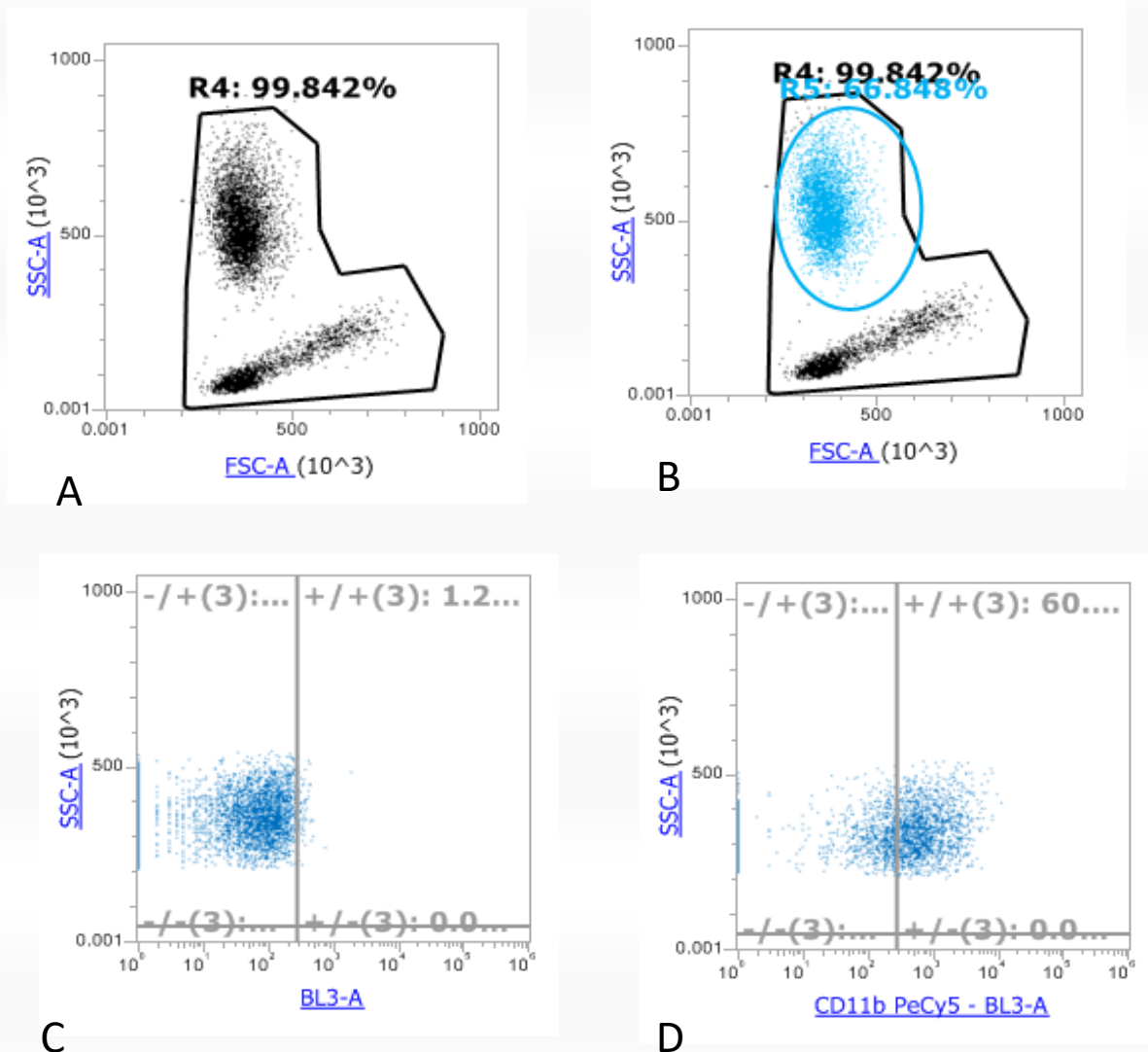


Figure 1. Dot plot showing reactivity to CD11b. A-Gate to include only leukocytes, excluding debris, B-Blue gate designed to select only Neutrophils, C-Neutrophils in third channel of fluorescence without antibody and D-Positive signal of Neutrophils against CD11b.

## CONCLUSIONS

The antibody CD11b can be used to identify goat Neutrophils with a satisfactory accuracy. However low number of cases can be a lack of this preliminary study. Thus further investigation are warranted to confirm these results.



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