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Research article

Interaction of Ti-6Al-7Nb alloy with simulated body fluid; a preliminary biocompatibility investigation

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Abstract

The current study aims to investigate the interaction of Ti-6Al-7Nb with simulated body fluid (SBF) in order to apply a preliminary biocompatibility investigation of this novel biomedical alloy, promising for orthopedic applications. Results of the static immersion tests conducted in SBF at body temperature demonstrated that Ca-P rich structures form on the Ti-6Al-7Nb alloy surfaces and the oxide layer formation-dissolution cycle reaches a stable state during immersion. Ion release levels were mostly below critical values except for the initial Al ion release level, which indicated the need for the presence of a stable protective layer on the alloy surface. The second set of static immersion experiments conducted in densified SBF demonstrated that, such a protective layer can rapidly form by biomimetic coating if followed by a preliminary surface treatment. Overall, Ti-6Al-7Nb alloys layers exhibit promising biocompatibility for orthopedic applications, especially with the presence of a stable protective layer.

Keywords: *Biomedical alloy; biomimetic coating; ion release; simulated body fluid; Ti-6Al-7Nb*

1. Introduction

Biomaterials have become an important material class that contribute to improving life quality. Among the many applications of biomaterials, orthopedic implants, which can replace or support bone tissue, are commonly needed due to various reasons such as bone and joint disorders, large and complex fractures, and bone tissue losses (Bauer et al., 2013; Behera, 2021). For orthopedic implant applications, usually metallic biomaterials are preferred, mainly owing to their mechanical properties as well as their biocompatibility (Bauer et al., 2013). Stainless steel, Cobalt-Chromium (Co-Cr) alloys, and Titanium (Ti) and Ti alloys, which are included in this group, are widely used biomaterials for bone tissue applications such as hip and knee implants, dental implants as well as bone screws and plates (Manam et al., 2017; Hazwani et al., 2022).

Among these biomedical alloys, Ti and its alloys (e.g. Ti-6Al-4V) have been favored over the recent years owing to their lower density which enables the manufacturing of low weight implants (Hazwani et al., 2022). Superior mechanical properties, corrosion resistance, and elastic modulus close to that of the bone tissue constitute other advantages of Ti and Ti alloys. Especially, the TiO_2 layer, which naturally forms on Ti alloys, provide remarkable corrosion resistance for these alloys. The chemical stability of the oxide layer on Ti enables chemical inertness, corrosion and erosion resistance as well as improved biocompatibility (Mahyudin et al., 2016; Hazwani et al., 2022). Moreover, this passive oxide layer provides protection against ion release from the alloy to the surrounding tissue and supports osseointegration by favoring the formation of hydroxyapatite like calcium-phosphate (Ca-P) rich structures on the surface of implant (Gedikoglu et al., 2021).

Owing to the aforementioned advantages, Ti and its alloys are preferred for many biomedical applications. Commercial grade Ti (Cp-Ti) is generally used in dental implants, while Ti-6Al-4V alloy is preferred for orthopedic applications such as hip and knee implants due to its superior mechanical properties (Toker et al., 2019). Ti-6Al-4V alloy is an α/β Ti alloy in which

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the α phase is stabilized by Aluminum (Al) and the β phase by Vanadium (V). Despite its advantages, the potential release of Al and V elements from Ti-6Al-4V alloy might cause some undesired effects for the patient (Mahyudin et al., 2016; Gedikoglu et al., 2021). Specifically, V element was shown to cause adverse reactions for the patient in long term use such as Alzheimer's disease, bone and nerve softening in addition to toxicity at cellular level (Toker et al., 2019; Badhe et al., 2021; Wei et al., 2023). On the other hand, Al element ion release above certain values may also cause various disorders such as changes in blood pressure, heart failure, parkinsonism including mild to severe symptoms in the body. Moreover, in the case of excessive Al ion release, this metal may accumulate in the kidney and damage different cells (Badhe et al., 2021). Therefore, the release of V and Al ions may cause significant health problems if they surround the tissues (Tuten et al., 2019).

Due to the aforementioned issues, β-Titanium alloys are being developed as an alternative by adding alloying elements such as Molybdenum (Mo), Tantalum (Ta), Zirconium (Zr), Tin (Sn) and Niobium (Nb) that can stabilize the β phase. These alloying elements, which can replace V, exhibit lower or no toxic effect when compared to V element; while maintaining the other properties (Bocchetta et al., 2021). Among these, Nb becomes prominent as an element that does not exhibit toxicity while contributes to high corrosion resistance (Fellah et al., 2014). Therefore, through the replacement of V with Nb, Ti-6Al-7Nb alloy has been developed as an alternative material to Ti-6Al-4V alloy for biomedical applications which exhibits as good mechanical properties while not causing toxic effects (Liu et al., 2013).

There are still various ongoing studies investigating the mechanical properties and biocompatibility of Ti-6Al-7Nb alloys to be used for biomedical purposes (Cingi and Cimenoglu, 2010; Fellah et al., 2014; Izmir et al., 2019). When exploring the biocompatibility of potential biomaterials, especially biomedical alloys, investigating the relationship between corrosion resistance and oxide layer formation of the metal in the body fluid environment is of utmost importance as metals are materials prone to corrosion. Specifically, it is crucial to examine the biomedical alloys' behavior in a synthetic body fluid environment at a pH value and ion concentration similar to the body part in which they will be used, especially for evaluating their corrosion resistance in the specific corrosive body environment and determining the possible corrosion reactions and toxic ion release behaviors (Yilmaz et al., 2020). Therefore, *ex situ* tests focusing on the correlation between corrosion, ion release and formation-dissolution cycle of the protective oxide layer of the metal in a simulated body fluid environment are important methods for understanding the preliminary biocompatibility response of metallic biomaterials (Toker et al., 2014; Toker and Canadinc, 2014; Gurel et.al, 2022; Li et al, 2023; Ozdemir et.al, 2023).

While there are few studies focusing on testing the biocompatibility of Ti-6Al-7Nb in synthetic body fluid environment, they are mostly focused on the bioactivity of this alloy and most of the studies on Ti-6Al-7Nb alloys for biomedical applications are focused on their mechanical properties. Specifically, studies investigating the various aspects of the biocompatibility of this novel alloy via static immersion tests where the corrosion–ion release relationship is investigated are relatively limited. Moreover, since Al, is a critical element for tissues due to the aforementioned reasons and is one of the constituents of Ti-6Al-7Nb alloy, it is especially crucial to investigate the ion release behavior of this alloy for potential biomedical applications. With this motivation, in the current study, the *ex situ* biocompatibility of Ti-6Al-7Nb alloy in synthetic body fluid environment is investigated as a function time. Specifically, the ion release behavior over time is examined in relation to the formation and dissolution of the oxide layer and Ca-P structures, which are important markers for osseointegration. The purpose of this study is to provide a preliminary biocompatibility investigation of the novel Ti-6Al-7Nb alloy for biomedical, specifically for orthopedic applications, via *ex situ* methods.

2. Materials and methods

The elemental composition of the Ti-6Al-7Nb alloy used in the study are given in Table 1 as weight percentage (%) value of each element (Titanium Ti-6Al-7Nb Data, 2022). The Ti-6Al-7Nb samples were in cylindrical geometry with a radius of 1 cm and height of 3 mm.

Table 1
The elemental composition of the Ti-6Al-7Nb alloy used in the study.

Element	Composition (weight %)
Titanium (Ti)	85.41-87.41
Aluminum (Al)	5.50-6.50
Niobium (Nb)	6.50-7.50
Iron (Fe)	max. 0.25
Oxygen (O)	max. 0.20
Carbon (C)	max. 0.08
Nitrogen (N)	max. 0.05
Hydrogen (H)	max. 0.009

In order to investigate of the behavior of Ti-6Al-7Nb alloy in the body fluid environment, the samples were statically immersed in a simulated body fluid (SBF) solution, which mimicked human blood plasma in terms of its pH and ionic content. The SBF solution was prepared according to the Kokubo protocol with the chemical content specified in Table 2 and the pH of the solution was fixed at 7.4 (Yilmaz et al., 2020).

Table 2
Chemical content of the SBF solution prepared for the static immersion experiments.

Adding order	Chemical	Amount (g/L)
1	NaCl	8
2	$NaHCO_3$	0.35
3	KCl	0.224
4	$K_2HO_4.3H_2O$	0.228
5	$MgCl_2.6H_2O$	0.304
6	HCl	40 ml
7	$CaCl_2$	0.278
8	Na_2SO_4	0.07
9	$(CH_2OH)_3CNH_2$	6.056

Surfaces of the Ti-6Al-7Nb samples were prepared by grinding and polishing prior to the static immersion experiments. Following surface preparation, the samples were placed in separate tubes where each sample was subjected to the SBF solution with a ratio of 1/10 of sample surface area (mm^2)/body fluid (ml) according to the Kokubo protocol. The sealed tubes containing alloys samples immersed in SBF solution were placed in a water bath where the body temperature environment of 37°C was provided. The immersion experiment

was applied for different time periods of 1, 7, 14, 21 and 30 days in order to investigate the ion release, passive oxide layer dissolution-reformation behavior as well as Ca-P rich precipitate formation on the Ti-6Al-7Nb samples in SBF as a function of time. At the end of each immersion period, the surfaces of the samples retrieved from the solutions were examined by scanning electron microscopy (SEM) (FE-SEM, Hitachi Regulus 8,230, Hitachi High-Tech Co., Tokyo, Japan,) coupled with Energy-Dispersive X-Ray Spectroscopy (EDX) (Ultim Extreme EDX instrument, Oxford Instruments, High Wycombe, UK) for the chemical analysis of the structures formed on the sample surfaces during static immersion. EDX analyses were conducted using both point and area spectrums at an accelerating voltage of 10kV and 10mm working distance. Potential ion release from the Ti-6Al-7Nb alloys into the SBF solutions were analysed via Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (ICP-MS, Thermo Fisher Scientific, iCAP RQ, MA, USA).

Finally, in order to test the biomimetic coating capability of the tested alloys, static immersion experiment was applied in a 3-times densified SBF (3X SBF) solution where the amount of each ingredient in the solution was multiplied by three. The immersion in 3X SBF was applied for periods of 1 and 7 days. For the sample-area/fluid ratio, Kokubo protocol was followed as in the procedure applied for the initial static immersion experiments. In order to prevent precipitate formation in the densified SBF environment, the 3X SBF solutions were renewed every two days. Following the immersion in the densified SBF solutions for 1 and 7 days, the samples were retrieved and analysed via SEM and EDX for the detection and elemental analysis of the newly formed structures, especially Ca-P products.

3. Results and discussion

3.1. Ex situ tests in SBF

The TiAlNb samples, which were immersed in SBF for 1, 7, 14, 21 and 30 days were retrieved from the fluids at the end of each immersion period and both the samples and fluids were thoroughly investigated. The initial examinations revealed that there were no significant changes on the pH values of the SBF solutions after the alloys were retrieved from the fluids following the determined immersion periods, which indicate the stability of the SBF solutions throughout the experiments.

The selected SEM images of the sample surfaces following immersion in SBF for 1, 7, 14, 21 and 30 days together with the control sample are given in Fig. 1. The initial visual observations from the SEM images indicate the formation of new structures starting from the 1st day of immersion in comparison to the control sample. The intensity and distribution of the new structures on the sample surfaces exhibit slight differences among the different immersion periods. These differences are due to the expected new structure formation-dissolution cycle that occurs throughout the immersion (Toker and Canadinc, 2014; Toker et al., 2014; Gurel et al., 2022; Li et al., 2023; Ozdemir et al., 2023).

The results of the EDX analyses obtained from the different areas of each sample paired with the corresponding SEM image are also provided in Fig. 2. Three or four different areas were examined via EDX on each of the samples, which were selected among the regions where formation of new structures was detected. In the EDX analysis of these selected regions, special attention was paid to Ca, P and O; as the changes in O signal are representative of the formation and dissolution of the protective oxide layer on the alloy surface while Ca-P signals are important in terms of providing information about the bone tissue formation tendency of the surface.

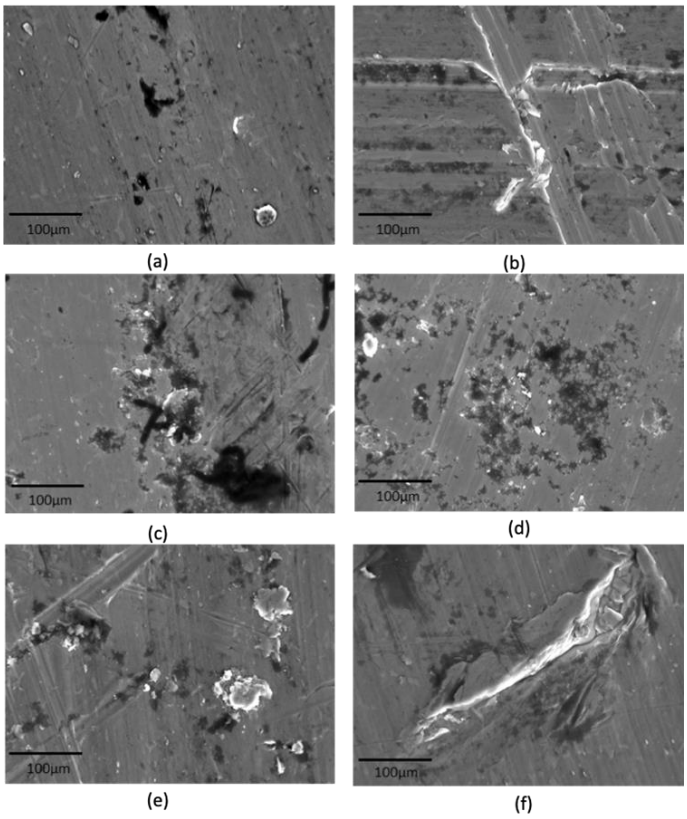

Fig. 1. SEM images of the Ti-6Al-7Nb sample surfaces; (a) control sample, (b) sample immersed in SBF for 1-day (c) sample immersed in SBF for 7 days, (d) sample immersed in SBF for 14 days, (e) sample immersed in SBF for 21 days, (f) sample immersed in SBF for 30 days.

In order to make a quantitative comparison, the maximum O, Ca and P levels measured on each surface were noted and organized as a graph of element weight percent vs. immersion period (Fig. 3 and Fig. 4.).

It is known that during the interaction of the implant materials with bodily fluids, the protective oxide layer goes into a continuous cycle of dissolution and reformation, which affects the ion release from the metal into the fluid (Toker et al., 2014). For the current samples, the change in O levels with respect to time exhibits a steady increase from the 1st day of immersion up to the 14th day, becomes stable around 21 days and followed by a decrease approaching to 30 days. Since this behavior is strongly correlated to the ion release behavior, it is important to examine this trend in relation with the ion release rates over time. The measured ion release levels of Al and Ti elements at each immersion period is provided as parts-per-billion (ppb), ie µg lt^{-1} in Fig. 5. According to this figure, the most striking observation is the sudden Al release on the 1st day immersion. This sudden increase is stabilized at the 7th day of immersion, followed by a slight decrease on the 14th day and afterwards the Al release levels fell below detectable levels. On the other hand, the detected Ti ion release levels over time are relatively low and close to each other. In terms of the critical levels of the ions, it is stated that the safe range in the body for the Ti element in the body is <1 ppb, and for the Al element is <5 ppb (Sahin et al., 2015). The current results exhibit that risky ion release levels

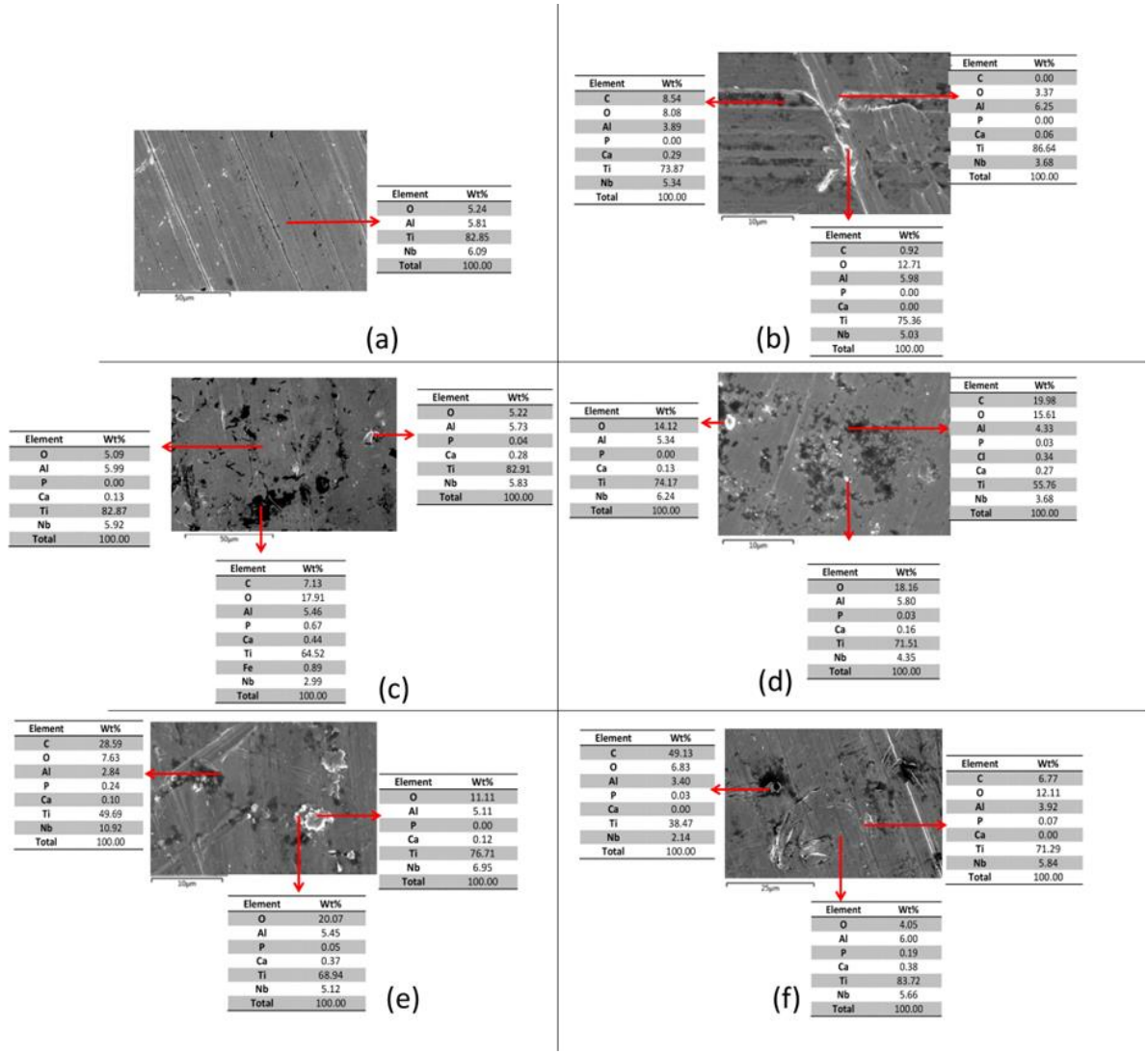

Fig. 2. EDX analysis results from selected areas of the Ti-6Al-7Nb sample surfaces; (a) control sample, (b) sample immersed in SBF for 1day (c) sample immersed in SBF for 7 days, (d) sample immersed in SBF for 14 days, (e) sample immersed in SBF for 21 days, (f) sample immersed in SBF for 30 days.

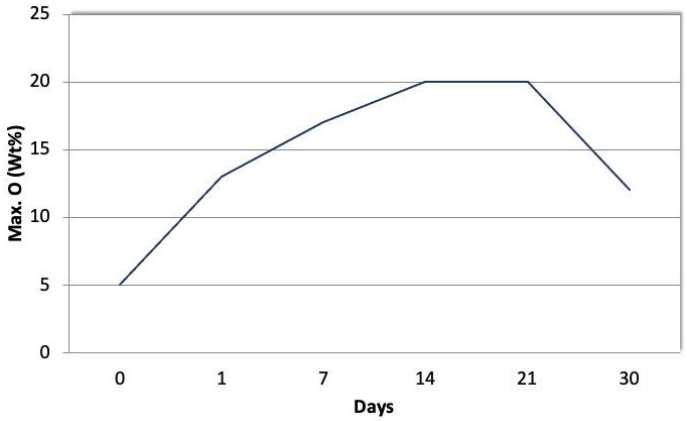

Fig. 3. The maximum O level measured on each sample surface throughout the different immersion periods. (Since the comparisons were based on the max O level observed on each sample, no standard deviation data was provided).

were observed for Al element, only on the 1st day of immersion while the measured Al ion release levels on the other days are much lower than the critically risky values (Sahin et al., 2015). Although O signal is slightly higher as compared to the control sample on the 1st day of immersion, it can be argued that the oxide layer is not sufficiently protective at this stage. Literature shows that, as the thickness of the oxide layer increases, the potential of crack formation within the oxide layer also increases, inducing higher ion release levels (Toker et al., 2014), which explains the observed result. However, the drastic decrease in the ion release levels and the steady state behavior following this decrease can indicate that oxide layer thickness reaches a protective level past the 1st day of immersion and preserves its protective nature despite the slight fluctuations in thickness over time. Therefore, it can be argued that, for this promising alloy, application of a process to ensure the stability of the oxide layer for the safety during initial interaction with bodily fluids is essential.

The changes in Ca and P levels detected by the EDX analyses were also recorded as a function of time since these observations are indicative for osseointegration which is the ability of bone tissue formation on the implant surface (Toker et al., 2020). Specifically, formation of Ca-P rich structures on the implant surface plays a supporting role in bone tissue formation by providing a surface where bone cells can adhere and multiply

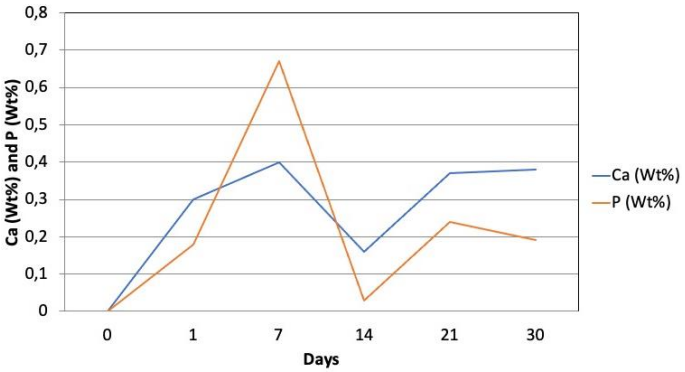

Fig. 4. The maximum Ca and P levels measured on each sample surface throughout the different immersion periods. (Since the comparisons were based on the max Ca and P levels observed on each sample, no standard deviation data was provided).

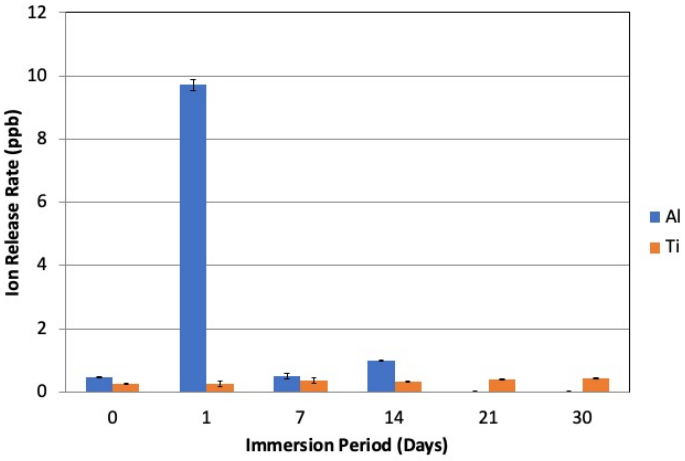

Fig. 5. The Al and Ti ion release levels at each immersion period (ppb: parts per billion). (Average value of the three measurements taken from each sample is provided with the error bar corresponding to the standard deviation).

in a healthy way and therefore play a critical role in terms of the biocompatibility of the implant material (Yilmaz et al., 2014). According to Fig. 4, significant increases are observed in both Ca and P signals from the 1st day up to the 7th day, which is followed by a decrease on the 14th day. Afterwards, both signals re-increase on the 21st day and remain stable until the end of the immersion period of 30 days. This fluctuating behavior of the time dependent Ca-P structure formation on the alloy surface can again be correlated to the dynamic dissolution and reformation cycle in corrosive body fluid environment over time (Toker et al., 2014; Ozdemir et.al, 2023). An important observation for the Ca-P structure formation behavior which exhibits similarity to that of oxide layer formation is the significant increase from the 1st day of immersion up to 7 days. Based on this similarity in the new structure formation over time, it can be suggested without any special surface treatment, bioactivity of the TiAlNb alloy increases and reaches a more optimum level for oxide and Ca-P rich structure formation following initial immersion. Although, this behavior may be considered promising for the overall biocompatibility of the alloy, the high ion release level of Al observed on the 1st day of immersion indicates that a stable layer should be present on the alloy surface prior to implantation. In other words, despite the relatively biocompatible components in this alloy, presence of a protective layer on the implant surface, in addition to the naturally formed passive TiO_2 layer, is still required to prevent Al ion release.

3.2. Determination of biomimetic coating capability in densified SBF

For orthopedic implant materials, usually hydroxyapatite-based coatings are used as protective layers, which also supports the bone formation ability of the implant with its chemical resemblance to the actual bone tissue. Among the commonly applied methods for hydroxyapatite coating, a simple and effective method is biomimetic coating, where the metal surface is subjected to a densified simulated body fluid environment (Yilmaz et al., 2014; Sarikayak et al., 2021).

For the current study, in order to test the biomimetic coating capability of the tested alloys, static immersion procedure was applied in a 3-times densified SBF solution for the immersion periods of 1 and 7 days. The reason 1 and 7 days were selected as biomimetic coating periods was based on the observations of the initial static immersion tests, where significant changes in new product formation occurred between 1 and 7 days. Both the visual observations from the SEM images and the quantitative results from the EDX analyses indicate that, biomimetic coating does not start on the 1st day of immersion (Fig. 6 and Fig. 7).

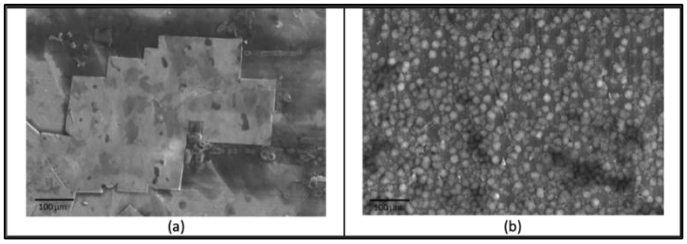

Fig. 6. SEM images of the sample surfaces following immersion in 3X SBF for (a) 1 day and (b) 7 days.

Specifically, following the 1st day of immersion in 3X SBF, formation of salt like precipitates rich in Na are observed, however Ca and P formation does not start yet. It is known that the formation of Na rich structures, specifically sodium titanate structures are favorable for the adhesion of Ca-P based structures (Sarikayak et al., 2021). As expected, Ca-P rich structure formation are prevalent on the alloy surface on the 7th day of immersion. The observed structures are widespread throughout the alloy surface and significant Ca and P signals are detected from these structures (Fig. 6 and Fig. 7). Normally, preliminary surface processing is necessary to achieve such rich Ca-P based structure formation (Hazwani et al., 2022; Wei et al., 2023). These findings indicate that, without any specific surface processing, such as alkaline procedures which are commonly applied prior to biomimetic coating, the TiAlNb alloy surface can be successfully coated with the biomimetic method in 7 days. However, in order to prevent any potential ion release, which may occur on the early stages of implantation, biomimetic coating procedure should be applied following a preliminary surface process such as alkaline treatment.

Overall, the findings of the current study support the opinions regarding the promising biocompatibility of the novel TiAlNb alloy for biomedical applications. However, in order to ensure the safe use of this alloy as orthopedic implant materials, presence of a stable protective layer is required, especially at the early stages of implantation. In that manner, results of the

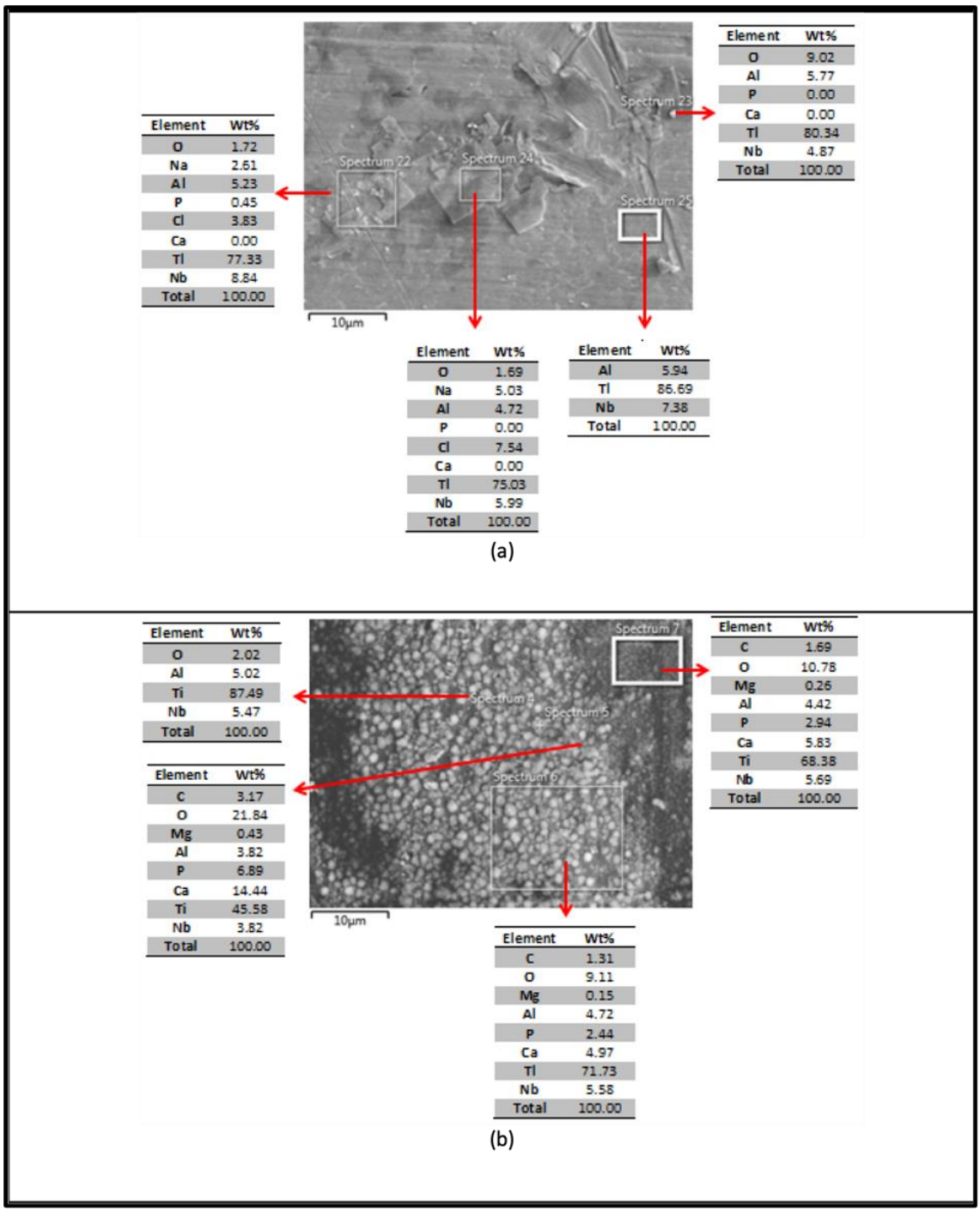

Fig. 7. EDX analysis results from the selected areas of sample surfaces following immersion in 3X SBF for (a) 1 day and (b) 7 days.

biomimetic coating test of this study indicate that, such a layer can be rapidly achieved by biomimetic coating applied a in densified simulated body fluid environment followed by the application of a preliminary surface treatment.

4. Conclusion

In the current study, it was aimed to systematically investigate the *ex situ* biocompatibility of Ti-6Al-7Nb, a promising alloy for biomedical applications. Results of the static immersion experiments conducted in synthetic body fluid environment indicated that even the untreated alloy surface is favorable the formation of Ca-P rich structures, starting from the early periods of immersion which is important for osseointegration. The oxide layer formation-dissolution cycle over time, which is important in terms of affecting ion release, was observed to reach a stable state around the 14th day of immersion. Although majority of the measured ion release levels were much lower than critically toxic or allergic values, the Al ion release level at the 1st day of immersion was above critical levels. This result indicated the need for the presence of a stable protective layer before implantation.

Findings of the static immersion in densified SBF part of the study, which aimed at the testing the biomimetic coating ability of the alloy exhibited that, formation of such a protective layer can be rapidly achieved by biomimetic coating applied in densified SBF environment. As compared to commonly used biomedical alloys whose bioactivity can be enhanced over complex surface treatments, rapid biomimetic capability of the tested TiAlNb alloy is another positive indication for its

biocompatibility (Hazwani et al., 2022; Wei et.al., 2023). Overall, the findings *ex situ* biocompatibility tests of the current study indicate that novel TiAlNb alloys are promising materials for biomedical applications, however in order to ensure their safe use in orthopedic implant applications, presence of a stable protective layer is required, prior to implantation.

Conflict of interest: The authors declare that they have no conflict of interests.

Informed consent: The authors declare that this manuscript did not involve human or animal participants and informed consent was not collected.

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Research article

Preparation and characterization of conductive blends of polyaniline with polyphenol red

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Abstract

Polymers are widely employed in biomedical applications, pharmaceutical product formulation, and drug delivery systems. Since every polymer has its own distinct properties, polymer blends will have novel chemical and physical properties. Functionally, the purpose of blending polymers is to improve, customize, or maximize material performance. In this study, polyaniline and polyphenol red polymer mixtures were prepared electrochemically and characterized with XPS imaging and SEM whether their distribution was homogeneous. The mixture of aniline and phenol red was deposited glassy carbon electrode (GCE) surface using the cyclic voltammetry technique in the potential range of -0.80 V to 2.00 V with 50 mV/s scan rate for 25 cycles. The phase separation of the two polymers was demonstrated by a combination of spectroscopic imaging and microscopy. For this purpose, the X-ray spot size and step number were set to 50µm. 1 x 1 mm^2 area scan of the polymer mixtures was performed, and spectra were obtained at each pixel in an array of 20 x 20 pixels. Chemical imaging was obtained by applying Principal Component Analysis (PCA) to collected XPS survey spectra. For the morphological characterization, scanning electron microscopy (SEM) was employed, and images were obtained at magnifications of 5000 x. The results obtained in the mixtures prepared with 5%, 10% and 25% were better compared to the mixture prepared with 50% polyphenol red. Since the X-ray spot size is limited, the desired image resolution could not be obtained. It was shown that XPS imaging studies could also be used for examining the distribution of different and unknown polymer mixtures together with SEM.

Keywords: *Chemical imaging; polyphenol red; polyaniline; polymer blend; principal component analysis; X-ray photoelectron spectroscopy*

1. Introduction

A true revolution in the field of polymers, conducting polymers are significant and novel materials. They have potential use in a variety of fields. Polyaniline (PANI) looks unique thanks to its exceptional electrical conductivity, biocompatibility, and lack of toxicity. PANI has some surprising properties, but its low solubility and processability have prevented it from being used in any practical applications so far. To overcome these limitations PANI-based composites, polymer grafting, and polymer blends have been developed (Pina and Falletta, 2022; Ghovvati et al., 2023; Morais et al., 2023; Nasir et al., 2023; Shokrollahi et al., 2023). All conductive blends of polymers (CPs) are prepared using two primary methods: chemical synthesis and electrochemical synthesis. The first method typically produces powders, but the second allows for the production of films. While chemical synthesis seems to be the best route for the large-scale production of CPs, the resulting polymers are difficult to work with and require structural modifications to improve their electronic properties. Conversely, conducting polymers represent a revolution in the field of polymers, since they represent an important and

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innovative class of materials. They qualify for employment in numerous industries. Polyaniline (PANI) is distinguished by its high electrical conductivity, biocompatibility, and low toxicity. Despite its outstanding characteristics, PANI has not yet found practical uses due to its poor solubility and processability. Electrochemical control of the polymerization process is more efficient, notwithstanding its ineffectiveness for large scale manufacturing (Li et al., 2012; Ogundele et al., 2023). PANI is composed of repeating aniline units. The presence of alternating -NH- groups and aromatic cycles provide compounds with distinct oxidation states. In contrast, phenol red (PR) is the usual pH indicator in many cell and tissue culture media since it allows for a rapid assessment of the culture's health. PR has also been utilized in several techniques to detect cellular hydrogen peroxide and human peroxidase enzyme activity (Morgan et al., 2019). The structures aniline and phenol red are presented in Fig.1.

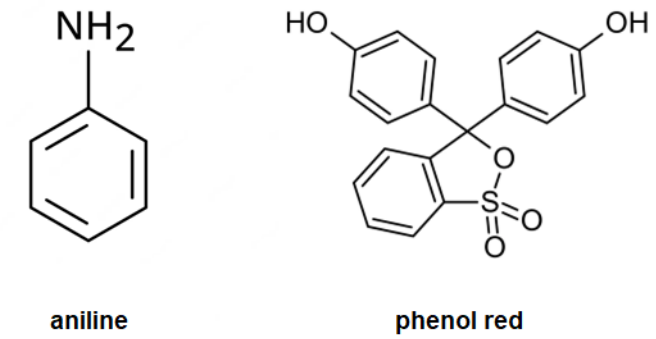

Fig. 1. Chemical structures and images of aniline and phenol red.

PANI can be used in a variety of biomedical contexts, including as an antioxidant, as an agent with antimicrobial and antivirus activity, as a drug delivery system, as a cancer therapy, and as biosensors etc. (Gizdavic-Nikolaidis et al., 2004; Boomi et al., 2014; Isakova et al., 2016; Zhao et al., 2017; Li et al., 2018; Liang et al., 2018; Ahmadkhani et al., 2019; Minisy et al., 2021; Ghovvati et al., 2023; Riaz et al., 2023). There is an ever-increasing demand for the creation of novel and more effective materials that can deliver drugs to the organs and tissues that are being targeted. One of the most important considerations relates to the requirement that there should not be an excessive amount of drugs or an insufficient amount of drugs in order to achieve a steady and well-controlled release of drugs over a specific amount of time (Pina and Falletta, 2022). Smart drug delivery systems are a promising tool in this regard because they integrate pharmaceutics, materials science, molecular biology, engineering, and other disciplines. The support can either actively contribute to the drug release mechanism or sit on the sidelines and play the role of an inert carrier. Among the various supports, CPs hold a significant position due to the extraordinary capabilities they possess, which are primarily associated with their one-of-a-kind characteristics, such as electroconductivity, pH sensitivity, light-sensitivity, and so on.

In very recent years nanofiber composites consisting of chitosan and PANI have been fabricated by in situ chemical polymerization (Minisy et al., 2021). Ketoprofen was able to be encapsulated within the composites due to the free volume space that the composites provided. It was demonstrated that the drug release is dependent on the pH of the medium, and that the release increased as the pH increased. On the other hand, working with a simulation of biological fluid yielded some encouraging results. By taking advantage of the sensitivity of PANI-based materials to changes in pH, a pH-electroactive bacterial cellulose/PANI hydrogel that allows for the controlled release of drugs was developed (Li et al., 2018). Chemotherapy for late-stage cancer is emerging. Accurate release control enhances drug accumulation in a specific target tissue in alternative cancer treatment. You et al. (2017) developed trastuzumab-modified PANI-mediated polymeric nanoparticles for tumour cell uptake. This was carried out to look into the possibility of a cis-platinum delivery that could be precisely controlled. CPs are utilized as transducers in biosensors due to the extraordinary properties that they possess, which include electroconductivity, redox properties, biocompatibility, and high levels of sensitivity. These properties allow for the CPs to detect changes in their environments with a high degree of accuracy. Shoaie et al. (2019) comprehensively discussed on PANI and PANI composite biosensor applications. Recent advances in PANI-based biosensors necessitate their use. Electrical stimulations are responsible for the regulation of most the human body's control mechanisms. In addition, electroactive materials have been extensively researched for potential applications in tissue engineering, with the goals of stimulating cellular responses and enhancing tissue regeneration. The properties of pure PANI are not suitable for use in any application involving tissue engineering.

The employment of a single polymer does not always meet the site-specific and time-controlled release profile requirements of a sophisticated drug delivery system, according to scientific literature. Even though polymers of various origins and of varying chemical natures have been introduced in biomedicine as carriers for the purposes of drug delivery, this remains the case. Polymer blends, from simple physical mixtures to more complex core-shell strategies and even polymeric block copolymers, will enable site-specific and rate-controlling drug delivery in pharmaceutical sciences. In spite of the fact that there is no one simple recommendation that can be applied in every situation, it would be helpful to apply various strategies that are relevant to the blending of polymers in order to overcome formulation limitations (Ghasemiyeh and Mohammadi-Samani, 2021; Barker et al., 2023; Yazie et al., 2023). Methods for blending polymers can lead to the formation of possible molecular interactions between the blended polymers, each of which has its own set of characteristics. The synthesis procedure needs to be improved to overcome cytotoxicity, processability, and physicochemical properties that prevent the clinical use of PANI and PANI-derivatives. When homopolymers are combined through the process of polymer blending, the resulting product may have altered physicochemical properties in comparison to those of the homopolymers by themselves. The production of miscible polymer mixtures is the primary benefit that comes from blending different types of polymers. Blended polymers' physical properties depend on both their homopolymer properties and their intramolecular interactions. Blending polymers can create a new product with a second functionality that can interact with other polymers and drugs (Jones et al., 2005).

In a wide variety of contexts, the surface properties of polymer blends are of critical importance. Because of the surface segregation of the component with the lowest surface energy, the physicochemical properties of polymer blends can be drastically different at the surface than they are in the bulk. In this work, X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM) have been used to

characterize surface and bulk properties since the surface properties and morphology of the prepared polymer blends directly affect their functionality.

2. Materials and methods

2.1. Chemicals and reagents

All reagents were of analytical reagent grade. Phenol red (PhR) ($C_{19}H_{14}O_5S$), aniline ($C_6H_5NH_2$) which is distilled two times for purification, perchloric acid ($HClO_4$), alumina (Al_2O_3) was supplied from Sigma Aldrich, Merck and BDH chemicals. All solutions were prepared using ultrapure water which produced from Millipore Milli Q system (18.2 Ω). All electrochemical experiments were performed at room temperature.

2.2. Preparation of polymer blends

GCE was polished alumina powders, rinsed and ultrasonicated in water prior to voltammetric measurements. Electrochemical cleaning was performed by keeping GCE in 0.1 M HCl, which was mechanically cleaned with alumina, at constant potential for 10 minutes. Cyclic voltammetric (CV) experiments were performed using Autolab 101 potentiostat/galvanostat and Nova Software. The electrochemical polymerization was carried out with a three-electrode system which consisted of homemade bare GCE, Pt wire and Ag/AgCl (sat. KCl) as working, counter and reference electrodes, respectively. Electrochemical polymerization of phenol red and aniline in 0.05 mmol L^{-1} $HClO_4$ solution was carried out by CV for 25 cycles between -0.5 V to 2.0 V (vs. Ag/AgCl) with 0.05 V s^{-1} scan rate. In order to obtain a polymer-coated nanocomposite surface, aniline and phenol red solutions using different ratios (5-10-25-50%) of monomer solutions were prepared.

2.3. CV, SEM and XPS instrumentation

Cyclic voltammetric (CV) measurements were carried out utilizing an Autolab 101 potentiostat/galvanostat and Nova Software. The electrochemical measurements were performed using a three-electrode system consisting of polyaniline and polyphenol red modified electrodes, Pt wire, and Ag/AgCl (sat KCl) as working, counter and reference electrodes, respectively.

To investigate the morphology of samples with SEM, the samples were coated with a gold (Au) layer that was few nanometers thick using the Leica EM ACE600 coating equipment (Leica Microscopes, Milton Keynes, United Kingdom). Since all the samples had previously been stored in a desiccator following the XPS studies, the typical drying step was not carried out. After being coated, the samples were analyzed using scanning electron microscopy (SEM, Thermo Scientific Apreo S LoVac SEM, Thermo Fisher Scientific, Waltham, Massachusetts, United States). The Everhart– Thornley detector (ETD) was used to record images of the scanned areas at magnifications of 5000 for each sample at 5kV.

Monochromatic Al Kα radiation (1486.7 eV) was employed in XPS (Thermo Scientific Model K-Alpha XPS - Thermo Fisher Scientific, U.K.) analysis of samples. The photoelectron binding energy scale referenced the 284.8 eV C 1s peak. Snap mode was used for each element and survey spectra scans were done at 200 eV with 1 eV step size. Analysis chamber pressure was 1 × 10^{-8} mbar. The electrodes were mounted on XPS sample holder (Ted Pella, Inc. USA) with double-sided sticky carbon tape. X-ray Spot and step sizes were 50 µm. The mapping captured 200 eV survey spectrum and 50 eV snapshot scans. Almost ~1 x 1 mm^2 area scan of the samples was made and spectra were obtained at each pixel in an array of 20 x 20 pixels. Chemical imaging was obtained by applying Principal Component Analysis (PCA) to the obtained data sets.

2.4. Chemical imaging by XPS using PCA

The Avantage Software, version 5.9925, was used to process each spectroscopic data with the aid of multivariate analysis. During XPS area analyses, both survey spectra and snapshot spectra for each element were obtained. Atomic percentages (at. %) were used to express the elemental composition of each pixel. After the data sets was quantified, principal component analysis (PCA) was performed on acquired survey-XPS spectra (~1000). Each pixel denotes the possibility of the data being processed as an XPS spectrum. The methodology that was presented by Erdogan et al., 2019; 2020; Erdogan, 2022 which was utilized for the purpose of identification. After that, an improvement was made to the overall image quality. The final images were layered on top of one another so that all the components in the sample could be seen as chemical images.

3. Results and discussion

3.1. Electropolimerization of aniline and phenol red

Fig. 2 shows multisweep CVs of electropolymerization on GCE surface in 0.05 molL^{-1} $HClO_4$ containing aniline and phenol red between -0.5 V to 2.0 V vs. Ag / AgCl (sat. KCl) at a scan rate of 0.05 V s^{-1} for 25 cycles. As seen in Fig. 2, two oxidation peaks, approximately 0.56V and 0.98V, were observed. The first one shows the transformation of PANI from the reduced leukoemaraldin (LE) state to the emeraldin (EM) state, while the second one, 0.98V, shows the transition from the

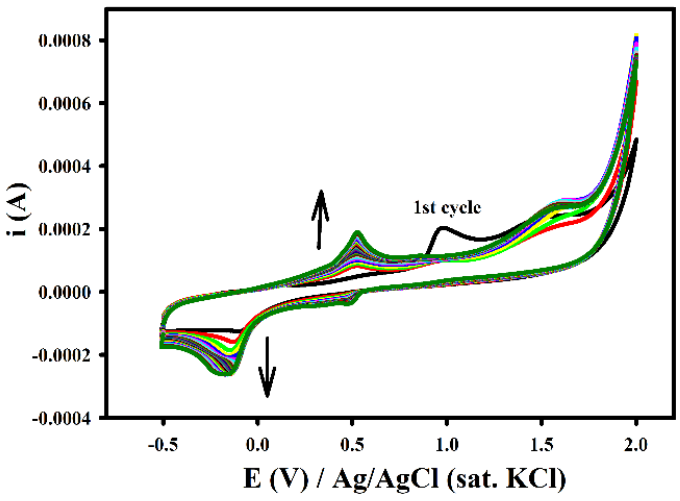

Fig. 2 Cylic voltammograms for aniline and phenol red in 0.05 molL^{-1} $HClO_4$ on bare GCE at 0.05 V s^{-1} scan rate with 25 cycles.

LE state to the pernigraniline (PE) state. It is also known that some dimers and intermediates such as p-benzoquinone, p-aminophenol are trapped in the polymer during polymerization

and the anodic bump at about 1.56V is attributed to the redox reaction of p-benzoquinone. The rise in redox currents throughout subsequent cycles indicates that the polyaniline film is growing successively (Hand and Nelson, 1974; Niu et al., 2003; Pournaghi-Azar and Habibi, 2007; Koluacik et al., 2018).

3.2. Surface analysis of polymer blends by XPS

The first information obtained from XPS is which elements are present on the surface of polymeric material. A survey, or wide-scan, spectrum over an energy range is recorded, with peaks for all elements (except H and He) in the periodic table. XPS survey spectra was collected for polyaniline and polyphenol red prior to mapping to discriminate the two polymers. In addition to survey spectra, valence band spectra are used to fingerprint polymers. Recently developed spectrometers with high counting rates and monochromators have made the study of valence bands extremely valuable for the identification of various materials. The valence-band spectrum comes from photoelectron emission from chemical bonding outer molecular orbitals. The valence band "fingerprint" energy ranges from 0 to 40 eV binding energy. Its complex band structure provides qualitative information about the chemical environment of the components that core line spectra cannot. These spectra may aid polymer identification. For this reason, both spectra were collected for two polymers. XPS survey spectra and valence band spectra for each polymer were presented in Fig.3.

According to the XPS measurements, polyaniline contains C, N and O whereas phenol red is composed of C, S and O as given in their chemical structures. Moreover, both polymers have unique valence band spectra which could be easily differentiated in area scan data processing. XPS area analyses were performed in the middle part of the electrode surface as depicted in Fig. 4. Based on the selected region, 1 x 1 mm^2 area was scanned.

The polymer blend compositions can thus be evaluated based on C, N, O and S percentages from the survey spectra, and the high-resolution C 1s, N1s and S2p photopeak shapes (data not shown). It is important to investigate the morphology of a polymer blend surface. However, the surface homogeneity can be confirmed by using XPS imaging which is also called "chemical imaging".

The use of PCA to do dimension reduction helps us simplify the entire spectrum data set by identifying the dimensions that are most prevalent within it. Following the use of PCA with the Avantage 5.9925 program in K-Alpha, one can obtain the PCA profiles (Fig. 5). PCA profiles can be designated in the form of images, as shown in Fig. 6. Photoelectron images at each individual pixel reveals the variations in concentration that occur in different locations. When PCA was applied to each polymer blend at varying concentrations, PCA1 corresponded to polyaniline and PCA2 to phenol red.

When the XPS images obtained for polyaniline mixtures are examined, although X-ray spot size is a limiting factor for XPS imaging, it has been quite successful in detecting whether polymer blends are homogeneously dispersed, especially at low concentrations.

The chemical images of polymer blends at different concentrations were designated in Fig.6. As the concentration increases the accuracy of distribution decreases due to resolution restrictions. However, with the development of new technology X-ray spot size could be decreased to 10 μm. Probably, in the future more detailed and high-resolution images will be obtained.

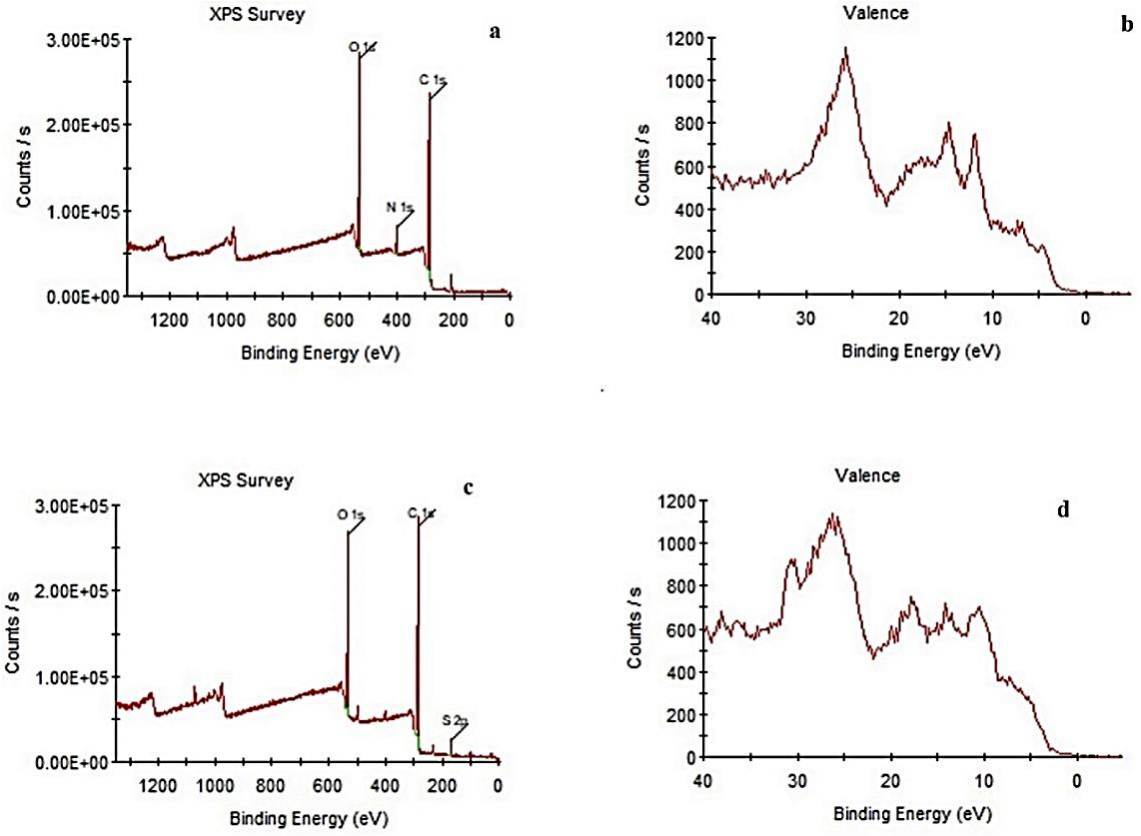

Fig. 3. XPS spectra for polymers (a, b) Survey and valence band spectra for polyaniline and (c, d) Survey and valence band spectra for polyphenol red.

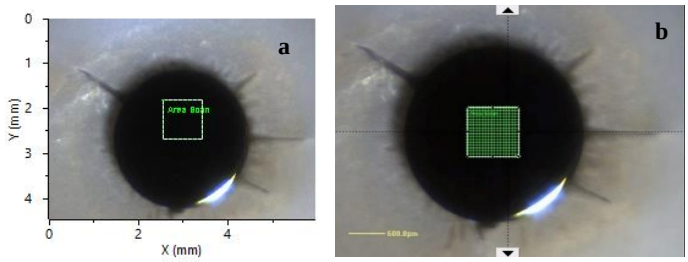

Fig. 4. Optical image of the scanned area (a) designated on a scale (b) pixel dimension (20 x 20).

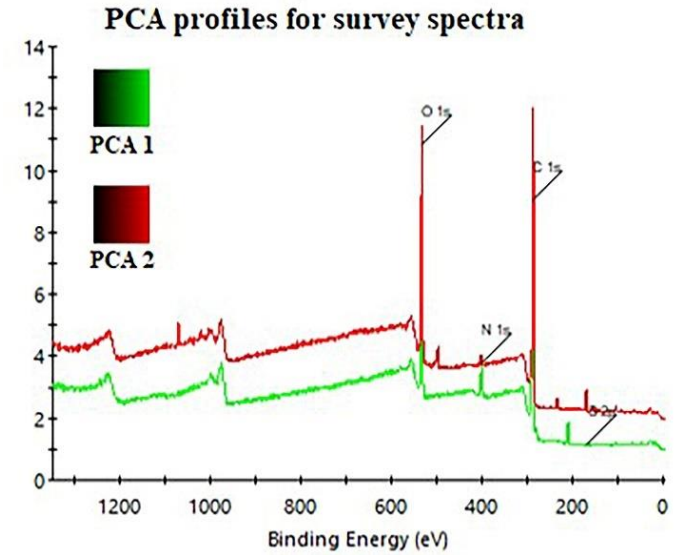

Fig. 5. XPS survey spectra of principal components for polyaniline and polyphenol red.

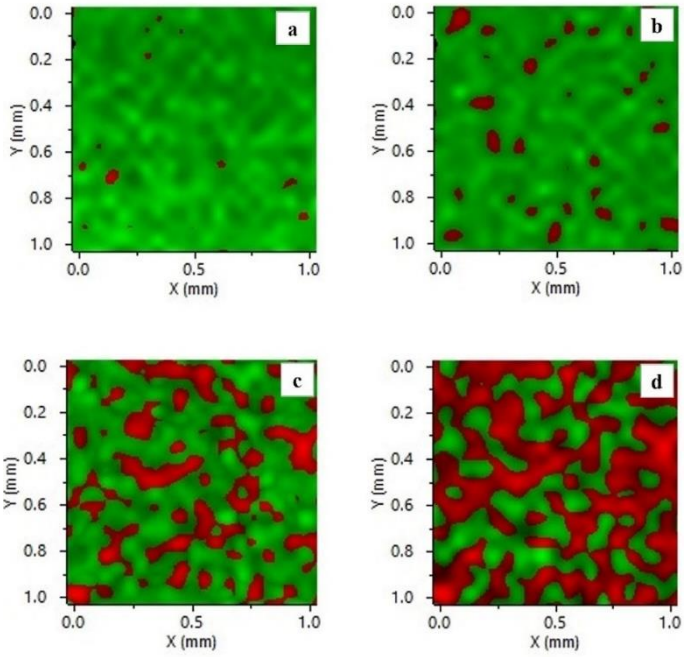

Fig. 6. XPS images of polyaniline containing phenol red (a) 5% (b) 10 % (c) 25% and (d) 50% by concentration (v/v).

3.3. Morphology of polymer blends by SEM

Due to its ease of use, specimen preparation, and image interpretation, SEM is the most popular microscopic technique. Owing to its ease of surface preparation, SEM can study all polymer structures. Surfaces of polymer blends frequently reveal important structural details and polymer distribution during preparation (Michler and Lebek, 2016). In the present study, SEM was used to give an idea about how accurate images were chemically obtained by XPS using PCA. In fact, SEM gives detailed information about the morphology of materials as well as their distribution on a small or large basis. Since XPS area scan was performed in the middle of the surfaces of the prepared samples, SEM images were also taken form the same region and with a magnification of 5000 x as presented in Fig.6. Based on SEM analyses, 5% and 10% phenol red added polymer blend areas tend to contain a significant amount of polyaniline and they are in accordance with the XPS images (Fig. 7). In addition, it is clear from these SEM images that there is phase separation occurring on the blend's surface. The data obtained from microscopy and spectroscopic imaging when combined demonstrate, as a result, that the two polymers in a mixture have separated into two distinct phases.

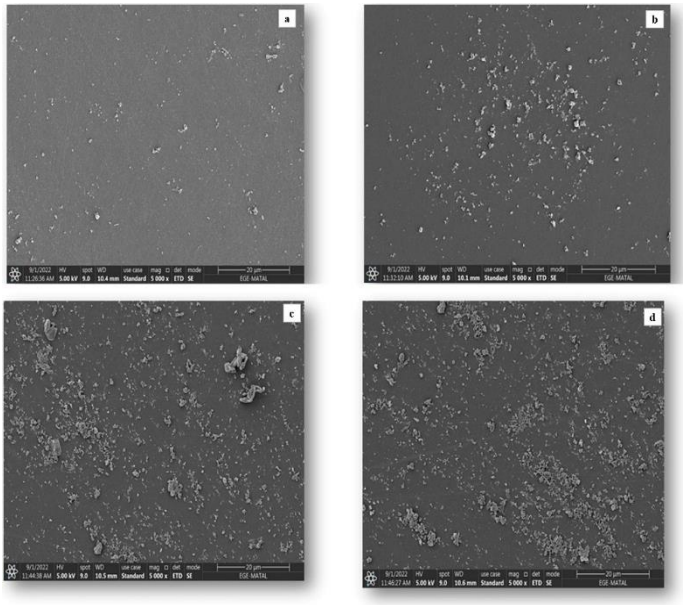

Fig. 7. SEM images of polyaniline containing phenol red (a) 5% (b) 10 % (c) 25% and (d) 50% by concentration (v/v).

4. Conclusion

In biomedical, pharmaceutical, and drug delivery applications, a single polymer often cannot perform a complex function. Blending polymers overcomes polymer limitations. Polymer blends enhance pharmaceutical and biomedical applications. Because many polymers are safe and have been used in pharmaceutical and biomedical products, many polymer blend combinations exist. To rationally select and use these polymer blends, polymer-polymer interactions must be well-understood.

This allows novel polymer blends to be designed and manufactured consistently to meet unmet needs. Pharmaceutical and biomedical applications of polymer blends and their characterization should be considered due to their localization and functionality.

In this study, chemical images of phenyl red-polyaniline mixtures prepared at different concentrations were analyzed by SEM and XPS. In addition, two different polymer mixtures were characterized by XPS and SEM. In addition, the distribution of

the polymer mixtures was determined by XPS imaging.

It should be noted that among the limitations of this technique is the X-ray spot size. This study demonstrated that XPS and SEM images acquired from the same areas on polymer blends could reveal whether the mixture is homogeneous or heterogeneous.

Both types of images were taken from the same locations on the polymer blends. The combination of the two methods results in a more comprehensive approach to the task of characterizing complex polymer blends that have distinct differences in their surface and bulk compositions.

Conflict of interest: The authors declare that they have no conflict of interests.

Informed consent: The authors declare that this manuscript did not involve human or animal participants and informed consent was not collected.

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Research article

The inhibitory effect of indisulam-coumarin combined therapy on glioblastoma multiforme

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Abstract

Cancer is a disease that occurs due to irregular growth and proliferation of body cells and can be caused by many factors. One of these factors is carbonic anhydrase 9 (CAIX). While its expression is high in malignant cells, it is a molecule whose presence is difficult to detect in healthy tissues. Glioblastoma multiforme (GBM) is one of the fast-spreading brain cancers, and unlike healthy tissues, overexpressed CAIX in its cell receptors. Indisulam, one of the new-generation drug candidates for the treatment of solid tumours, is a type of CAIX inhibitor that affects cell division progression in human tumour cells. Similarly, it is known that coumarin, as one of the new-generation drugs in cancer treatment, is used together with chemotherapy. In this study, combined treatment of indisulam and coumarin was investigated on glioblastoma multiforme cells to evaluate their cytotoxicity, cell migration and antiproliferative effects. The effects of combined treatment on cell migration and proliferation were investigated with the IC_{50} values determined after the cytotoxicity test. As a result of the wound healing assay, it was determined that the control cells were closed by 69.6%, while the combined treatment closed the wound by 32% and seriously prevented cell migration. The percentage of proliferative cell nuclear antigen (PCNA) positive cells decreased significantly after combined treatment, cell proliferation was 93% in the control group and 77% in the combined treatment group.

Keywords: *Cancer; carbonic anhydrase IX; coumarin; indisulam; glioblastoma*

1. Introduction

Glioblastoma multiforme (GBM) is a primary brain tumour with a genetically and phenotypically heterogeneous group of tumours (Lam et al., 2000; Karcher et al., 2006). GBM has aggressive characteristics such as rapid proliferation, invasion into brain tissue, necrosis, angiogenesis, microvascular proliferation, and migration. The development of GBM is associated with the deregulation of the G1/S checkpoint in cell division and the emergence of numerous genetic disorders in glioma cells (Robert and Wastie, 2008; Hanif et al., 2017). The histological characteristics of glioma models, such as foci of tumour necrosis, nuclear polymorphism, and a high mitotic index, are like those of human GBM and Wistar rat GBM. When C6 cells are implanted into the brains of newborn rats, they mimic human GBM (Giakoumettis et al., 2018). The C6 glioma models investigate several biological processes, including the development, dissemination, migration, angiogenesis, control of growth factor production, and disruption of the blood-brain barrier in tumours (Giakoumettis et al., 2018; Li et al., 2020).

CAIX is a transmembrane glycoprotein that consists of an ECD, a single-pass transmembrane region, and an intracellular tail. It is one of three exofacial CA isoforms, along with CAIX, CAIV, and CAXII, that is closely linked to cancer. It has a unique position in the enzyme family due to its expression pattern associated with hypoxia. Required for energy metabolism of most tumour cells during hypoxia, glycolysis results in accumulation of lactic acid, lowering of pH, and acidification of the environment. The acidic microenvironment, which is characteristic of solid tumours with hypoxic regions, is

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associated with tumour invasiveness and adversely affects anticancer therapy (Stubbs et al., 2000; Gatenby and Gillies, 2004; Kroemer and Pouyssegur, 2008).

The sulfonamides are bonded in a tetrahedral geometry. In its deprotonated forms, it interacts directly with catalytic zinc and inhibits CA activity by displacing zinc-bound water hydroxide ions. Due to its chemical structure, indisulam is one of the best examples of carbonic anhydrase inhibitors (Cuffaro et al., 2020). The inhibitory effect of indisulam, a new anti-cancer drug currently in phase II clinical development for the treatment of solid tumours, is associated with a cell division arrest in the G1 phase. Specific inhibitors such as indisulam establish a definitive pathway for CAIX in tumorigenesis, making the mechanism reversible. Therefore, selective CAIX inhibitors illuminate the role of CAIX in controlling pH imbalance in tumour cells in hypoxic cancers and developing diagnostic therapeutic applications for tumour management (Supuran, 2003).

Coumarins are polyphenolic chemicals that are members of the group of colorless heterocyclic compounds discovered in the plant *Dipteryx odorata* Wild. Coumarin-based carbonic anhydrase inhibitors are studied in association with the tumour-associated isoforms CAIX and CAXII. Apart from being a CAIX inhibitor, coumarin has a wide range of pharmacological effects such as anti-tumour, anti-coagulant, anti-inflammatory, antioxidant, anti-HIV and anti-bacterial. Recent studies highlight the potential for use of gambling CAIs in combination with standard chemotherapies in cancer therapy (Touisni et al., 2011; Sumorek-Wiadro et al., 2020).

CAIX inhibition is attributed to coumarin among the selected active ingredients (Ismail, 2023). A series of sulfonamide designs containing coumarin moieties have been discussed for combining these two active ingredients. Also, indisulam is an orally active agent that has anti-cancer properties and induces p53 and p21 through the downregulation of cognate cell division checkpoint molecules. It is a sulfonamide anti-tumour agent (Moncao et al., 2022). The combined use of indisulam and coumarin active ingredients has been evaluated by Chahal et al. (2022) with a database that predicts the most likely macromolecular targets of a presumed bioactive small molecule by using Swiss Target Prediction tool of Swiss Institute of Bioinformatics.

This study investigates whether the two compounds chosen are active on the CAIX enzyme. The dynamic table in the database provides the predicted target molecules for the queried molecule. The result obtained indicates the probability of targeting indisulam on CAIX and coumarin on CAIX (Teixeira et al., 2021; Pontecorvi et al., 2022). When the required scanning was performed for coumarin, the probability ratio against the CAIX enzyme was 0.86. As a result of scanning for indisulam, the probability ratio against the CAIX enzyme is 0.83. A probability value of 1 indicates that targeting is quite strong (Chahal and Kakkar, 2023).

In this study, indisulam+coumarin combined therapy was investigated on GBM cells in terms of cytotoxic effect, wound healing and antiproliferative properties. The main reason for the preference for the indisulam+coumarin combination was their anticancer activities. Carbonic anhydrase IX inhibition is attributed to coumarin among the selected active ingredients (Ismail, 2023). A series of sulfonamide designs containing coumarin moieties have been discussed for combining these two active ingredients. Also, indisulam is an orally active agent that has anti-cancer properties and induces p53 and p21 through the downregulation of cognate cell division checkpoint molecules. It is a sulfonamide anti-tumour agent (Moncao et al., 2022). When comparing the results of the combined treatment with indisulam and coumarin, the cytotoxic effect exhibited similarity to indisulam, surpassing the effectiveness of coumarin. Combined therapy decreased wound healing in cancer cells compared to the control group and significantly reduced proliferation. Indisulam+coumarin combined therapy was overall more effective than single-drug therapy for C6 GBM cells.

2. Materials and methods

Indisulam, coumarin, thiazolyl blue tetrazolium bromide %98 (MTT), trypsin EDTA and DMSO were obtained from Sigma Aldrich (St. Louis, USA). Gibco provided the Dulbecco's Modified Eagle Medium (DMEM) / F-12 Nutrient Mixture (Ham), L-glutamine, Penicillin-streptomycin (PEST), and Fetal Bovine Serum (FBS). The C6 glioblastoma cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Thermo Scientific™ supplied the proliferating cell nuclear antibody (MS-106-P). All the chemicals and solvents used were of analytical quality and were not purified further.

2.1. Cell culture studies

C6 glioma cells (ATCC-CCL-107) were cultured in a Dulbecco's Modified Eagle's Medium (DMEM)/Ham's Nutrient Mixture F-12 medium (1:1) with 5% fetal bovine serum (FBS), 100 µg/mL Penicillin-Streptomycin and 0.2 mM L-Glutamine. Cells were kept at 37°C in a humidified incubator with 5% CO_2. Fresh growth media was used to replenish the cells' growth medium 2 to 3 times each week. During the incubation period, the cells were passaged every two to three days.

2.2. Cytotoxic effect of indisulam, coumarin and indisulam+coumarin combine therapy

The MTT 3-(4,5-dimethyl-thiazolyl 2,5-diphenyltetrazolium bromide) is a sensitive reliable indicator of cellular metabolic activity and is used to determine and measure cellular metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity (Welder et al., 1991). To determine cell viability levels and cytotoxic activity of indisulam, coumarin and indisulam+coumarin combined therapy on C6 cells, MTT assay was used.

Confluent C6 cells were removed from the plate by trypsinization, and they were seeded at $1x10^4$ cells/mL in 96-well culture plates. The microplate was incubated at 37°C in a 5% CO_2 incubator for 24 hours. At the end of the incubation time, the medium was replaced with a fresh medium containing different concentrations (10, 25, 50, 100, 250, 500, µM) of indisulam, coumarin and indisulam+coumarin combinations, and no application was made to the control group. Microplates were incubated for 24 and 48 hours, and at the end of the incubation times, the medium was discarded from each well and 50 µL of MTT reagent was added. Cells were incubated for 3 hours at 37°C with 5% CO_2. After 3 hours, the MTT solution was removed from the cells and the formazan crystals were dissolved by adding 100 µL of DMSO. Absorbance values (OD) were measured with a microplate reader at 570 nm and the cell

viability value was calculated with the following equation after 3 repetitions.

Cell Viability (%) = OD570e / (OD 570b) * 100

OD570e: It is the absorbance value of the samples at 570 nm.

OD570b: It is the absorbance value given by the control group at 570 nm.

The concentration values were calculated that caused 50% inhibition (IC_{50}) of cells with the % cell viability results obtained.

2.3. Wound healing assay

A wound healing test is a common *in vitro* method for studying collective cell migration. In this test, a cell-free region is formed in a confluent monolayer either by physical, mechanical, thermal, or chemical damage (Jonkman et al., 2014). C6 glioblastoma cells were seeded in a 24-well plate as a $1x10^5$ cells/well and incubated for 24 hours until cells attached to the plate to form a confluent monolayer. A sterile 200 µL pipette tip was used to make a physical scratch in the center of each monolayer cell well. To eliminate non-adherent cells and debris, cells were gently washed with new media. The IC_{50} values for indisulam, coumarin, and indisulam+coumarin combinations were applied to the wells. Images were obtained immediately after injury (0 h), and 6 hours, 12 hours, and 24 hours afterward. GraphPad Prism was used to quantify the closure distance of the scratch after randomly selecting photos from each well using phase-contrast inverted microscopy (X10 magnification).

2.4. Determination of cell proliferation with PCNA method

The proliferating cell nuclear antigen is an evolutionarily well-conserved protein found in all eukaryotic species and Archaea (Strzalka and Ziemienowicz, 2011). PCNA was first shown to function as a transactivation factor of DNA polymerase δ, which is required for DNA synthesis during replication (Prelich et al., 1987). PCNA gene expression is involved with cell proliferation and consequently DNA synthesis during genome replication during the S phase of cell division in all species. PCNA forms a homotrimeric ring around the double helix of DNA and acts as a movable sliding clamp to attract other proteins involved in cell division control as well as DNA synthesis and repair (Yu et al., 2013). PCNA can stop damaged cell DNA replication and repair the damage. In cells that proliferate, that is, increase because of cell growth and cell division, PCNA levels rise in the middle of the G1 phase and remain high throughout the S phase. For PCNA, the DNA replication protein PCNA, which is synthesized in the G1 and S phases of the cell division, shows proliferation with the presence of anti-PCNA monoclonal antibodies. PCNA is a protein used in the analysis to show the proliferation rate. Cells in all phases of the cycle are marked with PCNA immunocytochemistry (Matsumoto et al., 1987). Cells in all phases of the cycle are marked with PCNA immunocytochemistry.

Proliferation was determined using the PCNA antibody and the Invitrogen Histostain-Plus Kit (Cat:85-9043). C6 cells were grown as $1x10^5$ cells/well on sterile coverslips in a 24-well plate and incubated for 24 hours until cells joined to the plate to create a confluent monolayer. The cells were then treated with 24h IC_{50} values of indisulam, coumarin, and indisulam+coumarin and incubated at 37°C for 24 hours. The media was taken from the wells at the conclusion of the incubation time, and the cells were rinsed with phosphate-buffered saline (PBS). For 5 minutes, the cells were fixed in ice-cold methanol. After the samples fixed with methanol, they were washed with PBS for 5 minutes, a blocking solution was added to each sample and incubated at room temperature for 20 minutes. After blocking, PCNA primary antibody prepared in PBS, and it was added to the samples at a ratio of 1/150. Antibody-added samples were incubated at room temperature for 2 hours and they were washed 3 times for 5 minutes with 1xPBS. Following washing with PBS, a biotinylated secondary antibody was added to the samples and incubated for 20 minutes at room temperature and washed 3 times for 5 minutes with 1x PBS. After the washing process, Streptavidin-peroxidase (HRP) was applied to the samples and incubated for 20 minutes at room temperature and washed again with 1x PBS for 3x5 minutes. After the last washing process, AEC chromogen (Invitrogen, USA) was added to the samples and incubated for 10-20 minutes at room temperature. Cells labelled with PCNA were stained and hematoxylin-eosin was used as counterstain. Coverslips, containing the PCNA positive and negative cells, was covered on the slides with a mounting medium and analysed under a light microscope. Proliferation levels were determined by identifying marked and unlabeled cells in 10 randomly selected areas at 100x magnification with an immersion oil objective. The percentage of immune-reactive cells was calculated with the formula.

Cell Proliferation (%) = (Number of PCNA positive cells)/(Number of total cells) *100

2.5. Statistical analysis

GraphPad Prism software was used for all statistical analyses (GraphPad Software, La Jolla, CA). Data were presented as mean standard deviation (SD), and ANOVA was used to compare groups, with $p<0.05$ deemed statistically significant. ns indicates that the p-value is greater than 0.05 and is statistically insignificant; *, **, ***, and **** represent increasingly significant statistical data, respectively.

3. Results and discussion

3.1. Cytotoxic effect of indisulam, coumarin and indisulam+coumarin combine therapy

In a study using indisulam as the active ingredient, the application of indisulam impaired a cell division progression of P388 murine leukemia cells in the G1 phase. Complete regression of advanced LX-1 tumours was observed in 80% of mice treated with E7070 (Ozawa et al., 2001).

The long-term survival of a patient with metastatic melanoma treated with indisulam is elaborated in the study conducted by Baur et al. (2007) supports this study and the patient with metastatic malignant melanoma was treated at varying indisulam doses. While the tumour burden was quite high at the start of treatment, after 2.5 years of treatment with Indisulam, the tumour appeared to shrink significantly. Finn et al. (2002) conducted a comparative trial of paclitaxel, doxorubicin and coumarin anti-cancer agents in HEp-2, HCT-8 and Caco-2 cell lines. Coumarin alone has been shown to be less effective in growth inhibition in all three tumour cell lines. However, it has been revealed that coumarin, which creates different mechanisms in each cell line, has lower cytotoxicity

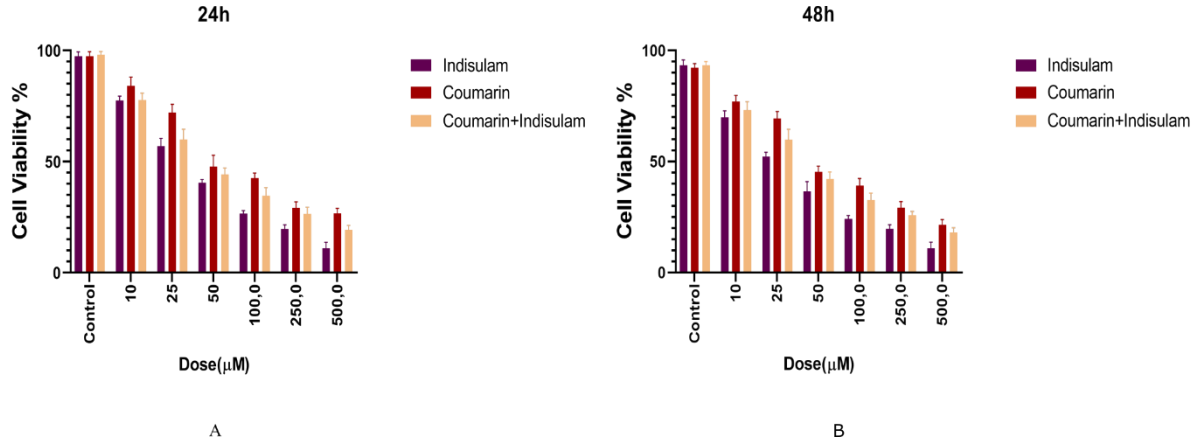

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Fig. 1. The IC_{50} values of indisulam, coumarin and indisulam+coumarin treatment on C6 glioma cells at the end of the 24th (A) and 48th (B) hour with the cytotoxicity. $IC_{50\ Coumarin}$: 45.98 µM, $IC_{50\ Indisulam}$: 32.64 µM, $IC_{50\ Combined}$: 39.11 µM for 24th hour; $IC_{50\ Coumarin}$: 39.80 µM, $IC_{50\ Indisulam}$: 28.34 µM, $IC_{50\ Combined}$: 31.41 µM for 48th hour.

compared to other anti-cancer drugs applied.

In this study, the IC_{50} values of indisulam in C6 Glioma cells were determined as 32.64 µM and 28.34 µM for 24 and 48 hours, respectively (Fig. 1A-B). In support of the experimental data obtained, it is seen that similar IC_{50} values were obtained because of MTT in the HCT116-C9 human colon cancer cell line (Oda et al., 2003).

The IC_{50} values of coumarin in C6 Glioma cells were determined as 45.9 µM and 39.80 µM for 24 and 48 hours, respectively (Fig. 1A-B). Experimentally obtained IC_{50} values are consistent with the study realized by Gkionis et al. (2021) comparing the cytotoxicity of coumarin analogues on breast cancer.

The combined application has been preferred for the purpose of providing both cytotoxicity balance and therapeutic effect. While indisulam alone has limited anti-tumour effects, studies evaluating its effects in combination with other chemotherapeutic agents have provided significant results. Because of this coumarin is included in this study as the before mentioned bioactive and chemotherapeutic agent (Touisni et al., 2011). The IC_{50} values of indisulam+coumarin combined treatment on C6 glioma cells were determined as 39.11 µM and 31.41 µM for 24 and 48 hours, respectively (Fig. 1A-B). In the literature, there is no study yet on the C6 GBM cells with combined treatment of indisulam+coumarin.

3.2. Cell proliferation assay with PCNA method

PCNA immunohistochemical analysis was performed to determine the effect of indisulam, coumarin and indisulam+coumarin combined treatment on the proliferation on C6 Glioma cells. In the application with 24-hour IC_{50} doses, it was observed that indisulam, coumarin and indisulam+coumarin treatment decreased the proliferation rate of the cells compared to the control group.

A study involving mice demonstrated that coumarin inhibits cell proliferation in skin papillom (Bhattacharyya et al., 2010). Another study reported that coumarin ameliorates benign prostatic hyperplasia by inhibiting the progression of G1/S phase cell turnover (Imani et al., 2021). It was also clarified that indisulam disrupts cell proliferation by targeting RBM39, a crucial mRNA splicing factor (Bussiere et al., 2020).

In this study, the percentage of proliferation was 93% in the control group compared to the indisulam and coumarin,

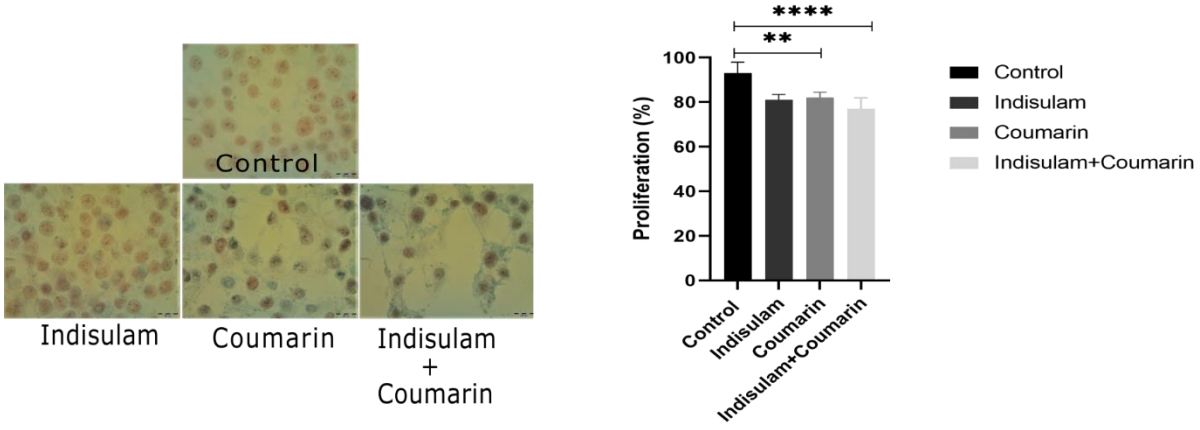

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Fig. 2. Indisulam, coumarin, and indisulam+coumarin therapy inhibited the proliferation of C6 Glioma cells using proliferating cell nuclear antigen (PCNA) labeling. Quantification of PCNA-positive immunostained cells demonstrated that the indisulam+coumarin combination therapy significantly reduced cell proliferation for 24 hours compared to the control group (****$p \leq 0.0001$) (B). PCNA-positive tagged cells (red) are visible in the brightfield microscope pictures (A). The data were reported as means standard deviations (n=3) for each group.

which were 81% and 82%, respectively. While these two compounds alone did not make a significant difference, when applied together, the percentage of proliferation decreased by 23% and was determined as 77% (Fig. 2B). This shows that the combined therapy suppresses the growth of cancer cells compared to single-dose therapy. According to the results obtained, the combined therapy of coumarin and indisulam on C6 glioma cells significantly reduces the proliferation rates of cells entering the progression of the cell division and causes the cells to be more susceptible to apoptosis after these active substances applications.

3.3. Wound healing

Wound healing assay has been preferred because it is low cost and the easiest test for cell migration *in vitro*. This method allows to simulate cell migration of an artificial wound created with a pipette tip and to examine cell-cell, active substance-cell interactions (Rodriguez et al., 2005). The most common information to come from the wound healing assay is the gap closure rate as a measure of the collective movement rate of cells. Wound healing was determined as 69.6% in the control group. Sayed et al. (2021) was stated that sulfonamides, which are the group including indisulam, can be used to reduce wound infection in addition to providing wound healing on rats. Lee et al. (2006) determined that certain coumarin molecules synthesized by different methods showed growth inhibition activity against HUVEC cells.

In this study, there was 57.38% wound closure with coumarin therapy, and 50.94% wound closure with indisulam therapy. The closure value of the combined application group was determined as 32% (Fig. 3). The reason for the almost complete closure of the wound created with a pipette tip in the control group in the C6 glioma cell line after 24 hours is the lack of an anti-tumour agent. The reason cells treated with indisulam and coumarin showed less closure at the end of 24 hours is that it causes inhibition of cell migration in a concentration-dependent manner. Microtubule binding agents are exemplary as anti-migration agents. In the study in which the effectiveness of migration inhibitors was measured, it was stated that active substances with anti-cancer activity such as doxorubicin and paclitaxel prevented wound healing compared to the control group (Wang et al., 2019). The combination of indisulam and coumarin can be classified as an anti-migration agent in tumour cells.

4. Conclusion

Indisulam inhibits cyclin-dependent kinases (CDKs), which control cell division progression and are frequently overexpressed in cancer cells. Inhibiting CDK causes cell division G1/S phase arrest, which can lead to induction apoptosis and tumour cell proliferation inhibition. It has been determined that coumarin induces apoptosis by causing morphological changes in human cervical cancer HeLa cell line studies (Chuang et al., 2007). The most important reason the coumarin active ingredient is preferred is that it is a natural inhibitor. Anti-tumour mechanisms of coumarin-derived compounds are remarkably diverse; inhibition of carbonic anhydrase (CA), targeting of PI3K/Akt/mTOR signaling pathway, inhibition of multi-drug resistance (MDR), and induction of apoptosis are some of them (Feitelson et al., 2015). A common effect of coumarin and indisulam is inhibition of CAIX. Inhibition of CAIX is another target because it is highly expressed in tumour cells (Fukuoka et al., 2001). The main goal is to identify less toxic and more effective anti-cancer agents. In these contexts, indisulam and coumarin were preferred in this study among the CAIX enzyme inhibitors, whose expression increased in the tumour microenvironment and in the tumour region (Abel and Baird, 2018).

According to the obtained data, indisulam and coumarin have anti-proliferative and cytotoxic activity in the C6 glioma cell line. But also, indisulam+coumarin combined therapy results showed us that combined therapy is more effective on C6 glioma cells. IC_{50} values were determined as 39.11 μM for 24th hour and 31.41 μM for 48th hour, respectively for combined therapy. As a result of the wound healing and proliferation studies with IC_{50} doses, it was determined in wound healing that the control cells closed at the rate of 69.6%, while the combined treatment closed the wound by 32% and seriously prevented cell migration. On the other hand, the percentage of proliferative cell nuclear antigen (PCNA) positive cells decreased significantly

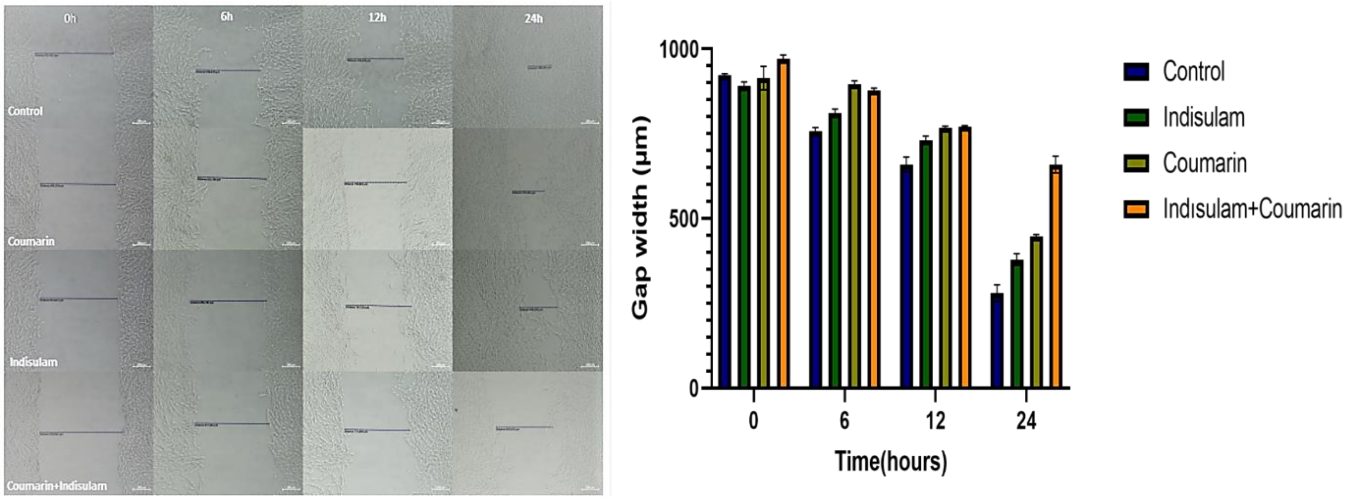

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Fig. 3. Effect of indisulam, coumarin and indisulam+coumarin combine treatment on C6 glioblastoma cell migration was determined by wound healing assay (A). Quantification of migration after 6, 12 and 24h treatment with indisulam, coumarin and indisulam+coumarin (n = 3) compared to control (B).

after the combined treatment, with cell proliferation regressed from 93% in the control group to 77% in the combined treatment group.

The results of the study show that the combined therapy of indisulam and coumarin can be used as an alternative chemotherapeutic therapy in the treatment of brain tumours. Inhibition of the CAIX enzyme, which participates in the common mechanism of action of indisulam and coumarin, in the tumour microenvironment may lead to original studies that will shed light on alternative treatments. The results of this study should be enriched with *in vivo* and clinical trials.

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Research article

Investigation of antimicrobial effects of zinc-based nanoparticles on food-borne pathogens

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Abstract

In this study, the antimicrobial effects of three different zinc-based nanoparticles, namely zinc oxide (ZnO), zinc chloride ($ZnCl_2$), and zinc ferrite ($ZnFe_2O_4$), on food-borne pathogen were investigated. ZnO and $ZnCl_2$ nanoparticles were obtained as commercially, but $ZnFe_2O_4$ nanoparticles were produced via sol-gel auto-combustion method. From the XRD results of $ZnFe_2O_4$ nanoparticle, it was found that all the peaks agreed with the literature. However, there was also small amount of the secondary phase peaks corresponding to the ferrite (Fe_2O_3) phases. Significant differences were observed between the inhibition effects of nanoparticles on bacteria in the disc diffusion method (p<0.005), except for the $ZnFe_2O_4$ nanoparticle, which has no effect on bacteria at the used dose. ZnO nanoparticle was observed to have the lowest inhibition zone on the Gram-negative bacterium *Campylobacter jejuni* of inhibition compared to other test bacteria. It was found that $ZnFe_2O_4$ had the highest value of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against Gram-negative bacteria.

Keywords: *Antibacterial activity; food-borne pathogen; nanoparticle; sol-gel; zinc oxide*

1. Introduction

Considered to be a new revolution in the 21st century, nanoscience and nanotechnology are seen at the forefront of modern and new studies (Sebastian and Gimenez, 2016; Al-Byati and Al-Duhaidahawi, 2023). Research and innovations in the field of nanotechnology contribute to many sectors such as medicine, health services, energy, defense industry, agriculture, food, textile, and cosmetics (Bhushan, 2010; Erkoc, 2012; Singh, 2017; Malik et al., 2023; Yalcin, 2022). It is thought that it is very important to produce functional foods with bionanotechnology and nanotechnology, which are advancing at a great pace. Nano-emulsion structures, nanoparticles, biopolymeric nano-composite materials, nanofibers, nanotubes, and nanosensors consisting of macromolecules in the structure of foods can also be used in applications on foods with different purposes. It has been reported that the substances in the structure of foods can have the desired properties at molecular levels. It is possible to develop different and new food products by using the mechanical and sensory properties of the food, and while the protection of the products and the extension of their shelf life are provided in the packaging with nano composites, the safety of the foods will be under control with the help of nanosensors (Tarhan et al., 2010; Ansari, 2023).

As an application area of nanotechnology science in the food industry, various topics such as detection of pathogens in foods, keeping food safety at a high level, development of antimicrobial packaging methods, controlled distribution with the transport of bioactive substances and production of functional foods can be listed (Robinson and Morrison, 2009; Siddiqui et al., 2023; Zhang et al., 2023).

Many different antimicrobial biomaterials are produced that are used in the prevention of diseases caused by infection, in the preservation of the long shelf life of the structures of medicines, food and cosmetics/dermo-cosmetic products, and in the prevention of biofilm formation in medical implants

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(Kanematsu and Barry, 2015).

In addition to being primary or secondary metabolite products of plants and animals or microorganisms, substances such as metal or nonmetal compounds, minerals, alloys, and mixtures may also have antibacterial properties. On the other hand, it has been possible to develop antibacterial carrier systems through chemically synthesized or purpose-built polymers, biopolymers, metal or metal oxide nanomaterials, carbon nanotubes, nanoemulsions and nanocrystalline structures (Bueno, 2015). It is known that the demand for high-quality and safe foods is increasing day by day. The presence of pathogens in food products is not desirable and is one of the leading causes of foodborne diseases worldwide (Mshelia et al., 2023). The presence of pathogens such as *Clostridium* spp., *Escherichia coli*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Campylobacter* spp., *Listeria monocytogenes* and *Salmonella* spp. are the most important microbial hazards found in food products. Pathogenic bacteria such as *Clostridium* spp., *Yersinia* spp., *Salmonella* spp., *Escherichia coli* and *Staphylococcus aureus* are the causes of numerous diseases related to gastric and intestinal symptoms such as vomiting and diarrhea (Friedman et al., 2002; Demirci et al., 2008; Negrut et al., 2020). Concerns about food safety are observed due to the increase in food-borne diseases caused by pathogenic microorganisms.

In addition, problems arising from chemical preservatives and artificial antibacterials used to inactivate or prevent the growth of pathogenic microorganisms that cause food safety risk are also observed (Turner et al., 2007; Chowdhury et al., 2023). Therefore, the use of natural antibacterials is important to keep microorganisms under control (Tajkarimi et al., 2010; Li et al., 2022). Although the presence and concentration levels of metals in the ecosystem, whose concentrations in the biological system produce toxic effects after a certain level, have been investigated by many scientists, their interaction with nanoparticles, which are increasing day by day, and the toxic effects they cause are a very important subject of research today (Lazar et al., 2022; Morais et al., 2022). Although heavy metals and nanoparticles have very common uses, they can also be found as mixtures in nature. Therefore, it is thought that it is very important to investigate the negative aspects of the pollutants in mixtures on the ecosystem and the lives of living things.

Zinc (Zn), which is an essential trace element for the normal development and growth of living things, is an essential food source for all living organisms and is a heavy metal that has duties in many enzymatic activities (Palmgren et al., 2008; Asad et al., 2023). Zn is used in the automotive industry and galvanizing to make casting molds. Apart from this, $ZnCl_2$ is also known to be used in the textile industry, as an activator in the tire industry, as a disinfectant and in the construction of battery bodies. ZnO structures in nanostructure are one of the favorite research topics of the researchers due to their optical and electrical properties (Chen et al., 2016; Khan et al., 2016; Sutradhar and Saha, 2016; Santhoskumar et al., 2017). ZnO nanoparticles are known to inhibit the growth of different bacterial strains and exert a cytotoxic effect against many cancer cells (Hanley et al., 2008; Nair et al., 2009; Premanathan et al., 2011). ZnO nanoparticle is known to be used as an additive in sunscreens, fertilizer, dyes, toothpastes, food, and food packaging (Baker et al., 2014; Shetty et al., 2015; Zhang et al., 2015; Kuang et al., 2016). Among the spinel ferrite compounds, $ZnFe_2O_4$ is used in soft magnet applications at high frequency values due to its different structures consisting of inverse, normal and mixed spinel structures and their high electromagnetic performance, excellent chemical stability, mechanical hardness, medium saturation magnetism property and their physical properties have been studied extensively (Naseri et al., 2012; Heiba et al., 2022; Badiger et al., 2023; Garg et al., 2023; Joshi et al, 2023). Recently, much research on Zn-based nanoparticles have been performed in the various field (Hatami et al., 2023; Kavitha et al., 2023; Senturk et al., 2023; Yalcin et al., 2023). The aim of this study was to evaluate the antimicrobial activities of different zinc nanoparticles (ZnO, $ZnCl_2$ and $ZnFe_2O_4$) on Gram-negative and positive food-borne pathogens.

2. Materials and methods

2.1. Bacteria and nanomaterials used in the study

Standard bacterial cultures such as *Yersinia enterocolitica* (NCTC 11175), *Listeria monocytogenes* (ATCC 19112), *Campylobacter jejuni* (ATCC 33560), and *Staphylococcus aureus* (ATCC29213) were used as food-borne pathogenic bacteria in the study. In the study, zinc chloride ($ZnCl_2$, Sigma Aldrich 229997) and zinc oxide (ZnO, Sigma Aldrich 209998) nanoparticles were used as stocked up, while $ZnFe_2O_4$ nanoparticles were prepared by sol-gel auto-combustion method (Ergin et al., 2023).

2.2. Analysis of antimicrobial activity

2.2.1. Agar well diffusion method

The levels of the minimum inhibition zone of Zn nanoparticles on food-borne pathogens were evaluated by the well diffusion method (Hwanhlem et al., 2017) using Mueller-Hinton Agar (MHA, Merck 1.05437, Darmstadt, Germany). The food-pathogenic bacteria were standardized to a Mcfarland cell density of 0.5 Mcfarland (10^8 cfu/mL) after 24 h growth of bacteria at 37°C in Nutrient Broth (Merck 1.05443, Darmstadt, Germany). Each bacterial cell culture (100 µL) was infused with a petri dish containing 20 mL of Muller Hilton agar. Three wells of 6 mm were formed in the solid medium. Both wells were inoculated with a 50 µL Zn nanoparticle stock solution. The petri dishes were then incubated at 37°C for 24 hours. After incubation, the zones of inhibition formed around each well were measured in mm with the help of a caliper.

2.2.2. Minimum inhibitory (MIC) and bactericidal concentration (MBC) of nanoparticles

In the study, minimum inhibitory concentration (MIC) was determined according to microdilution method suggested by Clinical and Laboratory Standards Institute (CLSI, 2006) Test microorganisms incubated at 37°C for 24 hours were standardized to 0.5 MacFarland cell density. Mueller-Hinton Broth (MHB, Oxoid, CM0405) was used as the medium. Nanoparticle stock solution was prepared as 50 mg/ml and diluted up to 0.19 mg/ml in sterile tubes. Only tubes containing stock solution or pure culture were evaluated as control group. Other tubes to which MHB was added contained microorganisms and diluted stock solutions used in the study. The tubes used in the study were prepared repeatedly and incubated at 37°C for 24 hours. The bacterial growths to be observed in the tubes were compared with the control group tubes and the tubes with the lowest inhibition in bacterial

proliferation were recorded as the MIC value. In line with the MIC results, Mueller Hinton Agar surface was grafted from tubes without bacterial growth and petri dishes were incubated at 37°C for 24 hours and MBC values were recorded.

2.2.3. Production of zinc ferrite ($ZnFe_2O_4$)

Sol-gel automatic burning method was used in the production of nanostructured zinc ferrite powders. Zinc and iron nitrate compounds, which are used as zinc and iron sources, were obtained from Alfa Aeser company. Zinc nitrate (1:2) and iron nitrate [$Fe(NO_3)_3.9H_2O$] weighed according to the stoichiometric ratio were mixed in 50 ml of pure water at room temperature for about 1 hour until completely dissolved in the magnetic stirrer. Citric acid supplied from Sigma Aldrich company with 99% purity was added to the homogeneous solution as fuel, again according to the stoichiometric ratio, and mixed in pure water in the magnetic stirrer for 30 minutes. After the solution preparation process, the pH of the solution was adjusted to 7 with the help of ammonia. The prepared solution was evaporated and combusted at about 350°C under atmospheric conditions. The nano-ferrite powders obtained in dry form were ground and homogenized for 20 minutes with the help of agat.

2.2.4. Statistical analysis

Statistical analyses were performed using SPSS 180 (SPSS Inc., Chicago, IL.USA). The ANOVA test was used to indicate significant differences, defined as $p<0.005$.

3. Results and discussion

Since the properties of ZnO and $ZnCl_2$ nanoparticles were previously made by the manufacturer (Sigma-Aldrich) and the results were presented, no structural analysis studies have been carried out on these samples. However, prior to investigating the effect of $ZnFe_2O_4$ nanoparticles produced by us, their structural analysis was also examined. Fig. 1. shows the XRD patterns of $ZnFe_2O_4$ nanoparticles. As can be seen from the Fig. 1, the XRD patterns was labelled according to the JCPDS Card No.: 82-1042 and all the peaks agreed with the literature (Sarala et al., 2020; Lakra et al., 2023). However, there was also small amount of the secondary phase peaks corresponding to the Fe_2O_3 phases in the structure. Fig. 2. shows the size and structure of $ZnFe_2O_4$ nanoparticles produced in this study using Transmission Electron Microscopy (TEM). From the TEM images, it is seen that many nanoparticles are agglomerated and spherical in shape. This agglomeration is due to the magnetic nature of the nanoparticles. In addition, particle sizes are distributed on average between 5 nm and 40 nm. This may be due to the inhomogeneous heat dissipation that occurs during the production of nanoparticles. Results obtained were consistent with literature findings (Abbasian and Afarani, 2019).

Table 1 shows the inhibition diameter zone of Zn-based nanoparticles on food-borne Gram-negative and positive pathogenic bacteria. Statistically significant differences were observed on inhibition zones of nanoparticles on pathogenic bacteria ($p<0.05$). $ZnFe_2O_4$ had no antibacterial effect on food-borne pathogenic bacteria at the administered dose. However, among the nanoparticles tested, the $ZnCl_2$ nanoparticle was found to have the highest inhibition zone on bacteria ($p<0.05$). The $ZnCl_2$ particle exhibited the highest zone of inhibition on

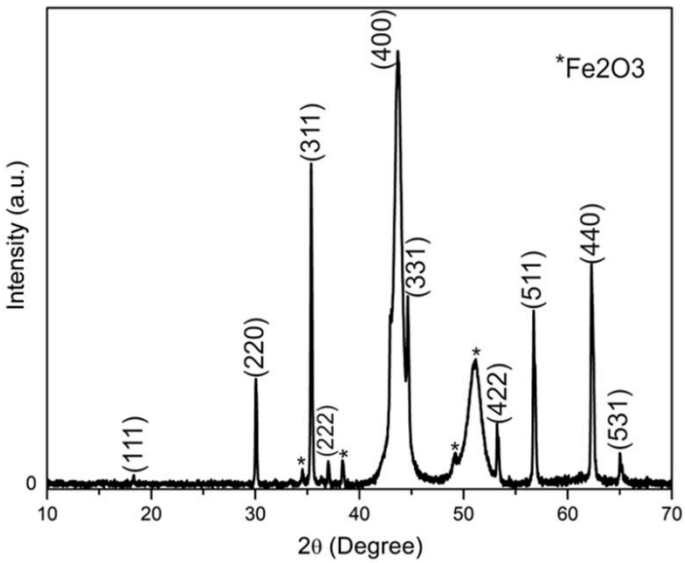

Fig. 1. XRD patterns of $ZnFe_2O_4$ nanoparticles.

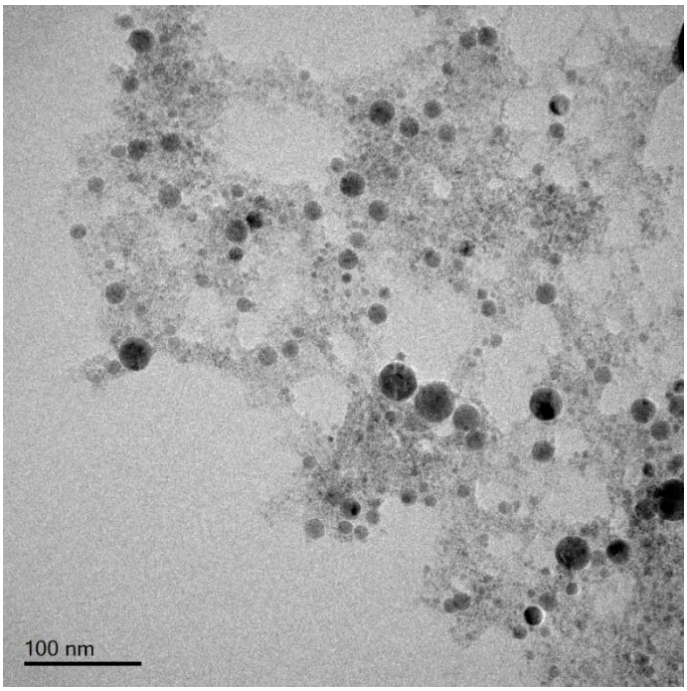

Fig. 2. TEM image of $ZnFe_2O_4$ nanoparticles.

Gram-positive *Listeria monocytogenes* (50 mm) and Gram-negative *Yersinia enterocolitica* (48.75 mm), while it showed statistically similar zone of inhibition (48 mm) on the other test bacteria. ZnO nanoparticle showed a lower zone of inhibition (12.25 mm) on *Campylobacter jejuni* compared to other test bacteria. *Staphylococcus aureus*, *Listeria monocytogenes*, and *Yersinia enterocolitica* had a statistically similar (19.25-19.50 mm) zone of inhibition against ZnO nanoparticles.

In the study, the minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) values of the test microorganisms against nanoparticles are given in Table 2. The MIC value of ZnO nanoparticle on bacteria ranged from 6.25 mg/mL (*Yersinia enterocolitica*) to 25 mg/ml (*Listeria monocytogenes*). ZnO nanoparticle exhibited a similar bacteriostatic effect (12.5 mg/mL) against *Staphylococcus aureus and* Campylobacter *jejuni bacteria*. Gunay et al. (2021) determined the MIC values of ZnO nanoparticles synthesized with *Nosturtium officinale* extract against *Aeromonas hydro-*

Table 1
Minimum inhibition zones of zinc nanoparticles against foodborne Gram-positive and negative pathogenic bacteria.

	ZnO	$ZnCl_2$	$ZnFe_2O_4$
Gram-positive bacteria			
Staphylococcus aureus	19.50±1.29*[a]	48.00±0.82[b]	-
Listeria monocytogenes	19.25±1.26[a]	50.00±0.00[a]	-
Gram-negative bacteria			
Campylobacter jejuni	12.25±1.00[b]	48.00±1.41[b]	-
Yersinia enterocolitica	20.13±1.03[a]	48.75±0.96[ab]	-

*Mean (n=3) ±Standard deviation. There is a significant difference ($p < 0.05$) between the groups in terms of different lettered (a-b) values in the same column.

Table 2
Minimum inhibitory (MIC) and bactericidal concentration (MBC) of zinc nanoparticles against foodborne Gram-positive and negative pathogenic bacteria.

	ZnO		$ZnCl_2$		$ZnFe_2O_4$	
	MIC	MBC	MIC	MBC	MIC	MBC
Gram-positive bacteria						
Staphylococcus aureus	12.5	25	12.5	12.5	12.5	25
Listeria monocytogenes	25	>50	25	25	25	>50
Gram-negative bacteria						
Campylobacter jejuni	12.5	50	6.25	12.5	25	50
Yersinia enterocolitica	6.25	>50	12.5	12.5	25	>50

phila and *Vibrio parahaemolyticus* strains as 15 and 7.5 mg/mL, respectively. In another study, it was determined that the MIC values of ZnO nanoparticles against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* were above 0.13 mg/mL (Erdogan et al., 2019). In this study, Campylobacter jejuni was one of the most sensitive bacteria to the $ZnCl_2$ nanoparticle (6.25 mg/mL), while Listeria monocytogenes was the bacterium with the highest MIC value (25 mg/mL) against the $ZnCl_2$ nanoparticle among the bacteria tested. $ZnFe_2O_4$ nanoparticle showed a MIC value of 25 mg/ml against test microorganisms other than S*taphylococcus aureus*. The bactericidal effect of the nanoparticles on the test organisms was generally 50 mg/ml or more for ZnO and $ZnFe_2O_4$, and 12.5 mg/ml for $ZnCl_2$.

On the other hand, studies with different nanoparticles and different nanoparticle production methods are also available in the literature. Eren and Baran (2019), synthesized silver nanoparticles (AgNP) by green synthesis method using the extract of *Pistacia vera lentiscus* plant and evaluated the antibacterial effect of the synthesized AgNPs in *Staphylococcus aureus* (ATCC 29213) *Escherichia coli* (ATCC 25922), and fungal strains *Candida albicans*. As a result, they stated that silver nanoparticles synthesized by the green synthesis method showed antimicrobial effects. They also reported that AgNPs from the leaves of the *Carica papaya* plant were >25 µg/mL of MIC and MBC against Gram-positive and Gram-negative bacteria in *Luria bertani* broth cultures. Moreover, the effects of the antimicrobial activities of biocompatible gold nanoparticles (AuNP) on food pathogens were evaluated by Baran et al. (2021). They reported that concentrations of 0.056 and 0.025 µg/mL were effective on gram-positive *Staphylococcus aureus* and *Bacillus subtilis*, whilst 0.50 and 0.25µg/mL concentrations were inhibitory effect on Gram-negative bacteria *Pseudemonas aeruginosa* and *Escherichia coli*, respectively.

4. Conclusion

As a result, statistically significant differences were observed on inhibition zones of nanoparticles on pathogenic bacteria ($p<0.05$). $ZnFe_2O_4$ had no antibacterial effect on food-borne pathogenic bacteria at the administered dose. However, the nanoparticle with the highest zone of inhibition on bacteria was identified as $ZnCl_2$.

On the Gram-negative bacterium *Campylobacter jejuni*, ZnO nanoparticle was observed to have the lowest zone of inhibition compared to other test bacteria, while other bacteria were determined to have a similar zone of inhibition against ZnO nanoparticle. It was determined that the minimum inhibitory (MIC) and bactericidal concentrations (MBC) values of zinc nanoparticles used in the study against foodborne Gram-positive bacteria were similar between the groups. The highest value of MIC and MBC against Gram-negative bacteria was found for $ZnFe_2O_4$ nanoparticle and has antibacterial effect. Apart from $ZnFe_2O_4$ nanoparticle, ZnO nanoparticle was determined to have an MBC value of <50 µg/mL against Gram-positive bacteria *Listeria monocytogenes* and Gram-negative bacteria *Yersinia enterocolitica*.

Conflict of interest: The author declares that she has no conflict of interests.

Informed consent: The author declares that this manuscript did not involve human or animal participants and informed consent was not collected.

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Research article

Comparison of the effects of different local thresholding techniques on noise: A potential for optical coherence tomography image binarization

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Abstract

This study aims to investigate the different local thresholding methods on various regions of noise images, which could be used for image binarization of optical coherence tomography images. In the methods one hundred 8-bit images of noise, 1000x1000 pixel in size, is generated using ImageJ/FIJI program. Images processed with four different auto local threshold method in ImageJ/FIJI program as Niblack, mean, median and midgrey, to perform binarization. Twenty-five different region of interest, 100x100 pixel in size, from different region in an image analyzed for area percentage (AP) measurement. Normality tests were performed via Saphiro Wilk Normality test, and Student's t test and one-way ANOVA were used to assess the continuous variables, and Bonferroni test for post hoc analysis, utilizing the IBM SPSS Statistics for the statistical analysis. In the results of this study mean AP for Niblack method was 42.08 ± 0.32%, for mean method was 50.00 ± 0.32%, for median method was 49.28 ± 0.16%, and for midgrey method was 49.63 ± 2.09%. One-way ANOVA analysis shows all the different subgroups of Niblack and mean, Niblack and median, Niblack and midgrey, mean and median, mean and midgrey, and median and midgrey measurements are significantly different from each other. In conclusion this study examined 100 noise images across 25 regions using four auto local threshold methods (Niblack, mean, median, and midgrey). Analyses indicated that Niblack having the lowest mean and there is significant difference between all the methods; researchers using auto local threshold methods in OCT image processing should select methods aligned with data properties, warranting further exploration of these methods' impact on diverse OCT image, especially taking into account the effect of the noise.

Keywords: *Biophysics; choroidal vascularity index; image processing; optical coherence tomography; thresholding*

1. Introduction

Image processing in the ophthalmological instruments have long been an important issue since first optical coherence tomography (OCT) has been introduced. In this very first explanation of advantages of the device itself, researchers discussed and emphasized the non-invasive nature of the technique for biological systems (Huang et al., 1991). Since then it is widely used for various kind of ophthalmological conditions including retinal and choroidal pathologies, acquired and inherited conditions (Adhi and Duker, 2013).

The basics of the device rely on low coherence interferometry, a concept similar to ultrasonic pulse-echo imaging. However, the key distinction is that it employs near-infrared light (NIR) instead of sound waves. The formation and visualization of images occur through the measurement of both the time and magnitude delay of the light that is scattered back from the retina. Due to the rapid velocity of the light beams, direct echoes cannot be detected directly and to overcome this, a reference mirror is employed which facilitates the comparison

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of reflections using a Michelson interferometer (Huang et al., 1991).

Recent improvements in the OCT technology allowed us to use spectral domain OCT (SD-OCT), as a high-resolution diagnostic tool (Nassif et al., 2004). High-resolution means more data to interpret and more need for the analysis the data of the image has itself. Thus, OCT and various ophthalmological images become a natural candidate for the various kind of image processing approaches (Agrawal et al., 2016a,c).

Empowering as a few micrometers of resolution to the ophthalmological images, nearly as a histological image section, new OCT technologies are even a better target for many of the image processing methods (Sull et al., 2010). Optical coherence tomography angiography (OCT-A) technology further improved our understanding of functional and anatomical vessel interactions and properties (Ferrara et al., 2016; Kashani et al., 2017). OCT-A technology has a complex computational background which mainly utilizes the signal based approaches (Kashani et al., 2017).

Many of the studies that utilize from OCT, measures mainly the thickness of the retina and choroid (Leitgeb et al., 2003; Spaide et al., 2008; Adhi and Duker, 2013). These thickness measurements were performed in different regions of the ocular structures (Manjunath et al., 2010). The image processing of the OCT images is not limited to the thickness measurement. The studies for retinal image analysis ranges from image enhancement, segmentation to the thresholding. Not only OCT images of the macula and fovea have been a subject for image processing approaches, but also other anatomical structures like vessels, optic nerve and other structures have been important for the ophthalmological image processing studies. Even there were different imaging modalities except OCT (Patton et al., 2006).

In the recent years, a new field closely related to the image processing gain importance which is artificial intelligence. Many of the artificial intelligence study techniques have been studied in both anterior and posterior segment analysis. Thus, there are numerous parameters and indexes that can be used in artificial intelligence architectures, in order to diagnose and follow up distinct diseases (Duan et al., 2022; Gozzi et al., 2023). Moreover, some of these studies showed that artificial intelligence may help the clinician in the process of deciding to the surgery (Gan et al., 2022). Artificial intelligence is studied for its role in screening of some ophthalmological conditions, and found to be a valuable and promising tool (Huang et al., 2022).

One of the most studied areas of image analysis research is choroidal vascularity index (CVI) (Agrawal et al., 2016a,c). Previous image processing studies paved the road for CVI calculations (Sonoda et al., 2014). It mainly questions if the thickness measurements are not sufficient for further evaluation of many diseases, whether ophthalmological and non-ophthalmological ones such as from Stargardt disease to panuveitis and from Vogt-Koyanagi-Harada disease to type-2 diabetes (Agrawal et al., 2016b,c; Kim et al., 2018; Ratra et al., 2018). In this particular method, CVI is measured with a Niblack binarization technique in OCT images (Agrawal et al., 2016a,c).

Image processing studies utilizing CVI as a tool has a broad spectrum of interest. Using image processing methods, particularly CVI, different regions of the retina has been investigated like macula and peripapillary region (Qi et al., 2023). Also, there are studies that investigate drug effects, like hydroxychloroquine, and these kind of image analysis tools are mentioned to be a candidate tool for decision making (Hasan et al., 2023). One other study found that primary open-angle glaucoma and primary angle-closure glaucoma had significantly different CVI values (Wang et al., 2023).

Although OCT is an advantageous method for many reasons, the noise is a concern while gathering the images and there were certain approaches to overcome this situation in the literature, even using artificial intelligence (Qiu et al., 2020). Deep learning approaches also used to denoise the OCT images of the different ocular anatomical regions (Devalla et al., 2019). Thus, because of the nature of the Niblack binarization method, noise could be very important, for the interpretation of the outcome results.

The need for the determining basic characteristics of widely used Niblack method and other methods in noisy images, simulating the noisy part of the OCT images, is increasing as the CVI research gain importance. Use of different thresholding methods in different image conditions can be used as the purpose of the study. For this purpose, in this study it is aimed to investigate the role of Niblack and three other methods on noise images, in 25 different regions of the noise images.

2. Materials and methods

2.1. Image acquisition

One hundred different noise image is generated in Tiff format with a frame of 1000x1000 pixel, a square shaped image, using ImageJ/FIJI program (version 1.52b) (Schindelin et al., 2012; Schneider et al., 2012). These images were in 8-bit gray scale as in the most binarization techniques have been performed previously (Agrawal et al., 2016c) (Fig. 1A).

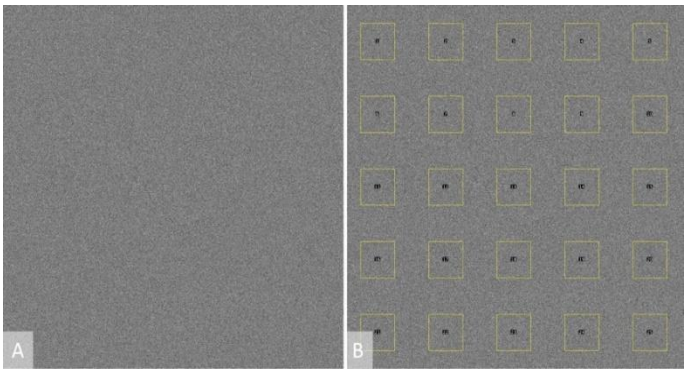

Fig. 1. (A) A sample noise image, (B) 25 Region of interest (ROI) that are measured in a single image.

2.2. Binarization process

Gray scale noise images have been employed in order to binarize via auto local threshold approaches. Binarization means rescaling two-digit value pixels, either 1 or 0, or simply white and black. Different techniques of local threshold methods for binarization are used as Niblack, mean, median and midgrey as in the previous studies (Fig. 2A-D, respectively). Auto local threshold is a plugin in the ImageJ/FIJI program, that are used to binarize images, which computes the threshold using different approaches such as Niblack, mean, median and midgrey as preset inbuilt methods (Agrawal et al., 2016c; Landini et al., 2017; Healy et al., 2018; Nichele et al., 2020).

2.3. Region of interests

After binarization of the image with a technique, new image has been saved and ROI measurements take place. Twenty-five independent regions of interest (ROI) drawn as shown in the Fig. 1B., in order to represent more of the noise area and randomize it. Dimension of the ROIs are 100x100 pixel square, 100 pixels apart each other, in an orientation of five horizontal and five vertical landing, a total of 25 per image. Thus, 25 different ROI for 100 image makes a total of 2500 ROI, which have been analyzed for binarization results with four different techniques.

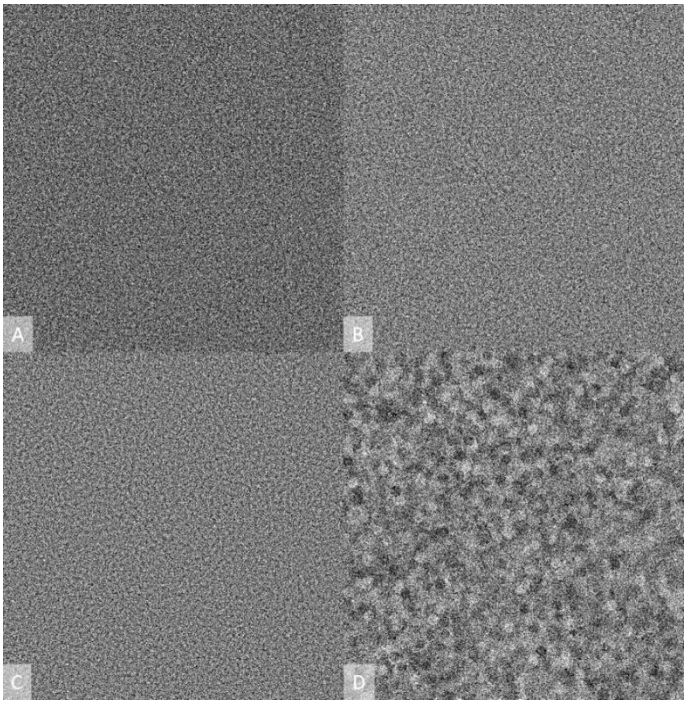

Fig. 2. (A) Binarized image using Niblack method, (B) Binarized image using mean method, (C) Binarized image using median method, (D) Binarized image using midgrey method.

2.4. Area percentage measurement

ROIs have been measured using measure function in the ROI manager tab of the program. In this section area percentage (AP) and mean pixel values were noted, and each ROI is investigated for the area fraction of white to the total area and showed in percentage. Histograms, boxplots and scatter plots of different methods have been drawn.

2.5. Statistical analysis

A total of 100 images with 25 different ROIs have been analyzed with four different auto local threshold technique. IBM SPSS Statistics, Version 28.0 is used for the statistical analysis. Techniques are compared between each other and for different regions with Student's T-test. One-way ANOVA has been employed for independent group assessment and Bonferroni post hoc analysis has been performed for further analysis. Normality tests were performed with Saphiro Wilk Normality tests. Continuous variables have been shown as mean and standard deviation. p value smaller than the 0.05 is accepted as significant.

3. Results and discussion

When the total measurements of all techniques analyzed, mean AP was measured as 47.75 ± 3.45% (41.11 - 55.84%, N = 10000). Mean AP for Niblack method was 42.08 ± 0.32% (41.11 - 43.21 %, N = 2500), for mean method was 50.00 ± 0.32% (49.02 - 51.28%, N = 2500) and for median method was 49.28 ± 0.16% (48.77 - 49.84%, N = 2500), for midgrey method was 49.63 ± 2.09% (42.34 - 55.84%, N = 2500) (Fig. 3).

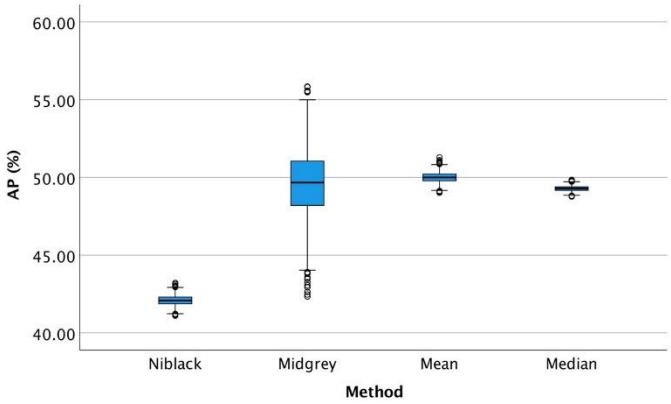

Fig. 3. Boxplot graphs of the four different auto local threshold methods: Niblack, midgrey, mean and median (AP: Area Percentage, %: Percent).

When Niblack and midgrey AP compared with each other, Niblack was found significantly lower than the midgrey (p< 0.001). The distribution width of midgrey, thus standard deviation is higher than the Niblack method, which can be seen in the Fig. 3 and Fig. 4. Niblack AP is significantly lower compared to both mean and median method APs (p<0.001 and p<0.001, respectively) (Fig. 3). AP of the midgrey is significantly lower than the mean and significantly higher than the median method (p<0.001 and p<0.001, respectively) (Fig. 3). And mean methods AP values are significantly higher than the median method (p<0.001) (Fig. 3). Although there is not a high difference between AP results, the high number of sampling rate makes small changes more significant. All the four methods results are normally distributed which can be seen at Fig. 4.

One-way ANOVA analysis showed that there were significant differences between 4 methods (p<0.001). Bonferroni post hoc analysis suggested that these differences were due to all of the sub groups of Niblack and mean, Niblack and median, Niblack and midgrey, mean and median, mean and midgrey, and median and midgrey (p<0.001 for all of the subgroup post hoc analysis).

Table 1
Mean values in different methods.

Method	Mean Pixel Value $\bar{x}\pm\sigma$	Minimum Pixel Value	Maximum Pixel Value
Niblack (n=2500)	107.31 ± 0.82	104.83	110.19
Mean (n=2500)	127.49 ± 0.82	125.00	130.76
Median (n=2500)	125.67 ± 0.41	124.36	127.09
Midgrey (n=2500)	126.55 ± 5.33	107.97	142.39

(n=Number, $\bar{x}$:Mean, σ:Standard Deviation)

Mean pixel value after binarization of different methods have been shown in the Table 1 which is typically between 0-255 in 8-bit images. The mean value of the Niblack method was

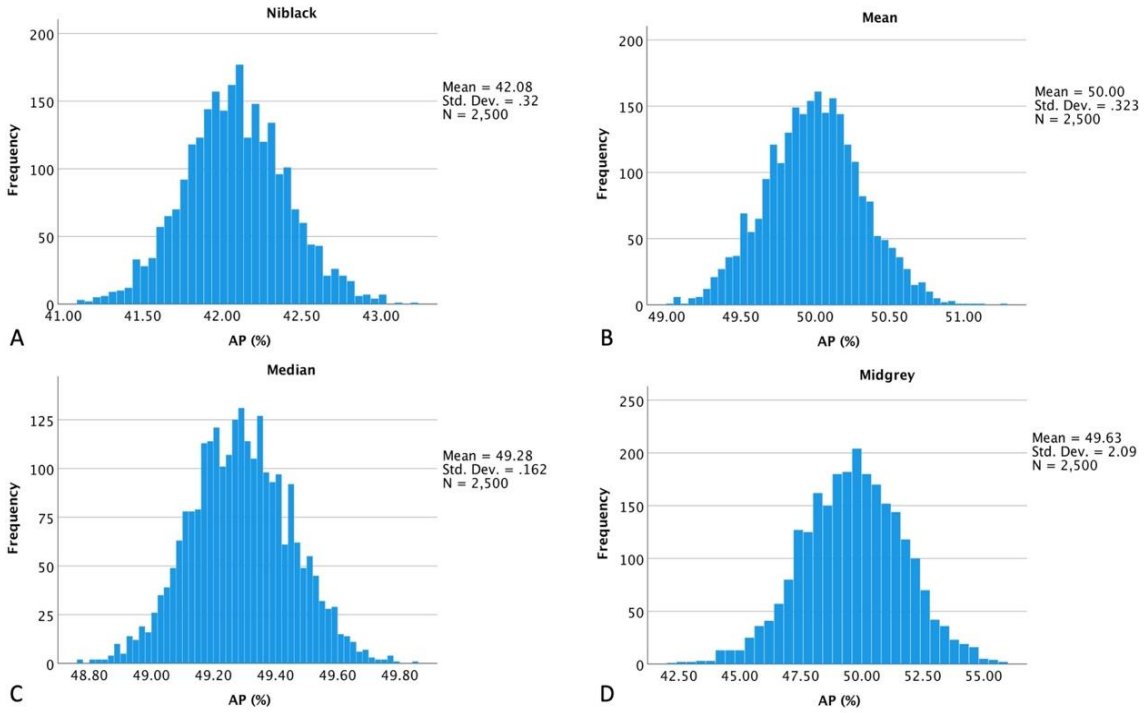

Fig. 4. Histogram graphs of the four different auto local threshold methods: (A) Niblack, (B) Mean, (C) Median, (D) Midgrey (AP: Area Percentage, %: Percent, Std. Dev.: Standard Deviation, N: Number).

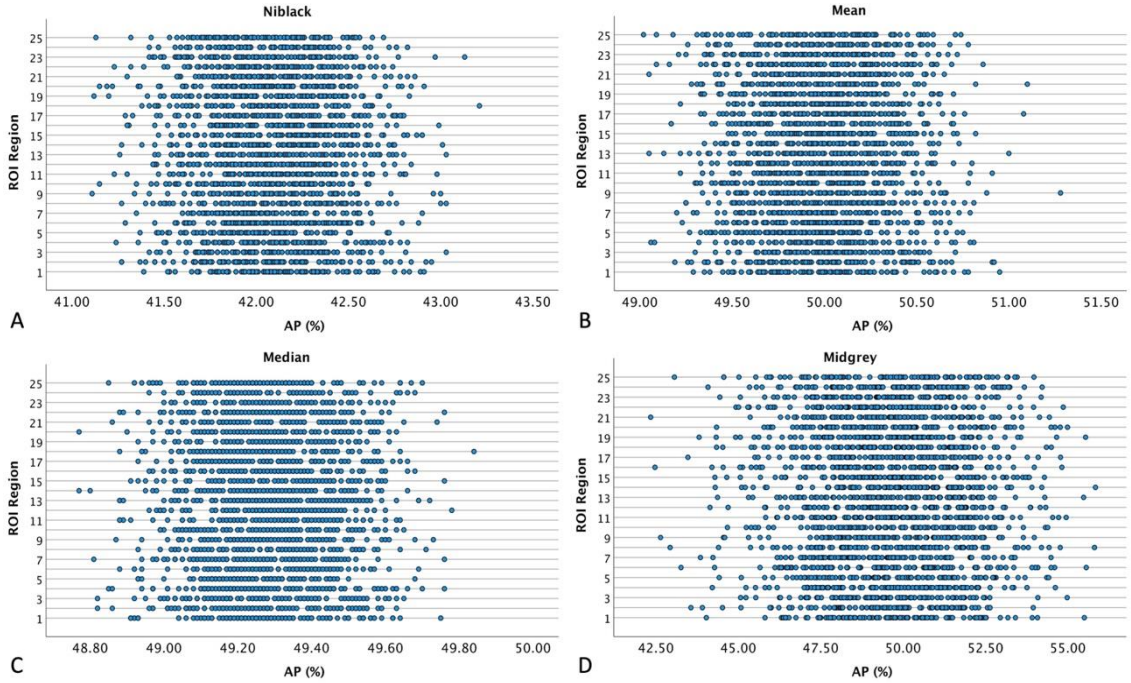

Fig. 5. Scatter plot graphs of the 25 different regions of the four different auto local threshold methods: (A) Niblack, (B) Mean, (C) Median, (D) Midgrey (AP: Area Percentage, %: Percent, ROI: Region of Interest).

found to be lowest among four different method, and mean method found to be highest (107.31 ± 0.82, 127.49 ± 0.82, respectively) (Table 1).

Scatter plot and box plot graphs showed that there are some outliers in all of the methods in different regions, which can be seen in Fig. 5 and Fig. 6.

These adaptive and local thresholding techniques have been used in a broad spectrum of images and studies including the CVI studies (Brocher, 2014; Agrawal et al., 2016a; Mohamed Razali et al., 2017; Healy et al., 2018; Nichele et al., 2020; Ban and Kweon, 2021). In a study investigating the adaptive thresholding methods in dental X-ray images, researchers compared the mean, median, midgrey, Otsu and Niblack methods and they have found that median method has the best thresholding range overall (Mohamed Razali et al., 2017). Another study in the literature mentioned the role of these methods in quantification of sweat areas in a mouse model (Ban and Kweon, 2021). Yet a study implies and investigates the role of these different methods on both experimental and synthetic confocal images of different bacteria types (Nichele et al., 2020).

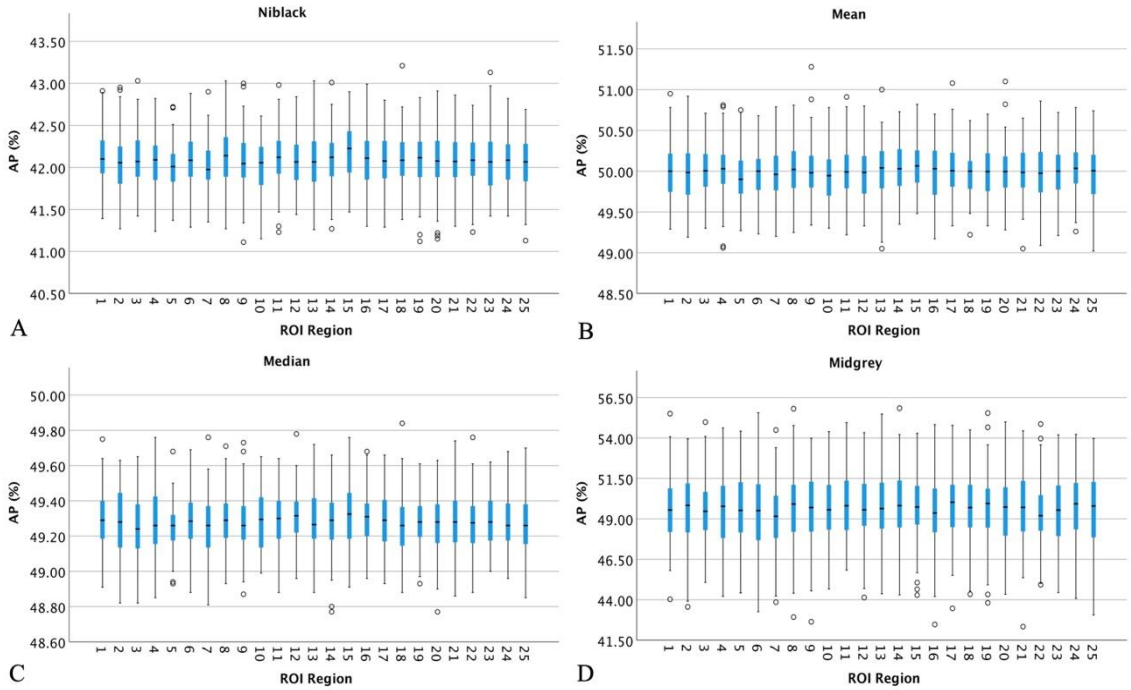

Fig. 6. Boxplot graphs of the 25 different regions of the four different auto local threshold methods: (A) Niblack, (B) Mean, (C) Median, (D) Midgrey (AP: Area Percentage, %: Percent, ROI: Region of Interest).

Different kind of images of astrocytes, microglia and oligodendrocytes were also evaluated using different thresholding methods including mean, median, midgrey, and Niblack (Healy et al., 2018). There were studies comparing new algorithms with pre-existing thresholding methods (Brocher, 2014).

Niblack method can be considered as a local thresholding method alongside many others like Sauvola (Niblack, 1986; Sauvola and Pietikäinen, 2000; Healy et al., 2018). Because Niblack method depends vastly on the mean and standard deviation in a local region around a particular pixel, the main aim of this method could be interpreted as making the thresholding process which takes into account that surrounding pixels; Thus, use of Niblack method could have both some advantages and disadvantages (Adeyanju et al., 2021).

In this study it is found that different adaptive thresholding methods has significantly different outcomes in the noise. Thus, especially images that has too much noise in it, can be vulnerable for that kind of different results when performed with different methods. Researchers should be careful about the methodology they chose, before they begin the study, depending on their dataset and the noise that contains. OCT images, especially that performed with an unsatisfied quality, should be considered for noise, if they will be included in studies that require for implementation of Niblack thresholding algorithm like CVI. There are some methods used for reducing the noise by reducing brightness of the OCT images, in order to get better results in the CVI assessment (Agrawal et al., 2016c). While the contrast disperse is uniform, global thresholding methods have advantages, whereas if the contrast is variable or noise is present, local thresholding methods gain importance (Singh and Mridula, 2014).

In this study it is performed analysis in different regions in a 1000x1000 pixel image with a ROI size of 100x100. A study in the literature performed a comparative analysis between different methods in confocal microscopic and synthetic images found that local thresholding techniques are very sensitive to the ROI size (Nichele et al., 2020). Thus, researchers should also consider the ROI size, when conducting a proper study that investigates utilizing local thresholding methods.

Considering its widespread investigation in recent years, CVI is a major research topic not only in medical but also in surgical and postsurgical conditions (Quiroz-Reyes et al., 2023). There are both advantages and disadvantages, strength and limitations of different thresholding algorithms (Nichele et al., 2020). Although CVI studies performed on the OCT images found Niblack method more reliable for showing luminal and stromal areas, other studies examining the dental X-ray images found median method more suitable for higher number of teeth segmentation (Agrawal et al., 2016c; Mohamed Razali et al., 2017).

Researchers should decide the correct thresholding method, for their study, considering the amount of noise in their datasets. This study sheds light on the basic characteristics in the noise images of various auto local thresholding methods.

4. Conclusion

In this study 100 noise images were investigated for 25 different regions and four different auto local threshold methods as Niblack, mean, median and midgrey. The findings indicated that the AP for all four methods followed a normal distribution. Additionally, there was a significant difference observed among subgroups, with the Niblack method exhibiting the lowest mean value. Although OCT is an advantageous imaging tool widely used in ophthalmology, noise in the OCT images could be a major problem, and researchers conduct image processing studies employing auto local threshold methods should consider their methods according to the properties of their data that they will work on. Future studies are needed for investigating the effects of different auto local thresholding methods during noise in various kind of OCT images.

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Research article

Investigation of hospitalization costs in orthopedics and traumatology clinic

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Abstract

The aim of the study was to analyze inpatient costs in the Orthopedics and Traumatology clinic of a public hospital. The hospitalizations in the Orthopedics and Traumatology clinic of the 525-bed AII group hospital in 2019, 2020 and 2021 were retrospectively evaluated. The study was planned as a cross-sectional descriptive study and data were obtained from the Hospital Management Information System. There were 27,330 inpatients in 2019, 18,604 in 2020 and 21,709 in 2021. The average number of days of hospitalization was 4.07 days in 2019, 2.99 days in 2020 and 2.34 days in 2021. The total annual inpatient cost of Orthopedics and Traumatology service was realized as 3,761,369.20 $ (21,326,963.36 TL) in 2019, 2,061,248.96 $ (14,428,742.72 TL) in 2020 and 1,231,753.09 $ (10,937,967.44 TL) in 2021. The average hospitalization cost of inpatients was 137.63 $ (780.36 TL) in 2019, 110.80 $ (775.60 TL) in 2020 and 56.71 $ (503.58 TL) in 2021. The decrease in total inpatient costs and average cost per patient over the years is a result of the Covid-19 pandemic. In calculating costs for cost control in hospitals, it is important to investigate the reasons for the change in costs over time as well as revealing the current situation. It is recommended to investigate the sources of changes in costs in future research.

Keywords: *Cost; hospitalization; orthopedics; traumatology*

1. Introduction

Effective use of limited resources is an important requirement for the improvement of health services. The cost of services provided in hospitals, which constitute the main axis of health service production, is quite high (Top and Aslan, 2017; Berk and Cercioglu, 2019). In order for costs to be used as an effective tool for increasing efficiency in health services, they must be detectable and auditable. In this direction, it is important to develop cost accounting systems in hospitals (Elif et al., 2015). Hospital-wide or clinic-based cost calculations serve the purpose of developing cost accounting systems.

Due to the increase in costs and limited resources in health service delivery, hospitals face financial difficulties. Therefore, the cost-effectiveness and efficiency of hospitals are closely monitored by both health care providers and health policy makers (Canbaz et al., 2015). However, hospitals consume a lot of resources as they are the institutions with the largest proportion of health service delivery. Costs are the main factor in terms of effective and efficient use of resources, directly affecting the provision of quality, efficient and timely healthcare (Carikci and Acar, 2017; Dirvar et al., 2020). In addition, it is important for decision makers how the Covid-19 pandemic, which has affected the whole world, has caused a change in healthcare resources. Therefore, decision makers can use this information to understand resource consumption in hospitals and both disease- and clinic-based costs, and to plan budget and resource allocation (Hussey et al., 2009).

Public hospitals play an important role in ensuring access to health services in the country and in protecting and improving

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public health. The costs of healthcare services offered in hospitals, including diagnosis, treatment, health education, surgery, birth, rehabilitation, and the protection and development of health, are quite high. High costs incurred in the clinics of these hospitals can become a factor limiting access to healthcare. In addition, personnel, medicine, serum, vaccine and medical consumables are among the important cost items in hospitals. As a matter of fact, many studies have found that personnel costs are the highest cost item (Yigit et al., 2003; Karasioglu and Cam, 2008; Esatoglu et al., 2010; Ozkan et al., 2014; Elif et al., 2015; Soylular and Agirbas, 2016; Buluc and Agirbas, 2017). In this context, it is possible to list the cost items in hospitals as follows: direct raw materials and materials, direct personnel and general production expenses (Esatoglu et al., 2010).

Systematic and continuous cost management is important in hospitals to optimize resource consumption and provide quality, effective and efficient healthcare. Cost management essentially focuses on planning, management and control of costs to achieve maximum benefit at minimum cost (Otlu and Karaca, 2005). As a matter of fact, cost information has a performance-enhancing effect for healthcare professionals (Cinquini et al., 2009). One of the cornerstones of cost management is "cost accounting". Cost accounting is an accounting and recording system that allows determining and monitoring the cost types that make up the cost of produced goods or services. Under cost accounting, which consists of recording and measurement systems, measurements are made with approaches such as traditional cost analysis, activity-based costing, order costing, phase costing, full costing, variable costing, actual costing and standard costing (Palteki, 2019). Depending on the scope of the research, performing cost analyzes with one of these methods is important for the management of increasing costs.

Reducing costs, especially in high-cost surgical clinics such as Orthopedics and Traumatology, will also facilitate access to healthcare. The costs of patients in surgical clinics may vary depending on factors such as the patient's diagnosis, interventional procedures and treatment method. High-cost treatment methods are known as factors that increase costs. Many studies in the literature focus on the medical and surgical treatment costs of diseases in the field of Orthopedics and Traumatology. These studies have helped to identify cost-effective treatment methods for clinicians. The aim of this study is to examine inpatient costs in the Orthopedics and Traumatology clinic of a public hospital.

2. Materials and methods

The aim of this study is to examine inpatient costs in the Orthopedics and Traumatology clinic of a public hospital. The study was conducted in the Orthopedics and Traumatology clinic of a 525-bed public hospital serving in the A2 service role in the Mediterranean Region in Turkey. A-II Group Hospitals are general hospitals operating in provinces in Turkey that have the status of regional health centers or in provinces affiliated to these centers and do not have education-research status. AII group hospitals meet the following criteria: second level, inpatient health facility status; having 6 or more specialist physicians from each of at least four branches (internal medicine, general surgery, gynecology and obstetrics, pediatrics) and being able to organize separate emergency and branch shifts; being able to provide follow-up and treatment of high-risk patients by hospitalization; having a Level III Emergency Service and a Level III intensive care unit; meeting the necessary examination and treatment services and imaging requirements (Ministry of Health, 2009).

The study was planned as a single-center, cross-sectional descriptive study, and all hospitalizations that occurred retrospectively between 01.01.2019-31.12.2021 were included in the study, and outpatients were excluded. The research period was determined as 2019-2021 because of make a more up-to-date comparison between costs and to see the results of the Covid-19 effect on costs.

Hospitalization costs were calculated based on the perspective of the reimbursement institution. From the perspective of the reimbursement institution, the hospitalization cost calculation includes the medical care costs paid by the reimbursement institution in exchange for the health care provided in this unit. Accordingly, the scope of the research is to examine the transaction costs incurred on patients hospitalized in the Orthopedics and Traumatology clinics of the relevant hospital. Thus, the cost of patients receiving inpatient treatment in the clinic will be determined. Data extracted from HMIS were examined on the basis of patient, procedure and diagnosis.

Cost findings are also calculated in US dollars according to the annual average effective selling rate of the Central Bank of the Republic of Turkey (1 $=5.67 TL in 2019 1 $=7.00 TL in 2020; 1 $=8.88 TL in 2021 (TCMB, 2023). M.S. Excel program was used for data analysis.

2.1. Research data

Data were obtained through the Hospital Management Information System (HMIS) of the relevant hospital, and the age, gender, diagnosis, number of days of hospitalization and detailed invoices of the inpatients in the relevant period were evaluated.

2.2. Institutional authorization

Institutional permission was obtained for the conduct of the study (19.12.2022/E71713619/11909), scientific, ethical and citation rules were followed in the writing process of the study; no falsification was made on the collected data and this study was not sent to any other academic publication environment for evaluation, and it was conducted in accordance with the principles of the Declaration of Helsinki.

2.3. Limitations

This research should be evaluated within the framework of certain limitations. The scope of the research is to calculate the cost of patients receiving inpatient treatment in the Orthopedics and Traumatology service of the relevant hospital from the perspective of the reimbursement institution. The cost calculation only covers the procedures applied to patients, and the lack of grouping such as direct raw material and materials, direct personnel and general production expenses is a limitation of the study. As a matter of fact, in this study, a detailed cost analysis such as traditional cost analysis or activity-based costing was not performed, and the costs of inpatient treatment in the relevant clinic were calculated on a transaction basis. In this context, it was not grouping in cost calculation. Additionally, due to the scope of the data obtained in the study,

the cost of inpatient treatment applied in the relevant clinic was calculated, and no classification was made according to sub-treatment methods. In addition, the inflation difference was not taken in the years in which the study was conducted.

3. Results and discussion

Within the scope of the research, 27,330 inpatients were treated in 2019, 18,604 in 2020 and 21,709 in 2021. The average age of these patients was 53.09 (0-21) in 2019, 52.63 (0-102) in 2020 and 53.44 (0-103) in 2021. In 2019, 58.35% of hospitalized patients were female and 41.65% were male; in 2020, 58.81% of hospitalized patients were female and 41.19% were male; and in 2021, 61.33% of hospitalized patients were female and 38.67% were male. The average number of days of hospitalization was 4.07 days in 2019, 2.99 days in 2020 and 2.34 days in 2021 (Table 1).

Table 1
Descriptive statistics.

		2019	2020	2021
Number of patients		27,330	18,604	21,709
Gender	**Male**	11,384	7,663	8,394
	Female	15,946	10,941	13,315
Average Age		53.09 (0-21)	52.63 (0-102)	53.44 (0-103)
Hospitalization day		4.07	2.99	2.34

Orthopedics and Traumatology service hospitalization costs are presented in Table 2. The total cost of hospitalization was 3,761,369.20 $ (21,326,963.36 TL) in 2019, 2,061,248.96 $ (14,428,742.72 TL) in 2020 and 1,231,753.09 $ (10,937,967.44 TL) in 2021. The average hospitalization cost of inpatients was 137.63 $ (780.36 TL) in 2019, 110.80 $ (775.60 TL) in 2020 and 56.71 $ (503.58 TL) in 2021. Details of hospitalization costs were analyzed based on the procedures performed on patients. In this context, the most billed expense item in 2019 was "graftless umbilical hernia repair" with a total cost of 1,874,980.25 $ (23,240 times, unit price: 80.68 $), "intra-articular injection, pain treatment" with a total cost of 50,180.53 $ (10,129 times, unit price: 4.95 $), and "plaster removal" with a total cost of 11,922.15 $ (5,719 times, unit price: 2.08 $). The most billed expense item in 2020 was "daily bed charge" with a total cost of 25,118.69 $ (16,541 times, unit price: 1.52 $), "intra-articular injection, pain treatment" with a total cost of 30,004.13 $ (7,477 times, unit price: 4.01 $), and "plaster removal" with a total cost of 6,620.89 $ (3,921 times, unit price: 1.69 $). The most billed expense item in 2021 was "daily bed charge" with a total cost of 23,487.47 $ (19,444 times, unit price: 1.21 $), "intra-articular injection, pain treatment" with a total cost of 35,546.62 $ (11,136 times, unit price: 3.19 $), and "plaster removal" with a total cost of 5,717.93 $ (4,257 times, unit price: 1.34 $).

Table 2
Orthopedics and Traumatology clinic hospitalization cost.

	2019	2020	2021
Total cost of hospitalization	3,761,369.20 $ (21,326,963.36 TL)	2,061,248.96 $ (14,428,742.72 TL)	1,231,753.09 $ (10,937,967.44 TL)
The average hospitalization cost	137.63 $ (780.36 TL)	110.80 $ (775.60 TL)	56.71 $ (503.58 TL)

Orthopedics and Traumatology clinic inpatient costs were analyzed based on ICD.10 (International Classification of Disease 10) diagnosis classification. In this context, 2,777 inpatients with different diagnoses were treated in the relevant service in 1,938 in 2020 and 2,296 in 2021. In 2019, the most common diagnosis was "M17.0-Primary gonarthrosis, bilateral". In 2019, 7.79% (2,129 people) of inpatients received this diagnosis and the total annual cost was 331,842.44 $ (1.881.546,66 TL). Second most common diagnosis was "M17-Gonarthrosis (arthrosis of the knee joint)" with total annual cost: 113,535.48 $ (643,746.19 TL) and third was "M19.8-Arthroses other, defined" with total annual cost: 26,389.15 $ (149,626.47 TL). In 2020, the most common diagnosis was "M17.0-Primary gonarthrosis, bilateral", which was seen in 8.69% of patients. The total annual cost of the diagnosis was realized as 170,075.73 $ (1,190,530.11 TL). Second and third most common diagnosis was "M77.3-Calcaneal spur" with total annual cost: 528,779.65 $ (3,701,457.53 TL) and "M19.8-Arthroses other, defined" with total annual cost: 24,850.79 $ (173,955.50 TL) respectively. In 2021, the most common diagnosis was "M17.0-Primary gonarthrosis, bilateral", which was seen in 11.16% of patients. The total annual cost of the diagnosis was determined to be 94,404.66 $ (838,313.38 TL). Second and third most common diagnosis was "M19.8-Arthroses other, defined" with total annual cost: 26,212.74 $ (232,769.09 TL) and "M17.9-Gonarthrosis, unspecified" with total annual cost: 23,818.97 $ (211,512.43 TL) respectively.

When the diagnosis with the highest annual total cost was analyzed, it was determined that the diagnosis seen in 994 patients in 2019 was "M77.3-Calcaneal spur". The rate of this diagnosis in total annual inpatient costs was 12.21% [458,957.14 $ (2,602,286.99 TL)]. In this year, this is followed by "M17.0-Primary gonarthrosis, bilateral" with total annual cost: 331,842.44 $ (1,881,546.66 TL) and "M17-Gonarthrosis (arthrosis of the knee joint)" with total annual cost: 113,535.48 $ (643,746.19 TL). In 2020, the diagnosis with the highest total cost was "M77.3-Calcaneal spur" seen in 1293 patients. The proportion of hospitalizations due to calcaneal spur in total annual inpatient costs was 25.64% [528,779.65 $ (3,701,457.53 TL)]. This is followed by "M17.0-Primary gonarthrosis, bilateral" with total annual cost: 170,075.73 $ (1,190,530.11 TL) and "S46.0-Shoulder rotator cuff tendon injury" with total annual cost: 87,518.14 $ (612,626.95 TL) respectively. The diagnosis with the highest annual total cost was "S46.0-Shoulder rotator cuff tendon injury" in 2021 with total annual cost: 99,276.75 $ (881,577.57 TL). This is followed by "M17.0-Primary gonarthrosis, bilateral" with total annual cost: 94.404,66 $ (838,313.38 TL) and "M17.1-Other primary gonarthrosis" with total annual cost: 53,876.18 $ (478,420.52 TL)) respectively.

In the study, patient hospitalization costs in the Orthopedics and Traumatology clinic of a public hospital were examined. Within the scope of the research, the number of patients hospitalized in the Orthopedics and Traumatology clinic decreased from 2019 to 2020 and increased from 2020 to 2021. This decrease in the number of patients is due to the Covid-19 pandemic. The fact that the Covid-19 pandemic started to be seen in Turkey in 2020 resulted in an increase in the demand for healthcare services in areas other than Covid-19 and urgent/non-emergency healthcare needs during this period (Genc, 2020; Sadyrbaeva and Chekirov, 2022).

In this context, as in other specialties, there has been a significant decrease in the number of inpatients in the field of Orthopedics and Traumatology. Anticipating and responding to

Covid-19 patients in need of hospital-based care, many countries have redesigned hospital discharge policies and delayed planned admissions for non-emergency care.

As a result, in many countries, total inpatient admissions declined between 2019 and 2020. In 2021, there was relatively greater adaptation to the Covid-19 pandemic, and deferred healthcare services translated into demand, resulting in increased inpatient admissions. This explains the increase in the number of inpatients from 2020 to 2021 (Azzolina et al., 2022; Chiba et al., 2023).

It is seen that the average age of the patients hospitalized in the relevant clinic was close in all years (≈53). Considering the gender distribution of the patients, it was determined that mostly female patients received inpatient treatment and the proportion of female patients receiving treatment increased over the years.

The average number of days of hospitalization decreased significantly over the years (from 4.07 days to 2.34 days). It is thought that this decrease in the number of hospitalization days is due to the fact that patients who received inpatient treatment in hospitals during the Covid-19 pandemic period were discharged as quickly as possible.

When the total annual inpatient cost in Orthopedics and Traumatology service is analyzed, a decrease occurred from 2019 to 2021 [2019: 3,761,369.20 $ (21,326,963.36 TL); 2020: 2,061,248.96 $ (14,428,742.72 TL); 2021: 1,231,753.09 $ (10,937,967.44 TL)]. This is seen as a natural consequence of the decreasing number of inpatients. While the average hospitalization costs per patient were close in 2019 and 2020, there was a significant decrease in 2021 [2019: 137.63 $ (780.36 TL); 2020: 110.80 $ (775.60 TL); 2021: 56.71 $ (503.58 TL)].

Kizilcec (2012) examined the inpatient costs in Cerrahpasa Medical Faculty Hospital in 2011 and concluded that the annual number of inpatients in the Orthopedics Clinic was 300 and the average hospitalization cost per patient was 24,598.44 TL (≈ 13,912.36 $). Yigit et al. (2003), in their research, found that the average cost per patient of the Orthopedics and Traumatology Service in 2002 was 1,201,789.837 TL (≈ 684,853.50 $). Elif et al (2015) conducted a branch-based cost analysis study in a public hospital and concluded that the annual number of inpatients in the orthopedics clinic was 205 and the average hospitalization cost per patient was 2,006.62 TL (≈736,374$). Sonsuz (2011) calculated the total cost of an Orthopedic clinic in a private hospital as 609,740.65 TL (≈369,898.48$) in 2009. Yilmaz (2018) determined that the total cost of the Orthopedics and Traumatology clinic in a public hospital was 361,215.60 TL (≈95,592,56$). Dogan (2022) determined that the total clinical cost of Orthopedics and Traumatology in a training and research hospital is 18,537,653.65 TL (≈3,410,028.93$) in 2019. Soylular and Agirbas (2016) determined in their research that the Orthopedics and Traumatology clinic is among the high-cost units. In this context, it is thought that the difference between the findings of this study and the findings of other studies may be due to the different types of hospitals where the studies were conducted.

As a matter of fact, this research was conducted in an AII group general hospital. Study of Kizilcec (2012) was conducted in a university hospital, while study of Elif et al. (2015) in a training and research hospital. Since these hospitals provide education services, it is considered normal that inpatient error costs are high (Kisakurek, 2010; Sonsuz, 2011; Ozkan et al., 2014; Ozkan and Agirbas, 2015; Buluc and Agirbas, 2017; Mut and Agirbas, 2017; Yilmaz, 2018; Dogan, 2022).

When the research findings were analyzed within the scope of expense items, it was determined that the most billed expense item was “Intra-articular injection, pain treatment” in 2019, while it was “Daily bed charge” in 2020 and 2021. It is thought that the reason for this situation is that orthopedic diseases are very painful and intra-articular injection treatment is often preferred in pain treatment. Based on the diagnosis-based inpatient cost findings, the most common diagnosis was “M17.0-Primary gonarthrosis, bilateral” in 2019, while it was “M19.8-Arthroses other, defined” in 2020 and 2021. The diagnosis type with the highest share in the total annual cost was “M77.3-Calcaneal spur” in 2019 and 2020 and “S46.0-Shoulder rotator cuff tendon injury” in 2021. Shoulder rotator cuff tendon injury is one of the most common orthopedic conditions (Bilen, 2016).

4. Conclusion

Musculoskeletal system diseases, which are included in the field of orthopedics, stand out as very common health problems today. Therefore, research on the cost of orthopedic clinics is important. Examining the cost of healthcare provided in public hospitals can have a significant impact on patients’ access to healthcare.

It is thought that the results of the research will provide valuable information for resource planning and cost analysis in hospital clinics. Based on the research findings, it was revealed that the number of patients hospitalized in orthopedics clinics decreased during the pandemic period. It is thought that this situation is caused by the health problems that were postponed due to the effect of the pandemic and the short hospitalizations in the hospitals.

There was also a significant decrease in the average cost of hospitalization per patient. The decrease observed is generally evaluated positively in terms of cost control. It is also important to calculate the costs for cost control in hospitals, to reveal the current situation, and to investigate the reasons for the change in costs. This is the limitation of this research. In future research on this subject, it is also recommended to investigate what causes the change in costs.

Hospital managers and policymakers should take precautions considering that hospital visits postponed due to the pandemic will increase further in the coming years and this will create an additional burden on the health system. In order to monitor and control costs, it is important to establish a cost accounting system in hospitals, to make regular cost division and unit cost analyses, and to use the analysis results in managerial decisions.

Conflict of interest: The authors declare that they have no conflict of interests.

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Research article

Antioxidant activities of plant species growing in different habitats (serpentine, gypsum and limestone)

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Abstract

In this study, plant species (gypsum, limestone, and serpentine) growing in different habitats in Erzincan province were investigated. Gypsum [*Verbascum alyssifolium* Boiss., *Tanacetum heterotomum* (Bornm.) Grierson, *Psephellus recepii* Wagenitz & Kandemir, *Gypsophila lepidioides* Boiss.], limestone [*Cyclotrichium niveum* (Boiss.) Manden. & Scheng, *Chrysophthalmum montanum* (DC.) Boiss, *Teucrium leucophyllum* Montbret & Aucher ex Bentham, *Phlomis oppositiflora* Boiss. & Hausskn] serpentine [*Fumana aciphylla* Boiss., *Convolvulus pseudoscammania* C. Koch., *Hypericum thymbrifolium* Boiss & Noé, *Salvia indica* L., *Gladiolus halophilus* Boiss. & Heldr.] were examined. The total phenolic contents (TPC), total flavonoid content (TFC), and the amount of antioxidant activity (DPPH, FRAP) were analyzed in different organs of the plants, including leaves, branches, and roots. According to the obtained data, when plant parts and habitats were taken into consideration, it was observed that the plant with the highest flavonoid content (29.71±0.57 mg QE g^{-1} extract) was *S. indica* growing in the serpentine area with its leaf parts. In terms of total phenolic content, it was determined that the root part of *S. indica* growing in the serpentine area had high values (91.53±2.48 mg GAE g^{-1} extract value). When evaluated in terms of the Iron (III) Ion Reducing Antioxidant Power (FRAP) method, it was observed that the highest value was the stem part of *F. aciphylla* growing in a serpentine area (100.35±1.60 mg TE g^{-1}). In terms of DPPH radical capacity, the highest value belonged to the leaf part of *Salvia indica* (15.75±1.74 µg mL^{-1}), which is also grown in the serpentine area. The results were evaluated utilizing the SPSS Statistical Program and differences were observed between habitats. A strong correlation was found between the phenolic and flavonoid contents of plants and their antioxidant activities. The findings showed that the phenolic, flavonoid content, and antioxidant activities of plants grown in different ecological conditions vary significantly.

Keywords: *DPPH; flavonoid; gypsum; limestone; phenolic; serpentine*

1. Introduction

Antioxidants are the first compounds to protect plants against free radicals. These compounds are not stable or active before free radicals attack cells (Percival, 1996; Saffaryazdi et al., 2020). Phenolic compounds are natural and complex containing antioxidants, flavonoids (anthocyanin, flavones, flavonols, and isoflavonoids), and commonly found tannins (Gentile et al., 2018; Williamson et al., 2018; Guven et al., 2019; Shen et al., 2022). Plants synthesize organic compounds such as

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tocopherol, flavonoid, phenolic compound, alkaloid, chlorophyll, polyfunctional organic acid, and carotene during their vital activities (Larson, 1988; Ergun, 2021). Phenolic compounds, defined as secondary metabolites, undertake the task of combating pests and shielding against external effects, as well as vital activities such as growth and reproduction in plants. The aroma and odor properties of plants are also due to phenolic compounds in the form of essential oil. In addition, these compounds are very important in determining the antioxidant capacity of the plant (Ergun, 2021; Ozyigit et al., 2023). In plants, these metabolites are affected by internal and environmental dynamics such as climate, soil, and genetics (Mikulajová et al., 2016; Tajik et al., 2019).

Türkiye is one of the richest countries in the temperate zone in terms of biodiversity, especially of floral diversity and endemism. Floristic diversity reflects the ecosystem and edaphic, climatic, topographic, and geological diversity. The contribution of edaphic diversity to biodiversity is considerable and important endemism in Türkiye is widely seen in serpentine, gypsum, or salty soils (Ozdeniz et al., 2017).

Rock and silicate minerals containing high amounts of Mg, Fe, and mafic minerals are olivine, pyroxene, and ultramafic rock (such as gabbro, basalt, and peridotite) while those containing <45% silica (SiO_2) are called ultrabasic rocks. The formation of serpentine by the reaction of olivine, the main mantle material, with water is called "serpentinization"/coiling. Soils containing serpentine are formed by the erosion of ultramafic rocks. At least 70% of these igneous or metamorphic rocks are composed of ferromagnesian or mafic (magnesium+ferric-mafic-) minerals (Kruckeberg, 2002; Ozdeniz et al., 2017).

Gypsum is in the form of calcium sulfate ($Ca_2SO_4.2H_2O$) containing water in crystalline form or anhydrite ($CaSO_4$) containing no water (Van Alphen et al., 1971; Herrero and Porta, 2000; Ozdeniz et al., 2016). When embedded at depths of several hundred meters, gypsum loses its water and turns into anhydrite. The solubility of gypsum is 2.6 gr/l at 25°C (Verheye and Boyadgiev, 1997; Ozdeniz et al., 2016).

While it can dissolve quickly in humid and rainy climates, in arid climates the rock may appear in the form of crystals with sand grains or the form of smaller and softer crystals (Ozdeniz et al., 2016). The efficiency of secondary metabolites is very important in the adaptation process of plants unique to these habitats since gypsum and serpentine soils have intemperate conditions (Politycka and Adamska, 2003). This study aimed to determine the effects of different habitats on antioxidant concentrations in plants.

2. Materials and methods

In this study, DPPH, total phenolic contents (TPC), flavonoids (TFC), and FRAP concentrations were determined in leaves, branches, and roots of plants grown in different habitats. The completely white and crystallized areas at the 3rd km to Kemah-İlic highway towards Yahşiler Village, the limestone rocks of Kocacimen Village (Kemaliye) and Eric Village (Kemah), and the serpentine regions around Yucebelen Village (Kemah) are the sampling areas (Fig. 1.).

In the present research, plant species specific to serpentine, gypsum, and limestone habitats were selected. Gypsum [*G. lepidioides* Boiss., *P. recepii* Wagenitz & Kandemir, *T. heterotomum* (Bornm.) Grierson, *V. alyssifolium* Boiss.], limestone [*C. montanum* (DC.) Boiss, *C. niveum* (Boiss.) Manden. & Scheng, *P. oppositiflora* Boiss. & Hausskn, *T. leucophyllum* Montbret & Aucher ex Bentham] serpentine [*C. pseudoscammania* C. Koch., *F. aciphylla* Boiss., *G. halophilus* Boiss. & Heldr., *H. thymbrifolium* Boiss & Noé, *S. indica* L.] native to their habitat were collected in an amount to represent the area where they bloom.

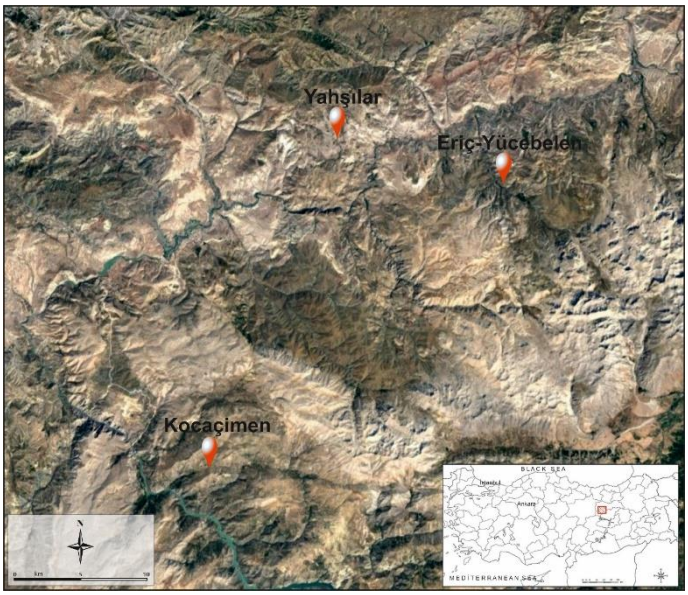

Fig. 1. Study areas.

2.1. DPPH free radical scavenging activity

The free radical scavenging activity of the extracts was determined using the method of Blois (1958), modified slightly. DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was dissolved with methanol at a concentration of 0.26 mM. For the test, stock solutions (1 mg/ml) were prepared of the extracts and standards. Concentrations of 20, 40, 100, 200, 300, 400, 800, and 1000 µg/ml were prepared from the stock solutions, and the final volume was completed to 3 ml with methanol, followed by the addition of 1 ml of DPPH solution. The mixture was vortexed and incubated for 30 minutes in the dark at room temperature. At the end of this period, each mixture's absorbance was measured at 517 nm, and the results were converted to % activity. Each extract's IC_{50} (µg/ml) was determined, and the findings were compared to Trolox, the standard antioxidant.

2.2. Iron (III) ion reducing antioxidant power method (FRAP)

The reducing power activity assay of plant materials was carried out with some modifications using the method developed by Oyaizu (1986). For the test, stock solutions (1 mg/ml) were prepared of the extracts and standards. 100 µl of the prepared stock solutions were placed into test tubes, and the volume was diluted to 1.25 ml using phosphate buffer (0.2 M, pH 6.6). This mixture was incubated at 50°C for 20 minutes after 1.25 ml of potassium ferric cyanide [$K_3Fe(CN)_6$] (1%) was added. Then, the absorbance of the final mixture was measured at 700 nm following the addition of 1.25 ml of 10% trichloroacetic acid (TCA) and 0.25 ml of 0.1% $FeCl_3$ solutions to the reaction medium. The results were given as mg TE/g extract using the calibration curve created from varied concentrations of Trolox used as a standard (Murathan and Ozdinc, 2018).

2.3. Determination of total phenolic content

The Folin-Ciocalteu method was used to determine total phenolic content (Singleton et al., 1999). For this purpose, 100 µl Folin-Ciocalteu, 4.5 mL of distilled water, and 300 µl (2%) Na_2CO_3 were added to 100 µl of the sample stock solutions at 1 mg/ml concentrations. After 2 hours of incubation at room temperature, the total phenolics content was measured in a spectrophotometer at 760 nm (Unico, S1205). The total phenolic content of the samples was measured as mg gallic acid equivalent to phenolic substance/g extract (Murathan and Ozdinc, 2018).

2.4. Determination of total flavonoid content

The total flavonoid content was determined using the method developed by Chang et al. (2002). 4.7 mL of methanol was added to test tubes containing 100 µL of the sample stock solutions prepared with methanol at 1 mg/ml concentrations. Following, 100 µL of 1 M NH_4CH_3COO solution and 100 µL of 10% $AlCl_3$ solution were added to the mixture, which was vortexed and incubated for 45 minutes at room temperature. After incubation, the total flavonoid content of the mixture was measured in a spectrophotometer at 415 nm. The total flavonoid content of the extracts was determined as mg quercetin equivalent/g extract by creating a calibration curve from different concentrations of quercetin used as standard (Murathan and Ozdinc, 2018). The data obtained in the study were statistically evaluated. In statistical calculations and comparisons, $p \leq 0.05$ was accepted as significant. The data were analyzed with SPSS 22 Package Statistical Program, ANOVA test at 95% confidence interval, and differences between habitats in multiple comparisons were determined by S-N-K and Tukey's B. The relationship between antioxidant activities and phenolic compounds was performed using the Pearson correlation coefficient (r). Differences at $p<0.05$ were considered significant by using the software (Chang et al., 2020).

3. Results and discussion

In this study, the antioxidant activities of root, branch, and leaf parts of 13 plants growing in different habitats were determined, leading to significant conclusions. In the study, when the data related to DPPH free radical scavenging activity were examined, the best activation in the leaves was 17.90±1.74 and 17.52±0.16 in the branch with *S. indica* in the serpentine region, and 33.19±1.54 in the root with *G. lepidioides* plant species growing in the gypsum region. When the data obtained for total phenolic content were evaluated, the highest amount was 55.97±0.51 in the leaves with *V. alyssifolium* in the gypsum region, 60.19±0.84 in the branch with *F. aciphylla* in the serpentine region, and 33.19±1.54 in the roots with 91.53±2.48 *S. indica* L. plant species grown in the gypsum region. When the data obtained regarding the total flavonoid content were examined, the highest amount was 29.71±0.57 in the leaves of the plant with *V. alyssifolium, S. indica* in the serpentine region, 26.86±0.86 in the branch, and 18.19±0.72 in the root with *H. thymbrifolium* in the serpentine region. Among the FRAP activity data, the highest amount was 76.51±3.83 in leaves of *H. thymbrifolium* 100.35±1.60 in branches, and 93.55±1.67 in roots of *F. aciphylla* in the serpentine region (Tables 1, 2 and 3).

When the data were analyzed statistically, significant differences were observed between habitats. Based on statistical evaluations, it was obvious that plants grown in the serpentine area were different in terms of antioxidant compounds compared to plants grown in other habitats (Table 4). It was determined that there were differences only in the correlation relationship within the habitat and in the correlation between all plants. According to the correlation made in the roots, branches, and leaves of all plants collected from different regions, a strong correlation was found between antioxidant compounds. A negative correlation was seen between antioxidant activity and phenolic, flavonoid content FRAP. The correlation between phenolic content and DPPH was strongly negative in leaves (r = -0.74), branches (r = -0.62), and roots (r= 0.62). The correlation has been reported in previous studies (Rumbaoa et al., 2009; Ghafar et al., 2010; Singanusong et al., 2015; Indradi et al., 2017; Fitriansyah et al., 2018; El Atki et al., 2019; Chang et al., 2020; Alizadeh and Fattahi, 2021) (Tables 5, 6, 7 and 8.) The data obtained in this study were compared with those in previous studies.

Their data on total phenolic, flavonoid contents, DPPH and

Table 1
Total phenolic, flavonoid contents and antioxidant activity in plant leaves.

Habitat	Plant Species and Families	DPPH IC_{50} (µg mL^{-1})	Total phenolics mg GAE g^{-1} Extract	Total flavonoids mg QE g^{-1} Extract	Reducing power mg TE g^{-1} Extract
Gypsum	*G. lepidioides* Boiss (Caryophyllaceae)	118.36±2.40	17.97±0.90	4.95±0.33	41.32±0.63
	P. recepii Wagenitz & Kandemir (Asteraceae)	59.07±3.01	29.08±0.71	15.14±0.57	52.79±2.88
	T. heterotomum (Bornm.) Grierson (Asteraceae)	>250	8.27±0.89	1.90±0.44	4.59±0.20
	V. alyssifolium Boiss (Scrophulariaceae)	27.57±1.47	55.97±0.51	13.14±1.03	72.98±2.44
Limestone	*C. montanum* (DC.) Boiss (Asteraceae)	>250	13.75±1.70	3.33±0.44	32.28±2.46
	C. niveum (Boiss.) Manden. & Scheng (Lamiaceae)	134.98±2.01	26.64±0.13	12.95±0.72	39.24±2.69
	P. oppositiflora Boiss. & Hausskn. (Lamiaceae)	149.98±0.69	19.01±0.84	3.52±0.82	49.08±1.90
	T. leucophyllum Montbret & Aucher ex Bentham (Lamiaceae)	110.14±1.24	22.41±1.80	3.43±1.03	51.37±1.42
Serpentine	*C. pseudoscammania* C. Koch (Convolvulaceae)	>250	17.90±0.68	7.14±3.97	22.04±0.29
	F. aciphylla Boiss (Cistaceae)	142.90±3.22	34.27±0.67	26.48±0.66	63.91±2.13
	G. halophilus Boiss. & Heldr (Iridaceae)	161.17±3.18	33.75±1.22	29.14±1.25	63.05±1.93
	H. thymbrifolium Boiss. & Noë (Hypericaceae)	61.19±2.59	42.93±1.11	29.62±0.82	76.51±3.83
	S. indica L. (Lamiaceae)	15.75±1.74	36.12±2.56	29.71±0.57	69.05±2.65
	Trolox	11.95±0.15			

Table 2
Total phenolic, flavonoid contents and antioxidant activity in plant branch.

Habitat	Plant Species and Families	DPPH IC_{50} (µg mL^{-1})	Total phenolics mg GAE g^{-1} Extract	Total flavonoids mg QE g^{-1} Extract	Reducing power mg TE g^{-1} Extract
Gypsum	*G. lepidioides* Boiss (Caryophyllaceae)	116.08±0.86	18.19±2.98	2.00±0.29	35.79±2.27
	P. recepii Wagenitz & Kandemir (Asteraceae)	56.30±1.20	13.16±1.56	9.90±0.16	41.66±1.90
	T. heterotomum (Bornm.) Grierson (Asteraceae)	>250	8.41±0.68	0.76±0.44	11.35±0.12
	V. alyssifolium Boiss (Scrophulariaceae)	54.63±0.69	27.30±3.22	5.43±0.29	70.93±3.81
Limestone	*C. montanum* (DC.) Boiss (Asteraceae)	>250	11.53±0.26	1.81±1.90	26.23±1.11
	C. niveum (Boiss.) Manden. & Scheng (Lamiaceae)	>250	16.19±1.78	1.90±0.44	19.86±1.47
	P. oppositiflora Boiss. & Hausskn. (Lamiaceae)	>250	9.45±1.05	0.38±0.44	27.94±0.32
	T. leucophyllum Montbret & Aucher ex Bentham (Lamiaceae)	>250	18.19±2.58	0.95±0.16	38.13±0.73
Serpentine	*C. pseudoscammania* C. Koch (Convolvulaceae)	248.56±60.03	15.08±1.85	4.86±0.57	31.14±0.41
	F. aciphylla Boiss (Cistaceae)	30.06±1.13	60.19±0.84	16.00±1.31	100.35±1.60
	G. halophilus Boiss. & Heldr (Iridaceae)	231.01±1.67	19.82±1.54	6.67±0.66	42.43±1.41
	H. thymbrifolium Boiss. & Noë (Hypericaceae)	44.64±1.83	37.45±2.28	26.86±0.86	72.38±1.56
	S. indica L. (Lamiaceae)	24.44±0.16	31.16±1.92	10.48±1.00	59.29±3.25
	Trolox	11.95±0.15			

Table 3
Total phenolic, flavonoid contents and antioxidant activity in plant roots.

Habitat	Plant Species and Families	DPPH IC_{50} (µg mL^{-1})	Total phenolics mg GAE g^{-1} Extract	Total flavonoids mg QE g^{-1} Extract	Reducing power mg TE g^{-1} Extract
Gypsum	*G. lepidioides* Boiss (Caryophyllaceae)	22.06±1.54	18.34±1.80	1.52±0.92	43.65±0.15
	P. recepii Wagenitz & Kandemir (Asteraceae)	147.10±0.74	14.64±3.30	3.71±0.76	41.89±0.92
	T. heterotomum (Bornm.) Grierson (Asteraceae)	>250	13.53±0.34	0.19±0.16	24.75±0.32
	V. alyssifolium Boiss (Scrophulariaceae)	30.31±1.31	33.82±2.52	8.86±0.29	37.05±3.08
Limestone	*C. montanum* (DC.) Boiss (Asteraceae)	>250	14.04±0.97	1.43±2.23	29.73±0.57
	C. niveum (Boiss.) Manden. & Scheng (Lamiaceae)	189.69±0.95	17.23±1.36	2.95±0.66	36.80±3.43
	P. oppositiflora Boiss. & Hausskn. (Lamiaceae)	>250	14.64±0.13	3.24±0.44	42.51±0.72
	T. leucophyllum Montbret & Aucher ex Bentham (Lamiaceae)	243.42±3.41	16.56±1.58	1.90±0.44	35.84±0.49
Serpentine	*C. pseudoscammania* C. Koch (Convolvulaceae)	205.16±20.55	17.97±0.68	1.62±0.16	44.53±0.47
	F. aciphylla Boiss (Cistaceae)	33.67±1.21	74.56±2.78	8.38±0.72	93.55±1.67
	G. halophilus Boiss. & Heldr (Iridaceae)	175.04±0.45	15.75±1.78	2.29±3.22	34.75±1.05
	H. thymbrifolium Boiss. & Noë (Hypericaceae)	92.85±2.64	27.60±0.44	18.19±0.72	57.29±1.84
	S. indica L. (Lamiaceae)	29.71±1.17	91.53±2.48	7.90±0.59	65.19±0.92
	Trolox	11.95±0.15			

Table 4
Statistical differences between habitats.

Antioxidant	Leaf			Branch			Root		
Compounds	Gypsum	Limestone	Serpentine	Gypsum	Limestone	Serpentine	Gypsum	Limestone	Serpentine
TFC	ab	a	b	a	a	b	a	a	b
TFP	a	b	c	a	a	b	a	a	b
FRAP	a	a	b	a	a	b	a	a	b
DPPH	a	b	ab	a	b	a	a	b	c

Letters indicate significant differences among the different habitats at $p \leq 0.05$.

FRAP in the leaves of *Achillea aleppica* D.C. subsp. *aleppica* were generally lower than the data obtained in this study, especially DPPH activity was lower (Colak et al., 2020). The total phenolic data of the leaves and stem parts of *V. major* subsp. *hirsute* was similar to the data obtained in this study, while FRAP and DPPH data were found lower (Saral et al., 2015). The data obtained from five *Isatis* species regarding total phenolic, flavonoid contents, DPPH, and FRAP in roots and stems are considerably lower than the data obtained in this study (Comlekcioglu, 2020). The antioxidant activity data obtained from *U. filipendula and V. album* plants are lower than the data obtained in this study (Yildiz et al., 2019).

Arituluk et al. (2016) determined the antioxidant activity and total phenolic and flavonoid contents of some *Tanacetum* L.

taxa growing in Türkiye. In their study, it was found that the data obtained in the aboveground parts of the plants were similar to the data obtained in some plants in this study. Antioxidant activity and phenolic compounds were examined in 10 selected plants from Serbia and it was reported that all plant species examined were rich in phenolic compounds and the data measured by two different methods used showed satisfactory antioxidant activity. A high correlation was found between antioxidant properties and phenolic compounds by Zugic et al. (2014). In their study, DPPH, FRAP, total phenolic (TP), and total flavonoid (TF) contents were analyzed to determine the antioxidant capacity of the methanol extract of *S. verticillata* subsp. *amasiaca*. As a result of the antioxidant tests, DPPH lC_{50}, FRAP, total phenolic and total flavonoid contents were determined as 11.47 ± 0.30, 22.22 ± 0.36 mmol TE/g extract, 140.18 ± 8.73 mg GAE/g extract and 51.56 ± 1.18 mg QE/g extract, respectively (Bayan and Genc, 2016).

Table 5
Correlation among total phenolic content (TPC), total flavonoid content (TFC), DPPH and FRAP of plants grown in serpentine habitat.

Correlation		Leaf	Branch	Root
DPPH	TPC	-0,92*	-0,69	-0,93*
TPC	TFC	0,93*	0,60	0,18
TPC	FRAP	0,99**	0,99**	0,77
TFC	FRAP	0,98**	0,67	0,38
DPPH	TFC	-0,94*	-0,63	-0,50
DPPH	FRAP	-0,95*	-0,73	-0,81

**Correlation is significant at $p \leq 0.01$; * correlation is significant at $p \leq 0.05$.

Table 6
Correlation among total phenolic content (TPC), total flavonoid content (TFC), DPPH and FRAP of plants grown in limestone habitat.

Correlation		Leaf	Branch	Root
DPPH	TPC	-0,83	-0,96**	-0,98*
TPC	TFC	0,76	0,31	0,29
TPC	FRAP	0,39	0,32	0,20
TFC	FRAP	-0,27	-0,56	0,90
DPPH	TFC	-0,34	-0,26	-0,23
DPPH	FRAP	-0,81	-0,51	-0,06

**Correlation is significant at $p \leq 0.01$; * correlation is significant at $p \leq 0.05$.

Table 7
Correlation among total phenolic content (TPC), total flavonoid content (TFC), DPPH and FRAP of plants grown in gypsum habitat.

Correlation		Leaf	Branch	Root
DPPH	TPC	-0,79	-0,71	-0,65
TPC	TFC	0,77	0,18	0,91
TPC	FRAP	0,92	0,93	0,18
TFC	FRAP	0,85	0,52	0,29
DPPH	TFC	-0,80	-0,66	-0,59
DPPH	FRAP	-0,97*	-0,82	-0,82

**Correlation is significant at $p \leq 0.01$; * correlation is significant at $p \leq 0.05$.

Table 8
Correlation among total phenolic content (TPC), total flavonoid content (TFC), DPPH and FRAP of plants grown plants.

Correlation		Leaf	Branch	Root
DPPH	TPC	-0,74*	-0,65*	-0,62*
TPC	TFC	0,69**	0,73**	0,46
TPC	FRAP	0,88**	0,94**	0,81**
TFC	FRAP	0,74**	0,75**	0,55*
DPPH	TFC	-0,54	-0,65*	-0,53
DPPH	FRAP	-0,82**	-0,75**	-0,59*

**Correlation is significant at $p \leq 0.01$; * correlation is significant at $p \leq 0.05$.

Calluna vulgaris (L.) Hull investigated the changes in the content of some phenolic groups and their biological activities in various parts of the plant during different growth periods. The data obtained in different parts of the plant are generally similar to the data obtained in this study (Chepel et al., 2020). They determined the phenolic content and antioxidant activity of purslane (*Portulaca oleracea* L.) during growth stages. They determined that FRAP, DPPH, phenol, and total flavonoid contents were higher in the generative period than in the vegetative period. As a result, they revealed that the growth stages of plants significantly affect antioxidant activity (Saffaryazdi et al., 2020). In this study, they determined the total phenolic content, flavonoid content, and antioxidant activities of hot water extracts of mint (*Mentha piperita*), lemon balm (*Mellissa officinalis*), marshmallow (*Althea officinalis*), chamomile (*Matricaria chamomilla*), green tea (*Camellia sinensis*) and sage (*Salvia officinalis*). The data obtained generally show similarity to those in this study (Karatas et al., 2019).

In their comparative study on phenolic, flavonoids, and *in vitro* antioxidant activities of wild edible plants obtained from the Wetland Ecosystem of Loktak Lake in the Himalayan Region of North East India, Singh et al. (2021) determined 3 plants with potential bioactivity among 28 plants species. They investigated the possibility of potential medicinal plants by determining the antioxidant activity and flavonoid content in relation to total phenolic compounds in plants collected in the Central Balkans. In their study, Stankovic et al. (2015) determined that *Statice gmelinii* and *Artemisia santonicum* species had high antioxidant activity among 16 halophyte plants collected from different families. The correlation between phenolic content and DPHH was found to be parallel with the correlation in this study.

They obtained different data on the phenolic contents and biological activities of two endemic plant species growing in Türkiye, *Corydalis oppositifolia* and *Senecio cilicius*, as well as ethanol, methanol, and ethyl acetate extracted from the subsoil parts of the plants. They determined that the antioxidant activities of the two plants were high (Acet et al., 2021).

They determined that the number of phenolic compounds varied significantly in different growth stages of *Rumex crispus* L. and *Rumex obtusifolius* L. species and that there were differences in antioxidant activity in different plant organs. They found that there was a high correlation between the fractions of phenolic compounds and antioxidant activity (Feduraev et al., 2019).

4. Conclusion

The biochemical and physiological structures of plants vary according to the ecological factors in the environment where they grow. In this study, the amount of some bioactive components and antioxidant capacities of the plants studied were compared. It was observed that the total phenolic, total flavonoid, DPPH, and FRAP activity values of the samples collected from various habitats were different from each other. In addition, the data obtained from leaves, branches, and roots of the same plant species differed. The correlation analysis showed a wide variation in the correlation coefficient between the studied parameters. The results show that there are significant differences in the parameters studied in the plants. In addition to the different morphological characteristics of the plants, it is evident that the antioxidant activities and phenolic

contents of the examined plants vary according to the geographical regions where they grow. Since Türkiye has a great diversity of endemic plants, it is very important to carry out research for more effective and efficient use of natural compounds obtained from plants.

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Derleme makalesi / Review article

Topraksız tarım teknolojileri gelecek için sürdürülebilir bir çözüm mü?

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Öz

Geleneksel tarımda bilinçsiz kimyasal kullanımının toprak yapısında değişikliklere yol açarak, toprak verimliliğini olumsuz yönde etkilediği belirtilmektedir. Ayrıca gittikçe artan dünya nüfusu nedeniyle gıda taleplerinin karşılanması için önlemlerin alınması gerekliliği de vurgulanmaktadır. Son yıllarda, alternatif bir çözüm olarak topraksız tarım araştırmaları; iş gücü, zaman, su tasarrufu sağlanması, herbisit/pestisit kullanımının azalması, mikrobiyolojik sıkıntıların ortadan kalkması gibi avantajları olduğu için artmaktadır. Öte yandan, dezavantaj olarak yüksek maliyetlerin düşürülmesi için de girişimlerde bulunulmaktadır. Bazı ülkelerde kentsel tarımın desteklenmesi amacıyla dikey tarım uygulamalarının yapıldığı merkezler bulunmaktadır. Son dönemlerde dikey tarım uygulamaları ile ilgili olarak uzayda bitki yetiştirilmesi, uzay çiftlikleri gibi başlıklar da popülerdir. NASA'nın yürüttüğü projelerde başarıya ulaşılmış olan ürünler bulunmaktadır. Mikro yerçekimi etkisi ile oluşabilecek hasarlar incelenerek, çalışmaların teknolojinin avantajlarıyla daha da ileri götürülmesi amaçlanmaktadır. Ayrıca, gastronomi alanında da topraksız/dikey tarım sürdürülebilirlik açısından son zamanlarda ön plana çıkmaktadır. Bu tekniklerin uygulanarak küçük ölçekli üretimlerin yapıldığı restoranların ön plana çıktığı görülmektedir. Bu derlemede bu konular detaylı olarak irdelenecektir.

Anahtar kelimeler: *Aeroponik; akuaponik; dikey tarım; gastronomi; hidroponik; uzay tarımı*

Are soilless agriculture technologies a sustainable solution for the future?

Abstract

It is stated that the unconscious use of chemicals in traditional agriculture causes changes in soil structure and negatively affects soil fertility. It is also emphasized that precautions must be taken to meet food demands due to increasing world population. Recently, soilless agriculture research as an alternative solution has been increasing due to its advantages such as saving labor, time and water, reducing use of herbicides/pesticides, and eliminating microbiological problems. However, attempts are being made to reduce high costs that are a disadvantage. In some countries, there are centers where vertical farming practices are carried out to support urban agriculture. Lately, topics such as growing plants in space and space farms regarding vertical farming applications are also popular. There are products that have been successful in projects carried out by NASA. It is aimed to take studies further with advantages of technology by examining damages that may occur due to the effect of microgravity. Moreover, in the field of gastronomy, soilless/vertical agriculture has recently come to the fore in terms of sustainability. It is seen that restaurants where small-scale production is carried out by applying these techniques come to the fore. In this review, these issues will be examined in detail.

Keywords: *Aeroponics; aquaponics; gastronomy; hydroponics; space farming; vertical farming*

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1. Giriş / Introduction

2050 yılına kadar dünya nüfusunun 10 milyara yaklaşması beklendiğinden, insanların ihtiyacı olan günlük ortalama 1600 kalorinin sağlanabilmesi için bitki yetiştirme alanlarının 2.1 milyar dönüm arttırılmasının gerekli olduğu bildirilmektedir (FAO, 2018; Arumugam, 2021). Birleşmiş Milletler Gıda ve Tarım Örgütü (FAO) raporuna göre, 2019 yılında 690 milyon insan açlığa maruz kalmıştır. Ayrıca ihtiyaç duyulan gıda talebinin yüzde 60 oranında artacağı tahmin edilirken, diğer yandan da küresel olarak her yıl 1.3 milyar ton gıdanın israf edildiği ve 750 milyar dolar ekonomik kaybın olduğu rapor edilmiştir (United Nations Report, 2011; Banerjee ve Adenaeuer, 2014). 2021 yılında yayınlanan Birleşmiş Milletler Çevre Programı (UNEP) Gıda Atıkları Endeks Raporuna göre, her yıl kişi başına düşen gıda israfının küresel bazda ortalamasının 74 kg olduğu belirtilmiştir (UNEP Gıda Atık Endeksi Raporu, 2021). Küresel ısınma, hastalıklar, ekonomik sorunlar, hızlı kentleşme, tarım alanlarının ve su kaynaklarının tahribatı gibi nedenlerle dünya nüfusunun çok daha ciddi bir açlıkla karşı karşıya kalacağı tahmin edilmektedir (Kalantari ve ark., 2018). Artan bu gıda talebinin karşılanması için pek çok araştırmacı mevcut tarımsal uygulamaların yanı sıra alternatif gıda kaynaklarının araştırılması ve sürdürülebilir bir şekilde şehirlere gıda tedarik etmenin yolları konusuna yoğunlaşmıştır (Al-Chalabi, 2015). Öte yandan, küresel ısınmaya bağlı olarak dünya genelinde gıda üretimi için kullanılabilecek alanların giderek azalacağı ve endüstriyel gelişme ile görülen iklim değişikliği ve çevresel tahribat sebebiyle de bitkisel ve hayvansal üretimin olumsuz yönde etkileneceği belirtilmiştir (Kim ve ark., 2019). Nüfus artışı gıda talebindeki önemli artışı beraberinde getirirken, nüfusun %70'inin de kentsel alanlarda yaşayacağı tahmin edilmektedir (United Nations, 2015). Bu durum kentsel alanlarda genişlemeye yol açabileceği için mevcut tarım arazilerinin azalabileceği düşünülmektedir (Beacham ve ark., 2019).

Konvansiyonel tarımda arazinin yanlış kullanımı, çölleşme, aşırı gübre kullanımı, fazla su tüketiminin vermiş olduğu zararların yanı sıra mahsul verimini artırmak için kullanılan herbisitlerin toprağın fiziksel, kimyasal ve biyolojik yapısında değişikliklere sebep olduğu ve bunun neticesinde de toprak ekosisteminde bozulmalar meydana geldiği bildirilmiştir. Böylelikle, toprak verimliliğinde ve ekilen ürünlerin kalitesinde olumsuz etkilerin görüldüğü belirtilmiştir (Despommier, 2013; Barbosa ve ark., 2015; Bingol, 2019). Ayrıca, konvansiyonel tarımda ortaya çıkan sera gazları, insektisidler, nitrojen ve fosfor gibi bitki besin maddelerinin çevreye salınımı birtakım olumsuzluklar ile sonuçlanmaktadır (Aune, 2012). Bu bağlamda, artan gıda talebine ve ekilebilir arazilerin yetersizliğine bağlı olarak tarımsal faaliyetler açısından acil bir çözüm getirilmesi gerekliliği ortaya çıkmaktadır (Pomoni ve ark., 2023). Bu olumsuz durumların ortaya çıkması sonucu, araştırmacıların teknolojinin gelişimine bağlı olarak hidroponik, aeroponik ve akuaponik sistemler gibi alternatif topraksız tarım tekniklerinin üzerinde yoğunlaştıkları görülmektedir (Bingol, 2019).

Yeni üretim arayışlarında toprak verimliliğindeki ve toprak besin rezervlerindeki azalma, sulama suyunun sınırlı olması ve iklim değişiklikleri dikkate alınması gereken parametrelerdendir. Bu açıdan bakıldığında, topraksız yetiştirme sistemleri üzerinde durulan sistemlerdendir (Mir ve ark., 2022). Bu sistemler sayesinde kontrollü bir şekilde ve yıl boyunca devamlı üretim yapmak mümkündür. Farklı teknik ve maliyetlere sahip olan topraksız tarım sistemleri ile çeşitliliğin artması ve maliyetlerin düşmesi öngörülmektedir Birçok ülke kullanılabilir tarım arazilerinin azlığı ve verimsizliği nedeniyle topraksız tarım uygulamalarına geçmiştir (Despommier, 2013; Barbosa ve ark., 2015). Bu derlemede topraksız tarım tekniklerinden, bu tekniklerin avantaj ve dezavantajlarından, şehirlerde topraksız tarım uygulamalarına örneklerden, sürdürülebilirlik kapsamında gastronomi alanında topraksız tarım uygulamalarından ve uzay tarımı ile ilgili gelişmelerden bahsedilecektir.

2. Dikey tarımın tanımı ve dikey tarım uygulamalarına yönelim sebepleri / Definition of vertical farming and reasons for tendency to vertical farming practices

Dikey tarım sistemleri, gıda güvenliğini ve gıda üretimini dünyanın sürekli artan kentsel nüfusuna hitap edecek şekilde şehirlerdeki sürdürülebilirliği sağlamayı amaçlamaktadır (Al-Kodmany, 2018). Bu noktada daha az su kullanımı ve atık üretimi ile özellikle şehirlere uzak bölgelerden mahsul taşınmasının önüne geçilmesi ve yıl boyunca daha sağlıklı ürünlerin üretilmesi hedeflenmektedir. Dikey tarım, şehir veya şehir merkezindeki bir binanın içinde, zeminlerinin hidroponik (besin içeren su) kullanılarak belirli mahsulleri üretecek şekilde tasarlandığı, meyve, sebze ve tahılların kentsel tarımıdır (Al-Chalabi, 2015). Bununla birlikte, ekonomik olduğu kadar çevresel fizibilitesi de derinlemesine bilimsel araştırma gerektirir (Banerjee ve Adenaeuer, 2014). Dikey tarım uygulamaları üzerinde son yıllarda yapılan çalışmaların sayısı giderek artmaktadır (Celebi, 2019). Bu açıdan, dikey tarımın endüstriyel ve bilimsel topluluklarda giderek geniş çapta tartışılan bir konu haline geldiği ifade edilmektedir. Araştırmalarda, dikey tarım yapılacak binada gereken enerji miktarı ile yenilenebilir enerji yoluyla binanın ihtiyacı olan enerjinin karşılanıp karşılanmayacağı ve geleneksel üretim veya dikey tarım uygulaması ile yetiştirilen ürünlerin karşılaştırmalı karbon ayak izinin belirlenmesi üzerine yoğunlaşmaktadır (Al-Chalabi, 2015). Dikey tarım, yüksek binalarda büyük ölçekli gıda üretimini içeren tarım tekniği olup, yıl boyunca meyve, sebze ve diğer gıda ürünlerinin (örn. çeşitli otlar, şifalı bitkiler) üretilebileceği bildirilmiştir. Böylece, yüksek nüfus yoğunluğu olan kentsel alanlarda (büyük şehirler) çok çeşitli bitkilerin yetiştirilmesi, hasat edilmesi, bu mahsullerin doğrudan şehirlerde satılması ve ulaşım masraflarının azaltılması mümkün olabilmektedir (Banerjee ve Adenaeuer, 2014).

Dikey tarımın uygulama alanları konteynırlar, depolar, fabrikalar, yer altı sığınakları ve otoparklar gibi çeşitli yapıların çatı veya faklı cepheleri olabilmektedir. Dikey tarım uygulamalarında genellikle terk edilmiş depo ve fabrika alanları tercih edilmektedir (Celebi, 2019).

3. Dikey tarımın kısa tarihçesi / Brief history of vertical farming

Dikey tarım kavramı son yıllarda popülarite kazanmış bir sistem olarak gözükse de aslında o kadar yeni olan bir kavram değildir. Deir el Bahari tapınağındaki resimler, 4000 yıl önce Mısırlıların büyük saksılarda ağaç yetiştirmeye ve nakletmeye çalıştığını göstermektedir (Raviv ve Lieth, 2007). M.Ö. 600'lü yıllarda Babil hükümdarı II. Nebukadnezar tarafından inşa ettirilen, dünyanın yedi harikası içinde kendine yer bulmuş Babil Asma Bahçeleri ve M.S. 1150'li yıllarda Aztek halkının bitki yetiştirmek için çeneampa adını verdikleri hidroponik tarım

tekniği uygulamaları öncü uygulamalar olarak değerlendirilmektedir. M.S. 1627'de Sir Francis Bacon, "Sylva Sylvarum" adlı kitabında ilk defa hidroponik yöntem ve tarım teknikleri üzerine bir teori yayınlamıştır. Bacon, kitabında karada yetişen bitkileri toprak olmadan da yetiştirmenin mümkün olduğunu savunmuştur (Swain ve ark., 2021; Bihari ve ark., 2023). 1699 yılında John Woodward'ın bitkilerin besinlerini topraktan mı yoksa sudan mı aldıklarını anlamaya çalıştığı ve bitki besleme araştırmaları üzerine yaptığı çalışmalar neticesinde ilk hidroponik sistemleri kurduğunu belirten yazılı kanıtlar bulunmaktadır. Uyguladığı yöntemde su kültürü kullanılmış ve bitki büyüme ve gelişimini izlenmiştir. Sonuçlar, toprak ve suyun bitki büyümesi ve gelişmesi için besin sağladığını göstermiştir (Jones, 1982). On dokuzuncu yüzyılda Fransız ve Alman bilim adamları bitki besin gereksinimlerini araştırmaya başlamışlar ve daha sonra yirminci yüzyılın ilk yarısında Amerikalı ve İngiliz bilim adamları tarafından bu çalışmalar geliştirilmiştir (Torabi ve ark., 2012). Dokuz element, bitki büyümesi ve gelişmesi için temel elementler olarak belirlenmiştir ve ardından 1860 yılında ise, iki Alman bilim insanları Julius von Sachs ve Wilhelm Knop bitkiler için besin solüsyonları geliştirmeye başlamışlardır. Araştırmacılar, besin solüsyonunda kullanılacak belirli elementlerin gerekliliğini ortaya koymuşlardır. Genellikle vasküler dokulara sahip olan bitkiler için gerekli olduğu düşünülen elementlerin sayısı, 1954'te klor eklendiğinde 16'ya çıkmıştır (Hershey, 1994). 1850'den 1900'lerin ortalarına kadar olan dönemde, yeşil bitkilerin toprak kültüründe ihtiyaç duyduğu temel elementler keşfedilmiştir. Wilhelm Knop'un oluşturduğu standart besin solüsyonu (içeriği: KNO_3, $Ca(NO_3)_2$, KH_2PO_4, $MgSO_4.7H_2O$, $FePO_4$) diğer araştırmalara temel oluşturmuştur. Bu solüsyonun kullanımı sırasında çökelti oluşmaması ve bitki büyümesinin stres olmadan gerçekleşmesi de aynı derecede önemli olarak değerlendirilmiştir (Jones, 1982). 1929 yılına kadar olan zaman diliminde ise hidroponik faaliyetler üzerine yapılan araştırmalar sadece bitki biyolojisi yönünden ele alınmıştır. 1929'dan sonra, besin solüsyonu yoluyla üretim, Berkeley'deki California Üniversitesi'nden William Frederick Gericke tarafından başlatılmıştır. Besin solüsyonun yalnızca araştırma için değil, mahsul üretmek için kullanılabileceğini bildirmiştir. Ardından diğer hidroponik sistemler geliştirilmiştir (Torabi ve ark., 2012; El-Kazzaz ve El-Kazzaz, 2017). California Üniversitesi'nden Dennis R. Hoagland ve Daniel I. Arnon, 1938'de tarım bülteninde "Growing plants without soil by the water-culture method" başlıklı yazıyı ve akabinde 1950 yılında "The water-culture method for growing plants without soil" adlı bir yazıyı yayınlamışlardır. Bu iki araştırmacı, Hoagland solüsyonları olarak bilinen mineral besin solüsyonları için çeşitli formüller geliştirmiştir (Hoagland ve Arnon, 1938; Hoagland ve Arnon, 1950; Pandey ve ark., 2009). Modifiye Hoagland solüsyonları bugün hala kullanılmaktadır (Fletcher ve ark., 2023; Germer ve ark., 2023; Sajiv ve ark., 2023). Gericke (1937) yılında su kültürü teriminin daha önce suda yaşayan bitki ve hayvanların yetiştirilmesi olarak tanımlandığından, tekniğin "hidroponik" olarak adlandırılacağını duyurmuştur. 1930'larda Pan American Airlines yolcuları için Wake Adası'nda sebze yetiştirdiği belirtilmiştir (Pandey ve ark., 2009). 1940'da II. Dünya Savaşında, Amerikan askerleri Pasifik'te sebze üretmek için ilk kez topraksız yetiştirme sistemleri kullanmış ve 8.000 tondan fazla taze sebze üretilmiştir (Dalrymple, 1973; Mhadhbi, 2012; Celebi, 2019). 1960'ların sonlarında İngiltere, Littlehampton'daki Glasshouse Mahsul Araştırma Enstitüsü'ndeki (GCRI) araştırmacılar, bir dizi müteakip iyileştirmeyle birlikte besleyici film tekniğini geliştirmişlerdir (Pandey ve ark., 2009). Bu araştırma, günümüzde kullanılan hidroponik sistemlerin ortaya çıkmasını sağlamıştır. 1964'te Viyana Uluslararası Bahçe Bitkileri Sergisinde, uzun bir cam kule şeklinde dikey bir çiftlik sergilenmiştir. 1980'de İsveçli bir çiftçi olan Åke Olsson, şehirlerde sebze üretimi için dikey çiftçilik önermiştir ve bitki yetiştirmek için spiral şekilli bir ray sistemi icat ettiği bilinmektedir (Al-Kodmany, 2018). Jensen ve Collins (1985), Avrupa ve Amerika Birleşik Devletleri'nde geliştirilen birçok yeni kültür sistemini vurgulayan, hidroponik sistemi yayınlamıştır. ABD Columbia Üniversitesi'nden Dr. Dickson Despommier 1999 yılında çok katlı binalarda kurulan dikey çiftliklerde bitki yetiştirilebileceğini önermiştir. Böylelikle, Despommier dünyanın önde gelen dikey tarım uzmanı ve dikey çiftlik savunucusu haline gelmiştir (Despommier, 1999; Chole ve ark., 2021). Modern dikey çiftliklerden biri olan Sky Green Farms'ın Singapur tesisi, her biri 9 metre yüksekliğinde, güneş ışığı kullanarak az enerji ile yeşil sebzeler yetiştiren ve yağmur suyunu özel tanklarda toplayarak sulama için kullanan çok fazla kuleden oluşmaktadır (Wang, 2021; Zareba ve ark., 2021). Hershey (1994), çözelti kültüründe bitki yetiştirmenin toprak kaynaklı hastalıkların olmamasından dolayı daha kolay olduğunu, sulamanın daha az sıklıkta yapıldığını, kök siteminin rahatlıkla takip edilebileceğini ve sulamanın otomatik olarak yapılabileceğini ifade etmiştir. NASA tarafından "Controlled Ecological Life Support System-Kontrollü Ekolojik Yaşam Destek Sistemi" (CELSS) için kapsamlı hidroponik araştırmalar yapılmaktadır. Mars'ta gerçekleşmesi amaçlanan hidroponiklerin çok daha az sıcaklık ile farklı renk spektrumunda gelişmeyi sağlamak için LED aydınlatma kullanmaktadır (Pandey ve ark., 2009). Literatürde 9300m² alana kurulu 30 kat yüksekliğindeki bir dikey çiftliğin 15.000 kişiye günlük 2000 kcal.'lik besin sağlayabileceği ifade edilmiştir (Pandey ve ark., 2022). Bu kadar çok insana yeterli gıdayı sağlamak amacıyla bir dikey kulenin sahip olması öngörülen özellikler olarak; kulenin 37 katlı olması (25'i yalnızca bitkisel üretim ve 3'ü su ürünleri yetiştiriciliği amaçlı) ve 0.93 hektarlık bir alanda yer alması gerektiği, ayrıca çevre ve atık yönetimi, paketleme ve ürün işleme gibi prosesler için de belirlenen katların olması gerektiği vurgulanmıştır (Banerjee ve Adenaeuer, 2014).

4. Dikey tarım sistemleri / Vertical farming systems

4.1. Hidroponik sistem / Hydroponic system

Hidroponik sistemler, pek çok üretici tarafından sıklıkla tercih edilen sistemlerden biridir (Waiba ve ark., 2020). Küresel olarak hidroponik endüstrisinin pazar payı 2022'de 4,65 milyar dolar olduğu ve 2023'ten 2030'a kadar %11,9'luk bileşik yıllık büyüme oranında (CAGR) büyümesi beklendiği rapor edilmiştir (Grand View Research, 2021). Hidroponik sistem, mahsullerin kum, çakıl, vermikülit, taş yünü, perlit, turba yosunu, Hindistan cevizi veya talaş gibi mekanik destek kullanılarak veya kullanılmadan besin solüsyonlarında (eriyik haldeki bitki besin maddeleri içeren) ve toprak kullanılmadan (topraksız kültür) yetiştirilebildiği bitki yetiştirme tekniğidir (Richa ve ark., 2020; Swain ve ark., 2021). Hidroponik terimi köken olarak, Yunancadan gelen bir sözcüktür ve "*Hidro*" ve "*Ponos*" sözcüklerinden türetilmiştir. Hidroponik ile ilgili diğer benzer terimler; "su kültürü", "hidrokültür", "besin kültürü", "topraksız kültür", "topraksız tarım", "tank çiftçiliği" veya "kimyasal kültür" dür. (Swain ve ark., 2021). Genel olarak bitki yetiştirme

yöntemlerinin hem olumlu hem de olumsuz yönleri bulunmaktadır (Richa ve ark., 2020). Geleneksel tarım yöntemlerine (toprak kültürü) kıyasla bu yenilikçi tarım tekniğinin başlıca avantajları şunlardır:

- Toprak türünden/kalitesinden bağımsız verimli üretim (hidroponikte 11±1,7 kat daha fazladır) yapılması,
- İklim kontrolünün sağlanması,
- Besin solüsyonu yoluyla büyümenin daha iyi izlenmesi,
- Kaynakları en üst düzeye çıkarma; su ve besin maddelerinin yeniden kullanılabilmesine olanak sağlaması, hidroponik sistemde geleneksel tarıma oranla %70 daha az su gereksinimi vardır.
- Kolay çevresel kontrol sağlanabilmesi; uygun sıcaklık, pH, bağıl nem vb. sağlanarak üretilen bitkilerin artan verimliliği,
- Hızlı büyüme ve mahsul artışı sağlaması,
- Toprak kaynaklı hastalıkları önlemek ve patojenleri kontrol etmek daha kolay,
- Herbisit ve pestisit kullanımının azalması,
- İş gücü ve zaman tasarrufu sağlanması,
- Bitki atıklarının yakılarak imhasının azaltması
- Mahsul yetiştirmek için daha az alan gerekmesi (Lee ve Lee, 2015; Bingol, 2019; Richa ve ark., 2020; Rakesh ve Javakrishna, 2022).

Hidroponik sistemin dezavantajları ise; maliyetli olması, hassas bir üretim sistemi olması, sistemin sürekli izlenmesi gerekliliği, teknik bilgi gerekliliği, sadece suyu seven bitkiler için uygun olması olarak sıralanabilir. Ayrıca nakliye maliyetleri geleneksel tarıma göre önemli ölçüde daha az olsa da, dikey çiftlikte aydınlatma ve iklim kontrolü için enerji tüketimi işletme maliyetlerini önemli ölçüde artırabilmektedir (Bingol, 2019). pH ve elektriksel iletkenliğinin analizi ile birlikte besin maddelerini doğru bir şekilde hazırlamak ve karıştırmak için eğitimli personele ihtiyaç vardır. Ayrıca elektrik arızalarını önlemek için sistemin izlenmesi gerekir. Yine de, hidroponiğin avantajlarının dezavantajlarından daha fazla olduğu ifade edilebilir (Richa ve ark., 2020).

Bu sistemde; marul (*Lactuca sativa*), nane (*Mentha piperita*), ıspanak (*Spinacia oleracea*), frenk soğanı (*Allium schoenoprasum*), salatalık (*Cucumis sativus*), domates (*Solanum lycopersicum*), çilek (*Fragaria*), dereotu (*Anethum graveolens*), kekik (*Thymus*), biberiye (*Rosmarinus officinalis*), anason (*Pimpinella anisum*), dolmalık biber (*Capsicum annuum* var. *annuum*), su teresi (*Nasturtium officinale*), fasulye (*Phaseolus vulgaris*), karpuz (*Citrullus lanatus*) ve kavun (*Cucumis melo*) yetiştirilebilmektedir.

Hidroponik sistemler temel olarak, açık hidroponik sistemler ve kapalı sistemler olarak ikiye ayrılabilir. Kapalı hidroponik sistemlerde, geleneksel tarımda kullanılan gübrelerde de bulunan nitrat ve fosfat kaynaklı su kaynaklarında meydana gelen kirlenme önemli ölçüde azalmaktadır. Öte yandan, bu sistemin kullanılmasının ana sorunlardan biri, sulama suyunun tuzlu (geri dönen besin solüsyonunda tuz iyonlarının birikmesi, yani yüksek NaCl konsantrasyonları) olmasıdır (Richa ve ark., 2020).

Martin ve ark. (2019), bira üreticilerinin kullandığı tahıllardan, geri dönüştürülmüş kâğıtlardan vs. besiyeri içeriğini desteklenmeye çalışmış ve sonuç olarak simbiyotik bir üretim süreci izleyerek hem sera gazı emisyonlarını yıllık olarak %60'ın üzerinde azaltılabileceğini hem de sürdürülebilirlik açısından yarar sağlanabileceğini tespit etmişlerdir.

4.2. Aeroponik sistem / Aeroponic system

Aeroponik sistem, toprak veya substrat kültürü içermeden iç mekânda yapılan bitki yetiştirme yöntemidir. Bitkinin yapay bir desteğin yardımıyla havada asılı büyüdüğü bitkiyi desteklemek için toprak veya substrat gerekmediği bir sistemdir (Francis ve ark., 2018; Lakhiar ve ark., 2018; Ikiz ve ark., 2018). Uluslararası Toprak Bilimleri Birliği Topraksız Kültür Çalışma Grubu (The International Union of Soil Sciences Working Group on Soilless Culture) ise, aeroponik sistemi "küçük damlalar (bir sis veya aerosol) ile doymuş bir ortamda köklerin sürekli veya süreksiz olarak büyütüldüğü bir sistem" olarak tanımlamaktadır (Balogun ve ark., 2014). Kökler havada asılı bir şekilde dururken, bitki büyümesi için gerekli olan besinler ve su ince sis halinde sarkan köklere püskürtülür (Francis ve ark., 2018; Ikiz ve ark., 2018). Besin çözeltisinin sis halinde bitkilere verilmesi genellikle yüksek veya düşük hava basıncı ile sağlanmaktadır. Besin çözeltisinin otomasyonunda sis/sprey/aerosol/damlacık boyutu için dikkate alınan nokta, yüksek basınçta 10 ile 100 mikron, düşük basınçta 5-50 mikron ve ultrasonik sisleyicilerde 5-25 mikron arasında olmasıdır (Lakhiar ve ark., 2018). Düşük basınçlı aeroponik sistemlerde, bitki kökleri besin çözeltisi rezervuarının üzerinde veya bir rezervuara bağlı bir kanalın içinde asılı olarak bulunmaktadır. Düşük basınçlı bir pompa besin çözeltisini ilettikten sonra rezervuara geri dönmesini sağlayabilmektedir. Bu tür üniteler genellikle tezgâh üstü yetiştirmeye uygundur. Yüksek basınçlı pompaların kullanıldığı aeroponik sistemlerde ise yüksek kurulum maliyetlerini telafi edebilecek yüksek değerli mahsullerin yetiştirilmesi söz konusudur (Choudhury ve Dutta., 2022). Bu sistemler hava-su kültürü olarak kabul edilmektedir. Bu bitki yetiştirme sistemi, B. T. Barker tarafından bir spreyle elma ağaçları yetiştirmesi ile gündeme gelmiştir (Barker, 1922; Clawson ve ark., 2000). Ayrıca sistem, 1957'de besin solüsyonunun sis şeklinde verildiği kökleri havada asılı olan domates ve kahve bitkilerini yetiştiren F. W. Went, tarafından "aeroponik" olarak adlandırılmıştır (Went, 1957; Clawson ve ark., 2000).

Aeroponik sistemde havada asılı olan kökler çok miktarda oksijen alabilmektedir ve bu durum bitkinin daha yüksek metabolizma ve büyüme hızına yardımcı olmaktadır. Çalışmalar, büyüme hızının toprakta olduğundan 10 kata kadar arttığını göstermektedir. Ayrıca, daha az buharlaşma nedeniyle su kaybı çok azalmaktadır (Francis ve ark., 2018). Aeroponik sistemler, mahsul verimini en üst düzeye çıkarırken su kullanımını yüzde 98'e kadar azaltabilmektedir. Aeroponik sistemlerde yetiştirilen bitkilerin daha fazla mineral ve vitamin alarak, daha sağlıklı ve potansiyel olarak daha besleyici hale geldiği de gösterilmiştir (Birkby, 2016; Chaudhry ve Mishra, 2019). Bitkilerin yetiştirildiği hazneler çok az yer kaplamaktadır. Bu durum ise aeroponik sistemin bir depoda, bodrumda veya herhangi bir alanda rahatça kullanılabilirliğini sağlar (Balogun ve ark., 2014). Bu sistemin hava/sis ortamında topraksız bir şekilde bitkilerin hızlı büyümesine izin verdiği için hidroponik sisteme göre daha avantajlı olduğu ifade edilmektedir (Chaudhry ve Mishra, 2019). Sistem su tasarrufunun yanı sıra %60'a varan besin tasarrufu sağlayarak, %100'e varan pestisit ve herbisit kullanımını azaltabilmekte ve bitki verimini hidroponik veya geleneksel tarıma göre %45 ila 75 arasında arttırabilmektedir (NASA, 2006; Lakhiar ve ark.,

2018). Sistem işçilik açısından da geleneksel tarıma göre daha avantajlıdır. Bu teknik, yapraklı sebzeler, kök sebzeler, aromatik otlar ve şifalı bitkiler için başarıyla uygulanmıştır. Aeroponik kültür ortamında yetişen bitkilerin fenolik ve flavonoid içerik miktarının, antioksidanlar ve vitaminler gibi verim ve besin kalitesinin toprakta yetişen bitkilere göre daha yüksek olduğu bulunmuştur (Böhme and Pinker, 2013; Chandra ve ark., 2014). Aeroponik sistemler, (1) büyüme odası, (2) bitki destekleme kısmı ve (3) besin besleme sistemi dahil olmak üzere temel olarak üç ana kısımdan oluşur (Lakhiar ve ark., 2020). Bu sistemin avantajları (1) hızlandırılmış bitki büyüme oranları ve daha kısa olgunlaşma süresi, (2) verimde önemli artışlar, (3) bitkiden bitkiye daha yüksek biyokütle ve ikincil metabolit tutarlılığı ve (4) kontaminant içermeyen ürün olarak sıralanmıştır (Pagliarulo ve Hayden, 2002). New York'ta domatesle yapılan aeroponik deneyde bir yılda mahsulün dört katına çıktığı gözlenmiştir (Al-Kodmany, 2018).

Literatürde aeroponik sistem ile domates, soğan, fesleğen, patates, tatlı patates, soya fasulyesi, mısır, marul, fesleğen, *Anthurium andreanum* ve *Acacia mangium* gibi sebzeler, meyveler, şifalı bitkilerin yetiştirildiği bildirilmiştir (Gopinath ve ark., 2017; Ikiz ve ark., 2018; Lakhiar ve ark., 2018; Khater et al., 2021).

Amerika Birleşik Devletleri'nin önde gelen dikey tarım şirketi olan AeroFarms Newark, New Jersey merkezli sürdürülebilir bir iç mekân tarım şirketidir ve ürün yetiştirmek için patentli bir aeroponik yetiştirme sistemi kullanmaktadır (Birkby, 2016).

4.3. GrowCube sistemi / GrowCube system

Topraksız tarım üzerine yapılan çalışmaların sayısı gün geçtikçe artmaktadır. Araştırmalar kapsamında aeroponik sistemin daha yüksek verim sağlayacak versiyonları üzerine de araştırmalar devam etmektedir. Son yıllarda, kentsel tarıma uygun olarak iç mekânlarda sebze yetiştirmek için yenilikçi bir teknik olarak GrowCube teknolojisi karşımıza çıkmaktadır. GrowCube, hem evlere hem de ticari operasyonlara her iklimde taze gıda yetiştirmek için tam otomatik bir yol sağlamayı amaçlayan bir aeroponik sistemdir. Bu sistemde geleneksel yetiştirme sistemlerine kıyasla büyük alan gerekmez. GrowCube mutfağa yerleştirilebilen ve ıspanak, lahana gibi yapraklı sebzelerin yetiştirilmesine uygun yetiştirme kutusu olup, ürünler her gün pişirmeden önce hasat edilebilmektedir. Böcek ilacı içermediğinden de ürün tüketimi açısından daha güvenlidir (Noh ve ark., 2021). GrowCube, bir tekerlek aracılığıyla dönen ve gerekli ışığı LED şeridi ile sağlayan beş hafif plastik plaka içeren yüksek teknolojili küp şeklinde bir havalandırma sistemi bulunan aeroponik bir prototiptir (Al-Kodmany, 2018; Noh ve ark., 2021). Yazılım geliştirici Chris Beauvois, bilgisayar programları ve topraksız tarım sistemlerini birleştirerek GrowCube sisteminin buluşunu gerçekleştirmiştir. Geleneksel tarım yöntemlerine oranla %95 daha az su kullanan bu sistem, bitkileri plakalarda ortama sis halinde su vererek büyütmektedir (Cooper, 2013). Patentli buğulanma teknolojisi, en karmaşık kök sistemlerine bile nüfuz edebilmektedir. Küp ve cihazlar, bilgisayar ve yazılım aracılığıyla kontrol edilir ve küpün içindeki sensörler, mikro iklimi optimize etmek için bilgisayarla iletişim kurar (Al-Kodmany, 2018). Günümüzde şirketler, online olarak çeşitli uygulamalar ve ürün yetiştirme kılavuzları geliştirmektedirler. Bu sayede yetiştirme süreçlerini tamamen uzaktan kontrol ederek optimize edilebilir hale getirmeyi sağlamış olmaktadırlar. GrowCube ile sebze veya otlar yetiştirilmiş olup, meyvelerin de üretilmesi hedeflenmektedir. GrowCube, bu yüksek teknolojili küplerden yüzlerce üreterek projeyi genişletmeyi planlamaktadır.

4.4. Akuaponik sistem / Aquaponic system

1980 yılından beri gün geçtikçe gelişen bir teknoloji olan akuaponik sistem, yenilebilir ve suyu seven bitkileri ve balıkları sadece balık yemi ve güneş ışığı girdisinin olduğu kapalı bir sistemde bir arada yetiştirmek amacıyla yapılan bitki + balık + bakteri + su ortamının dinamik etkileşimini içeren topraksız bir su kültürü şeklidir (Love ve ark., 2015; Shafeena, 2016; Krastanova ve ark., 2022). Dolayısı ile sistem su ürünleri yetiştiriciliği (balık yetiştiriciliği) ve hidroponik (topraksız bitki yetiştirme) yetiştiriciliğin kombinasyonu olup, simbiyotik üretim tekniği olarak da adlandırılabilmektedir (Kyaw ve Ng, 2017). Akuaponik sistem, bitkilerin büyümesi için gerekli olan besin maddelerinin geri dönüşüm aracılığıyla böcek ilaçları kullanılmadan yeniden kullanılması amaçlanmaktadır (Shafeena, 2016). Bu sistemler doğal biyolojik döngüler kullanılarak yenilenemeyen kaynakların kullanımını en aza indirildiği ve zamanla artabilecek ekonomik faydalar sağlayan sürdürülebilir bir tarım sistemi olarak hedeflenmiştir (Tyson ve ark., 2011).

Sistemin faydaları; geleneksel su ürünleri yetiştiriciliğine kıyasla suyun verimli kullanımı, sınırlı atık üretimi, sıfır atık su deşarjı, karbon ayak izinin küçük olması, daha az kaynak tüketimi, daha az çevresel etki, daha az metan emisyonu, güvenli gıda üretimi, sınırlı su kullanımına bağlı olarak çevresel kontaminant girişinin azalması, mahsul üretiminin yoğunluğunun artması, kalıntısız mahsul üretimi, nakliye masraflarının düşmesi, toprağa bağlı hastalıkların bertaraf edilmesi olarak belirtilebilmektedir (Love ve ark., 2015; Colt ve ark., 2021). Toprağa dayalı tarımdan daha verimli olduğu için ve sürekli su tasarrufu sağladığı için, kaynakların sınırlı olduğu ve iklim değişikliğinden etkilenen bölgelerde gıda üretimi için ideal bir üretim sistemi olduğu vurgulanmıştır (Pantanella, 2018). Akuaponik sistemden çıkan katı atıklar toprağı zenginleştirmek amacıyla geleneksel tarım çiftliklerine satılabilmektedir. Sistemin kurulacağı alanın su kaynağına yakın olmasına ya da drenaj sistemine ihtiyacı yoktur (Kargin ve Bilguven, 2018). Bu sistemin, geleneksel yöntemlere göre yüzde doksan daha az su tükettiği belirtilmiştir. Bir baş marulun toprakta üretimi için 50 gün gerekirken, bu sürenin akuaponik sistemde daha kısaldığı ve 35 gün sürdüğü belirtilmiştir. Yine araştırmacılar, 23.000 kg balığın 450.000 kg marul ve ot üretimine yardımcı olduğunu ifade etmişlerdir. Başka bir örnekte ise, 1 metrekare alanda 0,45 metreküp suya sahip bir sistemde 1 kg fesleğen yaprağının 60 günde yetiştirilebildiği bildirilmiştir (Hati ve Singh, 2021). Wilson (2005) akuaponik sistemde 1 kg balık yetiştiriciliği için harcanan yem ile 7 kg bitki üretilebildiğini ifade etmiştir. Florida'da akuponik bir sistemde hasat edilen ortalama balık ve bitki miktarının yılda sırasıyla 23-45 kg ve 45-226 kg olduğu belirtilmiştir (Colt ve ark., 2021). Rakocy ve ark. (2004), akuaponik sistemde dört yıl boyunca fesleğen ve bamya bitki türlerini, Nil Tilapia ve kırmızı Tilapia balık türleri ile birlikte yetiştirmişlerdir. Her iki balık türünün de hayatta kalma oranları yüksek bulunurken, aynı kültür suyunda bitkilerin yetiştirilmesi nedeniyle 4. hasattan sonra ortaya çıkan besin yetersizliğine bağlı olarak fesleğende bozulmalar görüldüğü ifade edilmiştir. Diğer yandan, bamyanın arazi üretimine göre 18 kat daha fazla üretilebildiğini belirtmişlerdir. Bahri ve ark. (2020) 4 metrekarelik bir alanda 120 adet nane

çeliği ile 90 adet balık yetiştirerek, bitkilerin köklerinde %50, yapraklarında %100 artış saptayarak hızlı büyüme gerçekleştiğini ve balıklarda %100'e yakın yaşama oranı ve büyüme oranı gözlemlemişlerdir.

Öte yandan, dünyanın bazı iklimlerinde veya bölgelerinde akuaponik sistem ile ilgili belirli ölçeklerde gıda üretimine yönelik çeşitli sınırlamalar vardır (Love ve ark., 2015). Birleşmiş Milletler Gıda ve Tarım Raporu (United Nations Food and Agriculture)'nda maliyetinin yüksek olması, enerji/kaynak gerektirmesi, günlük bakımların düzenli yapılması sistemin dezavantajları olarak vurgulanmıştır (Love ve ark., 2015; Sahin ve Kendirli, 2016). Yaşanabilecek elektrik kesintilerinin, borularda görülebilecek tıkanmaların sucul canlıların ölümüne yol açabildiği de ayrıca belirtilmiştir (Kargin ve Bilguven, 2018). Bu nedenle, gıda güvenliği ve sürdürülebilirlik konularında avantajlı olan sistem, balıkların ve bitkilerin sağlıklı büyümesi için tesislerin sürekli izlenmesi gerektiğinden işletimi zor olabilmektedir (Kyaw ve Ng, 2017). Bu noktada, çiftçi için ekonomik olması hususu dikkate alınmalıdır (Love ve ark., 2015).

Akuaponik sistem akuakültür kısım (canlılarının yaşayıp beslendiği, atık ürettiği kısım) ve hidroponik kısım sebze (lahana, kabak, salata, marul vb.) ve meyve (çilek, domates vb.) gibi bitkilerin yetiştirildiği kısım) olmak üzere iki kısımdan oluşmaktadır. Akuaponik sistemlerde bulunan atık arıtma sistemi aracılığıyla su, bitki ve balık tankları arasında devirdaim yapılarak biyolojik olarak filtre edilmekte ve su ürünleri yetiştirilmektedir (Love ve ark., 2015). Balık atıklarının yetiştirilmek istenen bitkiler için gübre işlevi gördüğü ve bitkinin ise balıklar için istenmeyen amonyağın diğer azotlu bileşiklerle birlikte sudan uzaklaştırıldığı bir sistemdir (Kargin ve Bilguven, 2018). Amonyak, balığın boşaltım ürünü olup, çok düşük dozlarda bile suyun pH'sına bağlı olarak balık için toksik olabilir (Yildiz ve Pulatsu, 2022). Temiz hale gelen su balıkların gelişimi için de uygun hale gelmektedir. Bu sistemde su ve balık atıkları bitki yetiştirme kabına pompalanır. Balık atıkları amonyak açısından zengin olup, bakteriler amonyağı önce nitrite, sonra da nitrata dönüştürür (Gosh ve Chowdhury, 2019; Hati ve Singh, 2021). Bu doğal biyolojik dönüşümü yapacak bakteriler yüzeylerde yaşarlar ve suda balıkların protein katabolizmasının son ürünü olan amonyak olduğu zaman gelişmeye başlarlar (Krastanova ve ark., 2022). Bakteri faaliyetleri sonucu oluşan nitrat bitkiler için besin maddesi görevi görür. Besinlerin bitki kökleri tarafından emilmesinden sonra temiz ve filtrelenmiş su akvaryuma geri döner (Hati ve Singh, 2021). Akuaponik sistemlerde bu dönüşümü gerçekleştiren nitrifikasyon bakterileri üzerinde çalışmalar yapılmaktadır. Özellikle amonyumu nitrite dönüştüren amonyum oksitleyici bakteriler üzerinde (*Nitrosococcus, Nitrosospira* ve *Nitrosomonas*) durulmaktadır. Nitritin nitrata dönüştürülmesinde nitrit oksitleyici bakteriler (*Nitrobacter, Nitrospira, Nitrococcus* ve *Nitrospina*) sorumludur. Son zamanlarda, *Nitrospira* sp.'nin amonyumdan nitrite tam nitrifikasyonu da gerçekleştirebildiği açıklanmıştır. Bununla birlikte, akuaponik sistemin farklı bölümlerindeki toplam mikrobiyal topluluk, omik teknolojileri kullanılarak karakterize edilmeye çalışılmaktadır. Akuaponik sistemlerin farklı tasarımlarda olması, bu sistemlerde farklı optimum koşullarda üretim yapılabilmesi ve sistemin farklı alt birimleri içerebilmesinden ötürü bu bileşenlerdeki mikrobiyal toplulukların da farklılık gösterebileceği belirtilmiştir (Schmautz ve ark., 2017; Kushwaha, ve ark., 2023). Örneğin, akuaponik sistemlerinin farklı bölümlerinden karakterize edilen taksonlar; bitki köklerinde *Proteobacteria, Planctomycetes, Acidobacteria, Bacteroidetes, Nitrospirae, Gemmatimonadetes, Methylophilales, Nitrosomonadales,* ve *Pseudomonas* sp.; biyofiltrelerde *Bacteroidetes, Verrucomicrobia, Fusobacteria, Planctomycetes, Chloroflexi, Nitrospirae, Proteobacteria, Microbacteriaceae, Rhodobacterales, Rhizobiales, Sphingomonadales, Nitrosomonadales, Burkholderiales ve Rhodocyclales*; balık tankında *Nitrospirae ve Nitrobacter* sp. olarak bildirilmiştir (Kasozi ve ark., 2021).

Mevcut akuaponik sistemlerde farklı balık türleri kullanılmaktadır. En yaygın olarak kullanılan balık türlerinin; tilapia, süs balıkları (koi, japon balığı ve tropikal balıklar), yayın balığı, sazan, bluegill, alabalık ve levrek olduğu belirtilmiştir. Yetiştirilen bitki türleri ise genellikle mutfak amaçlı kullanıma yönelik otlar ve baharatlar (fesleğen, kişniş, frenk soğanı, maydanoz, semizotu, nane, marul, ıspanak, pazı, Çin lahanası, su teresi vb.) olarak belirtilmiştir. Domates, biber ve salatalık gibi sebzeler daha yüksek besin gereksinimine ihtiyaç duydukları için iyi kurulmuş akuaponik sistemlere ihtiyaç duyulmaktadır (Yep ve Zheng, 2019; Krastanova ve ark., 2022).

Akuaponik çözeltide özellikle bazı bitkiler için K, Mg, Ca ve Fe'nin yetersiz olduğu ifade edilmiştir. Bu nedenle akuaponik sudaki besin seviyelerinin optimize edileceği detaylı araştırmalara ihtiyaç olduğu vurgulanmıştır (Yep ve Zheng, 2019). Akuaponik sistemlerde sıcaklık, nem, pH ve mineral konsantrasyonu gibi yetiştirme koşullarının yetiştirilecek sebze, bitki ya da balık türüne göre değişkenlik gösterebileceği belirtilmiştir. Örneğin, bitki türlerinin çoğu için pH bakımından optimal gelişimin pH 6-6,5; balıklar için ise pH 7-9 arasında gerçekleştiği ifade edilmiştir. Ayrıca, nitrifikasyon bakterilerinin gelişimi için pH seviyelerinin 7,5-8,3 arasında olmasının önemi literatürde belirtilmiştir. Bu nedenle tüm akuaponik sistem için geçerli olan ideal pH aralığı 6,0-8,0 olarak rapor edilmiştir (Gosh ve Chowdhury, 2019). Balıklar için en uygun sıcaklıkların yine türe bağlı olarak 25-30°C olduğu kaydedilmiştir (Rakocy ve ark., 2016). Krastanova ve ark. (2022) ise, akuaponik sistemde hem yetiştirilecek *Tilapia* balık türü ve hem de bitkiler için optimum su sıcaklığının 22-24°C, pH değerinin 5,6-7,3, çözünmüş oksijen miktarının ise 3-10 mg/L olması gerektiğini belirtmiştir.

4.5. Silindirik topraksız yetiştirme sistemleri / Cylindrical soilless growing systems

Silindirik Omega Garden veya Volksgarden olarak da bilinen son yıllarda gelişmiş hidroponik sistem örneği karşımıza çıkmaktadır. Bu sistemde bitkiler merkezi LED ışıklarının etrafında düşük beygir gücünde bir motor kullanılarak her 50 dakikada bir dönen çarkların içine yerleştirilmektedir. Çarkların dönüşünü güneş panelleri veya rüzgâr türbinleri ile de sağlamak mümkündür. Bu sistemlerde bitkiler bir merkez ünite etrafında dönerken belirli bir süre ve seviyede tüm bitkilerin eşit ışık alabilmesi mümkündür. Volksgarden sisteminde bitkilerin kökleri taş yünü içinde yetiştirilirken, bir yandan da geleneksel hidroponik sisteme göre daha hızlı büyümesi sağlanmaktadır. Sistem üniteleri üst üste istifleme yöntemiyle kapasitesini çoğaltmaya elverişlidir (Al-Kodmany, 2018). Volksgarden sisteminde her silindirin 80 bitki tutabilme kapasitesi vardır (Papadopoulos, 2021). Bu sayede tek seferde çok miktarda bitki yetiştirme durumu mümkün olabilmektedir. Volksgarden dikey çiftçilik yöntemi ile kullandığı bu sistemde 120 döner modül içermektedir. Kırmızı ve mavi LED aydınlatma kullanarak, hidroponik silindirik çok katmanlı bir sistemde marul ve

lahananın yetiştirildiğini ifade edilmiştir (Kaur ve Chawla, 2021).

5. Şehirlerde dikey tarım uygulamalarına örnekler / Examples of vertical farming applications in cities

5.1. Sky greens / Sky greens

Singapur bir ada ülkesi olarak tarımsal faaliyetler için sınırlı alana sahip olduğu ve tükettiği gıdanın yalnızca %7'sini üretebildiği için dikey tarıma oldukça önem vermektedir (Al-Kodmany, 2018). Singapur, gıda talebinin yüzde 90'ından fazlasını ithal ürünler ile karşılamaktadır. Bu sebeple Singapur'da dikey tarım uygulamaları ile yerel gıda üretiminin arttırılması ve ithal ürünlere olan bağımlılığın azaltılması hedeflenmiş ve 2012'de yüksek teknolojili şehir çiftliklerinden biri olan Sky Greens ticari olarak faaliyete başlamıştır (Gupta ve Ganapuram, 2019). Sky Greens, dünyanın düşük karbon ayak izine sahip hidrolik suyla çalışan yerçekimi destekli dönen bir yetiştirme sistemi ve enerji tasarruflu LED aydınlatma kullanan dikey tarım sisteminin kurucusudur (Sky Greens, 2018). Bu dikey çiftlikte, üretim sürecinde oluşan atıkların yeniden kullanılmasını sağlayan 3R prensibi (azalt, yeniden kullan ve geri dönüştür) takip edilmektedir. Çevre üzerinde olumsuz etkiye sebep olmadan, yüksek verim ve yüksek kaliteli ürünlerin üretimini sağlayan, ultra modern bir aydınlatma sistemi kullanan ve tamamen kontrolü sağlayan bir otomasyon sistemi ile çalışmaktadır (Zareba ve ark., 2021). Sky Greens, günde ortalama 0.5 ton sebze üretim yapabilme kapasitesine sahip, 9 metrelik bir boyutlarda ve 120 alüminyum kuleden oluşmaktadır (Gupta ve Ganapuram, 2019). Sky Greens'in avantajları; geleneksel tarıma göre birim alan başına 5 ila 10 kat daha fazla ürün üretilebilmesi, çevre dostu olması, düşük enerji ve düşük su kullanımı sağlaması, sürdürülebilir atık su yönetimine sahip olması ve yeşil teknoloji kullanması olarak belirtilmiştir (Sky Greens, 2018; Chaudhry ve Mishra, 2019). Sky Greens Solutions (Sky Greens'in sahibi olan şirket), 2015 yılında yenilikçi tasarımıyla INDEX Ödülü'nü kazanmıştır. Sky Greens arazi sıkıntısı olan kentlerde elektrik, su ve insan gücü tasarrufu ile dikey tarım sistemi olarak umut vadeden bir sistemdir (Wong ve ark., 2020). Bir veya birkaç tür mahsul (örneğin; marul, ıspanak, tropikal yapraklı sebzeler) büyük hacimlerde yetiştirilebilmektedir (Beacham ve ark., 2019).

5.2. Plantagon / Plantagon

Bir diğer dikey tarım şirketi olan Plantagon İsveç, Linköping'de faaliyetlerine başlamıştır. Plantagon, yerel enerji şirketleri ve yerel biyogaz fabrikaları ile kurulacak simbiyotik bir ilişkiyi sağlamayı hedeflemiştir. Dikey çiftliğin, fabrikaların organik atık, karbondioksit, gübre ve fazla ısısını toplayıp bunları ısıtma-soğutma için biyogaza dönüştürülmesini ve böylelikle gıda üretiminin yanı sıra sürdürülebilirlik konusunda da çözümsel yaklaşımı amaçlamıştır (Al-Kodmany, 2018). Plantagon diğer şirketlerden farklı olarak yerel üretime ek olarak üretilen ürünleri Asya pazarına da göndermeyi planlamıştır (Hallock, 2013). Küre şeklinde olan, dönen sarmal şekilli tasarımı ile otomatize bir sisteme sahip olması, karbondioksit ve oksijen alışverişini sağlaması, otel ve ofis gibi yerlere uygulanabilir olması sebebi ile avantajları olduğu bildirilmiştir (Chaudhry ve Mishra, 2019). Yapay aydınlatma ihtiyacının en aza indirilmesi hatta tamamen ortadan kaldırılması amaçlanmıştır (Möller Voss, 2013). Neredeyse tamamen makineleşen bu sistem gelecekte gıda sektörü açısından büyük bir trend olma potansiyeli taşımaktadır. Ancak bu durum, çalışan personelin yanı sıra teknolojiyi yönetebilecek bir profesyonelin ihtiyacını da ortaya çıkartmaktadır (Hallock, 2013). Ayrıca mahsülün üretimi, hasadı, atık yönetimi ile ilgili otomatik entegrasyon sistemi olmaması dezavantaj olarak belirtilmiştir (Chaudhry ve Mishra, 2019).

5.3. Çatı katı tarımı / Rooftop farming

Günümüzde kentsel yapı yoğunluğunun artması ile şehirlerde yeşil alan kaybı çok yüksek seviyelere ulaşmıştır. Bu nedenle büyük şehirlerde peyzaj mühendisliği alanında yeşillendirme çalışmaları yapılmaktadır. Özellikle New York, Singapur, Hong Kong gibi binaların yoğun olduğu şehirlerde, yeşil alan artırmak amacıyla yeşil çatı, yeşil duvarlar ve kentsel tarıma yönelim söz konusudur (Lee ve Chuang, 2017). Kentsel tarım arazilerinin yetersizliğine bağlı olarak, sürdürülebilirlik kapsamında kullanılmayan alanlar olarak çatılar eşsiz bir yetiştirme alanı olarak görülmektedir. Bazı şehirlerde çatılar ve balkonlar taze tüketim için çeşitli yapraklı yeşillikler, otlar ve baharatlar yetiştirmek için kullanılmaktadır. Bunun yanında, yeşil çatı çiftliklerinin ısı etkisinin azaltılmasında, yağmur suyunun akışının azaltılmasında ve binalara ısı yalıtımının sağlanmasında olumlu etkilerinin olacağı belirtilmektedir (Mir ve ark., 2022). Yeşil çatı çiftliklerinin gıda güvenliğini, atık yönetimini ve iş istihdamı sağlama yönünde olumlu sonuçlarının olduğu aşikârdır (Harada ve ark., 2018a). Üç tür yeşil çatı çiftliği bulunmaktadır. Bunlar, insanların organik gıdaya artan ilgisi neticesinde çatılarda kurulan hidroponik çatı sistemleri, pilot çalışmalar için veya eğitim amacı ile kullanılan çatı çiftlikleri ve ticari çatı çiftlikleridir. Ticari çatı sistemlerinin hedef kitlesi taze yetiştirilen ürünleri satın almayı talep eden müşteriler veya restoranlardır. Genellikle küçük ölçekli üretimlerdir ancak restoran kullanımını sürdürmek için yeterlidir. Hong Kong'daki HK çiftliği üretimin yanı sıra görsel olarak da şehre katkıda bulunmaktadır (Lawrence ve ark., 2022).

5.3.1. Brooklyn grange / Brooklyn dikey çiftliği

New York merkezli bir proje olan Brooklyn Grange 11 katlı bir binanın tepesinde 2010 yılında kurulan ve sebze yetiştirilen 0.6 hektarlık bir çatı çiftliği olup, her yıl 23.000 kg'dan fazla ürünü (domates, kale, salatalık, havuç, bezelye gibi) yetiştirebilme kapasitesine sahiptir. Üretilen ürünler çiftlik pazarlarında ve yerel restoranlara satılmaktadır (Royte ve ark., 2015; Harada ve ark., 2018a). Brooklyn Grange, işletmelere, okullara, kâr amacı gütmeyen kuruluşlara ve devlet kurumlarına yeşil çatılar, yeşil duvarlar ve organik kentsel çiftlik sistemlerini tasarlama ve kurma konusunda sürdürülebilirlik danışmanlığı hizmetleri de sunmaktadır (Plakias, 2016; Harada ve ark., 2018b). Çiftlik kurulduğundan bu yana tonlarca çöpü geri dönüşmüştür (Yang, 2022). Toplum Destekli Tarım (Community Supported Agriculture, CSA) programı uygulanan Brooklyn Grange'in yerel ekonomiyi ve istihdam yaratmayı teşvik etmesi ile çatı katı tarımında önemli bir yere sahip olduğu belirtilmektedir (Ghosh, 2021). Çatı katı tarımında, Rooflite® adı verilen yoğun yeşil çatılar, çatı bahçeleri, konteynır bahçelerinde sağlıklı bitki büyümesi için optimize edilerek tasarlanmış organik madde ve lignoselülozik materyal içeren toprak kullanılmaktadır. Aynı zamanda Brooklyn Grange, yağmur suyu (yaklaşık olarak 1 milyon galon) yönetimi ile mahsul yetiştirmeyi de mümkün hale getirmektedir. Günümüzde

ise Brooklyn Grange, faaliyetlerini giderek büyütmekte ve gelişmeye devam etmektedir (Nasr ve ark., 2014; Royte, 2015; Harada ve ark., 2018b).

5.3.2. Gotham greens / Gotham greens

New York, Brooklyn merkezli Gotham Greens iki katlı bir binanın tepesinde, 2011 yılında inşa edilmiş yeşil çatı çiftliğidir. Şirket gelişmiş hidroponik tarım tekniklerini kullanarak 40'tan fazla ABD eyaletine taze, yüksek kaliteli, böcek ilacı içermeyen ürünler yetiştirmektedir. %95 daha az su ve %97 daha az toprak kullanan tesis olarak Business Insider'ın "Amerika'daki En Harika 50 Yeni İşletme" listesinde yer almıştır. Gotham Greens, çok çeşitli yapraklı yeşillikler ve otlar yetiştirmekte ve satmaktadır (Gotham Greens, 2017; Goodman ve Minner, 2019; Gulati, 2022). Gotham Greens güneş enerjisi kullanmakta ve şirketin misyonuna uygun olarak 55 kW enerji üretmektedir. Böylelikle düşük karbon ve enerji ayak iziyle sürdürülebilir bir şekilde çalışmaktadır (Goodman ve Minner, 2019). 2023'te Gotham Greens'in dokuz adet yüksek teknolojili, iklim kontrollü hidroponik seraya ulaşmayı hedefledikleri belirtilmiştir. Gotham Greens'in; Teksas, Georgia ve Colorado'da devam eden yeni sera projelerinin olduğu ve Illinois ve Rhode Island'daki mevcut seraları genişlettiği de ayrıca ifade edilmiştir. Uzun mesafeli soğuk nakliyeye gerek olmadan, ürün kalitesi ve raf ömrünün muhafaza edilerek gıda israfını azaltan bir sistemle ürünlerin yetiştirilmesi ve tüketicilere ulaştırılması söz konusudur (Business Insider, 2022). Literatürde, Gotham Greens'in geleneksel tarıma göre daha fazla üretim yapılabilmesi, ısıtma, soğutma, sulama ve bitki beslenme gibi parametrelerin gelişmiş teknoloji ile kontrol edilebilmesi, enerji kullanımının azaltılabilmesi, ürünlerin besleyici özelliklerinin iyileştirilebilmesi gibi avantajları olduğu bildirilmiştir (Chaudhry ve Mishra, 2019). Ayrıca 100 civarında çalışan istihdamı sağlaması yönüyle de çatı katı tarımında önemli bir role sahiptir. Gotham Greens geniş bir dağıtım ağına sahip olup, perakende satış noktalarına, süpermarketlere, üst düzey marketlere ve restoranlara toptan tedarik sağlamaktadır (Goodman ve Minner, 2019).

6. Uzay tarımının gelişim süreci ve uzay çiftlikleri / Development process of space agriculture and space farms

Birleşmiş Milletler verilerine göre, yıl 2050'yi gösterdiğinde ekstra 2,3 milyar insanın daha gıdaya ihtiyacı olacağı belirtilmiştir. Konvansiyonel tarımın bu talebi karşılaması imkânsız gibi gözükmektedir (NASA, 2021). Tarımın uzayda insan hayatını desteklemek için kullanılması, uzay araştırmalarının uzun süredir devam eden alanlarından biri olmuştur (Wheeler, 2017). Özellikle NASA'nın bu yönde çalışmaları devam etmektedir. Dünya'da ve uzayda gıda üretimi için su kullanımını ve enerji tüketimini en aza indiren topraksız tarım sistemleri üzerinden araştırmalar gelişmektedir (NASA, 2021).

Öte yandan, Mars veya Ay gibi uzun süreli uzay görevlerinde astronotların yemek ihtiyaçları, donmuş veya konserve edilmiş gıdalardan karşılanmaktadır ancak bu gıdalar da kütleye ve buna bağlı olarak fırlatma ve yakıt tüketimi sırasında ortaya çıkan ekstra maliyetler nedeniyle fazla miktarda alınamamaktadır. Son zamanlarda astronotların beslenmelerini desteklemek için taze gıdaların üretimi ve bitki yetiştirme sistemlerinin keşfedilmesi gündeme gelmiştir. Ancak uzay çiftçiliklerinde yerçekiminin ve diğer koşulların dikkate alınmasının gerekliliği literatürde vurgulanmıştır (Nguyen ve ark., 2023). Bu bağlamda ilk denemeler ABD Hava Kuvvetleri ve NASA için Jack Myers ve diğerleri tarafından 1950'ler ve 60'larda alglerin (*Chlorella* sp., *Anacystis, Synechocystis, Scenedesmus, Synechococcus,* and *Spirulina*) test edilmesine dayanan çalışmalar ile başlamıştır. Uzay tarımı sistemlerinin temelinde, bitkilerin veya diğer fotosentetik organizmaların biyokütle (CH_2O) ve oksijen (O_2) üretmesi, havadan CO_2'yi uzaklaştırması vardır. İlk uzay bitkisi araştırması (Sputnik 4), 1960 yılında buğday, bezelye, mısır ve soğan çimlenmesi ile başlamıştır (Nguyen ve ark., 2023).

İlk uzay istasyonları olan Saylut ve Skylab 1970'lerde mikro yerçekiminin sitoplazmik akış üzerindeki etkisi gibi temel astrobotanik araştırmaların yapılabilmesine imkân sunmuştur (Reed ve ark., 2023). 1971'de Oasis 1'de keten, pırasa, soğan ve Çin lahanası yetiştirilmiştir. Daha sonra Biosatellite II'de biber ve Mir uzay istasyonunda turp ve lahana gibi sebzeler üzerinde ön çalışmalar yapılmıştır (Nguyen ve ark., 2023; Reed ve ark., 2023). Mir uzay istasyonundaki SVET büyüme odasında, tohumdan uzayda yetiştirilen ürünlerin ilk mahsulü elde edilmiştir (Reed ve ark., 2023). 1982'de Rus uzay istasyonu olan Saylut'ta *Arabidopsis thaliana* çiçekli bitkisi yetiştirilmiştir. Yakın zamanda ise, The International Space Station (Uluslararası Uzay İstasyonu, ISS)'de astronotlar The Vegetable Production System (Veggie-NASA tarafından uzay ortamlarında geliştirilen ve kullanılan bir bitki yetiştirme sistemi) ve Advanced Plant Habitat (APH- Uluslararası Uzay İstasyonunda bitki biyolojisi araştırması yapmak için kullanılan olan otomatik bitki yetiştirme tesisi)'ı kullanarak köklü ve yumrulu sebzeler, soğan, sarımsak, yeşil yapraklı bitkiler, domates ve salatalık gibi meyve veren sebzeler yetiştirmişlerdir (Nguyen ve ark., 2023; Reed ve ark., 2023). Veggie ve APH sistemlerinin teknolojik olarak farklı sistemler olduğu ifade edilmiştir. Veggie'nin, bitki yetiştirme ortamını kontrol etmek için yalnızca LED aydınlatma sistemine ve bir havalandırma fanına sahip olduğu ve kontrollerin minimal düzeyde kaldığı bildirilmiştir (Reed ve ark., 2023). Biyokütle üretim sistemi (BPS) uzayda bitki yetiştirme denemeleri için kullanılan 2002'de NASA tarafından çalıştırılan ilk yetiştirme odasıdır. BPS hem çevresel parametrelerin koşullarını kontrol etmiş hem de bu bilgiler ile birlikte görüntüleri Dünya'ya göndermiştir. NASA'nın yakın gelecek hedefinde Ohalo III'te daha karmaşık bitki yetiştirme büyüme sistemleri geliştirmek vardır (Nguyen ve ark., 2023).

Mikro yeşilliklerin uzay istasyonlarındaki mürettebat ve astronotların iştahlarını arttırmak ve vücutlarındaki homeostazı korumak için iyi bir gıda bileşeni olarak kabul edildiği ifade edilmiştir. Uzay tarımı mikro yerçekimi ortamından kaynaklanan birçok zorlukla karşılaşsa da çeşitli teknolojiler ve büyüme sistemleri geliştirilmeye devam edilmektedir (Teng ve ark., 2023). NASA, atıkları geri dönüştürebilen, yiyecek ve oksijen üreten öte yandan da karbondioksiti ortadan kaldıran bitkilerle biyorejeneratif yaşam destek sistemlerini araştırmaktadır. Kapalı Ekolojik Yaşam Destek Sistemi programı, NASA'nın Florida'daki Kennedy Uzay Merkezi'ndeki Yaşam Bilimleri Bölümü tarafından başlatılmış ve bitkilerin en ideal büyüme koşullarını belirlemek için büyüme odaları inşa etmiştir. NASA Biyokütle Üretim Odası 1980'lerde inşa edilmiş ve çok sayıda büyüme deneyi gerçekleştirilmiştir (NASA, 2021).

NASA'nın yayınladığı verilerden yola çıkarak, daha sonrasında Green Sense Farm Holdings, Bowery Farming gibi şirketler, NASA'nın sistemini yapay zekâ ve birkaç özellikler ile

geliştirerek Dünya üzerinde kullanmaya başlamışlardır. Özellikle Green Sense Farm'ın başkanı olan Robert Colangelo, bitkinin ihtiyacı kadar ışık dalga boyunun kullanıldığını ve bu kapsamda da elektrik konusunda tasarruf sağlandığını tespit ettiklerini açıklamışlardır. Bowery Farms ise, sistem ile entegre olan yapay zekânın kendi kendine öğrenebildiğini, binlerce veri ve fotoğrafı inceleyerek sorunu tespit edebildiğini açıklamıştır. Aynı zamanda yapay zekâ sürekli olarak denetleme yaparak, her bir detayı izleyebilme özelliğine de sahiptir (NASA, 2021; Nguyen ve ark., 2023).

Son yıllarda, uzay çiftliklerinde günümüz teknolojilerinin de kullanılarak bitkilerin yetiştirilmesine ilişkin öneriler de ortaya atılmıştır. Bunlardan biri Liu ve ark. (2021) tarafından bitki biyoteknolojisine dayanan Whole-Body Edible and Elite Plant (WBEEP) stratejisi olarak önerilmiştir. Bu strateji ile uzay çiftliklerinde daha fazla zengin besin içeriğine, yüksek verimliliğe ve yüksek mineral içeriğine sahip yenilebilir mahsullerin geliştirilebileceği öngörülmüştür. Bu amaçla da, patates (*Solanum tuberosum* L.) bitkisinin seçilebileceği ifade edilmiştir.

6.1. Veggie bitki üretim sistemi / Veggie plant production system

Veggie sistemi Expedition 39 mürettebatı tarafından geliştirilmiş ve Florida'da bulunan John F. Kennedy Uzay Merkezi'nde test edilmiş bir bitki geliştirme sistemidir. Temel amacı mikro yerçekimlerinde bitkilerin sağlıklı şekilde büyümesini sağlamak, atmosfer değişikliğine karşı bitkinin verdiği tepkiyi analiz etmek ve üretilen ürünleri daha da zengin bir içeriğe sahip hale getirmektir. Sadece bitki büyüme deneyleri için değil, aynı zamanda gıda üretmek için de tasarlanmış ilk bitki büyüme sistemidir (TechPort, 2018; Carillo ve ark., 2020). Veggie, Orbital Technologies Corporation (ORBITEC, Madison, ABD), tarafından NASA Small Business İnovasyon Araştırması (SBIR) programı bünyesinde inşa edilmiştir. Astronotlar Rick Mastracchio ve Steve Swanson 7 Mayıs 2014'te Columbus Laboratuvar Modülüne Veggie'yi kurmuşlardır (Herridge, 2017; John F. Kennedy Space Center, 2020; Carillo ve ark., 2020).

NASA, uzun süreli uzay uçuşları sırasında astronot mürettebatının gıda olarak tüketebilmesi ve biyorejeneratif yaşam desteği olması için taze salata bitkilerinin yetiştirilebildiği küçük sebze üretim odaları şeklinde olan Veggie'yi kullanmaktadır (Burgner ve ark., 2020; Koçkaya ve Un, 2022; Morsi ve ark., 2022). Veggie Uluslararası Uzay İstasyonu (ISS) için modüler, düşük kütleli ve düşük enerjili bir ünite olarak bahçe bitkileri yetiştirme olanağını sunmak üzere tasarlanmıştır (Massa ve ark., 2017). Veggie, düşük güç gereksinimleri ile "astronotlar için bir bahçe" olarak kabul edilir. Veggie'de yetişen bitkilerin, kendi LED aydınlatma sistemlerine ve hava akışına sahip oldukları belirtilmiştir (Carillo ve ark., 2020; Morsi ve ark., 2022).

Günümüze kadar bu sistem ile Çin lahanası, zinnia çiçekleri, marul, kırmızı lahana, Mizuna hardalı, kale, karnabahar gibi çeşitli bitkilerin üretimi başarıyla sağlanmıştır (Wheeler, 2017; Morsi ve ark., 2022; Reed ve ark., 2023). Özellikle uzayda yapılan tarımda patojen kontaminasyonu riskinden çok endişe edilmekte idi. Dr. Gioia Massa ve ekibi tarafından uzayda yetiştirilen Dünya'ya gönderilen marul numuneleri araştırıldığında ise, patojen bulgusu veya canlı organizma için zararlı bir etkene rastlanmamış ve yetişen ürünler güvenli olarak kabul edilmiştir (Wheeler, 2017).

6.2. BRIC çalışması / BRIC study

The Biological Research in Canisters (BRIC) adlı sistemin temel amacı, petri kabında gelişen mikroorganizmaların üzerinde uzay atmosferinin verdiği etkiyi incelemektir. Bunların yanı sıra BRIC-LED adı verilen sistem ise, algler ve yosunlar gibi ışığa ihtiyaç duyan canlıların yetişebilmesini sağlayan, LED ışıklardan yararlanarak hala geliştirme prosesleri devam eden, BRIC sisteminin daha gelişmiş versiyonudur. Araştırmacılar tarafından adaptasyon sürecinde yerçekimi kaynaklı genlere zararın oluşabileceği, reaktif oksijen türlerinin DNA yapısını bozabileceği gibi etkilerin ortaya çıkabileceği düşünülmektedir. Buna bir örnek olarak, Veggie'de üretilen Zinnia çiçeklerinde ani bir mantar enfeksiyonu gelişimi olmuştur (John F. Kennedy Space Center, 2020). Özellikle BRIC çalışmalarında, Florida Üniversitesinden Ph. D. Robert Ferl ve Ph. D. Anna Lisa Paul, bitkilerin genom dizilimi ve DNA'da gelişen değişiklikleri inceleme fırsatı bulmuşlardır. BRIC tekniği sayesinde, yaşam destek sistemlerinin nasıl sürdürülebilir hale getirilebileceği ve uzun süren uzay uçuşlarında gıda kalitesinin nasıl sağlanacağı hakkında detaylı veriler elde etmeyi başarmışlardır (NASA, 2013). Nicholson ve ark. (2021) BRIC tekniği ile Uluslararası Uzay İstasyonu (ISS) içindeki mikro yerçekimi ortamının Gram-pozitif bir bakteri olan *Bacillus subtilis* üzerine transkriptomik tepkisini değerlendirmişlerdir. Araştırmacılar, BRIC-21 ve BRIC-23 olarak adlandırılan iki farklı deney yaparak, kontrol suşları ile aynı *B. subtilis* suşlarını elde edebilmişlerdir. Diğer benzer çalışmalarda da, BRIC deneyleri ile *Staphylococcus epidermidis* ve *S. aureus* bakterilerinin de mikro yerçekimi ortamında geliştirilebildiği rapor edilmiştir (Nicholson ve ark., 2021). ISS'de mikro yerçekimine ve diğer koşullara maruz kalan bitkilerde kök eğriliği, gen ifadesinde değişiklikler ve hücre duvarı bileşiminde değişiklikler gibi durumların ortaya çıktığı belirtilmiştir. Mikro yerçekiminin hücresel organeller üzerindeki etkilerini ele alan sistematik çalışmaların eksik olduğu ifade edilmiştir. Bu amaçla Wang ve ark. (2022) *Arabidopsis zigzag*-1 (zig-1) ile yapmış oldukları çalışmada (BRIC-24), Wortmannin ile muamele edilen fidelerinde mikro yerçekiminin bazı hücrelerde hipokotil büyümesi ile vakuol füzyonunu azalttığı ve zig-1 fenotipini güçlendirdiğini göstermiştir.

6.3. Geliştirilmiş bitki habitatı (APH) / Advanced plant habitat

Sierra Space Corporation tarafından NASA KSC için inşa edilen APH, 2017 yılında ISS'ye fırlatılmıştır (Morrow ve ark., 2023). Veggie sistemine benzeyen APH sistemi, ISS üzerinde bitkilerde nesiller boyu denemeler yapılmak üzere geliştirilmiş, tamamen kapalı ve kompleks yapıya sahip büyük hacimli bir bitki yetiştirme alanıdır. APH, 180'den fazla kalibre edilmiş ve bitki açısından önemli hava hızı, bağıl nem, CO_2 gibi kapsamlı kontrollerin yapıldığı sensörlerle donatılmıştır. Ayrıca, bitki deneyleri için 135 güne kadar uygun bir büyüme ortamı (örn. sıcaklık, bağıl nem, karbondioksit seviyesi, ışık yoğunluğu gibi) sağlayan önceden programlanmış bir sisteme sahiptir). Ayrıca, APH sisteminin sahip olduğu sensörler ve kameralar sayesinde, bitkinin gelişim evreleri Dünya'dan izlenebilmektedir (NASA, 2013; Sempsrott, 2021; Levine, 2022; Reed ve ark., 2023). Buğday ve *Arabidopsis* bitkileri APH'de tohumdan başarılı bir şekilde üretilebilmiştir ve ISS'de 30 günlük büyümenin ardından hasat edilmiştir (Monje ve ark., 2018). Sistem, LED ışıklar ile gübrenin kontrollü salınımını sağlayan gözenekli bir kil substrat içermekte, sıcaklık, nem gibi çevresel faktörler

otomatik olarak kontrol edilmekte ve bu sayede günlük bakıma ihtiyaç kalmadan takipler yapılabilmektedir. Yetişen bitki örnekleri dondurularak incelenmek üzere Dünya'ya gönderilmektedir. Araştırmacıların cevabını bulmaya çalıştığı asıl soru ise, bitkilerin lignin içeriği ile mikro yerçekimi arasında olan bağ üzerinedir. Ligninler bitkilerin yerçekimine karşı gösterdiği direnç ve sertlik yapısını oluşturduğu için, insan kemiklerinin yapısına çok benzetilmektedir. Uzayın, insanların kemik ve kas yapısına uzun sürede zarar verdiği bilinmektedir. Aynı zamanda genetiği değiştirilerek daha az lignine sahip olan bitkilerin, uzay ile uyumu veya uzayda yaşamını sürdürebileceği de merak edilen konular arasındandır. Eğer böyle bir şey mümkün olabilirse, bitki tüketiminde daha yüksek besin verimi alınabileceği ve atıklardan kompost yapılmasının çok daha kolay olacağı düşünülmektedir (NASA, 2013; Sempsrott, 2021). Bugüne kadar APH'de yetiştirilen yenilebilir ürünler cüce buğday, Hatch biberi ve turptur (Reed ve ark., 2023).

6.4. Bitki habitat-04 (PH-04) deneyi / Plant habitat-04 (PH-04) experiment

NASA'nın Bitki Habitat-04 (PH-04) deneyi ile Uluslararası Uzay İstasyonunda ilk kez acı biber yetiştirildiği belirtilmiştir (Sempsrott, 2021). Deney, Temmuz 2021 yılında başlatılmıştır ve Kasım 2021'in sonunda sonlandırılmıştır (Massa ve ark., 2021). Biber tohumları, Ocak 2021'de klor gazı ile sanitize edildikten sonra Nisan 2021 yılında ekilmiştir (Khodadad ve ark., 2022). Biberlerin çimlenme süreleri 10-14 gün ve büyüme döngüleri 90-120 gün olarak rapor edilmiştir. Ayrıca, meyve verme dönemlerinin uzun olduğu bildirilmiştir (Spencer ve ark., 2020). Astronotlar iki hasattan ilkini gerçekleştirdiğinde biberler üç aydan fazla büyümüş ve 26 Kasım 2021'de ikinci bir hasat gerçekleştirilmiştir. Ekip, ilk hasattaki tüm biberleri yemiş ve ikinci hasadın bir kısmını yedikten sonra analiz için Dünya'ya 12 biber paketleyerek göndermişlerdir. PH-04, uzay istasyonunda ilk defa bu kadar uzun süreli bitki yetiştirme söz konusu olmuştur. Bu çalışma ile uzun süreli uzay görevleri sırasında gıda ürünleri yetiştirme konusunda bilgi elde edilmiştir. Ayrıca, gönderilen biber örneklerinde herhangi bir gıda patojeninin üremediği ve toplam heterotrofik bakteri/mantarların sayısının tespit edilebilir sınırların altında olduğu belirlenmiştir (Khodadad ve ark., 2022). Araştırmacılar, Hatch Valley'den farklı varyetedeki Hatch acı biberlerini seçmişlerdir. New Mexico Eyalet Üniversitesi tarafından Hatch Sandia ve Espanola biber türlerinin melezlenmesi ile elde edilen bir tür olan NuMex (Espanola Enhanced)'i elde etmişlerdir. Uzay tarım sistemleri ile iyi uyum yakalayan bu tür, Hatch biber türlerinin arasındaki en iyi biber olarak kabul edilmektedir (Sempsrott, 2021).

7. Gastronomi alanında topraksız tarım uygulamaları / Soilless agriculture applications in the field of gastronomy

Bilinçsiz tüketim ve kaynakların israfı sebebiyle sürdürülebilirlik kavramı günümüzün en temel konularından biridir. Nüfusun yoğun artışı, sanayileşme, teknolojik gelişmeler beraberinde tarım alanlarının tahribata uğraması problemini meydana getirmiştir. Bunların yanı sıra üretimde çok fazla su kaynaklarının tüketilmesi, kimyasal madde kullanımı, fazla miktarda atık oluşumu ve bu atıkların bilinçsizce doğaya bırakılması, çevreye ve canlılara zarar vermekte ve bu durum sürdürülebilir gastronomi konusunun önemini ortaya koymaktadır (Cekal ve Dogan, 2022, Cankul ve Toprak, 2022). Sürdürülebilir gastronomi bölgeye özgü gastronomik öğelere ve kırsal alanlara çevreye duyarlı bir şekilde yönelimi sağlayarak, gelecek nesillere aktarımı mümkün hale getirmektedir (Cilginoglu ve ark., 2022). Son zamanlarda toplumdaki bireylerin organik tarım ve sağlıklı beslenme konularına önem verdiği görülmekte ve sürdürülebilirlik konusunda daha fazla hassasiyet gösterdikleri de görülmektedir. Sürdürülebilir gastronomi kapsamında bazı yeşil restoranların kendi ürünlerini yetiştirdikleri ve tabaklarında da kullandıkları görülmektedir. Bu noktada topraksız tarım sistemleri gastronomi açısından da ele alınan sistemlerdir. Üretilen gıda maddesinin çevreye zarar vermeden nesiller arasında aktarılması sürdürülebilir gastronomi açısından oldukça önemlidir. Gastronominin sürdürülebilir olması da tarım uygulamalarının sürdürülebilir olmasıyla mümkün hale gelmektedir (Cankul ve Toprak, 2022; Cekal ve Dogan, 2022).

Sürdürülebilir gastronomi alanında kullanılacak dikey tarım tekniği ile doğal tarım ürünlerinin kullanımında bir teşvik oluşturulabileceği ve bu tarım ürünlerinin korunarak nesiller arasında aktarılacağı öngörülmektedir. Bu duruma bağlı olarak yerel üretimin destek alması ve üretimin gelişmesi sonucunda rekabet artışı ve müşteri memnuniyetinin oluşturulması sağlanabilecektir. Dikey tarım ile iklim koşullarında gerçekleşen değişkenlerden etkilenmeden 4 veya daha fazla kez ürün elde edilebilmektedir. Bunların yanı sıra bu şekilde elde edilen ürünlerin yüksek kalitelerde olması, ülkeler için son derece önem arz etmektedir. Gıda ürününe ulaşabilmek ve sürdürülebilir gıda güvenliği, sürdürülebilir gastronomi açısındanda oldukça önemlidir. Bütün bu taleplerin karşılanması ise dikey tarım ile mümkündür (Martin ve Molin, 2019; Cilginoglu ve ark., 2022; Goh ve ark., 2023).

Toskana (İtalya) yerel gastronomi kültüründe kullanılan yenilebilir yabani yapraklı bitkilerin dikey tarım ile birleşmesiyle yeni bakış açılarının oluşabileceği belirtilmiştir (Baldi ve ark., 2022). Gastronomi alanında, sürdürülebilir ekolojik tasarım anlayışı olan perma-kültür restoranlarda sıklıkla görülmektedir. Oluşturulan menülerde; akuaponik, hidroponik ve organik tarım teknikleri ile üretilen gıda ürünleri kullanılmaktadır. Özellikle hidroponik teknikte yatay ve dikey olarak oluşturulan alanlarda, doğal ve ekolojik ürünler rahatlıkla yetiştirilebilmekte ve maliyet açısından çeşitli avantajlar elde edilebilmektedir (Eren, 2018). Tarımın teknolojik hale gelmesi ve her geçen gün inovasyon ile gelişmesi, üretimin zor olduğu alanları efektif kullanmayı ve tarımsal verim sağlamayı insanlara kazandırmıştır. Türkiye'de faaliyet göstermekte olan Mutfak Sanatları Akademisi (MSA) ile Plant Factory ortak çalışma yaparak, MSA Bahçe olarak adlandırılan küçük bir dikey tarım uygulamasını hayata geçirmişlerdir. 2020 yılında faaliyete giren bu uygulama ile hem şehir tarımcılığı için bir tanıtım ve teşvik sağlanmakta hem de eğitim gören öğrencilerin ve eğitim veren şeflerin kapalı alanda dikey tarım ile tanışmasına ve bu ürünleri deneyimlemesine olanak sağlamaktadır. MSA bu teknik ile %95 daha az su tüketimi ve minimum düzeyde kayıp ile kıvırcık marul çeşitleri ile birlikte fesleğen gibi yeşil yapraklı ürünleri yetiştirebilmektedir. Gebze Teknik Üniversitesi ile HGT Tarım iş birliği yaparak üniversitenin kampüsünde 20 farklı tür bitki LED Destekli Dikey Tarım Laboratuvarı'nda (LAVFARM) pestisit veya herbisit kullanılmadan üretilmiştir (Kurum, 2021; Gastronomi Turkey by Rafine, 2021).

Omaha'da (ABD/Nebraska) bulunan Gather in Omaha isimli restoranın içinde yer alan bir dikey çiftlik bulunmaktadır. 61 adet aeroponik kuleden oluşan çiftlikte yetiştirilen fesleğen,

nane, roka, lahana, biberiye, marul ve hardal gibi çeşitli ürünler taze olarak hasat edilmekte ve menülerde bulunan yemek-kokteyl reçetelerinde kullanılmaktadır (Agrotonomi, 2023). Aynı zamanda bu uygulama ile tedarik zincirinde yaşanan kesintiler ve kontamine olmuş gıda ürünlerinin geri gönderilmesi gibi durumların da büyük ölçüde önüne geçilmektedir. Bunların yanı sıra 2020 yılında Poughkeepsie'de (New York) faaliyete geçmiş olan Farmers & Chefs isimli restoran konteyner çiftliği uygulamasını kullanmakta ve burada çeşitli üretimlerde bulunarak yetiştirdikleri ürünleri menülerinde bulunan reçetelerde kullanmaktadır (Ridden, 2021).

Son yıllarda mikro yeşillikler de yeni bir gastronomi trendi olarak karşımıza çıkmaktadır. Mikro yeşilliklerin çoğunlukla hem yaratıcı sunuma hem de lezzete önem verilen fine dining restoranlarda kullanıldığı görülmektedir (Kou ve ark., 2014; Mir ve ark., 2017). Mikro yeşillikler, bir bitkinin yaşaması için gerekli olan tüm unsurları içeren bir besin çözeltisi yardımı ile topraksız yetiştirme sistemleri kullanılarak üretilir. Mikro yeşilliklerin üretildiği en yaygın sebze türlerinin *Brassicaceae* (karnabahar, brokoli, lahana, Çin lahanası, lahana, su teresi, mizuna, turp, roka ve hardal gibi), *Asteraceae* (marul ve hindiba gibi), *Apiaceae* (dereotu, havuç, rezene, kereviz gibi), *Amarillydaceae* (sarımsak, soğan, pırasa gibi), *Amaranthaceae* (amaranth, pazı, pancar, ıspanak gibi) ve *Cucurbitaceae* (kavun, salatalık, kabak gibi) familyalarına ait olduğu görülmektedir. Ayrıca, tahıllar (yulaf, yumuşak buğday, durum buğdayı, mısır, arpa, pirinç), kinoa, baklagiller (nohut ve fasulye gibi), yağlı bitkiler (ayçiçeği) ve keten gibi lifli bitki türlerinin yanı sıra fesleğen, frenk soğanı, kişniş ve kimyon gibi birçok aromatik tür için de mikro yeşillikler üretilmektedir (Renna ve ark., 2017). Ancak restoranların mufağına ulaşana kadar nakil sırasında veya depolama esnasında mikro yeşilliklerin kalitesinde ve aromasında düşme meydana geldiği ifade edilmiştir (Xiao ve ark., 2014). Bu nedenle mikro yeşilliklerin yiyecek içecek işletmelerinde üretilmesi bazı şefler tarafından tercih edilen bir seçenek olmaktadır. Ritz-Carlton, Napoli'de Şef George Fistrovich, "The Grow House" adı verilen, iklim kontrollü bir ortamda mikro yeşillikler yetiştirmektedir (Ritz-Carlton, 2015).

8. Sonuç / Conclusion

M.Ö 600'lü yıllara kadar uzanan geçmişi ile topraksız tarım günümüzde insanlık için son derece önemli hale gelmiştir. İklim değişiklikleri, hızlı ve çarpık kentleşme, toprakların bilinçsiz kullanılması sonucu kullanılabilir verimli tarım arazilerinin miktarı her geçen yıl azalmaktadır. Ayrıca tarım arazilerinin azlığı nedeniyle toprakların sürekli kullanımı toprak verimliliğini azaltmasının yanı sıra ürün kalitesini de düşürmektedir. Bu yüzden kontrollü bir şekilde topraksız tarımın kullanılabilirliğinin artması hem yüksek verime sahip ürünlerin üretilmesi hemde yerel pazara sağladığı sağlıklı gıda desteği ile son derece olumlu bir etki göstermektedir. Ancak maliyet durumu oldukça fazladır. Ülkelerin hızlı davranarak topraksız tarım uygulamalarına yönelmesi ve bu teknikleri benimseyen şirketlere finansal destek sağlaması, bu maliyet probleminin önüne biraz da olsa geçebilecektir. Aynı zamanda bu sistemlerin uzay alanına taşınması durumu da gelecekte çeşitli araştırma konularını önümüze getirecektir. Ay ve Mars keşiflerinde bulunma, hatta burada yaşam alanı oluşturma gibi soruların bir kısmına cevap verebilme potansiyeline sahip olan topraksız tarım sistemleri, günümüzde uzay istasyonunda yapılan yetiştiricilik çalışmaları ile daha da ileriye taşınacaktır. Gastronomi alanında da sürdürülebilirlik anlayışı çerçevesinde topraksız tarım uygulamalarına her geçen gün daha fazla önem verildiği görülmekte ve bazı işletmelerde bu uygulamaların hayata geçirilerek hasat edilen ürünlerin daha standart ve taze bir şekilde tabaklarda kullanıldığı görülmektedir.

Çıkar çatışması / Conflict of interest: Yazarlar herhangi bir çıkar çatışması olmadığını beyan eder / The authors declare that they have no conflict of interests.

Etik beyanı / Informed consent: Bu çalışmada, yazarlar, hiçbir insan ya da hayvan denek kullanılmadığını ve Etik Kurul iznine gerek olmadığını beyan eder / The authors declare that this manuscript did not involve human or animal participants and informed consent was not collected.

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