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Chapter 1

A REVIEW ON THE PHYTOREMEDIATION TECHNIQUES OF POTENTIALLY TOXIC ELEMENTS: UNDERSTANDING OF PRINCIPLES AND PRACTICES

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1. Introduction

The global increase in human population has led to widespread industrialization and intensive agriculture, resulting in pollution of the ecosystem, particularly soil (Alori et al., 2017). The immunotoxic, cancer-causing, and mutagenic effects of soil pollutants change the physical, chemical, and microbiological properties of soil (Da Silva et al., 2020). These soil pollutants can also pass underground water, causing significant risk not only plants but also animals and humans (Alori et al., 2018). Therefore, it is important to recover contaminated soils. In this respect, various techniques have been used to clean up contaminated soils including chemicals, excavation and incineration. On the other hand, these techniques are extremely expensive and even do not completely solve the problem because they simply move the pollution from one place to another (Alori et al., 2015). Furthermore, they often also produce secondary pollutants that have more negative effects on the environment (Divya et al., 2015). Considering all these, it has become essential to develop new and more advantageous methods. Phytoremediation is one of the valuable acceptable, low-cost, easy-to-use, and common wastewater treatment methods.

Achieving sustainability in agriculture depends on the cleaning of soil and groundwater with appropriate conditions. In this sense, it is important to apply alternative methods rather than the utilization of chemicals. This chapter discusses phytoremediation and related techniques as safer and more practical alternative methods.

2. Phytoremediation

The word "phytoremediation" is a combination of the Greek prefix "phyto" meaning plant and the Latin root "remedium" which means to correct or remove something harmful. Phytoremediation is a method used to clean contaminated water, soil, and air. It utilizes the natural purifying abilities of plants and microorganisms to remove various types of pollution such as metals, pesticides, explosives, oil, and other contaminants. Therefore, phytoremediation is an environmentally friendly and cost-effective approach, gaining popularity in recent years (Raklami et al. 2022; Aguado et al., 2023).

Plants can help prevent the transportation of pollutants from one location to another by wind, rain, or groundwater. This non-destructive and cost-effective *in situ* technology is useful to clean contaminated soil, particularly in tropical regions where the climatic conditions promote plant growth and stimulate microbial activity (Bhat et al., 2022). For over 300 years, the potential of plants to eliminate pollutants from the environment has been utilized in agricultural areas. Nowadays, this practice progressed to include the creation of man-made wetlands and the planting of trees to combat air pollution (Mustafa et al., 2021; Watson & Bai, 2021).

Over time, several associated technologies have been developed to enable the practical use of higher plants to decontaminate soil and water. From 1993, these technologies were progressively documented in scientific literature, and the term "phytotechnologies" emerged as an expanded definition for the utilization of plants for environmental remediation (Latif et al., 2023).

3. Advantages and disadvantages of phytoremediation

Prevention of environmental pollution with phytoremediation has some advantages over other known methods. Phytoremediation is suitable for the phytoremediation process of various organic and inorganic compounds. This cost-effective method has the potential to recover and reuse precious metals. Moreover, plant-based remediation helps to provide safeguard topsoil, maintain soil fertility, reduce soil erosion, and even prevent metal leaching. Phytoremediation is a sustainable and eco-friendly approach to control pollution not only for soil but also for water and air (Khalifa et al., 2022; Zhou et al., 2023).

Despite its potential, phytoremediation has also some limitations. It is a time-consuming process, and the efficiency of metal hyperaccumulators is often limited due to the slow growth rate and low biomass of plants. Furthermore, mobilizing tightly bound metal ions from the soil can be challenging because of limiting the bioavailability of pollutants. Phytoremediation is only suitable for low to moderately-contaminated soils as plant growth cannot be sustained in heavily polluted soils. There is also a risk of food chain contamination if proper maintenance and management are not in place (Priya et al., 2023).

4. Phytoremediation Techniques

Phytoremediation can have more than one application method. These are phytoextraction, phytostabilization, phytodegradation, phytostimulation, phytovolatilization, and phytofiltration methods (Figure 1). These methods can be used as single or combinations to eliminate contaminants.

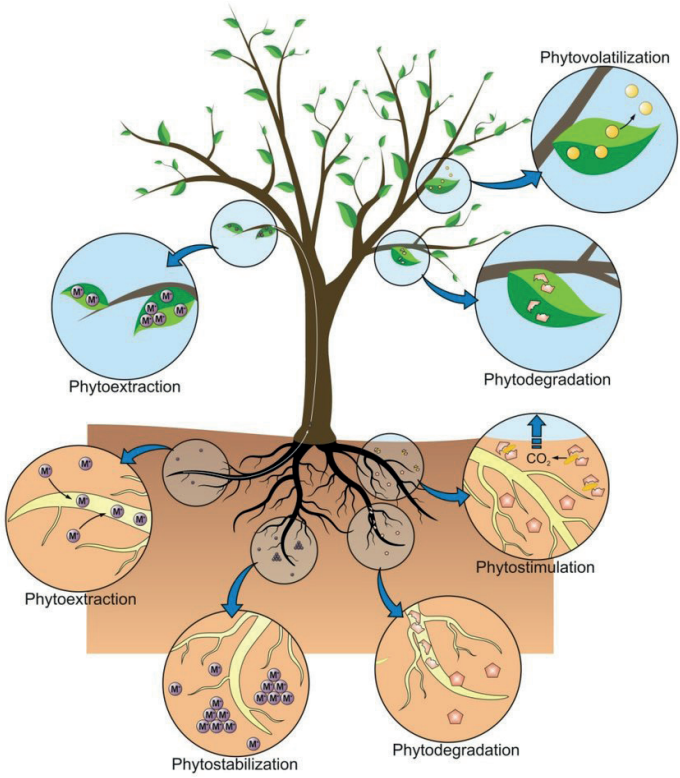

Fig.1. *Schematic representation of phytoremediation strategies (Favas et al., 2014).*

4.1. Phytoextraction

Phytoextraction describes the process of removing heavy metals or radioactive materials from the soil. Although some heavy metals are required for growth and development, others such as Cadmium (Cd), Chromium (Cr), Lead (Pb), Cobalt (Co), Silver (Ag), Selenium (Se), and Mercury (Hg) are not biologically necessary for plants (Baker et al., 1981; Breidenbach et al., 1997). Plants must maintain a balance between essential and non-essential metal uptake to protect cellular function and structures. They can use an avoidance mechanism that involves immobilizing metals in their cell walls and roots (Figure 2). Moreover, plants can tolerate heavy metals by sequestering them in vacuoles and even enzymes that operate effectively in the presence of high levels of metallic ions (Li et al., 2023).

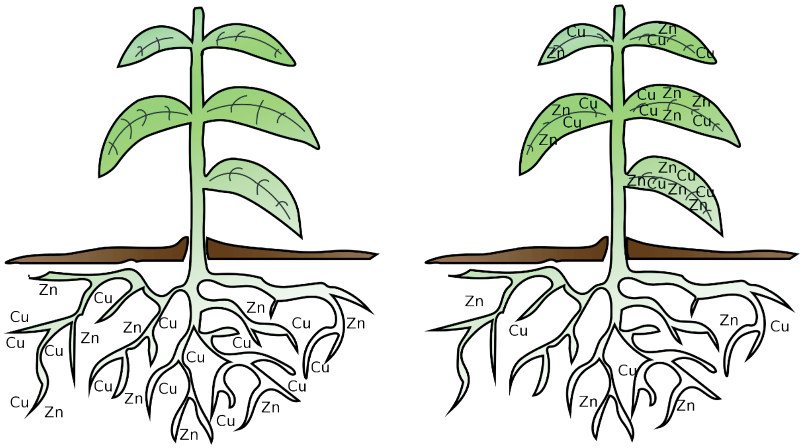

Fig. 2. *Uptake of heavy metals by phytoextraction (Xavier et al. 2016).*

An ideal plant for phytoextraction should possess certain characteristics such as the ability to tolerate high metal concentrations, accumulate large amounts of metals in the sections that will be harvested, grow rapidly, produce a significant amount of biomass in the field, and also have a deep root system. Some wild plants that grow in naturally mineral-rich soils have been discovered to contain high concentrations of metals in their leaves, leading to the concept of using plants for metal extraction from contaminated soils (Juel et al., 2022). Compared to crops, hyperaccumulators can accumulate metals which are 10-500 times higher than normal plants. Studies have shown that hyperaccumulators can accumulate metals such as Ni, Zn, and potentially Cu that cover 1-5% of plant dry weight (Ahmad et al., 2022; Kushwaha et al., 2022).

There are two methods of phytoextraction that involve either high-biomass plant species or hyperaccumulators along with metal-solubilizing agents. High-biomass plant species are more suitable for phytoextraction because they can absorb multiple heavy metals even at low concentrations. However, the main limitation of the effectiveness of phytoextraction is the availability of heavy metals to plant roots, regardless of the plant's high biomass (Bortoloti & Baron, 2022). The metal in the soil must be in a form that can be taken up by plants and translocated to the above-ground parts of the plant to achieve a high capacity for accumulating heavy metals. On the other hand, even if a plant has a high capacity for accumulating metals, the rate of metal extraction is limited by the product of the plant's metal concentration and the total biomass (Shen et al., 2021). Yu et al. (2022) studied the impact of (hyper)accumulator rhizospheres on field soils contaminated with multiple metals to monitor changes in microbial community diversity and composition over a six-year period. For this purpose, six types of

(hyper)accumulators were used in the field investigation, including *Solanum nigrum* L., *Bidens pilosa* L., *Xanthium strumarium* L., *Helianthus annuus* L., *Lonicera japonica* and *Pennisetum sinese* R. After two years of testing, *Bidens pilosa* L. and *Xanthium strumarium* L. were found to have superior metal(loid) phytoextraction capabilities and larger shoot biomasses compared to the other four (hyper)accumulators. It was also reported that the levels of DTPA-extractable Pb, Cd, and Zn in the rhizosphere soils of all six (hyper) accumulators decreased after following repeated phytoextraction. Furthermore, the research showed that planting hyperaccumulators alters the physiochemical characteristics of contaminated soils to assist rebuild and restore the composition and structure of the soil bacterial population.

In a different study, researchers investigated the use of *Phyllostachys pubescens* or *Moso Bamboo* to remove Cr from contaminated soil (Figure 3). The experiment was conducted in Mediterranean conditions where the soil was irrigated with water containing 180 mgCr/L at a flow rate of 600 mm/year. The study examined the phytoextraction of Cr from soil with a starting Cr level of 300 mg/kg, by using *Moso Bamboo*. After 6 and 12 weeks, the researchers found that 42% and 60.7% of Cr had been removed from the soil, respectively. The above-ground parts of the plant had a greater Cr content per gram of dry biomass compared to the underground parts. After 12 weeks of seeding, the Cr content was found to be 1.79 mg/g in roots and rhizomes and 2.49 mg/g in stems and leaves (Ranieri et al., 2022).

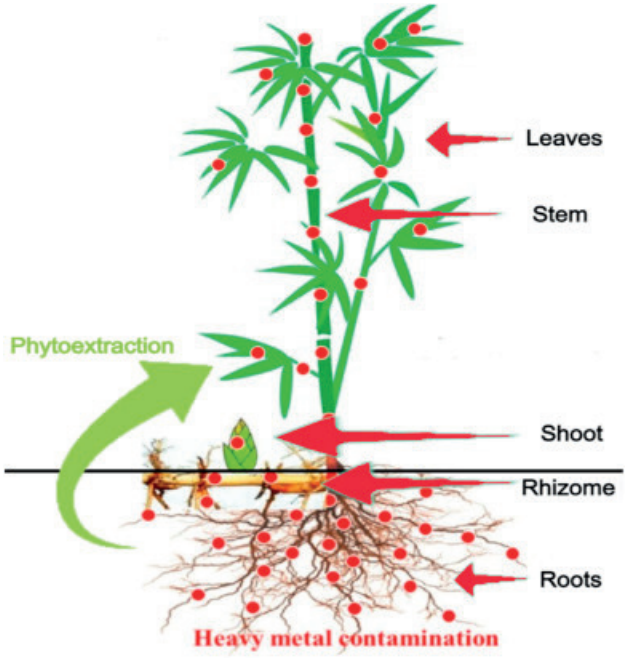

Fig. 3. *Phytoextraction of Cr by Moso Bamboo (Ranieri et al., 2022)*

4.2. Phytostabilization

The application of phytostabilization is to deposit pollutants in contaminated areas through root hairs. This procedure prevents contaminants from entering the food chain, restricting their mobility and ultimately reducing their bioavailability (Yan et al., 2020). It can be used to restore flora in polluted areas with high metal concentrations where it is difficult to grow native vegetation. Moreover, by using metal-tolerant plant species as phytostabilizers, the spread of various toxins into groundwater through air, water, and soil can be prevented (Sarath et al., 2022; Wyszkowska et al., 2022).

During phytostabilization, plants can stabilize metals in the substrate or soil and reduce their mobility or bioavailability. They can do this by collecting the metals within their roots. Metals can also be reduced in mobility or bioavailability by accumulating near the rhizosphere or within root tissue (Figure 4). The higher metal accumulation in roots may further reduce metal mobility in sediment (Zhang et al., 2023). Various factors that influence the basic mechanism of phytostabilization such as microorganisms present in the rhizosphere, root exudates, metal ion binding to cell walls, metal ion chelation through metal-binding molecules, and sequestration of metal ions within vacuoles (Bian et al., 2022; Kang et al., 2022; Nasir et al., 2022; Sharma et al., 2023). The central mechanism of phytostabilization is the mobility of trace elements in the rhizosphere, which is regulated by several soil characteristics, including pH, organic matter, texture, redox potential, temperature, and microorganisms (Tonelli et al. 2022). Biomass disposal is not necessary for phytostabilization. Effective immobilization lowers harmful metal ion leaching and bioavailability. It has benefits including enhancing ecosystem restoration, making the site more visually beautiful, and being less harmful than cleanup technology. Vegetation also stabilizes the soil physically (Radziemska et al., 2021; Kumar et al., 2023). Disadvantages of phytostabilization include the inability to remove pollutants from the environment and its usefulness is reduced where chemicals penetrate deep into the soil (Shackira & Puthur, 2019).

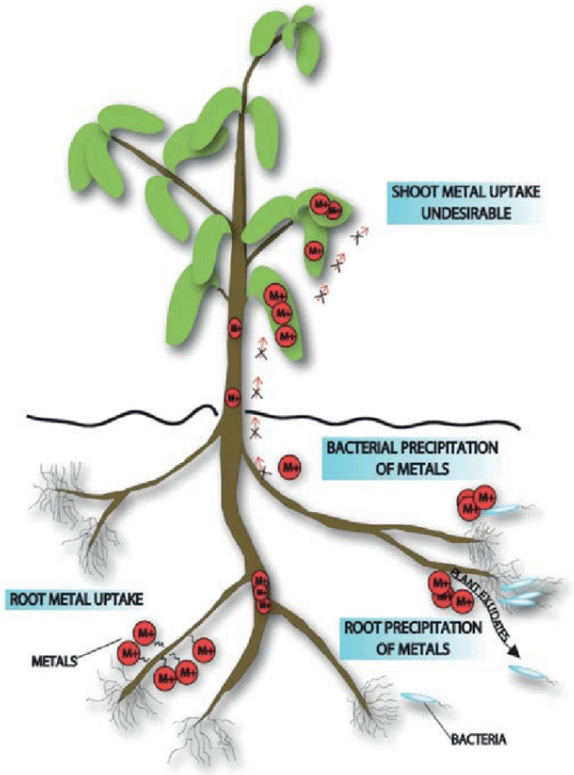

Fig. 4. *Schematic display of phytostabilization mechanisms (Mendez & Maier, 2008).*

Rhizobium and endophytic soil bacteria play a crucial role in enhancing phytostabilization. These beneficial bacteria support the process of phytoremediation by increasing the rate at which heavy metals are immobilized and accumulated (Alves et al., 2022). The immobilization of heavy metal ions and the prevention of their entry into the root system are facilitated by interactions between heavy metals and anionic functional groups such as amine and amide groups, sulfonate, sulfhydryl, hydroxyl, and carboxyl. These functional groups bind to extracellular polymers made up of protein and polysaccharides, which chelate the metals to detoxify them (Halim et al., 2020; Jeyakumar et al., 2022).

Different plants can be used for phytostabilization and *Miscanthus giganteus* is one of them. Zgorelec et al. (2020) aimed to determine how Cd and Hg affect *Miscanthus giganteus* (MxG) growing in polluted soil in terms of growth, biomass productivity, and phytoremediation ability. For this purpose, a random whole-block design was used to set up the experiment, and different concentrations of Cd and Hg (0, 10, and 100 mg kg^{-1}) and (0, 2, and 20 mg kg^{-1}) were applied to the soil. The study covered three vegetative years. The

yields of MxG ranged between 6.3 to 15.5 tons of dry matter per hectare while the Cd concentrations in plants varied from 45 to 6758 grams per kilogram, and Hg concentrations ranged from 8.7 to 108.9 grams per kilogram. There was significant variation in values between different treatments and years. MxG has a limited capacity to absorb and remove above-ground biomass from each container with a maximum of 293.8 grams of Cd and 4.7 grams of Hg per year. These findings suggest that MxG can be a useful choice for both phytostabilization and biomass production in soils that are moderately contaminated with Cd and Hg.

The phytostabilization potentials of five ornamental plants including *Osmanthus fragrans* (OF), *Ligustrum vicaryi* L. (LV), *Cinnamomum camphora* (CC), *Loropetalum chinense* var. rubrum (LC), and *Euonymus japonicas* cv. Aureo-mar (EJ) was examined in a greenhouse experiment. The study found that these plants could grow normally when the soil Cd level was less than 24.6 mg kg^{-1}. The roots of OF, LV, LC, and EJ were found to be heavily contaminated with maximum concentrations of Cd (15.76, 19.09, 20.59, and 32.91 mg kg^{-1}, respectively). The highest Cd content in the stems and leaves of CC were 12.5 and 10.71 mg kg^{-1}, respectively, and overall Cd levels in stems and leaves were comparable to those of other ornamental plants. The study found that these ornamental plants improved enzymatic activity in Cd-contaminated soil. Soil urease and sucrase activities increased while dehydrogenase activity decreased. Overall, the results suggest that attractive plants could be considered for phytostabilization of soil contaminated with Cd (Zeng et al. 2018).

4.3. Phytodegradation

Phytodegradation is the process by which plants take up pollutants and break them down into simpler and less toxic forms to grow more quickly. Phytodegradation can occur through the plant's metabolic pathways or with the help of enzymes (Chlebek & Hupert-Kocurek, 2019). Using particular secretory enzymes that catalyze and accelerate chemical reactions, the procedure can occur either internally or externally (Pandey et al., 2021; Kristanti & Hadibarata, 2023).

The method is effective for pesticides, other organic substances, chlorinated solvents, and numerous inorganic substances (Figure 5). The effectiveness of pollutants' uptake, their concentration in the soil, and the amount of groundwater all have an impact on phytodegradation. The phytochemical characteristics of the plants determine how well contaminants are absorbed. Plants affect the soil around them during the process with the production of root exudates, which draw numerous microbial species including bacteria and fungi that live in the rhizosphere. These microorganisms speed up the biodegradation of xenobiotics and promote a variety of advantageous interactions with plants (Khandare and Govindwar, 2015; Nebeská et al., 2021).

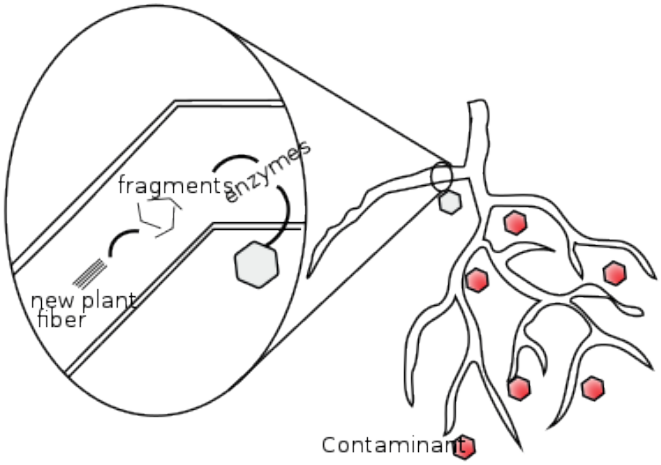

Fig. 5. *Phytodegradation of organic contaminants (Rona, 2019).*

In a study, the efficacy of *Azolla filiculoides* for eliminating Bisphenol A (BPA) from aqueous solutions was investigated. *Azolla* was cultivated in solutions containing 5, 10, 25, and 50 ppm of BPA, resulting in varying amounts of biomass (0.3, 0.6, 0.9, and 1.2 g). Samples were collected from each container every two days. The results revealed that *Azolla* is remarkably effective at eliminating BPA from aqueous solutions with a removal rate of 60-90%. Removal efficiency increased as BPA concentration decreased and biomass volume increased, and vice versa. When the BPA concentration was 5 ppm and the biomass amount was 0.9 g, removal efficiency exceeded 90% (Zazouli et al. 2014).

4.4. Phytostimulation

Phytostimulation relies on the secretion of root exudates by plants, facilitating the proliferation and metabolic functions of diverse fungal and bacterial populations in the rhizosphere (Sengupta & Pal, 2021) This process is also known as rhizospheric biodegradation (Figure 6). Natural fungi, bacteria, and actinomycetes can frequently break down organic pollu-tants in the soil into smaller products, and they can be entirely mineralized into inorganic pollutants like carbon dioxide and water (Ely & Smets, 2017). Microbial populations have expanded as a result of plant exudates which are substances made by plants and discharged from their roots. Nucleotides, sugars, sterols, organic acids, amino acids, fatty acids, growth factors, flavanones, enzymes, and other substances are present in exudates. Increased microbial activity and populations in the rhizosphere may boost the biodegradation of pollutants in the soil (Cristaldi et al., 2017).

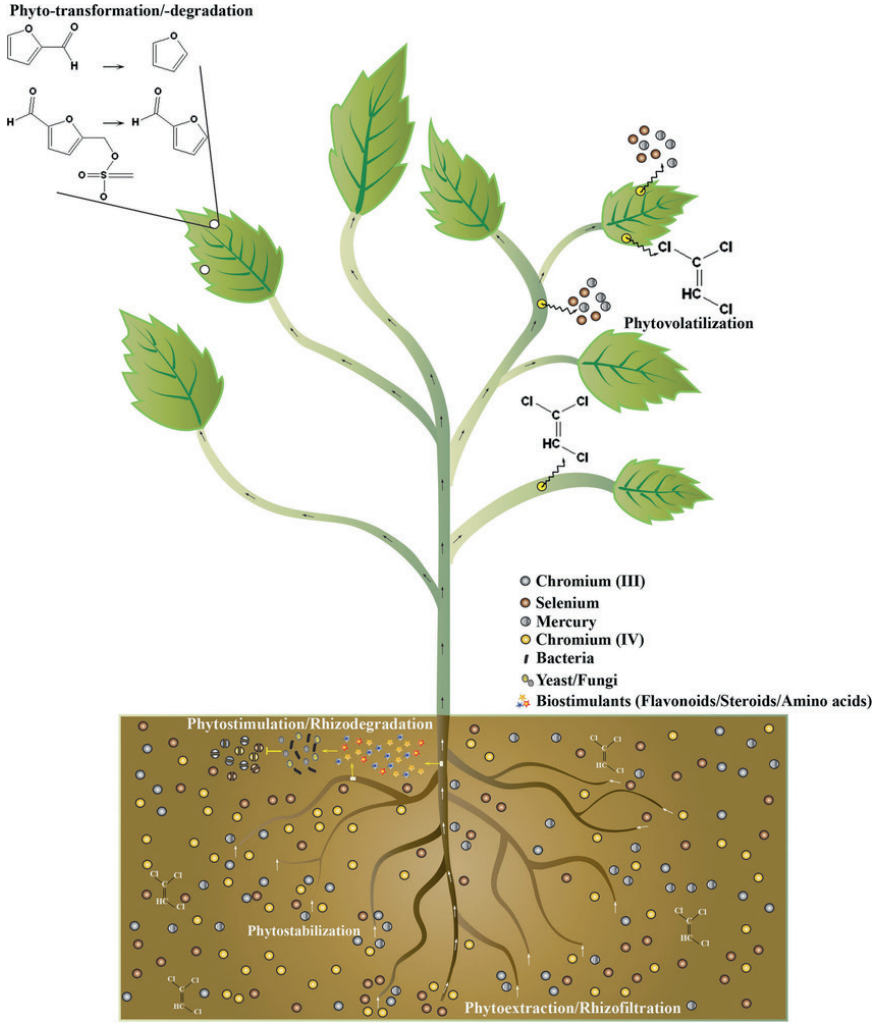

Fig. 6. *A variety of mechanisms of phytoremediation (Bedair et al., 2022).*

In a study, the pea cultivar Blauwschokker was investigated to stimulate the degradation of biodiesel in agricultural soil by assessing the impact of biological remediation on plant physiological parameters and soil microflora. The soil was spiked with 50 g of biodiesel per kilogram of dry soil mass. The study results showed that biodiesel degradation was more efficient in the soil with vegetation compared to natural attenuation. Moreover, the presence of biodiesel had a positive impact on the growth, development, and activity of soil bacteria and fungi. The study also demonstrated that the presence of biodiesel enhanced plant physiological characteristics, including an increase in chlorophyll and total chlorophyll concentration and higher relative water content in leaves (Hawrot-Paw et al., 2019).

4.5. Phytofiltration

Phytofiltration, also known as rhizofiltration, refers to the mechanism by which plant roots take up and deposit both organic and inorganic pollutants in order to eliminate them from polluted surface water, groundwater, and wastewater. Tolerance to metals, hypoxia, and a wide surface area of absorption are primary criteria for selecting a good plant for rhizofiltration. Compared to aquatic plants, terrestrial plants are more suitable for in situ or ex-situ rhizofiltration due to their better-developed roots and fibrous root structure which provides a larger surface area for absorption (Pang et al., 2023).

Pb, Cr, and Hg have been identified as the main probable metal contaminants for phytostabilization (Harms et al., 1986). Although some organic pollutants or their metabolic byproducts can become associated with plant components like lignin, this may offer an opportunity for the phytostabilization of organic contaminants. The process of phytolignification is used to describe this type of phytostabilization (Sharma, 2018). One important difference is that phytostabilization of organic pollutants through phytolignification can happen in the aboveground parts of plants, while phytostabilization of metals is usually thought to occur in the soil (Shackira & Puthur, 2019; Radziemska et al., 2023).

There are many reports related to the phytofiltration efficiency of different plants (Akhtar, 2019; Wei et al., 2021; Kafle et al., 2022). One of them was performed by Sandhi et al. (2017). They explored the feasibility of using aquatic moss, *Warnstorfia fluitans*, harvested from a wetland located near a mine tailings impoundment for phytofiltration of arsenic (As) in contaminated water. The total As content in the aquatic moss was analyzed after the treatment. During the experiment, the moss was subjected to different concentrations (0%, 1%, or 10%) of Hoagland nutrient solutions for 192 hours. These solutions contained arsenate ranging from 0 to 100 μM. The objective of the study was to examine the speciation and uptake of arsenic (As) in both living plant parts (through absorption and adsorption) and dead plant parts (through adsorption only). These plant parts were grown in water containing either 10 μM arsenate or arsenite. The findings indicated that in the absence of nutrients, W. fluitans removed up to 82% of As from the water within one hour when 1 mM arsenate was added. Higher concentrations of nutrients and As led to a longer elimination period. Up to 100 μM As had no detrimental effect on plant biomass. Almost 90% of the As which may be a defense against excessive As concentrations taken up was tightly linked to the tissue (Sandhi et al., 2017).

4.6. Phytovolatilization

Phytovolatilization is the process by which plants absorb organic contaminants from water and release these toxins into the atmosphere through their leaves. When a plant uptakes a contaminant, it may release a volatile degradation product or a modified volatile version of the original contaminant (Figure 7). In some circumstances, the release of contaminants into the atmosphere enables far more potent or quick natural degradation processes, like photodegradation. A risk study of the effects of this transfer on the environment and human health may be required since phytovolatilization entails the transfer of pollutants to the atmosphere. Because these areas would encourage the rapid discharge of volatile compounds, phytovolatilization should not be carried out close to densely populated areas and regions with unusual weather patterns (Guerra et al., 2021).

For phytoremediation to be effective, the modified volatile form or breakdown product must be less hazardous than the original contaminant. For example, harmful selenium (as selenate, Se) can be converted into the less toxic gas dimethyl selenide or toxic Hg can be reduced to less dangerous elemental mercury (Awa and Hadibarata, 2020). Many varieties of aquatic plants were used to remove Se from contaminated locations. The soil contains hazardous inorganic Se compounds that are more harmful than its volatile forms. Musk-grass and Indian mustard, two plant species, have the potential to absorb pollutants like Se and Hg, transform them into gaseous forms inside the plant, and then release these gases into the atmosphere (Hasanuzzaman et al., 2020).

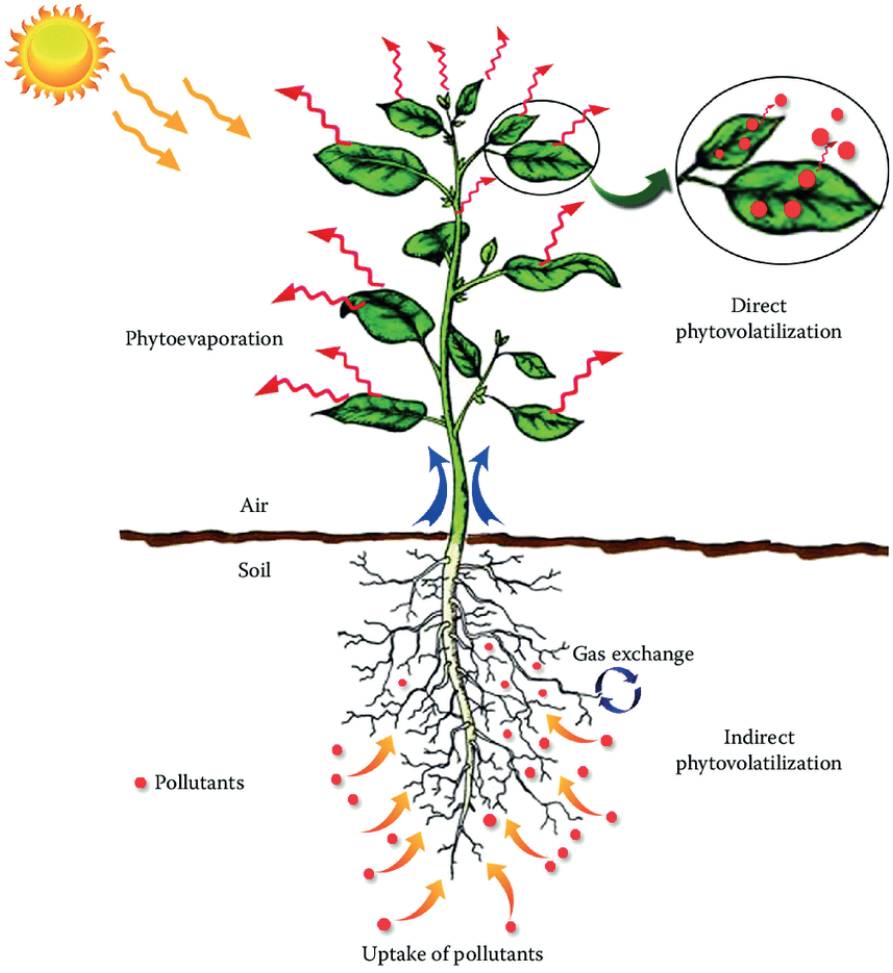

Fig. 7. *Phytovolatilization of pollutants (Chandra & Kumar, 2017).*

Phytoremediation techniques can be combined in various ways at a single site to remediate a particular contaminant or address different contaminants at different concentrations. For instance, soil contaminated with trichloroethylene (TCE) may undergo rhizodegradation at the root zone while being metabolized by plants through phytodegradation (Simmer & Schnoor, 2022). Both naturally occurring and synthetic sources can be found in the structural analogs 2,4-dibromophenol (2,4-DBP) and 2,4-dibromoanisole (2,4-DBA), which are widely found in environmental matrices. In a study about phytovolatilization, hydroponic exposure studies were used to assess their environmental fates, particularly volatilization including both direct volatilization from the cultivation fluid and phytovolatilization through rice plants. The findings demonstrated that 2,4-DBA showed a greater potential to volatilize and increased bioaccumulation in aboveground rice tissues. 2,4-DBA was

3.43 times more volatile than 2,4-DBP. 2,4-DBA and 2,4-DBP's phytovolatilization increased the amount of these pollutants that were released into the atmosphere from hydroponic solutions by 6.78% and 41.7%, respectively (Zhang et al., 2020).

Moreover, some metals or radionuclides in water can accumulate in the roots through rhizofiltration or be absorbed by the aerial part of the plant through phytoremediation. In the case of untreated contaminated areas, natural biodegradation may eventually occur, and abiotic processes, such as volatilization, or classical treatment methods can be implemented. The growth of vegetation in these areas can indicate that the pollutants are no longer toxic to native plant species. Alternatively, a plant that can tolerate the pollutant may preferentially grow and contribute to further loss of the contaminant through phytoremediation processes (Sharma et al., 2023b; Limmer & Burken 2016).

5. Conclusion

Phytoremediation is the utilization of plants to neutralize environmental pollutants. The concept of phytoremediation has evolved day by day related to the developments in phytotechnologies. Although phytoremediation has advantages such as low cost, soil fertility preservation, erosion, and metal leakage prevention, it also has disadvantages such as long cleaning time and the applications of lands with little or moderate metal contamination. However, some of these disadvantages have been tried to be solved with the help of gene engineering. Selecting or designing plants is more suitable for phytoextraction and increases the effectiveness of this technique. Research continues to improve phytoremediation and its techniques, which are promising for a cleaner environment. For this purpose, studies are carried out by combining multidisciplinary areas including molecular biology and genetics, biotechnology, biochemistry, and even engineering.

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Chapter 2

SOUTHEASTERN ANATOLIA REGION INSECT FAUNA II (ORDER HEMIPTERA III: SUBORDER AUCHENORRHYNCHA I: SUPERFAMILY MEMBRACOIDEA: FAMILY CICADELLIDAE) OF TURKEY

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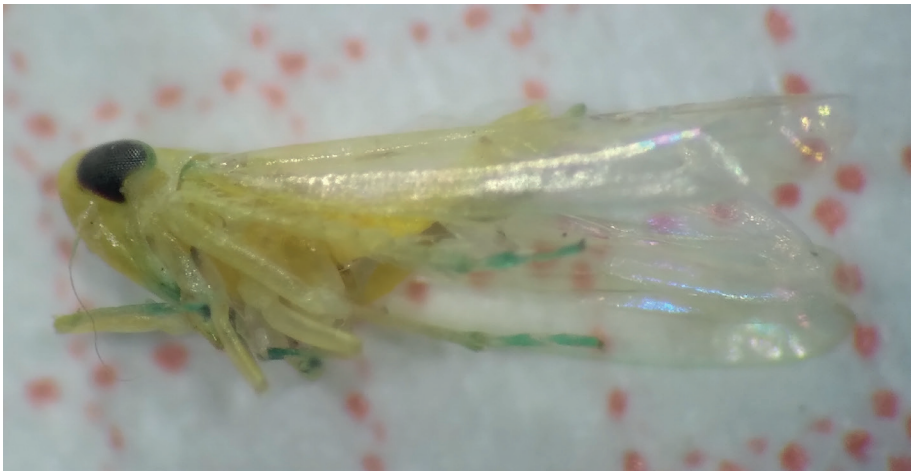

Introduction

Insects (Insecta) are the most numerous group of animals in the world, with over one million species that have been described (Price, 1997). Insects are difficult to study because they represent the most species-rich, yet one of the least known, of all taxa of living organisms, a problem that is compounded by a dearth of skilled entomologists. Although the number of described insect species is uncertain due to synonyms and the lack of a global list, most authorities recognize 900 000-1 000 000 named morpho-species, representing 56% of all species known on Earth (Groombridge, 1992; Anonymous, 2003). Sensible estimates of the number of insects yet to be discovered range from another 1 million to 30 million species (Erwin, 1982-1991), although most predict around 2-8 million more species (May, 1990; Gaston, 1991; Stork, 1997; Ødegaard, 2000). Conservative estimates suggest that 50-90% of the existing insect species on Earth have still to be discovered, yet the named insects alone comprise more than half of all known species of organism.

Insects constitute the most diverse form of animal life in terrestrial ecosystems. Most species are innocuous and essential components of natural ecosystems. Because they are cold-blooded, the rates of key physiological processes in their life cycles are determined by environmental conditions, especially temperature and precipitation. In general they have short generation times, high fecundity and high mobility (Moore & Allard 2008).

Auchenorrhyncha are a highly diverse group of hemimetabolous insects and a major component of the phytophagous insect fauna in most terrestrial ecosystems worldwide. They are treated here as a suborder of the Hemiptera and include the planthoppers (infraorder Fulgoromorpha), cicadas, froghoppers, spittlebugs, treehoppers, and leafhoppers (infraorder Cicadomorpha). With their piercing sucking mouthparts the majority of Auchenorrhyncha species

feed on phloem or xylem (plant sap) or plant cell contents (parenchyma or cell ruptures) although some species feed on mosses or fungi. This economically important group includes several plant pests pests and several vectors of plant pathogens, including phytoplasmas, viruses, and bacteria.

Turkey in fact seems to be like a small continent in terms of biological diversity. Despite the Anatolia is not a continent alone, it contains all properties of a continent that should have an ecosystem and habitat. Each of seven geographical regions in Turkey has a distinguishable climate, flora and fauna. This study aims to determine insect species found in various ecologies on Southeastern Anatolia Region.

This study aims to determine insect species found in various ecologies on Southeastern Anatolia Region of Turkey.

Material and Methods

Entomology studies on insect species of Southeastern Anatolia Region (Adıyaman, Batman, Gaziantep, Diyarbakır, Mardin, Siirt, Şanlıurfa, Şırnak) in different ecological provinces were made between the years 1948-2022 (Figure 1).

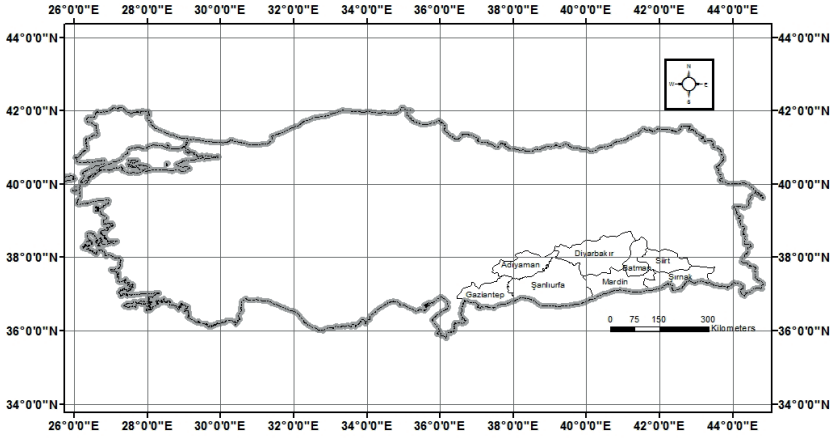

Figure. 1. Sampling localities in the Southeastern Anatolia Region of Turkey

In this study, I prepared for the inventory has reached the major advantage of the waterways:

-Currently in Turkey, published or unpublished entomology journals related to scanning,

-Giving more weight to faunistic studies, and in the meantime, the insect

fauna of our country foreign scientific journals that publishes articles about scanning,

-Faculty of Agriculture, Faculty of Science and Regional Plant Protection Research Institute in the library of books on insect fauna and the screening of the booklet,

-The doctorate (PhD) and the master's thesis of entomology in the region on the scanning,

-Review of other studies on the insect fauna in the area.

In this study, I evaluated the information as described above were obtained.

Results and Discussion

Surveys on insect species in various ecologies have been conducted in the provinces (Adıyaman, Batman, Gaziantep, Diyarbakır, Mardin, Siirt, Şanlıurfa, Şırnak) of Southeastern Anatolia region between the years 1948-2022. Almost 2600 species and subspecies almost 180 families belonging to 13 insect orders are defined owing to these studies. The distributions of determined insect species are as follows: Cicadellidae family included 126 speciess were recorded. Cicadellidae species in the study are given in alphabetical order.

ORDER HEMIPTERA

SUBORDER AUCHENORRHYNCHA

INFRAORDER CICADOMORPHA

SUPERFAMILY MEMBRACOIDEA Rafinesque, 1815

FAMILY CICADELLIDAE Latreille, 1802

***Aconurella prolixa* (Leitherry, 1885)**

Distribution of the studies area: Gaziantep, **Host plant:** Maize, Scrub and Grassland (Mutlu et al., 2008b; Önder et al., 2011).

***Agallia minuta* Melichar, 1896**

Distribution of the studies area: Gaziantep, **Host plant:** Agricultural area (Önder et al., 2011).

***Aglena ornata* (Herrich-Schäffer, 1838)**

Distribution of the studies area: Diyarbakır, **Host plant:** Scrub and Grassland (Önder et al., 2011).

***Alebra wahlbergi* (Boheman, 1845)**

Distribution of the studies area: Mardin, **Host plant:** Agricultural area, Woodland (Önder et al., 2011).

***Anaceratagallia laevis* (Ribaut, 1935)**

Distribution of the studies area: Mardin, Siirt, Şanlıurfa, **Host plant:** Cherry, *Medicago sativa*, *Sesamum indicum*, *Sinapis* sp., *Gossypium* sp. (Lodos & Kalkandelen, 1981; Çınar et al., 2004).

***Anaceratagallia ribauti* (Ossiannilsson, 1938)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Agricultural area, Scrub and Grassland and collecting with light trap (Önder et al., 2011; Bolu & Zeybekoğlu, 2022).

***Anaceratagallia venosa* (Fourcroy, 1785)**

Distribution of the studies area: Diyarbakır, collecting with light trap (Bolu & Zeybekoğlu, 2022).

***Aphrodes bicinctus* (Schrank, 1776)**

Distribution of the studies area: Southeastern Anatolia Region, **Host plant:** Legumes and Forage Crops (Akkaya, 1995).

***Aphrodes trifasciatus* (Fourcrcoy, 1785)**

Distribution of the studies area: Mardin, **Host plant:** Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009).

***Arboridia adanae* (Dlabola, 1957)**

Distribution of the studies area: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa, **Host plant:** Vineyard (Maçan, 1984; Özgen, 2008; Özgen & Karsavuran, 2009).

***Artianus manderstjernii* (Kirschbaum, 1868)**

Distribution of the studies area: Diyarbakır, Gaziantep, Şanlıurfa, **Host plant:** Scrub and Grassland (Önder et al., 2011;).

***Asymmetrasca decedens* (Paoli, 1932)**

Distribution of the studies area: Diyarbakır, Southeastern Anatolia Region, **Host plant:** Cotton, Maize, Pistachio and collecting with light trap (Göven, 1995; Bolu, 2002; Mutlu et. al., 2008a-b; Bolu & Zeybekoğlu, 2022).

***Austroagallia sinuata* (Mulsant & Rey, 1855)**

Distribution of the studies area: Diyarbakır, Gaziantep, Mardin, Siirt, Şırnak, **Host plant:** Agricultural area, Maize, Scrub and Grassland, *Gossypium* sp., *Sesamum indicum*, *Panicum miliaceum*, *Heliotropium* sp. and collect-

ing with light trap (Lodos & Kalkandelen, 1981; Mutlu et al., 2008b; Önder et al., 2011; Bolu & Zeybekoğlu, 2022).

***Balclutha frontalis* (Ferrari, 1882)**

Distribution of the studies area: Diyarbakır, Gaziantep, **Host plant:** Agricultural area (Önder et al., 2011).

***Balclutha hebe* (Kirkaldy, 1906)**

Distribution of the studies area: Diyarbakır, Gaziantep, Mardin, Şanlıurfa, **Host plant:** Agricultural area, Maize, Scrub and Grassland (Mutlu et. al., 2008a; Önder et al., 2011).

***Balclutha pellucens* Horváth, 1909**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Agricultural area, Scrub and Grassland (Önder et al., 2011).

***Balclutha punctata* (Fabricius, 1775)**

Distribution of the studies area: Adıyaman, Diyarbakır, **Host plant:** Agricultural area, Scrub and Grassland, Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009; Önder et al., 2011).

***Balclutha saltuella* (Kirschbaum, 1868)**

Distribution of the studies area: Gaziantep, **Host plant:** Meadow pasture (Önder et al., 2011).

***Balcanocerus alkani* (Wagner, 1958)**

Distribution of the studies area: Southeast Anatolia Region, **Host plant:** Pistachio (Bolu, 2002).

***Balcanocerus brusinae* (Horváth, 1891)**

Distribution of the studies area: Diyarbakır, Gaziantep, **Host plant:** Agricultural area, Scrub and Grassland, Woodland (Önder et al., 2011).

***Balcanocerus larvatus* (Herrich-Schäffer, 1835)**

Distribution of the studies area: Diyarbakır, **Host plant:** Agricultural area (Önder et al., 2011).

***Balcanocerus ramallahicus* Dlabola, 1965**

Distribution of the studies area: Gaziantep, **Host plant:** Agricultural area (Önder et al., 2011).

***Batracomorphus irroratus* Lewis, 1834**

Distribution of the studies area: Adıyaman, Diyarbakır, **Host plant:** Scrub and grassland (Önder et al., 2011).

***Batracomorphus signatus* Lindberg, 1923**

Distribution of the studies area: Mardin, **Host plant:** Agricultural area, Scrub and Grassland, Woodland (Önder et al., 2011).

***Chiasmus conspurcatus* (Perris, 1857)**

Distribution of the studies area: Diyarbakır, Şanlıurfa, **Host plant:** Scrub and Grassland (Önder et al., 2011).

***Chunrocerus larvatus* (Herrich & Schäffer, 1837)**

Distribution of the studies area: Diyarbakır, **Host plant:** Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009).

***Cicadulina bipunctella* (Matsumura, 1908)**

Distribution of the studies area: Diyarbakır, Gaziantep, **Host plant:** Agricultural area, Maize, Scrub and Grassland (Mutlu et al., 2008b; Önder et al., 2011).

***Cicadula placida* (Horváth, 1897)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009).

***Cicadella viridis* (Linnaeus, 1758)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Agricultural area, Maize, Scrub and Grassland, Woodland (Mutlu et al., 2008b; Önder et al., 2011).

***Cicadula divaricata* Ribaut, 1952**

Distribution of the studies area: Diyarbakır, Mardin, Şanlıurfa, **Host plant:** Agricultural area (Önder et al., 2011).

***Cicadula frontalis* (Herrich-Schäffer, 1835)**

Distribution of the studies area: Diyarbakır, **Host plant:** Agricultural area, Vineyard (Özgen et al., 2009; Önder et al., 2011).

***Circulifer haematoceps* (Mulsant & Rey)**

Distribution of the studies area: Adıyaman, Diyarbakır, Gaziantep, Mardin, Şanlıurfa, **Host plant:** Almond, Maize, Olive (Bolu et al., 2005; Mutlu et al., 2008b; Kaplan et al., 2011).

***Circulifer opacipennis* (Lethierry, 1876)**

Distribution of the studies area: Mardin, **Host plant:** Cherry (Çınar et al., 2004).

***Conosanus obsoletus* (Kirschbaum, 1858)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Agricultural area, Scrub and Grassland (Önder et al., 2011).

***Doratura concors* Horváth, 1903**

Distribution of the studies area: Siirt, **Host plant:** Scrub and grassland (Önder et al., 2011).

***Doratura exilis* Horváth, 1903**

Distribution of the studies area: Diyarbakır, **Host plant:** Maize (Mutlu et. al., 2008b).

***Doratura homophyla* (Flor, 1861)**

Distribution of the studies area: Diyarbakır, Gaziantep, **Host plant:** Maize, Scrub and Grassland (Mutlu et. al., 2008b; Önder et al., 2011).

***Doratura impudica* Horváth, 1897**

Distribution of the studies area: Gaziantep, **Host plant:** Scrub and grassland (Önder et al., 2011).

***Doratura salina* Horváth, 1903**

Distribution of the studies area: Siirt, **Host plant:** Scrub and grassland (Önder et al., 2011).

***Dryodurgades reticulatus* (Herrich-Schäffer, 1834)**

Distribution of the studies area: Diyarbakır, **Host plant:** *Pinus* sp., *Juniperus* sp., *Sesamum* sp., *Alhagi* sp., Scrub and Grassland (Lodos & Kalkandelen, 1981; Önder et al., 2011).

***Empoasca decipiens* Paoli, 1930**

Distribution of the studies area: Diyarbakır, Mardin, Southeastern Anatolia Region, **Host plant**: Almond, Cotton, Maize, Vineyard,and collecting with light trap (Göven, 1995; Bolu et al., 2005; Mutlu et. al., 2008a-b; Özgen, 2008; Özgen & Karsavuran, 2009; Bolu & Zeybekoğlu, 2022).

***Empoasca solani* (Curtis, 1846)**

Distribution of the studies area: Gaziantep, Şanlıurfa, **Host plant:** Agricultural area, Scrub and Grassland (Lodos & Kalkandelen, 1983; Önder et al., 2011).

***Eohardya fraudulenta* (Horváth, 1903)**

Distribution of the studies area: Diyarbakır, **Host plant:** Agricultural area, Scrub and Grassland (Önder et al., 2011).

***Euscelis alsius* Ribaut, 1952**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Maize, Vineyard (Mutlu et al., 2008b; Özgen, 2008; Özgen & Karsavuran, 2009).

Euscelis obsoletus (Kirschbaum, 1858)

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009).

***Eupelix cuspidata* (Fabricius, 1775)**

Distribution of the studies area: Adıyaman, Diyarbakır, Mardin, Şanlıurfa, **Host plant:** Scrub and grassland (Önder et al., 2011).

***Euscelis incisus* (Kirschbaum, 1858)**

Distribution of the studies area: Southeastern Anatolia Region, **Host plant:** Legumes and Forage Crops (Akkaya, 1995).

***Euscelidius mundus* (Haupt, 1927)**

Distribution of the studies area: Diyarbakır, Gaziantep, Mardin, Şanlıurfa, **Host plant:** Agricultural area, Scrub and Grassland, Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009; Önder et al., 2011).

***Euscelidius schenckii* (Kirschbaum, 1868)**

Distribution of the studies area: Diyarbakır, Mardin, Siirt, **Host plant:** Agricultural area, Scrub and Grassland, Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009; Önder et al., 2011).

***Euscelidius variegatus* (Kirschbaum, 1858)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Agricultural area, Vineyard collecting with light trap (Özgen, 2008; Özgen & Karsavuran, 2009; Önder et al., 2011; Bolu & Zeybekoğlu, 2022).

***Euscelis lineolatus* Brullé, 1832**

Distribution of the studies area: Diyarbakır, collecting with light trap (Bolu & Zeybekoğlu, 2022).

Erythroneura adanae Dlabola, 1957

Distribution of the studies area: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa, Şırnak, **Host plant:** Vineyard (Raşit, 1970; Günaydın, 1972).

***Exitianus fasciolatus* (Melichar, 1911)**

Distribution of the studies area: Diyarbakır, Gaziantep, Mardin, Şanlıurfa, **Host plant:** Culture plants, Meadow pasture (Lodos et al., 1984; Önder et al., 2011).

***Fieberiella florii* (Stål, 1864)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009).

***Goniagnathus bolivari* (Melichar, 1907)**

Distribution of the studies area: Şanlıurfa, **Host plant:** Scrub and Grassland (Önder et al., 2011).

***Goniagnathus brevis* (Herrich-Schäffer, 1835)**

Distribution of the studies area: Diyarbakır, **Host plant:** Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009).

***Goniagnathus guttulinervis* (Kirschbaum, 1868)**

Distribution of the studies area: Diyarbakır, **Host plant:** Maize (Mutlu et. al., 2008b).

***Handianus procerus* (Herrich-Schäffer, 1835)**

Distribution of the studies area: Şanlıurfa, **Host plant:** Scrub and Grassland (Önder et al., 2011).

***Hardya anatolica* Zachvatkin, 1946**

Distribution of the studies area: Adıyaman, **Host plant:** Agricultural area, Woodland (Önder et al., 2011).

***Hecalus glaucescens* (Fieber, 1866)**

Distribution of the studies area: Adıyaman, Diyarbakır, Siirt, **Host plant:** Agricultural area, Maize, Scrub and Grassland, Vineyard (Mutlu et al., 2008b; Özgen, 2008; Özgen & Karsavuran, 2009; Önder et al., 2011).

***Hecalus storai* (Lindberg, 1936)**

Distribution of the studies area: Mardin, **Host plant:** Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009).

***Hephathus freyi* (Fieber, 1868)**

Distribution of the studies area: Şanlıurfa, **Host plant:** *Artemisia* spp., especially on *A. herbaalba*, Scrub and Grassland (Lodos & Kalkandelen, 1981; Önder et al., 2011).

***Idiocerus (Sulamicerus) stali* Fieber, 1868**

Distribution of the studies area: Adıyaman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, **Host plant:** Pistachio (Sipahi, 1957; Günaydın, 1978; Yanık, 1997; Bolu, 2002).

***Idiocerus ustulatus* (Mulsant & Rey, 1855)**

Distribution of the studies area: Diyarbakır, **Host plant:** Vineyard (Öz-

gen, 2008; Özgen & Karsavuran, 2009).

***Idiodonus cruentatus* (Panzer, 1799)**

Distribution of the studies area: Mardin, **Host plant:** Scrub and Grassland, Vineyard (Özgen et al., 2009; Önder et al., 2011).

***Imbecilla imbecilla* (Linnavuori, 1962)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Agricultural area, Woodland, Scrub and Grassland (Önder et al., 2011).

***Iassus scutellaris* (Fieber, 1868)**

Distribution of the studies area: Adıyaman, **Host plant:** Woodland (Önder et al., 2011).

***Kyboasca bipunctata* (Oshania, 1871)**

Distribution of the studies area: Diyarbakır, Mardin, Siirt, **Host plant:** Agricultural area (Lodos & Kalkandelen, 1983; Önder et al., 2011).

***Limotettix striola* (Fallén, 1806)**

Distribution of the studies area: Diyarbakır, Şanlıurfa, **Host plant:** Agricultural area, Scrub and Grassland (Önder et al., 2011).

Macropsis graminae (Fabricius, 1798)

Distribution of the studies area: Adıyaman, Diyarbakır, **Host plant:** *Populus* sp., Salix sp., Woodland (Lodos & Kaldelen, 1981; Önder et al., 2011).

***Macropsis mendax* (Fieber, 1868)**

Distribution of the studies area: Gaziantep, **Host plant:** *Populus* sp., *Prunus avium*, *Pistacia vera*, Woodland (Lodos & Kalkandelen, 1981; Önder et al., 2011).

***Macrosteles alpinus* (Zetterstedt, 1828)**

Distribution of the studies area: Diyarbakır, **Host plant:** Scrub and Grassland (Önder et al., 2011).

***Macrosteles fieberi* (Edwards, 1889)**

Distribution of the studies area: Diyarbakır, **Host plant:** Maize (Mutlu et. al., 2008b).

***Macrosteles laevis* (Ribaut, 1927)**

Distribution of the studies area: Adıyaman, Diyarbakır, **Host plant:** Millet, Maize and collecting with light trap (Şimşek, 1988; Mutlu et. al., 2008b; Bolu & Zeybekoğlu, 2022).

***Macrosteles ossiannilssoni* Lindberg, 1954**

Distribution of the studies area: Adıyaman, Diyarbakır, **Host plant:** Agricultural area, Meadow pasture (Önder et al., 2011).

***Macrosteles quadripunctulatus* (Kirschbaum, 1868)**

Distribution of the studies area: Adıyaman, Diyarbakır, **Host plant:** Maize and Millet (Şimşek, 1988).

***Macrosteles sexnotatus* (Fallén, 1806)**

Distribution of the studies area: Adıyaman, Diyarbakır, **Host plant:** Agricultural area, Scrub and Grassland, and collecting with light trap (Önder et al., 2011; Bolu & Zeybekoğlu, 2022).

***Macydiopsis monticola* Remane, 1961**

Distribution of the studies area: Diyarbakır, **Host plant:** Agricultural area, Scrub and Grassland (Önder et al., 2011).

***Mogangella straminea* Dlabola, 1957**

Distribution of the studies area: Diyarbakır, collecting with light trap (Bolu & Zeybekoğlu, 2022).

***Megophthalmus scabripennis* Edwards, 1915**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009).

***Micantulina stigmatipennis* (Mulsant & Rey, 1855)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Almond (Bolu et al., 2005).

***Neoaliturus fenestratus* (Herrich-Schäffer, 1834)**

Distribution of the studies area: Diyarbakır, Mardin, Siirt, Şanlıurfa, **Host plant:** *Artemisia* sp., *Medicago sativa*, *Trifolium* spp., *Tamarix*, *Chenopodium*, *Hupericum*, Apple, Cotton, Maize, Vineyard, and collecting with light trap (Lodos & Kalkandelen, 1985; Mutlu et. al., 2008b; Özgen, 2008; Özgen & Karsavuran, 2009; Bolu & Zeybekoğlu, 2022).

***Neoaliturus haematoceps* (Mulsant & Rey, 1855)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009).

***Neoaliturus opacipennis* (Lethierry, 1876)**

Distribution of the studies area: Diyarbakır, collecting with light trap (Bolu & Zeybekoğlu, 2022).

***Neoaliturus pulcher* (Haupt, 1927)**

Distribution of the studies area: Diyarbakır, collecting with light trap (Lodos & Kalkandelen, 1985).

***Neoaliturus transversalis* (Puton, 1881)**

Distribution of the studies area: Diyarbakır, collecting with light trap (Bolu & Zeybekoğlu, 2022).

***Opsius cypriacus* Lindberg, 1958**

Distribution of the studies area: Diyarbakır, Siirt, **Host plant:** *Tamarix* sp. (Lodos & Kalkandelen, 1985).

***Orosius orientalis* (Matsumura, 1914)**

Distribution of the studies area: Diyarbakır, **Host plant:** Maize (Mutlu et. al., 2008b).

***Orosius is* (Matsumara, 1914)**

Distribution of the studies area: Diyarbakır, Şanlıurfa, **Host plant:** Agricultural area, Scrub and Grassland (Önder et al., 2011).

***Paluda flaveola* (Boheman, 1845)**

Distribution of the studies area: Mardin, **Host plant:** Vineyard (Özgen, 2008).

***Paradoryolium paradoxum* (Herrich-Schäffer, 1837)**

Distribution of the studies area: Diyarbakır, **Host plant:** Agricultural area, Scrub and Grassland (Önder et al., 2011).

***Parabolacratus storai* (Lindberg, 1957)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Vineyard (Özgen, 2008).

***Paramesodes lincaticollis* (Distant, 1908).**

Distribution of the studies area: Diyarbakır, **Host plant:** Scrub and Grassland (Önder et al., 2011).

***Paramesus major* Haupt, 1927**

Distribution of the studies area: Diyarbakır, Siirt, **Host plant:** Agricultural area, Scrub and Grassland (Önder et al., 2011).

***Phlepsius intricatus* (Herrich-Schäffer, 1838)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Vineyard and collecting with light trap (Özgen, 2008; Özgen & Karsavuran, 2009; Bolu & Zeybekoğlu, 2022).

***Phlepsius ornatus* (Perris, 1857)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Cherry, Scrub and Grassland, Vineyard (Çınar et al., 2004; Özgen, 2008; Özgen & Karsavuran, 2009; Önder et al., 2011).

***Planaphrodes trifasciatus* (Fourcroy, 1785)**

Distribution of the studies area: Mardin, **Host plant:** Agricultural area, Scrub and Grassland (Önder et al., 2011).

***Platymetopius cruentatus* (Haupt, 1927)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Agricultural area, Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009; Önder et al., 2011).

***Platymetopius hannelorae* (Abdul & Nour, 1987)**

Distribution of the studies area: Diyarbakır, **Host plant:** Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009).

***Platymetopius quercinus* (Dlabola, 1974)**

Distribution of the studies area: Adıyaman, Mardin, **Host plant:** Scrub and Grassland, Woodland (Önder et al., 2011).

***Platymetopius rostratus* (Herrich-Schäffer, 1834)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant: Maize,** Vineyard and collecting with light trap (Mutlu et al., 2008b; Özgen, 2008; Özgen & Karsavuran, 2009; Bolu & Zeybekoğlu, 2022).

***Platymetopius safavii* Dlabola, 1971**

Distribution of the studies area: Gaziantep, **Host plant:** Scrub and Grassland (Önder et al., 2011).

***Platymetopius undatus* (DeGeer, 1773)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Maize, Vineyard (Mutlu et al., 2008b; Özgen, 2008; Özgen & Karsavuran, 2009).

***Psammotettix alienus* (Dahlbom 1850)**

Distribution of the studies area: Diyarbakır, Mardin, Siirt, **Host plant:** Agricultural area, Woodland, Scrub and Grassland (Önder et al., 2011).

***Psammotettix confinis* (Dahlbom, 1850)**

Distribution of the studies area: Siirt, **Host plant:** Agricultural area, Scrub and Grassland (Önder et al., 2011).

***Psammotettix pictipennis* (Kirschbaum, 1868)**

Distribution of the studies area: Diyarbakır, Şanlıurfa, **Host plant:** Agricultural area, Scrub and Grassland, Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009; Önder et al., 2011).

***Psammotettix provincialis* (Ribaut, 1925)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009).

***Psammotettix striatus* (Linnaeus, 1758)**

Distribution of the studies area: Adıyaman, Diyarbakır, Mardin, Siirt, **Host plant:** Vineyard, Maize, Millet and collecting with light trap (Şimşek, 1988; Mutlu et. al., 2008a-b; Özgen, 2008; Bolu & Zeybekoğlu, 2022).

***Recilia schmidtgeni* (Wagner, 1939)**

Distribution of the studies area: Diyarbakır, Şanlıurfa, **Host plant:** Scrub and Grassland (Önder et al., 2011).

***Rhytidodus boluicus* Dlabola, 1970**

Distribution of the studies area: Diyarbakır, **Host plant:** Woodland (Önder et al., 2011).

***Rhytidodus decimusquartus* (Schrank, 1776)**

Distribution of the studies area: Mardin, Şanlıurfa, **Host plant:** Vineyard, Woodland (Özgen et al., 2009; Önder et al., 2011).

***Selenocephalus armeniacus* Lindberg, 1960**

Distribution of the studies area: Adıyaman, Diyarbakır, Mardin, Şanlıurfa, **Host plant:** Scrub and Grassland, Woodland and collecting with light trap (Önder et al., 2011; Bolu & Zeybekoğlu, 2022).

***Selenocephalus sirvadi* (Dlabola, 1965)**

Distribution of the studies area: Gaziantep, Şanlıurfa, **Host plant:** Scrub and Grassland (Önder et al., 2011).

***Stegelyta sororcula* (Dlabola, 1974)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Vineyard, (Özgen et al., 2009).

***Stenometopiellus angorensis* Zachvatkin, 1946**

Distribution of the studies area: Diyarbakır, Şanlıurfa, **Host plant:** Scrub and Grassland (Önder et al., 2011).

***Stenometopiellus fraudulentus* (Horvath, 1903)**

Distribution of the studies area: Diyarbakır, **Host plant:** Vineyard (Özgen et al., 2009).

***Stymphalus rubrolineatus* (Stål, 1855)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Scrub and Grassland (Önder et al., 2011).

***Sulamicerus ancorarius* (Dlabola, 1964)**

Distribution of the studies area: Diyarbakır, Siirt, **Host plant:** Agricultural area, Woodland (Önder et al., 2011).

***Sulamicerus stali* (Fieber, 1868)**

Distribution of the studies area: Diyarbakır, Southeastern Anatolia Region, **Host plant:** Pistachio (Bolu, 2002; Şimşek & Bolu, 2017).

***Tamaricella tamaricis* (Puton, 1872)**

Distribution of the studies area: Diyarbakır, **Host plant:** Scrub and Grassland (Önder et al., 2011).

***Thamnotettix creticus* (Dlabola, 1974)**

Distribution of the studies area: Diyarbakır, Mardin, **Host plant:** Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009).

***Thamnotettix zelleri* (Kirschbaum, 1868)**

Distribution of the studies area: Mardin, **Host plant:** Vineyard (Özgen, 2008; Özgen & Karsavuran, 2009).

***Ulopa trivia* (Germar, 1821)**

Distribution of the studies area: Mardin, Siirt, Şanlıurfa, **Host plant:** Scrub and Grassland (Lodos & Kalkandelen, 1981; Önder et al., 2011).

***Zyginella pulchra* Löw, 1885**

Distribution of the studies area: Diyarbakır, **Host plant:** Agricultural area, Woodland (Önder et al., 2011).

***Zyginidia sohrab* Zachvatkin, 1947**

Distribution of the studies area: Adıyaman, Diyarbakır, Gaziantep, Siirt, Şanlıurfa, **Host plant:** Agricultural area, Maize, Millet and collecting with light trap (Şimşek, 1988; Mutlu et. al., 2008a-b; Önder et al., 2011; Bolu & Zeybekoğlu, 2022).

***Zyginella ulchra* Löw**

Distribution of the studies area: Adıyaman, Gaziantep, Mardin, Şanlıurfa, **Host plant:** Olive (Kaplan et al., 2011).

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Chapter 3

DETERMINING THE EFFECT OF SOUND SYSTEMS ON CONTROLLING BIRD DAMAGES AT TRABZON INTERNATIONAL AIRPORT, TURKIYE

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INTRODUCTION

Most airports include extensive grasslands, scrubland, wetlands, and uninhabited areas. At many airports, the main areas used for birds are runways, taxiways and well-maintained short grasslands. Birds come to these areas to feed, rest, nest and roost (Eschenfelder, 2001; ATSB, 2002). Small birds may need a wide field of vision to detect predators, so they feel safer feeding in short grasslands (Devereux et al., 2004). The continuous cutting of grasslands means that the structure that hides insects and other invertebrates is destroyed, which can make prey easier to detect. It is also easier for birds to forage in short grasslands than in long grasslands (Butler and Gillings, 2004). Birds feeding on short grasslands may attract larger raptors to the site (Eschenfelder, 2001; ATSB, 2002). Places used as roosting or observation points for raptors are often aircraft hangars, runway markings, lights and trees. Another reason why airports are used by birds is that they can provide safety from larger predators, including humans (Wright, 1967). Birds can obtain water from drains and water channels, as well as from the standing water that forms at airports after rain. When it rains,insects may be forced to move from enclosed areas such as soil to hard surfaces such as runways, where they can feed more easily. Environments around airports, such as wetlands, dumps, scrubland and agricultural fields, can also contain resources that attract bird species (ATSB, 2002).

Flying species may be more likely to cause significant damage to aircraft in the event of a collision due to the potential for multiple collisions and ingestion by engines (Sodhi, 2002). Large migratory species have been observed to collide with aircraft more frequently than non- migratory species, especially since they often fly in large groups to conserve energy (Hummel, 1983; Weimerskirch et al., 2001). Migratory species may not be familiar with the dangers posed by aircraft and may be unable to detectapproaching aircraft (Sodhi, 2002). Some birds may be more likely to be involved in collisions due to fatigue after traveling long distances during migration (Sodhi, 2002).

Since the beginning of aviation, wild animal-aircraft collisions have caused 744 fatalities and 664 aircraft to become unusable. A total of 724 of the fatal collisions, 504 in civil aviation and 220 in military aviation, were caused by birds, while a total of 20, 9 in civil aviation and 11 in military aviation, were caused by mammals. Of the aircraft that became unusable, 166 in civil aviation and 421 in military aviation were caused by birds, while 70 in civil aviation and 7 in military aviation, 77 in total, were caused by mammals (URL-1, 2023). According to the International Civil Aviation Organization (ICAO), nearly 90% of bird-aircraft collisions occur during landing or take-off phases (ICAO, 2016). Since the power increases in the engines of aircraft can reach the highest values during the take-off andlanding phases, mass fatalities of lighter birds, especially at low altitudes, arehigh (Dukiya and Gahlot, 2013).

Despite the increasing number of studies on birds in Turkiye, scientific monitoring and data on birds and flight safety are still insufficient. The Eastern Black Sea Region has many different habitats for approximately 340 bird species identified to date (Kahraman et al., 2016). Başkaya (1994) conducted a study on migratory bird species in the Eastern Black Sea Region and Gülci (2011) investigated the effects of birds on flight safety at Trabzon International Airport (TIA). Sarı et al. (2022a) presented the first applied scientific study on bird damage at airports in Turkiye. In this study, a decrease of up to 85% in the number of domestic bird species, which negatively affect flight safety, was achieved by using trained dogs and adjusting the grass height according to migration seasons at TIA. Sarı et al. (2022b) conducted a study on bird species that threaten flight safety at TIA andtheir aviation risk values. In addition, Sarı and Mengen (2022) determined the control methods used on bird damages at TIA and conducted experiments using bioacoustic and ultrasonic sound systems.

In this study, the effectiveness of the methods applied in bird control at TIA and the responses of birds by testing various sound systems were investigated.

METHOD

Material

The surveys were conducted between August 13, 2021 and November 13,2022. During the field observations, two binoculars with 10x magnification, two telescopes with 20-60x magnification, and a digital camera with 125x optical magnification were used to collect remote information. Heinzel et al. (1995) "Birds of Turkey and Europe", Kiziroğlu, (2009) "Birds of Turkey Pocket Book" and Jonsson (2006) "Birds of Europe", were used to determine the species identification observed during field studies. After the areas used intensively by birds at the airport were identified, 5 cameratraps were placed in areas that would not threaten flight safety within the knowledge of the stuff of the airport. Images and video record of birds were tried to be obtained with cameratraps. Cameratraps are capable of taking infrared photos and videos. For recording the data obtained, 10 memory cards of different sizes were used according to the capacities of the devices. Although the energy needs of the cameratraps varied according to the model, they were provided with approximately 1000 batteries. While placing the cameratraps in the appropriate areas, 5 special wooden bars with a length of 50 cm and a thickness of 5 cm were prepared for the ground, and galvanized thin iron wire and pliers were used for fixing.

The golden jackal replica, which was made based on the original one-to-one measurements of the golden jackal (*Canis aureus*), one of the predators of Turkiye, was placed in the green areas of the airport that attract birds and the reactions of the animals were measured. The golden jackal replica was kept at

the airport during the study. The golden jackal replica creates the perception of a living creature with its three-dimensional design and movable tail. During the field studies in the areas where the golden jackal replica was placed, a remotecontrolled 38 cm high speaker with a sound power of 400-600 watts, which has a sound power of 400-600 watts and can last up to 5 hours of use, was placed and the reactions of the birds were measured again.

At the same time, along with the golden jackal replica, BirdXPeller®-PRO bird repellent, which has predatory bird sounds, can be programmed, emits sound waves over an area of 4 acres, and repels birds by emitting various distress calls and predatory sounds that confuse, scare or disorient birds, and Bird-K İmti 040 solar-powered multifunctional animal repellent device, which is effective in an area of 400 m^2 thanks to its ultrasonic signal and infrared flash light, were also placed in these areas. Bird-K Imti 040 was kept at the airport for the duration of the study.

A CTECHi 300 W portable power supply was used to meet the energy requirement during the activation of the BirdXPeller®PRO bird repellent device which was used when entering the field for field studies.

Determination of the Study Area

When the phases during which bird strikes occur are classified as acceleration, beginning of climb, climbing altitude, cruise, descent, maneuvering, beginning of approach, final approach and landing (FAA, 2010), the phases during which most bird strikes occur are 48% during climb, 30% during approach, 15% during flight, and 6% during landing (Maragakis, 2009; Gülci, 2011). Bird strikes are concentrated in the safe approach and climb area (ICAO, 2016; DGCA, 2017). For this reason, field studies were carried out in an area of approximately 3.5 km^2 within TIA and at most 1 km outside the airport (Figure 1).

Figure 1. *Study area (GoogleEarth, 2023)*

Installation of Devices in Study Areas

As a result of field observations, camera traps and devices were placed in 3 different areas determined to be used intensively by birds both during and outside the migration period.

After the first month of the study, the areas used intensively by birds were determined. Cameratraps were placed in these areas on September 13, 2021, which is the migration period, in the area shown as number 1 on the map to the east of the area known as the VOR road. This area is an area where especially gray heron species were found in groups of 60-80 during the study. The devices were kept in the area numbered 1 for about 3 months and after being used during the field studies, they were moved to the area named 1.1 critical area, which is the most intensively used by the birds, shown with the number2 on the map, on December 10, 2021. After being kept in area number 2 for 9months during the research, it was moved to area number 3 in critical area 1.1and followed here until the end of the research (Figure 2).

In these areas, the reactions of both migratory and resident species to the sound systems and the golden jackal replica were continuously monitored and recorded through direct observations and cameratraps. Cameratraps were used to monitor the presence of birds and their reactions to the golden jackal replica and the sound systems installed in the field. Bird-K Imti 040 was kept in the field continuously during the research period, staying in the line of sight of the cameratraps. BirdXPeller®PRO bird repellent was usually activated when the researchers arrived to the field, and sometimes it was left in the field without the practitioners in the field.

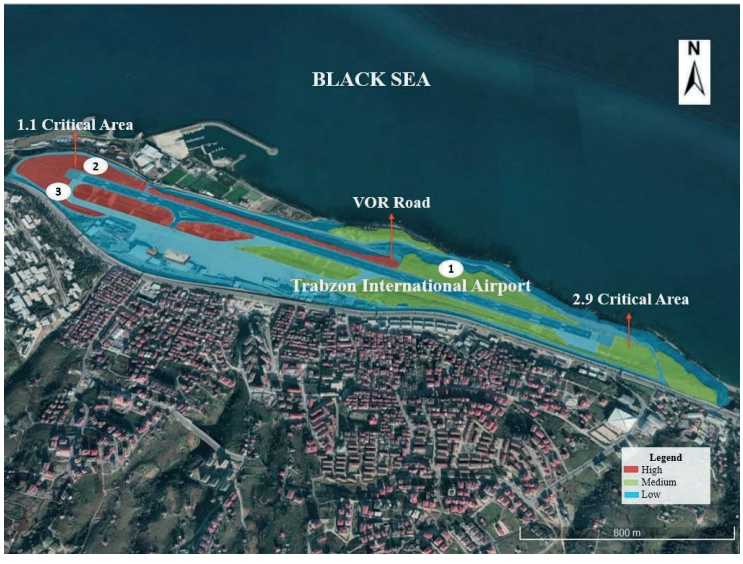

Figure 2. *Areas where cameratraps and sound systems were placed in the study area (GoogleEarth, 2023)*

RESULTS

Bird Control Methods Used at TIA in the Past and Present

One of the main methods used in the past to combat bird damage at TIA was the use of rifles, pistols and sound flares to scare birds away. Regular control of grass lengths, which is also used today, was also used in the past. Airport stuff, especially apron guards, were frequently used physically in the fight against birds. In addition, in the interviews with the managers of the airport, it was stated that an agreement was made with a company in the past years to install an ultrasonic sound system for testing purposes. It was stated that this ultrasonic sound system, which was quite expensive and tested, was ineffective on birds and that birds did not react to this system. Today, these and similar sound systems are not used in the vicinity of TIA due to the presence of residential neighborhoods.

Today, there is no comprehensive bird control methods either physically or systematically. Although weed control continues at TIA, the managers of the institution stated that the main purpose of this control is to protect against a possible fire hazard and that bird control is kept in the second priority. During the study period, it was observed that the weeds were mowed periodically in April, May, June, July, August, August, September and October by both tractors and airport teams. Otherwise, weed control is carried out taking into account the type and growth period of the weeds. In this struggle, 2 runway sweepers, 3 tractors, 7 motorized scythes, 1 drum type lawn mower, 1 garden type lawn mower and 1 weed collection vehicle are used.

Another method used at TIA today is the audible and visual physical control of birds by apron staff. In this method, apron guards try to drive away the birds on the runway and taxiways by driving vehicles and sounding sirens. In the grassland areas, apron officers try to ensure flight safety by getting out of their vehicles in a way that does not pose any risk, running over the flocks of birds in the grass and throwing foreign objects. It has been observed that when the bird density is very high, the fire trucks themselves and their sirens intervene with the birds several times a year. It was also observed that the apron attendants collect foreign objects on and near the runway with their hands. In particular, it was determined that gray carrion crows drop crusted food on the runway from a high distance in order tobreak it and then feed on it. These food residues left on the runway are also collected by the apron staff. During these physical struggles, it was determined that the apron staff had to exert considerable effort and difficulty.

Trials of Sound Systems for Bird Damage Control at TIA

Following the observations made inside the airport and outside the airport from the dominant points on the Karadeniz Technical University (KTU) Campus following the start of the study, cameratraps were first placed in the

area shown as number 1 on the map to the east of the area known as the VOR road during the migration period on September 13, 2021. This area offers temporary accommodation to many bird species, especially during migration periods, along with resident bird species. Among these species, one of the species that will adversely affect flight safety the most is the gray herons, which form groups of 60-80 in the area. Although the golden jackal replica and sound systems were used for about 3 months in area number 1, it was determined that they were not effective in any way, especially on large bird species from the beginning to the end. Again in the area number 1, Bird-K Imti 040 was kept in the field within the field of view of the cameratraps during the study, while the BirdXPeller Pro bird repellent was usually activated when the re- searchers arrived at the field and sometimes it was leftin the field without the practitioners by making the necessary adjustments andleaving it in the field of view of the cameratraps. All systems were tested individually in the field as well as in combination. During the study period, itwas determined that resident bird species did not use this area for a long timesince the grass lengths in the area number 1 were longer than the other areas and there were no trees near the area where birds could perch.

The areas where other resident bird species, especially gray carrioncrow, silver gull and rock pigeon, are seen most intensively at the airport are the green pasture areas in the area called "1.1 critical area" on the west sideof the runway. According to the observations made, it was determined that the trees located in the KTU Campus and in the north direction (the area where Trabzonspor facilities are located) are an important factor in the intensive use of this area. On the leafy tree species in these areas, especially resident species such as gray carrion crows stay in groups of 100-200 and constantly move between both directions. These low-height displacements and transitions pose a threat to airplane landings and take-offs. In this area, it was observed that rock pigeons use building roofs rather than tree branches for accommodation.

After the devices were tested during the field studies, they were moved to the 1.1 critical area, which is the most intensively used by birds, shown as number 2 on the map on December 10, 2021. During the study, all systems were kept in area number 2 for 9 months and then moved to area number 3 in critical area 1.1 and followed here until the end of the study.

Gray carrion crow, silver gull and rock pigeon, which pose the highest risk in terms of flight safety for TIA throughout the year and are among the resident bird species for the area, did not react to the stationary golden jackal replica in any way, while they reacted briefly to the sound systems. This short-term response was not in the form of flying away from the area, but in the form of short-term low-altitude flights over the devices for 10 to 40 minutes. During these short-term flights, it was even observed that the birds made attack-like peaks towards the systems used for bird control. It was determined that the

birds, which became accustomed to the systems in a short period of time, landed on nearby areas covered with short grass, on the sides of the runway or on the runway.

Figure 3. *Responses of birds to sound systems in the study area after the first 10 minutes*

It was determined that all the systems used for bird control (golden jackal replica, Bird-K Imti 040 general animal repellent, BirdXPeller Pro bird repellent and a loudspeaker with a golden jackal sound) gradually got used tothe systems after 10 minutes from the time they first started to work, and if the grasses were short, they landed near the devices, if not, they landed in suitable areas (Figure 3).

The golden jackal replica left at the airport during the study and the solar-powered multifunctional animal repellent Bird-K İmti 040 device, which is stated in the user manual to be effective in an area of 400 m^2 thanks to the

ultrasonic signal and flash light it emits, were observed that the birds got used to it in a short time and even landed on them without any discomfort (Figure 4).

Figure 4. *Acclimatization of birds to the methods used in the study area*

CONCLUSION AND COMMENTS

BirdXPeller®PRO bird repellent that has sounds of predatory birds was used in a study conducted by the University of South Africa on a pigeon population. It was found that there was a correlation between the number of pigeons observed at different time periods and the sound of BirdXPeller Pro bird repellent to pigeons. During the audible bird repellent trial, pigeons continued to disperse as if they were in a natural predator environment, moving away from the location during dawn and dusk counts. In contrast, when the pigeons did not see the presence of predatory birds causing them to fly away from buildings, they returned to their original locations (Harris et al., 2016). In a similar study, BirdXPeller®PRO bird repellent was used to repel pigeons from buildings. The sounds were played randomly so thatthe pigeons would not get used to the sounds. To measure the effect of the sounds, counts were made before and 10, 20 and 30 minutes after the sound was played. In this study, although there was no significant difference between the number of pigeons before and 30 minutes after the sound was played, there were differences in the number of pigeons between 0-30 minutes due to behavioral changes. In addition, since the pigeons did not have a warning call among themselves, the bioacoustics used to deter the pigeons were found to be insufficient (Harris et al., 2020). In this study, which was conducted by testing similar devices at TIA, it was observed that especially resident bird species got used to the systems in a short time and the effect of the systems was almost negligible.

Bioacoustic method, which is seen as a traditional method in Turkiye, is one of the most widely used methods in bird control. The use of this method alone causes the birds not to react in the long term as they get used to it after a while (Stenman, 1990). At TIA, the current bioacoustic control method

caused by apron guards during bird control is ineffective due to the fact that the sounds are not predatory or frightening.

Birds may give different results when confronted with ultrasonic sounds (Harris et al., 2020). It was observed that ultrasonic sounds were ineffective in scaring away birds at TIA. Bird species that were expected to leavethe area perched near the ultrasonic sound emitting devices, flew over them and even landed on them.

As a result of study conducted at airports around the world, it has been reported that leaving grasses at long (30-60 cm) lengths is effective in reducing the populations of dangerous bird species (geese, starlings, gulls) at airports (Hupf and Floyd, 1995), and that species such as ring-billed gulls, snow buntings, swallows and rock pigeons are more attracted to short grassland (5-10 cm) than long grassland (15-20 cm) (Potter, 1996). It has been observed that starlings, northern harriers and pigeons do not prefer tall grassland because tall grassland makes it difficult to see predators, be alert, foraging and social contact (Brough and Bridgman, 1980; Deacon and Rochard, 2000). When adjusting the height of the grassland, leaving it 25 cm and above may cause an increase in rodent and insect species, which may attract other predatory species to the site (Barras and Seamans, 2002; Clearyand Dolbeer, 2005). In this study, similar data were obtained and it was determined that the number of bird species was less dense in areas where grass height was 20-35 cm.

With this study carried out at TIA, it is aimed to determine the most appropriate control methods that can be used in Turkiye and the world airports against bird damage and to minimize the problem to the lowest levels. Within the scope of the studies, it has been clearly seen that especially resident bird species get used to the expensive sound systems preferred to increase flight safety and minimize economic losses at airports in a short time. In fact, it has been determined that they have no effect not only on resident birds but even on migratory birds. For this purpose, according to the results of the study, the measures that can be taken against bird damage at TIA are listed below.

- Assigning qualified stuff trained in their field at airports

- It was observed that apron guards are used in the fight against bird damage at TIA. Apron guards approach the birds with a vehicle and intervene by sounding the siren or getting out of the vehicle and running. Thisboth reflects on the enterprises as invisible expenses in economic terms and reduces the efficiency of the stuff. In developed airports that are successfulin combating wild animal-related damages in the world, teams of wildlife experts who have received training on the subject are among the permanent staff of airports. For this reason, it should be ensured that graduates of the “Wildlife Ecology and Management” department, which provides undergraduate education in Turkiye, are employed at the airport.

• Establishment of Wildlife control units

- In the airports of developed countries, there are bird control units where expert teams work full-time. Unfortunately, there are not yet any airports in Turkiye that have such units working directly in this way. Wildlife control units should be established as soon as possible, not only at TIA but also at allairports, especially within the body of General Directorate of State Airports Authority.

- Wildlife control units to be established should be staffed by wildlife experts (graduates of the "Wildlife Ecology and Management" department atthe undergraduate level) who have been trained in their field, and it should be ensured that the struggle is carried out by teams trained in this field. In Turkiye, apron guards or fire brigade units working at the airport are used in the fight against wild animal damage. However, the knowledge and experience of these stuff on wildlife, especially birds, is not sufficient. For example, birds are the most problematic wildlife class at airports. Knowing all kinds of information about birds (ecology, biology, migration periods, behavior, etc.) by the control team is essential for determining and implementing a successful control method.

• Continuous bird observations in all seasons of the year

- A total of 120 bird species were detected directly at TIA (Sarı et al., 2022a; Sarı et al., 2022b; Sarı and Mengen, 2022). However, considering that 340 bird species have been identified in the Eastern Black Sea Region to date, it is likely that the number of bird species identified in this study will increase with longer and continuous studies to be carried out in and around TIA. Continuous bird observations at the airport by wildlife experts trained in the field will also increase the success of the struggle. For this reason, bird observations should be made at the airport every day of the year.

• Use of trained dogs

- Although many different methods are used against bird strikes not onlyat TIA but also at airports in many countries around the world, the desired result cannot be achieved. Airport operators allocate high amounts of resources for these control methods. Birds, on the other hand, get used to almost all control methods applied in a short time and make the struggle unsuccessful. Therefore, alternative methods are constantly sought. In countries that minimize the problem in the fight against bird damage at airports, biological control methods are now used in addition to physical control. The most important of these methods is the use of trained dogs and predatory birds. The use of predatory birds is more difficult due to both the training of the bird and the lack of training stuff. For this reason, the use of dogs is more preferred.

- It has been found that the use of trained dogs is highly effective

evenin bird species such as silver gulls, gray carrion crows, storks and herons, which have limited response to sound systems and human intervention (Sarı et al., 2022a). Many countries in the Americas and Europe have employed trained predatory birds and dogs with trainers as permanent staff at airports. With this study conducted at TIA, it has once again been seen that almost all methods used against bird damage are ineffective. Therefore, in order to prevent further economic losses and, most importantly, mutual loss of life, trained dogs should be assigned to the airports of Turkiye on a permanent basis together with their trainers. For an area the size of TIA, 2 trained dogs shouldbe used together with wildlife experts.

• Regulation of grass lengths

- For TIA, except for migration periods, weed lengths should be kept between 20-35 cm and all weeds in the field should be reduced to the shortest length just before the start of the migration season (end of August - end of February). Weed control should be carried out continuously during migration periods. Instead of the existing grass species at the airport, spiny species that can be a food source for bird species and have a reducing effect on insect numbers should be selected, taking into account soil characteristics (Sarı et al., 2022a; 2022b).

• Plastic asparagus application

- Plastic asparagus, which is economically inexpensive and easy to apply and will not have a negative impact on electronic devices important for flightsafety, should be placed immediately on all structures used by birds during their temporary stay at TIA (electronic devices, building roofs within the campus, all electricity poles, wooden fences, lighting devices, plastic pontoons and antennas).

• Leaving suitable structures for natural predatory birds

- During the field studies, the hawk, which is a natural predator of small bird species, was seen in the airport at all seasons of the year. It has been determined that the hawk species has taken shelter in many structures in the area and poses a natural threat to other birds in the airport. For this reason, when plastic asparagus is applied, it would be appropriate not to apply this application on some fences and poles so that predatory birds such as falcons can land. In determining these structures, the opinion of experts who know the area and the requirements of the species should be taken into consideration.

• Felling of leafy, fruit-bearing trees and shrubs

- There are many coniferous and leafy tree species used by resident birds for accommodation and nesting at TIA. During the study, birds were detectedindividually on the tops, branches and interiors of coniferous species.

However, in leafy species, bird groups exceeding 100 were identified. Especially gray carrion crows were constantly seen on the leafy species in the airport in groupsof 100-200. In addition, fruity species such as cherries, figs and plums in the airport attract many bird species. Therefore, before the start of the vegetationperiod, the airport authorities should contact the relevant public institution (General Directorate of Forestry, Trabzon Regional Directorate) to cut all of these species within the scope of combating bird damage.

- Covering drainage channels

- During the study, it was observed that the birds formed large groups around the open drainage channels and all kinds of water accumulation areas in the airport. For this reason, it is necessary to cover the open drainage channels in the airport and to identify and level the pothole areas that cause water accumulation.

Acknowledgements

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Chapter 4

FORMATION OF FISH DISEASES AND USAGE OF MICROBIOME ANALYSIS METHODS IN THE DIAGNOSIS OF THE DISEASE

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1. INTRODUCTION

Todays, white meat consumption has increased gradually due to the increase in population density, decreasing agricultural areas, economic conditions and food deficiencies (Delgado et al., 2003). Due to the lack of high quality protein and natural resources, which is important for physical and mental development, a rapid development has been achieved in fish farming (Muir et al., 1994). Naturally, high-intensity fish farming, decrease in water quality, inadequate diet, manipulations cause disease problems intensively (Tolgay and Kaymaz, 2001, Özden and Gökoğlu, 1996) The importance of developing standard practical applications for an effective fight against the disease with correct diagnosis, drug administration when necessary and preventive measure strategies in effective fight against diseases in regions with variable geographical features has been highlighted once again (Zdanowicz et al., 2020, Das & Horton, 2016).

There are about 100 trillion bacteria in the community of microorganisms is microbiota and all of the genetic material of the microbiota is microbiome. Molecular techniques developed in recent years in research with fish pathogens are relatively more sensitive, reliable and faster than traditional methods. Techniques such as Restriction Fragment Length Polymorphism (RFLP), Polymerized Chain Reaction (PCR), Pushed Field Gel Electrophoresis (PFGE), and Multiplex PCR provide fast and reliable information on the detection and spread of fish pathogens (Karaalioğlu et al., 2019).

By preventing epidemics in aquaculture, the formation of antibiotic-resistant bacteria can be prevented and treatment expenditures can be reduced. Therefore, microbiome analyzes used instead of agent isolation and identification in fish are very important.

2. GENERAL INFORMATIONS ABOUT FISHES

2.1. Characteristics of Fishes

They are primitive vertebrates that are adapted to living in water, breathe with their gills, have mostly skin covered with scales, swim with fins and are poiclotherm. They are spread over large areas. They vary widely in number of species, size, external morphology and internal morphology, physiology and behavior. The oldest fish fossils ever found are 500 million years old. Today's fish are divided into cartilaginous fish (Chondrichthyes) and bony fish (Osteichthyes) (Alpbaz and Hoşsucu, 1979).

2.2.Body Structure of Fishes

The body of the fish is in accordance with the habitat and living conditions. Fast-swimming open sea fish are usually missile-shaped, with circular or elliptical cross-sections. Members of the Scombridae family

(mackerels) can be an example of this variety. Lateral flattened body shape is characteristic for stagnant water habitat with dense vegetation. Lepomis and Tilapia living in lakes and ponds are examples of this variety. More lateral flattening is seen in pelagic fish forming flocks as it is suitable for making short and quick turns. Members of the family Clupeidae (sardines) are an example of this variety. The bodies of demersal (bottom-dwelling) fish are very flattened. Members of the order Pleuronectiformes are examples of this variety. The elongated body shape is suitable for curling in and out of the soft ground, for crawling among the reeds, and for slowly getting into cavities and crevices. Anguilliformes members are also examples of this variety (Alpbaz and Hoşsucu, 1979, Alpbaz and Hoşsucu, 1980) (Figure 1).

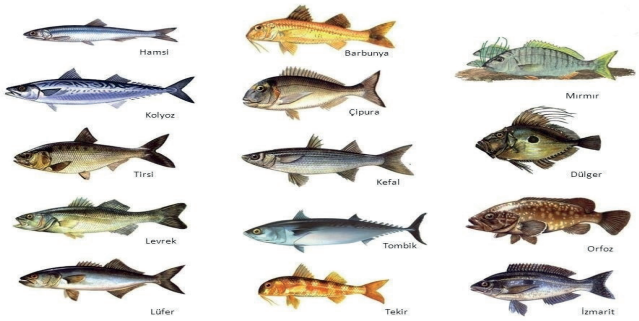

Figure 1. Body structure in fish

2.3. Skins of Fish and Their Characteristics

The skin is an important organ system that performs many different functions as an outer covering. The most obvious and vital importance; It can be expressed as protecting the body against various external factors, mechanical damage, various destructive substances and external parasites, and regulating the temperature (Fig. 2).

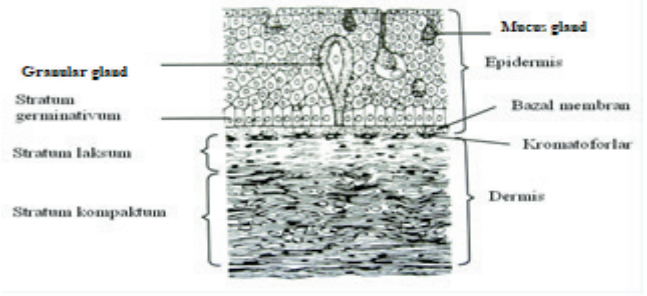

Figure 2. Skin structure in fish

As long as the skin is not injured, parasites and microbes cannot enter and thus protect the body. In fish, the skin consists of two layers: Outer skin (Epidermis) and Inner skin (Dermis) (Alpbaz and Hoşsucu, 1979, Alpbaz and Hoşsucu, 1980).yaralanmadığı sürece, parazit ve mikroplar giremez ve dolayısıyla vücudu korur. Balıklarda deri Dış deri (Epidermis) ve İç deri (Dermis) olmak üzere başlıca iki tabakadan meydana gelmiştir (Alpbaz and Hoşsucu, 1979, Alpbaz and Hoşsucu, 1980).

2.4. Mucus

Mucus consists of glycoproteins that can absorb large amounts of water. It gives the fish its characteristic slipperiness and smell. Smoothing the body surface reduces the friction between the ambient water and the body surface during swimming. It makes it difficult for water to enter the skin with osmosis. By removing the mucus, harmful microorganisms and irritants accumulated on the fish are also removed. Fish without scales and small scales generally produce more mucus than large scales (Alpbaz and Hoşsucu, 1979, Alpbaz and Hoşsucu, 1980).

2.5. Scales in Fishes

The scales originating from the dermis layer of the skin partially or completely cover the body in fish (Figure 3).

Figure 3. Scales in fish

Fishes, such as catfish (Silurisglanis) and blackfish (Clariaslazera), don't have scales on the skin. Since the scales are arranged from front to back, they make it easier for the fish to swim forward. In osteichties, the scales are composed of two layers. The part outside the skin is called the posterior region, and the part inside the skin is called the anterior region (Bostancı, 2005).

2.6. Linea Lateralis (lateral line) in Fish

In most fish, sensory cells are arranged on both sides of the body, with numerous microscopic holes (pores) extending in a single row from head to tail. All of these structures are called lateral lines (Figure 4).

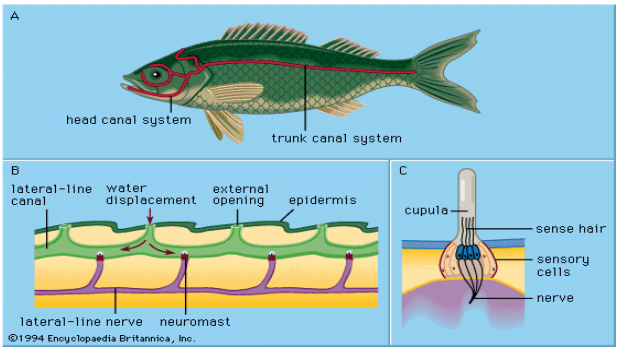

Figure 4. Linea lateralis in fish

Fishes, receive warnings of pressure changes in the external environment, determine their place in the herd, protect themselves by detecting the topographic features of their environment with linea lateralis (Alpbaz and Hoşsucu, 1979, Alpbaz and Hoşsucu, 1980).

2.7.Fins in Fishes

Fins generally allow fish to swim, balance and orient themselves to the environment (Figure 5).

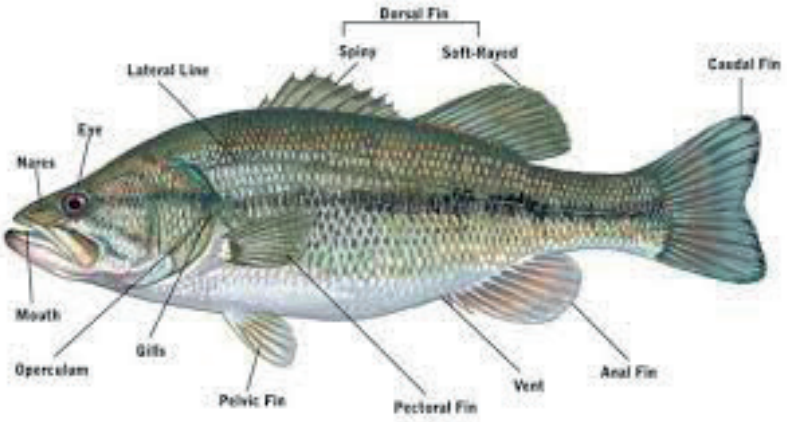

Figure 5. *Fins in Fishes*

There are two types of fins;

Single fins are Dorsal, Anal and Caudal fins. The double fins are the pectoral and ventral (pelvic) fins (Alpbaz and Hoşsucu, 1979, s. 19-29, Alpbaz and Hoşsucu, 1980).

3. FISH DISEASES

3.1. Mechanism of Disease Occurrence in Fishes

Many factors must be present in order for diseases to occur in fish. Diseases take their origin from internal or external factors. Internal factors include metabolic endocrine disorders, degeneration of organs and tumors. External factors causing disease (bacteria, viruses, fungi, parasites) and predisposing factors (physical and chemical, hitting, cold-heat, poisoning events, vitamin and mineral deficiency). The deterioration of one or more of the environmental factors causes stress in fish and initiates alarm reactions in the body. In this case, some biochemical and hormonal changes occur in the fish to adapt to the new conditions and the fish tries to adapt to the environment. Those who cannot adapt die, those who adapt are stressed. The blood cortisol level rises in fish under stress, and because of its immunosuppressive effect, the fish, whose general defense system is suppressed, cannot fight the agents and gets sick. Negative environmental conditions create stress in fish, causing the resistance of the host's defense mechanism to be broken and the disease to emerge. The virulence and amount of the agent is an important factor in the onset of the disease. The water in which fish live contains almost all kinds of microorganisms. Along with stress, opportunistic pathogens also cause disease formation (Erer, 1995).

3.1.1. Stress Factors in Fishes

Factors that cause stress in fish can be listed as follows. Changes in oxygen, pH, ammonia in the water, unsuitable water temperature and sudden changes in temperature, poor maintenance conditions and feeding, overstocking of fish, forcing fish in unsuitable conditions for movement, deterioration of feed, rancidity, mold, lack of hygiene, transportation or handling of fish Damage to fish during fishery, frequent disturbance of fish, frequent use of chemotherapeutic agents, supply of high flow water to pools, pathogenic microorganisms, toxic plankton, dense plankton, macroorganisms, ecdo-endo microorganisms (Erer, 1995).

3.1.2. Symptoms of Diseases in Fishes

Diseases occur as a result of the deterioration of the normal physiological structure of fish due to the deterioration of aquatic environment conditions and exposure to stress.

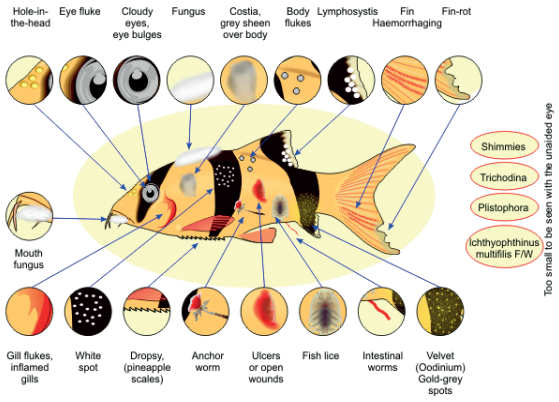

Figure 6. *Symptoms of Diseases in Fishes*

Diseases cause various symptoms in fish depending on the type and characteristics of the agent (Figure 6). Except for a few diseases, it is not possible to diagnose the diseases that occur in fish by looking at these symptoms. Exophthalmos (protrusion of the eye), ulcers just behind the head and on the body surface, saddle-like lesions including the dorsal fin, ulcers and bleeding in the mouth, abscesses and furuncles on the body, bruises in the dorsal and caudal fin, prolabsus in the anus (protrusion of the anus), and bleeding, abdominal swelling due to ascites, hemorrhages in the pectoral fin bases, change in color of the gill slits and ulcers (wounds with tissue loss) are common symptoms (Erer, 1995).

3.1.3. Prevention and Treatment of Diseases in Fishes

Disease prevention is essential in cultured fish. Careful care, development of disease-resistant species, adequate feeding and the addition of supplementary foods (vitamins, etc.), the use of vaccines, the use of chemotherapeutic substances (antibiotics and disinfectants), the prevention of the movement of factors that cause infection provide protection from diseases. For the prevention and treatment of diseases seen in fish, increasing water quality, removing pollutants, biological control, removing toxic substances that cause acute toxicity and chronic toxicity are important for fish in terms of the environment in which they live (Arda et al., 2005).

3.2. Bacterial Diseases in Fishes

Bacteria that cause bacterial diseases in fish are examined in two groups. These are Gr (+) and Gr (-) bacteria (Austin and Austin, 2007).

Gr (+) bacteria are examined in two groups.

Anaerobic Pathogens: Clostridium botulinum and Eubacterium tarantellus cause infections in fish.

Aerobic Pathogens: Streptococci causing lesions in the body, ***Renibacterium salmoninarum*** causing bacterial kidney disease, ***Staphylococcus epidermidis*** causing various lesions, **Nocardia** species causing Nocardiosis, **Mycobacterium** species causing Mycobacteriosis and ***Lactococcus garvieae*** causing Lactococcosis infections (Hielm et al., 1998).

Gr (-) Bacteria

Yersiniosis (Red Mouth Disease) is caused by ***Yersinia ruckeri***,

Furunculosis is caused by ***Aeromonas salmonicida***,

Motil aeromonas disease agents ***A. hydrophila, A. caviae and A. sobria***,

Myxobacteria infections agents; ***Flexibacter columnaris*** (Columnaris infection agent) ***Flexibacter psychrophila***, ***Flexibacter maritimus*** (Marine Flexibacteriosis agent),

Pseudomonas infections agents ***Pseudomonas anguilliseptica, P. chlororaphis*** and ***P. fluorescens***,

Vibriosis infections agents **Vibrio** species,

Pasteurellosis infection is caused by ***Photobacterium damselae subps. piscicida (Pasteurella piscicida***),

Edwardsiellosis agents ***Edwardsiella tarda*** and ***Edwardsiella ictaluri*** (Balta, 2020, Martin et al., 2005).

3.3. Viral Diseases in Fishes

Studies on viral infections in fish started with the definition of diseases as in human and veterinary medicine. With the development of fish tissue cultures in the 1950s, it was revealed that viral disease agents causing disease in fish could be diagnosed. In 1958, KenWolf was the first to isolate Infectious Pancreatic Necrosis Virus (IPNV). In 1960, Ross et al. reported a filter-passing agent as the cause of an epizootic seen in Chinook salmon at the Fishand Wildlife Service's Seattle laboratory. Two years after these studies, Ghittino mentioned 7 viral diseases in a booklet he published in Italy and mentioned the epizooty seen in the newly reported Chinook salmon. This infectious agent was later determined to be a Rhabdovirus and the infection was named Sockeye Salmon Virus disease. In the 1980s, the disease agent was named Infectious Hematopoietic Necrosis Virus (IHNV). In 1963, Jensen isolated Egved virus (Viral Hemorrhagic Septicemia Virus) in Denmark. It is reported that about 70 of the disease agents seen in fish today have viral etiology (Jensen, 1963, Jensen, 1965).

Infections with viral etiology seen in fish are examined in three groups;

Infections caused by isolated viruses.

Infections that could not be isolated but have virus particles seen in the electron microscope.

Those suspected of viral etiology due to the presence of virus-like particles in the infection (Alvarez-Pellitero et al., 2004, Castric, 1997).

4. MICROBIOME ANALYSIS METHODS

4.1. Polymerase Chain Reaction

PCR is based in-vitro amplification of nucleic acids under favorable conditions. This technique can produce millions or even billions of copies of the DNA molecule in a short time (Figure 7).

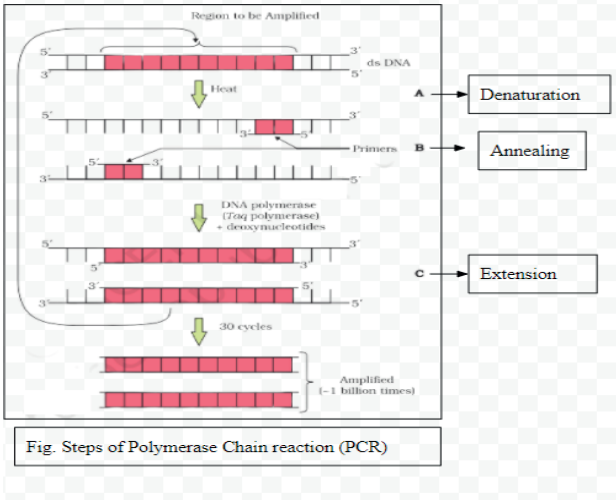

Figure 7. *PCR steps*

Denaturation: In the first stage, the dual helix structured of the DNA molecule is divided from each other with the help of more heat. It is mostly applied between 94°C - 97°C for 15-60 seconds. (Initial denaturation is applied as a single cycle up to 15 minutes)

Annealing: At lower temperatures following denaturation, oligonucleotide primers connect to their corresponding regions on the divided single-stranded DNA. It takes 30-60 seconds between 47°C and 60°C. (In areas with high G/C ratio, bonding temperature increased up to 68°C.

Elongation: Finally, the temperature increased to 72°C, the enzyme of DNA polymerase extends the complementary DNA. The extension time ranges from 30 seconds to 3 minutes according as the type of used polymerase and the length of amplified DNA (Kapley et al., 2000, pp. 1913-1918).

4.2. Sequence Analysis (Microbiome analysis)

Sequence analysis of a 74 nucleotide The tRNA molecule was invented in 1965 by Robert HOLLEY. In 1977, two different methods of DNA sequencing were discovered by Allan MAXAM-Walter GILBERT and Frederick SANGER. In 1982, Akiyoshi WADA suggested automatic DNA sequence analysis and robots began to be developed. In 1986, a fully automatic machine to be used in DNA sequence analysis was found by Leroy HOOD and Lloyd SMİTH from the California Institute of Technology (Caltech).

Two different methods are used in DNA Sequence Analysis today. These two methods;

Chemical cracking method of Maxam and Gilbert (Maxam et al., 1977).

Sanger-Coulson's chain termination method. (Sanger et al., 1977).

Of these two methods, Sanger - Coulson's method is more widely used today (Klug and Cummings, 2000, p. 745). The Sanger method is settled on enzymatic DNA synthesis. It is known as the most known DNA sequence analysis technique today. This method uses the DNA sequence to be sequenced as a template for newly synthesized strand. Klenov, Taq DNA polymerase, reverse transcriptase or sequenase enzymes can be used for DNA synthesis. The method is based on the use of Deoxy Ribonucleoside Triphosphates by DNA polymerase and Dideoxy Ribonucleoside Triphosphates without OH groups at the 3' position of deoxyribose as substrates. (Klug and Cummings, 2000). The 16S rRNA gene in bacteria is identified as a common target in molecular applications. Small subunit 16S ribosomal RNA gene sequence is used in the identification and classification of prokaryotes, and sequencing of these 16S ribosomal RNA genes directly amplified from the medium is used to predict microbial diversity (Rajendhran and Gunasekaran, 2011).

4.3. Next Generation Sequencing

New generation DNA sequencing systems can perform ultra-fast sequencing with high accuracy. The resulting microbial genome sequence using this method can provide rich and unique information that can't be obtained by any other empirical method. Such as, it can complete the 4.6 Mb E. coli genome in a single read. In one study, the genome of E. coli was sequenced de novo with 400,000 reads per run, and it was determined that the sequencing was done with an accuracy of 99.997% to 99.999% (Margulies et al., 2005).

4.4. Next Generation Sequencing Application Areas

With the new generation DNA sequencing technology, the genomes of different organisms such as bacteria, viruses, yeasts, plants, fungi and

Bacterial Artificial Chromosomes (BACs) can be sequenced ultra-fast and with high accuracy. In the de novo sequencing method, there are shotgun, De novo sequencing and double-ended sequencing methods. The Whole Genome Sequencing System is made by sequencing small DNA fragments and adapter sequences in picoliter volumes with large parallel sequencing (Margulies et al., 2005). One of the uses of next-generation sequencing technology is metagenomics. Metagenomics is the sequencing of 16s rRNA with the shotgun method to determine the microbiological diversity in a complex material. Environmental microbiological diversity can be revealed by random sequencing of all microbiological genomes (microbiome) in any environmental sample. With these studies, it gives information about the amount of microbes in the environment, as well as unknown or little-known bacteria that only reproduce in natural environments and cannot grow in culture. Usage areas: Fisheries research, Forest Research, Environmental Engineering, Dentistry, Basic Medicine and Clinical Research. With next-generation sequencing systems, it is possible to conduct extensive analysis of DNA obtained from fossils, frozen or preserved specimens such as hair and bone. As a result of sequencing, ancestral target sequences can be distinguished from environmental contamination. In a study, the ancestral DNA of a 38,000-year-old Neanderthal fossil was sequenced using next-generation sequencing and compared with modern-day chimpanzee and human DNA. It was determined that the Neanderthal DNA sequence diverged from the modern human DNA sequence 500,000 years ago. (Wheeler et al., 2008).

5. Studies with The Microbiome

5.1. Microbiome Studies

Although the metagenomic approach is used more frequently in areas where the results are of economic value such as sea water, wastewater, mineral deposits, it is also used to define infections of unknown origin in living things. As an example to this; Cox-Foster et al. revealed in 2007 that a virus called IAPV is at the root of the problem threatening the beekeeping industry with the mass death of millions of honey bees in the USA (Cox-Foster et al., 2007).

With the development of shotgun sequencing techniques, the Metagenomic approach has been used more and more to reveal the relationships between bacteria and humans. The first of these is the study of Turnbaugh et al., which revealed the relationship between bacteria in the stomach flora and obesity (Turnbaugh et al., 2006,). The spread of the H5N1 subtype, the pathogenic A virüs of avian influenza (HPAIV), requires a fast and reliable method for full-length sequencing analysis. A simple sensitive method was designed for preparation of sequence libraries from HPAIV diagnostic of RNA samples for sequencing with Genome Sequencer FLX instrument. Presented method integrates highthroughput presequencing with the Roche/454 into diagnostics

without the need standard PCR, Genome Sequencer FLX samples preparation and sequencing additional equipment (Höper et al., 2009).

All of the genome sequence of major food and bioenergy crop is critical in improving crop production to meet world's food and energy security need. Although next-generation sequence technologies have made major improvements in sequence output and cost, resequencing of the highly repetitive genomes found in most crops is not yet possible. A strategy is presented that combines state-of-the-art next-generation sequencing with legacy genomic resources, allowing for fast and cost-effective sequence of plant genomes (Rounsley et al., 2009).

Problems in honeybee health have not only affected the beekeeping industry but also created new risks for agricultural products due to pollination. Honeybee diseases can result from parasites and increased pressures of pathogens. Incidence of the microsporid pathogen Nosema ceranae has increase significantly in the last decades. A draft review of the N. ceranae genome is derived from prosequence data, with first models of gene and genomic checks with other members of the highly derive fungal lineage, is presented. N. ceranae has strong AT biased (74%) genome and few repetitive elements. It is predicted to have homologs in Encephalitozoon cuniculi, the most closely related genome sequence published. The shrunken N. ceranae proteome consists of new and replaceable essential proteins. Most supported gene models can have a conserved sense helix motif 15 bases upstream of starting codon. A previously undescribed version of motif is found in E. cuniculi. These comparisons guide research into Nosema - Honeybee interactions by providing insight into the organization and evolution of microsporid genomes (Cornman et al., 2009).

5.2. Studies in Fish with Microbiome

Asian silver carp's gut microbiota (SVCP) and native shad (GZSD) in the Mississippi river was compared by 16S rRNAgene pyro-sequencing. Analysis of filtered sequences (more than 440,000) from the anterior and posterior guts of SVCP and GZSD revealed the high microbial diversity of these samples. Differential microbial community was revealed between foregut and hindgut for individual SVCP and GZSD. Proteobacteria, Cyanobacteria, Actinobacteria and Bacteroidetes were found to be the dominant phyla regardless of fish - fish gut type (Ye et al., 2014).

The gastrointestinal (GI) microbiota of vertebrates is essential for development, nutrition, immunity and resistance to invading pathogens. Research on the mammalian (GI) microbiota provides a great deal of information about the microbiota and its relationship to disease. In addition, information on the fish gut microbiota is limited. The information obtained about the functions of zebrafish gut microbiota is discussed. It is thought

that understanding fish's intestinal microbiota will guide development of necessary prebiotics, probiotics and probiotic effector to improve fish health (Wang et al., 2018).

The microbiota in the fish gut contribute to digestion. It can affect fish growth, nutrition, reproduction, population dynamics and defense against disease. This microbial structure is very important in aquaculture applications. Recent advance in DNA sequence technologies and bioinformatics analysis are enabling us to gain understand of the complex microbial community, including the fish's gut microbiota. Recent advances have increased our knowledge of the bacterial community profile in the gut microbiota of various factors that affect fish: temperature, growth stage, salinity, digestive physiology and nutrition. The aim is to emphasize the potential of next generation sequence platforms in analyzing fish gut microbiota. Hopeful results in this area are presented with focus on new research aspects of fish gut microbial structure (Mahdi et al., 2015).

Recent technological advances in Next Generation Sequencing (NGS) have revolutionize the field of genomics, influencing viral research. In the study of aquatic (especially those that infect fish) viruses, technologies that provide larger amounts of molecular information in a shorter time, at a lower cost (NGS) have been greatly utilized. In applications in aquatic virology, the use of existing high-throughput sequencing platforms has been examined and specifically focused on challenges (related to sample preparation and bioinformatics) for:

(i) could it be associated with fish deaths,

(ii) can elucidate the mechanisms of pathogenesis

(iii) can examine the molecular epidemiology of these pathogens (Nkili-Meyong et al., 2016).

Species identification in food validation studies was sequences of mitochondrial genes isolated from food were developed by comparing them with NCBI - Barcode of Life Database (BOLD) data. These methods generally use the Sanger methodology for sequencing. The first research to recommend using next-generation sequencing (NGS) to identify fishes for food consumption appeare in 2012. Recently, several platform used NGS have demonstrated their capacity to identify 15 or more different fish species and processed fish product at a single high level (Carlos et al., 2021).

Meat adulteration has becomes a global problem that directly affect food consumer and producers. A tool to verify meat types is necessary to ensure the safety of foods products. Next Generation Sequence (NGS) coupled with the ribosomal RNA and mitochondrial DNA genes can used to analyze the mix in multiple meat samples. Therefore, studies aimed at combining NGS

with the rRNA gene have been conducted to identify the 4 meat species (beef, fish, chicken, and pork). Three primer set (12S-Ki, 16S-KH and 16S-Ki) were used to amplify DNA, from the four meat species in one study. 16SKH showed better detection effect in all species compared to other species. The library construct of all PCR amplicons was prepared and sequenced by NGS. NGS combined with 16SKH was then evaluated for susceptibility testing. Results from libraries produced from DNA from four meat species amplified separately for 3 different studies were compared. The number and rate of matched reads was consistent across the three different studies. Percentage of matched readings;

14.05% to 31.04% in study 1,

15.14% to 31.98% in study 2,

14.21% to 33.05% in study 3

This indicates that NGS combined with rRNA and mtDNA gene can be applied as a reliable test. This technique can applied to control and monitoring meat adulteration in halal meats production and industry (Mahama et al., 2022). Fish scale drop diseases virus (SDDV) is viral pathogen spread in farmed barramundi fish (Lates calcarifer (Bloch, 1790)) in Southeast Asia. Genomic information on SDDV is scarce. In the study, the microbiomes of brain tissues was investigate using Illumına HiSeq DNA sequence. The taxonomic analys show that SDDV was the main pathogens in the affected Barramundi fish. Comparison between the SDDV genome named 131 kb long and 135 ORF isolate TH2019 and the Singapore SDDV references genome revealed 99.97% of the nucleotide identities within the aligned regions. The genomes of SDDV TH2019 contained a unique 7,695 bp long genomic region containing six putative protein coding genes. This studies demonstrated the SDDV genome, despite its limited scope, can be directly sequenced using metagenomics analysis of an infected Barramundi fish sample (Kayansamruaj et al., 2020).

The gut microbiota of the rohu fish (Labeo rohita (Hamilton, 1822)) was investigate by shotgun metagenomic to understand their taxonomic compositions and functional abilities. The fish gut microbiota is quite diverses in nature. At the phylum levels, more than three-quarters of gut microbes belong to Proteobacteria. The very low prevalence of common probiotics (Streptococcus, Lactobacillus, Bacillus and Lactococcus) in fish guts has suggested the need to explore alternative probiotics in aquaculture. The most dominant (51%) biosynthesis pathways are (39%) degradation, (4%) energy metabolism and (2%) fermentation. In accordance with the herbivorous diet of L. rohita, the gut microbiome can degrade hemicellulose, cellulose, chitin, starch, pectin and other complex carbohydrates. Due to the high prevalence of Actinobacterias and antibiotic biosynthesis pathway in the fish gut microbiomes, new natural antibiotics have potential for biodiscovery.

51 different antibiotic resistance genes (ARGs) that can provide resistance to 24 antibiotic species have been identified in the fish gut. Some of ARGs for multidrug resistance were found to be located in plasmid-derived sequences. The presence of the pathogenic bacteria and ARG in plasmid arrays suggested the potential risks from horizontal genes transfer in the intestinal environment (Tyagi et al., 2019).

Vertebrates' gut microbiome play an integral role in hosts health by stimulating immune systems development, aiding nutrient uptake, and outrunning opportunistic pathogens. Next generation sequencing technologies have enabled researchers to explore complex microbiome communities with the microbiomes at minimal cost, resulting in an increase in studies investigating the bacterial diversity of fish. Most of these studies are on the microbial structure of economically important aquaculture species whose microbes are used to increase feeds efficiency and reduce susceptibility to diseases. Identifying key microbiome functions by resolving host-microbe symbiosis is essential for us to be able to use a healthy microbiomes to our advantages in fish culture, while also gaining an understand of bacterial role in vertebrate health. It aim to summarized the available knowledges about fish gastrointestinal community from metagenomic, including factors influencing community structure, the gut microbiologies of important aquaculture species, and the descriptions of the teleostean microbiomes (Tarnecki et al., 2017).

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Chapter 5

USE OF MEDICAL AND AROMATIC PLANTS IN FISH HEALTH

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INTRODUCTION

Cultivated organisms, including various marine and freshwater fish and crustacean species, form a significant industry with their increasing production each year (Maqsood et al., 2011). Aquaculture aims to efficiently bring the produced species to the market as quickly as and at a minimal cost. Research has been revealed the effects of numerous additives added to feeds on growth and feed utilization rates. These additives include growth-promoting and therapeutic, antibiotics, hormones that enhance metabolic efficiency, enzymes that facilitate the optimal utilization of essential nutrients, and immunostimulatory products. These additives play a crucial role in improving growth and feed efficiency by enhancing cultivated organisms' metabolism and immune response. The research in this field aims to optimize the use of these additives to achieve cost-effective and sustainable production (Turan et al., 2012; Cristea et al., 2012).

Due to the development of resistance in fish over time, the residues left in their meat, and the potential negative effects on consumers, the use of these substances has been restricted in our country. European Union banned the use of antibiotics in animal feeds in 2006. The rationale for this ban was based on the fact that 90% of antibiotics were used in human disease treatment, the excessive amounts of antibiotics used in feeds, the development of bacterial resistance, and the residues left in the consumed products, which could lead to allegic and carcinogenic reactions in humans. Antibiotic use has also been banned in our country following a similar trend. The prohibition of antibiotic use has led to an increase in previously controlled infections, a decline in growth performance, and a subsequent rise in production costs, prompting producers to consider alternative practices (Turan et al., 2012). Recently, there has been an increase in utilizing various medicinal plants, powdered forms, extracts, and essential oils, which offer numerous benefits (Yiğitarslan et al., 2011; Turan et al., 2012). The deficiency of existing studies, the absence of specific drugs for aquatic organisms in the market, the high cost of available drugs, and the potential disruption of ecological balance, and the reliance on imports highlight the importance of this issue (Yiğitarslan et al., 2011). Aromatic plants are widely used in various fields due to the presence of active chemical compounds in their seeds, fruits, leaves, or roots, which exhibit different modes of action (Turan et al., 2012). Many plants, their extracts, and essential oils possess antimicrobial, antioxidant, anti-stress, immunostimulant, therapeutic effects (Turan et al., 2012).

1. Antimicrobial Effects

Microorganisms such as bacteria, fungi, viruses, and protozoa act as agents for many infectious diseases, and effective compounds against these microorganisms are referred to as antimicrobial agents. These compounds

include disinfectants, antibiotics, vaccines, and chemotherapeutics. False use of chemotherapeutics can lead to mortality and other harmful side effects (Zheng et al., 2009; Harikrishnan et al., 2010). Although the effects vary according to the active ingredients, many studies have been investigated on the *in vivo* antimicrobial effects of medicinal plants in aquaculture (Table 1).

Table 1: *In vivo Studies on Antimicrobial Activity of Medicinal and Aromatic Plants*

Fish species	Pathogen	Medicinal plant	References
Oreochromis sp.	*Streptococcus iniae*	*Rosemarinus officinalis*	Abutbul et al. (2004)
Oreochromis niloticus	*Trichodina* sp.	*Allium sativum, Terminalia catappa*	Chitmanat et al. (2005)
Oreochromis mossambicus	*Aeromonas hydrophila*	*Eclipta alba*	Christybapita et al. (2007)
Oreochromis niloticus	*Aeromonas hydrophila*	*Astragalus membranaceus*, *Lonicera japonica*	Ardo et al. (2008)
Ictalurus punctatus	*Aeromonas hydrophila*	Thymol, carvacrol, Carvacrol+thymol, *Origanum heracleoticum* L.	Zheng et al. (2009)
Cyprinus carpio	*Aeromonas hydrophila*	*Astragalus radix*, *Ganoderma lucidum*	Yin et al. (2009)
Carassius auratus	*Aeromonas hydrophila*	*Azadirachta indica, Ocimum sanctum, Curcuma longa*	Harikrishnan et al. (2009)
Oreochromis mossambicus	*Aeromonas hydrophila*	*Tinospora cordifolia*	Alexander et al. (2010)
Carassius auratus	*Aeromonas hydrophila*	*Phyllanthus niruri, Aloe vera*	Ahilan et al. (2010)
Cyprinus carpio	*Aeromonas hydrophila*	*Zataria multiflora*	Soltani et al. (2010)
Cyprinus carpio	*Aeromonas hydrophila*	*Aloe vera*	Alishahi et al. (2010)
Orechromis sp.	*Streptococcus agalactiae*	*Rosemarinus officinalis*	Zilberg et al. (2010:361)
Cyprinus carpio	*Aeromonas hydrophila*	*Ocimum sanctum*	Pavaraj et al. (2011)
Oncorhynchus mykiss	*Aeromonas hydrophila*	*Allium sativum*	Nya & Austin (2011)
Oreochromis mossambicus	*Streptococcus iniae*	*Cuminum cyminum*	Yilmaz et al. (2012)

Oncorhyncus mykiss	*Aeromonas salmonicida*	*Oreganum vulgare* sp. *hirtum*, *Thymbra spicata*, *Satureja thymbra*	Okmen et al. (2012)
Poecilia latipinna	*Ichthyophtirius multifilus*	*Allium sativum, Matricaria chamomilla*	Sahandi et al. (2012)
Cirrhinus mrigala	*Pseudomonas aeruginosa*	*Nelumbo nucifera*	Sivagurunathan et al. (2012)
Oncorhynchus mykiss	*Yersinia ruckeri*	*Mentha piperita*	Adel et al. (2016)
Oncorhynchus mykiss	*Vibrio anguillarum*	*Artemisia vulgaris* L	Diler et al. (2018a)
Oncorhynchus mykiss	*Spironucleus salmonis*	*Artemisia campestris* L	Diler et al. (2018b)
Oncorhynchus mykiss	*Vibrio anguillarum*	*Rhus coriaria* L.	Diler et al. (2021)
Oreochromis niloticus	*Streptococcus agalactiae*	*Aegle marmelos*	Wangkahart et al. (2022)

2. Antioxidant and Anti-stress Effects

In intensive culture systems, fish are constantly exposed to stress-inducing factors (such as manual handling, stock density, therapeutic applications, poor water quality, and water temperature), and these factors cause significant changes at the biochemical and physiological levels in the organisms (Harikrishnan et al., 2010; Cristea et al., 2012).

Antioxidants have significant importance in terms of nutrition, particularly in recent years, due to their ability to reduce physiological stress in organs and cells. Resistance to diseases and immunological capability in animals and humans is associated with antioxidant mechanisms. Oxidation products include hydrocarbons, aldehydes, ketones, alcohols, organic acids, and peroxides, which are the initial products. These products negatively affect the nutritional value, sensory properties, and shelf life of animal products. Furthermore, the use of plant-based products as antioxidants has gained momentum as an alternative due to their potential to be expensive and leave residues. Plants have antioxidant effects that support organisms' coping with oxidative stress caused by free radicals, thereby improving the overall physiological condition of fish (Chakraborty & Hancz, 2011).

In organisms, chemical processes, particularly oxidation, lead to the generation of free radicals. These highly reactive species can readily interact with various molecules, causing cellular and organismal damage. Conversely, antioxidants interact with these free radicals by forming chemical bonds, thereby preventing their harmful effects on cells. Numerous substances with antioxidant properties have been identified, some of which are obtained from dietary sources, particularly plants, while others are naturally produced by

the body as a defense mechanism against free radicals. Catalase, glutathione peroxidase, and superoxide dismutase (SOD) are examples of enzymes produced by the body against free radicals (Dimitrios, 2006).

The formation of free radicals in biological systems is predominantly attributed to oxygen. Oxygen undergoes reduction to form the superoxide group (O_2) through the action of certain iron-sulfur-containing oxidation-reduction enzymes and flavoproteins. The highly reactive superoxide group, known for causing cellular damage, is converted to hydrogen peroxide (H_2O_2) and oxygen by the copper-containing enzyme superoxide dismutase (SOD). Hydrogen peroxide, being less potent than the superoxide group, is neutralized and transformed into less harmful byproducts such as water and oxygen by enzymes like catalase, peroxidase, and glutathione peroxidase (GPx) present in tissues. This cellular antioxidant defense mechanism involving H_2O_2 plays a critical role in protecting cells (Zheng et al., 2009).

Studies on the effects of medicinal and aromatic plants on stress response and antioxidant activity in fish are summarized in Table 2.

Table 2: *Studies on The Effects of Medicinal and Aromatic Plants on Stress and Antioxidant Activity in Fish*

Species	Medicinal plant	Dose and duration	Influence	References
Paralichthys olivaceus	*Massa medicata fermentata*, *Crataegi fructus*, *Artemisia capillaries*, *Cnidium officinale*	0.1, 0.3, 0.5, 1.0% 8 weeks	Hemoglobin and hematocrit levels-, plasma total cholesterol level-, alanine aminotransferase activity-	Ji et al. (2007)
Cyprinus carpio	Anthraquinone extract from rhubarb *Rheum officinale* Bail	0.5, 1.0, 2.0, 4.0% 10 weeks	Cortisol↓, glucose↓, MDA↓, SOD↑, CAT↑	Xie et al. (2008)
Oreochromis niloticus	*Allium sativum*	40 g kg^{-1}, 150 mg kg^{-1}, 32 g kg^{-1} 3 months	Gper↑, SOD↑, CAT↑, MDA↓	Metwally (2009)
Ictalurus punctatus	Thymol, carvacrol, Carvacrol+thymol, *Origanum heracleoticum* L.	0.05% 8 weeks	SOD↑, CAT↑	Zheng et al. (2009)
Oncorhynchus mykiss	Carvacrol Thymol	6, 12 g kg^{-1} 8 weeks	GR↑, GSTs↑, MDA↓, CAT↑	Giannenas et al. (2012)
Megalobrama amblycephala	*Rheum officinale*	0.1% 8 weeks	Serum cortisol content↓, SOD↓, MDA↓	Liu et al. (2012)
Oncorhynchus mykiss	*Origanum onites* L.	0.125, 1.5, 2.5, 3.0 mL kg^{-1} 90 days	SOD↓, CAT-	Diler et al. (2017a)

Oncorhynchus mykiss	*Rhus coriaria* L.	0, 1, 3, 5, 10 g kg^{-1} 60 days	SOD↑, CAT-, MDA-	Diler et al. (2021)
Oncorhynchus mykiss	Sage (*Salvia officinalis*), myrtle (*Myrtus communis*), a probiotic mixture	1% 60 days	SOD↑, CAT-, MDA↑	Özil et al. (2023)

(↑): significantly increases; (↓): significantly decreased; (-): no significant change

SOD: superoxide dismutase; NOS: nitric oxide synthetase; CAT: catalase; Gper: glutathione peroxidase; MDA: malonyldialdehyde; GR: glutathione reductase; GSTs: glutathione-S-transferase

3. Immunostimulant Effects

The immune system is a group of biological mechanisms that protect living organisms from invading pathogens. Therefore, any suppression of this system will certainly result in dramatic cell damage. The immune system in fish consists of two different complementary parts. The first is the natural (innate or non-specific) defense system which is formed by the cellular and humoral systems. The second is the induced (either acquired or specific) defense and is featured by the humoral response produced by the cellular response such as antibodies and T-lymphocytes (Ángeles Esteban, 2012).

Immunostimulants, which are used to develop the defense mechanism against opportunistic pathogenic microorganisms in the environment, are the most preferred method in the prevention of fish diseases. It is possible to improve the acquired immune responses of fish by vaccination. Unlike vaccination, immunostimulants primarily enhance the innate (non-specific) immunity of fish. The non-specific immune system can memorize potential microbial attacks and can rapidly provide protective effects during subsequent encounters. This approach has been extensively studied and recognized by researchers (Ardó et al., 2008; Harikrishnan et al., 2010; Maqsood et al., 2011; Cristea et al., 2012). Herbal products have shown significant potential in enhancing both the innate and adaptive immune responses of shellfish, as well as various marine and freshwater fish, against bacterial, viral, and parasitic diseases. These products can be administered through injection or oral methods at different concentrations (Harikrishnan et al., 2010; Maqsood et al., 2011).

Medicinal plants offer various forms of application, including injection, bath, and oral routes, for combating fish diseases. The efficacy of these plants depends on the dosage and the early developmental stages of the fish. Unlike antibiotics and chemotherapeutics, which can lead to the emergence of drug-resistant pathogens and environmental pollution, medicinal plants provide a more sustainable approach. Commercial vaccines, although effective, can

be costly for fish farms and have limited specificity against pathogens. In contrast, medicinal plants offer broader utilization options as they target both specific and non-specific defense systems (Table 3) (Ardó et al., 2008; Cristea et al., 2012).

Table 3: *Studies on The Immune Response of Medicinal and Aromatic Plants in Fish*

Species	Medicinal plant	Dose and Duration	Influence	References
Oreochromis niloticus	*Astragalus radix Scutellaria radix*	0.1, 0.5, 1.0% 4 weeks	Respiratory burst activity↑, phagocytosis↑, lysozyme activity↑	Yin et al. (2006)
Oreochromis mossambicus	*Solanum trilobatum*	4, 40, 400 mg kg^{-1} 2 weeks	Lysozyme activity↑	Divyagnaneswari et al. (2007)
Oreochromis mossambicus	*Eclipta alba*	0.01, 0.1, 1% 3 weeks	Lysozyme↑, antiprotease-, complement-, myeloperoxidase content↑, production of reactive oxygen and nitrogen species↑	Christybapita et al. (2007)
Oreochromis niloticus	*Astragalus membranaceus, Lonicera japonica,* Bor+Astragalus, Bor+Lonicera	0.1, 0.05% 4 weeks	Respiratory burst↑, phagocytic activities↑, plasma lysozyme↑, total protein-, total immunoglobulin level-	Ardó et al. (2008)
Oncorhynchus mykiss	*Nigella sativa*	1, 2.5 ve 5% 21 days	Hematocrit↑, leukocyte levels-, NBT positive cell activation-, serum protein↑, total immunoglobulin↑	Dorucu et al. (2009)
Cyprinus carpio	*Astragalus radix, Ganoderma lucidum*	5% 5 weeks	Respiratory burst activity↑, phagocytosis↑, lysozyme activity↑	Yin et al. (2009)
Oncorhynchus mykiss	*Allium sativum*	0.05, 0.1, 0.5, 1.0 g $100g^{-1}$ 14 days	Phagocytic activity↑, respiratory burst↑, lysozyme↑, anti-protease and bactericidal activities↑	Nya & Austin (2009)
Paralichthys olivaceus	*Punica granatum, Chrysanthemum cinerariaefolium, Zanthoxylum schinifolium*	5, 50 100 mg kg^{-1} 30 days	Phagocytosis activity↑, respiratory burst activity↑, alternative complement activity↑, lysozyme activity↑	Harikrishnan et al. (2010)
Cyprinus carpio	*Zataria multiflora*	30, 60, 120 ppm 23 days	Serum lysozyme-, bactericidal activity↑, total serum protein-, total albümin and globulin levels-	Soltani et al. (2010)
Oncorhyncus mykiss	*Lupinus perennis, Mangifera indica, Urtica dioica*	1% 14 days	Serum bactericidal activity↑, respiratory burst, and lysozyme activity↑	Awad & Austin (2010)
Macrobrachium rosenbergii	*Zingiber officinalis Cyanodon dactylon*	1.5, 2, 3, 4% 5 weeks	Hemocyte count↑, phagocytic activity↑	El-Desouky et al. (2012)

Oreochromis niloticus	*Nigella sativa*	3% 30 days	Phagocytic activity↑, total protein, and albumin↑	Elkamel & Mosaad (2012)
Mystus keletius	*Solanum trilobatum*, *Ocimum sanctum*	0, 3, 30 ve 300 mg kg^{-1} 60 days	Respiratory burst activity↑, serum bactericidal activity↑, lysozyme activity↑, serum protein, albümin, and globulin levels↑	Begum & Navaraj (2012)
Rutilus frisii kutum	*Mentha piperita*	0, 1, 2, 3% 8 weeks	Serum lysozyme activity↑, Serum immunoglobulin↑, Serum total protein↑, respiratory burst activity↑	Adel et al. (2015)
Oncorhynchus mykiss	Wormwood (*Artemisia vulgaris* L)	Powder; 0, 0.1, 0.5, 1, 2% Ethanol extract; 250, 1000 mg kg^{-1} 45 days	NBTassay↑, lysozyme↑, phagocytic activity↑	Diler et al. (2017b)
Rachycentron canadum	*Bidens pilosa, Lonicera japonica, Cyathula officinalis*	1, 5, 10% 10 weeks	Reactive oxygen species (ROS) production↑, phagocytic capacity↑, serum lysozyme activity↑, phagocytic activity↑	Lee et al. (2020)
Oncorhynchus mykiss	*Prunus domestica*	0.1, 0.5, 1% 21 days	Respiratory burst and potential bacterial killing activities↑, lysozyme activity-, myeloperoxidase activity↑	Terzi et al. (2021)
Cyprinus carpio	*Rosmarinus officinalis L.*	0.25, 0.5, 1% 60 days	Lysozyme↑, complement, respiratory burst↑, total protein, albumin, and glucose↑	Chelemal Dezfoulnejad, & Molayemraftar (2022)

(↑): significantly increases; (↓): significantly decreased; (-): no significant change

CONCLUSION

The selective behavior of today's consumers has created a trend among producers to use products in animal products that do not pose a risk to human health while enhancing both feed quality and product quality. In this context, the utilization of herbal products derived from natural and aromatic plants, which are harmless to human health, enhance performance, and prevent diseases, has emerged as an alternative feed additive in aquaculture. The use of herbal products offers advantages such as easy availability, affordability, minimal side effects, generally effective at low doses, and a broad spectrum of activity against pathogens, increasing their potential for preference today. Additionally, the fact that herbal products can be administered via the oral route, which is the most effective method for immunostimulant application, strengthens their effects. The lack of harm to the environment through the

biological recycling of herbal products is also among the reasons for their preference.

Despite extensive research into the benefits of using healthy alternative herbal extracts in aquaculture, their utilization remains limited in our country, particularly in the field of aquaculture. Conducting further research on the use of these products, both in terms of animal health and productivity enhancement, is crucial for the advancement of the aquaculture sector. Determining the effects of these products and expanding their application areas would yield significant contributions. The application of herbal substances has shown promising results in against viral, bacterial, fungal, and parasitic diseases in animals. However, these findings represent only a small portion of the potential effects of these plants, and as technology advances, their broader benefits will become apparent. Additionally, due to the variability of herbal product effects based on time and dosage, standardization of plant-derived products is essential for their integration into aquaculture practices.

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