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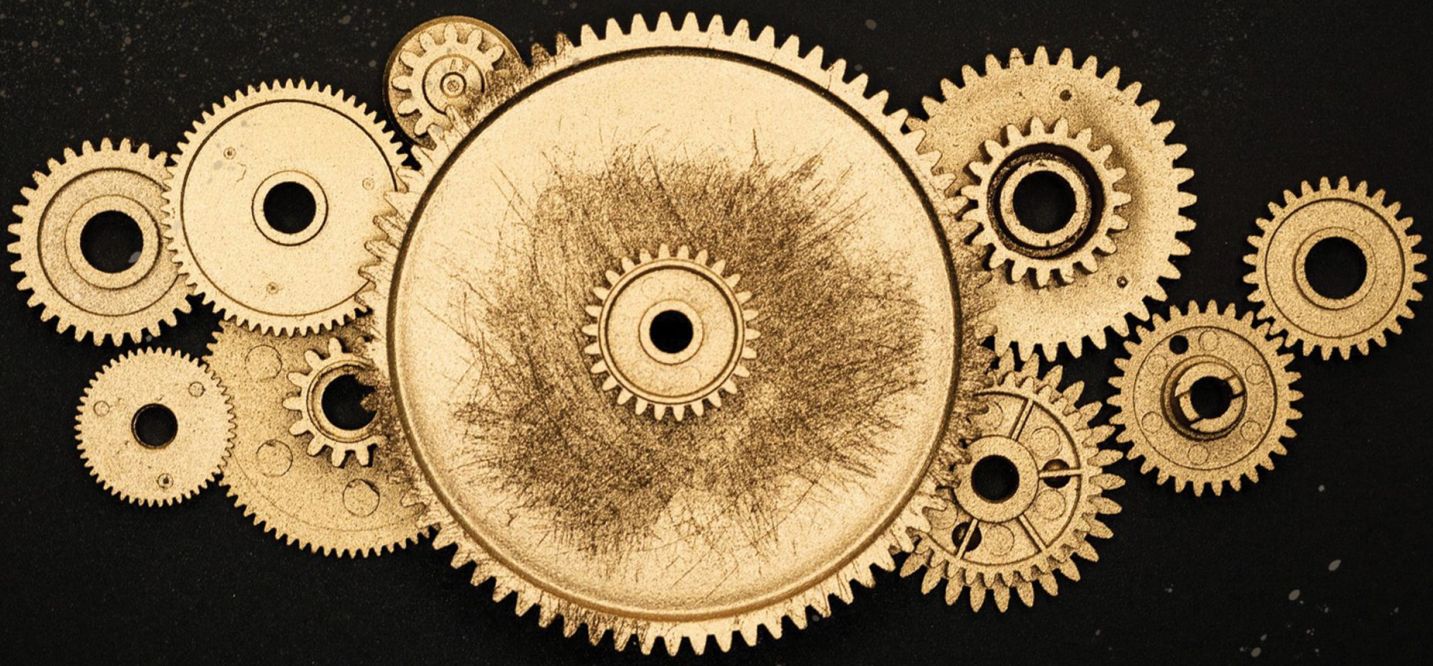

Editors

Assoc. Prof. Dr. Halil İbrahim KURT

Asst. Prof. Dr. Ali KILIÇER

LIVRE DE LYON

2022

Engineering Sciences

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Recent Researches and Practices in Engineering Sciences

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PREFACE

The researches have contributed to the development of education and training definitions in various periods. Although the importance given to science and data-based academic studies is mentioned today, it is observed that the studies conducted are far from the scientific method. Recently the growing interest in interdisciplinary studies has brought scientists together from Materials Science, Mechanical Engineering, Physics, Chemistry, Biology, Economy and Finance and increased the demand for these studies. Disciplinary and/or interdisciplinary studies allow for synthesis of ideas and the synthesis of characteristics from many disciplines. At the same time it addresses scientists, engineers and students' individual differences and helps to develop important, transferable skills. These skills, like critical thinking, communication, analysis, interpretation and discussion are important and continually developing at all stages of life.

One of the major aims of this book was to present evidence and gather together the results of research and development carried out on the engineering applications during recent years. The book project brought together scientists and engineers involved in assessing the various engineering areas, with particular emphasis on academic studies, applications and opinions.

The editor and editorial board hope that this book will be useful to engineers and to scientists working towards an understanding of and the resolution of the applications in the various fields of engineering that we have to face in near future.

October 2022

Assoc. Prof. Dr. Halil İbrahim KURT
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CONTENTS

CHAPTER I

RULE-BASED EVENT DETECTION SYSTEM FOR OBSTRUCTIVE SLEEP APNEA HYPOPNEA SYNDROME USING PHYSIOLOGIC PSG SIGNALS

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1. Introduction

Obstructive Sleep Apnea Hypopnea Syndrome (OSAHS) is a common sleep disorder and occurs as a result of apneic events (apnea and hypopnea) characterized by complete or partial cessation of breathing in sleep. This syndrome negatively affects the life of people around the world at an increasing rate (Peppard et al., 2013; Young et al., 1993) and cause serious diseases and even death (Huang, Guo, Shen, & Tang, 2017). Therefore, the diagnosis and treatment of OSAHS is important.

In the clinic, the gold standard for OSAHS diagnosis is polysomnography (PSG). Physiological signals of individuals thought to have OSAHS are recorded during the night (Xie & Minn, 2012). Recorded signals are interpreted by sleep specialists according to the American Academy of Sleep Medicine (AASM) guideline and the diagnosis decision is given by specialists based on the number of apnea and hypopnea events per hour of sleep, namely, the Apnea-Hypopnea Index (AHI). The diagnosis is person-dependent, laborious, boring, and exhausting (Collop, 2002; H. Lee, Park, Kim, & Lee, 2016). Therefore, alternative automatic OSAHS detection systems should be developed.

In the literature, several studies have performed automatic OSAHS detection using different physiological signals such as airflow (Huang et al., 2017; Koley & Dey, 2013; Nakano, Tanigawao, Furukawa, & Nishima, 2007; Rathnayake, Wood, Abeyratne, & Hukins, 2010), oximetry (SpO2) (Alvarez, Hornero, Marcos, & del Campo, 2010; Vazquez et al., 2000; Xie & Minn, 2012), snoring (Abeyratne, Wakwella, & Hukins, 2005; Ben-Israel, Tarasiuk, & Zigel, 2012), and ECG (Bsoul, Minn, & Tamil, 2011; Yilmaz, Asyali, Arikan, Yetkin, & Ozgen, 2010). While the some of these studies (Bsoul et al., 2011; Rathnayake et al., 2010; Xie & Minn, 2012; Yilmaz et al., 2010) detect apneic events on the basis of 30 or 60 sec, others focused on the direct detection of the events (Huang et al., 2017; Koley & Dey, 2013). In addition, one group of studies evaluated only a single signal for the event detection (Bsoul et al., 2011; Koley & Dey, 2013; Vazquez et al., 2000; Xie & Minn, 2012) while another group considered a combination of signals. When the results of the studies were examined, it was seen that the direct detection of apneic events and use of many signals combination provided diagnostic information closer to the clinical diagnosis (Al-Angari & Sahakian, 2012; Huang et al., 2017; Moret-Bonillo, Alvarez-Estevez, Fernandez-Leal, & Hernandez-Pereira, 2014). Also, according to Version 2.0 of the AASM manual (AASM, 2012), in order for an event to be defined as hypopnea, the decrease in the amount of oxygen in the blood must be taken into account in addition to the interruption in airflow. For all these reasons, it is necessary that subjects' both airflow and SpO2 physiologic PSG signals should be analyzed to detect apneic events and thus for OSAHS detection.

In this study, the direct detection of apneic events was performed with a developed system consisting of two stage. Firstly, apneic events were detected according to criteria given in the 2012 AASM manual (AASM, 2012): apneas are associated with a 90% decrease in airflow (breath) compared to the baseline value, hypopneas are defined by a reduction of at least 30% in the airflow compared to the baseline, the reduction of the airflow should last at least 10 sec for events, and hypopnea events should be accompanied by 3% oxygen reduction

(desaturation) in the blood or end with arousal. To detect airflow changes mentioned by AASM, an algorithm was developed based on both the Singular Value Decomposition (SVD) method and a set of rules. Then, desaturations in SpO2 signals were examined. Finally, the detectability of apneic events and the AHI computation and thus OSAHS diagnosis were evaluated. In addition, the severity of OSAHS for each subject was determined using AHI values. At the end of the study, rule-based event detection system for obstructive sleep apnea hypopnea syndrome was also clinically tested.

2. Data Acquisition and Preprocessing

In this study, PSG records of 145 subjects admitted to the Sleep Laboratory of Necmettin Erbakan University with complaints of sleep disorders were obtained. These records were stored by transferring them to a computer. The experimental protocol conformed to the principles outlined in the Declaration of Helsinki, with an approval statement confirmed by the Medical Ethics Review Board (Faculty of Medicine, Selcuk University, Konya, Turkey) for institutional, non-invasive clinical research. All participants provided proper informed consent. The demographic information of the subjects, obtained from the clinical reports, is shown in Table 1.

Table 1. Demographic information of subjects

AHI Range (Severity)	**Subject Number**	**Age**	**BMI**	**AHI**
AHI<5 (Healthy)	33	48.3 ± 12.1	31.3 ± 5.95	1.78 ± 1.24
5≤ AHI <15 (Mild)	33	51.6 ± 11.8	33.4 ± 6.4	9.0 ± 3.3
15≤ AHI <30 (Moderate)	25	53.4±13.3	32.9±7.4	26.3 ± 9.01
30≤ AHI (Severe)	54	49.0±14.3	33.9±7.3	71.4 ± 30.0

PSG records and reference files associated with the records were prepared according to the 2012 rules of the AASM (AASM, 2012). Each apneic event, with its onset and end times, was scored and indicated in the reference file. In addition, total sleep times, AHI values and subjects' disease status such as patient or healthy and severity of disease (mild, moderate, severe) was determined by specialists and recorded to reference file. Although the PSG records comprised multi-physiological polysomnographic signals, in accordance with the purpose of the study, nasal cannula airflow with a sampling frequency of 100 Hz and oximetry (SpO2) signals with a sampling frequency of 1 Hz were evaluated, which accurately reflect all respiratory events among the polysomnography records taken from all individuals included in the study.

All biological signals recorded from a person's body are corrupted by different types of artifacts and interference, such as power line interference, applied sensor contact noise, motion artifacts, muscle contraction, other noise signals inside the body, and so on. For the meaningful and accurate analysis of these signals, pre-processing is necessary. Therefore, appropriate filter was applied to all signals nasal cannula airflow signals used in this study by taking into account the literature. A third-order Butterworth bandpass filter with cut-off frequencies of 0.01–0.7 Hz was selected for airflow signals by trying different filters with various frequency ranges mentioned in references (Diaz, Arancibia, Bassi, & Vivaldi, 2014; Huang et al., 2017; Koley & Dey, 2013; Selvaraj & Narasimhan, 2013). In addition, SpO2 values between 0 and 50 were accepted as artifacts and removed from the SpO2 signals.

Long-term airflow signals can be affected by sensors, patients' body positions, patients' movements during the night, and therefore the values and amplitudes of airflow signals can change continuously during the night. For this reason, the filtered airflow signals of the all subjects were normalized with the adaptive normalization method proposed by the researchers in references (Choi et al., 2018; Tian & Liu, 2005). No normalization was performed on the SpO2 signal values in the preprocessing step.

After pre-processing of the signals, 145 subjects were randomly divided into two groups. 60% of subjects were evaluated as train group. Remaining 40% of subjects were assessed as test group. The records of training group were used to develop a system capable of detecting apneic events. The remaining records in the testing group were used to evaluate the developed system in terms of efficiency and accuracy.

After developing the apneic event detection system, 26 subjects were also included in the study for clinical test purposes. For these 26 subjects, following AHI ranges were determined by specialists.

- 21 subjects in $30 \leq AHI$
- 4 subjects in $15 \leq AHI < 30$
- 1 subject in $5 \leq AHI < 15$

All pre-processes were also applied to the nasal cannula airflow and oximetry signals of these subjects taken for clinical testing.

3. The Proposed Rule-Based System

In this study, a rule-based system depending on AASM (AASM, 2012) consisting of two stages is proposed to detect apneic events. For use in the first stage of

the system, a new algorithm is developed based on the single-channel nasal cannula airflow signal, SVD, and a set of rules. This algorithm carries out the automatic detection of apneic events. Events that provide at least 90% reduction (ie apneas) are considered apneic events. Others is considered as a candidate event. The second stage validate the candidate events as apneic or reject them as non-apneic With this aim, an oxygen desaturation analysis of the detected candidate events is carried out with the SpO2 signal in the second stage.

The general structure of this rule-based system is shown in Figure 1.

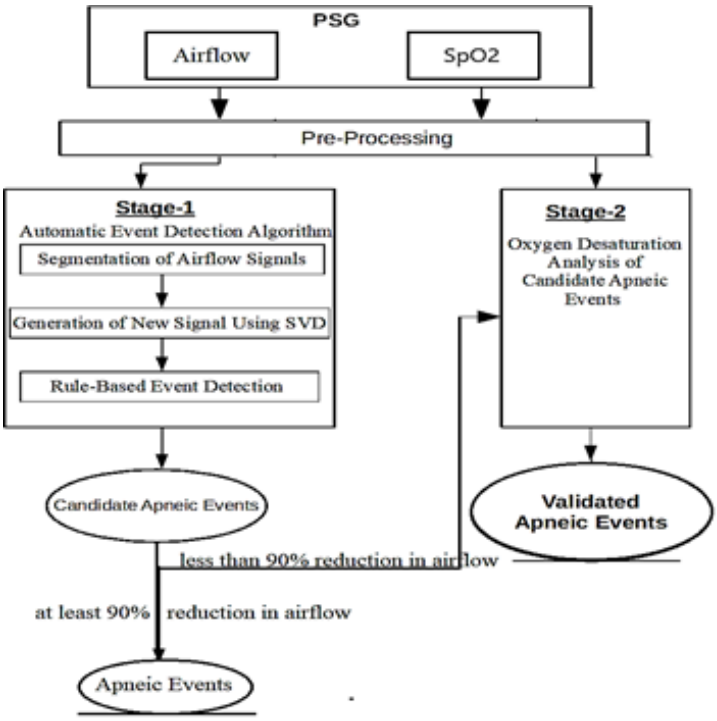

Figure 1: The general structure of developed rule-based system.

The development of the system and the determination of the parameter values used in the system were carried out according to the subjects in the training group. After the system was developed, the apneic events of the test group subjects were also detected with the same parameters, and the desaturations related to the events were determined. Finally, this system was also tested for 26 subjects in the clinical test group.

3.1. Stage 1: Automatic Event Detection Algorithm

In Stage 1, a new automatic event detection algorithm is developed to detect reduction in nasal cannula airflow signal. This stage consists of the segmentation

of the airflow signals, the generation of a new signal from these segments using SVD, and rule-based event detection through application of AASM rules.

In the classical definition of apneic events, events are called apneic, if they continue for at least 10 sec (AASM, 2012). For the detection of time intervals of at least 10 sec, it would be advantageous to examine the signal in smaller pieces rather than as a whole. Therefore, firstly, the airflow signals are segmented into shorter time segments. As previous studies have indicated that some disadvantageous situations occur when the duration of the segments is chosen as 10 sec or more, depending on the definition of an apneic event, the duration was chosen to be less than 10 sec in this study (Huang et al., 2017; Koley & Dey, 2013; Moret-Bonillo et al., 2014). Several trials with different durations shorter than 10 sec were carried out to decide on the optimum segment duration for each subject's airflow signal in the training group, and it was decided that approximately one breath cycle is suitable for the duration of a segment. When the duration of a segment was selected as longer than a breath cycle, fuzzy boundaries occurred for apneic events. Breath cycles were determined by using the traditional crossing technique (Schmidt, Foitzik, Wauer, Winkler, & Schmalisch, 1998) on the first two minutes of airflow waves, during which times OSAHS patients usually have no apneic events, and the duration of the segment was calculated as the mean of the durations of these cycles for each subject's airflow signal. Because each subject has a different duration of the breath cycle, it was seen that the duration of the segment for each airflow signal changes in the range of 2 to 6 sec (Huang et al., 2017; Rostig et al., 2005; Zhang et al., 2014). After determination of the duration of the segments, each airflow signal was divided into overlapping segments using a sliding window (with the specified segment duration), which shifts 1 sec at a time.

The properties of the airflow signals, such as amplitude, rate, energy, and so on, vary during apneic events. Therefore, in literature, many researchers have derived new signals such as instantaneous respiration amplitude (IRA) and an instantaneous respiration interval (IRI) signals from the airflow signals considering the changes in amplitude and breathing rate, and then detected apneic events by using these new signals instead of airflows (Huang et al., 2017; Koley & Dey, 2013; H. Lee et al., 2016; Moret-Bonillo et al., 2014). Just like in amplitude and rate changes, apneic events can also be detected by the determination of changes in the energy of the airflow signal. In this study, this situation was taken into consideration and new signals were generated from the airflow segments of each subject using the SVD method based on the evaluation of the signal energy information.

SVD is used as a useful method in many fields, such as dimensionality reduction, signal processing applications, statistical analysis, and so on (S. Lee & Hayes, 2004). The method is based on factoring a matrix. In this method, an m × n matrix A is factored into the product of three matrices U, Λ, and V as shown in Equation 1 (Xia, Zhou, Li, Yuan, & Geng, 2015; C. Yucelbas et al., 2018; Yucelbas, Yucelbas, Tezel, Ozsen, & Yosunkaya, 2018).

$$A=U\Lambda V^{T} \tag{1}$$

In Eq. 1, Λ is an $m \times n$ diagonal matrix. This matrix includes the singular values (SVs) of A_{mxn} as its diagonal components (C. Yucelbas et al., 2018; S. Yucelbas et al., 2018). The left and right SVs of A are the columns of U_{mxm} and V_{nxn}, which are orthogonal matrices (Xia et al., 2015; C. Yucelbas et al., 2018; S. Yucelbas et al., 2018). The SVs reflect natural characteristics of the matrix and indicate the energy information contained in each subspace, which means that the larger SVs contain more of the energy of the matrix (Hassanpour, Mesbah, & Boashash, 2004; C. Yucelbas et al., 2018). Moreover, the SVs are not affected by the variations in the matrix elements; in other words, these values have good stability. Sudden changes in the data will lead to a change in the SVs and to a redistribution of the energy in each subspace. So, the SVs can be regarded as useful features of biomedical signals (Hassanpour et al., 2004). For these reasons and because of the differences in the energy information between apneic and normal airflow patterns, the SVD method was preferred in this study. The SVD method was applied to each airflow segment and the SVs were generated.

Hassanpour et al. stated that the larger SVs have more information about the structure of patterns embedded in the matrix than the other SVs (Hassanpour et al., 2004). Taking this statement into consideration, the maximum values of SVs belong to every segment were selected to represent airflow segments and the new signal, called the SVD signal, was generated by merging the maximum SVs of all airflow segments of each subject.

In the generated SVD signals, some of the SVs are quite high because of the sudden opening of the upper airway succeeding a sleep apneic event (Ciolek, Niedzwiecki, Sieklicki, Drozdowski, & Siebert, 2015). This leads to incorrect decisions in apneic event detection. To overcome this constraint, significantly high values in the generated SVD signal should be ignored before the detection of apneic events. In order to decide whether the SVD values were high or not, the modified-Z scores as seen in Equation 2 were benefitted for the inequality (Iglewicz & Hoaglin, 1993). For SVD signals, the smallest of the signal values with a Z-score greater than 2 (Mi > 2) in absolute value was

accepted as threshold_value_1 (Equation 3) and SVD signal values greater than this threshold value were considered as outliers. These outliers were not taken into account during the detection of apneic events.

$$M_i = \frac{0.6745(s_i - \tilde{s})}{median(|s_i - \tilde{s}|)} \tag{2}$$

$$threshold\_value\_1 = min(s[M_i > 2]) \tag{3}$$

In Equation 2, s represents the SVD signal, si represents each sample of the signal, and $\tilde{s}$ represents the median of the signal.

After calculating the threshold_value_1 to determine the outliers in the SVD signal of each person, an automatic apneic event detection algorithm was developed to detect the apneic events of the individuals. This algorithm works by following the steps given below.

i. Determine the initial baseline value.
   AASM criteria indicate that the baseline value is the average of the stable airflow values over 2 min. People usually have no apneic events in the first 2 min in bed but have more regular and stable breathing. Hence, the initial baseline value was determined considering the signal samples in the first 2 min (120 sec). However, outlier values can be found in these 2 min and they can affect it. Therefore, it was checked whether each signal sample ($s[i]$) within the first 2 min was smaller than *threshold_value_1* or not and, if so, only small samples were accepted as elements of the *Base* [*j*] array in the calculation of the baseline value. Others were considered as outliers and were ignored for the calculation. After that, the baseline value (*Base_Val)* was calculated by taking the average of the accepted values (*mean*(*Base*[*j*])). Here, *s* represents the produced SVD signal, *i* is the sample number of the signal *s*, *Base*[] is the array consisting of the signal samples used to calculate the baseline, and *j* is the index value of *Base*, which increases as long as the samples within the first 2 min are smaller than *threshold_value_1*.

ii. The following steps are repeated for each sample after the end of the first two-minute interval.
   a. Set *apneic_thres* to 0.7**Base_Val*
      (*apneic_thres* is the second threshold of the study. This threshold is used to decide whether an apneic event has occurred. The coefficient of 0.7 used in the threshold calculation corresponds to at least 30% reduction according to the AASM.)

b. Two questions are asked for each signal sample ($s[i]$).

   First Question: Is the signal sample ($s[i]$) smaller than *over_thres*? If so, the second question is asked. Otherwise, the sample is considered as an outlier and is ignored.

   Second Question: Is $s[i]$ smaller than *apneic_thres*? If so, the example is labeled as an apneic event ('A') automatically. If not, the sample is added to *Base* [j] to calculate the base value and the index j increases. (The index j is incremented only when the signal sample is less than *over_thres* and the sample is not labeled as 'A,' in other words, does not belong to any apneic event interval.) An additional check is also performed in this section. Evaluate whether the sample labeled A is less than 10% of the baseline value (90% reduction). If small, this 'A' sample is further stored in a separate array called *Apnea []*. If it's not small, it doesn't need to be added to the *Apnea[]* array.

c. The baseline value (*Base_Val*) is updated based on the average of the samples belonging to the last 2 min in the array *Base*.

iii. After the examination of all the samples in SVD signals is complete, signal samples not labeled 'A' are labeled as normal 'N.'

iv. After all samples in the SVD signal have been labeled in previous steps as 'A' or 'N', labels are smoothed with the help of a set of rules.

   Rule 1: If there are one or two samples with different labels between the same labeled instances, the status of different labels should be changed.

   Rule 2: Any sample labeled 'A' must be part of a series of at least 10 sec of consecutive samples labeled 'A.' Otherwise, samples labeled 'A' should be relabeled as 'N.'

   Rule 3: If each sequential apneic ("A") sample sequence continues for 10 sec and longer, this sequence is considered an apneic event. The first apneic sample in this sequence determines the beginning time of the apneic event and the last apneic sample determines the end time of the apneic event.

   Rule 4: The duration of an apneic event is the difference between its beginning and end times.

As a result of the execution of the first 4 steps of the developed algorithm, firstly, each sample of the SVD signals belonging to individuals is labeled as normal 'N' or apneic 'A'. Then, with the application of the 4 rules, apneic events are detected together with their onset and ending times. Figure 2 shows how the events were detected in the first 4 steps of the developed algorithm. After apneic

events are detected with these steps of the algorithm, the samples of each apneic event are compared with the signal samples in the *Apnea[]* sequence. If 90% of the event contains consecutive samples from the *Apnea []* sequence, this event is considered and validated as apneic. Otherwise, events are assumed as candidate events.

One more case should be looked at in order to confirm or reject the candidate events detected by the algorithm as apneic events. The AASM (2012) criteria state that hypopneas among apneic events should be accompanied by at least 3% either oxygen desaturations or arousals. Therefore, the presence of oxygen desaturations accompanying the detected candidate events should be examined.

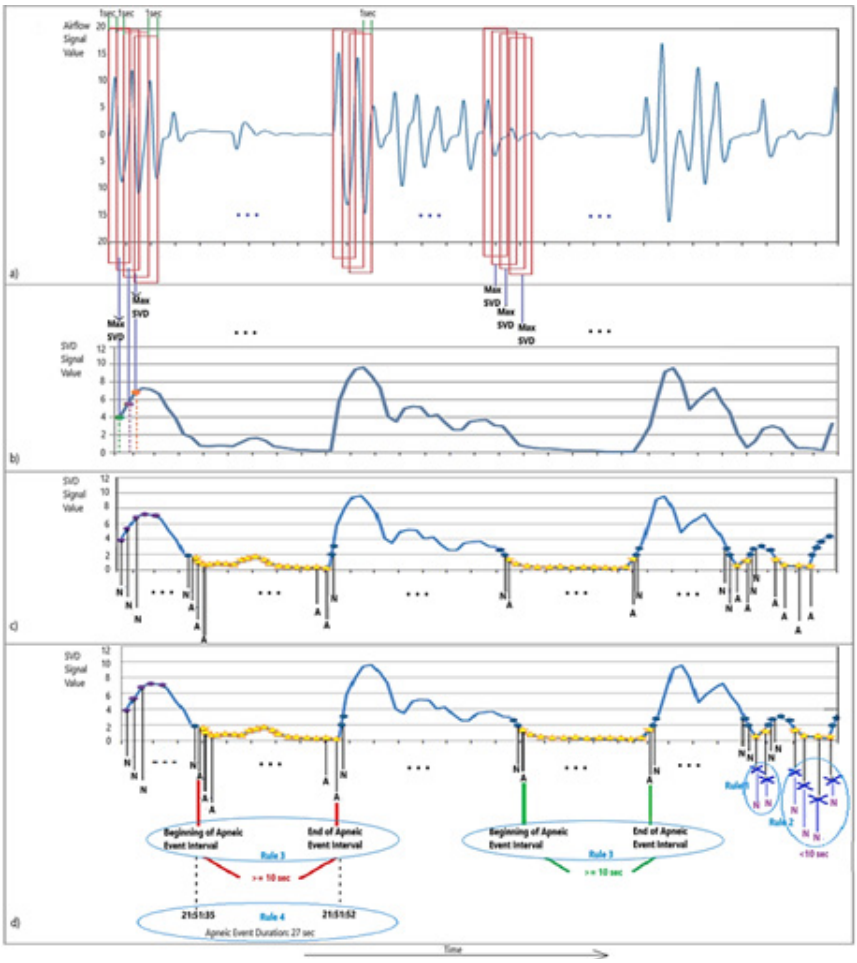

Figure 2: Automatic event detection a) segmentation of airflow signal and application of SVD to each segment, b) generation of new SVD signal, c) 'A' or 'N' labeling of each sample in SVD signal, d) determination of candidate apneic events

3.2. Stage 2: Oxygen Desaturation Analysis of Candidate Apneic Events

Oxygen desaturation begins with a certain time delay from the onset of an apneic event. Although there is no criterion for this delay time, desaturation is usually seen within 20-40 seconds after the onset of the apneic event (Moret-Bonillo et al., 2014). In other words, oxygen desaturation can occur within 20 seconds after the start of the event, but in some cases this period can be up to 40 seconds. Therefore, the 40 sec time interval in the oximetry signal is automatically determined from the onset of each apneic event detected as a candidate by the algorithm. Then, the 3% oxygen desaturation as specified by the AASM (2012) is sought within the ranges determined for each of the events identified as candidates. If this desaturation is available for candidates events, these candidates events are validated as apneic events. However, the remaining events cannot be considered as apneic because they do not meet the criteria of AASM (2012). For this reason, the label of each sample of these events is changed to "N".

Figure 3 shows an example of the confirmation and rejection of detected candidate apneic events as apneic events relative to desturation. The beginning of the desaturations is determined as the time of the first value where the oxygen decrease starts, and the end is the time of the value where the oxygen increase ends. Desaturation time is calculated by taking the difference between desaturation start and end times.

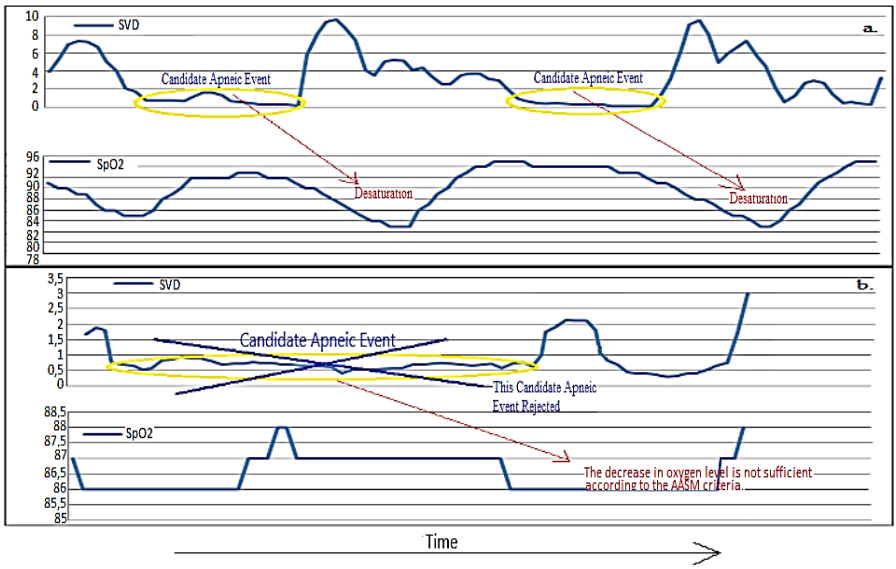

Figure 3: Synchronization of oxygen desaturations with candidate apneic events

4. Results

This study carried out apneic-event detection on 145 subjects with the developed automatic rule-based system. In addition, in this study, AHI values were calculated by dividing the number of apneic events detected for each subject by the total sleep time to determine disease severity. Performance evaluation of the system was performed using the accuracy (AC) as in Equation 4, the sensitivity (SN) as in Equation 5, the specificity (SP) as in Equation 6, the precision or positive predictive value (P or PPV) as in Equation 7, the F-score (FS) as in Equation 8, and Cohen's kappa satatictic value (K) as in Equation 9 and 10 were used. Also, statistical analysis was carried out to compare calculated AHI values and reference AHI values.

$$AC=\frac{TP+TN}{TP+TN+FP+FN} \tag{4}$$

$$SN=\frac{TP}{TP+FN} \tag{5}$$

$$SP=\frac{TN}{TN+FP} \tag{6}$$

$$P(PPV)=\frac{TP}{TP+FP} \tag{7}$$

$$FS=2*\left(SN * P\right) / (SN + P) \tag{8}$$

$$\mathrm{K}=\frac{P_o - P_e}{1 - P_e} \tag{9}$$

$$\mathrm{P_e}=\frac{[(TP+FP)\times(TP+FN)]+[(FN+TN)\times(FP+TN)]}{(TP+TN+FP+FN)^2} \tag{10}$$

In equations;

TP refers to true positive in confusion matrix. It indicates the number of positive examples classified accurately.

FN refers to false negative in confusion matrix. It is the number of actual positive examples classified as negative.

FP refers to false positive in confusion matrix. It shows the number of actual negative examples classified as positive.

TN refers to true negative in confusion matrix. It shows the number of negative examples classified accurately.

Table 2 shows the results of apneic event detection with the developed rule-based system.

Table 2: Apneic event detection results with the developed rule-based algorithm

Group	AAE	CDE	UDE	EDE	SN (%)	P (%)
Train	9521	9017	504	92	94.70	98.99
Test	4477	4220	257	39	94.25	99.08

*AAE: Number of Actual Apneik Event, CDE: Number of correctly detected events, UDE: Number of missing events, i.e. the number of undetected events, EDE: The number of extra detected events, that is, the number of events that are not actually detected by the system.

In the performance evaluation of apneic event detection, sensitivity refers to the ratio of the number of correctly detected apneic events to the total number of apneic events. According to Table 2, this criterion was found to be 94.70% for the training group and 94.25% for the test group. The precision criterion expresses the ratio of correctly detected apneic events to all detected apneic events. This criterion value is also quite high for both the training and test groups and is 98.99% and 99.08%, respectively.

After the detection of apneic events belonging to a total of 145 people in the training and test group, the AHI values of each person were calculated by the ratio of the total number of apneic events to the total sleep time according to AASM (2012).

Figure 4 shows the distribution (Figure 4.a) and Bland-Altman (Figure 4.b) graphs between the AHI values calculated according to the events detected in the study and the reference AHI values determined by the sleep experts.

The scatter plot (Figure 4.a) shows that there is a statistically significant correlation between the calculated and reference AHI values ($r = 0.9974$, $r^2=0.99$). The absolute mean difference between the calculated and reference AHI values according to the Bland-Altman plot (Figure 4.b) is 1.2.

Although there are undetected or extra-detected events in the apneic event detection process with the rule-based system, as can be seen from the graphs in Figure 4, their number is quite low, and they did not significantly affect the AHI values of the subjects.

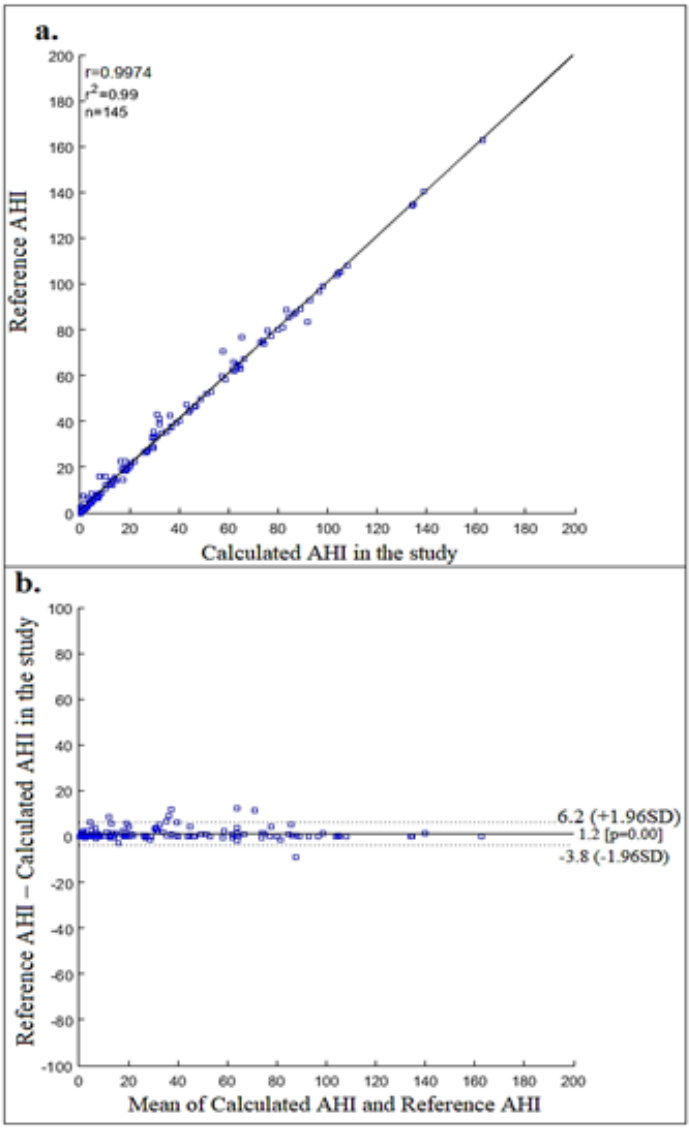

Figure 4. Between Calculated and Reference AHI values for 145 subjects, a. Distribution graphic, b. Bland-Altman graphic

In order to diagnose people with OSAHS and to determine the severity of disease (mild, moderate, severe), all subjects were divided into subgroups namely AHI<5 (Healthy), 5≤ AHI <15 (Mild), 15≤ AHI <30 (Moderate) and 30≤AHI (Severe) according to the calculated AHI values.

Table 3 shows the performance of people to be divided into AHI subgroups according to the calculated AHI values, Table 4 shows the confusion matrix.

Table 3: Results of dividing subjects into AHI subgroups

AHI subgroups	SN(%)	SP(%)	P (%)	FS (%)	AC (%)	K
AHI<5	100	98.21	94.29	97.06	96.55	95.26
5≤AHI<15	90.91	98.21	93.75	92.31		
15≤ AHI<30	92.00	99.17	95.83	93.88		
30≤ AHI	100	100	100	100		

Table 4: Confusion Matrix related to dividing subjects into AHI subgroups

		Predicted			
		AHI<5	**5≤AHI<15**	**15≤ AHI<30**	**30≤ AHI**
Actual	**AHI<5**	33	0	0	0
	5≤AHI<15	2	30	1	0
	15≤ AHI<30	0	2	23	0
	30≤ AHI	0	0	0	54

If the OSAHS diagnosis made according to the apneic event detection and the AHI values calculated accordingly is evaluated, as can be seen in Table 4, 110 of 112 patients with OSAHS have an AHI value greater than 5, and therefore these individuals correctly found as having OSAHS. AHI values of 2 of these 112 patients were found below 5, that is, these people were incorrectly evaluated as healthy even though they had OSAHS. 33 healthy individuals were also found to be healthy. Based on these findings, it can be said that OSAHS patients were distinguished from healthy individuals with 98.62% accuracy, 98.21% sensitivity, 100% specificity and 100% precision. In addition, as seen in Table 4, severity of OSAHS patients could be determined with high accuracy of 96.55% and K value of 95.26. Especially, sucjects suffered from severe OSAHS could be reached with 100% accuracy.

In order to determine whether 26 people included in the clinical evaluation phase of the study have OSAHS or not, apneic events of these people were determined and their AHI values were calculated.

Figure 5 shows the distribution (Figure 5.a) and Bland-Altman (Figure 5.b) graphs between the AHI values calculated according to the events detected in the study for 26 subjects and the reference AHI values determined by the sleep specialists.

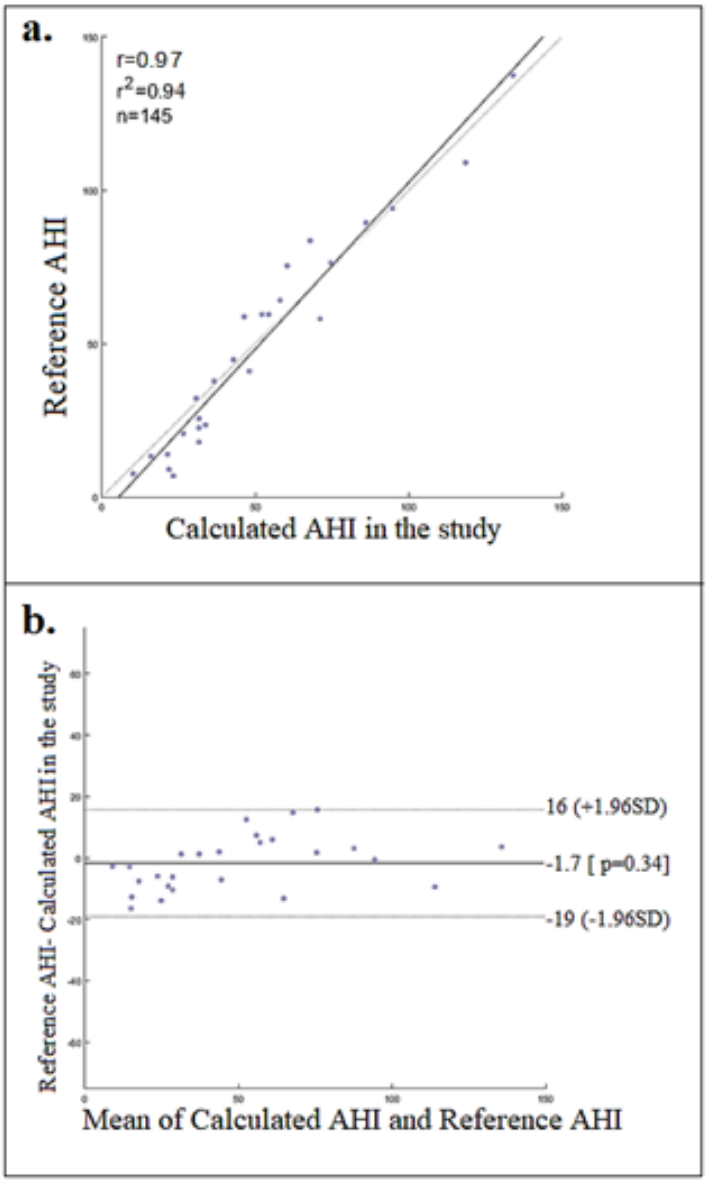

Figure 5. Between Calculated and Reference AHI values for 26 subjects, a. Distribution graphic, b. Bland-Altman graphic

As seen in the scatter plot (Figure 5.a), there is a statistically significant correlation between the calculated and reference AHI values ($r = 0.97$, $r^2=0.94$) for subjects included to clinical test. The absolute mean difference between the calculated and reference AHI values according to the Bland-Altman plot (Figure 5.b) is 1.7.

5. Discussion

The results obtained with the Rule-based system developed using physiologic PSG signals (nasal cannula airflow and SpO2) were compared with similar studies in the literature as shown in Table 5.

Table 5: Comparison of the results provided by the rule-based system with the literature

Researchers	**Signals**	**Performance**
Otero, Felix, and Alvarez (2011)	Airflow SpO2	For apneic events; Sensitivity: 95.7% False Negative: 1.6% Correlation for AHIs (r):-
Koley and Dey (2013)	Airflow	For apneic events; Accuracy for known data: 96.8 % Accuracy for unknown data: 96.1% Correlation for AHIs (r): 0.98
Moret-Bonillo et al. (2014)	Airflow SpO2	For apneic events; Sensitivity: 81.3% Specificity: 91.6% Correlation for AHIs (r):-
H. Lee et al. (2016)	Nasal Cannula Airflow	For apneic events; Sensitivity: 86.4% Precision: 84.5% Correlation for AHIs (r): 0.94
Huang et al. (2017)	Nasal Cannula Airflow SpO2	Sensitivity: 97.6 % Precision: 95.7 % Correlation for AHIs (r): -
Choi et al. (2018)	Nasal Cannula Airflow	For OSAHS detection; Accuracy: %94.9 Correlation for AHIs (r): 0.99
This Study	**Nasal Cannula Airflow** **SpO2**	**For apneic events;** **Sensitivity: %94.25 Precision: %99.08** **Between AHI values;** **r=0.99, r^2=0.99, p<0.0001** **Division of subjects into AHI subgroups:** **Accuracy=%96.55** **For OSAHS detection;** **Accuracy: %98.62** **Sensitivity: %98.21** **Specificity: %100** **Precision: %100**

As can be seen from the results in Table 5, this study is comparable to the studies in the literature in terms of both event detection, the relationship between AHIs, and its performance in the diagnosis of OSAHS.

Studies in the literature generally produced new signals from airflow signals, taking into account the changes in signal peak value, amplitude, tidal velocity and tidal volume, and performed apneic event detection in line with AASM (2012) criteria. In the Rule-Based System Approach carried out in this study, a new SVD signal representing the airflow signals was produced as a result of the use of the maximum singular value with the SVD method, and an algorithm that exhibits high performance based on certain rules was developed considering the AASM (2012) criteria. Thus, it was seen that the energy differences caused by apneic events in the airflow signals, just as in classical amplitude or respiratory rate changes, were revealed by the SVD method and these differences were detected with the developed rule-based system, helping the diagnosis of OSAHS.

5. Conclusion

This study demonstrates the detection of apneic events, calculation of AHI values and diagnosis of OSAHS. The developed rule-based system detects apneic events automatically using nasal cannula airflow signal, SpO2 signal, SVD method and some rules. In this way, the system calculates polysomnographic measurement namely AHI value with high accuracy for each subject. The system also determines whether subjects have OSAHS or not and the severity of OSAHS. These findings of the system can provide diagnostic information related to OSAHS to the specialists in a clinic. In fact, the proposed system can automate the manual scoring performed by specialists in the laboratory by eliminating dependency on a person.

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CHAPTER II

APPLICATION OF RESONANCE FREQUENCY PREDICTION USING CNN-LSTM APPROACH ON F-SHAPED MICROSTRIP PATCH ANTENNA

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1. Introduction

ISM Band (Industrial Scientific Medical) applications (2.40-2.485 GHz) used in Bluetooth devices, wireless phones, Wi-Fi computer networks, and NFC devices have gained importance with the development of today's wireless communication technologies. The use of patch antennas operating at different center frequencies and the easiest to manufacture; It is widely used in ISM band applications due to its advantages such as small size, low production cost, and lightweight. In addition to these advantages, they also

have disadvantages, such as being a narrow band and low gain, operating at low power capacities, and having poor insulation between supply points and radiation patches (Balanis, 2016).

In order to minimize the mentioned disadvantages and to design patch antennas that can operate in the desired frequency band, it is necessary to optimize the antennas according to variables such as feeding type/position, patch geometry, ground surface dimensions, and dielectric constant. In addition, various methods are used for optimization applications. Among these methods, the most widely used applications are based on patch and ground geometry configurations. It is necessary to optimize the antennas according to variables such as feeding type/position, patch geometry, ground surface dimensions, and dielectric constant. In addition, various methods are used for optimization applications. Among these methods, the most widely used applications are based on patch and ground geometry configurations. In the literature, rectangular (Uqaili et al., 2020; Palsokar & Lahudkar, 2020), triangular (Bhadoria & Kumar, 2018; Wong & Yan, 202), circular (Wong et al., 2019; Khattak, Sohail, Khan, Barki, & Witjaksono, 2019), pentagonal (Ferouani & Moulessehoul, 2021; Naik, Panda, Sahu, & Panda, 2022), E-shaped (Yin et al., 2019; Sheik, Sridevi, & Raju, 2020), L-shaped (Rawal & Rawat, 2021), H-shaped (Chatterjee et al., 2018) and C-shaped (Patel & Charotar University of Science and Technology, 2020) patch geometries are available. In order to increase the bandwidth and shift the resonant frequency to another frequency, slots are created on the ground plane with patch geometry (Awan et al., 2019; Gopi et al., 2021). In addition, short-circuit pins are added between the ground and the patch plane to increase the surface current density and homogeneity in patch antennas (Kumar et al., 2021). When optimizations on the specified patch geometries are applied, problems such as high-resolution times and power consumption arise in the analysis performed over full-wave electromagnetic simulation software. Artificial intelligence applications are used to solve these problems.

Applying optimization to various patch antenna design parameters using artificial intelligence applications has recently been an active research area. Recently, some case studies have been carried out in this area. Ustun et al. Conducted a study to calculate the resonance frequency of the E–shaped patch antenna (ESPA) using the deep neural network (DNN) in 2019. resonance frequencies of 144 ESPA were obtained using the 3D EM analysis program and used as training data. The APE error was calculated as 0.269 during the training phase using the K-fold cross-validation method. At the test stage, the APE value was calculated as 0.285 (Ustun, Toktas, & Akdagli, 2019). Sagik et al., 2019, performed the gain and directivity optimization of a microstrip patch antenna

with a metamaterial structure operating at 5.2 GHz using an artificial neural network approach (Sağık et al., 2021). Zhou et al. implemented TRPBO (Trust-Region Parallel Bayesian Optimization) on a U-slot array antenna with three broadband patch antennas. The proposed TRPBO was validated with seven different comparison problems of different geometric dimensions. According to the experimental results, it has been observed that it can reduce the calculation time by at least 33.53% while providing better design results than the standard BO-EI in the field of EM design (Zhou et al., 2021). Raya et al., in their study in 2019, applied optimization on the ground, feeding, patch, and dielectric layer dimensions of the Rectangular Microstrip Patch Antenna using a Deep Neural Network. As a result of the optimization, the input parameters converge with a 2.03 Mean Square Error and 1.51 mean absolute error for the test data set (Raya, Pal, & Ali, 2019). Khan et al. proposed using a convolutional neural network (CNN) to estimate the surface current in a rectangular microstrip-fed patch antenna. It predicts the desired surface currents due to the application with an average error of only 11% (Khan, Zekios, Bhardwaj, & Georgakopoulos, 2021). Özkaya et al., in their study in 2020, proposed a microstrip antenna consisting of C-shaped arrays that can operate in the C and X bands. Five different Long-Short Term Memory (LSTM) are applied to the proposed antenna, and it performs optimization with 1.0161 RMSE performance (Özkaya, Seyfi, & Öztürk, 2021).

In this study, a microstrip-fed patch antenna with F-shaped patch geometry that can operate in the ISM band is designed, and resonance frequency estimation application is performed using hybrid CNN-LSTM. The first section presents a literature review on why patch antennas operating in the ISM band are needed for optimization applications and how ANN applications take place in antenna design. The geometric dimension values, gain, directivity gain, and reflection coefficient values (S_{11}) of the F-Shaped Patch antenna designed are given in Section 2.1. In Section 2.2, F-Shaped antennas with 432 different geometric parameters are designed for the CNN-LSTM application. All 432 antennas were used in the data set. The flow diagram and content of the CNN-LSTM application are given in this section. Section 3 presents the training and test results of the CNN-LSTM application. This study provides a reference for fast and accurate resonance frequency analysis of microstrip patch antenna designs suitable for operation in the ISM band using CNN-LSTM.

2. Structure of Antenna and CNN-LSTM

2.1. F-Shaped Microstrip Patch Antenna

Geometrical dimensions of the F-Shaped Microstrip Patch Antenna (FSMPA) are given in Fig. 1. FSMPA consists of an F-Shaped patch with dimensions

$W_p \times L_p$ on a substrate with external dimensions W×L on a rectangular ground plane. F_1 and F_2 represent the two ledge lengths of the FSMPA on the patch. The mathematical relation between $W_p \times L_p$ and W×L is given in the formula below.

$$L = L_p + 10mm \quad (1)$$

$$W = W_p + 10mm \quad (2)$$

The length of the gap between the two ledge lengths is defined by W_x. The indentation length along the protrusions W_p is indicated by L_x. The designed F-shaped antenna operates on ISM (IEEE 802.11 b/g/n) 2400-2495 GHz bands. FR-4 material with relative dielectric constant ε_r =4.3 and tan δ=0.019≅0.02 is used as a substrate material to operate these bands. The thickness of the dielectric material is defined as "h" and determined as 1.6 mm.

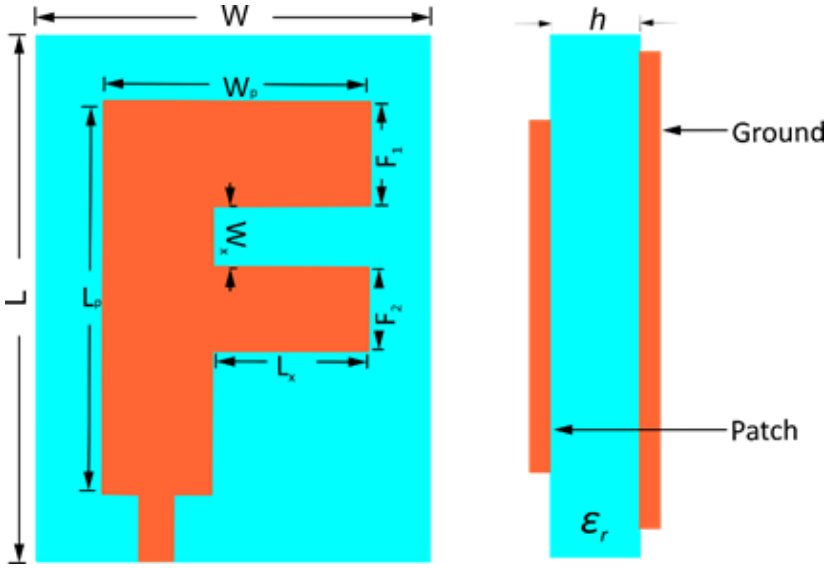

Figure 1. Dimensions of the F-Shaped Microstrip Patch Antenna

The antenna geometry of the FSMPA in Figure 1 is designed with the geometric size parameters in Table 1. The simulation results of the designed FSMPA are obtained using the HFSS 2021-R1 computer-aided simulation program. The simulation takes place on a system with an 8-core Intel Core i7 9700F processor, an Nvidia GeForce RTX 2060 graphics card with 6 GB of VRAM, and 16 GB of RAM.

Table 1. Geometrical parameters of proposed FSMPA

L	W	L_P	W_P	F_1	F_2	W_x	L_x
60mm	40mm	50mm	30mm	8mm	12mm	3mm	18mm

The values of the resonance frequency and the reflection coefficient S_{11} of the FSMPA, which were designed and simulated, are given in Figure 2. The designed antenna has a resonant frequency of 2.4168 GHz, a bandwidth of 54.7 MHz, and a return loss of -49.3572 dB between the frequencies of 2.3898 GHz-2.4445 GHz.

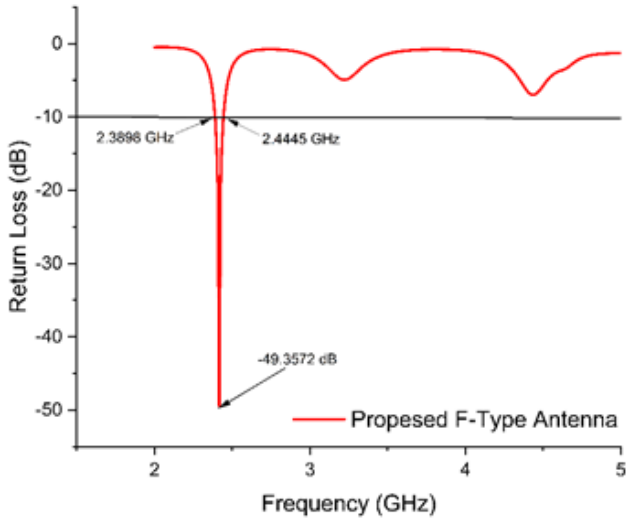

Figure 2. Resonance Frequency and Return Loss of the FSMPA

The Radiation Pattern showing the angular change of the power emitted by the FSMPA antenna in a given area at a constant distance is given with E-plane and H-plane in Figure 3.

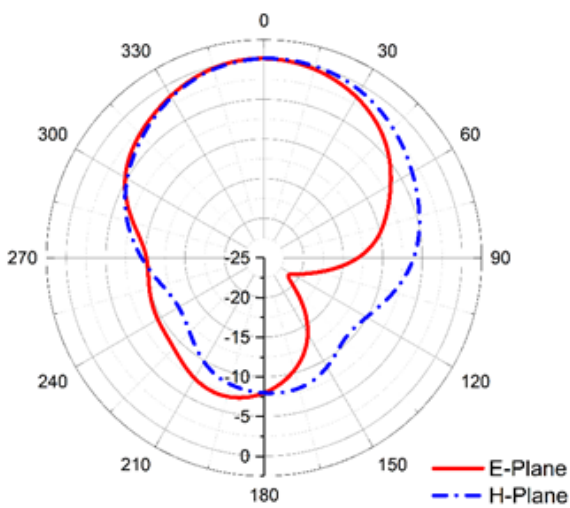

Figure 3. Radiation Pattern of the FSMPA

The graph of the designed FSMPA with gain and directivity for 2.4168 GHz is shown in three dimensions in Figure 4 and Figure 5, respectively. The antenna gain for 2.4168 GHz is 2.5 dB, and the directivity value is 4.12 dBi.

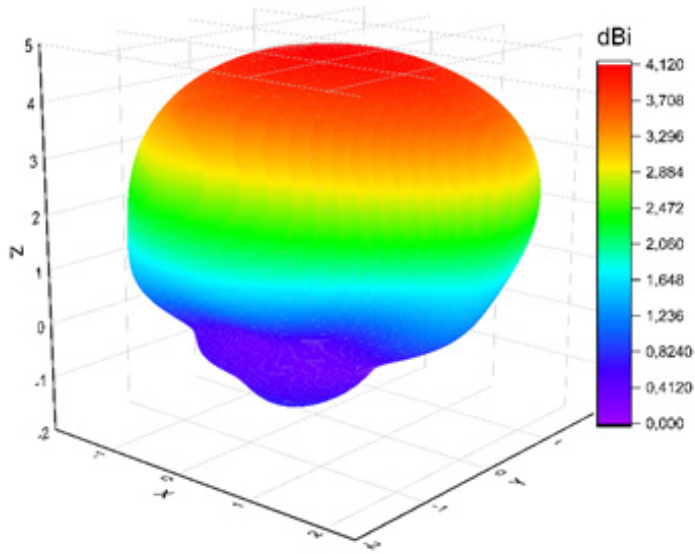

Figure 4. Directivity Gain of the FSMPA

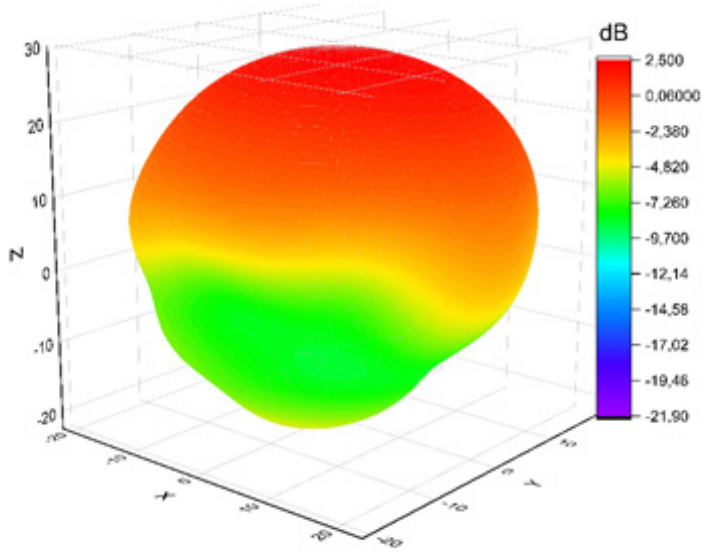

Figure 5. Gain of the FSMPA

2.2. CNN-LSTM Applications

In this study, it is aimed that the proposed FSMPA can operate in multiple frequency bands other than the ISM band. Therefore, resonance frequency results obtained from simulations using HFSS ® 3D EM are trained using Artificial Neural Networks (ANN) to obtain FSMPA geometries that can work for multiple frequency bands. As an ANN algorithm, the CNN-LSTM model, which is hybrid modeling of Long-Short Term Memory with a Convolutional Neural Network, is proposed for this study. Patch length (L_p), Patch width (W_p), two ledge lengths, the gap between the two ledge lengths (F_1 and F_2), the length of the gap between the two ledge lengths (W_x), and the indentation length along the protrusions patch width (L_x) are defined as input of CNN-LSTM. The resonant frequency is defined as the output of CNN-LSTM. The geometrical parameters of the FSMPA, defined as a dataset for CNN-LSTM training, are given in Table 2.

Table 2. Geometrical parameters of the simulated 432 FSMPAs by HFSS® 3D EM

Antenna Number	L_P (mm)	W_P (mm)	F_1 (mm)	F_2 (mm)	W_x (mm)	L_x (mm)
432	30	20	2:4:6:8	2:4:6:8	1:2:3	4:8:12
	40	25	3:6:9:12	3:6:9:12	1:2:3	5:10:15
	50	30	4:8:12:16	4:8:12:16	1:2:3	6:12:18

In Figure 6, the structure designed for the CNN-LSTM application is given. There are three Conv1D layers on the structure. The first is 128, the second is 256, and the third is 512. The MaxPooling layer, located between each Conv1D layer, filters the maximum size data between convolutions in 3x1 size. Data transformed into one-dimensional arrays in the Flatten layer is processed in the LSTM layer. LSTM has a longer resolution time than classical artificial neural network models. Dropout has been added between each LSTM layer to reduce this time and increase the matching of hidden units. Dropout values for this application are set to 0.25 for the first LSTM layer and 0.5 for the second LSTM layer. In the last step, the dense layer connects the data in the LSTM layer with the output data.

The training and testing phases are processed on Google Colab Cloud Services, having an Intel Xeon 3.2 GHz processor, 32 GB RAM, and Nvidia Tesla P100 16 GB graphics card.

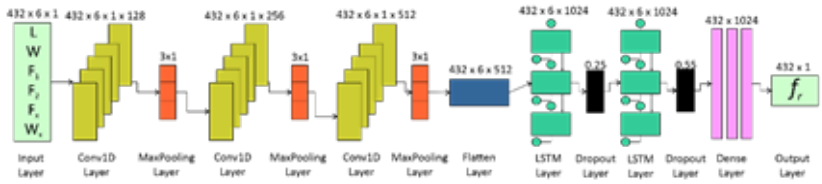

Figure 6. Topological illustration of the CNN-LSTM Application of the simulated 432 FSMPA.

3. Test and Training Results of the CNN-LSTM Application

In this section, simulation and CNN-LSTM data of 432 FSMPAs are compared. CNN-LSTM results in this study, the resonance frequency prediction criterion is based on training and test efficiency. Different physical and electrical parameters of the FSMPA can be calculated with high accuracy in a resonant frequency determined by the training section. To compare the simulation data with CNN-LSTM and decimate the numerical proximity between them numerically, some metrics are needed to evaluate the results obtained. The error analysis of the CNN-LSTM learning model as a metric is performed using the Mean Square Error (MSE) method and its general formula is given below.

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(y_i - \tilde{y}_i)^2 \tag{3}$$

Figure 7 shows the distribution of simulation data trained using the CNN-LSTM algorithm on the regression line. When 432 FSMPA data with distribution in line with the regression were examined, it was exposed that the CNN-LSTM algorithm was able to estimate the resonance frequency of the FSMPAs with an accuracy of 99,99981%.

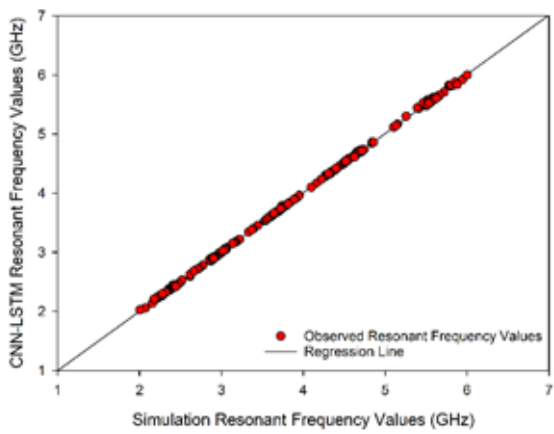

Figure 7. Regression-Based Comparison of Simulation Data and LSTN-CNN Data.

As can be seen from Figure 8, as a result of the 10942-epoch performed at the training stage, the saturation point was achieved with a minimum error value of 0.0002359259 MSE.

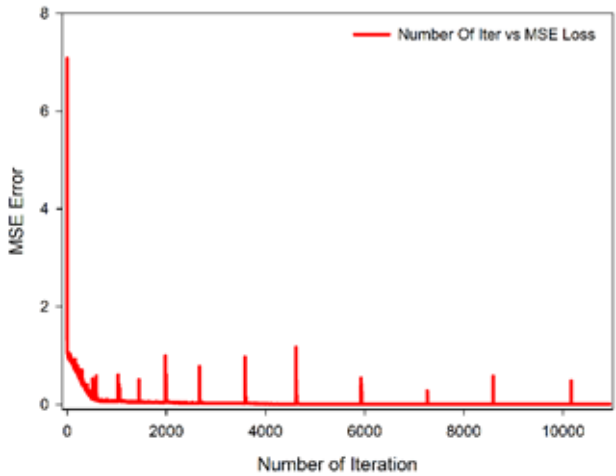

Figure 8. MSE Values in CNN-LSTM Application Iterations

After the completion of the training phase of 432 FSMPA, the CNN-LSTM algorithm is tested with the test data in Table 3. When the resonance frequency values are analyzed, prediction accuracy is calculated as 92.6706% by the CNN-LSTM test stage. The highest error rate was determined at the test stage of FSMPA No. 14 with 40.22%. The lowest error rate was determined at the test stage of FSMPA No. 12 with 0.1715%. When the Training and Test stages were compared, it was found that the accuracy rate at the training stage was 7.32921% higher than the accuracy rate at the test stage.

When Table 4 is examined, it has been determined that the CNN-LSTM application analyzes 432 antennas 914.800 times faster than HFSS™ 3D High-Frequency Electromagnetic Simulation Software in terms of time. The CNN-LSTM application can make inferences in FSKMA antennas in a short time with high prediction rates.

Table 3. Comparison of Simulation and CNN-LSTM Data for Resonant Frequency

No	L_P (mm)	W_P (mm)	F_1 (mm)	F_2 (mm)	W_x (mm)	L_x (mm)	HFSS (GHz)	CNN-LSTM (GHz)
1	30	20	2.3	7.3	2.4	7.1	3.9740	3.7438
2	30	20	2.1	4.2	1.2	4.7	3.6897	3.6690
3	30	20	2.7	3.7	1.3	8.1	3.6496	3.6618
4	30	20	4	6	1.8	5.2	3.6096	3.5889
5	30	20	6.2	5.4	2.5	7.8	3.5816	3.0735
6	30	20	7.8	5.2	2.4	11.5	3.5816	3.4812
7	30	20	3.5	3.5	2	10.8	3.1852	2.9077
8	40	25	4.7	2.3	1.1	6.3	3.5696	3.5820
9	40	25	5.1	5.8	1.7	7.1	2.8889	3.0586
10	40	25	3.1	3.7	1.7	8.2	3.1572	3.0823
11	40	25	8.7	3.1	1.2	14.3	2.5085	2.4747
12	40	25	9.6	11.3	2.3	5.2	3.6136	3.6074
13	40	25	11.6	5.2	1.7	9.6	2.9249	2.7786
14	40	25	11.8	8.8	1.1	14.8	2.8168	3.9498
15	50	30	4.1	4.8	1.5	7.1	3.0651	3.9763
16	50	30	8.7	9.2	2.6	8.2	3.1572	2.9444
17	50	30	9.7	13.7	1.7	7.2	3.1011	3.0614
18	50	30	10.6	14.1	2.6	8.7	2.4605	2.8928
19	50	30	15.4	13.8	2.8	11.7	4.7467	4.7139
20	50	30	14.1	7.6	1.2	6.1	2.9890	3.0479
21	50	30	11.1	8.3	1.9	7.8	3.1371	2.8975

Table 4. Comparison of HFSS Simulation Solution, CNN-LSTM Solution, and CNN-LSTM Training Time of 432 FSKMA

HFSS Simulation Solution Time of 432 FSKMA	CNN-LSTM Application Solution Time of 432 FSKMA	CNN-LSTM Application Training Time of 432 FSKMA
2592 Minutes	0.17 Seconds	892 Minutes

4. Conclusion

This study proposed an F-Shaped Microstrip Patch Antenna design operating at an ISM Band. According to the simulation results with HFSS™ 3D

Electromagnetic Simulation Software, the directivity gain is 4.12 dBi, the return loss of the proposed antenna is -47.25 dB, and a gain is 2.5 dB at a resonance frequency value of 2.4168 GHz. When the simulation data are examined, the proposed antenna is suitable for ISM band applications with high return loss and directivity values. Furthermore, 432 FSMPA simulations were performed for the CNN-LSTM application by deriving the proposed antenna's geometrical parameters L_p, W_p, F_1, F_2, W_x, and L_x. All 432 FSMPA data were used in the training phase. The trained CNN-LSTM application was validated on 21 FSMPA test data. As a result of the CNN-LSTM application, 99.99981% estimation accuracy was obtained in the training phase and 92.6706% in the testing phase. When the simulation times are compared with the CNN-LSTM application, the CNN-LSTM application provides approximately 9.148,235 times faster than the computer-aided electromagnetic simulation application HFSS. As a result of these data, the CNN-LSTM application is seen as an alternative to computer-aided simulation programs with its relatively high-power consumption, waiting times, and cost. In addition, it can be used to speed up resonance frequency calculations in patch antennas.

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CHAPTER III

PD-DEEPNET: VOICE ANALYSIS FOR PARKINSON'S DISEASE EARLY DETECTION VIA DEEP NEURAL NETWORK

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1. Introductıon

It is impossible to manage diagnostics only using human power in the health sector. Machine learning techniques, which are a type of artificial intelligence, are commonly used in medical predictions (Avci et al., 2016). PD is a neurological condition that causes a partial or total loss of motor reflexes and speech, as well as affects thinking, behavior, and other important nervous system processes. PD deteriorates speech and motor skills (writing, balance, etc.) in about 90% of its patients and is commonly found in the elderly. More

than 10 million people worldwide suffer from Parkinson's disease, according to the Parkinson's Foundation (Statistics, 2021). Many patients and their families regard dysphonia as one of the most painful features of Parkinson's disease. Approximately 9 out of 10 patients with Parkinson's disease have a speech or vocal issue (Avci et al., 2016). Speech signals can be used to classify between PD and healthy people due to speech abnormalities that arise during sickness. This is an effective method for diagnosing Parkinson's disease.

With the rise in Parkinson's disease cases, the difficult process of diagnosing the disease has become easier thanks to technological advancements and the application of new machine learning algorithms. Machine learning algorithms have been employed to treat Parkinson's disease in the literature due to their ease of use and high accuracy (Karapınar, 2020). About 90% of persons with this disorder have speech difficulties, which are one of the most common early-stage symptoms (Little et al., 2009).

Speech signals are recorded and subsequently, certain aspects are determined in different ways in investigations of early diagnosis of PD patients using voice analysis. Then, based on certain signal features, a classifier is used to diagnose PD patients. Although there are many uncontrolled variations, an effective classifier should diagnose the condition with as much precision as possible (Little et al., 2009).

1.1. Related Works

In Table 1, previous studies are analyzed and summarized in terms of feature extraction method, classification method, number of personal data used in the study, and classification accuracy. Many studies on speech assessment for voice abnormalities, in general, have been published in the literature (Karapınar, 2020; Little et al., 2009; Sakar et al., 2019).

The previous studies are examined in terms of dataset size, it is seen that the other studies, except for 8 research (Vital et al., 2021; Yücelbaş,2020; Polat et al., 2020; Majda et al., 2021; Almeida et al., 2019; Yaman et al., 2020; Tuncer et al., 2020) others contain 40 or less than 40 people's data (Sakar et al., 2010; Gürüler, 2017; Das, 2010; Peker et al., 2015; Sakar et al., 2017; Cai et al., 2017; Benba et al., 2016; Berus et al., 2019; Mittal et al., 2021; Wrobel, 2021; Benba et al., 2017; Tai et al., 2021). The success, reproducibility, and reliability of disease detection and classification studies in the field of health are only possible if they are developed with datasets with a high number of people.

Table 1. Performance of classification methods

References	Feature Extraction	Classification Method	Subject	Accuracy (%)
Karapınar, 2020	FS	SVM	31 *(PD:23, H:8)*	93.84
		ANN		91.54
Little et al., 2009	PPE	Kernel-SVM	31 *(PD:23, H:8)*	91.4
Sakar et al., 2019	Baseline, intensity, Mel Frequency Cepstral Coefficients (MFCC), Formant, Bandwidth, Vocal fold, Wavelet features and TWQT	RBF-SVM	252 *(PD:188, H:64)*	86
Vital et al., 2021	PNN	KNN	113 *(PD:62, H:51)*	98.85
		SVM		71.93
		RF		98.64.
		Naive Bayes		83.03
Yücelbaş, 2020	SLGS	SL	252 *(PD:188, H:64)*	88.71
Polat et al., 2020	OGA	LR	80 *(PD:40, H:40)*	84.3
		SVM		88.76
		KNN		89.46
Majda et al.,2021	LFCC	SVM	88 *(PD:44, H:44)*	95
Almeida, 2019	DET	KNN	99 *(PD:64, H:35)*	94.55
Yaman et al,2020	Relief	SVM	80 *(PD:40, H:40)*	91.25
Tuncer et al., 2020	Mam's	KNN	252 *(PD:188, H:64)*	**0.92**
		Linear SVM		0.84
		RBF SVM		0.85
		Log-Reg		0.84
		KNN		**0.92**
Tuncer et al., 2020	Mutual Information+mRMR	SVM	32 *(PD:24, H:8)*	92.75
Gürüler, 2017	KMCFW	CVANN	31 *(PD:23, H:8)*	99.52
Das, 2010	SAS	NN	31 *(PD:23, H:8)*	92.9
Peker et al., 2015	mRMR (ASA)	CVANN	31 *(PD:23, H:8)*	98.12

Sakar et al., 2017	UPDRS	Kernel-SVM	50 *(PD:42, H:8)*	-
Cai et al., 2017	RFS	BFO-SVM	31 *(PD:23, H:8)*	97.42
Benba et al.,2016	MFCCs	Kernel-SVM	40 *(PD:20, H:20)*	82.5
Berus et al., 2019	A-MCFS	ANN	40 *(PD:20, H:20)*	86.47
Mittal et al., 2021	PCA	SVM	40 *(PD:20, H:20)*	89.36
		KNN		90.3
Wrobel, 2021	SBS	SVM	32 *(PD:24, H:8)*	87.5
		DT		90.6
		KNN		90.6
		Naïve Bayes		90.6
		RF		87.5
Benba et al.,2017	HFCC	KNN	40 *(PD:20, H:20)*	87.5
Tai et al., 2021	PCA	SVM	36 *(PD:20, H:16)*	88
		LR		71
		RF		82
Ramzi et al.,2019	FS	ANN	31 *(PD:23, H:8)*	100
Ma et al., 2021	L1-RFS	SVM	40 *(PD:20, H:20)*	98.02
Lahmiri et al., 2018	PEM (Performance Evaluation Method)	SVM	195 *(PD:147, H:48)*	92.9

1.2. Contribution and Novelty

One of the biggest problems of current studies in the literature is the limited data use in studies. In addition, it is seen that basic ML algorithms are used in the studies. In this study;

- A large voice dataset collected from 252 participants was used (Sakar et al., 2019).
- The most efficient 256 out of 752 feature sets were determined by the NCA feature selection method.
- With the developed one-dimensional deep neural network (DNN) model, classification success has been increased compared to the literature.

The remainder of the article is divided into three main sections. The Materials and Methods section contains detailed information about the PD dataset, feature

set, feature selection, and deep neural network architecture. In the experimental results and discussion section, performance metrics and confusion table are presented and the results are compared. Finally, the important outputs of the research are summarized in the "Conclusion" section.

2. Material and Method

In this study, the processes carried out in the method designed with deep learning in order to increase the accuracy of the diagnosis of Parkinson's disease are explained as Figure 1.

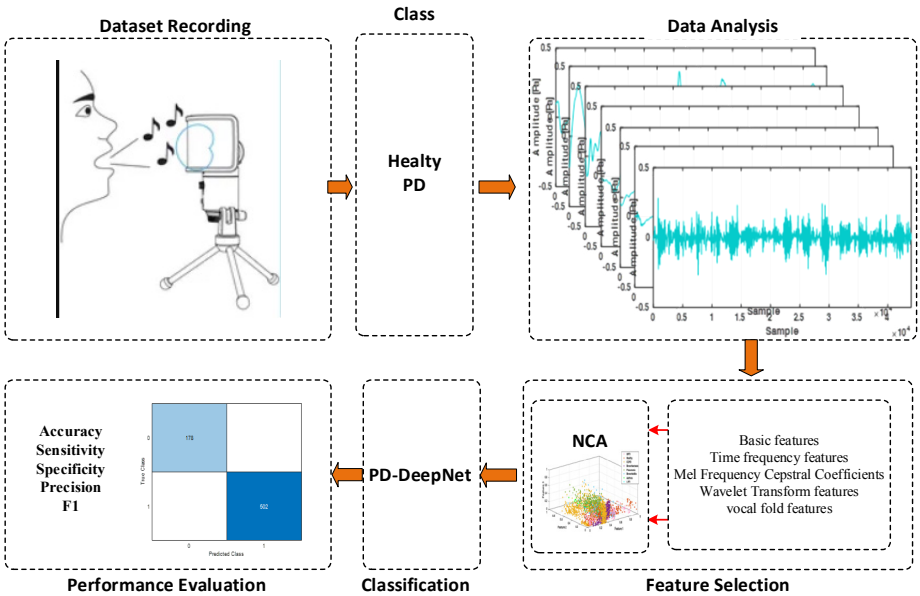

Figure 1. Methodical flow chart of the study

2.1. Parkınson's Dısease (PD) Dataset

The Parkinson's disease dataset, was recorded by Sakars et al. 2019, used in this study. In total, voice data were collected from 252 subjects. These subjects consisted of 188 Parkinson's disease patients (107 men and 81 women) aged between 33 and 87 years, and 64 healthy individuals (23 men and 41 women) aged between 41 and 82 in the Neurology Department of Istanbul University Cerrahpasa Faculty of Medicine. The microphone was adjusted to 44.1 kHz during the data collection process, and each subject's continuous phonation of the vowel 'a' was collected three times after the doctor's examination (Sakar et al., 2019).

2.2. Features and Feature Selection With NCA

The PD dataset used in this study includes voice data collected from 252 subjects in total (Sakar et al., 2019). These data were collected from 188 Parkinson's disease patients (107 men and 81 women) aged between 33 and 87 years and 64 healthy individuals (23 men and 41 women) aged between 41 and 82 by specialists in the Neurology Department of Istanbul University Cerrahpaşa Faculty of Medicine. Data collection was done with a microphone at a sampling rate of 44.1 kHz. During data collection, each subject was asked to vocalize the vowel 'a' three times and the data were recorded (Sakar et al., 2019). 752 features were extracted from the collected audio data (Table 2). Methods used in feature extraction; baseline, intensity, Mel Frequency Cepstral Coefficients (MFCC), Formant, Bandwidth, Vocal fold, Wavelet properties, and TWQT properties (Sakar et al., 2019).

Table 2. Features in Sakar's dataset (Sakar et al., 2019)

Main Features	Sub-Features	Account of featuressı
Key features	Jitter variants	23
	Shimmer variants	
	Fundamental frequency parameters	
	Harmonicity parameters	
	Recurrence Period Density Entropy (RPDE)	
	Detrended Fluctuation Analysis (DFA)	
	Pitch Period Entropy (PPE)	
Time frequency features	Intensity Parameters	10
	Formant Frequencies	
	Bandwidth	
Mel frequency cepstral coefficients	MFCCs	84
Wavelet transform based features	Wavelet transform (WT) features related with F0	181
	TQWT Features	431
Vocal fold features	Glottis Quotient (GQ)	23
	Glottal to Noise Excitation (GNE)	
	Vocal Fold Excitation Ratio (VFER)	
	Empirical Mode Decomposition (EMD)	

2.3. NCA Based Feature Selection

On the retrieved features, NCA was used to choose discriminative features. NCA is one of the most used feature selection techniques. The significance of each attribute in NCA is described using these weights. The NCA uses an exponential kernel function and a Manhattan distance-based fitness function. All feature weights are given a fixed positive integer number at the beginning (usually one). Utilizing stochastic gradient descent, weights are modified (SGD). Characteristics with a positive weighting are generated by NCA. The fact that each feature is given a positive weight is the NCA algorithm's biggest benefit. The NCA algorithm is detailed in Equation (1) (Yaman et al., 2021).

$$S = \{(x_1, y_1), \ldots, (x_i, y_i), \ldots, (x_N, y_N)\} \tag{1}$$

It is a multi-class classification with N observations in the training set. where c denotes the number of classes, $y_i \in \{1, 2, 3, \ldots, c\}$ represents the class labels, and x_i is the feature vectors. It is conceivable that the NCA procedure, which employs random selection, will use all S elements as a reference point. The probability $P(Ref(x) = x_j \mid S)$ of choosing point x_j from S as the reference point for x is higher if x_j is closer to x as measured by the distance function d_w. d_w is explained in Equation (2).

$$d_w(x_i, x_j) = \sum_{r=1}^{p} w_r^2 \left| x_{ir} - x_{jr} \right| \tag{2}$$

In Equation 14 w_r are the feature weights. $P(Ref(x)$ is explained in Equation 3. When $d_w(x_i, x_j)$ is tiny, a kernel or similarity function with the value k given in Equation (3) takes high values.

$$P(Ref(x) = x_j \mid S) = \frac{k(d_w(x, x_j))}{\sum_{j=1}^{n} k(d_w(x, x_j))} \tag{3}$$

With 752 features, Sakar's PD dataset was utilized to pick the most discriminative 256 features with the NCA feature selector. Experiments were used to determine how many features to provide. A total of 256 characteristics were employed to arrive at the most accurate results.

2.4. Classification methods

For the sound detection of Parkinson's disease, a deep neural network (DNN) architecture was used and the results were compared with the five most commonly used basic machine learning algorithms (LD, NB, DT, DVM, RF) in the literature.

2.4.1. Deep Neural Network Algorithm (PD-DeepNet)

A one-dimensional Deep Neural Network (DNN) model was created for the classification of the PD sound feature dataset. The network consists of 7 layers in total. The input layer is the layer where the selected features are entered. Data were normalized using z-score normalization at the input layer. Next, a fully connected layer with an output size of 50 was added, followed by a bulk normalization layer and a ReLU layer. The bulk normalization layer stabilizes the learning process and significantly reduces the number of training cycles required to build deep networks (Rosario et al., 2013). Table 3 gives a breakdown of the hyperparameter of the classifiers.

Table 3. Hyperparameters using PD-Deep Neural Network

PD-Deep Neural Network
Feature Input Layer (numFeatures,'Normalization', 'zscore')
Fully Connected Layer: 50
Batch Normalization Layer
Relu Layer
Fully Connected Layer: numClass
Softmax Layer
Classification Layer

2.4.2. Traditional ML Methods

Support Vector Machines (DVMs) are learning machines that use the inductive principle of Structural Risk Minimization (SRM) to achieve a high level of generalization over a small number of learning models. SVM is an effective learning method for identifying patterns in complex datasets that are difficult to evaluate (Pal et al., 2010). The support vector machine algorithm looks for a hyperplane in N-dimensional space (N — number of features) that clearly classifies the data points. There are many possible hyperplanes that can be used to separate classes of data points. The aim is to find planes with the largest margin, that is, the largest distance between data points in each class (Pal et al., 2010). A supervised machine learning technique called a decision tree (DT) continuously separates data based on a given parameter. Two entities can be used to describe the tree: decision nodes and leaves. Decision trees are one of the most widely used methods in classification models. Because it is a simpler technique to configure and grasp, it gives the model transparency and has a visual presentation (Yaman et al., 2021). The K-Nearest Neighbor (KNN) method is a non-parametric classification algorithm. The KNN model is easy as it is based on basic mathematical foundations. And it is widely used in many

industries. The basic principle is based on the assumption that the class of an unknown variable will be the same as that of its nearest neighbors. The average of the current states of the k nearest elements in the training dataset is used to calculate the prediction result. The number of neighbors is indicated by the letter "k" in the method name. The k number is very important when it comes to determining the optimum classification or estimation. It can use trial-and-error or cross-validation approaches to choose the correct number k (Yiğiter et al., 2018). The data class is determined by averaging the k data points calculated as the closest distance to the training set. The threshold value is calculated before the found value is interpreted. Linear Discriminant (LD) is to describe a relationship between a categorical dependent variable and more than one independent variable. Two-group problems in LD analysis are relatively easy. To distinguish subjects between two groups, a linear discriminant function is defined passing through the means of the two groups. When there are more than two groups, a function definition is made for the classification problem minus the number of groups. Linear discriminant analysis is evaluated separately for each group. Explanatory variables are assumed to have a normal distribution with equal covariance matrices. In each case, the estimated coefficient for an independent variable is multiplied by the event's score on that variable, and these results are added to the constant. The result gives the discriminant score for the condition. The Random Forest (RF) model is a method of creating a decision ensemble (forest) consisting of multiple decision trees. The RF model is a combination of hundreds of decision trees, and to obtain a comprehensive result, the decision results from all trees are evaluated by the majority voting method to produce the final result of the decision tree (Raizada et al., 2013). Table 4 gives a breakdown of the hyperparameter of the classifiers.

Table 4. Design parameters used in ML classification algorithms

DT	**KNN**	**DVM**
Type: Fine	Type: Fine	Type: Linear
SplitCriterion:Deviance	Distance : Cityblock	Standardize :True
Maximum Splits: 100	Number of Neighbors : 1	Kernel Fonk : Polynomial
Surrogate: off	Distance Weight : Equal	Polynomial Order : 3
	Standardize: True	Kernel Scale: Auto
		Box Constraint Level : 1
LD	**NB**	
Multiclass method : One vs One	Kernel Fuction: Gaussion	
İteration limit: 1000		

2.5. Performance Evaluatıon Methods (PEM)

We used various standard evaluation methodologies such as sensitivity, precision, accuracy to compare the performance of machine learning algorithms for early detection of PD. The performance evaluation criteria used are given in Table 5 (Yaman et al., 2021). An estimate is made for performance metrics for true positive (TP), true negative (TN), false positive (FP), and false negative (FN).

Table 5. Performance evaluation metrics used in this study

Performance Metric	**Acronym**	**Equation**
Positive Predictive Value	PPV Precision	$\frac{TP}{TP+FP}$
Negative Predictive Value	NPV	$\frac{TN}{TN+FN}$
True Positive Rate	TPR Sensitivity	$\frac{TP}{TP+FN}$
True Negative Rate	TNR Specificity	$\frac{TN}{TN+FP}$
Single class Accuracy	ACC	$\frac{TP}{TP+FN}$
Multi-class Accuracy	ACC	$\frac{TP+TN}{TP+TN+FP+FN}$
F1-Score	F1	$2x\frac{PPV*TPR}{PPV+TPR}$

3. Experimental Results and Dıscussıon

A computer with 3.60 GHz Intel i7-7700 CPU, Windows 10, and 16 GB RAM was used in the study. The Matlab 2020 program was used to implement the proposed methodological approach. 10% cross-validation of this dataset was applied. And by running each classification algorithm 100 times, the best, minimum, mean, and standard deviation values for accuracy, sensitivity, specificity and sensitivity values were calculated and presented in Table 6.

Table 6. Performance of classification methods

Classification	Metric	Accuracy	Sensitivity	Specificity	Precision	F1
LD	**Best**	84.656	79.586	80.956	80.606	80.265
	Min	81.216	75.219	75.731	74.906	75.474
	Average	82.746	77.200	77.882	77.248	77.539
	Std	0.681	0.880	0.8770	0.954	0.854
KNN	**Best**	94.179	92.699	91.805	91.678	92.250
	Min	91.931	90.324	89.284	89.009	89.971
	Average	93.099	92.528	90.979	90.793	91.747
	Std	0.473	0.274	0.286	0.299	0.270
DT	**Best**	83.068	77.679	77.487	76.653	77.583
	Min	76.984	69.827	69.919	67.741	70.156
	Average	79.640	73.162	73.065	71.813	73.111
	Std	1.174	1.546	1.479	1.693	1.467
SVM	**Best**	90.211	88.832	84.851	84.149	86.653
	Min	87.698	84.811	81.621	80.440	83.185
	Average	89.230	87.347	83.254	82.359	85.250
	Std	0.460	0.679	0.712	0.809	0.649
RF	**Best**	87.830	83.400	86.425	86.400	84.765
	Min	83.730	78.429	81.366	81.224	79.870
	Average	85.521	80.581	83.719	83.634	82.119
	Std	0.829	0.994	1.054	1.080	1.002
NB	**Best**	78.703	71.745	70.090	68.006	70.738
	Min	76.719	68.935	67.564	64.804	68.242
	Average	77.706	70.336	68.826	66.415	69.573
	Std	0.412	0.586	0.556	0.682	0.560
PD-DeepNet	**Best**	100	100	100	100	100
	Min	99.705	100	100	100	100
	Average	99.926	100	100	100	100
	Std	0.103	0	0	0	0

In the PD early diagnosis study, LD, KNN, DT, DVM, RF, NB and DNN classification methods were obtained as 82.746%, 93.099%, 79.640%, 89.230%, 85.521%, 77.706%, 99.926%, respectively. The highest success was obtained with DNN and KNN methods. In addition, confusion matrices were obtained for each classifier showing the number of correct and incorrect predictions of our classification model and are given in Figure 2.

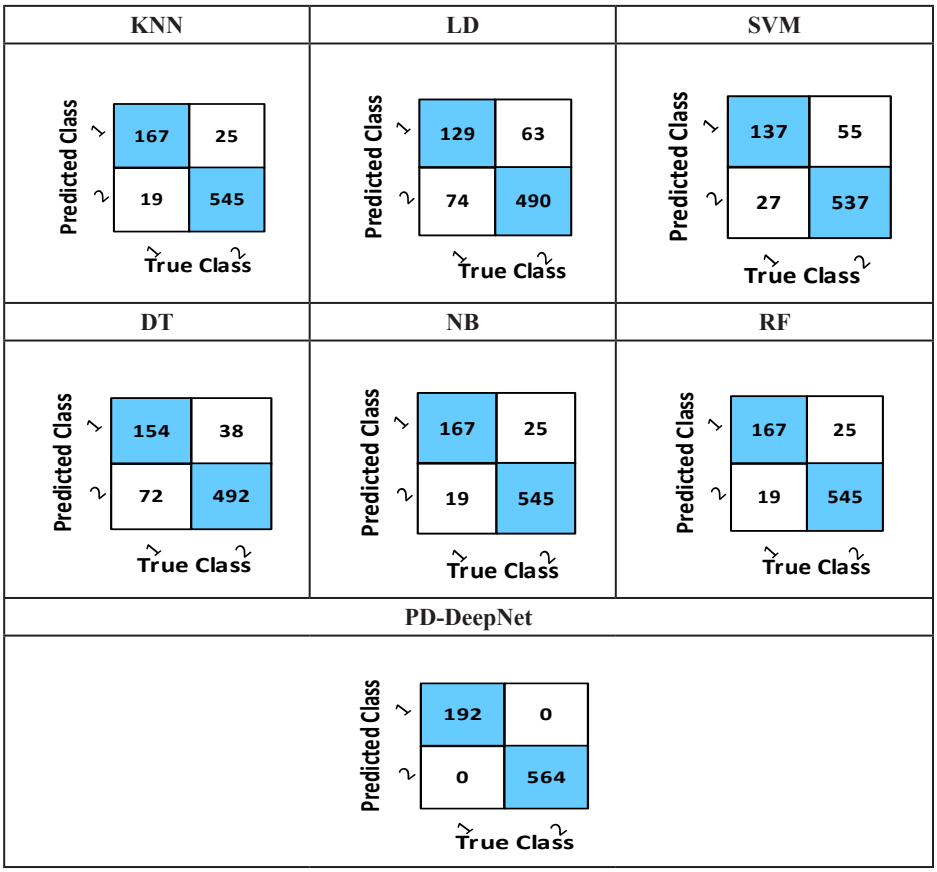

Figure 2. Confusion matrices of classification

The performance of the proposed method was demonstrated by Sakar (Sakar et al., 2019) using the same dataset. (Sakar et al., 2019) and (Tuncer et al., 2020). It was compared with the studies of al. (Table 7). Sakar (Sakar et al., 2019) and Tuncer (Tuncer et al., 2020) used KNN, Log-Reg, linear SVM, and RBF SVM classifiers as classification algorithms in their studies. Sakar (Sakar et al., 2019) achieved the highest success with the RBF–DVM method with 86% accuracy. Tuncer, on the other hand, has the highest success rate with the KNN classifier, with an accuracy of 92%. In this study, the highest success was obtained with the DNN method with an average accuracy of 99.926%.

Table 7. Performance comparison of classification methods

Reference	Classification Method	Accuracy (%)	F1 Score
Sakar [5]	KNN	0.84	0.83
	Linear DVM	0.83	0.82
	RBF DVM	0.86	0.84
	Log-Reg	0.85	0.84
Tuncer [12]	KNN	0.92	0.90
	Linear DVM	0.84	0.82
	RBF DVM	0.85	0.78
	Log-Reg	0.84	0.78
In this study	PD-DeepNet	99.926	100

Table 7 shows that the DNN method proposed in this study is 13.926% higher than the method with the highest accuracy suggested by Sakar, and 7.926% higher than the method suggested by Tuncer. Therefore, our proposed methodical approach has shown to be more effective in detecting PD. The developed methodical approach has proven to be highly successful, which can be a decision support mechanism that helps experts in clinical practice.

The advantages of the proposed method are:

- The most basic features with less computational load are selected and calculated. Of all the features, the most distinctive 256 features were selected using the NCA method.
- Although there is not the same number of data for each person in the data set, that is, the data are heterogeneous, high classification performance has been achieved with the proposed approach.
- The proposed method can be used to support specialists in the clinical setting as it is computationally faster and easier and has a high success performance.
- Weight update or meta-heuristic optimization is not used in the proposed method. High classification ability is achieved without adjusting any parameters. Therefore, the system is not parametric.
- The disadvantage of this study is that even when the largest PD dataset in the literature is used in the study, this data includes 252 people and is considered small to develop reliable and reproducible machine learning algorithms.

3. Conclusion

Table 7 shows that the DNN method proposed in this study is 13.926% higher than the method with the highest accuracy suggested by Sakar and 7.926% higher than the method suggested by Tuncer. Therefore, our proposed methodical approach has shown to be more effective in detecting PD. The developed methodical approach has proven to be highly successful, which can be a decision support mechanism that helps experts in clinical practice. All of the disease detection decision support software/platforms in the field of health are still in the laboratory-scale testing phase, they are not actively implemented in hospitals. There is no commercialized software for detecting PD from audio data yet. However, we will continue to work on converting the proposed method into a phone application. Thanks to our software, people will have a simple preliminary PD diagnosis kit in daily life by using their phone microphones, so it will be quickly applicable to life. Thanks to this application, if the person is determined as PD as a result of the application, an incentive will be created for them to apply to the experts. Early diagnosis will pave the way.

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CHAPTER IV

AN OVERVIEW OF BIODEGRADABLE POLYMERS FOR FOOD PACKAGING APPLICATIONS

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1. Introduction

In order for human beings to survive, they need to consume food constantly, and food consumption is increasing rapidly with the increasing world population. After producing food in the desired quality and quantity, the packaging, storage, transportation, and sale of the produced food constitute the most critical steps of the food safety chain. Packaging plays an essential role in delivering the food to the consumer economically and reliably without spoiling, protecting the food product inside, increasing the durability of the food product, facilitating its promotion, and providing ease of use in terms of loading, unloading and storage. Food packaging material also acts as a barrier against moisture and gases (CO_2, O_2) and flavor, minimizes mechanical damage and may even have antimicrobial properties to improve the shelf life of foods (Rocha et al., 2018). Therefore, it is necessary to use suitable packaging materials to protect the food from production until it is consumed by the end user.

In general, the package can be made from different materials such as glass, tin and wooden materials, as well as paper of various types, thicknesses and weights, cardboard, aluminium foil and plastics, or flexible foils obtained from the lamination with each of these products, and coextrusion of plastics. Plastics produced from petroleum-derived materials are the most used materials in conventional food packaging. Plastics, defined as synthetic or semi-synthetic

organic polymers with good barrier properties against moisture, oil and gases, improved strength properties, and good resistance to stress and corrosion, are also known to be cheap, lightweight, and easy to carry and handle (Derraik, 2002; Gupta et al., 2022). The usage of plastics for manufacturing food packaging materials for daily use has incredibly increased. Various types of plastic food packaging such as wraps, films, coatings and bottles have emerged rapidly since the early 1900s.

Plastic films are packaging materials that are lightweight, transparent, unaffected by biological agents, resistant to chemical substances and atmospheric conditions, printable, resistant to corrosion, unbreakable and unaffected by temperature changes between 60 and 200 °C. Plastic films, which have a thin and elastic structure, are the most widely used plastic packaging materials due to their low permeability, transparency, and smooth surface. The films used as packaging materials in the industry are generally produced from polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET) and polyamide (PA), and they can be used alone or as a mixture depending on the characteristics of the product.

It is known that more than 35 million tons of waste from various food plastic products are produced worldwide every year. The immediate disposal of used food packaging constitutes the largest share of the total plastic waste generated (Barone at al., 2021). Unfortunately, only 7% of this plastic waste is recycled, and the rest poses a threat to the environment (Porta, 2017). Most plastic products such as coatings, plastic bags, cutlery and packaging materials used in the food industry are single-use packages, and unconsciously throwing these products into nature causes severe environmental pollution. These synthetic solid plastic materials take hundreds of years to degrade after their end-use. Since they are resistant to microbial degradation, their accumulation in ecosystems seriously affects environmental pollution, human health and climate change (Madera-Santana et al, 2014; Shankar and Rhim, 2015). In addition, the availability of limited petroleum resources that could be used for other necessary usages is in danger due to the increased demand for packaging applications. The packaging industry faces significant challenges due to the limited availability of petrochemical resources, rising raw material prices, and the persistence of these materials in the environment beyond their practical uses. Despite the advantages of traditional food packaging, such as being abundant, inexpensive, durable and convenient, these significant disadvantages of conventional plastic packaging materials have shifted the attention to new solutions for producing renewable, environmentally friendly and recyclable

biodegradable food packaging materials (Shankar and Rhim, 2015; Moreno et al., 2015).

Proteins and polysaccharides, renewable raw materials from marine, agriculture and animal sources, are known as biodegradable materials. Biodegradable polymers degrade into CO_2, CH_4, biomass, water, humus and other natural substances in the bioactive environment due to the optimum soil moisture, oxygen, and enzymatic activities of microorganisms such as algae, bacteria and fungi, and thus do not cause any environmental problems (Rocha et al., 2018; Gupta et al., 2022). Due to environmental concerns, many biodegradable polymers have been developed to date, such as polyhydroxyalkanoates (PHAs), poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL), poly(butylene adipate-co-terephthalate) (PBAT) and poly(butylene succinate) (PBS), which can be easily converted into small molecules (water, carbon dioxide, etc.) by open media or microorganisms. While biopolymers are reported to biodegrade within a few days or months, petroleum-based plastic materials are known to take more than 30 years to biodegrade (Yamada-Onodera et al., 2001).

Recently, there has been a growing interest in using sustainable natural polymers to produce environmentally friendly food packaging materials with no adverse impact on human health and the environment. This chapter examines the types, properties and detailed studies of renewable, biodegradable, biocompatible and eco-friendly biopolymers used as packaging materials.

2. Biodegradable polymers

The biodegradability of a polymer molecule mainly depends on its chemical structure, physical properties, source, and environmental conditions. Degradable plastics significantly change physicochemical properties such as shape, color, and mechanical strength under several environmental conditions such as humidity, heat, light or biological activity. Under these effects, breaking the bonds in the plastic structure and the occurrence of various chemical transformations may cause a decrease in the construction and functionality of the plastics (Kumar et al., 2011). The degradation of polymers can be examined in 4 ways: hydrolytic degradation, photodegradation, oxidative decomposition and composting. The breakdown of polymers can occur in three stages; biodeterioration, fragmentation by biotic or abiotic reactions, and bio assimilation by microorganisms. Biodegradable polymers can be categorized as i) natural biopolymers such as polysaccharides or proteins, ii) bacterial polymers such as PHAs, and iii) a mixture of natural biopolymers with synthetic polymers (e.g., PE) (Rocha et al., 2018; Gontard et al., 1992).

2.1 Biomass-derived biodegradable polymers

The search for new materials in the food packaging industry has shifted the studies to the production of biodegradable materials from renewable natural polymers, such as polysaccharides (e.g. starch, cellulose, chitosan), proteins (e.g. gelatin, collagen, zein) and lipids. These natural biopolymers can be easily obtained from microorganisms, algae or agricultural products such as potatoes, corn, and soybean. Generally, polysaccharides are used as gas barriers, while water transmission can be reduced by lipids, and mechanical stability can be achieved with proteins. However, the use of natural polymers alone as film or coating materials has been restricted due to their low thermal and mechanical strength, higher water solubility and poor barrier properties, and various strategies such as the chemical modification or blending with synthetic polymers have been developed to overcome these disadvantages (Kumar et al., 2011; Gupta et al., 2015).

2.1.1. Polysaccharides

Food packages derived from low toxicity polysaccharides are edible and degradable and have good thermal and mechanical strength and barrier properties. However, their highly hydrophilic character increases their water solubility, and the brittleness of their films restricts their usage in food packaging applications. The main polysaccharides used to create edible food packaging are starch, cellulose and derivatives, alginate, chitosan, agar, pectin and carrageenan. They consist of repeats of the same or more monosaccharides and exist in linear or branched form. Depending on the chemical functionality of the monosaccharides, they can be in a neutral, negatively or positively charged condition, which directly influences the type of and strength of the intermolecular bonds between the polymer chains and, therefore, the final properties of the packaging material.

2.1.1.1. Cellulose

Cellulose is the most common, abundant, and economical natural polymer in the world and a promising packaging material due to its cost-effectiveness and biodegradability. However, its highly hydrophilic character, high crystal structure and low solubility create difficulties in the production of packaging materials. The hydroxyl side chains in the structure of cellulose cause low moisture barrier properties in cellulose-based packages, and this causes the brittleness of the films obtained from cellulose with a highly crystalline structure. Cellulose acetate, one of the cellulose derivatives, shows good clarity, gloss, printability,

and dimensional stability (Rocha et al., 2018; Kumar et al., 2011). Cellulose acetate films are puncture-resistant, but they can be easily torn. However, the use of cellulose acetate in food packaging is limited due to having poor gas and moisture barrier properties. To use cellulose derivatives as bioplastics, economical and practical process technologies should be developed.

Cellulose is a linear polymer of covalently linked hydroglucose units with β-1,4-glycoside bonds. Derivatives of cellulose can be obtained by chemical modification of cellulose via introducing chemical groups such as carboxyl, methyl or hydroxypropyl onto the hydroxyl groups of β (1–4) glycosidic units, and they have improved properties depending on the type and degree of derivatization. Among cellulose derivatives, methylcellulose (MC) and hydroxypropyl methylcellulose (HPMC) have good film-forming properties and mechanical strength and exhibit thermogelation properties. HPMC forms odorless, tasteless, transparent, water-soluble, and oil-resistant films with medium flexibility, making them promising candidates for edible food packaging materials. Although both MC and HPMC have a nonionic nature, linear structure and high solubility, MC films with good oxygen barrier properties are known to be stronger than HPMC films (Akhtar et al., 2013). Moreover, cellulose derivatives such as methylcellulose, hydroxypropyl cellulose (HPC) and ionic carboxymethyl cellulose (CMC) are widely used in edible coatings.

2.1.1.2. Starch

Starch, along with cellulose, is one of the most common natural polymers found in nature. It can be used as an alternative to petroleum-derived materials because being cheap and degradable in nature. Starch is a heterogeneous substance consisting of two different macromolecules: amylose, a linear chain of d-glucose, and amylopectin. The branched structure and high molecular weight of amylopectin reduce the mobility of the polymer chains (Tzia et al., 2016). Starch has a very complex microstructure, and it is difficult to process alone due to the higher melting point of starch compared to its thermal decomposition temperature. Therefore, plasticizers can be used as auxiliary agents to weaken the strong hydrogen bonds between the molecular chains of starch and to lower the melting point. Thermoplastic starch with low glass transition temperature obtained in the presence of plasticizers can be easily processed by conventional methods.

Odorless and transparent biodegradable films can be obtained from abundant, cost-effective and renewable starch, but the hydrophilicity, brittleness, poor water-barrier properties, low oxygen permeability and low mechanical

properties of these films limit the widespread use of starch-based food packaging materials (Cano et al., 2014). Muscat et al. reported that the mechanical and oxygen-barrier properties of the biodegradable films obtained from starch with high amylose content are improved compared with native starch due to the linear nature of amylose, which tends to crystallize and is insoluble in water (Muscat et al., 2013). However, the transparency, flexibility and strength of these films are insufficient to be used as a packaging material, and the undesirable properties of these starch-based films must be enhanced by chemical, physical or enzymatic modification of starch, crosslinking of starch or blending starch with different biopolymers.

2.1.1.3. Chitosan

Chitosan is a hydrophilic polysaccharide obtained from chitin, which is the main component of the exoskeletons of crustaceans such as shrimp, hermit clam, oyster and lobster, by the application of chemical processes including demineralization, deproteinization, decolourization and deacetylation stages. The copolymer produced as a result of the alkaline deacetylation process based on the removal of acetyl groups from the molecular structure of chitin is defined as chitosan. Since the N-deacetylation process has not fully occurred, chitosan is considered a partial N-deacetylated product of chitin. The solubility and activity of chitosan vary depending on its biological source, molecular weight and degree of deacetylation (Rocha et al., 2018; Elsabee et al., 2013).

The biocompatibility, non-toxicity, bioadhesiveness, excellent film-forming properties, biodegradability, antibacterial effect, excellent workability and extraordinary mechanical properties of chitosan allow it to be used as a film-forming and coating material. It has been determined that chitosan coatings inactivate the tyrosinase enzyme that causes rot, delaying the decay or deterioration of vegetables and fruits such as tomatoes and strawberries, thus prolonging their shelf life. Chitosan can also be used in film form as a food preservative for controlling psychotropic pathogens in fresh, processed or packaged meat and fish products. Membranes prepared with chitosan have moderate water permeability, low oxygen, nitrogen and carbon dioxide permeability and antimicrobial properties, which provide severe advantages in terms of use in packaging. Moreover, it has recently been used as an alternative compound instead of sulfites as a browning inhibitor in foods (Chaudhary et al., 2020).

Chitosan is known as an edible film component due to having excellent oxygen barrier. Also, semi-permeable chitosan films are rigid, durable, flexible

and not easily torn materials which are considered a significant advantage, and these properties can compete with most commercial polymers. Chitosan films are good oxygen barriers and protect food against fungal decay. They are used to delay the ripening of green peppers, cucumbers and tomatoes and to reduce their shelf life. The antimicrobial effect of chitosan plays an essential role in extending the shelf life of foods. Studies have shown that chitosan inhibits the growth of many microorganisms such as staphylococcus sp., escherichia coli, salmonella sp. and bacillus sp. Although the exact mechanism of this effect is not known, various theories have been proposed, such as i) the positively charged chitosan molecules bind to the negatively charged cell membrane and impair its function; ii) promoting the leakage of intracellular contents and inhibiting the transport of nutrients into the cell; iii) binding to trace elements as a chelating agent and thereby to inhibit toxin production via microbial growth; iv) inhibiting enzymes by binding water; v) binding with DNA and stopping reproduction by inhibiting mRNA synthesis (Elsabee et al., 2013; Chaudhary et al., 2020).

2.1.1.4. Alginate

Alginate, sodium salt of alginic acid, obtained by the reaction of brown seaweed with alkali, is a water-soluble polysaccharide that is widely used in many applications in the industrial field, especially in the food industry. Alginates are one of the commonly used materials in water and meat products. In addition, it is used as a stabilizer in ice cream and cheese, as a gelling agent in the pudding and gel-formed juicy desserts, as a thickener by forming a suspension in fruit drinks and other watery beverages, as a foaming agent in beer and as an emulsifier in mayonnaise (Tavassoli-Kafrani et al., 2016). Alginate has a natural structure and is a non-toxic and edible biopolymer. Its usage areas are diversified based on the three essential properties of alginate. Firstly, it can increase the viscosity of the solution due to its water solubility. Secondly, by adding calcium salt to the sodium alginate solution prepared with water, the replacement of Na ions with Ca ions results in gel formation by keeping long alginate chains together. The third one is that it can be prepared in biodegradable film form. Alginate has a wide range of usage areas since it is a natural polysaccharide with all these properties (Kocira et al., 2021).

Alginate-based edible films and coatings have been used in the packaging of many food products for a long time. Transparent and glossy biodegradable films can be easily prepared from alginate with high water vapor permeability and impermeability to oils and fats. The major advantages of alginate-based films can be classified as moisture retention, reduced shrinkage, and protected

food color and odor. These films could delay lipid oxidation and senescence and reduce the weight loss of fruits and vegetables due to their excellent barrier properties against gases. In addition to reducing the moisture loss of the food product on which it is applied, it also has the feature of preventing rancidity caused by lipid oxidation. Thus, it prevents darkening in fruits and vegetables and rancidity in animal products (Tavassoli-Kafrani et al., 2016). Chen et al. prepared edible coatings with 1.5% sodium alginate, 0.7% citric acid and 1.0% sucrose ester, with or without Ficus hirta fruit extract, to preserve the postharvest quality of nanfeng tangerines. They reported that weight loss, degradation rate, respiration rate and maleic dialdehyde content were much lower in Ficus hirta sodium alginate coated samples compared to the uncoated group. They determined that the Ficus hirta-sodium alginate coating process increased the activities of antioxidant and defence-related enzymes and stimulated the accumulation of phenolic compounds (Chen et al., 2016).

Alginate-based films have not been found to have any allergic effects, and the fact that they are obtained naturally makes alginate both environmentally friendly and economically advantageous.

2.1.1.5. Pectin

Pectin, a heteropolysaccharide, is a polygalacturonic acid chain formed by α-D-galacturonic acid molecules linked by α-1,4 glycosidic bonds. Pectin is a plant-based and water-soluble polysaccharide. Along with cellulose and hemicellulose, pectin is found in the cell walls of plants and is the main compound that binds cells together and gives tissue rigidity. Pectins, a biopolymer of acidic polysaccharides found in agricultural wastes, are complex carbohydrate structures with white color and an amorphous structure found in fruits and some vegetables. The white part is called “albedo” in the peel of citrus, and the pulp and peel of apples are the most widely used raw materials for commercial pectin production. Pectin is a secondary product of juice, sunflower oil production, and sugar manufacturing (Otoni et al., 2014).

Pectin has been extensively used in food products, such as yoghurt drinks, ice cream and jams, mainly as a thickener, gelling agent or stabilator. Furthermore, biocompatible and biodegradable pectin-based films can be prepared by solution casting, spraying or extrusion; however, they cannot meet the desired properties of conventional packaging materials due to the poor barrier and physical, mechanical and antimicrobial properties (Espitia et al., 2014). Since pectin-based films have good moisture barrier properties, they are used as packaging films for the preservation of low-moisture foods and to increase their shelf

life. The methyl ester content in the structure of pectin determines the gelation property and degree of esterification of pectin. Studies have shown that pectin-based films reduce shrinkage and bacterial growth in meat. In a study conducted by Ravishankar et al., pectin-based edible films obtained from sources such as apples, carrots and hibiscus enriched with carvacrol and cinnamaldehyde were studied on ham and Bologna-type sausages, and films containing carvacrol and pectin obtained from apple has been reported to show better antimicrobial activity than those obtained from other materials (Ravishankar et al., 2012).

2.1.1.6. Carrageenan

Carrageenan, obtained from water extraction from some species of red seaweed, is a natural anionic polysaccharide with a linear chain. Carrageenan-based films and coatings used in food technology are produced from kappa-(κ), iota-(ι) and lambda-(λ) polymers. Carrageenan is a combination of some polysaccharides and is used in food technology for gelling, thickening and improving the viscosity of foods. Carrageenan has a large or small molecular structure, and large molecule carrageenan used in the food industry is preferred since it has been determined in studies on mice that small molecule carrageenan affects the intestinal functions of mice negatively. The solubility of carrageenan in water is due to the high ester sulfate content and the existence of different cations (sodium, calcium, magnesium, potassium), which cause solubility at low temperatures and aggregation of carrageenan helices depending on the cation type, respectively. For example, lambda-carrageenan is readily soluble in cold water, and kappa-carrageenan can be dissolved at high temperatures, such as 82°C, while iota-carrageenan solubility is among them (Rocha et al., 2018; Tavassoli-Kafrani et al., 2016).

Carrageenan films and coatings used in food applications generally increase the shelf life and quality of chicken products and fish. Edible films prepared from iota carrageenan with improved mechanical strength can effectively protect vegetables and fruit against moisture loss, ageing processes and the oxidation of compounds by reducing the transfer of oxygen, limiting the dehydration of the surface and keeping the fruit flavor. Although there is not enough work on carrageenan-based edible films in the literature, there is extensive information about the application of carrageenan as a coating material. Highly hydrophilic carrageenan coatings have a limited barrier to moisture, while their barrier properties against oxygen, fats and oils are pretty good (Tavassoli-Kafrani et al., 2016). Furthermore, it can protect against lipid oxidation. Rhim et al. synthesized a new multilayer film comprised of agar, kappa-carrageenan,

clay and PLA. They observed that the barrier and optical properties, thermal stability and mechanical strength of the prepared films were enhanced with this combination (Rhim et al., 2011).

2.1.1.7. Agar

Agar, a polysaccharide obtained from red seaweed, is comprised of a mixture of agarose and agaropectin, forming the gelling and nongelling fractions, respectively. Agarose is the unbranched gelling segment of the biopolymer, while agaropectin is slightly branched and contains substituent groups of methyl ethers, sulfate esters and pyruvate acid ketals. However, commercial food grade agars have only an agarose fraction because most of the agaropectin fraction is removed from the structure during processing. Agarose can form a highly swellable physical gel which shows outstanding reversibility with the temperature by forming hydrogen bonds. Agar can be dissolved in water or other solvents at higher temperatures (90–100°C), and it protects its stability at high temperatures and low pH conditions compared with other polysaccharides (Rhim, 2011).

Agar can be used in various application areas, such as gelling agents in food applications, pharmaceutical products or medicine, cosmetics and biotechnology. Due to being biocompatible, biodegradable and non-toxic, edible agar-based films with excellent properties such as being homogeneous, flexible, transparent, mechanically durable and moderate barrier properties against O_2 and CO_2 have been widely used in food packaging applications. However, its usage in food packaging applications has been restricted due to the highly hydrophilic character of the film, just like other biopolymers. The poor aging property of agar, photodegradation and fluctuations in the temperature and humidity of the medium leads to the formation of microfractures and polymer embrittlement by altering the crystallinity of the biopolymer. Several methods can be applied, such as incorporating nanoparticles in the film structure to enhance the tensile strength and modulus, blending agars with other biopolymers to get films with good water resistance and mechanical properties, and producing multilayer films to modify the barrier and mechanical properties (Rhim et al., 2011, Atef et al., 2014).

2.1.2. Proteins

Proteins from vegetable-derived (zein, corn, wheat gluten, soy protein, peanut protein, sunflower protein) and animal-derived (collagen, keratin, gelatin, casein, egg white protein, whey protein and fish myofibrillar protein) can be used in

the production of packaging film and coatings. Protein-based films generally have good mechanical and optical properties, high water vapor permeability and good barrier properties against oxygen, carbon dioxide, flavor and lipid transfer. The good mechanical and barrier properties of these films can be attributed to the hydrophilic character of the proteins and can be adjusted depending on the protein composition. The permeability properties of the protein-based films may differ depending on the changes in the isoelectric point due to the interactions between the amino and carboxyl groups of the proteins. Moreover, the source of the protein, pH of the protein solution, plasticizer, film thickness, preparation conditions and structures included in the film solution can directly affect the film properties. It has been observed that the pH value of the film solution causes differences in the film properties such as color, structure and tensile strength (Paylath et al., 2009).

Among the film-forming biopolymers obtained from renewable sources, proteins have an excellent film-forming ability, good barrier properties and high mechanical strength comparable to petroleum-based polymer films. This is because they have a specific structure that gives them more potential properties than uniform polysaccharides. The most important advantages of protein-derived films and coatings are their high physical stability, providing the desired shape to the product. It is known that protein-based edible films and coatings also enrich the food in terms of nutritional value (Zink et al., 2009).

In producing protein-based films and coatings, mostly animal proteins such as casein, whey protein, collagen and gelatin, and vegetable proteins zein (corn), soy and gluten are frequently used (Zink et al., 2009).

The most widely used animal-derived protein is collagen, abundant in skin and connective tissue components in animals. Collagens are widely used commercially as films and coatings. Mainly, collagen casings take on the same task as the natural intestine in sausage casings and are used more often. Collagen casings used in the coating of sausage and meat products can be eaten with the product as long as they are not produced too thick or removed before consumption. Such films and coatings have a flexible structure that can remain intact during product processing and have high stress. They are transparent in appearance, healthy and increase the weight of the food they cover.

Milk proteins are very suitable for producing edible films in terms of controlling permeability due to their stable structure. Milk protein-based films, which consist of casein and whey proteins, can be used alone or combined with other coating materials in specific proportions. These proteins, known as milk proteins, are commercially available and depending on the extraction methods,

they can be the source of films with different properties. Casein is a water-soluble phosphoprotein. Films obtained from casein are transparent, odorless and flexible. Oil permeability and oxidation resistance can be increased by adding oil to the structure of casein-based films and coatings used to preserve fruits, vegetables and frozen fish (Zink et al., 2009). Whey protein is an essential source of milk protein, which is released in large quantities during cheese production. Although the whey protein film has superior properties in preventing oxygen permeability, its moisture barrier property is not good enough due to its hydrophilic nature. Films produced from whey proteins are clear, odourless, and highly elastic. Whey protein coatings provide antioxidant properties in frozen fish and are used in baked goods, chocolate and biscuit products. They are also used in breakfast cereals to reduce moisture permeability and the stickiness of raisins (Halal et al., 2016).

Similar to other edible films, gelatin coatings act as antioxidant carriers. Edible films produced from water-soluble proteins of fish have less water vapor permeability than many other protein films. Similar to fish proteins, creatine films have low water vapor permeability. Egg white (albumin) protein has not been studied much as other proteins in developing films and coatings.

Wheat gluten, a combination of gliadin and glutenin polypeptides, is the water-insoluble protein of wheat flour. Its high molecular weight, nonpolar character and structural diversity are the most important features. Also, it has high moisture permeability and low oxygen and carbon dioxide permeability. Wheat gluten-based films are known to be homogeneous, transparent, mechanically strong, and relatively water resistant. These films and coatings are edible when food-based additives and natural substances are used. However, due to the widespread gluten sensitivity, these films cannot be among the raw materials accepted by everyone(Zink et al., 2016). Gluten coatings provide the coating of salt and flavorings on cookies and are also used to encapsulate flavor and color factors in bakery products.

Coatings based on corn zein, one of the vegetable-derived protein films with excellent oil barrier properties., form a hard, shiny, durable and protective layer for microorganisms. To improve the flexibility of the zein film and to prevent ruptures in the film, which has a fragile structure, plasticizers such as glycerol, sugars and organic acids were added. It was found that the films plasticized with glycerol have five times more water absorption capacity than the unplasticized films at high water activity due to the hydrophilic nature of the glycerol. Zein film coatings have been used in many foods, especially nuts, candy, small fruit pieces, dried nuts and some freeze-dried foods. It has also been applied as an

alternative to collagen in sausage casings and as a plant-derived biopolymer in producing water-soluble packaging in dry foods. It is known that tomatoes covered with zein reduce the brightness and moisture permeability and delay color change (Tzia et al., 2016).

Soy protein is widely used in the food industry as a raw material. It is produced mainly in Far East countries. Compared to other plant-derived proteins, soy protein forms more flexible, smooth and transparent films. Peanuts are an essential component of biopolymeric films. Cottonseed proteins can be used as packaging to protect products, cover seeds and prevent water loss.

2.1.3. Lipids

Lipid compounds contain glycerides, glycerol and fatty acid esters of neutral lipids, and natural and synthetic waxes, long chain monohydric alcohols and fatty acid esters. Acetylated monoglycerides and natural waxes are widely used as coating materials due to their low polarity and good barrier properties against moisture loss. Natural waxes containing high amounts of long chain alkanes and fatty alcohols are the most effective materials that reduce moisture permeability due to their high hydrophobicity. Waxes are generally more durable than many other lipid-based or lipid-free coatings. Lipids are mostly hydrophobic compounds that act as good moisture barriers, and lipid-based coatings have a low affinity for water due to their low water vapor permeability. This feature is mainly used to preserve white and red meats. Moreover, there are many studies on its use as a coating on fresh fruits and vegetables to control drying. Lipids also increase the visual appeal of food products, making them shiny (Muscat et al., 2013; Tzia et al., 2016).

Lipid-based coatings have a low affinity for water, which explains why they have low water vapor permeability. Since each hydrophobic substance has physicochemical properties, each lipid-based coating behaves differently regarding moisture transfer. It is known that liquid phase lipids show less resistance to gas and vapor passage than solid phase lipids. However, wax and oil-based films and coatings can cause problems in application due to their thickness and, slippery surfaces, waxy and bitter taste. Direct application of any lipid to a hydrophilic or wet surface causes a weak force of attraction between the food and the film interface. Therefore, better barrier properties can be achieved by making a double-layer coating (Muscat et al., 2013). The main disadvantages of lipid-based films and coatings are instability, brittleness and opacity. In producing films from lipids, solvent and high temperature are required, and lipid-based films show poor mechanical properties.

2.2. Microorganism-derived biodegradable polymers

2.2.1. Polyhydroxyalkanoate (PHA)

Polyhydroxyalkanoates (PHAs) are linear polyesters formed due to bacterial fermentation of sugar and fat molecules in the structure of lipids or carbohydrates. Bacteria produce them to store carbon and energy. In a PHA chain, the carboxyl group of a 3-hydroxy fatty acid monomer and the hydroxyl group of the following monomer make an ester bond, and polymers with molecular weights ranging from 50 kDa to 1000 kDa are formed, depending on the producing microorganism. PHAs can be either thermoplastic or elastomeric according to their monomer structures, and their melting temperatures vary between 40 and 180°C (Rocha et al., 201; Tzia et al., 2016).

Polyhydroxyalkanoates are completely natural biopolyesters with physical and thermal properties similar to synthetic thermoplastics such as PE, PP and PS. Thanks to their valuable properties, such as biodegradability, biocompatibility, and thermoplasticity, they have become the most suitable candidates to replace fossil fuel-derived synthetic thermoplastics. The physical, chemical and thermal properties of PHA polymers mainly depend on the structures and molecular weights of the monomeric components, which are more than 150 and their combination, and also the natural structure of the carbon sources used as substrate in fermentation. Although being biodegradable, PHA is moisture resistant and stable during use and storage conditions. PHAs are resistant to UV light, have low moisture permeability, and are comparable to low-density polyethylene, which is widely used in the food industry (Paylath et al., 2009).

PHAs, naturally produced by bacteria, are easily degraded when left in the soil. The biodegradation rate of PHAs depends on material properties, such as crystallinity, molecular weight, surface area, and environmental conditions, such as microbial activity, temperature, and pH. It has been reported that biodegradation is faster at 55% humidity and 60°C temperature, and 85% of the material is destroyed in 7 weeks under these conditions. In the study carried out in Lake Lugano in Switzerland, PHA-based packaging films and plastic bottles were placed at specific depths, and it was reported that the biodegradation of plastic bottles took 5-10 years. In contrast, the biodegradation of PHA films was completed in 254 days (Rocha et al., 2018; Paylath et al., 2009).

2.2.2. Poly-β-hydroxybutyrate (PHB)

Poly-β-hydroxybutyrate (PHB) is an entirely biodegradable polymer produced by bacteria from glucose or starch. PHB, synthesized and deposited as

an intracellular storage granule, is a long polymer consisting of repeating hydrophobic units containing short-chain β-hydroxy fatty acids in its structure. The molecular weight of PHB is between 60,000 and 2,000,000 Da and varies according to the type of bacteria, growth conditions and place in the cell's life cycle. It is reported that PHB is resistant to UV radiation but has poor resistance to acids and bases. On the other hand, polymers with long side chains produced only by certain bacteria have lower melting points and glass transition temperatures (Dhar et al., 2015; Anbukarasu et al., 2016).

Since the mechanical properties of PHB and its copolymers are similar to commercial plastics such as PE and PP, they are known as thermoplastic polyesters and can be easily pressed and shaped. PHB, with a melting temperature of 175-180°C, is similar to PP in its mechanical properties, but it is harder, more brittle and has poor solvent resistance than PP. It is identical to low-density polyethylene with its low water vapor permeability, which is important for the food packaging industry. PHB copolymers, on the other hand, are more flexible and have a lower melting temperature, making them more beneficial for the manufacture of pressed products. In addition, the use of PHB expands as the water and airtightness of the polymer provide resistance to hydrolytic degradation.

Poly-β-hydroxybutyrate is preferred as a food packaging material due to its biodegradability and easy shaping. Due to the excellent gas barrier properties, PHB has been used in coatings in the form of films. PHB films are mainly used in the inner surface coating of bags, sachets, plastic trays used in food services, soft drink bottles and milk cartons. Packaging films have excellent gas barrier properties, and a PHB film with 25 μm thickness has an oxygen permeability of 45 $cm^3/m^2/day$ (Anbukarasu et al., 2016). Because of its biodegradability, PHB has been turned to for the manufacture of disposable products, and PHB is often used to make biodegradable coated paper and high-quality film. PHB, which is very similar to polypropylene due to its physical properties, can also be used to produce many products such as washable containers, crinkle packages and ropes. Moreover, PHB polymers can be completely degraded by bacteria and fungi in marine and marine sediments without producing toxic products. PHB polymer decomposes to water and carbon dioxide in aerobic conditions, while it forms carbon dioxide and methane gas in anaerobic conditions (Usurelu et al., 2022).

2.3. Biotechnologically synthesized biodegradable polymers

2.3.1. Poly(lactic acid) (PLA)

Poly(lactic acid) (PLA) is an aliphatic poly(α-hydroxy acid) polymer obtained from natural and sustainable sources such as corn, starch and sugar cane. PLA

can also be synthesized from polycondensation of the lactic acid or ring-opening polymerization of high molecular weight lactide. PLA is a thermoplastic biopolymer as some of its properties, such as mechanical strength, elasticity, recyclability and thermal adhesion, are similar to synthetic thermoplastics. In contrast, some properties such as biodegradability, barrier properties and paintability are similar to biobased polymers (Carmona et al., 2014). The main properties of PLA, melting point, mechanical strength and crystallinity, strongly depend on the structure of PLA polymer (the ratio of L-lactic acid, D-lactic acid or L-, D-lactic acid) and molecular weight. In addition, the end-use properties of PLA depend on the blending and process conditions, like other plastics (Moran et al., 2016).

The ratio of D- and L-lactide determines the morphology of the polymer, and accordingly, PLA can be produced in amorphous or crystalline nature. PLA exhibits a semi-crystalline structure at 93% or more L-lactide acid composition, while an amorphous structure at 50-93% L-lactide acid composition. Racemic PLA has atactic, high amorphous and optical inactivity properties, and amorphous PLA is transparent. PLA exhibits high transparency and low turbidity properties, and its optical properties vary depending on the additives and production conditions. It shows high transparency at low crystallinity and poor optical properties at high crystallinity. Since PLA is a more polar material than polyolefins, it has high surface energy, providing convenience in printing and dyeing processes (Balla et al., 2021).

PLA is used in many areas such as packaging, agriculture and the pharmaceutical industry due to its biodegradability, compostability, thermoplastic processability and environmental friendliness. PLA has advantages and disadvantages compared to other commercial polymers as a packaging material. The UV light barrier property of PLA films, which is required for food packaging to protect the product from UV rays, is better than low-density polyethylene but worse than cellophane, PS and PET.

PLA films, suitable for injection molding and vacuum forming, have low moisture permeability and high barrier property to prevent flavor loss of the product. Besides their biological properties, PLA films are often used because of their strong sealing properties, low-temperature adhesion, heat sealing to paper or cardboard, stability, transparency and easy processing. However, the main disadvantages of unmodified PLA packaging films are their brittleness and their limited use in hot product applications due to low melting temperatures of around 60 °C. PLA packaging is widely used in products such as beverage glasses, fresh pasta, bread and salad bags, thermoform containers for bakery products, agricultural covers and boxes. In addition, PLA is preferred in bread

and bakery products as it prevents the formation of steam inside the packaging films. There are studies in which plasticizers, nano reinforcement or both are added to obtain the properties required for using PLA as packaging material. For example, in a study conducted by Moran et al., it was found that polylactic acid/ cellulose nanowhisker nanocomposites showed better mechanical properties than neat PLA (Moran et al., 2016).

2.4. Petrochemical-derived biodegradable polymers

2.4.1. Polycaprolactone (PCL)

Polycaprolactone (PCL), an important member of the aliphatic polyester family, is a thermoplastic biodegradable semi-crystalline polyester obtained by ring-opening polymerization of ε-caprolactone. PCL, which has attracted great interest in recent years, is a biocompatible polymer that can be easily synthesized and dissolved in many organic solvents, with a high molecular weight, low melting point (55-60˚C) and glass transition temperature (-60˚C), which can be completely disposed of by the body. The average molecular weight of PCL can generally range from 3.000 to 90.000 g/mol, and its crystallinity tends to decrease with increasing molecular weight. The degradation time of PCL is over one year, which is much less among biodegradable polyesters and even natural biopolymers due to its repeating moiety of hydrophobic CH_2 units (Thakur et al., 2021).

PCL is an important polymer used in many applications due to its biocompatibility, non-toxicity, biodegradability, superior mechanical properties and miscibility with various other polymers. There is a growing interest in using non-toxic PCL in the production of biodegradable packaging materials. Therefore, PCL is widely used in foam containers, disposable cutlery, plates, straws, fruit and vegetable nets, compostable bags and coatings due to its ability to come into contact with food. Due to their highly hydrophobic nature, PCL films, produced from crude oil, have good resistance to water, oil and chlorine. In a semi-crystalline polymer such as PCL, gas mass transfer is mainly a property of the amorphous phase because the crystalline phase is assumed to be impermeable. As the percentage of crystallinity of a polymer increases, its oxygen permeability decreases. However, the high crystallinity of PCL directly affects its properties such as stability, strength, barrier, permeability and biodegradation. On the other hand, its high crystallinity negatively affects the biodegradability of PCL and to optimize its crystallinity, organic and inorganic additives can be added to the PCL matrix (Mohamed et al., 2016).

The major disadvantage of PCL that restricts its commercial use in food packaging is its high price and complex manufacturing process. To overcome

this limitation, PCL can be blended with low-cost polymers to give an excellent biodegradable PCL composite with enhanced chemical and physical properties such as adhesion, stress crack resistance and dyeability. Thanks to its chain structure with polar and nonpolar units, PCL is compatible with other natural and synthetic polymers such as PEG, PVA, PLA, PLGA, chitosan and starch (Thakur et al., 2021). Moreover, the addition of PCL to the structure of non-biodegradable polymers makes them biodegradable.

Conclusion

Food safety is crucial to protecting the well-being and health of consumers. Packaging is essential in protecting food against external contamination, physical damage, moisture, heat, and much more during its shelf life. Synthetic packaging films derived from traditional petroleum-based plastics with desired transparency, softness, flexibility, and lightness have supplied most of the common packaging materials for a long time. However, these single-use non-biodegradable plastics are harmful to the environment and human health and cause serious ecological problems. With the increasing environmental awareness, there has been a great interest in replacing these non-biodegradable materials with renewable, environmentally friendly, biocompatible, and biodegradable packaging films, thereby reducing pollution. The biodegradable biopolymers, associated with renewable materials, can be classified as biomass-derived biopolymers, microorganism-derived biopolymers or biotechnologically synthesized biopolymers. These biopolymers can degrade into simple molecules, water and carbon dioxide, and biomass with exposure to microorganisms, moisture or oxygen. Although extensively researched, the commercial usage of these environmentally friendly packaging films is not very common, and the widespread use of these packaging materials can solve the waste problem, at least to some extent. Comprehensive studies are needed on selecting suitable films and coating materials, process optimization and industrial suitability, and adapting low-cost applications to develop and commercialize such packaging materials.

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CHAPTER V

EFFECTIVE ROLE IN WASTEWATER TREATMENT USING HYBRID MEMBRANE BIOREACTOR-MOVING BED BIOFILM REACTOR

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1. Introduction

Water is an extremely important resource for living things to continue their vital activities (Damptey et al., 2022). Although our world is surrounded by water, only 0.1% of the world's fresh water resources are drinkable. However, it is a factor in the decrease of fresh water resources due to the development of industries, increase in population and increasing global warming in recent years (Faroque & South, 2021). In addition, the development of industries and the increase in the human population have brought a serious water pollution on a global scale, as well as the effect of the reduction of fresh water resources (Dai et al., 2022).

Water pollution is caused by humans, industrial and agricultural practices. These wastewaters from industry and domestic are among the sources of water pollution (Afolalu, Ikumapayi, Ogedengbe, Kazeem, & Ogundipe, 2022). With the discharge of wastewater from these sources to receiving environments without treatment, it causes serious damage to the environment (Shindhal et al., 2021). Therefore, these wastewaters need to be treated. With the treatment of these wastewaters, environmental pollution will be prevented and thanks to the reusability of this treated wastewater, it is used in agricultural irrigation and

irrigation of parks and gardens, preventing the reduction of water resources (Ciobanu, Teodosiu, Almeida, Agostinho, & Giannetti, 2022).

2. Wastewater Treatment Processes

Wastewater treatment processes are divided into three as physical, chemical and biological treatment. The flow diagram of a wastewater treatment process is shown in Figure 1. Generally, wastewater treatment processes consist of pre-treatment, primary treatment, secondary treatment and advanced (tertiary) treatment (Figure 2).

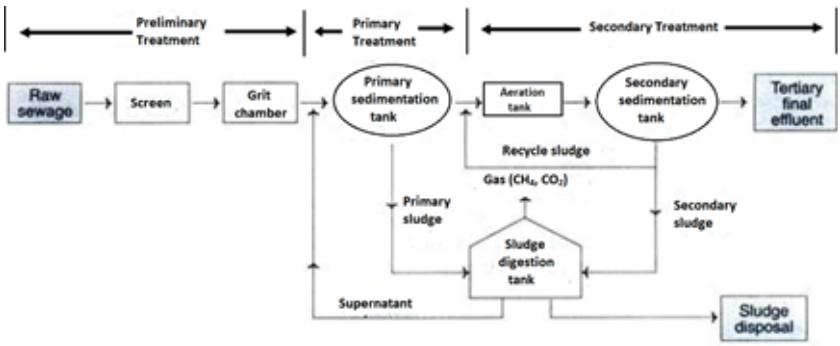

Figure 1: Wastewater treatment process flow diagram (Jasim & Aziz, 2020)

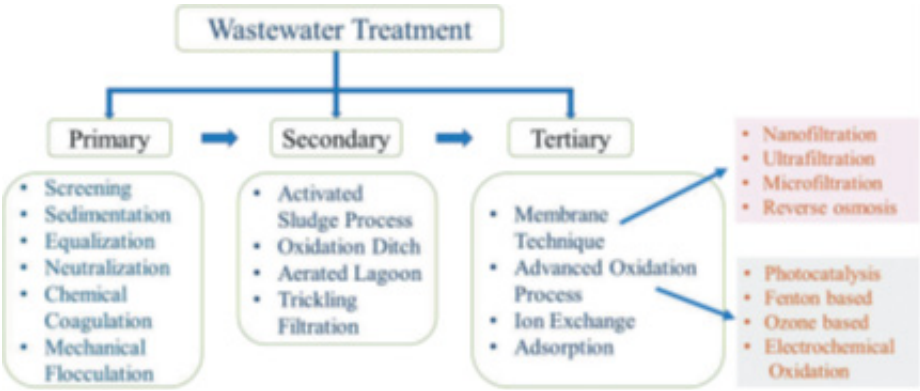

Figure 2: Classification of wastewater treatment processes (Shindhal et al., 2021)

2.1. Pre-Treatment

The purpose of pre-treatment is the separation of particulate organic substances from wastewater by their own weight (Sawyer et al., 2013).

2.2. Primary Treatment

In primary treatment, physical methods such as sedimentation and flotation are used to remove organic and inorganic solids from wastewater. In this treatment, approximately 25-50% of the biochemical oxygen demand (BOI_5), 70% of the total suspended solids and 65% of the oil and grease are removed (Sonune & Ghate, 2004).

2.3. Secondary Treatment

It is used in the treatment of easily degradable organic substances and suspended solids that cannot be removed in primary treatment. In addition, nitrogen and phosphorus nutrients are removed in secondary treatment (Sonune & Ghate, 2004).

2.4. Advanced (Tertiary) Treatment

The purpose of advanced treatment is to remove both more organic matter and solids. In addition, advanced treatment is needed to reuse the treated wastewater (Sawyer et al., 2013).

3. Membrane Bioreactor (MBR)

Membrane bioreactor (MBR) is a new technology used in wastewater treatment in recent years (Khastoo, Hassani, Mafigholami, & Mahmoudkhani, 2021). MBR is a process that consists of a combination of both biological and physical treatment for the treatment of domestic and industrial wastewater. The working principle of this process is to firstly decompose degradable organic matter using activated sludge, then separate microorganisms and macromolecules from wastewater thanks to the membrane module (W. Zhang et al., 2022).

MBR process has the advantage of operating at higher suspended solids (MLSS) concentrations than activated sludge systems. In order to reduce high MLSS concentrations in conventional processes, these solids concentrations are removed by using the secondary clarifier after the activated sludge systems. However, in MBR systems, organic substances in high concentration are removed without the need for a secondary clarifier. In this case, since there is no secondary clarifier, it reduces both the operating cost and the required space requirement of the facility (Rodriguez-Sanchez, Leyva-Diaz, Gonzalez-Lopez, & Poyatos, 2018).

Compared to other conventional processes, MBR has advantages such as less space requirement, high quality of effluent, removal at high MLSS concentrations, and high mass transfer effect (W. Zhang et al., 2022).

4. Moving Bed Biofilm Bioreactor (MBBR)

Moving bed biofilm reactor (MBBR) technology was invented by the Norwegian company KALDNES in the late 1980s and they have patented this new technology (Madan et al. 2022). The MBBR flow diagram is shown in Figure 3.

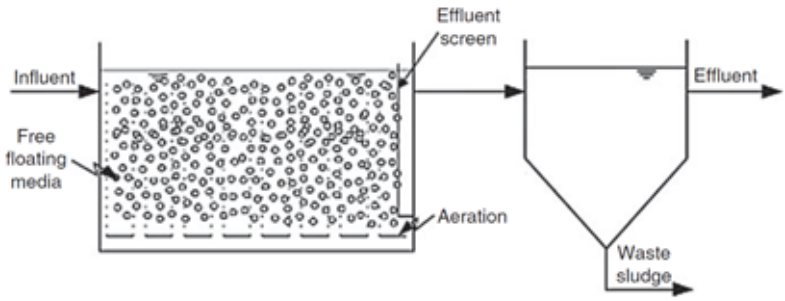

Figure 3: MBBR flow diagram
(Esfahani, Zeidabadi, Bazargan, & McKay, 2018)

MBBR combines with the combination of biofilter and activated sludge process (Santos, Martins, Quinta-Ferreira, & Castro, 2020). By placing the biomass in the reactor, it is ensured to move freely with the biocarriers. These biocarriers are usually made of hollow plastic material. The biofilms in the reactor live attached to these biocarriers. This biofilm plays an active role in the removal of pollutants in wastewater (Patel, Patel, & Nerurkar, 2021).

MBBR process provides effective removal of organic substances and nitrogenous compounds in wastewater (Alarjani et al., 2021). In addition, it is thought that a benefit will be provided to the economy due to the high nutrient removal and the qualified effluent quality that the wastewater can be reused after treatment (Saidulu, Majumder, & Gupta, 2021).

MBBR process has many advantages, these advantages are listed as follows;

i) the space requirements are low,
ii) occurs at low hydraulic residence time,
iii) effective in removing organic substances and nutrients (Madan, Madan, & Hussain, 2022).

5. Hybrid Membrane Bioreactor-Moving Bed Biofilm Bioreactor (MBR-MBBR)

In recent years, hybrid membrane bioreactor-moving bed biofilm reactor (MBR-MBBR) process, which is a new technology in the treatment of wastewater, has come to the fore. The hybrid MBR-MBBR process is based on the combination of an activated sludge system and a membrane bioreactor (X. Zhang et al., 2020). This technology was created by refluxing from the MBR region to the MBBR region (Rodriguez-Sanchez et al., 2018).

Membrane fouling is one of the most important disadvantages of the MBR process. The developed hybrid MBR-MBBR system is an effective wastewater treatment technology to prevent this membrane fouling in the MBR (Leyva-Diaz & Poyatos, 2015).

This hybrid MBR-MBBR process provides effective removal efficiency in the treatment of wastewater. It is also effective in the nitrification / denitrification process (Sohail, Ahmed, Chung, & Nawaz, 2020).

As a result, hybrid MBR-MBBR

i) have higher organic loadings,
ii) low sludge production rate,
iii) have both efficient oxygen transfer and higher biological reaction rate as a result of biomass accumulation at high concentrations,
iv) It has been a preferred system in the treatment of wastewater because it has advantages such as reducing membrane fouling in MBR (Sohail et al., 2020).

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CHAPTER VI

EXAMPLE OF RISK ANALYSIS STUDY FOR HAZARDOUS WASTE STORAGE AREAS

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1. Introduction

At present, the implementation of heavy sanctions because of work accidents has forced businesses to pay more attention to legal regulations and give more importance to human health. Big businesses are more sensitive about taking the necessary measures by detecting the risks that may occur in order to fulfill their obligations related to occupational health and safety. Risk analysis can be defined as the identification of all kinds of potential hazards that may occur during the activities in the workplace and the determination of the risks related to them (Birgoren, 2017). Risks cannot be eliminated, but they can be reduced to acceptable levels by establishing a systematic framework to identify, assess, control, prevent, and reduce risks (Jamali et al., 2017; Mensi et al., 2015; Bakhshi et al., 2021). Thus, the negative effects on human health will be reduced (Ceylan and Bashelvaci, 2011).

There are a limited number of risk analysis studies for different sectors in the literature. Ozgur (2021) performed risk analysis evaluating 14 hazards and the risks they pose for the disinfection unit of a drinking water treatment plant with 5×5 matrix risk analysis. At the end of the study, the sources of risks that may occur in the disinfection units were determined as insufficient training of employees, not using appropriate personal protective equipment, and exposure to disinfectant chemicals. He stated that raising awareness among the workers by giving training at regular intervals can minimize occupational accidents (Ozgur, 2021).

Bayram and Kaya (2022), using the Fine–Kinney method, carried out risk analysis within the scope of the port of Trabzon. A total of 72 risks were evaluated, 13 of which were "Intolerable Risk", 19 "Major Risk", and 40 "Significant Risk". The measures to be taken to reduce the risks to an acceptable level are listed as follows: (1) Hanging warning signs, (2) Periodic checks of loading vehicles, (3) Use of personal protective equipment by employees, (4) Checking electrical installations, (5) Periodic health checks of personnel (Bayram and Kaya, 2022). Yalcinkaya et al. (2020) investigated the possible environmental risks that may occur during the transportation of dangerous goods on highways and made a process evaluation with the help of fault tree analysis in 4 different scenarios that may occur. At the end of the study, scenarios such as fire and explosion, hazardous material leakage or traffic accident, poisoning and explosion, or other chemical reactions are shown. The measures to be taken for risks are businesses making practices more effective, being aware of potential risks, providing regular training to employees, and keeping near-miss records (Yalcinkaya et al., 2020).

In the 21st century, cement production especially has become a leading sector in the global economic recovery due to the acceleration of infrastructure construction and the creation of wide employment opportunities and chain services in the sectors that depend on it (Xi and Liu, 2022). With the increase in the world's population in the last 10 years, it is estimated that approximately 626 kg of cement has been produced per person (Rocha et al., 2022). Cement production stages are in the form of quarrying, raw material preparation, clinker production, cement grinding, packaging, and shipping (EUM, 2021). Limestone is ground by being transported from marl quarries to crushers and from there to mills (Li et al., 2022). Raw meal is obtained. The raw meal is passed through the preheaters (DOPOL) and sent to the rotary kiln to be cooked. The rotary kiln is cylindrical, mounted with 3-4% inclination, and built from refractory bricks (Atmaca and Yumrutas, 2014). The raw meal cooked in the oven at 1400-1450

°C comes out as clinker from the rotary kiln (Cormos, 2022). The clinker is passed through the cooler and poured into the closed clinker silo (Santos et al., 2022). It is then ground in a cement mill with the addition of gypsum and stored in silos (Her et al., 2022).

The increasing importance of ensuring sustainability in the cement industry and the improvement of production and storage areas in terms of occupational health and safety are important for both employees and prevention of environmental pollution (Balci, 2016). While different environmental problems arise at every stage of cement production, dust and gas release occurs in the processes related to the operation of the machines and the quarry (Cakir, 2020). Wastes in the cement sector, mineral oil and grease from machinery and equipment, packaging of mineral oils (metal barrels), used tire and motor oils, discarded electronic wastes, batteries used in machinery equipment, acid and base waste, X-ray tube, radioactive sensors wastes such as casting wastes and insulating liquids in workshops, insulating oils in transformers, frying oils, medical wastes, batteries and fluorescent lamps from offices, used occupational safety materials are hazardous, and packaging wastes, paper wastes, food wastes and food packaging wastes of materials coming to the factory are non-hazardous wastes. can be counted among (Viczek et al., 2021). The wastes that can be incinerated in the cement factory are burned and the wastes with energy recovery and calorific value (waste-derived fuels, various domestic or industrial wastes containing components such as wood, textile, plastic, different types of treatment sludge with appropriate calorific value, waste oils, bilge wastes, end-of-life wastes tires and solvents) are used as an alternative fuel in the industry (Tosun, 2006). Wastes generated in the facility (hazardous waste, non-hazardous waste, waste oil, contaminated wastes, oil and air filters, waste batteries, waste batteries, waste cartridge-toner, medical wastes, etc.) sent for conversion (Tuncez, 2021).

Besides fuel production from solid wastes, there is also growing interest in sustainable material use which professionals are oriented towards the use of more cost effetive and environmentally sound products for construction and decorating. Within this approach, waste tires (Sahin et al., 2022) and wood could be one of the well choice considering very durable materials even in outdoor conditions and could be provided varsilite properties (Sahin et al., 2021; Sahin et al., 2022).

In the present study, the risk factors for the temporary hazardous waste storage area of a selected cement factory were determined, the risk values for each risk factor were calculated by the Fine–Kinney method, and their effects on

the environment and employees were investigated. The Fine–Kinney method is a comprehensive method for quantitative assessments to assist in the control of hazards, in which the risk value is calculated by considering the frequency and probability of occurrence of a hazard event that may lead to an accident (Pariyani and Reniers, 2018). The first step of the risk assessment is to implement the control measures by following the prepared risk analysis studies, the second step is to monitor the reduction of the determined risks to an acceptable level, and finally to control the continuity of the measures so that the probability and severity of the risks do not increase (Jiang and Zhao, 2022). Risk analysis studies are a current subject of study in terms of both the cement industry and hazardous waste storage areas, but the literature resources are quite limited. It is thought that the risk factors and working method mentioned in the present study can be a guide for other similar sectors. It is aimed to contribute to the literature and increase the risk analysis studies. The results of the study include measures that can be taken in terms of occupational health and safety, especially in temporary hazardous waste storage areas in the cement sector.

2. Materials and Methods

2.1. Facility data

A cement factory located in the Mediterranean region was selected for the study. The field of activity of the selected factory is to produce clinker, cement, and ready mixed concrete using advanced technology and full automation systems. Clinker (the raw material of cement) Portland cement (the final product), Portland composite cement, pozzolanic cement, and sulfate resistant cement are produced. The facility, operating with 650 people, has an annual clinker production capacity of 3,150,000 tons and an annual cement production capacity of 5,068,800 tons.

Alternative raw materials are used in the selected cement factory in two ways. First, natural raw materials (mud, gypsum, gypsum waste, bleaching waste, mud waste, foundry sand, iron powder, scale, gypsum, fly ash, iron slag and excavation soil mud, land, etc.) are used. Second, alternative raw materials are used in cement production, without contravening the cement quality norms, as well as the natural additives added to the clinker in certain proportions. In the cement sector, where the use of raw materials is intense, it is possible to significantly reduce greenhouse gas emissions and natural resource consumption, and to further improve the existing mine life and mineral quality. Therefore, replacing fossil fuels and natural materials with alternative materials

is not only an environmentally friendly method of waste management, but also a cost-saving and viable way to conserve fossil fuels and natural resources.

The facility is usually rated at significant risk and acceptable risk. Therefore, with some arrangements in the working environment, in a short time risk that occur can be removed and a safe environment can be created for employees. The waste management system of the cement factory started in 2008 and ensures the collection, temporary storage, and disposal of all hazardous and solid wastes in accordance with the regulation. The temporary waste storage site permit has been obtained from the Provincial Directorate of Environment and Urbanization, and the wastes arising from the routine activities of the facility are stored in accordance with the legislation according to their types. The wastes generated in the facility (non-hazardous and hazardous waste: contaminated wastes, waste oil, air filters, waste batteries and fluorescent lamps, waste cartridge toner and medical wastes, etc.) are collected in the relevant compartments in the waste storage area. The stored wastes are sent to environmental permit and licensed facilities for disposal or recycling. The wastes that cannot be sent but can be incinerated are burned together in the factory under the waste license, and energy recovery is performed. Alternative energy use is important for climate change, ecosystem quality, and human health.

2.2. Method

The "Mathematical Evaluations for Controlling Hazards" method was developed by W. T. Fine and revised and published by Kinney and Wiruth in 1976 under the name "Practical Risk Analysis for Safety Management". Nowadays it is known as the Fine–Kinney method (Oturakci and Dagsuyu, 2017).

The Fine–Kinney method is used to prioritize jobs according to their results, which determines the degree of risk of the hazard through a mathematical product of three risk parameters, called the probable outcome of a potential accident (Gul et al., 2018). It is decided to combine the risk assessment information by calculating the weight ratios of the risks, to calculate the risk level, and to rank the risk priority (Ozcelik, 2013). The method gives realistic results in terms of the experience of undertaking a duty in risk assessment studies on the occupational health and safety of technical workers in the workplace, the result of risk assessment, the preparations that need to be planned, and the stages of interpretation (Aker and Ozcelik, 2020). While determining the hazards and risks in the study, risks such as electricity and lighting that may occur in any business are not considered; only the dangers and risks specific to the temporary hazardous waste storage area are emphasized.

Fine-Kinney risk assessment method (Kinney and Wiruth, 1976);

R= L x E x C

In this place;

L = Likelihood (Table 1),

E = Exposure (Table 2),

C = Consequences (Table 3),

R = Risk level (Table 4).

Table 1: Likelihood

Likelihood	**Probability of damage**
10	Expected, certain
6	High, quite possible
3	Possible
1	Possible but low
0.5	Unexpected but possible
0.2	Unexpected

(Kinney and Wiruth, 1976)

Table 2: Exposure

Exposure	**Repeated exposure in hazard damage**
10	Almost constantly (several times an hour)
6	Frequent (once or several times a day)
3	Occasionally (once or several times a week)
2	Infrequently (once or several times a month)
1	Infrequent (several times a year)
0.5	Very rare (annually or less frequently)

(Kinney and Wiruth, 1976)

Table 3: Consequences

Consequences	**Estimated damage to humans and/or the environment**
100	Multiple deaths - environmental disaster
40	Death – serious environmental impact
15	Permanent damage, disability, long-term treatment – significant environmental impact
7	Significant damage, injury, medical treatment – wide environmental impact
3	Minor damage, injury, first aid – limited environmental impact
1	Near miss – no environmental damage

(Kinney and Wiruth, 1976)

Table 4: Risk Score

Risk score	Risk situation
400<R	Unacceptable risk – interruption of work until recovery.
200<R<400	Serious risk – must be cured soon.
70<R<200	Significant risk – must be improved in the long term.
20<R<70	Possible risk – should be kept under surveillance.
R<20	Minor risk – acceptable.

(Kinney and Wiruth, 1976)

3. Results and Discussions

There are 23 hazards identified for the temporary hazardous waste storage area in Table 5. No activity in the category of unacceptable risk (400 < R) or serious risk (200 < R< = 400) was detected in the temporary hazardous waste storage area. Transport of waste oils is included in the significant risk (70 < R< = 200) group. Many activities in the study area were evaluated in the acceptable risk group (20 < R< = 70). These activities include storage of waste oils; transportation and storage of contaminated wastes such as bag filters, contaminated drums, and drums; storage of contaminated wastes with cement, paint, and waste oils; storage of waste printer cartridges and toners; and collection, transportation, and storage of hazardous wastes such as waste fluorescent. Transportation of waste tires can be listed as loading hazardous wastes into waste transportation vehicles. Storage of oil filters and air filters is an activity in the insignificant risk (R< =20) group.

Table 5: Hazardous waste temporary storage area risk identification

No	Activity	Routine	Non-routine	Source of Danger	Possible Impact	Affected	Current Situation / Measures
1	Storage of Waste Oils	x		Waste Oils	Skin Ailments, Slip, Fire	Employees, Subcontractors, Interns	PPE, Instruction, Environmental Trainings, OHS Trainings, Extinguishing Equipment
2	Transport of Waste Oils	x		Working with Removal Tools	Traffic Accident, Injury, Ailments, Death	Employees, Subcontractors, Interns	PPE, Instruction, OHS Trainings, Periodic Controls of Lifting Vehicles
3	Storage of Waste Batteries	x		Acids and Bases	Chemical Burns	Employees, Subcontractors, Interns	PPE, OHS Trainings, Material Safety Data Sheets
4	Transport of Contaminated Waste (Oil, bag filters, drums)	x		Working with Removal Tools	Crush, Jam, Crash, Death	Employees, Subcontractors, Interns	PPE, Instruction, OHS Trainings, Periodic Controls of Lifting Vehicles
5	Storage of Contaminated Waste	x		Waste Oils	Skin Ailments, Slip, Fire	Employees, Subcontractors, Interns	PPE, Instruction, Environmental Trainings, OHS Trainings, Extinguishing Equipment
6	Storage of Contaminated Waste	x		Chemicals	Poisoning, Skin Irritations, Burns	Employees, Subcontractors, Interns	PPE, OHS Trainings, Material Safety Data Sheets

7	Storage of Contaminated Waste	x		Paint	Skin, Respiratory, Eye Disorders, Burning	Employees, Subcontractors, Interns	PPE, OHS Trainings, Material Safety Data Sheets
8	Storage of Contaminated Waste	x		Cement Mortar	Skin Disorders	Employees, Subcontractors, Interns	PPE, OHS Trainings, Environmental Trainings
9	Storing Waste Cartridges and Toner	x		Paint	Skin, Respiratory, Eye Disorders, Burning	Employees, Subcontractors, Interns	PPE, OHS Trainings, Material Safety Data Sheets
10	Storing Oil and Air Filter	x		Waste Oils	Skin Disorders	Employees, Subcontractors, Interns	PPE, OHS Trainings, Material Safety Data Sheets, Environmental Trainings
11	Storing Oil and Air Filter	x		Slip	Injury, Fall, Slip, Trip	Employees, Subcontractors, Interns	PPE, OHS Trainings
12	Storing Oil and Air Filter	x		Dust	Respiratory, Eye, Chest Disorders	Employees, Subcontractors, Interns	PPE, OHS Trainings, Instructions
13	Storing Oil and Air Filter	x		Fire	Burn, Drowning, Death	Employees, Subcontractors, Interns	PPE, Instruction, Environmental Trainings, OHS Trainings, Extinguishing Equipment
14	Storing Hazardous Waste	x		Fire	Burn, Drowning, Death	Employees, Subcontractors, Interns	PPE, Instruction, Environmental Trainings, OHS Trainings, Extinguishing Equipment
15	Collection and Transport of Waste Fluorescents	x		Mercury Vapor	Skin Disorders, Acute Poisoning	Employees, Subcontractors, Interns	PPE, OHS Trainings, Material Safety Data Sheets
16	Collection and Transport of Waste Fluorescents	x		Break	Injury	Employees, Subcontractors, Interns	PPE, OHS Trainings

17	Storage of Waste Fluorescents	x		Mercury Vapor	Skin Disorders, Acute Poisoning	Employees, Subcontractors, Interns	PPE, OHS Trainings, Material Safety Data Sheets
18	Storage of Waste Fluorescents	x		Break	Injury	Employees, Subcontractors, Interns	PPE, OHS Trainings
19	Storage of Waste Tires	x		Fire	Burn, Drowning, Death	Employees, Subcontractors, Interns	PPE, Instruction, Environmental Trainings, OHS Trainings, Extinguishing Equipment
20	Transport of Waste Tires	x		Working with Removal Tools	Crush, Jam, Crash, Death	Employees, Subcontractors, Interns	PPE, Instruction, OHS Trainings, Periodic Controls of Lifting Vehicles
21	Loading of Wastes to the Hazardous Waste Transport Vehicle and Exit of the Vehicle	x		Working with Removal Tools	Crush, Jam, Crash, Death	Employees, Subcontractors, Interns	PPE, Instruction, OHS Trainings, Periodic Controls of Lifting Vehicles
22	Loading of Wastes to the Hazardous Waste Transport Vehicle and Exit	x		Manual Handling, Lifting, Putting, Loading, Forcing	Joint, Low Back Disorders, Impingement, Crushing	Employees, Subcontractors, Interns	PPE, Instruction, OHS Trainings, Breaks
23	Loading of Wastes to the Hazardous Waste Transport Vehicle and Exit of the Vehicle	x		Non-Ergonomic Equipment/ Motion	Injury, Joint and Muscle Ailments	Employees, Subcontractors, Interns	PPE, Instruction, OHS Trainings, Breaks

Activities where waste oils are the source of danger include waste oil storage, contaminated waste storage, and oil and air filter storage. Possible effects are skin ailments, slipping, and fire. Activities whose source of danger is working with lifting equipment include transportation of waste oils, transportation of contaminated waste, transportation of waste tires, loading of wastes onto the hazardous waste transportation vehicle, and exit of the vehicle. Possible effects affecting the health of employees such as illnesses, colds, crushing, entrapment, and death can be listed. Activities where the source of danger is chemicals, cement mortar, mercury vapor, and paints include the storage of waste batteries because of the storage of contaminated waste. Employees may experience effects such as chemical burns, poisoning, skin irritations, and burns. Activities where fire is the source of danger include hazardous waste storage and oil and air filter storage. Possible effects for workers are burns, suffocation, and death. It may result in possible effects such as injury to employees, falling, slipping, and tripping due to breakages that may occur during the transportation, collection, and storage of waste fluorescents. Waste fluorescent lamps are also hazardous wastes containing mercury (Coskun and Civelekoglu, 2014; 2015; Ozgur et al., 2014; 2015). Care should be taken to avoid breakages in storage areas and during transportation. Among the activities that are the source of danger with non-ergonomic equipment and movements are the loading of wastes on the hazardous waste transport vehicle and the possible effects that may occur from the exit of the vehicle, which include injuries and joint and muscle disorders.

Necessary precautions must be taken for activities with significant risk values ($70<R<=200$). Possible effects are traffic accidents, injuries, respiration, etc. that will occur with lifting vehicles during the transportation of waste oils. It has been observed that this situation, which can often occur with the undertaking of the work by unauthorized persons and not performing the periodic inspection of the lifting tools, can have serious consequences. In another study in which risk analysis was calculated according to the L matrix (likelihood and severity) in the cement sector, similar results were obtained by Karahan (2016). The risk value of injuries as a result of traffic accidents with lifting vehicles is 16 (serious), and it has been stated that studies should be carried out in a short time to reduce the risks (Karahan, 2016). If the risk is not large enough to require stopping the work, it should be managed by authorized persons in a controlled manner. Periodic checks of the machines should be conducted regularly. In addition, there must be people who have received and documented training in the use of construction equipment. The fact that the training received by the employees cannot increase their level of knowledge and awareness about their

working life can be prevented by making the training to be given practical. With the establishment of Occupational Health and Safety Management Systems (OHSAS 18001) in the facilities, even small accidents will be detected, and necessary precautions will be taken (Serin and Cuhadar, 2015; Ghahramani and Salminen, 2019; Subhi and Septiawan, 2020).

Table 6 shows that the hazards related to waste oils, acids and bases, chemicals, paint, cement mortar, dust, fire, mercury vapor, breaking of waste fluorescents, manual handling, non-ergonomic equipment, and lifting tools have acceptable risk values in the temporary hazardous waste storage area. According to Karahan (2016), the risk values vary between R=21 and 60. The possible effects of equipment lifting and manual handling activities are musculoskeletal disorders according to Karahan (2016). Studies should be started to reduce the risks and should be concluded in at least 6 months (Karahan, 2016). Employees' awareness should be raised by providing regular occupational health and safety training. In addition, paying attention to the harmony between the requirements of the job and the physical and mental qualities of the employees by ensuring individual–job harmony with the selection of employees can prevent occupational accidents that may occur due to the inadequacy of the employee's qualifications at the initial stage. If the fire hazard grows, it is possible that it will pass to the surrounding enterprises, and both the spread of harmful gases and damage to the enterprise and health problems of the employees will be inevitable (Maditinos and Vassiliadis, 2020; Pence et al., 2003). Building materials produced and used in accordance with the standards will protect people and the environment from harmful factors.

Table 6: Hazardous waste temporary storage area risk analysis

	Unacceptable Risk		**Serious Risk**	**Significant Risk**		**Acceptable Risk**		**Minor Risk**	
	400 < R		**200 < R< = 400**	**70 < R< = 200**		**20 < R< = 70**		**R < = 20**	
	Current Risk Analysis			**Precautions to be Taken to Reduce the Risk to an Acceptable Level**		**Acceptable Risk**			
Activity No	**Likelihood**	**Consequences**	**Exposure**	**Risk Score**		**Likelihood**	**Consequences**	**Exposure**	**Risk Score**
1	2	1	15	**30**					
2	2	1	40	80	Maintenance and control of lifting vehicles, not assigning unauthorized personnel	1	1	40	**40**
3	1	1	40	**40**					
4	1	2	15	**30**					

5	1	1	40	**40**					
6	1	1	40	**40**					
7	3	1	15	**45**					
8	1	1	40	**40**					
9	3	1	15	**45**					
10	1	1	15	15					
11	1	1	15	15					
12	3	1	7	**21**					
13	1	1	40	**40**					
14	1	1	40	**40**					
15	1	3	15	**45**					
16	1	3	15	**45**					
17	1	3	15	**45**					
18	0,5	3	15	**22,5**					
19	3	0,5	40	**60**					
20	1	2	15	**30**					
21	0,5	1	40	**20**					
22	3	1	15	**45**					
23	3	1	15	**45**					

Waste oils and slip hazards are insignificant risk values (20>R). According to the table, the risk value was R= 15. According to Karahan (2016), the risk value in slip hazards is 12 (moderate), and necessary activities should be initiated to reduce the risks and completed within at least 6 months. In order to prevent accidents such as slipping, tripping, and falling, the working environment should be organized and slippery floors should be cleaned immediately (Pavlovic-Veselinovic et al., 2016; Rose et al., 2013). Equipment used for work should be moved from the working environment to the equipment warehouse it belongs to after the end of the work. Passways and gates must be clearly marked. In case of taking these precautions, both the health of the employees and the possible pollution by the leaked oils of the soil (health of living creatures), air (harmful gases), and water (possibility of mixing with rivers) will be prevented.

The ideal physical conditions for temporary hazardous waste storage areas to prevent harm to the employees and the facility are given below (Anonymous, 2015; Maowei et al., 2021).

- Waste producers producing less than one thousand kilograms of hazardous waste per month will be exempt from temporary storage permits for areas/containers where they temporarily store/store their hazardous waste. Waste producers producing one thousand kilograms or more of hazardous waste per month should obtain a temporary storage permit from local authorities for their temporary storage areas/containers. In the case of a change in the temporary storage area, the temporary storage permit is renewed.
- Financial liability insurance is taken out for temporary storage areas that are liable for insurance, regardless of the amount, and it is renewed every year and presented to local authorities.
- Producers of waste can temporarily store their wastes on their land for a maximum of 180 days, provided that the amount of collected hazardous waste does not exceed six thousand kilograms. Non-hazardous wastes can be stored temporarily for a maximum of 1 year. Hazardous and non-hazardous wastes should be collected separately and sent to licensed waste processing facilities before the specified time.

The characteristics of the temporary storage area are that the floor is of impermeable material, absorbent material should be available to prevent leakage or spillage, and it should be surrounded by a grid. It is obligatory to provide recovery/disposal with the appropriate method by collecting the

accumulated liquids, and they cannot be directly discharged into the receiving environment (KTU, 2022). In the temporary storage area, security measures (fire extinguisher, etc.) should be taken against all kinds of emergency such as fire. In the temporary storage area, appropriate partitioning should be created according to the hazardous nature of the wastes, and they should be stored separately. A sign indicating the code of the waste should be hung (EUM, 2022).

The purpose of hazardous waste management is to both reduce waste generation and provide effective waste control. Waste such as used tires, waste oils, waste solvents, paint sludge, inks, and other organic wastes can be used as alternative fuels (Unlu, 2006; Navia and Bezama, 2008; Murray and Price, 2008). Another suggestion might be to explore alternative cements. The alternative cement should be based on abundant raw materials and low carbon emissions, energy consumption, pollutants, and waste by-products. In order to take the necessary measures, the risks in the working environment should be determined (risk assessment), responsibilities should be regularly checked, and potential deviations from the legislation should be dealt with quickly via a proactive approach.

4. Conclusions

The vital importance of cement in the formation of concrete for use in the construction industry cannot be denied. However, activities for cement production can create risks both in terms of occupational health and safety and in terms of the environment, living things, and climate change. It is possible to reduce the undesirable effects of the cement manufacturing industry on personnel and the environment through risk analysis studies on the environment and occupational health and safety and the measures to be taken afterwards. In the present study, risk analysis of the selected cement factory's temporary hazardous waste storage area in the Mediterranean region was conducted using the Fine–Kinney method.

Occupational accidents in the temporary hazardous waste storage area in the enterprise were analyzed according to the field of activity and risk values were calculated. The actions with the highest risk value out of 23 dangers were detected: (1) during the transportation of waste oils with lifting vehicles, traffic accidents, injuries, respiratory and so on, ailments, and death (R= 80), (2) burns, suffocation, and death during the storage of waste oils (R= 60), (3) the loading of wastes into the hazardous waste transport vehicle and work accidents

experienced at the exit of the vehicle, joint and back ailments, compression, and crushing (R= 45).

As a result of the study, the measures to be taken in order to reduce all activities to an acceptable risk assessment level are given below:

(1) Periodic checks of the machines should be performed regularly.
(2) Construction equipment operators must be trained and documented. The fact that the training received by the employees cannot increase their level of knowledge and awareness about their working life can be prevented by making the training to be given practical.
(3) Employees' awareness should be raised by providing regular occupational health and safety training.
(4) The use of building materials produced and used in accordance with standards should be encouraged against fire hazard, and mandatory standards should be taken under control.
(5) In order to prevent accidents such as slipping, tripping and falling, the working environment should be organized, and the slippery floor should be cleaned immediately. Equipment used for work should be removed from the working environment to the equipment warehouse it belongs to after the end of the work.
(6) Pass ways and gates must be clearly marked.
(7) Occupational health and safety provisions are scattered in various laws. The inclusion of provisions in the law alone is not enough, these provisions must be implemented.
(8) By emphasizing the importance of precautionary duties rather than compensation duties to business managers, prevention should be done before diseases and accidents occur.

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CHAPTER VII

ENVIRONMENTAL RISK ASSESSMENT OF ROSE OIL PRODUCTION

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1. Introduction

Industrial facilities consider environmental risk factors not only because of increased regulatory pressure, but also because of the economic benefits associated with reducing material and energy consumption. Environmental risk factors with the capacity to pose a threat to the environment and public health are additional criteria that should be focused on the design and operation of industrial processes (Sonmez, 2021; Sahin et al., 2021; Hao and Nie, 2022). Risk assessment is the scientific component of risk analysis, which consists of four basic steps: hazard identification, hazard characterization, exposure assessment and risk characterization (Mahoney, 2022). Environmental risk assessment is the process of identifying, evaluating, selecting, and implementing actions towards ecosystem (Celik, 2000). In developed countries, studies such as the creation of emergency plans and data banks for the assessment of environmental risks are carried out. (Sunar, 1998).

There are a limited number of environmental risk analysis studies for different sectors in the literature. According to the research of Kuleyin and Asyali (2007), the environmental risk analysis for the port of Aliağa was created by L-type matrix method. A 5-point risk checklist was established, and measures were taken for the eight identified hazards. Air pollution is caused by transportation and stationary sources such as ships, trains, other freight vehicles, cargo handling equipment, oil and gas storage facilities, open coal piles. Domestic wastewater from ships, bilge waters, rainwater flowing to the surface in the port, ship paint and oil spills, and pollutants from other port activities cause water pollution. Dredging in ports causes habitat damage, changes in the rate of siltation and deoxygenation of in-port waters. In addition, many sources such as ship engines, fans, cranes, tractors, trucks cause noise pollution in port areas. Herva et al. (2012), in their research, two environmental assessment methodologies (EF and EMFA) applied to evaluate performance of a tailoring factory as a complementary, and to develop a simplified tool for it's environmental assessment. For a detailed study of EMFA, energy and material flow, it has been proposed to replace energy with clean energy sources. EF has been researched for resource consumption. Excluding emissions, the most impacting input stream was cotton fabric; otherwise, kerosene has become the most important factor. Therefore, substitution for cleaner energy sources has been recommended.

Ciftci and Beyhan (2021) carried out and compared the environmental risk assessment of ready-mixed concrete plants in Denizli and Adana with the L-type matrix method. The environmental risk of the ready-mixed concrete plant in Denizli is less and it has been determined that all environmental risks in the facility have been minimized with the implementation of the recommended measures. It has been observed that the unacceptable risks are higher in the ready mixed concrete plant in Adana. The recommendations in the research are as follows: establishment of an integrated recycling system, recycling of all wastewaters produced by using advanced technologies in the ready-mixed concrete sector, construction of waste areas, regular storage of wastes and disposal / recovery of wastes through licensed companies and employee trainings.

Rose oil production industry is still among the leading sectors worldwide as it provides raw materials for cosmetics (soap, cream, powder, perfume, etc.), perfumery, pharmaceutical and food industries (paste, sugar, Turkish delight, confectionery, ice cream, etc.). (Gungor, 1990; Baydar, 2006; Anonymous, 2020). The biggest share in Turkey's rose oil export is followed by France with 68% and Germany, Switzerland, Ireland and England respectively (Demircan,

2005). Raw material for rose oil, rose flower is taken into the boiler and boiled for a certain time by adding water and steam from the steam boiler (Slavov et al., 2017; Agaoglu, 2014). The oily rose water obtained here is taken into the tank to be collected. It is transferred from the tank to the veronika boiler. The cold-water pipes in the boiler and the added steam are separated into rose oil (Uysal and Ekinci, 2021; Kovacheva et al., 2010). For the concrete, the extractors are filled with flowers and n-hexane and washed with fresh n-hexane at regular intervals. The n-hexane extracts from each extraction are collected in a tank and from there filtered and subjected to vacuum distillation (Sismanoglu, 2005). Here, all of the n-hexane is evaporated and pumped into the tank for reuse. What remains is a statement called "concrete". Concrete is an intermediate raw material used to produce absolutes (Katekar et al., 2022).

Considering the increasing demand of industries for environmental performance evaluation and the need for sector-specific environmental performance indicators, a rose factory was selected in this study and risk factors were determined for environmental risk analysis, risk values were calculated for each risk factor with L-type matrix method and their effects on the environment were investigated. The L-type matrix method identifies potential hazards and events that may lead to an accident, sorts the detected accident events according to their severity, and identifies the necessary hazard controls and countermeasures (Ozfirat et al., 2017). The first step in risk assessment is to implement control measures by following the prepared risk analysis studies, and the second step is to monitor the reduction of the identified risks to an acceptable level, and finally to control the continuity of the measures and control the risks (Jiang and Zhao, 2022). Environmental risk analysis studies are a current study topic for the rose oil production sector and literature resources are quite limited. It is thought that the risk factors and working method mentioned in the study can be a guide for other similar sectors. As a result of the study, it is aimed to contribute to the literature and increase environmental risk analysis studies. The results of the study include measures that can be taken for environmental risks in the rose oil production sector.

2. Materials and Methods

2.1. Facility data

The facility operates in the production of rose oil has from 10 to 20 employees seasonally (in May, June, July). The facility is in Isparta/Turkey, where rose oil is produced throughout the country. The annual production capacity is 437 kg/

year for rose oil and 765 kg/year for concrete. Within the framework of the Waste Management System at the facility, it provides the collection, temporary storage, and disposal of all hazardous and solid wastes in accordance with the legislation. Temporary waste storage area permission has been obtained from the Provincial Directorate of Environment and Urbanization, and the wastes arising from the routine activities of the facility are stored in accordance with the legislation according to their types. Waste generated at the facility (alkaline batteries, waste fluorescent lamps and mercury lamps, packages containing residues of dangerous substances or contaminated with dangerous substances, absorbents contaminated with dangerous substances, filter materials (oil filters unless otherwise specified), cleaning cloths, protective clothing, plant pulp and non-hazardous mixed municipal wastes) are collected in the relevant compartments in the temporary waste storage area. Wastes are sent to environmental permit and licensed facilities for disposal or recycling.

2.2. Method

Since the L-type matrix method can be applied in small and large facilities, it is both an easy method and the most applicable risk analysis method in the occupational health and safety sector (Selcuk and Selim, 2018). It is also widely used for environmental risk analysis calculation.

L matrix method, which is a two-dimensional matrix graph, has different meanings for horizontal and vertical coordinates. Horizontal coordinate shows the risk severity (C), and the vertical coordinate shows the probability (L) of the risk (Wang and Wang, 2020). The reason why the L-type matrix method was preferred in the study is that this method is suitable for environmental risk analysis (Gul et al., 2014). In this method, the risk score is calculated for each environmental element. According to the result of the risk score, suggestions were made to the facility according to whether the risk is acceptable risk, significant risk, high risk and very serious risk. Risk score was calculated with the following formula (MSANZ, 2004).

Risk (R) = Likelihood (L) x Consequences (C) (Wang and Wang, 2020; Gul et al., 2014)

In this place;

L = Likelihood (Table 1),

C = Consequences (Table 2),

R = Risk level (Table 3 and Table 4).

Table 1: Likelihood

	Likelihood	**Classification**
1	Very Small	Hardly ever
2	Small	Very little (once a year), only in abnormal situations
3	Middle	Few (several times a year)
4	High	Often (monthly)
5	Very High	Very often (once a week, every day)

(MSANZ, 2004)

Table 2: Consequences

	Consequences	**Classification**
1	So Light	Insignificant environmental impact
2	Light	Minor operational rash
3	Middle	Significant environmental damage
4	Serious	Environmental life suffers serious losses
5	So Serious	Disaster

(MSANZ, 2004)

Table 3: Risk level classification

Risk Score		**Consequences**				
Likelihood		5	4	3	2	1
		So Serious	Serious	Middle	Light	So Light
5	Very High	25	20	15	10	5
4	High	20	16	12	8	4
3	Middle	15	12	9	6	3
2	Small	10	8	6	4	2
1	Very Small	5	4	3	2	1

Table 4: Risk Score

Risk score	**Action (activity)**
15,16,20,25	Unacceptable risk
8,9,10,12	Considerable risk
1,2,3,4,5,6	Acceptable risk

3. Results and Discussions

Environmental risk analysis of the selected company was made using the L-type matrix method. The environmental risk assessment of the rose factory is given in Table 5. In Table 5, there are 8 hazards identified for environmental risk analysis. In the environmental risk analysis of the rose factory, no activity was identified in the notable (8, 9, 10, 12 = R) category. Paper waste, non-hazardous mixed municipal waste, and wastewater generation from the use of printers are included in the acceptable risk group (1, 2, 3, 4, 5, 6 = R). Significant risk (8, 9, 10, 12 = R) is plant pulp waste and wastewater generation. Many activities in the study area were evaluated in the unacceptable risk group (15, 16, 20, 25 = R). These hazards can be listed as battery-powered devices, lamps and bulbs used in lighting, contaminated packaging, cleaning cloths and protective clothing. Effects as a result of printer use and correspondence; Environmental resources are harmed by the use of paper. One of the possible environmental effects in battery-operated devices is that alkaline batteries can get into the soil and harm living things. Waste fluorescent lamps are also hazardous wastes containing mercury (Coskun and Civelekoglu, 2014; Coskun and Civelekoglu, 2015; Ozgur et al., 2014; Ozgur et al., 2016). Mercury content of waste fluorescent lamps and mercury bulbs is harmful to living things by mixing with nature. Environmental risks arising from cleaning detergents are contaminated packaging, cleaning cloths and protective clothing harming the environment. When non-hazardous mixed municipal waste is stored irregularly, it harms nature and living things. Plant pulp wastes formed because of rose oil produced in the factory create odor problems in the environment. Since the use of water in the boilers and office is connected to the sewerage, it is sent to the treatment plant. Necessary measures should be taken for activities with unacceptable risk value.

Table 5: Rose oil production plant risk analysis

No	Activity	Source of Danger	Possible Impact	Likelihood	Consequences	Risk Score	Prevention
1	Office	Printer usage and correspondence	Damage to environmental resources due to paper use	5	1	5	Waste should be sent to licensed companies. Employees should be trained.
2	Office	Battery powered devices	Mixing of alkaline batteries with the soil and harming living things	4	4	16	Waste should be sent to licensed companies. Employees should be trained.
3	Office	Lamps and bulbs used in lighting	The mercury content of waste fluorescent lamps and mercury lamps mixes with nature and harms living things.	4	4	16	Lighting should be turned off when not in use. Automatic lighting can be used. Waste should be sent to licensed companies. Employees should be trained.
4	Business and Office	Cleaning detergents	Contaminated packaging harming nature	4	5	20	Contaminated packages should be collected and sent to licensed companies on a regular basis. Employees should be trained.
5	Business and Office	Cleaning detergents	Cleaning cloths, protective clothing harming nature	4	5	20	Cleaning cloths and protective clothing should be collected and sent to licensed companies on a regular basis. Employees should be trained.
6	Business and Office	Non-hazardous mixed municipal waste	Waste harming nature	5	1	5	Waste should be sent to licensed companies. Employees should be trained.
7	Business and Office	Plant pulp waste	Odor problem in nature	5	2	10	The pulp accumulated in the pool should be cleaned regularly. Employees should be trained.
8	Business and Office	Water use in boilers and in the office	Wastewater generation	5	1	5	There is a sewage permit document, and it is sent to the wastewater treatment plant.

In another study in which the environmental risk analysis was calculated according to the L matrix (probability and severity) in the biological wastewater treatment plant, the same results were obtained by Guner (2018), and the risk value is 18 (unacceptable risk), and work should be done immediately to reduce the risks. Another danger is the possible effects of battery powered devices; is the mixing of alkaline batteries with the soil and harming living things. According to the study of Ciftci and Beyhan (2021), whose environmental risk analysis in ready-mixed concrete plants was carried out using the L-type matrix method, the risk value of waste batteries was determined as 12 (considerable risk). It is recommended that risks be addressed as quickly as possible. According to the regulation, it should be sent to licensed companies in sealed and labeled bags, in case of leakage, within 6 months (Anonymous, 2015). In addition, the fact that the trainings received by the employees cannot increase their level of knowledge and awareness about their working life can be prevented by making the trainings practical.

In the remarkable risk group, odor problem that will occur with plant pulp wastes, etc. are possible effects. It has been observed that not cleaning the plant pulp from the pool regularly and not sending it to the necessary sectors (fields, ceramics industry) may cause odor problems that can occur frequently. In another study in which the environmental risk analysis was calculated according to the L matrix (probability and severity) in the biological wastewater treatment plant, the same results were obtained by Guner (2018), and the risk value was 9 (considerable risk), and it was stated that the studies aimed at reducing the risks should be intervened quickly (Guner, 2018). In the regulation for waste producers; It is obliged to take the necessary measures to minimize the waste production and to keep the municipal wastes ready for collection by keeping them closed in such a way that can be useful for the environment and human health in places such as residences, workplaces, as determined by the institutions and organizations that have been given the responsibility of collection, transportation and disposal within the scope of the relevant legislation (Anonymous, 2015).

In Table 5, paper waste, non-hazardous municipal waste and wastewater generation are among the risks as acceptable risk values. According to Guner (2018), risk values vary between R = 2 - 6. The possible effects of wastes are stated as environmental pollution according to Guner (2018). It has been stated that there is no need for urgent measures to reduce risks (Guner, 2018). Awareness of the employees should be provided by giving regular trainings. In addition, if it is collected regularly and sent to licensed companies, damage to nature can be prevented. Connecting the wastewater to the sewer and sending

it to the wastewater treatment plant is one of the appropriate measures. Thus, while the risk value is in the unacceptable risk group, the acceptable risk value decreases. In case of taking measures in accordance with the legislation and regulation, possible pollution of both human health and the environment will be prevented. Necessary measures will be taken by identifying even minor risks with legislation and regulations at the facilities (Serin and Cuhadar, 2015; Ghahramani and Salminen, 2019; Subhi and Septiawan, 2020).

The aim of waste management is to both reduce waste generation and provide effective waste control. In order to take the necessary precautions, the risks in the working environment should be determined (environmental risk assessment), responsibilities should be checked regularly, and the deviations from the legislation should be intervened quickly with a proactive approach.

4. Conclusions

Rose oil production industry is among the important sectors as it provides raw materials in the perfumery, pharmaceutical and food industries, especially in cosmetics. However, activities in the rose industry may pose risks in terms of environment and climate change. It is possible to reduce the undesirable effects of the rose industry on personnel and the environment with environmental risk analysis studies and the measures to be taken afterwards. In this study, the environmental risk analysis of the rose plant selected in the Mediterranean region was determined by the L-type matrix method.

Environmental risks in the facility were analyzed and risk values were calculated. Actions with the highest risk value out of 8 hazards were determined in order; (1) the mercury content of the lamps and bulbs used in lighting mixes with nature and harms living things (R= 20), (2) the contaminated packaging contaminated with cleaning detergents harms nature (R= 20), cleaning cloths contaminated by cleaning detergents, protective clothing harms nature (R= 20), (3) alkaline batteries mixed with the soil and harming living things (R= 15).

As a result of the study, the measures to be taken to reduce the activities to an acceptable risk assessment level can be listed as follows:

(1) Wastes collected in the temporary storage area; If it is dangerous, it should be sent to licensed companies within 6 months, if it is not dangerous, within 1 year.
(2) Lighting should be turned off when not in use. It is recommended to use automatic lighting.

(3) Employees' awareness should be raised by providing regular environmental trainings.
(4) Contaminated packaging, cleaning cloths, protective clothing should be collected regularly.
(5) Pulp accumulated in the pool should be cleaned regularly and sent (it can be poured into the fields for fertilizer, it can be used in the ceramic industry).

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CHAPTER VIII

THE STRATEGIES OF LACTIC ACID BACTERIA ON ACRYLAMIDE MITIGATION - A REVIEW

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1. Introduction

Lactic acid bacteria (LAB) constitute a phylogenetic group of gram-positive bacteria united by a constellation of features, including gram-positive, facultatively anaerobic, and non-spore-forming. *Lactobacillaceae* contains 26 phylogenetic groups (Zheng et al., 2020). LAB has a unique mode of sugar fermentation. Mainly in relation to these metabolic systems of theirs, they produce many functional compounds. LAB-produced bioactive compounds can be used as natural food bio-preservatives. Organic acid (lactic, acetic, propionic, linoleic, and butyric acids), vitamins, and aroma compounds

are the most important and very much evaluated metabolites of LAB. On the other hand, exopolysaccharides (EPS), peptidoglycans, and enzymes produced by LAB have attracted much attention with their functionality. Moreover, the target-specific metabolites of LAB are able to combat pathogens, oxidative stress, toxins or toxins receptors and other linked infections. In general, the bio-detoxification mechanisms of LAB and its applications in food as bio-protective agents are of great interest (Nasrollahzadeh et al., 2022). One of them is that LABs show an inhibition feature against acrylamide formation in heat-treated foods or provide elimination of acrylamide.

Acrylamide (2-propenamide; AA) is a monomer unsaturated hydrophilic amide. As for AA production in food; no acrylamide is present in raw food, and AA is defined as a food-processing-induced contaminant (Krska et al., 2012). It is commonly formed in foods rich in carbohydrates at high heat. Also, the amount of acrylamide varies according to the natural content of the foods and the way of cooking. It has been proven by animal experiments that continuous and high doses of this chemical compound cause cancer. The European Union has methods and guidelines to monitor acrylamide levels in foods and to develop production processes that prevent its formation. Average daily exposure values to AA were reported as 0.5-1.0 µg/kg for 18-65 years old and as 0.44 - 0.95 µg/kg for < 2 years old, respectively, by European Food Safety Authority (EFSA, 2015) and Food and Drug Administration (2006). When many studies are examined together, it has been observed that the average daily dietary acrylamide intake is between 0.02 and 1.53 µg/kg (bw). The major acrylamide contenting foods are potato crisps and chips, cakes, and biscuits (EFSA, 2015). These products are mostly consumed by children. Considering the level of acrylamide taken into the body against the kg body weight of children, children are exposed to acrylamide three times more than adults. Therefore, applying effective approaches to reduce the level of acrylamide in foods is the common point of view of the whole world (Khorshidian et al., 2020). Also, Supported by scientific studies in recent years, scientists have found that food processes, predominantly roasting, baking, and frying, produce acrylamide (Bin-Jumah et al., 2021). The highest concentrations have been identified in fried potato products, breakfast cereals, and coffee (Bagheri et al., 2019; Capuano & Fogliano, 2011).

2. Emergence and Spread of AA

AA was first synthesized in a laboratory in 1949 (Sarion et al., 2021). Acrylamide was classified as a probable human carcinogen by International Agency for Research (IARC) in 1994 and Environmental Protection Agency US (EPA) in 1988. Later, in April 2002, AA was discovered in foodstuffs by scientists working at Stockholm University in Sweden.

Typically, AA is formed as a result of interaction between free amino acids (main asparagine) and reducing sugars (Mollakhalili-Meybodi et al., 2021). The acrylamide generation depends on 1) exogenous factors and 2) endogenous factors. Exogenous factors include temperature, frying time, pH, water activity, raw food origin, and cooking method (Albedwawi et al., 2021; M. Bin-Jumah et al., 2021; Liyanage et al., 2021). The endogenous factors are the precursors content in the food (Emadi et al., 2021; Xu et al., 2014). The main precursors in the acrylamide formation pathway are 3-aminopropionamide (3APA), decarboxylated Schiff base, decarboxylated Amadori product, acrylic acid, and acrolein (Maan et al., 2020; Perera et al., 2021).

While the exact acrylamide formation pathways have not been fully understood. The most relevant and probable acrylamide formation two pathways are Asparagine-Glucose [Asn-G] pathway and the Acrolein pathway. In the Asn-G pathway, the α-amino group of free asparagine (Asn) is interaction with the carbonyl group of reducing sugar. A key intermediate in acrylamide synthesis (Schiff base) converts into acrylamide. This reaction is called an acrylamide-forming Maillard reaction. In the Acrolein pathway, acrylamide is formed by the reaction of an acrolein compound with acrylic acid, which is formed as a result of oxidative degradation of fats and subsequent dehydration of glycerol (Udomkun et al., 2021). Fig. 1, it was summarized the important steps and intermediates of the AA formation pathways.

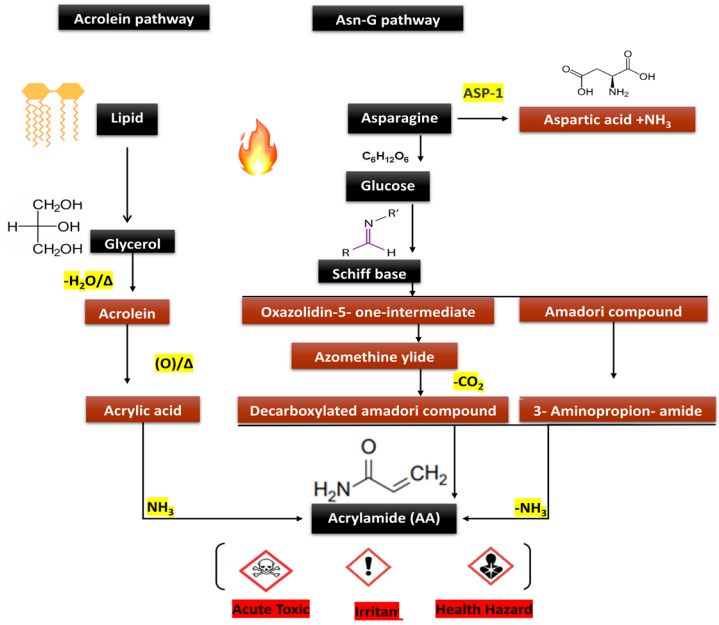

Figure 1. The Asn-G/ Acrolein pathway for AA formation in food

2. Biotransformation of AA and Microbial Mitigation

Results of in vitro and in vivo experiments indicated that a number of microorganisms, particularly lactic acid bacteria (LAB) and yeast, can bind dietary carcinogens. As a result of these experiments, it was determined that some LAB strains and some yeasts (some *S.cerevisiae* strains etc.) may act as potential carcinogenic mitigation within the food matrix or directly in the intestinal lumen (Cuevas-González et al., 2022). Some studies have been conducted on this. For example, Microbial dextran (MD) synthesized by *Leuconostoc mesenteroides* NCIM-2198 strain was successfully used to mitigate acrylamide formation in quinoa biscuits (Mousa, 2022). A blue-green alga *Spirulina platensis* was reported to have protective effects against acrylamide toxicity in rats (Bin-Jumah et al., 2021). In another study, the acrylamide removal capabilities of several lactic acid bacteria and yeasts were assessed. AA-removing-LAB under either bind or degrade acrylamide was evaluated (Albedwawi et al., 2021).

Various microorganisms that reduce AA with microbial cell enzymes are given in Table 2. When the studies were examined, it was determined that L-asparaginase enzyme synthesized from *Aspergillus terreus* strains could be used up to ~93% together with physicochemical pretreatment techniques for acrylamide reduction (Paul & Tiwary, 2020). In another study, a lactase enzyme from a strain of *Bacillus subtilis* species and an amidase enzyme from a strain (N-771) of *Rhodococcus* genus have been reported to cause biodegradation of anionic polyacrylamide (HPAM) (F. Wang et al., 2021). With a general evaluation, it can be concluded that microorganisms with potential amidase producers play an important role in reducing AA because it amidase activity is able to convert acrylamide into acrylic acid (Khasanova, 2021; Prabha & Nigam, 2020). In other studies, AA content in the treated French fries with L-Asparaginase enzyme (L-ASNase, EC 3.5.1.1) from a *Palaeococcus ferrophilus* species was significantly reduced by 79% (Wang et al., 2021). In another, a specific LAB strain in conjunction with glucoamylase from Aspergillus niger was used as an acidic regulator and AA content mitigation in dough and bread (Bartkiene et al., 2013). In a study related to this, AA in dough containing *Lactobacillus acidophilus* + *Saccharomyces cerevisiae*, *Lactiplantibacillus plantarum* + *S. cerevisiae*, and *L. acidophilus* + *L. plantarum* + *S. cerevisiae* decreased with 46 %, 77 %, 83 %, and 90 %, respectively (Abedi et al., 2022).

3. LAB - AA Mitigations

Some LAB has been postulated as AA formation inhibitors owing to strategies inholding 1- utilize C/N sources, 2- possess enzyme genes, and 3- binding

techniques. In Fig. 2, it was summarized the AA reduction strategies of LAB. However, this feature of LAB also depends on different conditions. It was known that an effect of the concentration of AA in the removal capacity of AA by the lactic acid bacteria. In a rich nutrient medium, probiotic strains prefer easily available C/N nutrients, so utilization of AA will not occur (Petka et al., 2022). In a study conducted by Onacik-Gür et al. (2022), it was found that rye bread made by the direct method using acidification by lactic acid had 3.5 times lower AA content than those made by the indirect method with a starter culture. Torres-Gregorio et al. (2021) stated that both individual probiotic strains and probiotic consortia could play an important role in reducing the intestinal availability of acrylamide. For example, *Lacticaseibacillus casei* was used to ferment mixed rye and removed 20.2% and 29.4% of AA in bread samples of 500 g and 1000 g, respectively (Bartkiene et al., 2013). LAB for acrylamide removal in biscuits, fried potatoes, and potato chips was assessed. AA was reduced by the use of strains of *Pediococcus acidolactici* (78% ↓AA), *Latilactobacillus sakei* subsp. *sakei* (83.40%↓AA), *L. acidophilus* (60%↓AA), and *L. casei* Shirota (65-73%↓AA) (Bartkiene et al., 2016; Mousavinejad et al., 2015; Rivas-Jimenez et al., 2016). It should also be noted that the acrylamide removal ability of LAB depends on factors such as their bacterial cell density, acrylamide concentration, bacterial viability, incubation or treatment time, and temperature (Wu et al., 2021).

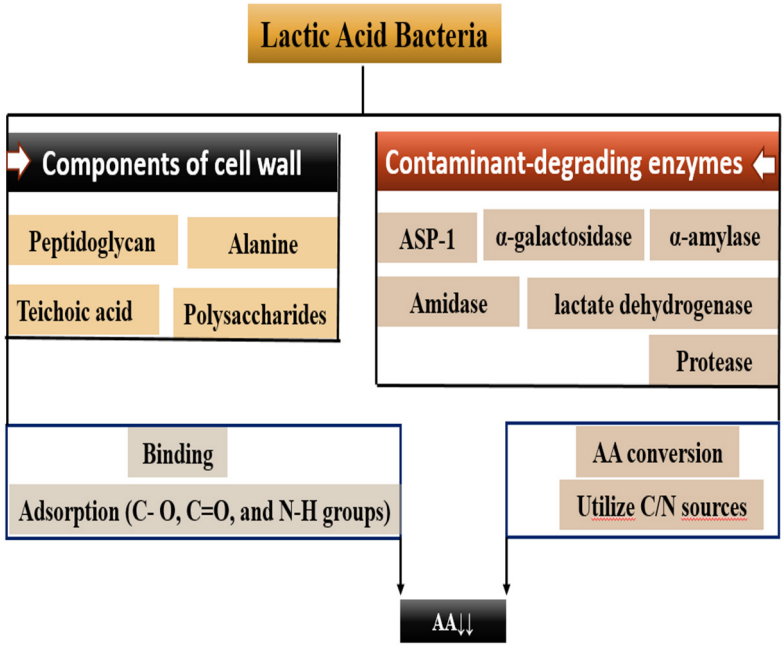

Figure 2. Acrylamide reduction strategies of LAB

3.1. AA Mitigation Profiles of LAB: Genes

Some strains of probiotics (some strains of *L. casei* and *Limosilactobacillus reuteri* subsp. *reuteri)* have asparaginase genes known to be associated with AA reduction techniques via binding of peptidoglycan compounds or L-asparagine conversion as possible mechanisms (Khorshidian et al., 2020). Recently, it has been highlighted that some LAB species have an ASNase gene (Aishwarya et al., 2019; Amer et al., 2013). In a study conducted by Phetsri et al. (2019), it was determined that the ASNase gene from *Streptococcus thermophilus* was amplified with F 5'AGTAGTACATATGATTAAAAAAATCCTAG3' and R 5'TTTCTCGAGCCCTTCAATATAATC 3' primers. Phetsri et al. (2019) demonstrated that the St-ASNase gene is a good candidate for AA reduction.

3.2. AA Mitigation Profiles of LAB: Enzymes

Acrylamide can stimulate the growth of *L. acidophilus* and *Kluyveromyces lactis*. *L. acidophilus* LA-5 strain can utilize acrylamide as a source of carbon and nitrogen if they lack them in the environment. This is probably due to acrylamide degradation by amidases. These findings provide a basis for future work on the enzyme degradation of AA.

Phytase is an enzyme that has a synergic effect in amylase and asparaginase activity due to broken down phytic acid and an increase in the mineral content (Abedi et al., 2022). Furthermore, the asparaginase enzyme offers a clear advantage or benefit over the formation control strategy, which is the potentially little effect on sensory traits as well as the quality of the final product. Depending upon the pH and amylase activity, the amount of acrylamide in wheat bread can adequately be reduced by LAB (Shao et al., 2021). LAB with amylolytic activities caused a dramatic reduction of the precursors for AA formation because of its starch-glucose breakage action within a mild acid sourdough system (Abedi et al., 2022). Although α-amylase and α-galactosidase could accelerate acrylamide formation by increasing its precursors, it simply doesn't happen. It may be attributed to making them easily consumed by LAB. Additional investigations have shown that in order to avoid adverse effects, LAB with lower enzymatic activities, protease as well as amylase should be used in the fermented bread-making process (Bartkiene et al., 2013). The most common strategy of acrylamide reduction using LAB enzymatic activity is reducing its precursors in a glucose/asparagine system.

3.3. AA Mitigation Profiles of LAB: Cell Extracts

Studies indicated that the cell wall of LAB had a crucial role in AA adsorption (Albedwawi et al., 2022; Ge et al., 2017; Shen et al., 2019). Both *S. lutetiensis*

and *L. plantarum* strains removed more acrylamide in the range of 35–46% and 45–55%, respectively. After being exposed to a simulated gastrointestinal tract environment, at the gastric stage and intestinal stage. *S. lutetiensis* and *L. plantarum* removed more than 30 and 40% of the acrylamide (Albedwawi et al., 2022). The authors of the study represent that the acrylamide adsorption capacity correlated with cell wall thickness and functional groups C=O, C-O, and N-H (Albedwawi et al., 2022). Serrano-Niño et al. (2014) reported that in 5µg/mL AA for 12 h of incubation the *L. reuteri* and *L. casei* Shirota were the most efficient AA binders.

Several studies revealed that carbohydrate components of Lactic acid bacteria (LAB) played an important role in decreasing harmful substances (Hernandez-Mendoza et al., 2009; Zhang et al., 2017). Mechanism of binding involved physisorption and peptidoglycan of LAB has attracted researchers as an area of interest (Shao et al., 2021). LAB strains have the ability to cope with dietary carcinogens that act through different mechanisms, including the adsorption of toxic compounds to the cell wall and peptidoglycans (Yousefi et al., 2021).The cell wall composition of LAB strains consists of polysaccharides, teichoic acid, and peptidoglycan structures that play a crucial role in complex binding to contaminants. In a vivo assay, the AA binding rates of peptidoglycan obtained from L. plantarum 1.0065, L. casei ATCC393 were 87.05 and 75.05%, respectively (Zhang et al., 2017). Also, it is stated that besides peptidoglycan components of specific strains of LAB, AA binding ability of them is associated with the presence of alanine (Khorshidian et al., 2020). As for teichoic acid, it is an anionic polymer in the gram-positive LAB cell wall. A significant correlation was observed between its content and the percentage of AA-binding (Serrano-Niño et al., 2015).

4. Nonmicrobial Innovative Mitigation Approaches of Acrylamide

Although enzymatic pretreatment is quite expensive, the enzyme-addition method with assistant techniques sounds like a practical and economical venture for AA reduction. Therefore, a combination of conventional methods and emerging techniques within the food industry should be promoted. Nonmicrobial innovative mitigation approaches to acrylamide are represented in Table 3. A new application of protein-polysaccharide biopolymer particles such as zein–alginate could reduce acrylamide at high temperatures in chemical and food models (Champrasert et al., 2022). It was determined that low concentrations of sodium alginate and pectin successfully inhibited acrylamide formation in the chemical model (Champrasert et al., 2021). AA was inhibited in French fries pretreated with acids such as CA, HAc, SAPP (Y. Huang et al., 2022). Using

thiol compounds to reduce acrylamide levels has been reported in the literature (Cerit & Demirkol, 2021). An effective strategy to control acrylamide is by using low-gluten flour and maltitol in the product ingredients, combined with infrared-assisted deep-frying (Han et al., 2021). The usage of fermentation of sprouted cereal products caused lower acrylamide formation (Yiltirak et al., 2021). The pretreatment of potatoes with the plant extracts of ginger, borage, and fennel succeeded in reducing acrylamide formation, particularly in air frying (AF) (Haddarah et al., 2021).

5. Health Risks of AA

Acrylamide is a problematic contaminant. AA-rich foods have raised considerable public health concerns worldwide (FAO/WHO, 2020). The continued consuming acrylamide-containing foods lead to serious health consequences (Nachi et al., 2018; Shen et al., 2019). Acrylamide concentrations exhibit great variation among products, countries, and even brands. Table 4 represents the estimation of acrylamide concentration in a variety of food products, countries, and brands. Numerous findings on the effects of acrylamide on human health are available in the literature. These data confirm that AA is a direct-acting in the dysfunction of various organs and the immune system. The effect of AA depends on several factors I) Amount of acrylamide in the diet. II) Dose level of AA mg/kg b.w. per day. II) Absorption and distribution of AA. III) The internal dose and bioavailability of GA.

Acrylamide is an electrophilic molecule. Upon absorption in vivo, AA is rapidly and extensively absorbed from the gastrointestinal tract (European Food Safety Authority (EFSA), 2015). It passively diffuses throughout the body, therefore, all tissues (European Food Safety Authority (EFSA), 2015; Salimi et al., 2021). In the body, AA intoxication (AA → GA (glycidamide) or detoxication (AA → AA-GSH (glutathione)) (Y.-S. Luo et al., 2022). Glycidamide (GA) is a DNA-reactive epoxide of acrylamide. Acrylamide causes oxidative DNA damage in target organs (Mori et al., 2022). AA and GA can be further detoxified through glutathione (GSH) conjugation in the liver and excreted in urine (Y.-S. Luo et al., 2022; Ngo-Thanh et al., 2021).

AA had a negative effect on the motility and viability of human spermatozoa (Kashani et al., 2021). AA is selective neurotoxicity; however, its underlying mechanisms remain largely unknown (Hassan et al., 2021). AA and GA may disrupt testicular function, thereby adverse effects on reproductive function and fertility in the male (Aydin, 2018; Kashani et al., 2021). The target organs of AA are the brain and reproductive system (Cuevas-González et al., 2022). As

a limitation in the current study, a tolerable daily intake (TDI) of acrylamide in food cannot be set. Therefore, experimental models such as animal studies are still highly needed. Taking on attention to the adverse effect of AA on human health, lowering its level in food is evaluated as efficient risk management.

6. Challenges and Obstacles

Although dietary AA intake is a health concern, the estimation of the AA intake is uncertain. In recent years, various conventional chemical-based methods with different principles have been published for the detection of AA content in different food matrices. The time-consuming and skilled labor-intensive are the major disadvantages of this strategy. Therefore, a simple yet accurate and fast-tracking time method is a major need. There is a need to develop different methods in this regard. The noted reduction strategies of akrilamidin have a negative impact on heated foods' pleasant sensory characteristics. Researchers need in terms of efficiency of AA- reduction, food properties stability, and cost-effect. The mechanism of acrylamide removal by microorganisms such as LAB and yeast is not fully explored, understood, and still highly studied. To the best of our knowledge, limited information is available regarding acrylamide removal by LAB under the in vitro digestion condition (Albedwawi et al., 2022; Shen et al., 2019). It is crucial to investigate the acrylamide removal conditions surrounding the microorganisms. The conducted response surface methodology indicated that pH as well as incubation time and temperature, significantly influenced the amount of ammonia released from acrylamide by the bacteria (Petka et al., 2021). The viability and efficacy of selected LAB strains during preprocessing, and post-process of AA mitigation should be taken into consideration as an important challenge.

7. Conclusion

The results indicate that LAB can eliminate acrylamide with several strategies. These strategies included contaminant-degrading enzymes (asparaginase and amidase) and components of cell walls (adsorption and binding). LAB is considered a safe and inexpensive practice for dealing with AA-contaminated food. Despite the scarcity of information about the mechanisms of acrylamide removal by Lactic acid bacteria in vitro, it remains a promising field and may achieve scientific precedent, especially in the case of combining LAB strains with probiotics properties and mitigation abilities of AA. Continuous AA monitoring and risk analysis within processed foods to generate data that can

help to establish affirmative elimination strategies should be taken as urgent steps. Even with the gaps in knowledge regarding acrylamide mitigation using LAB, Further studies should conduct to elucidate the mechanisms of AA removal by LAB.

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Table 1: The Raw Result for Acrylamide from Different Studies

References	S. Area	S.Year	Applicability	S. Size (g)	Analysis Method	Detection Range
Zhuang et al., 2022	China	2021	Cookies, white bread, potato products, coffee	3	AA-GSH-Au NPs- TMB	0.5 - 175 µM
Schouten et al., 2022	Italy	2021	Biscuit	1	HPLC-MS/MS	275 µg/ kg (175 °C / 26 min).
Onacik-Gür et al., 2022	Poland	2021	Rye bread	-	GC/MS	7.36 - 75.76 µg/ kg
Demir & Agaoglu, 2021	Turkey	2021	Ready-made potato chips	-	HPLC	536.21 mu g/kg
Pogurschi et al., 2021	Romania	2021	Pretzels	-	LC-MS/MS	113.95 - 130.8 µg/kg
Mojska et al., 2021	Poland	2021	Breast Milk	-	LC–MS/MS	<0.1 -1.0 µg/L
Basheer, 2021	Saudi Arabia	2021	Pet Food Samples	-	Electrospun Membrane	-
Abdel-Haleem & Abdel-Aty, 2021	Egypt	2021	Ground coffee Substitutes Brewed coffee substitutes	2.0 g 1.25 mL	GC-MS	<10 - 242.8 µg kg^{-1} 0.26 - 1.70 µg 70 mL^{-1}
Smeesters et al., 2021	Belgium	2021	Potato	-	Reflection spectroscopy (400–1700 nm) and machine learning	-
Ghazouani et al., 2021	Tunisia	2020	Bsissa	1	RP-HPLC-DAD	Not detected
Cheng et al., 2021	China	2020	Baked and Fried Foods	-	UHPLC-QqQMS/MS	0.5 - 500 ng/mL
Wei et al., 2021	China	2020	Bread crust	1	Fluorescent biosensor/ CQDs/ssDNA	1 x 10^7 -5 x 10^3 M
Maurya et al., 2021	India	2020	Potato chips	-	Computer-aided automatic detection	-/+

Bahrami et al., 2021	Iran	2020	Date syrups	1	HPLC	141–554 ppb
Wei et al., 2020	China	2020	White bread	2.0	Carbon quantum dots	5×10^{-4} - 5×10^{-6} M
Barón Cortés et al., 2021	Colombia	2020	Coffee	1.5 g/100 mL	HPLC	3.3 - 9.5 µg/kg
Luo et al., 2021	China	2020	Drinking Water	-	fluorescence immunoassay	82.3 - 93.5 % Recovery
Deribew & Woldegiorgis, 2021	Ethiopia	2020	Roasted coffee powder	500 g	HPLC	135 -1139 µg/kg
Zhao et al., 2021	China	2020	Potato chips and biscuits	4 g	MIP-PEC-ZnO-PPy	10^{-1} - 2.5×10^{-9} M
Roszko et al., 2020	Poland	2019	bread	-	GC/MS	3.6 - 163 µg kg^{-1}
Kruszewski & Obiedziński, 2020	Poland	2019	Dark Chocolate		GC/MS	6.7 - 20.3 µg/kg
Galuch et al., 2019	Brazil	2018	Brewed coffee	40 g/L	UPLC-MS/MS	0.9- 3.0 µg/L

Table 2: The Control of Acrylamide (AA) by Microbial Cell Enzymes

Microbial	**Enzyme type**	**Activity**	**°C/min**	**Reduction(%)**	**Product**	**References**
T. zilligii AN1 TziAN1_1	ASNase	10 U/mL	80 / 4	80.5 %	French fries	(Zuo et al., 2015)
B. aryabhattai	ASNase	18 U	30 min	90%	Food model system	(Alam et al., 2018)
R. eutropha AUM-01	Amidase	ND	30 - 70 / 10–30	60%	Coffee	(Cha, 2013)
B. tequilensis (BITNR004)	Amidase	ND	50˚C.	~60%	-	(Prabha & Nigam, 2020)
A. niger	Glucoamylase	500 GU/kg	30/3h.	16 %	Rye bread	(Bartkiene et al., 2013)
R. miehei	ASNase	10 U/g	ND	90%	Flour	(Huang et al., 2014)

Table.3: Nonmicrobial Innovative Mitigation Approaches of Acrylamide

Techniques	Assistant Tec.	Reduction(%)	Product	Explanation/mechanism	References
Asparaginase	High Pressure Processing (HPP)	26–47%	Fried potatoes	Preprocessing /Enhance diffusion of ASNase	(Dourado et al., 2020).
	Conventional blanching	86%	Potato chips	Pretreatment (85 ◦C/3.5 min)/Enhance diffusion of ASNase	(Jia et al., 2021).
	Magnetic nanoparticles	90%	Food model system	Pretreatment /↑Catalytic efficiency	(Alam et al., 2021).
	Steam	59%	Coffee beans	Pretreatment /↑Enzyme's acces	(Oliveira Correa et al., 2021).
Blanching	Distilled Water	19–59%	Potato chips	Prior to frying (65 C for 5 min)	(Liyanage et al., 2021).
Soaking	%1 acetic acid solution	%73	Jerusalem Artichoke tea	Adding before roasting (1h- 20 °C) / Suppressed acrylamide formation	(Jo et al., 2021).
	Ultrasound pretreatment	~95%	Potato slices	↑The reducing sugars extraction	(Pedreschi et al., 2021)
Alternative baking technologies	Vacuum baking (10 mbar) Combined baking	up to 98% up to 95%	Potato chips	Lower temperatures	(Akkurt et al., 2021).
	Tyrosyl Acetate (TyAc),	90 %	Fried potato	Added to dough /Higher K_0/W value	(Pantalone et al., 2021)
	Arabic Gum(AG)	83%	Cake	1% GA / Gelling, thickening effect, or acidic pH value of AG solution	(Mousa, 2021)

Table 4: Occurrence of Dietary Acrylamide in Various Food Products, Countries, and Brands.

Food Stuff	**Country**	**AA CONC (µg/kg)**	**References**
Potato chips	Spain	325	(Bermudo et al., 2006)
	Sweden	4000	(Tareke et al., 2002)
	Turkey	433 µg/g	(Şenyuva & Gökmen, 2005)
	Chile	1000<	(Barrios-Rodríguez et al., 2021)
Coffee	Colombia	960	(Mousavi Khaneghah et al., 2020)
	Poland	212.10	
	Japan	9.40	
French fries	North Macedonia	214.3-657.4	(Dimitrieska-Stojkovikj et al., 2019)
	Korean	101.6 - 1174.8 ng/ g	(Jeong et al., 2020)
Arabica ground roasted coffee	Romania		(Pogurschi et al., 2021)
Brand 1		69.5 - 101.7	
Brand 2		118.5- 173.5	
Brand 3		321- 427	
Filter coffee	Turkey		(Basaran & Aydin, 2020)
Brand1		16.3–29.7 ng/mL	
Brand2		12.4–20.9 ng/mL	
Brand 3		6.5–7.9 ng/mL	

CHAPTER IX

FISH DISEASES DOCTRINE

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1. Introduction

"Bacterial resistance and the grounds on which resistance thrives are a common threat to humanity..." (Nurcan, 2010). Hundreds of fish pathogens are included in the doctrine of fish diseases. also included in hundreds of non-infectious diseases in fish diseases doctrine. Consumption of fish as food, fishery activities, zoonosis (human-transmitted diseases), fish farming, etc. It is also included in the public health doctrine as epizootiologically because this creature is a source of contamination and is a source of contamination. From this point of view, prevention of pathogenesis is of vital importance for all living things in the world. All living and non-living beings on Earth interact. In the past, the focus of fish disease research was on various aspects of fish pathogens (biochemical, serological, virulence etc.) rather than the disease itself. Currently, this situation has changed and all aspects of the disease have begun to be investigated. Stress diseases is the first of the field of study. Stress is the inability to maintain the normal physiological state of the fish due to adverse factors. Stress arises when any change begins and continues in favorable conditions for the living thing. The basis of stress, which reduces resistance to diseases, is the weakening immune system of fish (Wish et al., 2022).

Proven that bacteria communicate with social behavior via signaling molecules, monitor whether they reach a certain majority, and trigger critical gene expressions such as virulence factors as soon as they reach a sufficient

number. Thus, by not stimulating the host's immune system before time, it creates a successful disease process (Nurcan, 2010).

The similarities between the signals used by bacteria and artificial neural networks are striking. There may even be low intelligence matter for bacteria, based on the finding that bacteria have many of the properties of a neural network (Hellingwerf, 2005).

The aim of this chapter, is to compile some dispersed literature published about different aspects of the most threatening diseases occurring in fish survived and cultured in marine waters worldwide such as Vibriosis, Furunculosis, Yersiniosis, Edwardsiellosis, Viral Hemorrhagic Septicemia, Whirling Diseases, Saprolegniasis, and Mycobacteriosis. A wide range of diseases of the clinical symptoms, diagnosis, treatment and vaccines are also discussed in this chapter. Therefore, the geographic distribution of each disease and the main host species affected, together with the biochemical and antigenic diversity existing in the aetiologic agents are described. Also, the purpose of this report is to attempt to fill this gap in the literature by analysing the usage of a point-of-care decision support tool.

2. Fish Autopsy

An autopsy is the most essantial piece at Fish medicine. An autopsy (post-mortem examination, obduction, necropsy, or autopsia) is a surgical procedure that consists of a thorough examination of a died fish by dissection to determine the cause, mode, and manner of death or to evaluate any disease or injury. Autopsies are unremarkably conducted by a specialized doctor (Rothenberg, 2008). Autopsy and ancillary diagnostics is an essential diagnostic in fish medicine. Due to rapid autolysis, fish autopsies should be performed promptly after death. Samples of autopsy should be conserved in 10% Neutral Buffered Formalin (NBF). Squash preparations, tissue imprints, mycology, parasitology, microbiology, and virology are also useful in obtaining a diagnosis (Weber and Govett, 2009). General autopsy procedure is applied. opercular incision, lateral incision, cranial incision, ceraprial incision are taken and internal organs are examined. Microbiological culture of kidney, brain, liver, spleen, and affected visceral organs, and histopathology tissue samples are also essantial aspects of the fish autopsy (Yanong, 2003). Autopsy may be required to investigate fish disesase by assessing visceral organs. Diagnosis can be made through, skin cytology, fecal examination, or autopsy (Weber and Govett, 2009).

3. Virulence

Virulence is the disease-causing capability of pathogens bacteria, virus, fungi, and protozoa). Virulence factors are produce by some microorganisms and cause

disease in the host (Cobo, 2022). Specific pathogens possess a widely array of virulence. Some virulences are intrinsic to the bacteria and chromosomally encoded, whereas other virulences are obtained from mobile genetic elements like bacteriophages and plasmids (Mohanraju et al., 2022).

A major category of virulence are bacterial toxins. These are exotoxins and endotoxins. Hyphal is an example of a fungus virulence from *Candida albicans* (Bien et al., 2018). Also important some virulence factors are integrins (e.g. beta-1 and 3) and biofilm (Bülbül and Filik, 2019). Examples of virulence for *Staphylococcus aureus* are protease, lipases, deoxyribonucleases and enterotoxins. Examples for *Streptococcus pyogenes* are exotoxins, M protein, some acids, capsule and destructive enzymes. *Listeria monocytogenes* include of internalin A, internalin B and, listeriolysin O are used to in the pathogenesis. Examples for *Yersinia pestis* are type three secretion system, YopE and YopJ pathogenicity (Bien et al., 2018).

4. Fish Diseases Category

Fish diseases are two widespread categories of disease that affect fish be on the point of being infectious and non-infectious diseases (Banrie, 2013).

4.1. Infectious Diseases

Generally categorised as parasitic, bacterial, viral, or fungal diseases. The percentage distribution of the causative agents of the major causes of ınfectious diseases is on average as follows: bacteria (54.9%), virus (22.6%), parasite (19.4%), fungus (3.1%) (Banrie, 2013). Infectious diseases are a significant fish health risk. It is significant to treatment, management and diagnose with these diseases (Walsh et al., 2019). Utilising the clinical decision support tool to aid their decisions in management, treatment and diagnosisof diseases and that veterinary needs help in the medical history taking (anamnesis), conducting an examination, ordering tests and ruling in or out differential diagnoses (Gov. Uk, 2018). Bacterial diseases are often internal infections and require treatment with medicated feeds containing antibiotics which are approved for use in fish by the Food and Drug Administration. Typically fish infected with a bacterial disease will have haemorrhagic spots or ulcers along the body wall, necrotic wound and around the eyes and mouth. They may also have an enlarged, fluid-filled abdomen, and protruding eyes. Bacterial diseases can also be external, resulting in erosion of skin and ulceration. Viral diseases are diagnosed by special laboratory tests, however they are difficult to

diagnose (Banrie, 2013). All viral diseases are notifiable diseases (Walsh et al., 2019). Fungal diseases are the fourth type of infectious disease. Fungal welded spores are common in the marine water, however do not usually cause disease in healthy fish. (Banrie, 2013).

4.2. Non-Infectious Diseases

Non-infectious diseases classed as genetic, nutritional and environmental. Nutritional diseases is difficult to diagnose. A classic example of a nutritional disease is "Broken Back Disease," due to vitamin C deficiency. The lack of dietary vitamin C contributes to improper bone development, resulting in deformation of the spinal column. Genetic diseases include conformational oddities such as lack of a body area or presence of an extra. Environmental diseases are the most essential. Environmental diseases include low dissolved oxygen, natural or man-made toxins or high ammonia in the water. Managing water quality will prevent this diseases (Banrie, 2013).

5. Common Diseases in Fish

5.1. Dropsy

Dropsy is caused by *Pseudomonas punctata*. Dropsy (dropsical or ascites) is a condition in fish caused by the buildup of fluid inside the body cavity, visceral organs or tissues. As a symptom rather than a disease in its own right, it can indicate a number of underlying diseases (Alderton, 2003).

5.1.2. Symptoms

Symptoms are include swelling of the visceral organs and abdomen, exophthalmos, scales that starting to point outward instead of lying flush with their body giving a "pine cone" appearance, abnormality of the fins, a curve in their spine, pale gills, pale feces, a loss of appetite, swelling surroundings of anüs, a lack of energy and movement, accumulation of body fluid in the body, in cavity or in scale pockets, scales become loose, abdomen bulge largely and pressing on it water comes out through the mouth etc. in fish (Vajargah, 2022).

5.2. Motile Aeromonas Septicemia

Aeromonas hydrophila causes Motile Aeromonas Septicemia (MAS) disease (Rahman et al, 2022). *A. hydrophila* virulence is a very potent fish pathogen (Filik, 2020). General characteristics of motile aeromonas; They are Gram-negative, bacillus and coccobacillus in size 0.5-1.0-1.5 microns, facultative

anaerobic, non-sporeform, acting only with polar flagella, positive for catalase, oxidase, fermentative and resistant to 0/129 vibriostate. The causative agents of Motile Aeromonas Septicemia are *A. hydrophila*, *Aeromonas caviae* and *Aeromonas sobria* bacteria (Durmaz, 2022).

5.2.1. Symptoms

A. hydrophila disease welled fish with may have many different clinic sings. These symptoms range from sudden death pale gills, difficulty breathing, bloated appearance, swimming abnormalities, generally acts abnormalities and skin ulcerations. Commonly affected with this disease internal organs and skeletal muscle (Swann and White, 1991). At autopsy; hemorrhage in the internal organs, enlargement of the spleen and kidney, and acidic fluid accumulation in the abdominal cavity mixed with blood are observed (Durmaz, 2022).

5.3. Vibriosis

Many different names of the disesase however the internationally accepted name Vibriosis (Austin and Austin, 2016). Vibriosis is cause by *Vibrio anguillarum* (Onuk, 2022). In hard cases, animal hospitalization may be required. The most important and best of fish grown in salt water. One of the known diseases is Vibriosis. There are 63 species in the genus Vibrio. Within the Vibrionaceae family, the species that cause serious disease include *V. anguillarum*, *Vibrio ordalii*, *Vibrio alginolyticus*, *Vibrio salmonicida*, *Vibrio vulnificus*. They are Gram-negative, aerobic, straight or curved rods. They move actively with sheathed flagella. The reproductive requirements of most strains are mineral matter, salt and glucose. It is tolerant of pH up to 10 in alkaline conditions. All strains breed at 20°C, most at 30°C and those that infect mammals at 37°C. In solid media, they form smooth-edged, convex, off-white S-type colonies within 18-24 hours (Onuk, 2022). Polar flagella antigen is common to all vibrio genus, lateral flagella antigens may differ between species. Somatic antigens are used for typing clinical strains. *V. ordalii*: Previously identified as *V. anguillarum* biotype II, it has been isolated from infections seen in many fish species. *V. salmonicida*: It is the etiological agent of 'Hitra' disease' or 'cold water vibriosis'. It is characterized by severe anemia and widespread haemorrhage, particularly in structures around the internal organs (abdominal fat, kidney). They breed only in environments enriched with blood. *V. vulnificus*: consists of two biotypes. Biotype 1 is an oppurtunistic human pathogen. Biotype 2 is virulent for eels (Austin and Austin, 2016).

5.3.1. Symptoms

Microorganisms that enter the body continue to reproduce by reaching the dermis from here to the muscle tissue and veins. Hemorrhages occur, especially in the ventral region, with damage to the vessel walls. The bacteria spreads throughout the body through the blood. In cases disease progresses, bubbles filled with blood, leukocytes and bacteria form on the body surface. These enlarge and mature, forming oval or rounded ulcers. In this state, the infection shows the character of ulcerative hemorrhagic septicemia. While vibriosis causes death at a rate of 40-60% in marine fish (Onuk, 2022). Clinical symptoms in sick fish are swelling of the gill lamellae, lethargy, hemorrhage around anus and fins. In peracute cases, loss of appetite, darkening in color and sudden death are seen in fry, in acute form, intense hemorrhagic lesions around the anus and fin bases, ulcers and swellings in the muscles are seen. In chronic cases, intense hemorrhages, pectoral fin, operculum, around the anus, ventral of body and mouth have been reported hemorrhages (Austin and Austin, 2016). There are bleedings all over the body. Sometimes there is swelling and ulcerous appearance in the skin-muscle. In chronic case, been seens large deep necrotic lesions in the muscle. The gills are pale, anemic, and there may be fibrinous peritoneal adhesions in the peritoneum. Hemorrhages are seen in all tissues, especially in muscle, kidney, liver, intestine and heart tissues. All internal organs are hyperemic and erythema. In the presence of exophthalmos, areflexia is seen in the eye (Ziarati et al., 2022). At autopsy findings of fish infected with the causative agent, enlargement, necrosis, hemorrhage and hyperemia in the internal organs, petechial hemorrhage in the muscle walls, and bloody exudate accumulation in the peritoneal cavity were reported (Austin and Austin, 2016).

5.4. Yersiniosis

Yersiniosis is a significant bacterial septicaemia caused by *Yersinia ruckeri*. Gram-negative, bacillus, 1-0.75 µm in diameter and 1.0-3.0 µm in length, sporeless, encapsulated, its motility is variable because its flagella are not always present (Zwoliński, 2022). Universal title of diseases is enteric red mouth or ERM. Acute infections by caused ‘Hagerman’ strain are usually florid (Carson and Wilson, 2009).

5.4.1. Symptoms

Yersiniosis occur in fish and infection is manifest by poor feeding response in recently transferred smolt, rising levels of mortality and appearance of exophthalmos and blood spots in the eye (Carson and Wilson, 2009). At autopsy;

The most characteristic finding is diffuse petechial hemorrhages in the muscles, large intestine, liver, and caeca. Kidney and spleen are enlarged. Intestines are hemorrhagic and inflamed. The intestines are enteritis, with edema and filled with a yellow colored liquid. There are hemorrhages in the muscles, air sac and gonadal tissues (Durmaz 2022). It is seen circumoral. Congestion of the vessels in the mouth region are its main clinical features. There are no external signs. Fibrinous peritonitis occurs in internal organs. Sometimes, erythema and hemorrhages in jaw and mouth, opercula and anus region of the fish. Internally, petechial hemorrhages may be seen in peritoneum, body fat, swim bladder, and other organs in acute situations. The intestinal tract is usually filled with erythemic and bloody mucus. Kidney and spleen enlarge. There is unilateral or bilateral exophthalmos. The spleen and kidney are much more swollen. A serous fluid may accumulate in the viscera area. The fish have no with poor appetite. Petechiae on the abdominal organs, redness and swelling in the spleen and kidney, histologically necrotic points in the some visceral organs; A dense leukocyte infiltration is seen with granuloma in chronic cases, but with hemorrhage in acute cases. The distinguishing feature is the accumulation of blood in the eye, in the vessels and especially in the brain capillaries (Pajdak-Czaus et al., 2019).

5.5. Edwardsiellosis

Genus Edwardsiella, named after Bacteriologist P.R. Edwards, was suggested in 1965. The causative pathogen of Edwardsiellosis is *Edwardsiella tarda*. The pathogen a Gram-negative, flagellated, rod-shaped bacteria (Ewing et al., 1965). This bacteria forms green colonies with black centricals on Rimler-Shott agar. *E. tarda* is strong H_2S producer (Acharya et al., 2007). Disease is emphysematous due to putrefactive.

5.5.1. Symptoms

E. tarda is zoonosis. It causes chronic encephalitis and acute septicemia. In chronic forms of the disease, bacteria infect the olfactory sac and reach the brain by following the olfactory nerves. It causes grunulomatous inflammatory reactions in the brain. Meningoencephalitis developing in the brain affects the behavior of the fish negatively and changes its normal swimming characteristics. In acute cases of infection, disorders of the intestinal mucosa occur. Bacteria pass from here to the body and cause bacteremia. Anemia, exophthalmos and inflammatory reactions occur in the gills. In chronic cases, small 2-4 mm wide cutaneous abscesses and lesions develop in the dorso-lateral regions. Lesions on the skin gradually increase and may widespread to the all body. In autopsy,

enlarged liver and abscess foci, accumulation of fluid in the abdomen dropsy, enlargements in the visceral organs are among the findings that can be observed. A general hyperemia is also observed in the internal organs. At autopsy; there are white nodules on the entrails organs. Abscesses occur in internal organs in eels (A'yunin et al., 2020).

5.6. Furunculosis

Furunculosis is a major severe, contagious and important disease in fish worldwide (Baset, 2022). *Aeromonas salmonicida* is the agent bacteria of the so-called "typical" furunculosis (Toranzo et al., 2005). Furunculosis develops as a chronic or acute haemorrhagic septicaemia, liquefactive necrosis. Deep ulcerative lesions appear be seen in the acute cases. *A. salmonicida* perform typical brown pigmented colonies generally 48 h on microbiological agar (Cipriano et al., 1992).

5.6.1. Symptoms

Fishes colors darken. They have no appetite. External symptoms; Erythema around the percula and anus, external hemorrhages on the erythema and double fins are seen. Internally; There may be erythema in internal organs and adipose tissue. Petechial hemorrhages may be present in adipose tissue, gonads, stomach wall, pericardium, swim bladder, peritoneum, and muscles. Externally, fruncules is seen on the dermis. In chronic or subacute conditions, there may be grayish areas in the organs. A watery bloody liquid comes out from ulcers formed on the fruncles of sick fish (Baset, 2022). It occurs as a result of focal localization of fruncles bacteria in dermis or epidermis. Debridement (fruncles) is defined as 'the removal from the wound bed until underlying healthy tissue is exposed' and debridement is removed surgically (De Decker et al., 2022). Furunculosis is acute, subacute and chronic in fish. Acute cases are more common in young fish. The incubation period is very short. Animals die within 2-3 days without clinical signs. In subacute and chronic cases, deaths begin slowly. In fish, stagnation, exophthalmos, bleeding from the nostrils, and numerous small lesions between subcutaneous tissue and muscles occur. Furuncles on the body are generally seen in chronic large fish, and when these furuncles are opened, ulcers with pus-microbe discharge and raised edges are formed. In acute cases, generalized hemorrhagic septicemia, hemorrhages on the internal organs, fins, mouth and lower scales are observed. In furunculosis, no swelling kidneys. At autopsy, a critical dropsy, bleeding body wall and heart, fullness of the vessels

and hemorrhage in the intestines are observed. Intestinal contents are bloody (Baset, 2022).

5.7. Bacterial Kidney Disease (BKD)

Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum*, is a chronic systemic disease, which causes mortality in fish. *R. salmoninarum* is a motile, Gram-positive diplobacillus. It is a chronic disease characterized by formation of ulcers with pus on the skin, degeneration and necrosis of the kidneys. The disease usually progresses chronically, but becomes acute at 13-18ºC, especially at low temperatures. BKD can be transmitted horizontally and vertically. The source of the infection is shed by latent carrier salmonids or the feces of subclinically infected. It can also progress subacutely due to intense stress and bad environmental conditions (Kristmundsson, 2022).

5.7.1. Symptoms

The infection is characterised by a systemic infiltration of the viscera by the bacteria causing granulomatous lesions specially in the kidney. Abscesses tend to multiply resulting in enlargement and necrosis of the whole kidney, which appears swollen with irregular greyish areas. The external signs are exophthalmia, abdominal distension and pethechial haemorrhage. (Bruno, 1986). BKD disease is systemic and has a slow course. Because of anemia, pallor of the gills and fins, darkening in color, abdominal swelling, hemorrhages around the anus, slow swimming are seen. Exophthalmos and loss of one eye are noteworthy. Skin blisters, superficial ulcers formed as a result of their bursting, small hemorrhagic furuncles on the lateral line, hemorrhage, ascites are observed. The disease also progresses asymptomatically. Unopened lesions contain necrotized tissue, a (cream-red) fluid made up of bacteria. Kidneys are organs that are frequently affected. There are white areas in the kidneys that contain swelling, cell debris, bacteria and leukocytes. In advanced cases, kidney tissue is destroyed. Clinically, there is hemorrhage in the body wall and testicles, and fluid accumulation in the peritoneum. Fluid due to the amount of hemorrhages in the pericardial spaces, membranous layers in the internal organs characteristically, there are cream-white granulomatous lesions (Durmaz, 2022). At autopsy; acidic fluid accumulation is seen in the abdominal cavity. Most disorders are observed in the kidney. In typical cases, there is swelling of the kidneys and white-gray nodules filled with a white fluid. This organ is enlarged and degenerated. Gray-white lesions are position on the ventral surface and on

the kidney. A pus material was found in them. An overgrowth of the spleen may ocur (Jansson et al., 2022).

5.8. Streptococcosis

Streptococcosis is diseases caused by a group of bacteria called Streptococcus (strep-TOE-coccus) (CFSPH, 2022). Streptococci are microorganisms with round or oval structure, characterized by forming long chains. They cause infections in fish. Streptococci become pathogenic when the resistance is broken. Streptococci are Gram-positive, 0.6-0.9 micron in diameter, beta-hemolytic and appear in short chains. They are catalase negative. If the water temperature rises above 20°C, the disease increases. The agents enter through the pores in the skin and cause infection. Diseased fish have darkening of color, exophthalmos, protrusion of the eyes (pop-eye, burst eye syndrome), corneal opacity and later blindness. *Streptococcus iniae* is a zoonotic pathogen (Durmaz, 2022).

5.8.1. Symptoms

Streptococci; cause hemorrhagic septicemia in fish. Infection manifests itself with blisters in the dorsolateral regions. Skin darkening, slow swimming, stagnation and loss of appetite, exophthalmos, ascites, hemorrhages in the eyes, tongue, around the mouth, operculum, fin bottoms and under the abdomen are seen in sick fish. Dying fish swim close to the water surface. Hemorrhages on the fins and gills cause the disease to be confused with vibriosis. At autopsy; It has a shiny appearance like gold. There is hemorrhage in the abdominal wall, dropsy in the abdominal, congestion in the intestine and anus, and a bloody-mucoid content in the intestine. The kidneys are enlarged and the liver has a bloody appearance. At the same time, necrotic foci are encountered in these organs and in the heart. The spleen is enlarged (Avrilia et al., 2022).

5.9. Whirling Disease (WD)

The agents are found in the non-ossified cartilage parts and the spine. Trophozoites settle in cartilage tissue. The agent disintegrates the motoric parts of the spinal cord and nervus sympathicus. The trophozoites are located in the cartilage tissue. If they are found in the vertebrae, due to the destruction of the sympathetic nerves that control pigmentation around the 26th vertebra, darkening and shrinking of the tail and deformations in the form of bending-bending are seen in the tail. The chin takes the shape of a parrot's beak. Spores are of severe vital in diagnosis of *Myxobolus cerebralis*. Spores have sporplasm inside, they

appear in two or three parts (Stroud, 2022). This disease, caused by Myxosoma (Myxobolus) cerebralis, is a protozoan disease that settles in the cartilage of fish and causes rotational disease. WD is transported by *Tubifex tubifex*. It is very dangerous for fingerling fish. After the caps of the spores of *M. cerebralis* open in the stomach of young trout, they enter the intestinal wall and begin to develop by entering the organs through the circulatory system, especially the cartilage in the upper part of the head and backbone (James et al., 2021). Formation of spores is completed 8 months after infection. Mature spores usually remain in the lesions and are found in fish. In young fish, the disease occurs when the spores of *M. cerebralis* settle in the cartilage tissue, bone and brain. If the factors are in large numbers and settle in the brain, death occurs. If the factors are few and located in the brain, although the fish will live, their growth will be different. Bending and deformations are formed in the spine. Deformations also develop and the upper jaw remains shorter than the lower jaw. Again, the shrinkage and bending of the caudal fin and the formation of black pigmentation in the 1/3 of the body on the tail side attracts attention. Pressure exerted by *M. cerebralis* on the sympathetic nerves under the spine, the color-forming nerves become inoperable, and accordingly, melanistic regions and blackness on the skin occur. Short gill cover formation and muscle atrophy may also be observed. Since the skull and some bone formations in young individuals are cartilage tissue in the early stages, young individuals are most affected by the disease (James et al., 2021). Abnormalities in the jaw, curvature of the spine, pits behind the eyes and skull are seen. Fish affected by *M. cerebralis* cannot swim well. The causative agent lodged in the skull and vertebrae of the fish causes a swimming disorder and typically a swirling motion in the fish. As a result of these movements, the fish get tired and die. The spores in the dead fish disperse and infect the surrounding area. Triactinomyxon, Sporoplasm and Myxospora forms are seen in their development (Baxa and Nehring, 2022).

5.9.1. Symptoms

Histopathologically, it is characteristic for the disease that fish are extensively infected with myxosporean trophozoites throughout the skull bones and cartilages, and that the same trophozoite infections are seen in the gill arches and vertebrals. Whirling disease affects juvenile fish and causes skeletal deformation and neurological damage. Fish performs “whirl” forward in an awkward, corkscrew-like pattern instead of swimming normally. The mortality rate is high for fingerlings, up to 90%, and those that do survive are deformed by the parasites residing in their bone and cartilage (Stroud, 2022). *M. cerebralis*

was the first myxosporean whose both pathology and symptoms were identified scientifically (Lisnerová, 2022).

5.10. Hexamita (Octomitus)

Its causative agents are *Hexamita (Octomitus) salmonis* and two flagellated protozoan parasites named *Hexamita intestinalis*. They are small protozoa of 7-10 microns in size. Thanks to their whips, they move very quickly. They escape from the microscope field as they move rapidly in scrapings from the intestines or stool controls (Moon et al., 2006).

5.10.1. Symptoms

It usually settles in intestines, however, it is abundant in the gallbladder. It is important in aquaculture. In heavy infections, it causes intestinal enteritis in young trout and salmon. A picture of enteritis with blood and mucus (catarrhal and hemorrhagic) occurs in the intestines and thus causes significant damage. The active cyst forms. It occurs when the infectious agents are taken together with feed and water. Sudden swimming movements, weakening, darkening in color and retraction in the abdomen are seen in infected fish. At autopsy, the agents are found in the entrails organs. The scrapings or content taken from these places for diagnosis native staining (with Giemsa) is required (Farmer et al., 2021).

5.11. Infection with Intracellular Bacteria

Agents are *Piscirickettsia salmonis, Hepatobacter penaei* Necrotising hepatopancreatitis (NHP), *Francisella noatunensis* and *Chlamydia* spp. The host range is quite wide. Diagnostics is special media needed. Management is standard. Disease aren't zoonotic potential (Haenen, 2017).

5.12. Mycobacteriosis

Mycobacteriosis is characterized by tubercles in organs and tissues in fish, with a chronic and usually sporadic course. There are three main species isolated from disease cases. *Mycobacterium marinum* is Gram-positive, acid-resistant bacilli, aerobic, non-sporeless, encapsulated. It grows as lemon yellow, photochromogenic colonies on Glycerin Agar and LJ medium GA at 18-25 °C for 2-3 weeks. It produces pink colonies singly or in groups in Ziehl-Neelsen staining. *Mycobacterium fortiutum* grows more quickly on media.

Colonies produce a cream or brown color (variable pigmentation) pigment. In epizootiology, contaminated food, canibalismus (eating sick fish), entry of the agent in injuries or skin loss, and external parasites, transovarian transmission have been reported in the transmission routes (Onuk, 2022).

5.12.1. Symptoms

Progressive weakening, loss of appetite, stagnation, discoloration, flaking, ulcers on the skin and fins, exophthalmos, deformations of the vertebrae and mandible, swimming disorders, small, many gray-white nodules in the internal organs and muscles, anemia, and fluid collection in the abdomen (Onuk, 2022).

5.13. Bacterial Gill Disease (BGD)

Bacterial gill disease is pestiferous and mortal infection that occurs in fish with gill congestion, hyperplasia of epithelial cells, adhesion of gill lamellae, degeneration and necrosis. In etiology, infection cases are usually caused by *Flavobacterium branchiophila* and less commonly by *Flavobacterium aquatilis*. Gram-negative, aerobic, non-sporeless, encapsulated, immobile. Colonies are S type, convex, with smooth edges. Catalase, oxidase, phosphatase are positive. Its morphology may vary. It varies from short rods to filamentous forms. Flexirubin pigment, 20% KOH, changes to brown color. In epizootiology, the disease can be found in fish of all ages. Their numbers increase during infection. Infection occurs at temperatures of 2°C and 20°C. Irritations caused by the overgrowth of bacteria settled on the gills cause hyperplasia of the gill epithelial cells and secretion of excess mucoid. The gills are initially hyperemic and later turn pale. Mucoid secretion causes the gill lamellae to stick together. Ends of filaments thicken up and in advanced cases may be necrosis. Mucoid secretion also prevents gas exchange, fish can die depend on Anoxia (Onuk, 2022; Bakhtiyar et al., 2022).

5.13.1. Symptoms

Sick fish move slowly and on the surface, are sluggish and have no appetite, have difficulty breathing and want to swim in the direction the water comes from. There is highly mucus secretion on surface of gills. It is seen that gill covers are open and cannot be closed. The gills are hyperemic, swollen, filaments and lamellae adhered to each other, and even grains of sand and extraneous may be present on them. Infection is typically localized in the gills and no apparent macroscopic disorder in the visceral organs. Consequently isolation is conducted

form the gills. Fungal agents (especially Saprolegnia) may subsequently settle on the damaged gills and change prognosis of infection. Pantothenic acid deficiency in fish, non-ionized acid in water and other causes may cause similar diseases (Bakhtiyar et al., 2022).

5.14. Pasteurellosis

Agents are *Photobacterium damselae* subs. *piscicida* and *Photobacterium damselae* subs. *damselae*. It is an intracellular facultative, halophilic and Gram-negative bacteria responsible for fish photobacteriosis (Osorio et al., 2018). The disease is highly pathogenic for fish and widespread the world. Due to the tubercules in entrails organs of fish, the disease is also called "Pseudotuberculosis". Disease agents enter the fish through the mouth and skin. There is a vertical transmission in fish. In addition, some aquatic invertebrates are also included in the transmission chain as vectors. The disease is zoonosis. Transmission of infection to humans is principally via the skin. General precautions should be followed during treatment. Vitamin and mineral supplements added to feeds are beneficial. Vaccination is used effectively. Various antibiotics can be used. Oxytetracylin, chlorophenikol, sulfadiazine, sulfamerazine are used (Rivas et al., 2013).

5.14.1. Symptoms

In addition to the general clinical findings in sick fish, darkening of the skin is evident. The disease progresses in acute and chronical. In acute usually results in the death innumerable fish without clinical signs. Enlarged spleen and blackberry appearance at autopsy are typical findings for this disease. There are numerous necrotic lesions in the liver. The kidney is pale. General clinical findings in fish with chronic disease are hyperemic areas on the head and gills (Rivas et al., 2013).

5.15. Staphylococcosis

The causative agent is *Staphylococcus aureus*. Gram-positive cocci are in the form of a bunch of grapes. There is no specific symptom, rather a general disease picture is encountered (Onuk, 2022).

5.16. Botulism

It is a lethal toxication disease seen sporadically. The causative agent is *Clostridium botulinum*. Unlike other microorganisms, *C. botulinum* is not a single bacterial species. All microorganisms that produce the characteristic

protein called botulinum neurotoxin (botulin) are defined as *C. botulinum*. While these microorganisms were previously classified in the Bacillaceae family, new taxonomic studies have now included the Clostridia class, the Clostridiales team, the Clostridiaceae family. They are Gram-positive, rod-shaped, spore-forming, anaerobic bacteria. Spores are found in terminal or subterminal form (Dodds and Austin, 1997). In addition, consumption of toxin-containing feeds is another way of infection. The agents are concentrated in sediment and sludge in anoxic environments. Irregular swimming, balance disorder and paralysis are seen in sick fish. Diagnosis is made by laboratory findings. Currently, there is no effective treatment (Wei et al, 2022).

5.17. Pseudomonas Infections

Its agents are *Pseudomonas anguilliseptica* and *Pseudomonas fluorescens* bacteria. The causative agents are found on the body surface, gills, intestines as well as fish eggs in fish. *P. anguilliseptica* infections cause a disease with petechial hemorrhage and septicemia called "red spot disease" in fish. There are bleeding lesions mouth, anus, and operculum. *P. fluorescens* causes infection in fish characterized by necrotic, hemorrhagic septicemia known as "Bacterial Hemorrhagic Septicemia Disease". Low water temperature is important at the exit of the infection. The causative agent is an opportunistic pathogen. Intensive fish death occurs within 2 weeks following the appearance of hemorrhages, especially in young and juveniles, the loss can reach 100%. There are hemorrhagic lesions especially in the fin and tail area (Easwaran et al., 2022).

5.18. Nocardia

In various fish species and is characterized by anorexia, inactivity, discoloration of the skin, weakening, and in advanced cases, caseous and nodular lesions on skin and fluid accumulation in abdomen. It is Gram-positive, weak acid-resistance, aerobic, sporeless, encapsulated. It reproduces in mycelial style in 4-5 days at 20-30°C (Onuk, 2022).

5.19. Infectious Pancreatic Necrosis Virus (IPNV)

Infectious Pancreatic Necrosis Virus (IPNV) is a viral fish disease. A IPNV is virus of Birnaviridae family. The virus, has been isolated sick fish either diseases or asymptomatic (Dopazo, 2020). This disease fundamentally affects young fish, though adult may carry the virus asymptomatic. Resistance to disease develops step by step more rapidly in hot water. It is found worldwide and

highly contagious. The disease is normally spread horizontally and vertically. It is incapacitate to infect mammals. Diagnostic methods of disease: PCR, indirect fluorescent antibody testing, virus culture, ELISA and characteristic histological pancreatic lesion. Virus titers can be isolated from carrier and sick fish. Currently, no treatment is available. But, vaccines are available. In order to eradicate the disease, very strict protocols are applied on movement (Aedo et al., 2022).

5.19.1. Symptoms

Highly mortality is seen depending on virulence of the disease. Clinical symptoms include abnormal swimming and acts, darkening of the skin, anorexia, dropsy. At autopsy, seens viral necrosis in pancreas and intense mucus in intestines. Surviving fish should recover within two to three weeks (Aedo et al., 2022).

5.20. Infectious Hematopoietic Necrosis Virus (IHNV)

Infectious Hematopoietic Necrosis Virus (IHNV), is negative-sense single-stranded RNA virus that is from Rhabdoviridae family (Rudakova et al., 2007). IHNV is transmitted following shedding of the virus in the external mucus, sexual fluids, urine, and feces. Virions consist of a nucleocapsid and an envelope. Virions are bullet-shaped (Chong, 2022).

5.20.1. Symptoms

The clinical signs include faded gills, abdominal distension (dropsy), exophthalmia and darkened skin. Long and semi-transparent fecal casts exit the anus. Affected fish are lethargic, with bouts of hyperexcitability and abnormal activity, frenzied. Petechial hemorrhages commonly occur the muscles at anüs, at fins, the mouth, the skin posterior to the skull above the lateral line, and the yolk sac in sac fry. In sac fry, the yolk sac often swells with fluid. Surviving fish often have scoliosis. In post mortem lesions the abdomen, stomach, and intestines often contain white to yellowish fluid, but food is usually absent from the digestive tract. The visceral organ as kidney, heart, spleen, and liver are typically very pale. Necrosis is common in visceral organs. Hemorrhages may occur in the kidney, peritoneum and swim bladder. Petechial hemorrhages are often found in the membranes surrounding the heart, and brainand the internal organs including the pyloric caeca, spleen, peritoneum, intestines (Chong, 2022).

5.21. Viral Hemorrhagic Septicemia Virus (VHSV)

Viral Hemorrhagic Septicemia (VHS) is a mortal and dangerous disease caused by virus (Maj-Paluch et al., 2022). VHSV is also known as Egtved virus. VHSV

is a negative-sense single-stranded RNA virus of the family Rhabdoviridae (NCBI, 2007). The viral disease was discovered in 1963 by M. H. Jenson. In molecular virology genome of VHSV is consists of approximately 11-kb of single stranded RNA, which contains six genes that are located along the genome in the 3′-5′ order: 3′-N-P-M-G-NV-L-5′ (Nombela et al., 2018).

5.21.1. Symptoms

Sick fish have wounds which can look like lesions from lamprey attacks. Fish shows hemorrhaging of their all body and internal organs. Some fish show no external symptoms, but show internal symptoms as exophthalmos, dropsy, bruised-looking reddish tints to the all body. The disease may also be a nervous disease where fish due to perpetually showing abnormal behaviour (Maj-Paluch et al., 2022).

5.22. Saprolegniasis

Saprolegniasis is dangerous fungal and infectious a diseases. The agent is destructive oomycete *Saprolegnia parasitica* pathogen. Saprolegnia's life cycle begins with hyphae. Saprolegnia filaments (hyphae), millions of hyphae come together to form the mycelium. With the formation of the mycelium, the disease fully initiates its virulence effect. As the mycelium matures, the tip of the hyphae takes a rounded form and the tip of each hyphae is filled with zoospores. Zoospores grow out of the hyphae and form young hyphae (Bonansea et al., 2019).

5.22.1. Symptoms

In clinical signs saprolegnia lesions are focal white patches on the skin which have a cotton wool-like appearance under water when the hyphal elements extend out. The fungus invading the stratum spongiosum of the dermis and then the epidermis, causing erosions as it spreads. Numerous hyphae will be seen, and underneath, dermal necrosis and oedema. There is slightly inflammatory response in clinical table (Durborow, 2003). In more severe cases, cotton-like growths can extend into muscle tissue.

6. Discussion and Conclusion

In this chapter, we have submitted dispersed literature published on several fish diseases associated with fish. Acute infections caused by pathogenic bacteria should be studied extensively. These infections have killed millions of fish in

the past and today, but are effectively being combated with the development of modern medicinal approaches and measures. However, the development of alternative treatment and control approaches in the fight against infection is an important expectation.

Much of the research into bacterial pathogenesis has focused on acute infections, but these diseases are now supplemented by a new category of chronic infections caused by bacteria growing in biofilms. Biofilm infections such as chronic wounds, fibrinous peritonitis infections affect millions of fish in the world every year and cause many deaths (Bjarnsholt, 2013; Filik, 2019).

Some of the pathogens may be contact-zoonotic, good hygiene is crucial for aquafarmers, field technicians, and processors. Proper diagnosis of bacterial diseases is crucial. Many of the diseases can be prevented by good management, including use of appropriate vaccines. In case antibiotic treatment is necessary, we recommend to always make an antibiogram. Countries please should be pay attention to this list of bacterial pathogens (Haenen, 2017).

Dr. Olga Haenen presented on the most important bacterial diseases from a list drafted at the FAO expert meeting in December 2016 in Frascati, Italy using the following criteria: economic importance of affected species, socio-economic impact and zoonotic potential. Researcher discussed important aspects of diagnosing fish bacterial diseases and the need to identify the infection as primary or secondary, the necessity of antibiograms and methods of isolation and identification such as diagnostic polymerase chain reaction (PCR), 16s ribosomal ribonucleic acid (rRNA) typing, enzyme-linked immunosorbent assay (ELISA)-based serological field tests and the matrix-assisted laser desorption/ionization-time of flight (MALDI TOF) protein method. Important fish vaccines for prophylaxis were identified. Emphasized proper diagnosis of bacterial diseases, the necessity of antibiograms and the fact that many of the important diseases in aquaculture can be prevented by good management, including the use of vaccines (Haenen, 2017; FAO, 2020).

First, a fish anamnesis is taken. In anamnesis questions, information about the first appearance of the disease, fish (species, age, weight, etc.), characteristics of the environment (water temperature, weather, culture medium, etc.), last manipulations, loss of appetite, daily mortality, symptoms, swimming, gills, body information such as color, other symptoms are sought. Has the same disease been seen before in the anamnesis? Which antibiotics were used in the treatment? Has antibiotic resistance developed? Important medical questions should also be included.

The virulence forces and pathogenicity of bacteria, which are agents of fish diseases, reveal serious consequences in fish. The most important of these serious

consequences is that it becomes almost impossible to stop the bacteria. Faced with a post-antibiotic era in which our ability to fight bacteria is diminished, the need for new strategies to deal with disease has increased. While the strength of diseases has increased so much, the most effective strategy is the concept of "super antibiotic".

Scientists have succeeded in developing a "super antibiotic" that bacteria cannot develop resistance to. According to the article published in the journal Nature Chemical Biology, scientist Vern Schramm and his team from the Albert Einstein Faculty in New York tested the newly developed antibiotic on cholera and *Escherichia coli* bacteria. According to result of research, it was noted that even the 26th treatment with this antibiotic gave results as in the first treatment. Stating that the new antibiotic does not kill the bacteria, scientists reminded that this method causes the bacteria to develop resistance. The superantibiotic interrupts the communication of bacteria with each other by blocking an enzyme, so that bacteria cannot develop biofilms in which they are protected from the immune system (Gutierrez et al., 2009). It is opinioned that this chapter will guide further research in order to bring the "super antibiotic", which has proven successful even in the 26th treatment developed by Vern Schramm and his team, to the field of fish diseases researches.

If the subject is evaluated in terms of Quorum Sensing System (Interbacterial communication) (QS), it is crucial to focus on the strategies of preventing the AHL molecules of the QS, which gives strength to the bacteria and is very effective in pathogenicity, and the virulence in the management of the system. Therefore, stopping the communication in the bacterial world draws attention in the control of fish diseases. Stopping AHL signal molecules, which are communication tools, before they can shape the disease, brings up the concept of early diagnosis, and in this case, it is aimed to break new ground in prophylaxis. Although fish mucus, fins, scales and skin protect the fish, proper management, nutrition, use of immunostimulant, vaccination, and improvement of environmental conditions are of vital importance in the fight against diseases.

There are clear messages in topic of the chapter. These include the sections on "approach to diagnosis", "history and examination", "differential diagnosis", '' fish health management'' "investigations", "diagnosis: step-by-step" and "case history". Of the remaining, three relate to issues in management. These include the sections on "treatment options", "treatment details", and "approach to management".

Fish diseases, both infectious and non-infectious, seriously affect the life of living things in the aquatic environment. It causes serious symptoms internally and externally and increases the life-threatening risk of the fish, leading to

death. Treatment strategies to target virulence factors and the genes encoding them have been proposed. It is vital to improve the immune systems of fish in order to maintain their health and protect them from diseases. Also fish diseases management need to be developed furthermore. Having sufficient knowledge on fish diseases, developments in multidisciplinary animal medicine fields, preventing diseases before they occur, increasing fish vaccines and preventing fish diseases with vaccine development in the light of new technological developments are promise fish diseases literary, fish diseases doctrine and fish medicine literaure for the future.

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CHAPTER X

DRIFT PRECAUTIONS WHEN SPRAYING PESTICIDES

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1. Introduction

The increase in population and decreasing agricultural areas in the world make the importance of agriculture more felt every day. In addition, with the effect of population growth, agricultural areas are getting closer to residential areas day by day. The fact that people are adversely affected by this situation and the resulting food and environmental pollution have led to an increase in awareness, especially due to the use of pesticides. The negative effects of sensitive areas such as wetlands, habitats and vegetation around agricultural areas, such as the release of pesticide residues in foodstuffs, has revealed the term Pesticide Drift.

According to the definition given in the ISO 22866 Standard, "Drift is the transport of pesticides out of the sprayed area by the effect of air currents during application". As a result, unwanted pesticide accumulation can be encountered in sensitive areas (waterways, natural parks, children's playgrounds, wetlands, etc.), even in urban areas. In particular, accumulation in neighboring fields can cause contamination of unauthorized active ingredients or direct damage (phytotoxicity) to adjacent crops.

The last European Directive 128/2009/EC on the sustainable use of pesticides defined specific indicators for the prevention of environmental risks associated with pesticide drift. In particular, item 11th of this directive entitled "Special measures to protect water areas and drinking water"; In order to

prevent pesticide drift, it recommends using application equipment with low drift risk and planting high plants around the application area (Anonymous, 2009). In addition, in order to prevent pesticide accumulation in undesirable areas due to drainage and surface runoff, it recommends the use of buffer zones of appropriate sizes in order to protect ground or surface waters used as drinking water in places where there are unused or stored pesticide packages.

It is possible to collect the main source points of environmental pollution caused by pesticides during application in two groups as Point sources and Spread sources. Filling, cleaning and waste or residue applications on the farm can be expressed as Point sources. The drift of pesticides in the area due to reasons such as flow and drainage is in the group of spread sources.

When the factors affecting pesticide drift are examined, we encounter some factors that we can have a direct and indirect effect on (Figure 1). We can arrange them as shown in Figure 1. High temperatures, low air humidity can cause evaporation. For this reason, spraying should not be done at temperatures higher than 25 degrees and in air with humidity less than 40%. Spraying should not be done if the wind speed is more than 5 m/sec as a factor affecting the drift. Applications should be managed according to the location of sensitive areas, taking into account speed and direction.

Figure 1: Factors on which we can be directly or indirectly affected

Plants in the application area are very important in terms of drift risk. While applications made on bare soil have a higher risk of drifting compared to soils with plants, the risk increases when spraying tall plants. Especially in orchards, while the number of leaves decreases, the risk of drift decreases when the leaf area increases.

1.1. Precautions

The measures to be taken to reduce the drift can be grouped under two groups as direct and indirect measures. Direct measures aimed at reducing pesticide drift at the source are mainly based on application technologies, sprayer accessories designed to reduce pesticide drift generation, and correct sprayer setup. These measures depend on factors such as drop size, velocity, direction. Indirect measures include creating buffer zones to trap entrained pesticides, leaving unsprayed areas and installing windbreaks. It is essential that the operator follow the recommendations regarding the optimum weather and environmental conditions for spraying.

It is important to develop BMP (The Best Management Practices), known as drift prevention measures, especially in pesticide applications. In many countries, studies on pesticide drift produce different recommendations. Many plant protection products are tested and classified to reduce the risk of pesticide drift. Recently, many manufacturers have focused on the design of hydraulic spray nozzles. In fact, many researchers are working on changing the droplet spectrum (Tuck et al. 1997; Arın and Çelen, 1999; Çelen et al., 2007; Nuyttens et al., 2009; Martin and Carlton, 2012; Önler et al, 2015; Liao et al. 2020; Cerruto et al. , 2021; Li et al., 2021).

In addition, the tests and classification of sprayers used in orchards are quite complex. In orchards, techniques are tried to be developed on the whole design, not on the direction of spraying as in field sprayers. Today, it is seen that sprayer types that reduce pesticide drift are recommended in a few developed countries. However, in all applications, success will be more effective with the creation of reference information or the joint action of all countries.

It is crucial to have a harmonized framework of recommendations for pesticide drift, to establish a common basis for implementation between countries and to develop the levels of confidence necessary for their implementation. Collaboration and mutual trust between countries or researchers is essential to avoid pesticide drift, as the immediate benefits of investing in a changing practice or new technology are not always clear and the long-term benefits are not always properly valued. Studies at this point (BMPs) should provide practical and consistent guidance to operators, sprayer manufacturers and other stakeholders in order to make the use of plant protection products more sustainable.

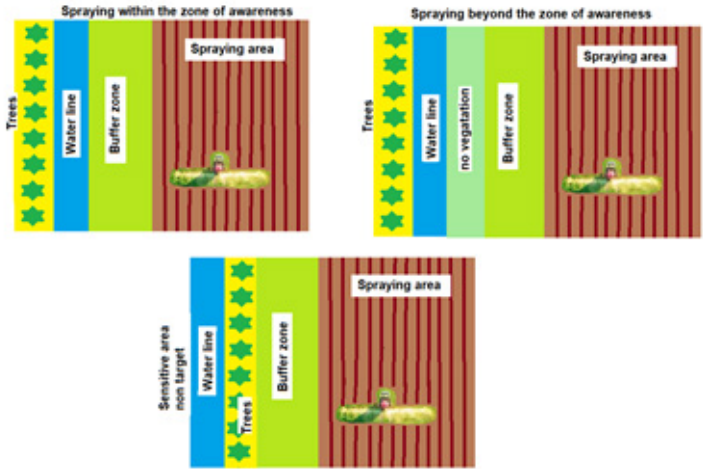


Figure 2: The existence of different buffer zones

Here, BMP applications were evaluated in five categories as environmental factors, meteorological factors, spraying, sprayers and equipments, and sprayer calibration.

It is recommended to carry out a risk assessment of pesticide drift in the fields and orchards to be sprayed before any application is made. In this context, information such as the distance to sensitive areas, the existence and location of buffer zones, the characteristics of wind, air and application area, whether anti-drift measures are taken, plant heights and progression speed should be calculated and recorded in detail. These factors allow the operator to assess drift risk, taking into account certain parameters and mitigation measures. The factors are based on practical and scientific experience and provide practicality for operators. Increases their awareness of pesticide drift, including potential risk reduction measures.

The first step in applications should be the creation of a buffer zone. The distance of these regions can be expressed as 20 m in field areas and 5 rows in orchards according to various research results (Brown et al., 2004, Wenneker et al.; 2012).

In the second step, key variables affecting pesticide drift need to be identified. We can list these as wind direction and speed, air temperature and humidity, application conditions related to vegetation in the field and vegetation type next to the field. Canopy density, sprayer type/nozzles, spraying and airflow scenarios etc. in orchard and vineyard applications. parameter may need to be taken into account.

In the third step, the sprayer configuration is selected by selecting the measures that can be taken. Such as nozzle type, pressure, spray rate, feed rate, height of nozzle from ground. Nowadays, it has become easier to access information online or offline, such as mobile phones and computers. It will be useful to follow new measures by making use of this technology.

1.2. Environmental factors

Before starting an application, environmental factors related to the risk of pesticide drift should be considered. The most important thing is to know the distance of any sensitive area from the area being sprayed. Maps or images documenting such information and showing the buffer zone or the indirect risk mitigation measures taken will be useful in this context. In addition, the leaf structure or density of the crop, the spacing between plants along the row, the growth period and condition of the crop or plant should also be known. The critical point is leaf density and leaf area that can capture pesticide drops and keep them in the target area. Environmental factors do not change rapidly and therefore are useful to know for any application plan and pesticide drift reduction strategy.

In plants with a low leaf density or leaf area, it may be difficult for the spray liquid to be retained by the plant. For example, when applying herbicides to bare soil before emergence in perennial plants, it should be noted that the efficiency of catching spray droplets in the early stages of woody plants is very low.

In cases where environmental conditions are effective, anti-drift spray nozzles should be used, and separate calibrations should be made for each application. The distance of the spray nozzles from the target should be reduced.

Water wells in the application areas should be constructed taking into account pesticide drift precautions and should be covered. It is necessary to construct new wells with cover safely and away from potential flood areas, following national and local regulations. As mentioned before, you should have field maps and the location of the wells should be marked on the maps. Safe areas should be created around the wells.

In addition, when examining environmental factors, local regulations regarding buffer zones and warnings on the labels of Plant Protection Products should be well understood. Sometimes local regulations may contain changes, especially regarding the safe distance, according to the Crop Protection Product.

In addition to preserving the existing vegetation between sensitive areas and pesticide application areas, the creation of structures such as windbreaks

(curtains or trees) is of great importance in preventing drift due to environmental factors. In this case, the height of the plant in the area to be created in order to catch the entrained pesticide; for orchards: 6 to 8m, for field crops: 2 to 3.5m. Considering the density of the canopy of the vegetation to be established in this planning, conifers that develop before the application plant or plants that are permanent throughout the season can be selected. It can even be used in containment artificial nets such as artificial plants. Hail nets can reduce drift by limiting the entrained liquid cloud traveling farther.

1.3. Meteorological factors

Weather conditions are one of the main factors affecting pesticide drift. These conditions cannot be directly changed and cannot be predicted. Wind speed, wind direction, air humidity and temperature are the main variables to be considered. If one of the key variables exceeds the specified limit, it is recommended not to spray. These limits differ between countries and should always be considered and adhered to (Deveau, 2009).

Wind speed affects the amount of fine droplets carried from the target area. Wind direction determines the direction of the spray plume and whether it is drifting towards a sensitive area.

In low humidity conditions, the heat evaporates the water in the sprayed droplets. This effect increases the amount of fine droplets. Therefore, it increases the risk of drift. If the air temperature is too high, the thermal effects weaken the small droplets and delay the precipitation (thermal drift) of the pesticide. Therefore, the spray cloud is subject to wind entrainment over a longer period of time.

Weather forecasts from local agencies should be checked when planning spraying applications. Wind direction and speed, air temperature and humidity should be followed for different times of the day. Spraying should be planned in the most suitable weather conditions possible. Under low wind speed (below 2.5 m/s), medium temperature (10 - 25 °C) and high air humidity (above 50%) conditions, the estimated wind direction should be determined away from sensitive areas. In the calmest winds (morning/evening), spraying should be done on areas adjacent to sensitive areas.

Before starting the plant protection application, it should be checked whether the weather conditions (wind direction, wind speed, air temperature, air humidity) are in accordance with the forecasts. It should be decided to start the application according to the evaluations regarding the weather conditions. After

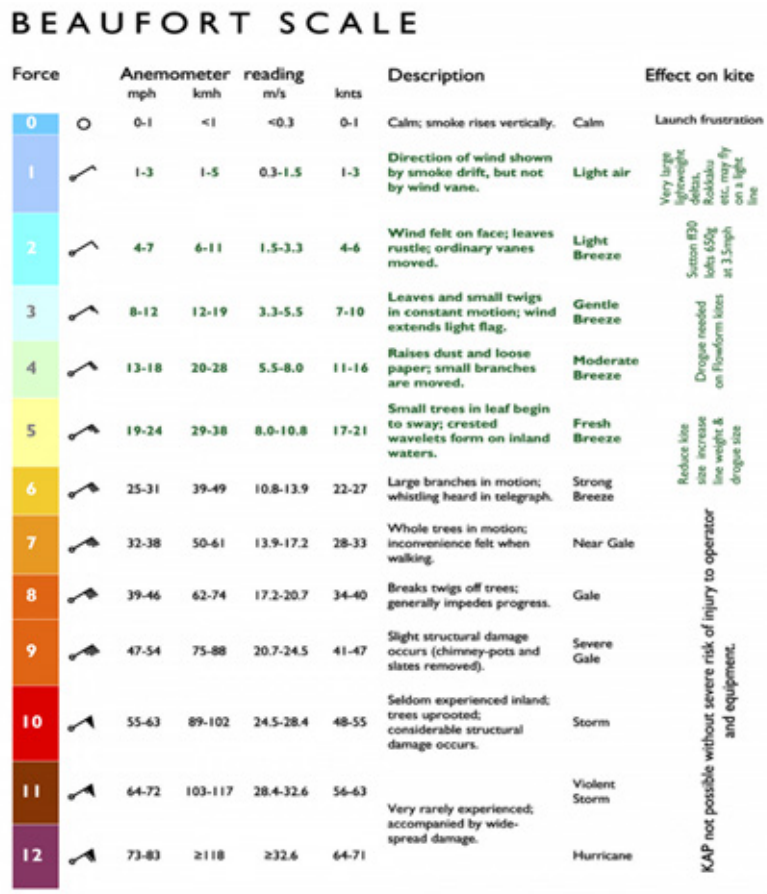
the sprayer is calibrated according to the current conditions, spraying should be done.

Spraying should not be done when the wind speed exceeds the locally recommended values or the general guidance specified in the regulations should be followed. If no legal requirements regarding wind speed are specified, spraying can be done at the recommended spray height, preferably at low to medium wind speeds (0.5 - 3.0 m/s). At high wind speeds (3.0-5.0 m/s), spraying should be stopped until the wind speed decreases. If timing is a critical factor, in other words, if application is unavoidable, it is necessary to take the highest possible drift prevention measures. Spraying at very high wind speeds (>5.0 m/s) should definitely be avoided.

Spraying should be done in stable atmospheric conditions. Spraying should be avoided on hot and calm summer evenings to avoid thermal drift. Spraying should be done during cooler hours of the day (morning) if possible. If urgent spraying is required, it is appropriate to take other entrainment measures by reducing air movement and sprayer speed, using coarse or very coarse spray nozzles.

Choosing the right time to spray can be tricky. A lot of research tell us that spraying when it's calm is wrong. One question is very important " when can you actually spray?". Spray applications are always recommended in a little wind. because the wind helps disperse the spray up and down and dilutes the spray cloud fairly quickly. It also tends to be reasonably constant in the direction and speed of the winds. so that downwind areas can be identified and potential effects can be known or predicted. sunny weather further improves cloud dispersion.

It is important to define windy weather. The classic wind scale is the Beaufort scale (fig 3), which was designed for the sea but is also used on land. The upper limit for spraying is probably Force 3 or Force 4 and upper limits are around 20 – 25 km/h. The Beaufort Scale calls these "Gentle or Moderate Strokes".



BEAUFORT SCALE

Force	Anemometer reading mph	kmh	m/s	knts	Description		Effect on kite
0	0-1	<1	<0.3	0-1	Calm; smoke rises vertically.	Calm	Launch frustration
1	1-3	1-5	0.3-1.5	1-3	Direction of wind shown by smoke drift, but not by wind vane.	Light air	Very large lightweight delta, Rokkaku etc. may fly on a light line
2	4-7	6-11	1.5-3.3	4-6	Wind felt on face; leaves rustle; ordinary vanes moved.	Light Breeze	Sutton B30 lofts 650g at 3.5mph
3	8-12	12-19	3.3-5.5	7-10	Leaves and small twigs in constant motion; wind extends light flag.	Gentle Breeze	Drogue needed on Flowform kites
4	13-18	20-28	5.5-8.0	11-16	Raises dust and loose paper; small branches are moved.	Moderate Breeze	
5	19-24	29-38	8.0-10.8	17-21	Small trees in leaf begin to sway; crested wavelets form on inland waters.	Fresh Breeze	Reduce kite size increase line weight & drogue size
6	25-31	39-49	10.8-13.9	22-27	Large branches in motion; whistling heard in telegraph.	Strong Breeze	
7	32-38	50-61	13.9-17.2	28-33	Whole trees in motion; inconvenience felt when walking.	Near Gale	KAP not possible without severe risk of injury to operator and equipment.
8	39-46	62-74	17.2-20.7	34-40	Breaks twigs off trees; generally impedes progress.	Gale	
9	47-54	75-88	20.7-24.5	41-47	Slight structural damage occurs (chimney-pots and slates removed).	Severe Gale	
10	55-63	89-102	24.5-28.4	48-55	Seldom experienced inland; trees uprooted; considerable structural damage occurs.	Storm	
11	64-72	103-117	28.4-32.6	56-63	Very rarely experienced; accompanied by wide-spread damage.	Violent Storm	
12	73-83	≥118	≥32.6	64-71		Hurricane	

Figure 3: Beaufort scale (Wolf, 2021)

1.4. Spraying

In the application of Crop Protection Products, basically three main techniques are used to disperse the pesticide solution: hydraulic spraying, which produces drops that break down with pressure energy, pneumatic atomization that creates drops by tearing the liquid film at high air speed, and rotary disk atomization, where the liquid is broken down with the help of centrifugal force to form droplets.

Hydraulic spray nozzles can come in different designs. They can generate different droplet spectra. The correct selection of these Easy-to-replace nozzles by farmers is an important factor in reducing drift. Although they are classified according to their effects on pesticide drift, many countries make their own classification. For this reason, there are differences in the determination of the dimensions of the buffer zones.

With the technology available in pneumatic atomization today, it is difficult to change droplet spectra under practical conditions. Larger drops will form if the air velocity is reduced. On the other hand, air velocity and air volume are important for transporting the droplets to the target and ensuring the necessary penetration of the pesticide solution into the canopy.

In case of risk of entrainment, lower pressures should be used and spray nozzles that produce less small droplets (< 100 µm) should be preferred. In fact, spray nozzles that prevent drift should be selected. In high wind (3.0 - 5.0 m/s) and/or high spray speed (> 8 km/h) use, the use of drift reducing spray nozzles is required.

If there is a risk of drift, spray nozzles in the class of spray nozzles that reduce drift must be selected. Most researchers categorize drift-reducing spray nozzles by comparing them to a standard spray nozzle. While this varies in most countries, if a nozzle classification is not available or not in practice, the following indicators can help select the best nozzle type.

Many researchers have conducted studies on drift and its reduction using different nozzles Gil et al., 2014; Celen et al., 2020. When using Even Flat or Hollow cone spray tips (1-4 bar), drift reduction success was 10-20%, while these values were 30-50% in pre-orifice flat fan spray tips (2-5 bar). In the new type of spray nozzles compared to a standard spray nozzle tips, 70-90% and 50-75% drift reduction was observed in Air induction Flat fan tips (2-8 bar) and hollow cone nozzle types (10-15 bar), respectively. In their study, Satallinga et al.(2014) reported that at 2.0~3.0 m, DG11004 (black line) provided an aberration reduction of approximately 30% compared to XR11004. Especially in field applications, air induction spray nozzles tips are recommended. Flat Fan and Hollow Cone spray nozzle tips produce larger droplets with air induction. These are less prone to drift. When you choose this type of spray nozzle tips, the pressure adjustment specified in the catalog must be made for success.

Air induction spray nozzle tips are recommended to reduce pesticide drift in orchard applications. Air introduction spray nozzle tips with narrow spray angles should be used to avoid collisions between adjacent spray jets. In case of a short distance (<50 cm) between the spray nozzle and the plant canopy, an air introduction spray nozzle tips with a wider spray angle should be selected.

In rotary disc sprayers, the disc speed should be reduced to reduce drift. In rotating disc atomizers, liquid is delivered at low pressure to the center of a rotating disc, which produces a fine drop due to its rapid rotation. The faster the rotation speed, the finer the droplets produced. In practice, droplet size adjustment can be difficult as penetration may be affected.

Permitted drift reducing additives may be used if recommended by the chemical manufacturer for drift. However, these drift-reducing additives can change the physical properties of the spray solution. Changes in the viscosity of the spray solutions can have an effect on the droplet spectrum created and the nozzle flow rate. The correct additive concentration is a critical factor in reducing drift. The hygroscopic substance can reduce the volatility of small droplets in low humidity environments. Most Crop Protection Product formulations are optimized and additives are not recommended. For this reason, the label of the Crop Protection Product and the manufacturer's recommendations should be taken into account.

Another class of spray nozzles that reduce the risk of pesticide drift are twin flat spray nozzle tips. They allow the flow rate and droplet size to be changed independently. These provide the possibility of adjustment to form coarse drops on the field edges. However, if the droplet size is increased too much, there is a risk that the spray cross-distribution will become more uneven in twin spray nozzle tips.

For application on bare soil it is recommended to use deflector spray nozzle tips that produce coarser droplets. This type of spray nozzle tips has a wide spray beam and good overlap between the spray jets. The boom height can therefore be lowered easily.

1.5. Sprayers and equipments

Besides the correct use of Crop Protection Products, spray equipment is the most important element in the effort to reduce drift. Especially for pneumatic sprayers, the risk of pesticide drift is very high. Therefore, it is an issue that needs attention. Three characteristics of the application; Droplet spectrum, Sprayer application technique and adjustability (including air support), Sprayer modification according to environmental factors and product properties. It must be taken into attention.

Some countries have also begun to classify sprayers according to their potential to reduce pesticide drift. Sprayers are divided into pesticide drift reduction classes such as 25%, 50%, 75%, 90%, 95% or 99% (ISO 22369-1).

Known as pesticide drift reduction technology, SDRTs are classified separately for different crop types (field, fruit, vineyard, etc.). In some countries the use of SDRT has required distance regulations for applied Crop Protection Products. Local agencies provide recommendations on drift reduction measures where there is no SDRT classification. These recommendations should be followed.

Following pesticide prevention technologies from various sources, being aware of new technologies has a special importance in terms of success in studies against drift. Equipping your spraying machines and tools with these technologies will be beneficial in reducing drift. An inventory of the technologies (nozzle type, spray nozzles, sprayer setting options, air support (speed, volume, direction), other features (protective devices, sensors, etc.)) should be created. Thus, it will ensure that deficiencies are always noticed.

While applying pesticides, attention should be paid to the use of techniques and technologies that have been inspected and comply with standards. While regular sprayer testing is required in some countries, there are many countries where mandatory testing is not yet required (ISO 16122 and EU Directive 128/2009)(Martini et al., 2019). In summary, with or without inspection, equipment related to drift reduction (sprays, spray nozzles, hoses, pump, boom stabilizers, etc.) should not be used without being specifically tested.

Consideration should be given to the use of sprayers with harmonized EN standards. It should be noted that while compliance with EN standards is not mandatory at the moment, harmonized EN standards have already been set and will be mandatory. Unbranded equipment, purchased or self-made or modified, used to apply Crop Protection Products must conform to the same standards as EN certified equipment supplied by machine manufacturers. For new machines to be purchased, those that reduce drift and are environmentally friendly should be preferred.

In applications, closing different parts of the boom when needed is included in the drift measures. These systems carry a pressure regulator (relief valve) on them. In this way, when a section is closed, the pressure in the system remains constant. With the use of this technology, off-target spraying can be prevented in areas where pesticide disposal is not desired or in small area applications.

The use of multi-nozzle bodies is successful in practice in order to prevent drift. The multi nozzle body equipped with different types of spray nozzles allows selecting nozzles with different droplet spectrum (Groot, 2012). Nozzle replacement can be manual or automatic, as well as nozzle holders that hold up to five nozzles today. It is recommended to use multi nozzle bodies to easily adjust the droplet size according to distance requirements to reduce drift.

The colors of most nozzles are standardized by ISO according to flow rate and pressure. ISO colors determine the characteristics of nozzles according to the relationship between output (l/min) and pressure (bar).

Air assisted sprayers produce an uncontrollable spray cloud that is exposed to the wind, with a high risk of drift. They should not be used in areas where

transport of droplets could pose a risk. If it is necessary to use such a sprayer, the specified anti-drift measures must be taken.

The use of sprayers with adjustable air direction is important in terms of drift. Cross-flow sprayers with air deflectors or towers with air spouts, directional air-jet ejectors with flexible air ducts and adjustable air vents have targeted features (Figure 4).

It is necessary to use the sprayer's control system and adjustment features correctly to apply the spray precisely according to the canopy size, geometry and density. By spraying over or under crop canopies, off-target spray loss is prevented.

Figure 4: Some types of sprayers with controllable air outlets

Using sprayers with adjustment possibilities such as the setting of the appropriate nozzle position and direction, the adjustment of the air flow direction and speed will be beneficial in reducing the risk of drift. Even sprayers with nozzle on/off automation should be preferred. With this type of sprayers, it will provide uniform accumulation and less drift, as well as give the opportunity to spray the necessary parts of the plants. It is important to adjust the nozzle position and direction to achieve uniform spray distribution throughout the canopy profile.

To prevent spray droplets from being blown (drifted) out of the canopy, it is necessary to adjust the airflow angle and speed according to the canopy width and density. It can be seen whether we have adjusted the air jet direction correctly, when the spray has completely penetrated the plant canopy and no spray clouds are visible on the other side of the crop row. Visual inspection of the airflow setting in the plantation with clean water prior to application is required to check penetration.

Generally, deflectors are used to direct the air flow in auxiliary air flow nozzles. In narrow and open canopies, in low wind situations, the airflow should be much more backwards during the early growth stages. In stronger winds, very high air velocity is used, while for tall and dense plants the air direction should be directed little or no backwards.

The use of sprayers with adjustable airflow rate will reduce the risk of drift. The target plant, its size and geometry should be considered when adjusting the airflow rate. While changing the air flow rate, the blades of the fan propeller must be adjusted to the appropriate angle and the rotation speed of the propeller must be adjusted with the appropriate gear box setting.

In sprayers, the PTO is generally used while power is taken from the tractor. The air flow rate should be adjusted in relation to the advance rate of the sprayer to ensure air penetration within the canopy volume. No spray clouds are observed on the other side of the plant row when the air has fully penetrated the crop canopy with the spray liquid. In narrow and open canopies, lower airflow rates should be used during early growth stages. For larger and denser plant canopies, higher airflow rates should be preferred at higher sprayer speeds and stronger winds. In the case of crosswind, the sprayer should be used close to the downwind row of the product.

The fact that the sprayer has systems that can close the air flow on both sides is an important advantage in terms of the risk of drift. To prevent spraying from being blown out of the target area, out of the crop canopy, it is recommended to use a sprayer that can close the air outlets on both sides when spraying on the outer row of the plantation (Figure 5).

Figure 5: Sprayers that prevent spray from going outside the target area

Sprayers with individually controllable control system provide high success in preventing drift and reducing pesticide consumption. It is important to be able to adjust the number of active spray nozzles according to canopy development. Spray nozzles should not spray the area where there is no crop, either manually or automatically. It should be noted that by closing some of the spray nozzles, the spray volume changes and this value needs to be readjusted.

It is necessary to adjust the spray profile according to the target characteristics. A spraying profile should be obtained as appropriate for the plant profile as possible. Sampling surfaces such as Water Sensitive Papers (WSP) can be used to obtain information about the canopy's interior, exterior, and vertical profile penetration with certain nozzle outlet settings. Vertical collectors (sampling surfaces) can be used to select or adjust the most suitable spray profile. It is also necessary to adjust the position and direction settings of the nozzles on the sprayer according to the plant growing system and growth stage.

Air flow rate and direction must be adjusted according to application conditions. Excessive airflow and velocity that causes a high risk of drift should be avoided in crops with low leaf cover or early stage crops. By changing the gear settings, the selection of a lower propeller speed allows the airspeed to be changed. Correct orientation must be made by changing the angle of the blades on axial fan sprayers, the air deflectors to match the airflow canopy profile. When spraying pesticides when plants are in their early growth stages (leafless), the air support should be turned off.

Airflow counteracts the effects of windy conditions and wind generated from driving. For this reason, it is recommended to use air assisted systems in field sprayers. Air assist can be used if necessary for good penetration. Air curtain sprayers have a spray arm equipped with a fan and air sleeve that produces a downward airflow of 1400 to 2000 $m^3/h/m$, which supports the transport of droplets to the target. In this type of machines, there is a reduction of pesticide drift up to 75%. In some studies, this value has been reported as 50% for air-induction and conventional spray nozzles.

With the use shielded boom field crop sprayers, the droplets are protected from the wind for a certain distance, thereby reducing the effect of the wind. Shields are also designed to deflect airflow and direct droplets towards the ground.

Shielded boom sprayers are particularly useful for applications on grain crops where deep penetration of the chemical is required. These tilt the plants below the boom to create an opening for the spray liquid to penetrate. In systems designed as a sliding shield over the canopy, air induction spray nozzles have a 90% drift reduction potential, while conventional flat fan nozzles have a 75% drift reduction potential.

Tape sprayers can be used to minimize the pesticide rate per unit area. If possible, sprayers that apply to the band should be used. Normally these sprayers are combined with seeders or implements for mechanical weed control. Special even spray nozzles with a angle of 60^0-80^0 are usually used for belt sprayers.

It would be beneficial to use shielded band sprayers for row crops. In order to minimize the use of Plant Protection Products per unit area, only sprayers with the shield that keep the product in line can be used. The use of these apparatus in non-selective weed control between rows ensures the protection of the crop on the row.

Especially, the use of field sprayers with sensors, which have been commercialized recently, reduces pesticide consumption. Sensor sprayers equipped with target identification systems such as GreenSeeker can detect target plants/area by leaves. The sensors are only able to turn on the spray nozzles one by one when the leaf area is detected. Using sensor target detection (presence/absence of leaf area) prevents spraying into gaps and exposing the spray cloud to the wind. Advanced sensors that determine canopy geometry and density adjust the spray volume according to the actual canopy structure, reducing the risk of drift.

Another control technique is the sprayers controlled by GPS support. Automatic nozzle shut-off when turning headland with the use of GPS allows specific sprayer settings such as pressure, nozzle type, number of active nozzles, airflow rate to be automatically adjusted according to sprayer position in the field.

Weed wipers that spread the pesticide by rubbing on weeds can eliminate the risk of drift (Figure 6). This technique can be used especially if the weeds are taller than the cultivar. Since droplets are not formed in this system, drift is eliminated.

Figure 6: Systems in which the pesticide applied to the ropes is rubbed onto the plant

Tunnel sprayers with a recycling system reduce spray drift by reducing the effect of wind on droplets during application. These; tunnel sprayers are classified as Conventional tunnel sprayers, Tunnel sprayers with spray separators (lamella filters), Over-the-row sprayers with spray separators (lamella filters), Over-the-row sprayers with reflector shields. These sprayers can also be equipped with recirculation systems that minimize soil losses and save spraying. Recovery of

the pesticide mixture is high during early growth stages when using a protected recycling sprayer. In addition, if it is thought that there are missing plants on the row, the losses here can be recovered.

Tunnel sprayers equipped with panels reduce the volume of spray applied and the risk of drift. Especially the use of fan jet air induction nozzles increases the success.

As a disadvantage, it can be said that using a tunnel sprayer will leave high volumes of residual pesticide residues in the tank, since it is not easy to estimate the amount of pesticide mixture to be prepared. Therefore, using a Tunnel sprayer requires efficient residual spray management to prevent drift reduction from being translated into point source pollution.

The use of systems that completely cover the row by closing the spray nozzles from all sides (multi-row sprayer) shows success in drift. In this technique, which increases the accumulation inside, all sides of the breast may not be closed. The same success can be achieved by closing only two sides.

Figure 7: Shielded sprayers

1.6. Sprayer calibration

Sprayer calibration is largely the responsibility of the operator and the setup options of the sprayer. According to EU directive 2009/128 EC on the sustainable use of Crop Protection Products, operators are obliged to calibrate their sprayers regularly. Calibration means making sure the sprayer can be operated according to good agricultural practice requirements. Sprayer parameters must be adjusted and controlled to apply the correct amount of Pesticide to the product. Correct adjustment of the sprayer means minimizing potential losses of Pesticide to the environment. These checks should be done several times during the season as product specifications change. It should be noted that spray nozzles can also be subject to corrosion or clogging.

If standard spray nozzles are used on field sprayers, the forward speed should not exceed 6 km/h. For higher forward speed (> 6 km/h), coarse spray nozzles (air induction nozzles), air-assisted nozzles or other drift reduction techniques should be used. The boom height should not exceed 50 cm.

Orchard sprayers should optimize calibration by adopting the optimal number and configuration of spray nozzles that match the target profile. To minimize losses, airflow, direction and velocity should be adjusted to match the size and geometry of the target. The calibration of sprayers used in orchards should be checked visually when working by spraying clean water. As seen in Figure 8, we can use only 60% of the air. We can use 100% with the deflector.

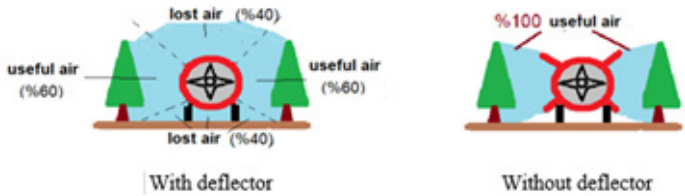


Figure 8: Use of air direction with and without deflectors.

It is necessary to use the lowest effective distance between the spray nozzles on the sprayers and the spray target. The optimum distance for flat fan nozzles in field sprayers is the distance between full overlap from the nozzle tip where the spray fan exits and the surface where full wetting occurs. The closer the nozzles are placed on the boom, the shorter the effective distance to the target.

The distance to the target depends on the spray angle produced by the spray nozzle. In general, spray nozzles with an angle of 110 degrees are used at a distance of 50 cm from the target, while spray nozzles with a spray angle of 80 degrees are used at a distance of 70 cm. It is necessary to constantly check the distance of the boom to the target before and during spraying. For band and row sprayers, the nozzles are arranged to cover the row or band and are kept at the lowest possible distance from the target.

It is necessary to reduce the distance between the spray nozzles and the target as much as possible, using specific and optimized settings on the orchard/vineyard sprayers. For each application, calibration should be performed according to the crop growth characteristics. In plants such as vineyard, it is more appropriate to reduce the number of sprayed rows to reduce the risk of drifting in early growth stages.

In pesticide applications, low forward speed should be preferred as much as possible. At higher forward speed, the effective distance of droplets to targets increases. In other words, the droplets are exposed to the wind for a longer time. Increasing speed also increases the wind and turbulence around the sprayer. This will leave more droplets behind the sprayer. It can be observed in the air as a “spray fog” cloud. In applications, the smallest possible droplet mobility should

always be ensured. If it is desired to increase the speed, negative effects should be prevented by other measures to be taken for the field.

In field sprayers, droplet size should be increased by using spray nozzles that reduce drift, and low boom height should be used. air-assisted sprayers should be preferred, and even apparatus that tilts the product or shields that surround and cover the spray nozzle should be used.

In the sprayers used in areas such as orchards or vineyards, coarse droplet size should be used and the air flow rate should be adjusted correctly.

In pesticide applications, the lowest effective pressure should be used with hydraulic spray nozzles. The specifications specified in the nozzle manufacturer's catalog must be observed. It should be noted that larger droplets are produced at low pressures, very fine droplets are minimized, and thus the risk of drift is reduced.

Buffer zones and other non-target areas should never be sprayed. It is absolutely necessary to examine the label of the Plant Protection Product to be used for the required distance to water bodies and other sensitive areas.

When spraying the outer row in the orchard, it is imperative that the nozzles on the side of the canopy of the sprayer be closed. Also, spraying should not be done while turning on headland.

In field sprayers, closing the boom sections outside the target area is important in terms of drift.

For vineyard/garden sprayers (especially multi-row sprayers), the number of sections should match the shape of the spray pattern given by the sprayer and the size of the field. Care should be taken at field margins and drift technology should be used.

In particular, sprayers with control systems or apparatus that reducing or control the horizontal and vertical movements of the boom should be used. Booms without effective stabilization tend to swing relative to the uneven field surface (Çilingir and Çelen, 1995). The higher the boom swings, the greater the risk of drift. Booms with shock absorbers, motion dampers or anti-yaw system should be preferred. It should also reduce the pressure of the tires to reduce the negative impact of the rough soil surface.

Air flow rate should be adjusted according to application conditions in air assisted sprayers. In air assisted sprayers used on bare soil or soil with weak vegetation, turbulence and dust formation must be minimized by reducing the air velocity. As the importance of spray liquid penetrate the crop canopy grows, it is necessary to increase airflow. User guides should be read carefully during air velocity adjustments.

Spray nozzles angle and air support direction should be adjusted according to application conditions. The nozzle angle should be changed towards the direction of advancing in head winds. If it is downwind, it should be against the direction of travel. In cases where there is no side wind or wind, the angle should be adjusted vertically or against the direction of travel. Only at high forward speed can it be angled forward.

Where there is no vegetation or low vegetation, it should be angled backwards to prevent reflection of spray liquid. When changing the angle in dense crops, it should look at the tilt of the plant. In such cases, the penetration can be improved by opening the product with the product bending systems.

If the wind speed, wind direction or forward speed changes, the optimum angle of the nozzles will likely change as well. Therefore, it is always necessary to pay close attention to the application conditions.

The advancing speed is adjusted according to the airflow volume and speed. The amount of air hitting the target should be adjusted to maximize spray penetration into the canopy. Thus, the risk of entrainment of the spray liquid due to the droplets passing through the spraying rows will be reduced.

Wind velocities hitting the target should be set at 6-8 m/s in the vineyard (full leaf development) and 10-12 m/s in the orchard (full leaf development). The airflow rate is determined by the advance rate of the sprayer. The sprayed liquid should completely penetrate the product canopy, and no spray clouds should be visible on the other side of the product row.

When spraying on the edge of the plantation or sensitive areas, it is necessary to turn off or reduce the outward airflow. When approaching field borders or sensitive areas, the air shut-off systems located on the side of the sprayer are used to prevent droplets from spilling out of the sprayed area. It will be advantageous to use automatic systems to independently manage the air flow rate in closed or non-closed sprayers.

2. Conclusion

Pesticide drift off-target can harm living things, the environment, or urban settlements. It is very important to understand, reduce or prevent the problems that may arise as a result of drift.

The fact that operators comply with the manufacturer's recommendations and chemical product label instructions and take responsibility is an indication that pesticides spraying is safe. It should be noted that the application of products without following the label instructions is always considered illegal.

The manufacturer's instructions on the label actually serve a vital purpose. It increases the risk of the chemical reaching its target.

Implementation success should always be in the hands of the operator. It should be able to be stopped when necessary. The operator's knowledge of the application technique should always be up to date. It would be beneficial to attend field days organized by equipment manufacturers.

It should also be noted that for crop protection products, specific spray drift management instructions regarding optimum weather conditions and spray equipment are included on the labels.

Sensitive plants and areas around the area you plan to spray should be determined and the spraying operator should have knowledge about these areas. In fact, it should be ensured that good relations are maintained by negotiating with neighbors about the applications to be made.

If possible, a buffer zone should be created between the application areas and the areas where there is a risk of damage.

The selection of equipment that minimizes drift, maximizes efficiency and gives the right droplet size range is the most important factor to consider in terms of drift risk. Besides the selection, the necessity of calibration should not be forgotten.

For spray nozzles, reading the correct boom height from the label and maintaining it during application is an important factor in both application success and drift risk.

It is important in terms of risk to measure and record the weather conditions before and after the applications. Considering that the weather conditions have changed, the application should be stopped. Spraying should be done only in suitable weather conditions. Continuous wind is good during applications. Because light, variable or strong winds make it difficult to predict where the spray cloud will go. In addition, spraying should not be done in calm or still conditions. Under calm conditions, droplets are more likely to remain suspended in the air.

Spraying should be avoided at temperatures close to or above 30°C and when humidity is low, as droplet size may be reduced during spraying, which may increase the risk of drift. Especially in the case of inversion, spraying should not be done.

The use of pesticides is inevitable in today's modern agriculture. When using pesticides, both the protection of the product against diseases, pests and weeds and its negative effects on humans and the environment should be evaluated together. In order to benefit only from the advantageous aspects of

pesticide use, which is an integral part of the agricultural system, and to avoid its negative effects, pesticide applications should be done deliberately in line with IPM principles, by examining and using new techniques.

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