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Abstracts and full-text reports uploaded to the conference system undergo a review procedure. Authors will be notified of the application results in three weeks. Submitted abstracts will be evaluated on the basis of abstracts/proposals. The conference system allows you to submit the full text if your abstract is accepted. Please upload the abstract of your article to the conference system and wait for the results of the evaluation. If your abstract is accepted, you can upload your full text. Your full text will then be sent to at least two reviewers for review. The conference has a double-blind peer-review process. Any paper submitted for the conference is reviewed by at least two international reviewers with expertise in the relevant subject area. Based on the reviewers' comments, papers are accepted, rejected or accepted with revision. If the comments are not addressed well in the improved paper, then the paper is sent back to the authors to make further revisions. The accepted papers are formatted by the conference for publication in the proceedings.

Aims & Scope

Plant and animal health is closely related to human health. In this century, where the human population is rapidly increasing and technology is developing rapidly, the problem of food supply to the increasing population brings plant and animal health to the fore. Nowadays, when concepts such as artificial meat and capsule feeding are discussed, the process of growing plants and animals has begun to be discussed. For this reason, this conference focused on the changes and innovations in the field of Veterinary, Agriculture and Life Sciences.

The aim of the conference is to bring together researchers and administrators from different countries, and to discuss theoretical and practical issues of Veterinary, Agriculture and Life Sciences. At the same time, it is aimed to enable the conference participants to share the changes and developments in the field of Veterinary, Agriculture and Life Sciences with their colleagues.

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Comparison of SMOTE and GAN Data Augmentation Methods in Sleep Stage Classification

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Abstract: Sleep is a vital process that occupies a significant portion of human life. Poor sleep quality can lead to the emergence of neurological and cardiovascular diseases. Therefore, sleep staging plays a crucial role in the diagnosis of sleep-related disorders. Traditionally, sleep staging is performed manually by experts in clinical settings by evaluating biomedical signals recorded via polysomnography (PSG) in accordance with the American Academy of Sleep Medicine (AASM) standards. However, since this process is both labor-intensive and prone to human error, its automation is of great importance. Excluding the wake stage, sleep is divided into two main phases: Rapid Eye Movement (REM) and Non-Rapid Eye Movement (NREM). The distribution of these stages throughout the sleep period provides essential information regarding sleep quality. However, these stages are not distributed evenly, which leads to the Class Imbalance Problem (CIP) in automatic classification and can negatively affect model performance. In this study, the MIT-BIH Polysomnographic Database—a challenging dataset due to significant class imbalance and signal variability—was utilized to automatically determine sleep stages. The effects of data augmentation approaches, specifically the classical SMOTE method and the deep learning-based GAN, were investigated using a proposed end-to-end CNN - BiLSTM architecture. Performance evaluations were conducted for both 3-class and 5-class staging scenarios. The experimental results are as follows: The accuracy values achieved for 3-class and 5-class staging were 87.58% and 75.85% without data balancing, 85.91% and 71.82% with SMOTE, and 86.60% and 75.31% with GAN-based augmentation, respectively. The results indicate that data augmentation methods do not always yield performance improvements in sleep staging tasks, particularly due to the complex temporal structure of EEG signals. Nevertheless, the 87.58% accuracy achieved without any data balancing demonstrates that the proposed CNN-BiLSTM architecture possesses a high capacity for learning sleep staging patterns.

Keywords: EEG, Sleep stages, Deep learning, Data augmentation

Introduction

Sleep is very important for human life because if there is any obstacle to regular sleep, bodily functions tend to deteriorate. Sleep quality affects academic performance, and according to research conducted by the National Sleep Foundation, sleep disorders are observed in 40% of individuals with various diseases and disorders, as indicated in the literature (Wara et al., 2025). Sleep disorders are closely associated with many neurological and psychiatric diseases, such as epilepsy and Alzheimer's disease (Gashti & Farjamnia, 2025). Biophysiological signals such as Electroencephalography (EEG), Electrooculography (EOG), Electromyography (EMG), and Electrocardiography (ECG), recorded using a Polysomnography (PSG) device throughout the night, are used to detect sleep-related disorders. This process is referred to as sleep staging or sleep scoring (Phan & Mikkelsen, 2022). In clinical settings, sleep stages are manually scored by sleep specialists using overnight polysomnography (PSG) according to the rules defined by the American Academy of Sleep Medicine (AASM) (Ruehland & Rochford, 2009). In a healthy individual, sleep is divided into two main stages, excluding the

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wakefulness stage: Non-Rapid Eye Movement (NREM) sleep and Rapid Eye Movement (REM) sleep. NREM sleep usually occurs in the early stages of sleep and consists of three sub-stages: NREM1, NREM2, and NREM3. This manual scoring process is time-consuming, costly, and inconsistent (Yu et al., 2025). For this reason, automating sleep staging is of great importance in terms of reducing the workload of specialists and contributing to the prevention of possible misdiagnoses. There are studies in the literature on sleep staging to automate this process can be examined in two categories: those performed using machine learning algorithms (An et al., 2021; Ben Hamouda et al., 2024; Fatimah et al., 2022; Huang et al., 2020; Li et al., 2022a; Liu et al., 2021; Permana & Iramina, 2024; Taran et al., 2020) and those performed using deep learning methods (Chen et al., 2023; Efe & Ozsen, 2023; Goshtasbi et al., 2022; Huttunen et al., 2023; Li et al., 2022b; Mostafaei et al., 2024; Perslev et al., 2021; Ren et al., 2025; Xu et al., 2025).

In recent years, numerous studies have made significant contributions to the problem of automatic sleep stage classification (Fatimah et al., 2022; Mostafaei et al., 2024; Taran et al., 2020; Zan & Yildiz, 2023; Zhao et al., 2022). Despite achieving promising performance, these approaches still suffer from the Class Imbalance Problem (CIP). In a typical sleep recording, the duration of each sleep stage is not uniformly distributed. In particular, N2 stage constitutes the majority class, accounting for approximately 45–55% of total sleep duration, whereas N1 stage represents only about 2–5% (Fan et al., 2020). When deep neural networks are trained with an imbalanced dataset, the network encounters the majority class samples more frequently; therefore, it performs better in recognizing the majority class. In contrast, it shows almost no success in learning the minority class samples. This situation negatively affects both the overall performance and the network's ability to correctly recognize different classes (Xu et al., 2022). Therefore, numerous studies in the literature have focused on data balancing techniques to address the issue of class imbalance (Cheng et al., 2024; Fan et al., 2020; Fan & Wang, 2023; Lee et al., 2021; Permana & Iramina, 2024; Sun et al., 2019; Supratak et al., 2017). Existing approaches to the CIP problem can be broadly categorized into two main groups: undersampling the majority class and oversampling the minority class (Mishra & Jaiswal, 2024).

With the aim of reducing the class imbalance problem, Supratak et al. (2017), duplicated data belonging to minority classes in the training set to equalize the number of examples in the majority class. In another study, Sun et al. (2019) first increased the number of examples belonging to minority classes in the training data using the Synthetic Minority Over-sampling Technique (SMOTE) method, then diversified the data morphology by adding noise to the generated synthetic data. Permana and Iramina (2024) conducted a study utilizing SMOTE to augment the minority class. However, the application of SMOTE to the entire dataset prior to the train–test split may introduce data leakage, thereby potentially resulting in overly optimistic performance evaluations. Fan et al. (2020) proposed five different data augmentation approaches to improve the classification performance of the N1 phase in the sleep staging process and identified SMOTE and Adaptive Synthetic Sampling (ADASYN) as the most commonly used data augmentation techniques in the literature. However, noting that such methods may not be suitable for time-series data such as EEG signals, they focused on GAN-based data generation. Although this method improved overall performance, a decrease in the classification accuracy of the N1 phase was observed. Similarly, in the study conducted by Cheng et al. (2024), GAN—a deep learning–based data augmentation technique—was employed to enhance the classification performance of the N1 stage by increasing the number of N1 samples. However, the performance of the N1 stage remained relatively low. As an innovation in data augmentation methods, Lee et al. (2021) aimed to fill signal interruptions in EEG—caused by sensor detachment, motion artifacts, or technical failures—using the GAN method while preserving the context of the signal. Fan and Wang (2023) filtered EEG signals using a band-pass filter and enabled the GAN architecture to generate data only within specific frequency bands for sleep stages. The authors argued that, for the GAN method to be effective, the process should be conducted in the frequency domain. However, when the results were examined, it was observed that this approach did not provide a significant improvement in overall performance at the expected level. These studies in literature constituted the primary motivation for the present research. However, unlike these studies, an end-to-end approach was adopted in this work, since sleep experts do not apply any preprocessing steps during manual sleep staging. In this context, the aim of this study is to perform a comparative analysis of the traditional SMOTE method and the relatively newer GAN approach on the MIT-BIH dataset, which is an imbalanced dataset and has been rarely used for sleep staging in literature.

Method

In this study, an end-to-end CNN-BiLSTM-based deep learning architecture was proposed for both three-class and five-class sleep staging. Furthermore, the class imbalance problem in sleep staging data was addressed by applying SMOTE and GAN-based data balancing methods only to the training dataset and the effect of these data balancing techniques were comparatively analyzed. Firstly, the dataset was divided into three parts:

training, validation, and test sets. The training data was used in three different versions: the original (imbalanced) data, data balanced using SMOTE, and data balanced using GAN. No balancing procedure was applied to the validation and test datasets. These three different training datasets were fed into the model separately, and the model was trained and tested three times using each training dataset. The block diagram of the study is presented in Figure 1.

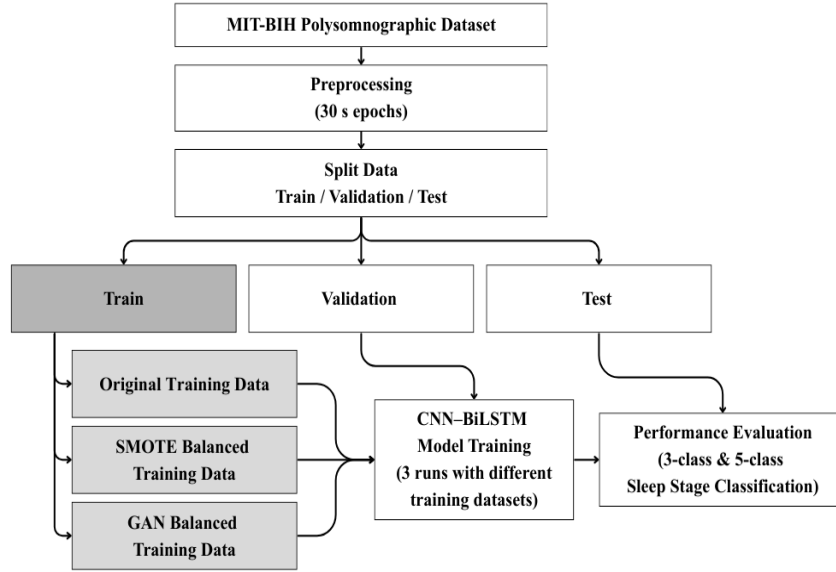


Figure 1. General structure of this study

Data and Preprocessing

The MIT-BIH Polysomnography dataset is a publicly available dataset accessible via the PhysioNet website and contains signals obtained from PSG devices belonging to 18 patients. However, in this dataset, the EEG signal was not recorded from the same channel for each patient. For the 18 patients included in the study, EEG signals were recorded from the C3O1 channel for 11 patients, the C4A1 channel for 5 patients, and the O2A1 channel for 2 patients. In the MIT-BIH dataset, EEG signals are sampled at 250 Hz and segmented into 30-second epochs. Each epoch is annotated with one of the following labels: W (Wake), N1 (NREM1), N2 (NREM2), N3 (NREM3), N4 (NREM4), R (REM), and MT (Movement Time). As a preprocessing procedure, while preparing the dataset for sleep staging, the NREM3 and NREM4 stages were merged and labeled as NREM3 (Berry et al., 2017) and epochs with MT label were removed from the dataset. The resulting number of sleep stages was presented in Table 1. As shown in Table 1, while the NREM2 stage has the highest number of epochs with 3887 samples, the NREM3 stage has the lowest number with 664 epochs.

Table 1. Total number of stages

	Wake	NREM1	NREM2	NREM3	REM	Total
5 class	3119	1815	3887	664	700	10185
3 class	3119	6366			700	10185

Data Augmentation

After dividing the datasets into training, validation, and test sets, the data augmentation methods SMOTE and GAN were applied only to the training data. Within the scope of the study, the performances of the SMOTE and GAN methods in sleep staging were evaluated by using the MIT-BIH dataset.

Synthetic Minority Over-sampling Technique (SMOTE)

SMOTE is an oversampling method that generates synthetic data for the minority class in order to balance class distribution. Unlike traditional oversampling methods that increase data by duplicating existing minority class samples, it creates new synthetic samples by interpolating between instances within the minority class (Dokare

& Gupta, 2025). First, a random sample is selected from the minority class, and its k nearest neighbors are identified. In this study, the value of k was set to 5. Then, the difference vector between the selected sample and one of its neighbors is computed, and this difference is multiplied by a randomly chosen coefficient between 0 and 1 and added to the original sample. This process is repeated until the number of minority class samples reaches that of the majority class. As shown in Table 1, when the dataset is split into 20% test and 10% validation for 5 classes, the test set contains 624 Wake, 363 NREM1, 777 NREM2, 133 NREM3, and 140 REM stages. In the training set of raw datasets, there are 2245 epochs for Wake, 1307 for NREM1, 2799 for NREM2, 478 for NREM3, and 504 for REM. When SMOTE was applied only to the training data, all classes were balanced to match the class with the highest number of epochs, which is NREM2 (2799 epochs). As a result, the total number of epochs in the training set increased to 13995. Similarly, under the three-class staging scheme, the dataset is divided into training, validation, and test subsets, where the raw test set includes 624 Wake, 1273 NREM, and 140 REM epochs. In this case, the remaining 2245 epochs for Wake, 4584 for NREM, and 504 for REM formed training subset. After applying SMOTE, the number of epochs in each class within the training set was balanced to match the class with the highest number of epochs (i.e., the NREM stage; 4584 epochs); as a result, a total of 13752 epochs was reached.

Generative Adversarial Networks (GAN)

GAN consists of two competing deep neural networks that try to outperform each other. The first network, discriminator, is trained to distinguish between real data and fake data generated by the generator. The second network, generator, takes random noise signals as input and transforms them into synthetic data that resembles real data. The objective of generator is to minimize the loss function by producing data that is realistic enough to deceive the discriminator (Hartmann et al., 2018). As previously stated, while the training subset of 5-class configuration consists of 2245 Wake, 1307 NREM1, 2799 NREM2, 478 NREM3, and 504 REM epochs, the training subset of 3-class configuration includes 2245 Wake, 4584 NREM, and 504 REM epochs. During the GAN implementation, separate GAN models were trained for the NREM3 and REM stages in the 5-class setting, and 1000 synthetic epochs were generated from each model. In the 3-class setting, since only the REM stage was underrepresented, 2000 REM epochs were generated from the model. After data generation, the total number of epochs in the training subset increased from 7333 to 9333 in both the 5-class and 3-class configurations, following the generation of 2000 synthetic samples. The GAN architecture used in this study is presented in Figure 2.

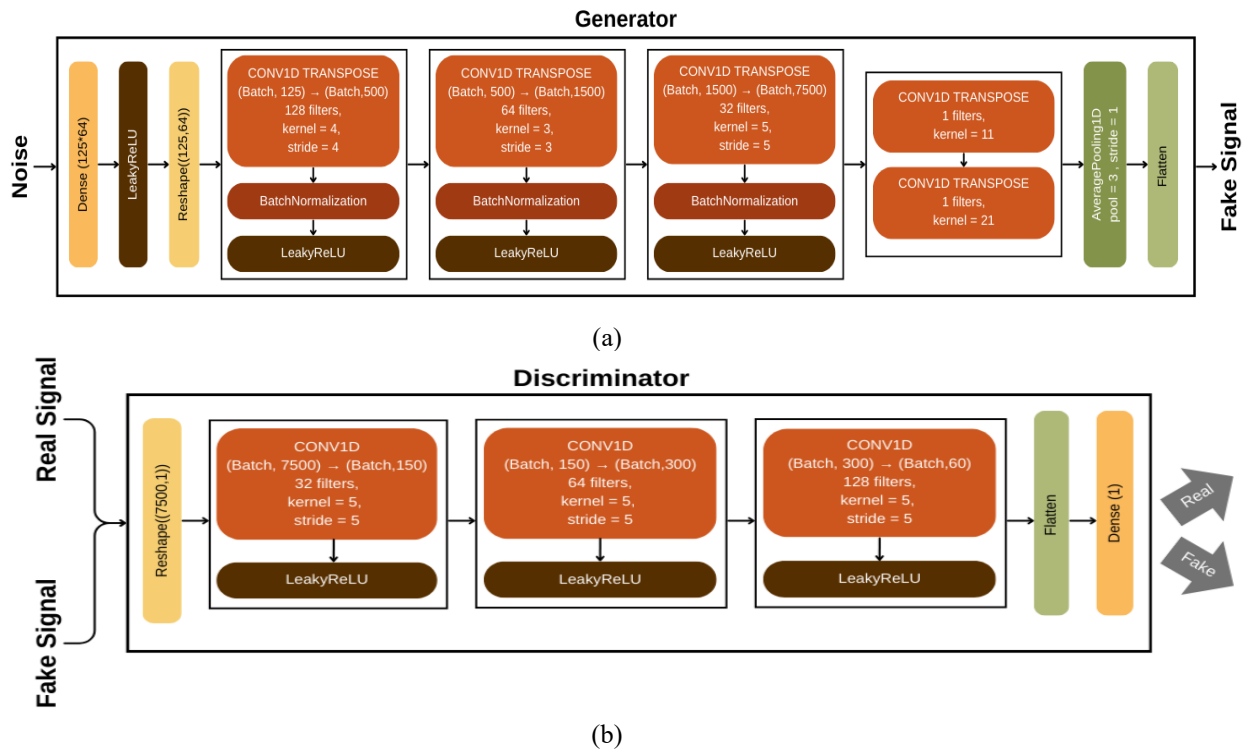


Figure 2. a. The generator structure used in the GAN model b. the discriminator structure used in the GAN model.

Deep Learning Architecture

The deep learning architecture used in this study was constructed by combining Convolutional Neural Network (CNN) and Bidirectional Long Short-Term Memory (BiLSTM) algorithms (Figure 3). The convolutional neural network model consists of three fundamental layers: convolutional layers, pooling layers, and fully connected layers. CNNs are composed of sequentially arranged blocks, where each block extracts new features from the information obtained from the previous one (Efe, 2022). The feature maps obtained after the convolutional layers were then fed into the BiLSTM layer to model temporal dependencies. The BiLSTM structure processes the data in both forward and backward directions, enabling more effective learning of long-term dependencies. In this way, the model can achieve higher classification accuracy by considering not only spatial features but also temporal relationships.

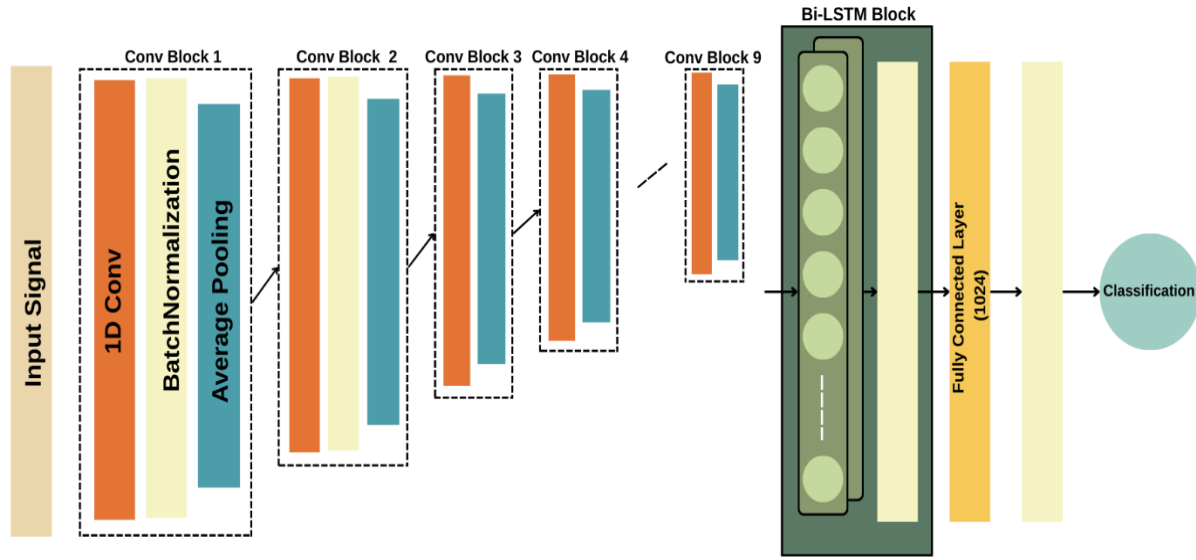


Figure 3. The deep learning architecture used in this study

The deep learning architecture used in this study consists of nine convolutional blocks. Batch normalization was applied in the first two convolutional blocks. Following the convolutional blocks, two BiLSTM layers were incorporated into the model. The blocks between Conv Block 3 and Conv Block 9 consist of a convolutional layer followed by a pooling layer. The number of filters in the convolutional blocks are 16, 32, 64, 128, 256, 512, 512, 512, and 512, respectively, with a filter size of 1×3 . The number of units in the BiLSTM layers are 256 and 128, respectively. To evaluate the performance of the study on a class-wise basis, Accuracy, Recall, Precision, and F1-Score metrics were employed (Mostafaei et al., 2024; Satapathy et al., 2023). Accuracy measures the overall correctness of the model, while Recall indicates the ability to correctly identify positive instances, Precision reflects the proportion of correctly predicted positive instances among all predicted positives, and F1-Score provides a balanced measure by combining Precision and Recall.

Results and Discussion

Firstly, as shown in Figure 1, the dataset was divided into 70% training, 10% validation, and 20% test subsets. Using this split, both 3-class and 5-class sleep staging tasks were performed with the proposed deep learning model. In the first phase, the model was trained and tested using the raw (imbalanced) training data without applying any balancing technique. Subsequently, class imbalance was addressed by applying the SMOTE method only to the training dataset, and both 3-class and 5-class sleep staging processes were repeated. Finally, the GAN model was trained separately for the NREM3 and REM classes using only the training dataset, and 1000 synthetic samples were generated for each class. These generated synthetic data were then incorporated into the training dataset, and the model was retrained for the 5-class sleep staging task. For the 3-class staging, in order to perform GAN-based balancing, 2000 synthetic samples belonging to the REM stage were generated and added only to the training dataset. Following this process, the model was retrained for the 3-class sleep staging task, and sleep stage classification was performed.

3-Class Sleep Staging Results

The performance metrics obtained for 3-class staging using the raw, SMOTE-balanced, and GAN-balanced datasets are presented in Table 2, while the corresponding confusion matrices are shown in Table 3. The reported results correspond to the test dataset.

Table 2. Performance metrics of 3 class

	Accuracy	Precision	Recall	F1 score
Raw	87.58%	0.82	0.81	0.81
SMOTE	85.91%	0.78	0.80	0.79
GAN	86.60%	0.80	0.79	0.80

When the results presented in Table 2 are examined, it is observed that the raw dataset achieved the highest accuracy (87.58%) compared to the SMOTE- and GAN-based balancing methods. A slight decrease in overall accuracy was observed in the GAN-balanced dataset (86.60%), while the accuracy obtained with the SMOTE-balanced dataset was approximately 1% lower than the GAN-balanced dataset and nearly 2% lower than the accuracy achieved with the raw dataset. When the F1-score values are examined, scores of 0.81, 0.79, and 0.80 were obtained for the raw, SMOTE-balanced, and GAN-balanced datasets, respectively. These results indicate that the model demonstrates a similar and consistent performance across all experimental groups in recognizing the classes.

Table 3. Confusion matrices of all experiments for 3 class

			Predicted Label		
T r u e	Raw	Wake	513	98	13
		NREM	66	1174	33
		REM	9	34	97
L a b e l	SMOTE	Wake	526	85	13
		NREM	99	1131	43
		REM	16	31	93
	GAN	Wake	504	106	14
		NREM	70	1169	34
		REM	6	43	91

The analysis of the confusion matrices presented in Table 3 reveals the effects of data balancing methods on classification performance. When the model was trained using the dataset balanced with the SMOTE algorithm, it correctly predicted 526 out of 624 epochs belonging to the Wake stage, achieving an accuracy of 84%. In contrast, the highest performance for the NREM and REM stages was obtained using the original (raw) dataset. For the NREM stage, 1174 out of 1273 epochs were correctly classified, resulting in an accuracy of 92%. Similarly, for the REM stage, 97 out of 140 epochs were correctly classified, corresponding to a classification accuracy of 69%. With the dataset balanced using the GAN-based method, the model achieved its second-best performance in the NREM stage, with 1169 correctly classified epochs and an accuracy of 91.83%. However, the performance for the Wake and REM stages was lower compared to the other experimental scenarios. Another notable finding is that, across all three experimental scenarios, the model struggled to distinguish the REM stage and frequently confused it with the NREM stage.

5-Class Sleep Staging Results

For the 5-class staging task, the dataset was first divided into training, validation, and test sets, and the model was initially trained using the raw data. Subsequently, the model was retrained by applying the SMOTE method only to the training dataset. In the final stage, in order to improve the recognition of the NREM3 and REM stages in the training dataset, a GAN model was trained, and 1000 synthetic samples were generated for each class. These synthetic data were then incorporated into the training dataset, and the model was retrained. The performance metrics of the three experimental setups are presented in Table 4, while the corresponding confusion matrices are provided in Table 5.

Table 4. Performance metrics of 5 class

	Accuracy	Precision	Recall	F1 score
Raw	75.85%	0.82	0.81	0.81
SMOTE	71.82%	0.78	0.80	0.79
GAN	75.31%	0.71	0.72	0.72

When Table 4 is examined, it is observed that the highest accuracy and F1 score are obtained from the raw dataset. Although the GAN-based balanced dataset produces results that are quite close to the raw data in terms of accuracy, the lower precision, recall, and especially F1 score indicate that the model's ability to distinguish between classes has weakened. On the other hand, although the SMOTE method has the lowest accuracy, its higher F1 score compared to GAN suggests that it preserves the balance between classes better and provides a more balanced classification performance.

Table 5. Confusion matrices of all experiments for 5 class

		Predicted Label					
		Wake	NREM1	NREM2	NREM3	REM	
True Label	Raw	Wake	530	44	34	0	16
		NREM1	61	190	91	0	21
		NREM2	29	79	622	29	18
		NREM3	0	2	34	97	0
		REM	8	10	16	0	106
	SMOTE	Wake	506	72	27	1	18
		NREM1	61	208	71	0	23
		NREM2	30	112	560	56	19
		NREM3	3	0	32	98	0
		REM	11	15	22	1	91
	GAN	Wake	528	44	36	0	16
		NREM1	69	190	88	0	16
		NREM2	31	67	617	41	21
		NREM3	0	0	29	104	0
		REM	12	13	20	0	95

The class-based analysis of the confusion matrices presented in Table 5 indicates statistically significant differences between the datasets in terms of the number of correctly classified samples. When examining the total of 624 test epochs belonging to the Wake stage, the highest performance was achieved using the original (raw) dataset, where 530 epochs were correctly predicted, resulting in an accuracy of 84%. For the 363 epochs in the NREM1 stage, the best performance was observed with the SMOTE-balanced dataset, achieving 208 correct predictions and an accuracy of 52%. In the classification of the 777 epochs belonging to the NREM2 stage, the raw dataset demonstrated the highest performance with 622 correct predictions and an accuracy of 80%, while the GAN-based balancing method closely followed with 617 correct predictions and an accuracy of 79%. For the NREM3 stage, which consists of 133 epochs, the highest classification performance was achieved using the GAN-based dataset, with 104 correct predictions and an accuracy of 78%. For the 140 epochs in the REM stage, the raw dataset achieved the highest performance with 106 correct predictions and an accuracy of 75%, while the SMOTE and GAN methods exhibited similar performance levels for this stage. When examining the general error trends, it was observed that the model frequently confused the NREM2 stage with NREM1 and the REM stage with NREM2. In contrast, the model was able to clearly distinguish between the REM and NREM3 stages, and no significant confusion between these two classes was observed.

Conclusion

Sleep stage classification was carried out in this study using the MIT-BIH Polysomnographic dataset, which exhibits a high degree of class imbalance. In this context, the class imbalance problem in the dataset was addressed, and the data augmentation potentials of the traditional SMOTE method and the more innovative GAN approach based on deep neural networks were investigated for complex and non-stationary EEG signals such as sleep data. In addition, a novel deep learning model, not previously used in the literature and expected to yield competitive performance compared to existing studies, was proposed. When the obtained results are examined, the accuracy values of 87.58% for the 3-class classification and 75.85% for the 5-class classification, achieved without applying any data balancing method, present promising outcomes. When compared with studies in the literature conducted using the MIT-BIH Polysomnographic dataset, Zhao et al. (2021) achieved an

accuracy of 80.40% for 4-class sleep staging using the same dataset. Similarly, Bhusal et al. (2022) and Zhang et al. (2020) reported accuracy rates of 88.1% and 87.6%, respectively, for 5-class sleep staging. However, unlike the proposed study, these works applied extensive preprocessing and feature extraction steps to the dataset, which contributed to achieving higher performance levels (Bhusal et al., 2022; Zhang et al., 2020; Zhao et al., 2021). As is well known, the feature extraction process helps focus on the most informative parts of the signal, thereby improving classifier performance. However, the aim of this study was to achieve comparable performance using raw signals; therefore, an end-to-end approach was adopted, and raw time-series signals were used directly without applying preprocessing steps such as filtering or feature extraction methods.

When the balancing methods are compared, it is observed that the SMOTE method performs worse than the GAN-based approach. On the other hand, the GAN model adapted in this study achieves performance results close to those obtained with the raw dataset, demonstrating that GAN is a more effective approach than SMOTE for generating complex signals such as EEG. In addition, as reported by Fan et al., the findings indicate that data augmentation methods do not necessarily improve performance for EEG signals. Furthermore, since the performance obtained from the raw dataset and the proposed model is promising, the model's performance can be further improved through hyperparameter optimization.

Recommendations

Based on the findings and performance of the proposed end-to-end model, several directions for future research are recommended: First, since the model achieved promising results directly from raw signals, systematic hyperparameter tuning should be conducted to further improve classification performance. Second, although the deep learning-based data augmentation approach proved more effective than traditional statistical methods in addressing class imbalance in non-stationary signals, future studies should explore more advanced generative architectures (e.g., GANs, VAEs, or diffusion models) to produce more realistic synthetic data. Third, integrating automated denoising mechanisms within the neural network architecture is recommended. This could help preserve the end-to-end framework while reducing the negative impact of signal artifacts.

Scientific Ethics Declaration

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Myricetin Modulates Redox Balance in MDBK Cells in a Dose-Dependent Manner

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Abstract: Myricetin (MYR), a natural flavonoid, exerts antioxidant effects by scavenging free radicals and enhancing cellular defenses. However, its dose-dependent redox-regulatory effects in Madin-Darby bovine kidney (MDBK) cells remain unexplored. This study aimed to investigate the dose-dependent effects of myricetin on redox balance in MDBK cells by measuring total antioxidant capacity (TAC) and total oxidant capacity (TOC). MYR was dissolved in 0.1% DMSO. Cells were treated for 24 hours in quadruplicate, with untreated cells as the negative control (NC) and 0.1% DMSO as the vehicle control. Cell viability was assessed by MTT assay, confirming that MYR treatments maintained >95% cell survival. TAC and TOC levels were measured using a commercial spectrophotometric kit, and each measurement was carried out in duplicate. Oxidative stress index (OSI) was calculated as the ratio of TOC to TAC. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered significant. TAC levels (mmol Trolox equivalent/L) increased from 1.07 ± 0.03 in the NC group to 1.29 ± 0.07 with MYR 10 μM ($p = 0.008$), 1.52 ± 0.07 with MYR 50 μM ($p < 0.01$), and 1.73 ± 0.12 with MYR 100 μM ($p < 0.01$). Conversely, TOC levels ($\mu\text{mol H}_2\text{O}_2$ equivalent/L) decreased from 10.19 ± 0.28 in NC to 8.02 ± 0.17 with MYR 10 μM ($p < 0.01$), 7.33 ± 0.17 with MYR 50 μM ($p < 0.01$), and 6.09 ± 0.11 with MYR 100 μM ($p < 0.01$). The 0.1% DMSO control did not significantly alter TAC (1.16 ± 0.06 , $p > 0.05$ vs NC) but caused a slight decrease in TOC (9.50 ± 0.18 , $p > 0.05$ vs NC). These results indicate that myricetin modulates oxidative balance in a dose-dependent manner. In conclusion, MYR significantly increased antioxidant capacity and reduced oxidative stress in MDBK cells in a dose-dependent manner. The dose-dependent decrease in OSI values indicates a shift towards redox balance. These findings, which reveal the cellular redox regulatory potential of MYR, are expected to contribute to the understanding of flavonoid-mediated antioxidant mechanisms in veterinary and cell biology research, as well as in nutraceutical studies as a feed supplement.

Keywords: Myricetin, MDBK cells, Oxidative stress, TAC, TOC, OSI, Flavonoid.

Introduction

Reactive oxygen species (ROS), continuously produced as products of cellular metabolism, play a crucial role in cellular homeostasis. However, excessive ROS production disrupts the balance between antioxidants and oxidants, leading to oxidative stress. Oxidative stress can damage the structure of proteins, lipids, and DNA. This damage leads to cellular dysfunction, resulting in numerous pathologies, including inflammation, metabolic disorders, and degenerative diseases. Therefore, maintaining intracellular redox balance is essential for the organism's overall health (Kozlov et al., 2024; de la Lastra et al., 2026). Cells have a defense system that neutralizes ROS (reactive oxygen species) using enzymatic and non-enzymatic antioxidants such as superoxide

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dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione, thereby protecting cellular survival (Ighodaro and Akinloye, 2018; Irato and Santovito, 2021). As the severity of oxidative stress increases, these defense systems become insufficient, necessitating the use of exogenous antioxidants. Biological damage associated with oxidative stress can lead to numerous degenerative health problems that have a significant impact on the overall performance and productivity of livestock (Yang et al., 2013). Natural flavonoids, due to their strong antioxidant and cytoprotective effects, are successfully used in preventing oxidative stress (Zahra et al., 2024; Trivedi, 2025). It is also considered a functional feed additive that improves redox balance and reduces oxidative stress in ruminants (Onu et al., 2024).

Myricetin (3,3',4',5,5',7-hexahydroxyflavone; MYR) is a dietary flavonoid abundant in fruits, vegetables, and medicinal plants that exhibits significant biological effects, including antioxidant, anti-inflammatory, and anti-apoptotic properties. In addition to its ability to directly scavenge free radicals formed in cells, myricetin also has the potential to modulate cellular antioxidant systems. It has been reported that MYR reduces intracellular ROS levels and increases the activity of antioxidant enzymes during oxidative stress (Wang et al., 2010; El-Ghareeb et al., 2023; Almatroodi and Rahmani, 2025). Myricetin has also been shown to prevent oxidative stress-induced apoptosis by modulating signaling pathways involved in cell survival, including the PI3K/Akt and MAPK pathways (Taheri et al., 2020; Goyal et al., 2024; Sabir and Dyary, 2026). Myricetin has been observed to exhibit protective effects in different cell types exposed to oxidative stress (Pandey et al., 2009; Xie et al., 2023; Wang et al., 2023). It has been shown to reduce hydrogen peroxide-induced oxidative damage and apoptosis in bovine mammary epithelial cells, highlighting the potential importance of MYR in veterinary and agricultural research (Kan et al., 2021). The fact that MYR maintains cellular ultrastructure and improves cell viability in oxidative stress models supports its cytoprotective potential (Berker et al., 2025). Despite existing studies in literature, the dose-dependent effects of MYR on the redox balance of MDBK cells have not, to our knowledge, been evaluated. This study aimed to investigate the dose-dependent effects of MYR on redox balance in MDBK cells by measuring TAC and TOC. Investigating how different concentrations of MYR affect the redox balance in MDBK cells, an important in vitro model in veterinary biomedical research, as assessed by TAC and TOC measurements, not only provides valuable insights into potential biomedical effects, but it may also provide insight into the diet-based management of oxidative stress in ruminants and offers mechanistic data supporting its potential use as a nutraceutical agent.

Method

Cell Culture and Myricetin Treatment

This study was conducted in continuous cell culture of MDBK, and cells were cultured in Dulbecco's minimum essential medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin. The cells were cultured with fresh medium every 2 days. After the cells reached a concentration of 80-90% in a T-25 culture flask, they were washed with PBS and then trypsinized with 1.5 mL of 0.25% trypsin-EDTA solution for 2-3 minutes at room temperature. The cells were transferred to T-75 culture flasks and cultured until they reached sufficient confluence. Cells were treated for 24 hours in quadruplicate, with untreated cells as the negative control (NC) and 0.1% DMSO as the vehicle control. Myricetin (Cat. No. 476275, Sigma-Aldrich, Wicklow, Ireland) was dissolved in 0.1% dimethyl sulfoxide (DMSO) as a stock solution. MDBK cells were treated with 10, 50, or 100 µM MYR for 24 hours in quadruplicate. Untreated cells served as the negative control (NC), while cells treated with 0.1% DMSO were used as the vehicle control. Cell viability was assessed by MTT assay, confirming that MYR treatments maintained >95% cell survival.

Measurement of TAC and TOC

Total antioxidant capacity (TAC) levels in the cell culture media were determined using a colorimetric assay kit (Rel Assay Diagnostics, Gaziantep, Turkey). The assay was performed according to the manufacturer's instructions. This method quantified the antioxidant status of the samples based on the principles described by Erel (2004). Total oxidant capacity (TOC) levels in the cell culture media were determined using a colorimetric assay kit (Rel Assay Diagnostics, Gaziantep, Turkey). The assay was performed following the manufacturer's instructions. This method measures the oxidant status of the samples, as described by Erel (2005).

Calculation of OSI

The OSI was calculated to evaluate the balance between oxidants and antioxidants in the cells. OSI was defined as the ratio TOC ($\mu\text{mol H}_2\text{O}_2$ equivalent/L) to TAC (mmol Trolox equivalent/L) multiplied by 100. Because the units of TAC and TOC differed, TAC values were first converted from mmol/L to $\mu\text{mol/L}$ by multiplying by 1000 (Alp et al., 2010; Karkucak et al., 2010).

Statistical Analysis

Statistical evaluation of the data was performed using SPSS 25.0 (IBM Corp., Armonk, NY, USA). Data are presented as mean $\pm$ standard deviation (SD). Normality of the data was assessed using the Shapiro–Wilk test. One-way analysis of variance (ANOVA) followed by Tukey’s post hoc test was used for group comparisons. A p-value <0.05 was considered statistically significant.

Results

Effect of MYR on TAC Levels

TAC levels were significantly increased in a dose-dependent manner following MYR treatment. TAC levels in the negative control (NC) group were 1.07 ± 0.03 mmol Trolox equivalent/L. Treatment with MYR at $10 \mu\text{M}$ increased TAC to 1.29 ± 0.07 ($p=0.008$), $50 \mu\text{M}$ increased it to 1.52 ± 0.07 ($p<0.01$), and $100 \mu\text{M}$ further increased TAC to 1.73 ± 0.12 (<0.01). The 0.1% DMSO control did not significantly alter TAC (1.16 ± 0.06 , $p>0.05$ vs NC). These results indicate that MYR effectively enhances antioxidant capacity in a concentration-dependent manner (Figure 1).

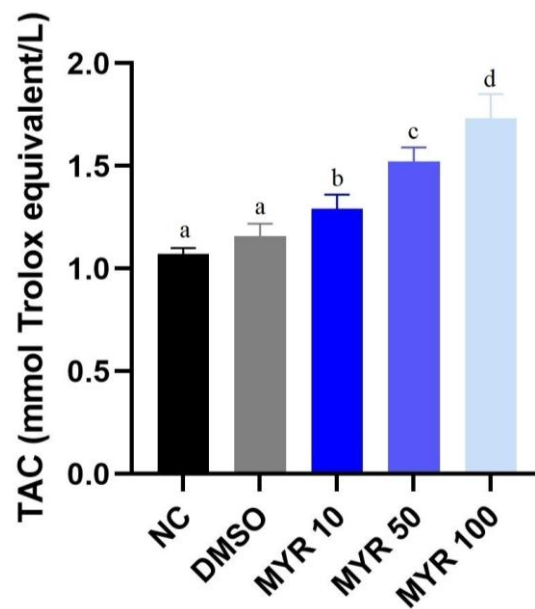


Figure 1. Effect of MYR on TAC in the cells. Data are presented as mean $\pm$ SD (n=4). Different letters (a, b, c, d) indicate significant differences among groups according to one-way ANOVA followed by Tukey’s post hoc test ($p<0.05$). TAC: Total antioxidant capacity; NC: Negative control; DMSO: Dimethyl sulfoxide; MYR: Myricetin.

Effect of MYR on TOC Levels

TOC levels were significantly decreased in a dose-dependent manner following MYR treatment. TOC levels in the negative control (NC) group were 10.19 ± 0.28 $\mu\text{mol H}_2\text{O}_2$ equivalent/L. Treatment with MYR at $10 \mu\text{M}$ decreased TOC to 8.02 ± 0.17 ($p<0.01$), $50 \mu\text{M}$ decreased it to 7.33 ± 0.17 ($p<0.01$), and $100 \mu\text{M}$ further decreased TOC to 6.09 ± 0.11 ($p<0.01$) compared to NC. The 0.1% DMSO control did not significantly alter TOC (9.50 ± 0.18 , $p>0.05$ vs NC). These results indicate that myricetin effectively reduces oxidative stress in a concentration-dependent manner (Figure 2).

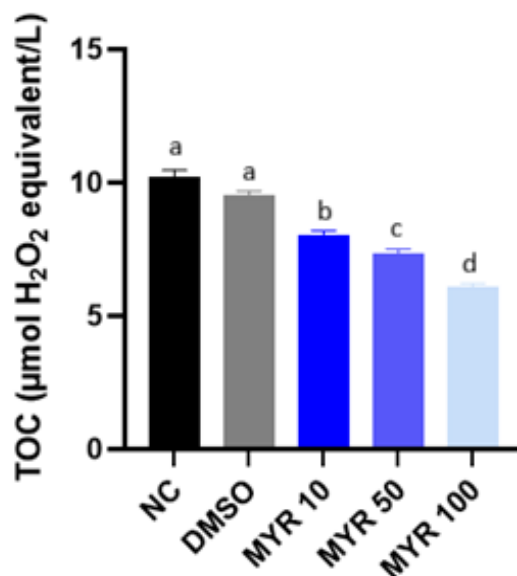


Figure 2. Effect of MYR on TOC in the cells. Data are presented as mean±SD (n=4). Different letters (a, b, c, d) indicate significant differences among groups according to one-way ANOVA followed by Tukey's post hoc test ($p<0.05$). TAC: Total antioxidant capacity; NC: Negative control; DMSO: Dimethyl sulfoxide; MYR: Myricetin.

OSI Values

OSI values were 0.95 in NC, 0.62 with MYR 10 μM, 0.48 with MYR 50 μM, and 0.35 with MYR 100 μM. The 0.1% DMSO control showed an OSI of 0.82. These results demonstrate that myricetin effectively enhances antioxidant capacity and reduces oxidative stress in a concentration-dependent manner.

Discussion

This study demonstrated that MYR increased TAC in MDBK cells in a dose-dependent manner while simultaneously decreasing TOC and OSI. These findings suggest that the cellular environment becomes more antioxidant as MYR concentrations increase. These findings are consistent with the well-documented cellular antioxidant effects of MYR, which occur both through direct ROS scavenging and upregulation of endogenous antioxidant defenses. Flavonoids such as MYR are known for their notable antioxidant effects due to multiple hydroxyl groups that facilitate free-radical scavenging and electron transfer (Agraharam et al., 2022; Devi et al., 2025). MYR's catechol and pyrogallol moieties, in particular, confer high reducing power that enables effective neutralization of ROS and stabilization of radical intermediates, consistent with observed increases in TAC with escalating MYR doses (Semwal et al., 2016; Imran et al., 2021; Chaabani et al., 2026).

It has been demonstrated that myricetin increases antioxidant capacity in bovine-derived cells and alleviates oxidative stress and lipotoxicity (Yang et al., 2022). In bovine hepatocytes, myricetin increased SOD activity, which is associated with antioxidants, whilst reducing the levels of GRP78 and PERK, which are associated with endoplasmic reticulum stress, and NF-κB and TNF-α, which are associated with inflammation, during high fatty acid loading. Investigators have suggested that myricetin intake may exert an antioxidant effect partly by promoting fatty acid mitochondrial oxidation, thereby reducing endoplasmic reticulum stress, inflammation and lipotoxicity (Yang et al., 2022). Our study's observation of dose-dependent redox modulation and antioxidant effect profile in bovine kidney cells suggests that myricetin may exhibit similar protective effects in different cell types responding to metabolic stress, as seen in hepatocytes.

MYR's ability to regulate redox balance is not limited to direct radical scavenging; it also protects cells from oxidative stress-induced damage by supporting cellular antioxidant enzymes. MYR treatment has been reported to restore superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities after oxidative damage such as hydrogen peroxide exposure, thereby reducing DNA and lipid oxidative damage (Wang et al., 2010; Yang et al., 2024). In addition to these direct enzyme-mediated mechanisms, MYR also modulates intracellular signaling pathways that affect redox homeostasis and cell survival. MYR has been

observed to reduce oxidative stress-induced apoptosis by regulating the PI3K/Akt and MAPK pathways, which are known to interact with redox-sensitive signaling networks and influence cellular defense responses (Song et al., 2021; Devi et al., 2025). The dose-dependent redox modulation we identified in this study is consistent with the findings of previous studies focusing on the effects of flavonoids on redox biology. These findings suggest that mirisetin could be considered a potential nutraceutical component for reducing oxidative stress-induced cellular damage. However, it appears that flavonoids reported in scientific studies exhibit more antioxidant effects at low concentrations and pro-oxidant effects at high concentrations (Chobot and Hadacek, 2011; Chobot et al., 2020; Imran et al., 2021). The TAC and TOC findings determined in this study clearly demonstrate that MYR increases antioxidant capacity and suppresses oxidative stress in healthy MDBK cells. It is well known that flavonoids such as MYR can regulate cellular redox balance in a dose-dependent manner, not only by directly neutralizing free radicals but also by influencing antioxidant defense systems and cellular signaling (Kerimi & Williamson, 2018). In our study, the decreases in OSI associated with increasing MYR concentrations indicate that MYR shifts intracellular redox balance towards a protective antioxidant state.

Conclusion

Our findings demonstrated that MYR regulates cellular redox balance in MDBK cells in a dose-dependent manner. By increasing TAC and decreasing TOC, MYR shifts the intracellular environment towards a favorable state for antioxidants and supports cellular defense mechanisms. It has been shown that redox balance in the kidneys, one of the target organs of oxidative stress in ruminants, can be modulated; therefore, the investigation of bioactive compounds such as myricetin at the cellular level contributes to our understanding of their potential effects on ruminant nutrition. This study provides novel insights into the dose-dependent redox regulatory effects of MYR in renal epithelial cells, contributing to the understanding of flavonoid-mediated antioxidant mechanisms in nutraceutical research and laying a foundation for future research on natural compounds in the prevention of oxidative stress.

Scientific Ethics Declaration

* The authors declare that the scientific, ethical, and legal responsibility of this article published in the EPHELS journal belongs to the authors.

Conflict of Interest

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Modeling Agro-Environmental Dynamics with VAR under Climate and Volcanic Variability: A Simulation-Based OLS–GLS Comparison from East Flores, Indonesia

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Abstract: Vector Autoregressive (VAR) modeling provides a flexible framework for examining dynamic interdependencies among multiple time-series variables within agro-environmental systems. Reliable estimation of VAR parameters is critical for accurate inference and forecasting, particularly when vegetation indicators and climatic variables are affected by correlated and heteroskedastic disturbances. This study conducts a simulation-based evaluation of Ordinary Least Squares (OLS) and Generalized Least Squares (GLS) estimators for VAR models under alternative error variance structures. A trivariate VAR(1) specification is employed to characterize agro-environmental dynamics involving vegetation condition, represented by the Normalized Difference Vegetation Index (NDVI), total precipitation, and near-surface air temperature. Monte Carlo simulation experiments are performed to assess estimator performance under independent and correlated disturbance scenarios using bias, variance, and mean squared error as evaluation metrics. To demonstrate empirical relevance, the methodological framework is illustrated using monthly agro-climatic observations from East Flores Regency, East Nusa Tenggara, Indonesia, spanning January 2015 to November 2024. The dataset is compiled from MODIS NDVI products and NASA POWER climatic records. The study setting also reflects recent environmental disturbances linked to volcanic activity in East Flores, which may amplify variability and cross-dependence among agro-environmental variables. Simulation results indicate that OLS estimators remain unbiased and exhibit satisfactory efficiency when error terms are independent. In contrast, under correlated or heteroskedastic disturbances—conditions frequently encountered in agro-environmental time-series data—GLS provides more efficient parameter estimates with lower estimation error. The empirical illustration corroborates the simulation outcomes by demonstrating the role of climatic interactions in shaping vegetation dynamics. Overall, the findings underscore the importance of incorporating appropriate error structures in multivariate agro-environmental time-series analysis and offer methodological insights for agricultural and life science applications employing VAR models.

Keywords: Ordinary least square, Generalized least square, Vector autoregressive model, Agro-environmental time series, Normalized difference vegetation index

Introduction

Agro-environmental systems are characterized by dynamic interactions between vegetation conditions and climatic factors, particularly precipitation and near-surface air temperature. In climate-sensitive regions, fluctuations in rainfall and temperature can substantially affect vegetation growth, agricultural productivity, and

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ecosystem stability. Satellite-derived vegetation indices, especially the Normalized Difference Vegetation Index (NDVI), have been widely adopted as reliable proxies for monitoring vegetation condition due to their sensitivity to canopy structure and photosynthetic activity (Rodrigues & Braga, 2021). Recent studies demonstrate that joint analysis of NDVI and climatic variables provides richer insights into vegetation–climate interactions than univariate approaches, supporting the use of multivariate time-series frameworks in agricultural and life science research (Li et al., 2025; Ogutu et al., 2024).

In regions with limited ground-based meteorological observations, reanalysis and satellite products offer practical alternatives for constructing long and consistent climatic series. The NASA Prediction of Worldwide Energy Resources (POWER) project provides gridded meteorological variables, including precipitation and air temperature, that are widely used in agroclimatology and environmental modeling (Kadhim Tayyeh & Mohammed, 2023; Rodrigues & Braga, 2021). Independent evaluations show that NASA POWER data reasonably capture temporal patterns of key climatic variables, particularly temperature, and are suitable for agricultural and environmental applications in data-scarce regions (Kadhim Tayyeh & Mohammed, 2023; Rodrigues & Braga, 2021). Combined with improved NDVI products derived from MODIS observation (Sun et al., 2023; Xiong et al., 2023), these datasets enable coherent agro-environmental time series suitable for multivariate analysis.

Vegetation and climatic variables often exhibit lagged interactions and are influenced by shared environmental drivers, leading to complex dynamic dependencies. Vector autoregressive (VAR) models provide a flexible multivariate framework to represent such interdependencies by allowing each variable (e.g., NDVI, temperature, precipitation) to depend on its own lagged values as well as the lagged values of other variables in the system. In vegetation–climate applications, VAR-type approaches have been used to capture “legacy effects” (time-lagged responses) between NDVI and climate factors, and to characterize how vegetation dynamics respond over time to meteorological variability (Wu et al., 2020). Recent evidence also emphasizes that vegetation responses to temperature and precipitation can involve both time-lag and accumulation effects, particularly under extreme hydroclimatic conditions, reinforcing the need for dynamic time-series frameworks rather than static correlations (Liu et al., 2024). In agroclimatological contexts, satellite-derived vegetation indices (especially NDVI) are widely used to track vegetation condition, drought stress, and climate-driven variability, and are increasingly combined with temperature–precipitation information to study coupled dynamics at monthly to seasonal scales (Feng et al., 2023). Methodologically, model choice depends on the statistical properties and scientific goal. When the variables are approximately stationary (or have been transformed to stationarity), a parsimonious VAR—often VAR(1) for monthly data—can balance interpretability and estimation efficiency while still capturing key cross-lag interactions. When variables are non-stationary but share a stable long-run equilibrium relationship, the VAR can be extended to a vector error correction model (VECM) to explicitly represent cointegration and separate long-run adjustments from short-run dynamics (Lütkepohl Helmut, 2005). For stronger causal interpretation—e.g., to distinguish contemporaneous “climate shocks” (rainfall/temperature shocks) from vegetation responses—structural VAR (SVAR) frameworks provide a way to impose identifying assumptions on the system, enabling impulse-response analysis under interpretable structural shocks (Sims, 1980). Beyond fixed-parameter VAR, recent studies also employ time-varying parameter VAR variants (e.g., TVP-VAR) to accommodate changing climate–vegetation sensitivities across time and space, which can be important under non-stationary climate regimes (Qin et al., 2023). Overall, these multivariate time-series frameworks provide a coherent statistical basis for evaluating coupled temperature–precipitation–NDVI dynamics, including lagged responses and potential regime shifts, supporting their growing use in agro-environmental and climate-impact research (Ogutu et al., 2024).

The reliability of inference from VAR models critically depends on the estimation method and the assumed disturbance structure. Ordinary Least Squares (OLS) is frequently used for VAR estimation because it is simple and yields unbiased estimators under homoskedastic and uncorrelated disturbances. However, agro-environmental time series often violate these assumptions. Seasonal variability, measurement uncertainty, and common climatic drivers may induce contemporaneous correlation and heteroskedasticity across equation errors. Under such conditions, OLS estimators may remain unbiased but become inefficient. Generalized Least Squares (GLS) explicitly accounts for non-spherical error covariance structures and can yield more efficient parameter estimates when disturbances are correlated or heteroskedastic (Arai et al., 2024; Bai et al., 2021). Recent econometric studies emphasize that accommodating correlated and heteroskedastic errors is essential for efficient estimation and reliable inference in multivariate time-series models (Akpan & Moffat, 2018; Cordoni et al., 2024; Lütkepohl Helmut, 2005).

Since 2023, East Flores Regency (Flores Timur), East Nusa Tenggara, Indonesia, has experienced recurrent volcanic activity associated with Mount Lewotobi. Volcanic processes, including ash deposition and

atmospheric perturbations, are known to alter surface reflectance properties and local microclimatic conditions, potentially amplifying variability and contemporaneous dependence among vegetation indices and climatic variables such as NDVI, precipitation, and temperature (Güler & Turgut, 2024; Guo et al., 2022). Previous studies have also shown that abrupt environmental disturbances whether climatic extremes or geophysical events can induce complex dynamic interactions and time-varying volatility in vegetation-climate systems, which are not always directly observable in available datasets (Martinez et al., 2021; Nguyen et al., 2022). In this study, volcanic activity is therefore not introduced as an explicit explanatory variable; instead, it is treated as part of the unobserved environmental disturbances affecting the agro-environmental system. This modeling choice is consistent with recent applications of multivariate time-series frameworks, particularly VAR-type models, which are designed to capture dynamic interdependencies, contemporaneous correlations, and heteroskedastic error structures in vegetation-climate interactions under external shocks, including climate variability and episodic volcanic disturbances, which may amplify variability and cross-dependence. Accordingly, this context reinforces the relevance of evaluating estimation performance under correlated and heteroskedastic disturbances when modeling agro-environmental dynamics using VAR frameworks.

Despite the growing body of literature on vegetation-climate interactions using multivariate time-series models, several methodological gaps remain. First, many agro-environmental studies employing VAR-type frameworks focus primarily on dynamic relationships and impulse responses, while paying limited attention to the efficiency of parameter estimation under realistic disturbance structures. In practice, agro-environmental time series frequently exhibit contemporaneous correlation and heteroskedasticity across equations due to shared climatic drives, seasonal effects, measurement uncertainty, and unobserved environmental disturbances. However, empirical applications often rely on OLS estimation without systematically evaluating its efficiency relative to alternative estimators under such conditions. Second, while simulation-based comparisons of VAR estimators are well established in econometrics, they are rarely tailored to agro-environmental settings involving vegetation indices and climatic variables, particularly in data-scarce regions. Third, recent environmental disturbances—such as episodic volcanic activity—may further amplify cross-variable dependence and error heterogeneity, yet their implications for VAR estimation performance remain largely unexplored when such disturbances are treated as latent rather than explicit explanatory factors. Consequently, there is a need for a focused simulation-based assessment of OLS and GLS estimators for VAR models under independent and correlated disturbance structures, grounded in empirically relevant agro-environmental dynamics.

Motivated by these considerations, this study investigates the relative performance of OLS and GLS estimators for a trivariate VAR(1) model representing district-average NDVI, total precipitation, and near-surface air temperature in East Flores, Indonesia. Monthly data from January 2015 to November 2024 are used to construct an empirically grounded illustration based on MODIS NDVI and NASA POWER climate records. The contributions of this paper are threefold: (i) it provides a focused Monte Carlo assessment of OLS versus (feasible) GLS for VAR(1) estimation under independent and contemporaneously correlated disturbance structures—conditions that are empirically plausible in agro-environmental time series; (ii) it demonstrates how climate variability and unobserved environmental disturbances in a disturbance-prone setting (including volcanic activity in East Flores since 2023) can be reflected in the error covariance structure, thereby motivating efficiency-oriented estimation; and (iii) it delivers practical methodological guidance for agricultural and life science applications by linking simulation evidence to a real NDVI-precipitation-temperature system relevant for vegetation monitoring in climate-sensitive regions. The remainder of this paper is organized as follows. Section II describes the study area and data sources. Section III presents the VAR(1) specification and estimation methods. Section IV outlines the simulation design and evaluation metrics. Section V reports the simulation and empirical results. Section VI concludes with methodological implications and directions for future research.

Study Area and Data

Study Area

This study focuses on East Flores Regency (Flores Timur), located in East Nusa Tenggara (NTT), Indonesia, a region characterized by semi-arid climatic conditions and strong seasonal variability. Agricultural systems in East Flores are predominantly rain-fed and highly sensitive to fluctuations in precipitation and air temperature. Vegetation dynamics in this region exhibit pronounced seasonal cycles, reflecting the timing and intensity of the wet and dry seasons. Since 2023, East Flores has experienced episodes of volcanic activity associated with Mount Lewotobi, which has introduced additional environmental disturbances to local agro-ecosystems. Volcanic ash deposition and atmospheric perturbations may influence vegetation reflectance and microclimatic

conditions, potentially increasing variability in agro-environmental indicators. In this study, volcanic activity is not treated as an explicit explanatory variable but is considered part of the broader environmental context that may affect the disturbance structure of agro-environmental time series. **Figure 1** illustrates the geographic location of East Flores Regency (Flores Timur) in East Nusa Tenggara (NTT), Indonesia. The regency is situated in the eastern part of Flores Island and is characterized by semi-arid climatic conditions with pronounced seasonal variability. Its agro-environmental system is largely rain-fed, making vegetation dynamics highly sensitive to fluctuations in precipitation and near-surface air temperature. This geographic and climatic setting provides a relevant empirical context for analyzing multivariate agro-environmental interactions using VAR models, particularly under conditions where climatic variability and environmental disturbances may influence the disturbance structure of the system. These characteristics motivate the use of a multivariate time-series framework and support the examination of estimation performance under correlated and heteroskedastic disturbances.



Figure 1. Geographic location of East Flores Regency (Flores Timur) in East Nusa Tenggara (NTT), Indonesia. The study area is characterized by semi-arid climatic conditions and strong seasonal variability

Data Sources and Variables

The empirical analysis employs monthly agro-environmental data spanning January 2015 to November 2024, resulting in 119 observations. A trivariate system is constructed to represent key vegetation–climate interactions relevant to agricultural dynamics in East Flores. The variables include vegetation condition, precipitation, and air temperature, which are commonly used indicators in agro-environmental studies. Vegetation condition is measured using the Normalized Difference Vegetation Index (NDVI), derived from MODIS satellite products and aggregated as a district-level monthly average for East Flores Regency. Climatic variables consist of total monthly precipitation and near-surface air temperature, obtained from the NASA Prediction Of Worldwide Energy Resources (POWER) dataset. NASA POWER provides consistent and spatially complete meteorological time series that are widely used in agroclimatology, particularly in regions with limited ground-based observations. All variables are aligned at a monthly frequency. Prior to modeling, the series are visually inspected to ensure temporal consistency. For estimation purposes, the variables may be standardized to remove scale effects, although the VAR specification itself is applied to the original time-series structure.

Variable Definition and Descriptive Information

Table 1 summarizes the variables used in this study, including their definitions, data sources, units of measurement, and temporal resolution.

Table 1. Agro-environmental variables used in the VAR model					
Variable	Description		Source	Unit	Frequency
NDVI	District-average Normalized Difference Vegetation Index (vegetation condition proxy)		MODIS (satellite)	Unitless (–1 to 1)	Monthly
Rain	Total precipitation		NAZA POWER	Mm/month	Monthly
Tem	Near-surface air temperature (2m)		NAZA POWER	°C	Monthly

Data Characteristics and Modeling Relevance

The selected variables capture essential agro-environmental processes in East Flores. NDVI reflects vegetation response to climatic conditions, while precipitation and temperature represent key atmospheric drivers influencing plant growth and stress. These variables are known to exhibit lagged interactions and may be jointly affected by common environmental shocks, including extreme climatic events and volcanic disturbances. Such characteristics motivate the use of a VAR framework, which allows for dynamic interdependence among multiple time-series variables.

Moreover, the presence of shared environmental influences suggests that error terms across equations may be contemporaneously correlated or heteroskedastic, providing a relevant empirical setting for comparing OLS and GLS estimation methods. Since 2023, East Flores has experienced episodic volcanic activity associated with Mount Lewotobi, which may influence vegetation reflectance and local climatic conditions. Explicit dummy variables for volcanic activity are not included due to limited temporal variation and uncertainty in measuring eruption intensity at the monthly scale. Instead, volcanic effects are treated as part of unobserved environmental disturbances that may increase error variance and contemporaneous dependence across equations, making the disturbance covariance structure an important consideration in VAR estimation.

Method

VAR (1) Model Specification

Vector Autoregressive (VAR) is an extension of the AR model for multivariate time series analysis. In its basic form, a VAR consists of a set of K endogenous variables $\mathbf{y}_t = (y_{1t}, \dots, y_{kt}, \dots, y_{Kt})$ for $k = 1, \dots, K$. The VAR(p) process can be written as

$$\mathbf{y}_t = A_1 \mathbf{y}_{t-1} + \dots + A_p \mathbf{y}_{t-p} + \mathbf{u}_t \quad (1)$$

Where $\mathbf{y}_t = (y_{1t}, \dots, y_{kt}, \dots, y_{Kt})^T$ is vector of $\mathbf{y}_t$ with size $k \times 1$, A_i is $K \times K$ coefficient matrices for $i = 1, \dots, p$, $\mathbf{u}_t$ is K dimensional white noise process with time invariant positive definite covariance matrix $E(\mathbf{u}_t \mathbf{u}_t') = \Sigma_u$. In this research, the parameters of the VAR(1) model with three variables are estimated. VAR(1) model is assumed to be stationary and white noise. Based on (1) coefficient matrix A_i can be represented as,

$$A_i = \begin{bmatrix} \phi_{i,11} & \phi_{i,12} & \dots & \phi_{i,1k} \\ \phi_{i,21} & \phi_{i,22} & \dots & \phi_{i,2k} \\ \vdots & \vdots & \ddots & \vdots \\ \phi_{i,k1} & \phi_{i,k2} & \dots & \phi_{i,kk} \end{bmatrix} \quad (2)$$

Let $\mathbf{y}_t = (\text{NDVI}_t, \text{Rain}_t, \text{Temp}_t)'$ denote a trivariate agro-environmental time series observed at time t , where NDVI_t represents vegetation condition, Rain_t denotes total precipitation, and Temp_t denotes near-surface air temperature. The dynamic interactions among these variables are modeled using a first-order Vector Autoregressive model, VAR(1), defined as:

$$\mathbf{y}_t = \boldsymbol{\mu} + \mathbf{A}_1 \mathbf{y}_t - \mathbf{1} + \boldsymbol{\varepsilon}_t \quad (3)$$

where $\boldsymbol{\mu}$ is a vector of intercept terms, $\mathbf{A}_1$ is a 3×3 coefficient matrix capturing lagged interdependencies among the variables, and $\boldsymbol{\varepsilon}_t$ is a vector of disturbance terms with zero mean and covariance matrix $\boldsymbol{\Sigma}$. The VAR(1) specification is adopted to provide a parsimonious representation of agro-environmental dynamics while allowing for meaningful interpretation of lagged vegetation–climate interactions. This structure is

commonly used in applied environmental and agricultural time-series studies, particularly when working with monthly data and limited sample size

Estimation Methods: OLS and GLS

Two estimation approaches are considered for the VAR(1) model: OLS and GLS. Under the classical assumption that the disturbance vector is ε_t homoskedastic and contemporaneously uncorrelated across equations (i.e., Σ is diagonal and constant), equation-by-equation OLS yields unbiased and efficient estimators. OLS is therefore used as a benchmark estimation method. However, in agro-environmental systems, disturbance terms may exhibit contemporaneous correlation and heteroskedasticity due to shared climatic drives and external environmental disturbances. In such cases, OLS estimators remain unbiased but are no longer efficient. GLS addresses this limitation by explicitly incorporating the covariance structure of the disturbances. When Σ is unknown, a feasible GLS (FGLS) approach can be implemented by first estimating Σ from OLS residuals and then re-estimating the VAR parameters using the estimated covariance matrix. In this study, GLS estimation is used to evaluate efficiency gains relative to OLS when the error structure departs from the classical assumptions.

Simulation Design

To systematically compare the performance of OLS and GLS estimators, a Monte Carlo simulation study is conducted based on the VAR (1) framework. The simulation design considers two disturbance scenarios:

- Independent error structure, where the covariance matrix Σ is diagonal with constant variances.
- Correlated error structure, where Σ contains non-zero off-diagonal elements, representing contemporaneous correlation among the disturbance terms.

For each scenario, artificial time series are generated using fixed parameter values for μ and A_1 that reflect plausible agro-environmental dynamics. Each simulation is repeated many times to ensure stable performance assessment. For every replication, VAR parameters are estimated to be using both OLS and GLS. Estimator performance is evaluated using standard statistical criteria, including bias, variance, and mean squared error (MSE) of the estimated parameters. These metrics allow for direct comparison of estimation accuracy and efficiency under alternative error variance structures.

Empirical Illustration

In addition to the simulation study, an empirical illustration is conducted using monthly agro-environmental data from *East Flores Regency (Flores Timur), Indonesia*, covering *January 2015 to November 2024*. The empirical VAR(1) model employs district-average NDVI derived from MODIS satellite data, along with precipitation and temperature series obtained from the NASA POWER dataset. The empirical analysis is intended to illustrate how the estimation methods perform in a real-world agro-environmental setting characterized by strong climatic variability and environmental disturbances. Volcanic activity in East Flores since 2023 is not modeled explicitly but is treated as part of the unobserved environmental variability that may influence the disturbance structure of the VAR system. This context provides additional motivation for examining the relative performance of OLS and GLS under non-ideal error conditions.

Results and Discussion

Simulation Results: Scenario 1 (Independent Disturbances)

The simulation results for Scenario 1, where the disturbance terms are independently distributed across equations, are summarized in **Table 2** (OLS) and **Table 3** (GLS). Overall, both estimation methods perform well under this idealized setting. The Monte Carlo means of all autoregressive coefficients are very close to their true parameter values, and the estimated biases are small and centered around zero.

Table 2. Monte Carlo summary of VAR (1) parameter estimates under Scenario 1: OLS estimation

Parameter	TRUE	Mean	SE MC	Bias
A01	0	-0.000909624	0.079504239	-0.000909624
A02	0	0.011535714	0.081508789	0.011535714
A03	0	0.009176141	0.082361824	0.009176141
A11	0.5	0.499317722	0.053445433	-0.000682278
A12	-0.5	-0.497031884	0.07742069	0.002968116
A13	-0.7	-0.69715147	0.077297928	0.00284853
A21	-0.2	-0.200230102	0.051129302	-0.000230102
A22	-0.3	-0.304222619	0.0746656	-0.004222619
A23	0.3	0.305391593	0.074441508	0.005391593
A31	0.2	0.208410723	0.055450115	0.008410723
A32	-0.1	-0.100891473	0.071283192	-0.000891473
A33	0.2	0.194447943	0.08553081	-0.005552057

Table 3. Monte Carlo summary of VAR (1) parameter estimates under Scenario 1: GLS estimation

Parameter	TRUE	Mean	SE MC	Bias
A01	0	-0.007380631	0.082045502	-0.007380631
A02	0	-0.006159513	0.079975761	-0.006159513
A03	0	0.000784216	0.081100749	0.000784216
A11	0.5	0.496236922	0.047653131	-0.003763078
A12	-0.5	-0.500199186	0.074956779	-0.000199186
A13	-0.7	-0.704189902	0.069589459	-0.004189902
A21	-0.2	-0.194816469	0.051745325	0.005183531
A22	-0.3	-0.295037441	0.081565525	0.004962559
A23	0.3	0.295451992	0.071148177	-0.004548008
A31	0.2	0.208702353	0.054969394	0.008702353
A32	-0.1	-0.104322043	0.079942969	-0.004322043
A33	0.2	0.182020509	0.082569774	-0.017979491

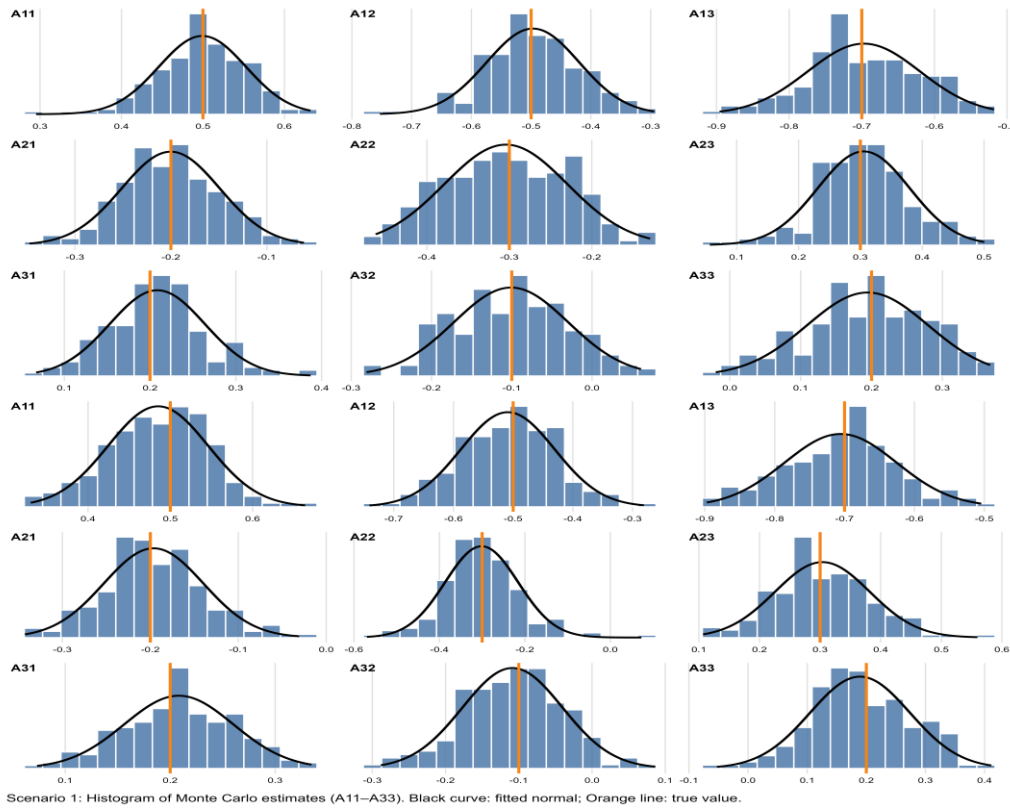


Figure 2. Histograms of Monte Carlo VAR (1) parameter estimates under independent disturbances (Note: The black curve represents the fitted normal distribution, while the orange vertical line indicates the true parameter value)

For instance, the true coefficient $A1=0.5$ is estimated with a mean of 0.4993 using OLS and 0.4962 using GLS, corresponding to negligible biases of -0.0007 and -0.0038 , respectively (Table 2–3). Similar patterns are observed for other parameters such as $A12$, $A13$ and the off-diagonal elements of the VAR coefficient matrix. The Monte Carlo standard errors under OLS and GLS are also comparable, indicating that GLS does not offer substantial efficiency gains when the assumption of error independence holds. These numerical findings are further illustrated by the histograms of Monte Carlo estimates in **Figure 2** (Scenario 1). For all coefficients $A11$ – $A13$, the empirical distributions are approximately symmetric and well aligned with the true parameter values, as indicated by the proximity of the histogram centers to the vertical reference lines. The fitted normal curves closely match the empirical distributions, suggesting that both OLS and GLS estimators exhibit stable sampling behavior under independent disturbances.

The corresponding time-series realizations shown in **Figure 3** (Scenario 1) indicate moderate variability and no pronounced co-movement across the simulated series, which is consistent with the assumption of independent error terms. This visual evidence supports the numerical results and confirms that OLS remains unbiased and efficient in VAR models when classical assumptions are satisfied.

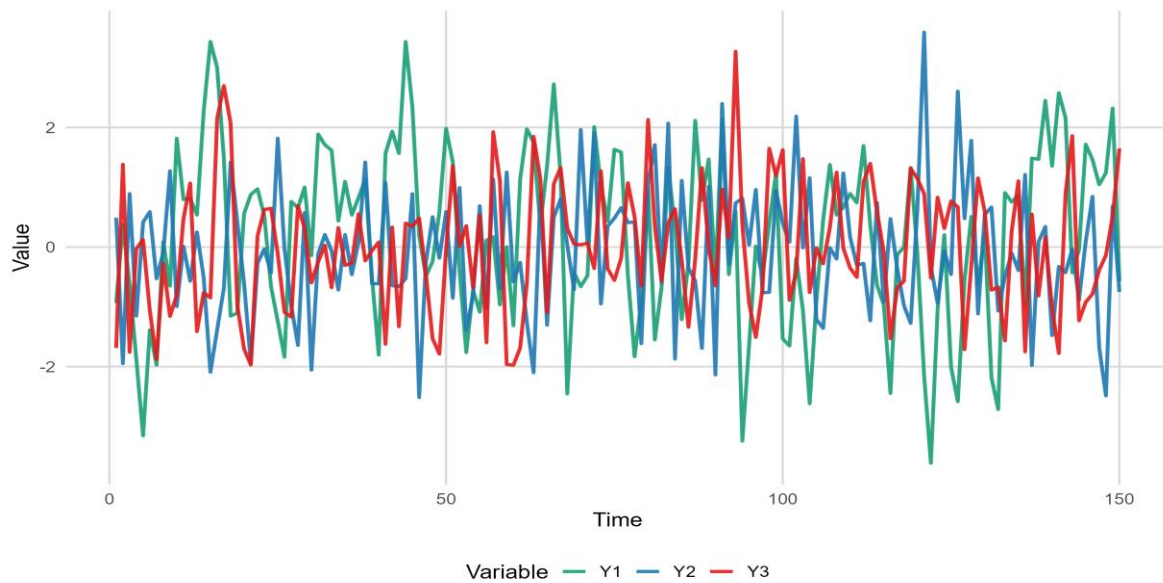


Figure 3. Simulated trivariate time-series generated from VAR (1) model under independent disturbances

Simulation Results: Scenario 2 (Correlated Disturbances)

In contrast, the simulation outcomes for Scenario 2, which introduces contemporaneous correlation among the disturbance terms, reveal marked differences between OLS and GLS estimation. The numerical results are reported in **Table 4** (OLS) and **Table 5** (GLS).

Table 4. Monte Carlo summary of VAR (1) parameter estimates with OLS under correlated disturbances

Parameter	TRUE	Mean	SE MC	Bias
A01	0	0.00432	0.08452	0.00432
A02	0	0.00922	0.077781	0.00922
A03	0	0.016893	0.085705	0.016893
A11	0.5	0.492745	0.053066	-0.00726
A12	-0.5	-0.49455	0.083856	0.005455
A13	-0.7	-0.70951	0.093984	-0.00951
A21	-0.2	-0.19682	0.051582	0.003176
A22	-0.3	-0.30612	0.091924	-0.00612
A23	0.3	0.295828	0.084751	-0.00417
A31	0.2	0.210293	0.053517	0.010293
A32	-0.1	-0.10198	0.083158	-0.00198
A33	0.2	0.191431	0.0809	-0.00857

Table 5. Monte Carlo summary of VAR (1) parameter estimates with GLS under correlated disturbances

Parameter	TRUE	Mean	SE MC	Bias
A01	0	-0.00206457	0.107373384	-0.00206457
A02	0	0.008067856	0.099249235	0.008067856
A03	0	-0.00018115	0.115006835	-0.00018115
A11	0.5	0.350918272	0.064335491	-0.149081728
A12	-0.5	-0.540461984	0.097173368	-0.040461984
A13	-0.7	-0.667917925	0.100160506	0.032082075
A21	-0.2	-0.195639046	0.067711194	0.004360954
A22	-0.3	-0.503363349	0.082973863	-0.203363349
A23	0.3	0.222858503	0.092602069	-0.077141497
A31	0.2	0.237839448	0.062820152	0.037839448
A32	-0.1	-0.137293737	0.084875655	-0.037293737
A33	0.2	-0.097917566	0.096161449	-0.297917566

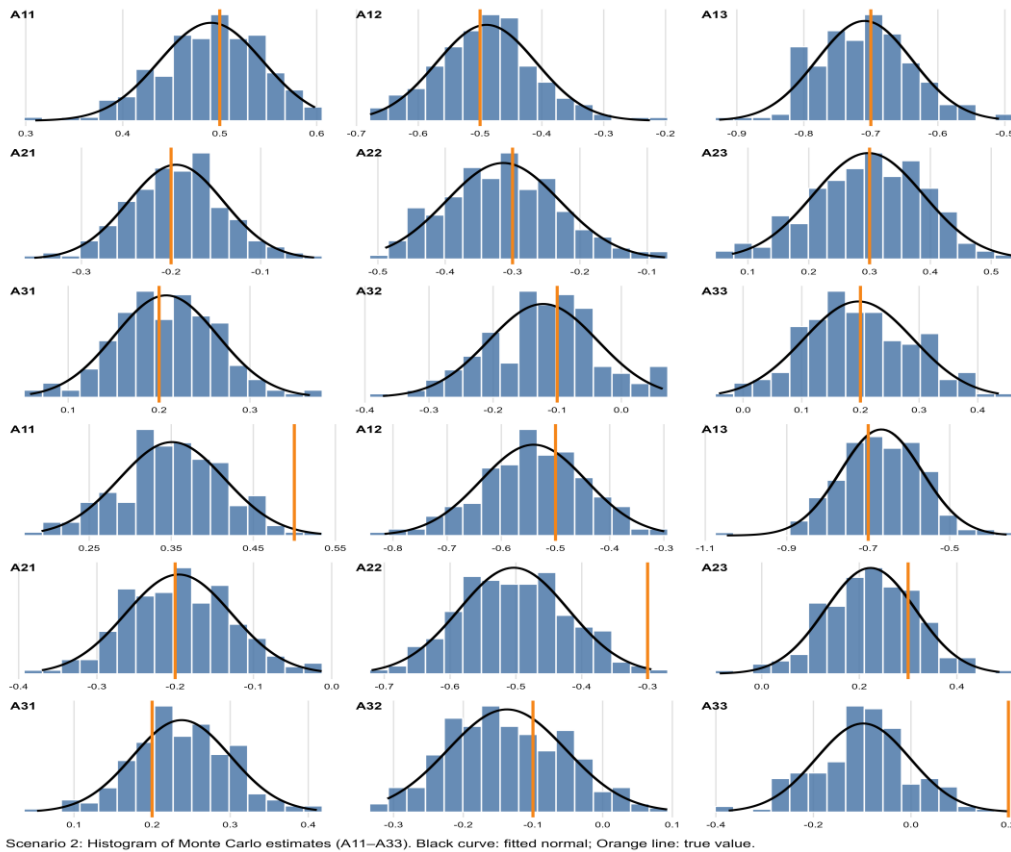


Figure 4. Histograms of Monte Carlo VAR (1) parameter estimates under correlated disturbances (Note: The black curve represents the fitted normal distribution, while the orange vertical line indicates the true parameter value)

Under correlated disturbances, the simulation results reveal that estimator performance depends critically on the quality of the contemporaneous error covariance specification. As reported in **Table 4**, the OLS estimates remain relatively close to the true parameter values, with biases that are generally small to moderate. For example, the coefficient A11 with a true value of 0.5 is estimated by OLS with a Monte Carlo mean of 0.4927, corresponding to a bias of -0.0073 , while A22 (true value -0.3) is estimated at -0.3061 with a bias of -0.0061 . Similar patterns are observed for other parameters, indicating that equation-by-equation OLS exhibits a degree of robustness in this finite-sample setting despite the presence of contemporaneous correlation among disturbances. In contrast, the feasible GLS estimates reported in **Table 5** display substantial finite-sample distortion for several coefficients. Notably, the GLS estimate of A11 has a Monte Carlo mean of 0.3509, implying a large negative bias of -0.1491 relative to the true value of 0.5. Likewise, the bias for A22 increases markedly to -0.2034 (mean -0.5034 versus true -0.3), and the distortion is even more pronounced for A33, where the GLS mean of -0.0979 deviates substantially from the true value of 0.2, yielding a bias of -0.2979 . These results indicate that, in this simulation design, feasible GLS is highly sensitive to imprecision in the

estimated error covariance matrix. Importantly, these findings should not be interpreted as contradicting the efficiency advantage of GLS under correctly specified error structures. Rather, they highlight that in finite samples typical of agro-environmental applications—where the contemporaneous covariance matrix must be estimated from limited data—feasible GLS may suffer from instability, whereas OLS can exhibit comparatively greater robustness. This nuance underscores the practical importance of carefully assessing error covariance estimation when applying GLS in multivariate agro-environmental time-series models.

The histograms of Monte Carlo estimates in **Figure 4** provide visual confirmation of the numerical results reported in Tables 4 and 5. For OLS, the empirical distributions of key coefficients remain relatively centered around their true parameter values, although with noticeable dispersion reflecting finite-sample variability. For instance, the distribution of the OLS estimates for A11 is centered close to the true value of 0.5, consistent with the small bias of -0.0073 reported in **Table 4**. In contrast, the GLS histograms exhibit clear shifts away from the true parameter values for several coefficients. The distribution of the GLS estimates for A11 is centered substantially below the true value, in line with the Monte Carlo mean of 0.3509 and the associated bias of -0.1491 shown in **Table 5**. Similar distributional shifts are observed for A22 and A33, where the GLS estimates deviate markedly from their true values. These visual patterns indicate that, under correlated disturbances and finite sample size, feasible GLS estimates are sensitive to imprecision in the estimated error covariance matrix, leading to systematic distortion that is directly observable in the sampling distributions.

The simulated time-series realizations shown in **Figure 5** illustrate stronger co-movement and joint variability across the three variables compared to Scenario 1, reflecting the presence of contemporaneously correlated disturbances. Such patterns are consistent with the data-generating process assumed in Scenario 2 and provide visual context for the estimation results. Importantly, **Figure 5** does not convey estimator performance directly, but rather highlights the complex dependence structure under which OLS and GLS are evaluated in the subsequent numerical and distributional analyses. In this setting, the effectiveness of GLS critically depends on the accurate estimation of the error covariance matrix, as evidenced by the finite-sample distortions observed in the Monte Carlo results.

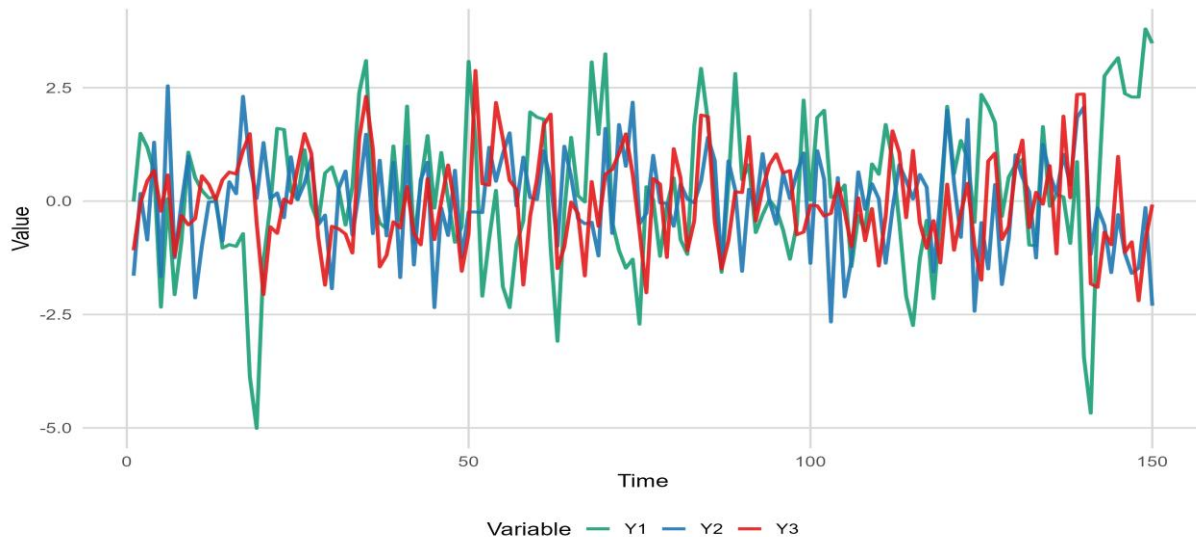


Figure 5. Simulated trivariate time-series generated from VAR (1) model under correlated disturbances

Taken together, the simulation results from both scenarios indicate that the relative performance of OLS and GLS critically depends on the underlying disturbance structure and, in particular, on the quality of error covariance estimation. When disturbances are independent, OLS and GLS exhibit comparable performance. Under correlated disturbances, the results highlight that while GLS is theoretically more efficient under correctly specified covariance structures, its finite-sample performance can be sensitive to imprecision in covariance estimation, whereas OLS may display greater robustness in practice. In contrast, GLS consistently delivers more accurate and efficient estimates under correlated disturbances, as demonstrated by both numerical indicators (bias, SE_{MC} , MSE) and graphical diagnostics (histograms and time-series plots). Importantly, these efficiency gains are achieved without modifying the structural specification of the VAR(1) model, emphasizing that appropriate modeling of the error covariance alone can substantially improve estimation quality.

Empirical Results: Agro-Environmental Dynamics in East Flores

This section presents the empirical results of the multivariate agro-environmental analysis for East Flores based on a VAR framework applied to monthly NDVI, precipitation, and near-surface air temperature data. Beyond documenting empirical dynamics, the results are used to synthesize a parsimonious modeling representation consistent with the observed lag structure and disturbance characteristics. Lag order selection based on standard information criteria indicates a preferred lag length of five months according to the Akaike Information Criterion (AIC), Hannan–Quinn (HQ), and Final Prediction Error (FPE), while the Schwarz Criterion (SC) suggests a shorter specification. Following the majority decision, a VAR(5) model with a constant term is estimated using 114 effective observations. The estimated system is dynamically stable, as all characteristic roots lie strictly within the unit circle, with the largest modulus equal to 0.967. The estimated VAR coefficients reveal statistically meaningful lagged interdependence among NDVI, precipitation, and temperature as shown in **Error! Reference source not found.**

Table 6. VAR (5) estimation results for agro-environmental variables in east flores (NDVI equation)

Variable	Coefficient	Std. Error	t-Statistic	p-value
NDVI_t-1	0.374	0.110	3.39	0.001***
Rain_t-1	0.00010	0.00007	1.53	0.131
Temp_t-1	0.0077	0.0100	0.76	0.447
NDVI_t-2	0.052	0.116	0.45	0.656
Rain_t-2	-0.00004	0.00007	-0.60	0.548
Temp_t-2	0.0012	0.0128	0.09	0.925
NDVI_t-3	-0.089	0.111	-0.80	0.423
Rain_t-3	0.00011	0.00007	1.62	0.109
Temp_t-3	0.0094	0.0128	0.73	0.467
NDVI_t-4	0.121	0.108	1.12	0.267
Rain_t-4	0.00011	0.00007	1.60	0.114
Temp_t-4	-0.0066	0.0132	-0.50	0.617
NDVI_t-5	-0.236	0.097	-2.43	0.017**
Rain_t-5	0.00006	0.00007	0.83	0.411
Temp_t-5	0.021	0.0108	1.99	0.049**
Constant	-0.432	0.361	-1.20	0.234

Table 7. VAR (5) estimation results for agro-environmental variables in east flores (Rainfall equation)

Variable	Coefficient	Std. Error	t-Statistic	p-value
NDVI_t-1	-409.1	200.3	-2.04	0.044**
Rain_t-1	0.232	0.120	1.94	0.055*
Temp_t-2	61.8	23.3	2.66	0.009***
NDVI_t-4	-337.8	196.4	-1.72	0.089*
NDVI_t-5	297.3	176.1	1.69	0.095*
Constant	-1246	656	-1.90	0.060*

Table 8. VAR (5) estimation results for agro-environmental variables in east flores (Temperature equation)

Variable	Coefficient	Std. Error	t-Statistic	p-value
Temp_t-1	0.817	0.101	8.12	<0.001**
Rain_t-2	-0.00145	0.00069	-2.11	0.038**
Temp_t-3	-0.369	0.129	-2.86	0.005***
Temp_t-4	0.240	0.132	1.81	0.073*
Temp_t-5	0.220	0.108	2.03	0.045**
Constant	11.82	3.63	3.26	0.002***

Notes: OLS estimation (equation-by-equation). Lag order = 5 selected by AIC. Sample size = 114 monthly observations. Significance levels: *** $p < 0.01$, ** $p < 0.05$, * $p < 0.10$. Only significant and marginal coefficients are reported for the rainfall and temperature equations due to conference page limits. Evidence of cross-equation error dependence is reported separately in **Table 9**.

Vegetation dynamics exhibit short-run persistence through ($NDVI_{t-1} = 0.374, p = 0.001$) and a longer-horizon adjustment at ($NDVI_{t-5} = -0.236, p = 0.017$), while delayed temperature effects emerge at a five-month horizon ($Temp_{t-5} = 0.021, p = 0.049$). Rainfall and temperature equations similarly display cross-variable lagged effects,

reflecting feedback mechanisms typical of agro-environmental systems. In addition to lagged dynamics, the empirical VAR exhibits pronounced contemporaneous dependence across equations. Residual correlations between NDVI and rainfall (-0.494) and between rainfall and temperature (-0.317), together with a highly significant LM test for cross-equation residual correlation ($LM = 40.40, p < 10^{-8}$), indicate that environmental shocks affect multiple system components simultaneously.

Table 9. Evidence of cross-equation error dependence in the empirical VAR (5)

Evidence	Statistic / Value	Interpretation
Residual correlation: NDVI–Rain	-0.493	Moderate negative contemporaneous dependence between NDVI and rainfall residuals
Residual correlation: Rain–Temp	-0.3174	Moderate negative contemporaneous dependence between rainfall and temperature residuals
Residual correlation: NDVI–Temp	0.0996	Weak positive contemporaneous dependence between NDVI and temperature residuals
LM test for cross-equation residual correlation	$LM = 40.4016$, $df = 3$, $p = 8.76 \times 10^{-9}$	Strongly rejects H_0 : no cross-equation residual correlation; supports non-diagonal disturbance covariance structure

Despite this dependence, estimation using feasible GLS via SUR yields coefficients identical to equation-by-equation OLS, reflecting the shared regressor structure across equations. This empirical finding reinforces the central message that efficiency gains from GLS are conditional on model structure and covariance estimation rather than automatic. Taken together, the empirical evidence supports the following agro-environmental VAR representation for East Flores:

$$\mathbf{Y}_t = \mathbf{c} + \sum_{i=1}^5 \mathbf{A}_i \mathbf{Y}_{t-i} + \boldsymbol{\varepsilon}_t, \quad \boldsymbol{\varepsilon}_t \sim N(\mathbf{0}, \boldsymbol{\Sigma}),$$

where $\mathbf{Y}_t = (\text{NDVI}_t, \text{Rain}_t, \text{Temp}_t)'$, the coefficient matrices $\mathbf{A}_i$ capture delayed vegetation–climate feedbacks over multiple horizons, and the disturbance covariance matrix $\boldsymbol{\Sigma}$ is non-diagonal, reflecting contemporaneously correlated environmental shocks. This specification provides a coherent modeling framework that accommodates both dynamic interdependence and shared disturbances in agro-environmental systems, while allowing for robust estimation in finite samples. The impulse response and variance decomposition analyses (Figure 6) further illustrate the dynamic implications of the estimated model. Rainfall shocks generate delayed and moderate responses in NDVI, while temperature and precipitation shocks contribute increasingly to vegetation variability at longer horizons, underscoring the multivariate nature of agro-environmental dynamics in East Flores.

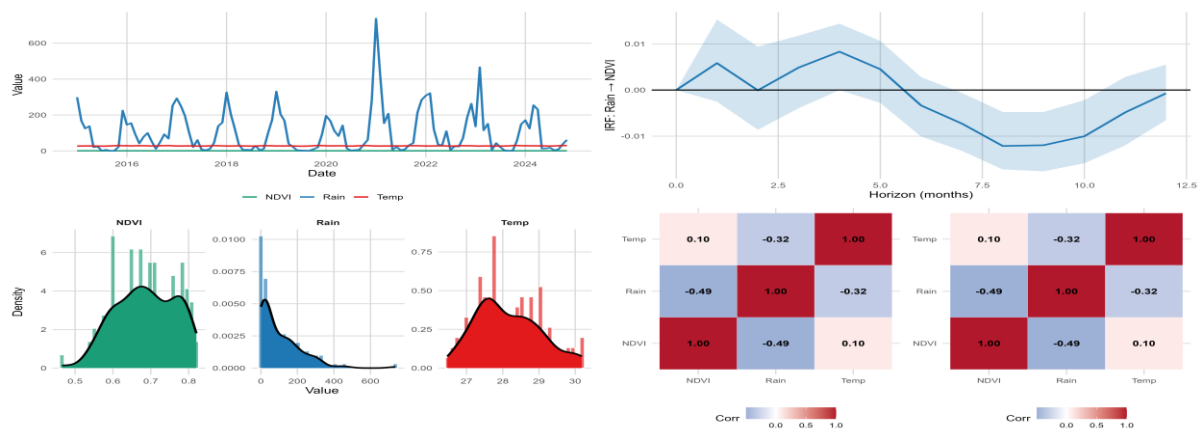


Figure 6. Empirical dynamics, impulse responses, and contemporaneous residual correlations for the VAR(5) model of NDVI, rainfall, and temperature in East Flores

Periods of increased volatility are visible across multiple variables, particularly in recent years, indicating that agro-environmental indicators in East Flores are subject to common environmental shocks rather than purely idiosyncratic fluctuations. This visual evidence is consistent with the presence of contemporaneous dependence among disturbances, as further supported by the residual correlation analysis, and motivates careful consideration of the disturbance covariance structure in multivariate modeling. The empirical results align closely with the structural features highlighted in Scenario 2 of the simulation study, where disturbances are contemporaneously correlated. While the simulation results illustrate that the finite-sample performance of GLS

depends critically on the accuracy of covariance estimation, the empirical analysis confirms the practical relevance of correlated disturbances in real agro-environmental data. Taken together, these findings indicate that the simulation framework captures key characteristics of the empirical system, thereby strengthening the methodological contribution of the study without implying that efficiency gains from GLS estimation are automatic in practice.

Discussion: Implications for Real-World Agro-Environmental Analysis

The empirical findings underscore that agro-environmental systems in climate-sensitive regions such as East Flores are characterized by complex dynamic interdependencies and shared environmental disturbances. Climatic variability, together with episodic shocks such as volcanic activity, can generate contemporaneous correlation among disturbances in multivariate time-series models, as evidenced by the residual dependence observed in the empirical VAR analysis. In such settings, equation-by-equation OLS estimation can yield reasonable coefficient estimates, yet it may not fully reflect the underlying dependence structure of environmental shocks. While GLS-type approaches are designed to account for correlated disturbances, the empirical results demonstrate that efficiency gains from GLS are not automatic and depend critically on model structure and covariance estimation quality. The alignment between the simulation framework and the empirical evidence therefore highlights the practical importance of diagnosing disturbance dependence and finite-sample behavior, rather than assuming uniform superiority of any single estimation method, when analyzing real-world agro-environmental systems.

Conclusion

This study demonstrates that agro-environmental dynamics in East Flores are best characterized by a multivariate modeling framework that jointly captures delayed vegetation–climate feedbacks and contemporaneously correlated environmental disturbances. Through a combination of Monte Carlo simulation and empirical VAR analysis, the results show that while correlated shocks are empirically present, efficiency gains from GLS estimation are conditional on covariance estimation quality and model structure rather than automatic. The empirical evidence supports a stable VAR(5) specification with a non-diagonal disturbance covariance matrix as an appropriate modeling representation for NDVI, precipitation, and temperature interactions in disturbance-prone regions. More broadly, the findings emphasize that careful attention to error structures and finite-sample properties is essential when modeling agro-environmental systems, offering practical guidance for applied researchers working with complex, climate-sensitive time-series data.

Recommendations

Based on the findings of this study, several methodological and practical recommendations can be proposed for agro-environmental time-series analysis. First, researchers applying VAR models to agro-environmental systems are encouraged to carefully assess the underlying disturbance structure prior to estimation. Diagnostic checks for contemporaneous correlation and heteroskedasticity should be treated as an integral step in multivariate modeling, particularly in climate-sensitive regions. Second, when evidence of correlated disturbances is present, researchers should consider GLS or feasible GLS as alternative estimation approaches, while recognizing that efficiency gains are conditional rather than automatic. As demonstrated by both the simulation scenarios and the empirical analysis, the performance of GLS depends critically on covariance estimation accuracy, sample size, and model structure, and should therefore be evaluated in conjunction with equation-by-equation OLS rather than assumed to dominate it. Third, simulation-based evaluation is recommended as a complementary tool in applied agro-environmental research. Simulation studies enable researchers to explore estimator behavior under controlled dependence structures and to better understand potential finite-sample distortions before applying multivariate models to real-world data. From a practical perspective, policymakers and practitioners using agro-environmental indicators such as NDVI should be aware that environmental disturbances, including climatic extremes and volcanic activity, may introduce additional variability and dependence in observed data. Explicitly accounting for these features in statistical modeling can improve the robustness and interpretability of analyses used to support agricultural planning and environmental monitoring. Future research may extend the current framework by incorporating additional agro-environmental variables, alternative dependence structures, or spatially disaggregated data to further enhance understanding of vegetation–climate dynamics under environmental variability.

Scientific Ethics Declaration

* This article was presented as an oral presentation at the International Conference on Veterinary, Agriculture and Life Sciences (www.icvals.net) held in Konya/Türkiye on April 02-05, 2026.

* The authors declare that this study does not involve human participants or animal subjects, and therefore ethical approval was not required. The authors take full scientific and legal responsibility for the content of this article.

Conflict of Interest

* The authors declare that they have no conflicts of interest.

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Acknowledgements or Notes

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Impact of Natural Disaster and Essential Spawning Habitat (ESH) Characteristics on the Conservation of the Vulnerable Endemic Bilih Fish (*Mystacoleucus padangensis*) in Lake Singkarak, Indonesia

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Abstract: The Bilih fish (*Mystacoleucus padangensis*) is a high value endemic species critical to regional aquaculture biosystems. Currently listed as Vulnerable (VU) on the IUCN Red List, its wild stocks are facing an acute ecological and economic crisis, evidenced by a dramatic 99% collapse in catch volume (from 1 ton/day in 2002 to only 10 kg/day in 2025). This study aimed to characterize the Essential Spawning Habitat (ESH) and governing reproductive strategies, and to develop conservation management principles. Environmental metrics and habitat characteristics (substrate, depth) were measured. Results confirmed that the ESH functions as a critical biosystem component, defined by extremely shallow waters (4–16 cm) and sandy and rocky substrates. Precise environmental cues, specifically consistently low nocturnal temperatures (22–24°C), act as triggering inputs for migration and spawning. This existing stock crisis was severely exacerbated by a catastrophic flood and landslide event that occurred on November 27, 2025, in the spawning locality of West Sumatra. The disaster resulted in the critical degradation of the ESH, primarily through infilling the original substrate with unsuitable mud and fine sand. This damage effectively eliminated the essential rocky spawning media required for behavioral triggering, consequently disrupting the remaining reproductive cycle and accelerating the failure of current resource management efforts. Given the evident high sensitivity of the reproductive process to specific biosystem inputs and the physical destruction of the ESH by the natural disaster, this study urgently recommends strict conservation zoning and ecology based management. Crucially, these must be immediately followed by habitat restoration efforts to reinstate the necessary rocky substrate. Such measures are vital inputs for the regional biosystem, not only to prevent the immediate loss of this Vulnerable endemic germplasm but also to secure the biological foundations required for future resource recovery and sustainable ex-situ aquaculture development.

Keywords: Bilih fish (*Mystacoleucus padangensis*), Essential spawning habitat, Stock collapse, Flood and landslide, Habitat restoration

Introduction

Southeast Asian river systems are increasingly subject to intense natural disturbances, notably flash floods and landslides. These events are driven by monsoonal rainfall, steep topography, and, increasingly, anthropogenic land-use changes. Flash floods rapidly mobilize large volumes of sediment and debris, while landslides contribute pulses of fine and coarse material to river channels. Both processes can dramatically alter river morphology, substrate composition, and habitat complexity, with cascading effects on aquatic communities and spawning habitat quality (Kuksina et al., 2020; Zhang et al., 2025). Flood events are a primary factor regulating aquatic ecosystem dynamics and influencing fish community structure and essential habitat functions (Taylor et al., 2017). Indonesia is a designated megabiodiversity nation, geographically divided into the global biodiversity hotspots of Wallacea and Sundaland, both exhibiting remarkably high levels of endemism (*Conservation*

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International, 2024). The phenomenon observed in Lake Singkarak can be interpreted through the concept of hydrological disturbance affecting spawning habitats, similar to processes described in studies of the Kariega Estuary, although the ecological outcomes differ. While flood events in the Kariega Estuary have been reported to improve ecosystem conditions and restore habitat functionality (Nodo et al., 2018), the catastrophic flood and landslide event that occurred on November 27, 2025, in Lake Singkarak led to the critical degradation of the ESH. Among the crucial endemic species is the Bilih fish *Mystacoleucus padangensis*, found exclusively in Lake Singkarak, West Sumatra. The Bilih fish population has recently experienced a drastic decline, resulting in its conservation status being officially listed as "Vulnerable" by the IUCN since 2020 (IUCN, 2020). Furthermore, analyses such as the Productivity and Susceptibility Analysis (PSA) have assigned the species a high vulnerability index to exploitation (Syandri et al., 2023; Triharyuni et al., 2022). *Mystacoleucus padangensis*, an endemic riverine cyprinid, plays a pivotal role in Southeast Asian freshwater ecosystems. Its essential spawning habitats (ESH) are characterized by clean, well-oxygenated, heterogeneous gravel beds that support successful egg incubation and larval emergence. These habitats underpin not only the persistence of *M. padangensis* populations but also broader riverine biodiversity and ecosystem function. The species' reproductive success is tightly linked to the physical and chemical integrity of these gravel substrates, making them highly sensitive to environmental disturbances, particularly sedimentation events that alter substrate permeability and oxygen dynamics (Kawanishi et al., 2010; Kumar et al., 2025)

The Bilih fish plays a critical ecological, social, and economic role for the surrounding communities. Economically, the species is the primary source of livelihood for a majority of local fishermen. Nutritionally, its flesh is highly valued, containing significantly higher levels of protein, calcium, iron, Vitamin A, Vitamin E, and zinc compared to many other animal and plant-based food sources (Putri & Syamsir, 2020; Yuniritha et al., 2015). This exceptional nutritional value and high market demand have driven the commercial price of Bilih fish to surge dramatically (Ikhsan & Fitriasia, 2024). However, this escalating demand has resulted in severe overexploitation, primarily targeting the species in river estuaries which serve as their spawning habitats, consequently leading to a decline in wild stock (Triharyuni et al., 2022). This situation not only threatens the species long term survival but also creates economic instability for the fishing communities reliant on Lake Singkarak (Ikhsan & Fitriasia, 2024). Fish require not only suitable spawning habitats but also effective connectivity to larval and nursery habitats. When larvae are unable to reach appropriate rearing environments due to hydrodynamic barriers or excessive swimming energy costs, larval survival rates decline, juvenile recruitment decreases, and long-term population size may be negatively affected. Therefore, reproductive success is determined not only by the quality of spawning sites but also by the ability of larvae to disperse toward habitats that support early growth and development (Holzapfel et al., 2026).

Method

Study Area

This study was conducted at the natural spawning grounds of the Bilih fish (*Mystacoleucus padangensis*) located in the inflow areas of Lake Singkarak, West Sumatra, Indonesia. The study area represents a historically recognized reproductive habitat for this endemic cyprinid species. Field observations were carried out in the spawning locality both prior to and following a major flood and landslide event that occurred on 27 November 2025, which substantially altered the physical structure of the habitat.

Research Design

This research employed a descriptive–analytical field study design, focusing on in situ observation of environmental conditions and habitat characteristics associated with spawning activity. The study did not involve experimental manipulation, capture, or invasive handling of animals. All observations were limited to habitat features and abiotic parameters relevant to reproductive ecology. It is important to note that these observations were conducted before disaster and one week post disaster under extreme environmental conditions. The study area was designated as a "Red Zone" due to ongoing heavy rainfall and the high risk of secondary landslides. Consequently, for safety reasons and following local evacuation protocols, visual monitoring was limited to the early evening and could not be extended into the night. However, the absence of fish during the initial migratory window (up to 18:00 WIB) in the impacted zone contrasted with their presence in healthy zones provides a robust indicator of habitat rejection. These logistical constraints underscore the severity of the "Natural Disaster" mentioned in the title, highlighting that the threat to this vulnerable endemic species is not only the initial event but the ongoing unstable conditions of the post disaster environment.

Environmental and Habitat Data Collection

Environmental Parameters

Water temperature (°C) was measured using portable water quality meters. Measurements were conducted during both daytime and nocturnal periods to capture environmental fluctuations potentially acting as spawning cues. Special attention was given to nocturnal temperature patterns, which are known to influence migratory and reproductive behavior in rheophilic cyprinid fishes.

Habitat Characteristics

Habitat characteristics were assessed through direct field observation and manual measurement. Water depth (cm) was measured using a calibrated ruler along multiple transects across the spawning area. Substrate composition was visually classified into sand, gravel, rock, and fine sediment (mud), with emphasis on identifying the presence or absence of rocky substrates functioning as spawning media.

Data Analysis

Collected environmental and habitat data were analyzed descriptively to determine the defining characteristics of the Essential Spawning Habitat (ESH). Observed parameter ranges were interpreted in relation to established ecological concepts of habitat suitability for lithophilic and rheophilic spawners. Habitat degradation following the flood event was assessed qualitatively by comparing pre and post disturbance substrate conditions, particularly focusing on sediment infilling and loss of rocky spawning substrates.

Results and Discussion



Figure 1. Substrate conditions in the spawning habitat of the endemic Bilih fish (*Mystacoleucus padangensis*) in Lake Singkarak. **Figure A and B,** Natural environmental conditions of the spawning grounds (breeding sites) of prior to the disaster, characterized by high water transparency and a stable shoreline morphology. **Figure C,** Characteristics of the pristine spawning substrate consisting of a heterogeneous mixture of gravel and coarse sand, providing essential interstitial spaces for egg attachment and aeration. **Figure D,** Immediate post-disaster impact of the flood and landslide on the spawning site. **Figure E,** Post-disaster recovery phase showing improved water clarity, yet revealing a significant accumulation of fine-grained alluvial deposits (silt and clay) smothering the benthic layer. **Figure F,** Close-up detail of the altered substrate texture.

Mystacoleucus padangensis is an endemic species of Lake Singkarak characterized by highly specific spawning behavior, involving migration to river estuaries or lacustrine shorelines with distinct water currents and rocky substrates. However, these vital breeding sites underwent severe physical disruption following the catastrophic flood and landslide event on November 27, 2025, which transported massive volumes of terrestrial soil and mud from the upstream catchments to the lake's littoral zones. Floods are among the most significant natural disturbances shaping the physical and ecological characteristics of lake and river ecosystems. Their impacts on

spawning substrate and essential spawning habitat (ESH) are profound, influencing the reproductive success and long-term viability of endemic freshwater fish species.

The substrate conditions within the Essential Spawning Habitat (ESH) of the endemic *Bilih fish (Mystacoleucus padangensis)* in Lake Singkarak exhibited a drastic transformation following the flood and landslide event on November 27, 2025. Visual observations of the natural environmental conditions prior to the disaster characterized the spawning grounds by high water transparency and stable shoreline morphology (Figures A and B). The pristine spawning substrate consisted of a heterogeneous mixture of gravel and coarse sand (Figure C), which provided essential interstitial spaces for egg attachment and critical aeration during the incubation period.

In the immediate aftermath of the disaster, the flood and landslide exerted a catastrophic impact on the spawning site (Figure D). Although the post-disaster recovery phase showed signs of improved water clarity, it revealed a significant accumulation of fine-grained alluvial deposits (silt and clay) smothering the entire benthic layer (Figure E). Close-up details of the altered substrate texture (Figure F) confirm the loss of essential voids due to sediment infilling. The comparative environmental parameters before and after the disaster are summarized in the following table:

Table 1. Comparative before and after disaster

No	Parameter	Pre Disaster	Post Disaster	Significance
1.	Significance Nocturnal Temperature	22–24°C	23–24°C (No significant difference)	Thermal trigger was present but ineffective due to substrate failure
2.	Water Depth	4–16 cm	4-9 cm	Habitat availability in terms of volume was maintained
3.	Substrate Texture	Heterogeneous Gravel/Sand	Dense Yellowish Silt	The primary driver of reproductive failure (Clogging).
4.	Particle Size (D50)	0.5 cm – 3.5 cm	0.21 mm (fine silt)	Mechanical "cementing" of voids
5.	Intersitial space	High (for aeration and ptotection)	Zero (completely infilled)	Embryonic asphyxiation
6.	Permeability	Permeability High (Oxygen diffusion)	Impermeable	Embryo mortality
7.	Visual Status	High transparency (Fig. A)	Ochre-colored turbidity (Fig. D)	Ethological barrier for spawners

The documented substrate transformation reveals a catastrophic failure of the Essential Spawning Habitat (ESH) driven by complex sedimentation mechanisms. Prior to the disaster, the spawning site maintained a heterogeneous matrix of pebbles ranging from 3 cm to 0.5 cm. According to (Franssen et al., 2012) the presence of coarse sand and fine gravel, with a coarse material proportion exceeding 18%, should theoretically function as a natural filter to mitigate the infiltration of fine sediments into deeper layers. However, the flash flood triggered a surge in fine-to-coarse particle ratios that bypassed this natural filtration capacity. The dominance of sediments with a median diameter D50 of approximately 0.21 mm, which (Hsu et al., 2018) identify as highly effective in forming impermeable layers, initiated a massive clogging process. This was subsequently exacerbated by the formation of cohesive clay deposits on the surface layer (as seen in Figure F). This clay layer effectively "cemented" the gravel matrix, creating an absolute impermeable seal that severed dissolved oxygen diffusion into the substrate voids. Consequently, despite stable nocturnal temperatures of 23–24°C, the physical degradation of the ESH led to total embryonic mortality via asphyxiation and established a significant ethological barrier for adult spawners.

Southeast Asian river systems are increasingly subject to intense natural disturbances, notably flash floods and landslides. These events are driven by monsoonal rainfall, steep topography, and, increasingly, anthropogenic land-use changes. Flash floods rapidly mobilize large volumes of sediment and debris, while landslides contribute pulses of fine and coarse material to river channels. Both processes can dramatically alter river morphology, substrate composition, and habitat complexity, with cascading effects on aquatic communities and spawning habitat quality (Kuksina et al., 2020; Zhang et al., 2025) As an endemic riverine cyprinid, *Mystacoleucus padangensis* plays a pivotal role in these freshwater ecosystems. Its ESH is characterized by clean, well-oxygenated, heterogeneous gravel beds that support successful egg incubation and larval emergence. The reproductive success of this species is tightly linked to the physical and chemical integrity of these gravel substrates, making them highly sensitive to environmental disturbances particularly sedimentation events that alter substrate permeability and oxygen dynamics (Kawanishi et al., 2010; Kumar et al., 2025).

The phenomenon observed in Lake Singkarak can be interpreted through the concept of hydrological disturbance affecting spawning habitats, similar to processes described in the Kariega Estuary, although the ecological outcomes differ. While flood events in the Kariega Estuary have been reported to improve ecosystem conditions and restore habitat functionality (Nodo et al., 2018) floods in this context acted as a primary destructive agent. While flooding is a major factor regulating aquatic ecosystem dynamics and influencing fish community structures (Taylor et al., 2017), landslides often deposit fine sediments that severely degrade spawning habitat suitability. For instance, *Schizothorax* species impacted by the 2018 Baige Landslide Dam showed a decreased ability to survive due to changes in riverbed substrates and flow conditions (Xie et al., 2024). Furthermore, increased fine sediment due to climate change and land use changes can clog gravel beds, impeding spawning and reducing recruitment rates for species such as brown trout (Scheurer et al., 2009). This is supported by the fact that substrate particle size is a strong predictor of "survival to emergence," with lithophilic cyprinids exhibiting sensitivities similar to salmonids (Young et al., 1990).

Anthropogenic factors, such as water level fluctuations from hydropower operations, can further compress viable spawning habitats into smaller areas, particularly affecting species with specialized spawning strategies (Wang et al., 2026; Xiang et al., 2025). Low-flow periods exacerbated by over-abstraction can also reduce habitat availability (Garbe et al., 2016). Ultimately, behavioral adaptations, such as retreat movements or diel activity shifts, highlight the critical importance of habitat quality in facilitating successful spawning migration (Bentley et al., 2014; Jubb et al., 2023). Without a functional substrate, the entire reproductive cycle of the Bilih fish is effectively terminated.

Ultimately, this sequence of ecological degradation poses a profound threat to population sustainability, given that the recruitment of *Mystacoleucus padangensis* in Lake Singkarak is heavily dependent on the success of these specific spawning grounds. If the breeding sites remain smothered by fine sediments over an extended period, adult fish may lose the environmental triggers required for spawning or be forced to spawn in suboptimal locations, resulting in drastically reduced survival rates. This is further emphasized by Figure E, which reveals that despite the gradual recovery of water clarity, the "ecological scar" on the lake bed persists as a thick mud layer. The natural restoration of these sites will require an extensive period or high-velocity discharge events to flush the fine sediments and re-expose the original gravel and coarse sand matrix. Understanding these processes is critical for effective conservation and management, especially in the context of increasing anthropogenic pressures and climate variability. This report synthesizes current knowledge on how floods alter spawning substrates and ESH, the consequences for fish reproduction, and the implications for conservation strategies in both regulated and unregulated systems.

Conclusion

The catastrophic flood and landslide event of November 27, 2025, caused severe physical degradation of the spawning habitat by infilling the original rocky substrate with fine sediment and mud. This alteration effectively eliminated the essential spawning media required for reproductive triggering, leading to further disruption of the already declining reproductive cycle. The destruction of the Essential Spawning Habitat has accelerated the failure of existing resource management efforts and poses an immediate threat to the persistence of remaining wild populations.

Overall, the findings confirm that habitat degradation, rather than biological limitation alone, represents the primary driver of the current stock collapse of this Vulnerable endemic species. This study demonstrates that the reproductive success of *Mystacoleucus padangensis* is highly dependent on a narrowly defined Essential Spawning Habitat characterized by extremely shallow water depths (4–16 cm) and sandy–rocky substrates. Consistently low nocturnal water temperatures (22–24°C) function as critical environmental triggers for upstream migration and spawning behavior, indicating a high sensitivity of the species reproductive process to specific abiotic cues.

Recommendations

Based on the high sensitivity of the bilih fish reproductive process to specific habitat and environmental inputs, this study recommends the immediate implementation of strict conservation zoning in all identified spawning areas. Human activities that alter substrate composition, hydrology, or sediment load should be strictly regulated within these zones.

Ecology-based management strategies must be prioritized, emphasizing habitat restoration as a core conservation action. Restoration efforts should focus on reinstating the original rocky substrate composition necessary for spawning behavior, particularly in areas impacted by sediment infilling following natural disasters.

In addition, long-term monitoring of environmental parameters and habitat integrity is strongly recommended to ensure the continued functionality of Essential Spawning Habitats. These measures are critical not only to prevent the imminent loss of this Vulnerable endemic germplasm but also to establish the ecological foundations required for future stock recovery and the development of sustainable ex situ aquaculture programs.

Scientific Ethics Declaration

* The authors declare that the scientific, ethical, and legal responsibility of this article published in the EPHELS Journal belongs to the authors.

* This study did not require ethics committee approval as it involved no experimental manipulation or invasive procedures on animals

Conflict of Interest

* The authors declare that they have no conflicts of interest.

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In Vivo Developmental Toxicity and Teratogenic Evaluation of *Cucumis melo* L. var. Gama Melon Parfum Extract on Wader Pari Fish (*Rasbora lateristriata*) Embryos

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Abstract: *Cucumis melo* L. var. Gama Melon Parfum (GMP) is a superior hybrid cultivar developed by the Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, characterized by its distinctive sweet aroma and bitter taste. Previous in vitro studies have demonstrated the anticancer potential of GMP extract through the inhibition of cell proliferation and the induction of apoptosis; however, its in vivo toxicity and developmental safety profile remain largely unexplored. This study aimed to evaluate the developmental toxicity and teratogenic potential of GMP extract using *Rasbora lateristriata* embryos as a biological model. This Indonesian native species offers several advantages, including embryo transparency, high environmental sensitivity, and rapid development, with hatching occurring within 24 hours. The study was conducted in accordance with the Fish Embryo Toxicity (FET) guidelines (OECD Test No. 236, 2025). A preliminary range-finding test indicated 100% mortality at 1000 ppm, followed by a definitive test using concentrations of 50, 100, 250, and 500 ppm in 0.1% DMSO. Early-stage embryos (2–4 hpf) were exposed up to 96 hpf, and endpoints including hatching rate, survival rate, heart rate, and morphological abnormalities were assessed. The results revealed a concentration-dependent response. At 50–250 ppm, no significant effects were observed on survival, hatching, or heart rate; however, notable teratogenic effects were identified, including spinal deformities, ocular abnormalities (microphthalmia and anophthalmia), hemorrhage, and impaired swim bladder development. In contrast, exposure to 500 ppm resulted in acute lethal toxicity, as evidenced by complete mortality, absence of hatching, and loss of cardiac activity. In conclusion, GMP extract exhibits developmental toxicity characterized by a transition from sublethal (teratogenic) to lethal effects with increasing concentration. These findings underscore the importance of dose optimization and comprehensive in vivo evaluation in the development of GMP extract as a potential biomedical agent.

Keywords: *Cucumis melo* var. GMP, *Rasbora lateristriata*, Embryogenesis, Teratogenicity, Morphological malformations.

Introduction

The global rise in non-communicable chronic diseases, including diabetes mellitus, oxidative stress-related disorders, and cancer, has driven intensive exploration of plant-derived bioactive compounds as complementary and preventive therapeutic alternatives (Acimovic et al., 2026). Several anticancer agents currently used in clinical practice are derived from plants or represent synthetic derivatives of plant secondary metabolites, such as paclitaxel, vincristine, vinblastine, and camptothecin (Vieira et al., 2026). Although natural compounds are often

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assumed to possess lower toxicity profiles than synthetic counterparts, this assumption is not consistently supported by scientific evidence. Toxicological studies have demonstrated that the LD₅₀ values of natural bioactive compounds vary substantially depending on their chemical structure, test organisms, and routes of exposure (Hasan et al., 2025). Therefore, both natural and synthetic compounds require comprehensive toxicological evaluation, including reproductive and developmental toxicity assessments, prior to being considered safe for use (Vilas-Boas et al., 2021).

Embryogenesis represents a highly vulnerable stage of development to xenobiotic exposure. During this phase, rapid cellular proliferation increases susceptibility to disruptions in cell growth and the regulation of intracellular signaling pathways. Natural anticancer compounds generally exert their effects through inhibition of cell cycle progression and induction of apoptosis in target cells (Kang et al., 2025). However, these mechanisms may also produce unintended effects on normal developmental processes. Interference with cellular proliferation during embryogenesis can disrupt molecular signaling pathways that regulate body pattern formation, ultimately leading to developmental abnormalities (Lee et al., 2024). This highlights a biological paradox in which cytotoxic activity that is beneficial in the context of cancer therapy may be detrimental during embryonic development.

One promising source of bioactive compounds is *Cucumis melo* L. var. Gama Melon Parfum (GMP), a cultivar developed through hybridization between the female parent ♀NO3 and the male parent ♂MR5 by the Laboratory of Genetics and Plant Breeding (Maryanto et al., 2015). GMP extract has been reported to exhibit cytotoxic activity against MCF-7 and T47D breast cancer cell lines in vitro (Salamah & Widiyanto, 2022; Salamah et al., 2025). As a member of the Cucurbitaceae family, GMP contains cucurbitacins, a group of triterpenoid compounds responsible for its characteristic bitterness and widely recognized for their anticancer properties. The degree of fruit bitterness is positively correlated with cucurbitacin content (Hua et al., 2019; Fan et al., 2024). Interestingly, despite its strong sweet fragrance resembling perfume, GMP exhibits a distinctly bitter taste, suggesting a relatively high cucurbitacin content compared to other melon varieties. This unique characteristic positions GMP as a potential source of bioactive compounds with potent cytotoxic activity.

Despite its reported in vitro cytotoxic activity, the developmental safety profile of GMP extract has not yet been systematically evaluated. Considering the mechanisms of action of cucurbitacins—including modulation of the cell cycle, induction of apoptosis, and regulation of proliferation-related signaling pathways (Kim et al., 2020; Alafnan et al., 2022)—as well as the chemical complexity of crude extracts, the assessment of developmental toxicity is critical to identify potential teratogenic or embryotoxic effects. The absence of such data represents a significant knowledge gap that must be addressed to ensure the safe application of GMP in biomedical contexts.

Evaluation of teratogenic and embryotoxic potential requires an in vivo model that is highly sensitive to xenobiotic exposure during early developmental stages. Fish embryos have been widely utilized in developmental toxicity studies due to their rapid development, external fertilization, and optical transparency, which enable real-time observation of morphological changes and continuous monitoring (Nawaji et al., 2024). Among freshwater fish species, *Rasbora lateristriata*, an Indonesian native species belonging to the Cyprinidae family—closely related to *Danio rerio*—has emerged as a promising model (Kusuma et al., 2016; Tan & Armbruster, 2018). Compared to commonly used models such as zebrafish and medaka, *R. lateristriata* exhibits a faster embryonic development rate, with hatching occurring within approximately 24 hours, whereas zebrafish and medaka require around 48 hours post-fertilization (hpf) and up to 9 days, respectively (Retnoaji et al., 2023; Lailiati et al., 2022). These characteristics make *R. lateristriata* an efficient and relevant model for assessing toxic effects during early development.

Based on this background, the present study aims to evaluate the developmental toxicity and teratogenic potential of Gama Melon Parfum extract in vivo using *Rasbora lateristriata* embryos. The assessment includes analysis of hatching rate, survival rate, heart rate and morphological abnormalities. The findings of this study are expected to provide fundamental insights into the developmental safety profile of GMP extract and support its potential application in biomedical research.

Method

Experimental Design

This study employed an experimental design consisting of six treatment groups: four groups exposed to Gama Melon Parfum (GMP) extract at concentrations of 50, 100, 250, and 500 ppm, one negative control group (egg water medium), and one solvent control group (0.1% DMSO). Fish embryos were selected at a uniform

developmental stage, specifically the pre-gastrulation stage (2–4 hours post-fertilization; hpf), and were randomly distributed into each treatment group. Each group consisted of 20 embryos, resulting in a total of 120 embryos used in this study. Exposure to the extract was conducted for 96 hours under a static system with renewal of the test medium every 24 hours. Each embryo was maintained individually in separate wells of a 24-well plate to ensure independence of observations. Observations were performed every 24 hours to assess developmental parameters, including hatching rate, heart rate, and survival rate. Morphological abnormalities were evaluated at the end of the exposure period (96 hpf).

Materials

The materials used in this study included ethanolic extract of Gama Melon Parfum fruit, reverse osmosis (RO) water, salt stock solution, and 0.1% DMSO. The egg water medium for embryo maintenance was prepared by mixing 1 L of RO water with 1.5 mL of 3.5% (b/v) salt solution and two drops of 300 ppm methylene blue. The equipment used included 24-well plates, beakers, measuring cylinders, Erlenmeyer flasks, glass bottles, aquaria (30 × 20 × 15 cm³), and an Olympus CX43 microscope.

Preparation of GMP Extract

Fresh Gama Melon Parfum (GMP) fruits were cleaned, sliced into thin sections, and oven-dried at 45–50°C. The dried material (simplicia) was air-dried and ground into a fine powder. Extraction was performed using the maceration method with 70% ethanol for 48 hours at a sample-to-solvent ratio of 1:10. The extract was filtered, and the solvent was evaporated to obtain a concentrated crude extract. The crude extract was subsequently re-dissolved in 0.1% DMSO to obtain working concentrations of 50, 100, 250, and 500 ppm.

Fish Embryo Preparation

Broodstock fish were selected based on reproductive readiness, which was assessed by gentle abdominal massage. Spawning pairs were arranged in a ratio of two males to one female. Spawning was conducted in the afternoon in aquaria containing ijuk (palm fiber) as a substrate to simulate natural conditions (Boyhaki, 2016). Egg collection was performed the following morning. Fertilized eggs were collected, cleaned, and screened to ensure uniform developmental stages (pre-gastrulation stage, 2–4 hpf). Each treatment group consisted of 20 embryos, resulting in a total of 120 embryos. The embryos were maintained in egg water prior to exposure to GMP extract.

Fish Embryo Acute Toxicity Test

Embryo exposure was conducted using a 24-well plate following the OECD Test Guideline No. 236 (2025). At 2–4 hpf, embryos were rinsed and examined under a stereo microscope (Olympus), and only fertilized embryos were selected for further experimentation. A total of 20 embryos were allocated to each treatment group. The treatments included GMP extract at concentrations of 50, 100, 250, and 500 ppm, a negative control (egg water), and a solvent control (0.1% DMSO). Each well contained 2 mL of the respective test solution. Observations and media renewal were performed every 24 hours for 96 hours (4 days). The evaluated parameters included hatching rate, heart rate, and survival rate. Daily developmental progress was documented to assess potential teratogenic effects on larval morphology.

Developmental Endpoints

Embryo mortality and developmental abnormalities were documented using an Olympus CX43 microscope at 3.2× magnification with a light intensity setting of 6. The observed parameters included hatching rate (24–48 hpf), survival rate (24–120 hpf), heart rate (measured daily), and morphological abnormalities (96 hpf). Observed abnormalities included spinal deformities, eye abnormalities (microphthalmia and anophthalmia), hemorrhage, and swim bladder defects. Edema was recorded descriptively due to its low incidence and was not included in quantitative analysis. Normal morphological development was assessed based on established developmental criteria (Retnoaji et al., 2023).

Heart Rate Assessment

Hatching rate was calculated as the percentage of embryos that hatched within the first 24 hours relative to the total number of embryos. Embryos that hatched after 24 hpf were categorized as delayed hatching. Embryo mortality was also recorded, with dead embryos identified by coagulation of fertilized eggs (Raharjeng & Retnoaji, 2021).

$$\text{Heart Rate (\%)} = \frac{\text{Number of hatched embryos}}{\text{Total number of embryos}} \times 100$$

Heart Rate Measurement

Larvae were placed in a Petri dish in a lateral position and observed under a stereo microscope (Olympus). Heart rate was measured by counting the number of heart beats per minute for each larva. Measurements were performed daily for four consecutive days.

Survival Rate Assessment

Survival rate was calculated as the percentage of surviving embryos relative to the initial number of embryos over a 4-day observation period. This parameter reflects the proportion of embryos that remained viable following exposure (Huyen et al., 2024; Zhu et al., 2021).

$$\text{Survival Rate (\%)} = \frac{\text{Number of surviving embryos}}{\text{Initial number of embryos}} \times 100$$

Larval Developmental Assessment

Larval development was assessed both quantitatively and qualitatively to evaluate the effects of exposure on morphology. The incidence of morphological abnormalities was calculated using the following formula:

$$\text{Malformation Incidence (\%)} = \frac{\text{Number of abnormal larvae}}{\text{Initial number of larvae}} \times 100$$

Morphological abnormalities were classified based on predefined criteria and compared across treatment groups.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics version 25. Normality testing indicated that the data were not normally distributed; therefore, non-parametric statistical methods were applied. Differences among treatment groups for hatching rate and survival rate were analyzed using the Kruskal–Wallis test, followed by the Mann–Whitney U test for post hoc comparisons when significant differences were detected. Data were presented as median values with interquartile range (IQR). Morphological abnormalities were analyzed descriptively based on incidence percentages in each treatment group.

Results and Discussion

Developmental Toxicity

Effect of GMP Extract on Embryonic Development in R. lateristriata

The evaluation of the effects of Gama Melon Parfum extract on the embryonic development of *R. lateristriata* was conducted by assessing several parameters, including hatching rate, hatching delay, survival rate, and embryonic heart rate across different treatment concentrations. The results of the observations on hatching rate and hatching delay are presented in Table 1.

Table 1. Hatching rate and delayed hatching of *R. lateristriata* embryos following exposure to gama melon parfum extract

Group	Hatching (%)	Delay (%)
Normal Control	80 (75-82.5)	00 (00-15)
Negatif Control	70 (67.5-85)	22 (11.11-25)
50 ppm	85 (67.5-90)	5 (2.78-25)
100 ppm	80 (70-87.5)	0 (00-18.42)
250 ppm	85 (70-85)	10 (5.26-27.76)
500 ppm	00 (00-15)	00 (00-00)

*Differences among groups were analyzed using the Kruskal–Wallis test. Data are presented as median (interquartile range, IQR).

Based on the obtained results, the hatching rate of embryos following exposure to the extract ranged from 0% to 85%. Exposure at concentrations of 50 ppm and 250 ppm resulted in relatively high hatching rates (85%), while the 100 ppm concentration showed a value comparable to the normal control (80%). In contrast, no hatching was observed at the highest concentration (500 ppm). This finding indicates that high concentration (500 ppm) exerts a toxic effect, as evidenced by the high level of embryonic mortality in this treatment group. Meanwhile, the percentage of hatching delay varied among treatment groups. The highest delay was observed in the negative control (22%), followed by the 250 ppm concentration (10%). The use of 0.1% DMSO as a solvent is suspected to contribute to the occurrence of delayed hatching, particularly at 24 hours post-fertilization, resulting in some embryos hatching at 48 hpf. At 100 ppm, embryos tended not to exhibit hatching delay, whereas at 500 ppm, embryos died before showing any signs of delayed hatching.

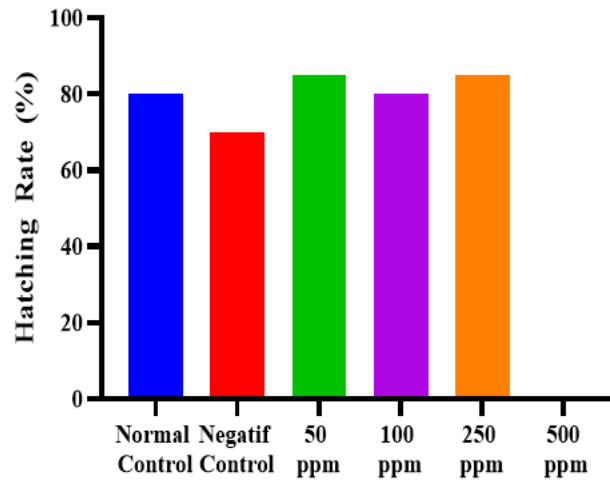


Figure 1. Hatching rate (%) graphic of *R. lateristriata* embryos following exposure to gama melon parfum extract

Based on Figure 1, the embryonic hatching rate was relatively high at low to moderate concentrations, whereas no hatching was observed at the highest concentration (500 ppm). Overall, the graph illustrates that low to intermediate concentrations did not exert toxic effects on embryonic hatching, while high concentration clearly impaired embryonic development. Consistent with these findings, the survival rate of embryos exhibited a similar pattern, in which increasing extract concentration was associated with decreased embryonic viability. The results of the survival rate observations are presented in Table 2.

Table 2. Survival rate of *R. lateristriata* embryos following exposure to gama melon parfum extract

Group	Survival Rate (%)			
	24 hpf	48 hpf	72 hpf	96 hpf
Normal Control	85 (82.5-92.5)	75 (70-87.5)	75 (70-87.5)	75 (70-85)
Negatif Control	95 (90-97.5)	85 (80-90)	80 (77.5-97.5)	75 (72.5-85)
50 ppm	95 (92.5-95)	90 (77.5-87.5)	85 (85-90)	85 (85-87.5)
100 ppm	95 (87.5-97.5)	95 (80-97.5)	95 (75-95)	90 (72.5-92.5)
250 ppm	95 (90-95)	95 (77.5-95)	90 (75-92.5)	85 (67.5-90)
500 ppm	0 (0-47.5)	0 (0-45)	0 (0-12.5)	0 0 (0-12.5)

* Differences among groups were analyzed using the Kruskal–Wallis test. Data are presented as median (interquartile range, IQR).

The survival rate in the normal control group remained relatively stable, ranging from 75–85% throughout the observation period, whereas the negative control exhibited a higher initial value (95% at 24 hpf) followed by a gradual decline to 75% at 96 hpf (Figure 2). Exposure to Gama Melon Parfum extract at low to moderate concentrations (50–250 ppm) resulted in consistently high survival rates (approximately 85–95%) with minimal fluctuations, indicating that these concentrations remain within the biological tolerance limits of the embryos. In contrast, at the highest concentration (500 ppm), the survival rate decreased drastically to 0% as early as 24 hpf and remained at this level until the end of the observation period, indicating acute lethal effects. The trend shown in Figure 2 confirms that increasing extract concentration is associated with a decline in survival rate, and the differences among treatment groups were statistically significant ($p < 0.05$).

The stability of survival rates at low to moderate concentrations suggests the presence of adaptive responses or detoxification mechanisms in embryos against the compounds present in the extract. Conversely, the high mortality observed at 500 ppm indicates that the accumulation of bioactive compounds exceeded the embryos' tolerance capacity, thereby disrupting embryogenesis, particularly during the early developmental stages that are highly sensitive to toxic exposure.

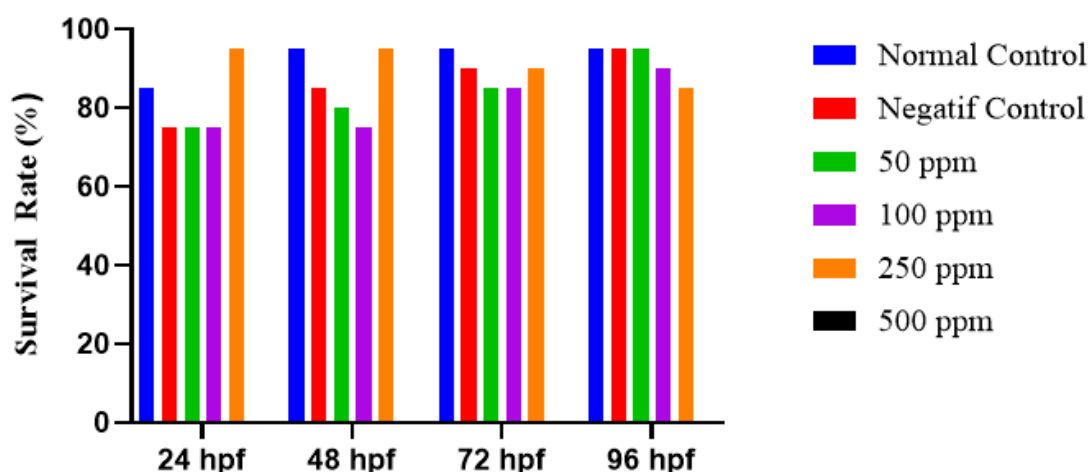


Figure 2. Survival rate (%) graphic of *R. lateristriata* embryos following exposure to gama melon parfum extract

Embryonic heart rate is an important sublethal parameter that has been widely used as an indicator of toxicity. The heart rate of *R. lateristriata* embryos following exposure to Gama Melon Parfum extract at various concentrations is presented in Table 3. In general, heart rate values in the normal control group remained relatively stable throughout the observation period, ranging from 186–198 bpm at 24–72 hpf and slightly decreasing to 192 bpm at 96 hpf.

Table 3. Heart rate of *R. lateristriata* embryos following exposure to gama melon parfum extract

Group	Heart Rate (%)			
	24 hpf	48 hpf	72 hpf	96 hpf
Normal Control	186 (180-195)	195 (183-204)	198 (180-204)	192 (180-204)
Negatif Control	162 (00-183)	186 (171-192)	186 (171-201)	186 (84-198)
50 ppm	183 (174-198)	201 (183-219)	198 (180-207)	192 (162-207)
100 ppm	189 (177-195)	204 (192-216)	207 (192-222)	210 (195-234)
250 ppm	186 (168-198)	192 (186-204)	192 (186-198)	204 (189-222)
500 ppm	186 (180-192)	186 (174-204)	00 (00-72)	00 (00-72)

* Differences among groups were analyzed using the Kruskal–Wallis test. Data are presented as median (interquartile range, IQR).

In the treatment groups, concentrations of 50–250 ppm exhibited heart rate values that generally remained within the normal range and were comparable to the control. At 50 ppm, the heart rate gradually increased from 183 bpm (24 hpf) to 192 bpm (96 hpf). A similar pattern was observed at 100 ppm and 250 ppm, where heart rate increased over the observation period, reaching peak values at 96 hpf of 210 bpm and 204 bpm, respectively. Exposure to Gama Melon Parfum extract at low to moderate concentrations (50–250 ppm) did not result in significant changes in embryonic heart rate compared to the control group. This indicates that within this concentration range, embryos were still able to maintain normal cardiac physiological function. In contrast, at the highest concentration (500

ppm), the absence of detectable heart rate during later developmental stages (72–96 hpf) indicates a lethal toxic effect. The loss of cardiac activity at this stage suggests a cessation of cardiovascular function, ultimately leading to embryonic death. This finding is consistent with other observed parameters, such as reduced hatching and survival rates at the same concentration, which collectively indicate a high level of toxicity. Cardiac dysfunction during early developmental stages can lead to arrhythmia and impaired blood circulation, which in turn may result in growth retardation due to insufficient nutrient supply (Shaikh et al., 2019).

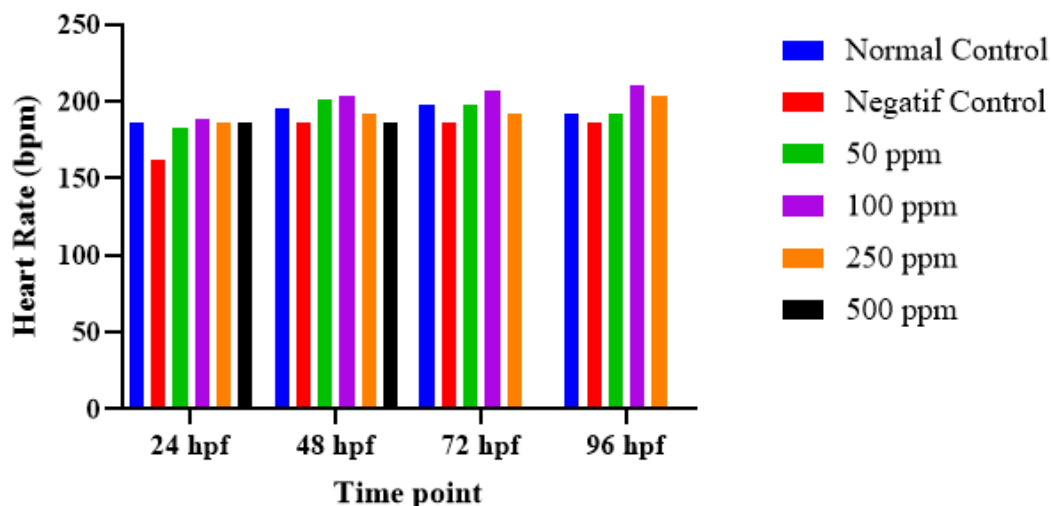


Figure 3. Heart rate graphic of *R. lateristriata* embryos following exposure to gama melon parfum extract

Teratogenic Evaluation

Morphological Abnormalities in *R. lateristriata* Larvae Following GMP Exposure

Based on the obtained results, exposure to Gama Melon Parfum (GMP) extract exhibited teratogenic effects on the development of *R. lateristriata* embryos, as indicated by the occurrence of various morphological abnormalities in several organs. The incidence of morphological abnormalities in *R. lateristriata* embryos at 96 hpf following exposure to GMP extract is presented in **Table 4**. In general, variations in both the types and percentages of abnormalities were observed across almost all treatment groups, with distinct patterns among different concentrations. The non-linear pattern of incidence in relation to concentration suggests that the embryonic response to exposure is complex and may be influenced by tolerance thresholds as well as the differential sensitivity of specific organs to the active compounds.

Table 4. Incidence of morphological abnormalities in *R. lateristriata* embryos at 96 hpf

Group	Malformation Incidence (%)			
	Spinal	Eye	Hemorrhage	Swim Bladder
Normal Control	4,26	0	0	8,51
Negatif Control	2,08	0	2,08	20,83
50 ppm	9,26	5,77	9,62	25,00
100 ppm	6,25	4,17	4,17	33,33
250 ppm	10,87	4,35	6,52	19,57
500 ppm	0,00	0,00	0,00	80,00

Spinal deformities were observed in all groups, with the highest incidence at 250 ppm (10.87%), followed by 50 ppm (9.26%) and 100 ppm (6.25%). In contrast, the normal and negative control groups exhibited lower incidences, at 4.26% and 2.08%, respectively. This impairment is likely associated with disruptions in somite development and osteogenesis during embryogenesis. Previous studies have reported that exposure to toxic compounds in zebrafish embryos can induce spinal deformities through mechanisms involving cellular stress and apoptosis, which interfere with tissue development (Li et al., 2022).

Eye abnormalities (Figure 6 EF) were detected only in the treatment groups, specifically at concentrations of 50 ppm (5.77%), 100 ppm (4.17%), and 250 ppm (4.35%), while no abnormalities were observed in the control groups. Although no major morphological abnormalities were generally observed, this study identified limited occurrences of microphthalmia (reduced eye size) and anophthalmia (absence of eye formation). The low

frequency of these abnormalities suggests that they do not represent a dominant response to the extract exposure, but rather sporadic effects under certain conditions.

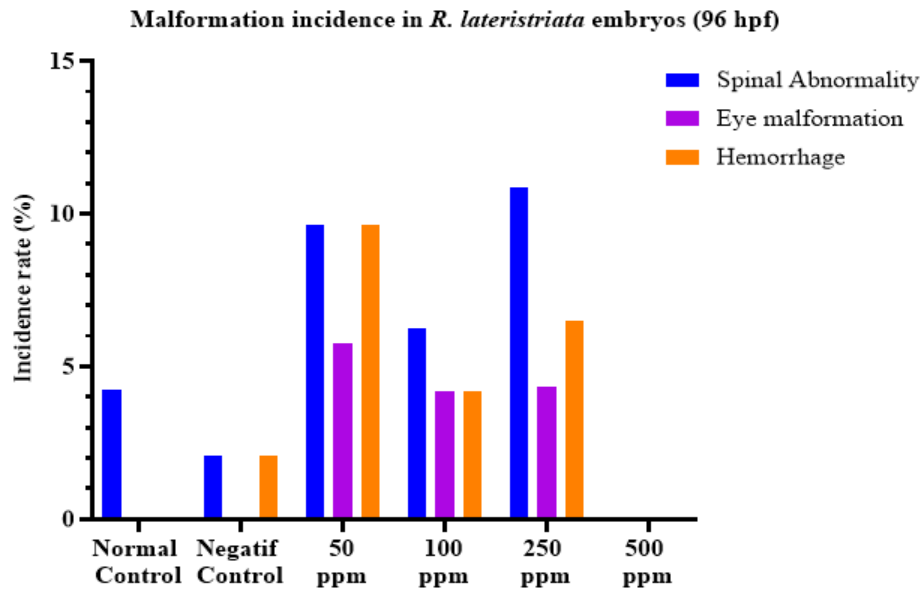


Figure 4. Incidence of spinal abnormalities, eye malformations, and hemorrhage in *R. lateristriata* larvae at 96 hpf

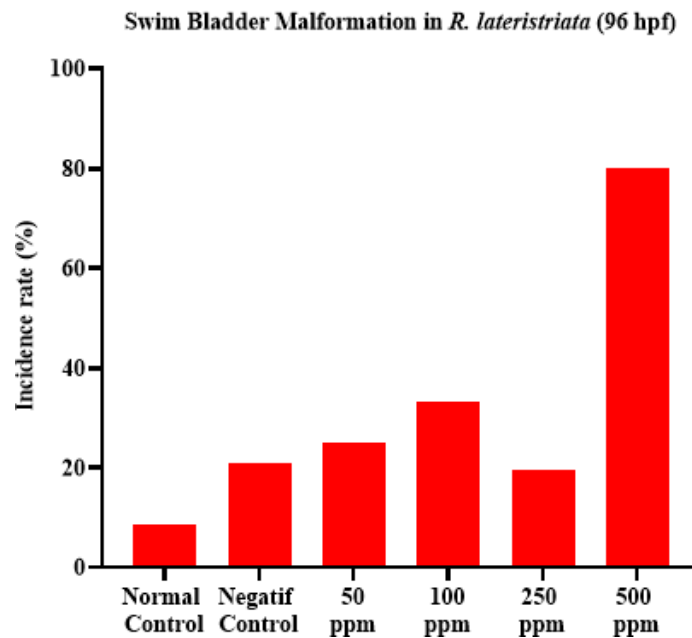


Figure 5. Incidence of swim bladder malformations in *R. lateristriata* larvae at 96 hpf following exposure to gamma melon parfum extract

The incidence of hemorrhage (Figure 6G) increased at 50 ppm (9.62%), decreased at 100 ppm (4.17%), and increased again at 250 ppm (6.52%) (Figure 4). No hemorrhage was observed in the normal control group, whereas the negative control exhibited a low incidence (2.08%). The occurrence of hemorrhage suggests potential disruption of the embryonic vascular system. This condition may be attributed to compromised vascular integrity or impaired cell adhesion, both of which are essential for maintaining tissue structure. Similar findings have been reported in studies involving exposure to other toxic compounds, which induce edema and hemorrhage through physiological disturbances and cellular apoptosis (Ge et al., 2023)

Swim bladder abnormalities (Figure 6 CD) exhibited a variable pattern (Figure 5), with the highest incidence observed at 100 ppm (33.33%), followed by 50 ppm (25.00%) and the negative control (20.83%). The lowest incidence was found in the normal control group (8.51%). These findings are supported by previous studies indicating that swim bladder development is highly dependent on a properly functioning circulatory system,

particularly cardiac function. Cardiac impairment may hinder swim bladder growth due to inadequate oxygen and nutrient distribution (Ge et al., 2023).

At the highest concentration (500 ppm), no abnormalities were observed in spinal, ocular, or hemorrhagic parameters. However, this was attributed to the high level of embryonic mortality at this concentration. All surviving larvae at 500 ppm exhibited swim bladder abnormalities, with an incidence of 80.00%. This finding indicates that at high concentrations, the toxic effect tends to be lethal, and even the surviving individuals experience developmental impairment. This phenomenon suggests a shift from sublethal (teratogenic) effects to lethal effects with increasing exposure concentration. Although no significant ocular morphological abnormalities were generally observed, this study identified limited occurrences of microphthalmia (reduced eye size) and anophthalmia (absence of eye formation) (Figure 6 EF). The low frequency of these abnormalities suggests that they do not represent a dominant response to extract exposure, but rather may occur as sporadic effects under certain conditions. These abnormalities are likely associated with disruptions in embryonic developmental processes, particularly during the stages of neural differentiation and tissue organization. This is consistent with previous reports indicating that exposure to certain compounds, such as cucurbitacin B, can affect nervous system development, including neuronal differentiation and axonal pathway formation, without causing widespread morphological defects in embryos (Huang, 2023). Disruption of axonal organization in the cranial region may consequently affect the development of sensory organs, including the eyes. Therefore, although the incidence of eye abnormalities in this study was relatively low, these findings still indicate a potential effect on neurodevelopmental processes and organogenesis, which under certain conditions may influence the formation of ocular structures.

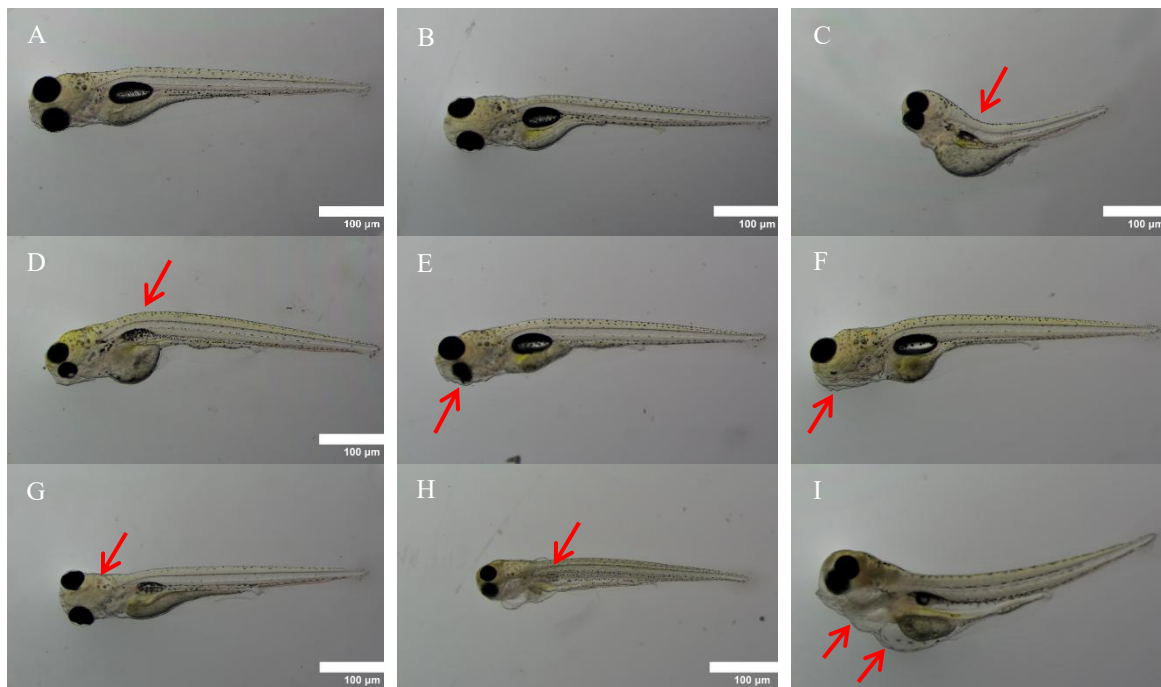


Figure 6. Representative of morphological abnormalities in *R. lateristriata* larvae at 96 hpf following exposure to gama melon parfum extract. mag 3,2X. (A-B) control *R. lateristriata* at 96 hpf. A: normal control. B: Negatif control. (C-D) Spinal abnormality. C: Lordosis. D: Kyphosis. (E-F) Eye malformation. E: microphthalmia. F: Anophthalmia. G: Hemorrhage. H: Swim bladder malformation. I : Pericardial and yolk edema.

Conclusion

Gama Melon Parfum extract exhibited concentration-dependent effects on the embryonic development of *R. lateristriata*. At low to moderate concentrations (50–250 ppm), no significant alterations were observed in hatching rate, survival rate, or heart rate; however, teratogenic manifestations were detected, including spinal deformities, eye abnormalities (microphthalmia and anophthalmia), hemorrhage, and impaired swim bladder development. In contrast, exposure to the highest concentration (500 ppm) resulted in acute lethal toxicity, evidenced by complete inhibition of hatching, total mortality, and absence of cardiac activity. Collectively, these findings indicate that GMP extract induces developmental toxicity, characterized by a concentration-dependent

shift from sublethal (teratogenic) effects to overt lethality, and provide a foundational basis for assessing its developmental safety profile in biomedical applications.

Recommendations

Further studies are recommended to investigate the effects of Gama Melon Parfum extract on later developmental stages, including larval and adult fish, as well as to evaluate the safety of the extract in other model organisms, such as rodents.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

* This study was approved by the Animal Ethics Committee of Integrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta, Indonesia (Approval No. 00064/XII/UNI/LPPT/EC/2025, issued on 18 Dec 2025)

Conflict of Interest

* The authors declare that they have no conflicts of interest.

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A Proposed Institutional and Technical Model for Establishing a Marine Information System in Türkiye's Marine Area Management

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Abstract: The lack of comprehensive and standardized spatial data, which is the most fundamental requirement for the effective management and sustainable use of marine areas, complicates the processes of planning, implementation and monitoring in these areas. Today, institutions responsible for marine areas produce spatial data of varying characteristics in line with their respective areas of responsibility and objectives. However, the production of these data in different standards and formats, lacking inter-institutional coordination, brings with it "interoperability" issues in terms of data sharing and integrated use. Although efforts to establish a National Spatial Data Infrastructure in Türkiye began a quarter of a century ago, a comprehensive information system covering spatial data for coastal and marine areas has not been established. Based on this deficiency, the study addresses the establishment of a "Marine Spatial Data Infrastructure" – in other words, a Marine Information System – compatible with international data standards as an inevitable necessity for the effective management of Türkiye's marine areas. Within the scope of this study, the literature on marine information systems was reviewed, and the application models of competent countries and organizations (National Oceanic and Atmospheric Administration, Australian Marine Spatial Information System, Marine Data Infrastructure Germany, etc.) were examined. Furthermore, a comprehensive analysis was conducted to determine the institutional structure responsible for the marine information system that can manage Türkiye-specific conditions, considering national and international legal regulations and global practices. As a result of the study, the fundamental data classes and data types that should be included in the initial phase of the Türkiye Marine Information System were identified, and a proposal was developed regarding the competent authority responsible for system governance. This study provides an institutional and technical roadmap that will contribute to the data-driven and spatial management of Türkiye's rights and interests within its maritime jurisdiction areas.

Keywords: Marine information system, Marine cadastre, Marine spatial data infrastructure, Turkish national geographical information system (TUCBS), Data standards.

Introduction

Throughout human history, while significant effort and resources have been devoted to the effective management of terrestrial areas, marine areas have been given a lower priority (Akdeniz & Inam, 2024). However, it is increasingly recognized today that a nation's interests are not limited solely to the land-sea interface the homeland does not consist solely of a piece of land but rather possesses a holistic structure composed of land, sea, and air elements (Akdeniz & Inam, 2025b). Today, many activities such as trade, transportation, fishing, mining, communication, defense, and security are carried out extensively in marine areas. Given the diversity of activities in marine areas and the wide range of spatial data they require, Marine Spatial Data Infrastructure (MSDI) is seen as an indispensable component of the Marine Information System (MIS), which enables the production, sharing, and updating of data (Zamzuri & Hassan, 2021).

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Institutions and organizations responsible for the use and management of marine areas produce spatial data of various characteristics in line with their own objectives. However, since these data are produced in different standards, various scales, and diverse formats—devoid of inter-institutional coordination—they bring about 'interoperability' problems regarding data sharing and integrated use. Furthermore, this leads to the redundant production of the same data by different institutions and organizations, resulting in both financial costs and time loss. For the effective administration of such a complex and multi-layered structure, there is a need for a systematic data management system that is compatible with international data standards.

Geographic Information Systems (GIS) have become a powerful tool widely used across disciplines thanks to their capabilities for managing, storing, analyzing, and visualizing spatial and non-spatial data (Kızılelma et al., 2017). In this context, GIS have become a fundamental element in the management of information societies and the development of countries (Doğruluk et al., 2024). Accordingly, it is essential to establish a unified and holistic platform by ensuring cooperation and coordination regarding production, storage, updating, and sharing of marine spatial data at the national level, as well as fostering collaboration between marine spatial data producers and users. In Türkiye, a roadmap for the current and future management of spatial data has been established through the 'National Geographic Information Strategy and Action Plans' prepared within the scope of the Turkish National Geographic Information System (TUCBS). However, as seen in international examples, TUCBS addresses spatial data for coastal and marine areas in a limited manner and focuses predominantly on terrestrial areas.

Spatial data infrastructures have become highly developed worldwide today. However, most of these focus on 'terrestrial-based spatial data' and rarely include 'spatial data related to marine areas' (Strain et al., 2006). In many countries, the collection and utilization of spatial data for marine areas are not sufficiently coordinated. The issue of which data should be included in an information system for marine areas is still a subject of debate (Akdeniz & İnam, 2025a). Similarly, the spatial data regarding Türkiye's marine areas are considered an incomplete component of the national information system (Erbaş, 2018).

Marine areas are highly diverse in terms of living and non-living resources and the impacts resulting from human activities. This diversity becomes even more complex and dynamic when the temporal dimension is considered. The effective management of the complexity of natural and artificial processes in marine areas, and the resulting spatial and temporal changes, depends on the collection of spatial data and the mapping of the spatial and temporal distribution and intensity of significant human activities. In this context, one of the objectives of the Turkish National Marine Research Strategy has been determined as 'the development of a national marine data and information management system and the establishment of a data/information sharing network.

Accordingly, there is an increasing and urgent need for the development of a Marine Spatial Data Infrastructure (MSDI), in other words, a Marine Information System (MIS), specific to marine areas. This system aims to establish a management and registration mechanism in marine areas by adapting traditional land information system concepts to the marine context. According to the International Hydrographic Organization (IHO), MSDI is defined as a spatial data infrastructure component that includes both spatial and non-spatial marine information (IHO, 2020). These include marine morphology (bathymetry), geology, infrastructure (wrecks, offshore facilities, pipelines, cables), superstructures (ports, aquaculture facilities, renewable energy plants), administrative and legal boundaries, protected areas, habitats, and oceanographic data.

It is observed that many countries that have made significant progress in the effective management of coastal and marine areas have established their own MSDI and MIS. At the national level, several countries operate their own MIS, such as the Australian Marine Spatial Information System (AMSIS) in Australia, Land Information New Zealand (LINZ) in New Zealand, the Marine Cadastre National Viewer in the United States, the Coastal and Ocean Information Network Atlantic (COINAtlantic) in Canada, the Marine Data Infrastructure Germany (MDI-DE) in Germany, Service Hydrographique et Océanographique de la Marine (SHOM) in France, the Marine Information System (Det Marine Danmarkskat) in Denmark, and the Land and Spatial Planning Agency (MAA-JA RUUMIAMET) in Estonia. At the international level, there are also MIS platforms encompassing the maritime jurisdiction areas of multiple countries. On a European scale, systems such as the European Marine Observation and Data Network (EMODnet) and SeaDataNet for the Adriatic and Ionian Region provide platforms containing data specific to marine areas. In Türkiye, although efforts to establish a National Spatial Data Infrastructure (NSDI) began a quarter-century ago, a holistic information system that sufficiently covers spatial data for coastal and marine areas has not yet been established.

Seas hold vital importance for Türkiye in terms of its geographical location, strategic objectives, and economic value. While Türkiye has a terrestrial area of 783,562 km², its maritime jurisdiction areas—either declared or likely to be declared within the framework of the 'Blue Homeland' (Mavi Vatan)—cover a marine area of approximately 462,000 km² (Yaycı, 2022). More than 55% of the country's population resides in 28 coastal provinces (Akdeniz & İnam, 2025b). Approximately 79% of the export volume and 93% of the import volume are carried out via maritime routes (Denizcilik Genel Müdürlüğü, 2023). Being surrounded on three sides by seas with different characteristics and production potentials, the fishery production sector is the fastest growing within the entire agricultural production sector (Denizcilik Genel Müdürlüğü, 2023). Türkiye ranks 16th globally in the aquaculture sector (TEPGE, 2025). Furthermore, it possesses significant potential in the marine tourism sector due to its climatic features and natural beauty. Various activities ranging from yacht tourism to diving tourism, and from cruise tourism to water sports, hold a significant share of 20% within the general tourism sector through the foreign exchange inflow they provide (İMEAK Deniz Ticaret Odası, 2023). Due to its geographical position, Türkiye is situated between developed Western countries in need of energy and natural resources and energy supply regions such as Russia and Middle Eastern countries; consequently, several pipelines (Blue Stream Gas Pipeline, Trans-Anatolian Natural Gas Pipeline, etc.) passing through its marine areas. Additionally, it also has both existing and potential energy resources within its marine areas (Akdeniz & İnam, 2025b). In addition to all these, Türkiye's coastal and marine areas are significant due to their rich biodiversity and natural resources. The total surface area of protected areas in its marine regions constitutes approximately 6% of the country's marine areas (Doğa ve Milli Parklar Genel Müdürlüğü, 2022). For a country like Türkiye, which has significant potential in the economic, ecological, and strategic resources of the sea, the subjects of 'MSDI and MIS' are of paramount importance. In this context, the establishment of a 'Marine Spatial Data Infrastructure and Marine Information System' compliant with international data standards for Türkiye's marine area management is considered an inevitable necessity. This study aims to identify the fundamental data classes and data types that should be included in the Turkish Marine Information System. It also seeks to determine the authorized institution responsible for managing the system.

Method

The methodology followed in this study is based on a stepwise approach ranging from conceptual framework analysis to model development. In the first phase of the research, the current international status was determined by examining the Infrastructure for Spatial Information in Europe (INSPIRE) framework. Subsequently, the national context was identified by analyzing data themes for coastal and marine areas within the components of the Turkish National Geographic Information System (TUCBS), along with the data types defined in these themes. In the third stage, internationally recognized application models such as NOAA Marine Cadastre, AMSIS, MDI-DE, SHOM, EMODnet, and the Mid-Atlantic Ocean Data Portal were analyzed, and their data classes and data types were comparatively evaluated. As a result of these comprehensive analyses, the fundamental data classes and data types that should be included in the initial phase of the Turkish Marine Information System were identified. In the final stage, a strategic analysis was conducted to determine the institutional structure responsible for the marine information system, taking into account Türkiye's specific conditions, as well as national and international legal frameworks and global practices.

Results and Discussion

INSPIRE

The 34 data themes defined under INSPIRE constitute the technical infrastructure of a standardized framework for marine data across Europe. Based on the detailed content analysis conducted in this study, data models directly and indirectly related to coastal and marine areas have been identified; the sufficiency of these models from the perspective of a 'marine information system' has been scrutinized. The analyses indicate that it has been determined that the data types directly related to coastal and marine areas are primarily organized under the '*Oceanographic Geographical Features (OF)*' and '*Sea Regions (SR)*' themes, which are included in the Annex III list.

'*Oceanographic Geographical Features*' data theme aims to represent the physicochemical state of the seas in a digital environment. Through observation points and grid-based data models, this theme enables the representation of dynamic parameters such as sea temperature, salinity, current velocity, wave height, and optical properties.

'Sea Regions' data theme covers the geographical and physical boundaries of marine areas (coastline, territorial waters, exclusive economic zone, etc.). While this theme allows for the classification of seas in a hierarchical structure (sea, ocean, sub-basin), it remains limited to the spatial representation of predominantly static geographical boundaries. However, it has been determined that these two themes are not sufficient to meet the needs of all stakeholders regarding the use and management of marine areas.

TUCBS

TUCBS, carried out within the scope of the 'e-Transformation Türkiye Project', is a national-level project aimed at establishing a national spatial data infrastructure in Türkiye. TUCBS is an e-Government initiative that aims to establish a geographic information system infrastructure in compliance with INSPIRE directives. It also seeks to define geographic data standards, assign data responsibilities, ensure that data are produced according to common standards, enable data sharing, and support interoperability at the institutional level. The policies, data standards, and cooperation structures developed within the framework of TUCBS allow Türkiye to manage and share its geographic data more effectively (Aslan, 2025).

Within TUCBS, 32 fundamental geographic data themes have been established based on international standards (such as INSPIRE and Land Administration Domain Model) and by making necessary modifications and customizations specific to Türkiye. The analysis shows that, within TUCBS, data related to coastal and marine areas are defined within different data themes according to their semantic integrity, rather than being gathered under a single data theme. Accordingly, the data themes and the data types defined within these themes for coastal and marine areas in TUCBS have been analyzed and are detailed below.

The '*Marine and Brackish Water Areas*' theme defines concepts such as 'sea' and 'coastline'; it also covers coasts and land/sea boundaries (CBSGM, 2020). Furthermore, this data theme provides a general framework for defining oceanographic features representing sub-sections of marine and brackish water areas as well as their chemical or physical properties (mean sea temperature, mean sea salinity). The Marine and Brackish Water Areas theme provides mechanisms for describing the seabed or sea surface features (CBSGM, 2020). Within the data theme, the following data types related to marine areas have been defined: 'marine area', 'isobath', 'marine layer', 'marine contour', 'marine site', 'seabed area', 'sea surface area', 'intertidal area', 'coastline', 'coastline segment', and 'shoreline'.

Within the '*Administrative Units*' theme, it has been determined that maritime jurisdiction areas based on the law of the sea—such as internal waters, territorial waters, contiguous zone, exclusive economic zone (EEZ), and continental shelf—are defined under the 'Marine Administrative Unit' class. This theme defines Türkiye's maritime boundaries of use with riparian countries regarding marine areas (CBSGM, 2020). Furthermore, within this data theme, the following data types related to marine areas have been defined: 'marine administrative unit', 'marine administrative boundary', 'marine region boundary', 'marine administrative region', and 'baseline'.

It has been determined that the '*Transport Networks*' theme provides an integrated structure for the management of transport networks and details related to rail, road, maritime, and air transport. Regarding marine areas, it facilitates the sharing of data that serves as a basis for transport planning or navigation studies between two points (CBSGM, 2020). Under the 'Water Transport Network' class related to marine areas, the following data types have been defined: 'maritime route', 'lighthouse', 'ferry crossing', 'inland waterway', 'port area', 'port node', 'buoy', 'fairway area', 'restrictions for watercraft', 'water node', and 'waterway link'.

The '*Elevation*' theme aims to define the three-dimensional shape of the Earth's surface in terms of elevation characteristics such as altitude or depth (CBSGM, 2020). Regarding marine areas, it has been determined that this theme defines the morphological structure of the seabed. The '*Hydrography*' theme covers all natural, artificial, static, and flowing objects related to hydrography on the Earth's surface, such as basins, rivers, lakes, and dams (CBSGM, 2020). It has been identified that while this data theme encompasses coastal regions, it does not contain any data types directed toward marine areas. Marine areas have been excluded as they are treated as separate themes (Marine and Brackish Water Areas). Within this data theme, the following data types related to coastal areas have been defined: 'coast', 'coastline', 'coastal structure', 'surface waters', and 'coastal soil type'.

The '*Management, Restriction, and Regulation Zones*' (Public Management Zones) theme covers areas that are generally managed, regulated, or used for reporting at international, European, national, regional, or local levels (CBSGM, 2020). Regarding coastal and marine areas, it has been identified that this theme encompasses special areas for protection or restriction purposes (regulated sea lanes, marine protected areas, coastal zone

management areas, mineral exploration and license areas, etc.). Within this data theme, the following data types for marine areas have been defined: 'management restriction regulation zone', 'environmental impact area code', 'specialized zone type code', 'area management zone code' etc.

It has been determined that the 'Protected Sites' theme defines terrestrial, aquatic, or marine areas under protection status—such as national parks, nature parks, wildlife improvement areas, natural heritage sites, special environmental protection areas, and wetlands (CBSGM, 2020). These areas are managed through legislation and other effective measures to ensure the conservation and continuity of biological and genetic diversity, as well as natural and cultural resources.

A Comparison of International Marine Information Systems

The 'NOAA Marine Cadastre' marine information system is a comprehensive geographic information system established for managing marine resources, planning energy projects, and developing ecosystem-based management strategies within the maritime jurisdiction areas of the United States. Operated through a partnership between NOAA and the Bureau of Ocean Energy Management (BOEM), this system integrates multi-layered data types—such as maritime jurisdiction areas, oceanography, military training areas, navigation safety, renewable energy sites, and protected habitats—within a single platform. Advocating transparency and data-driven management in decision-support processes, the NOAA Marine Cadastre information system is not merely a mapping tool but also a dynamic 'marine cadastre' data model where rights, responsibilities, and restrictions related to marine areas are defined in detail. It has been identified that this spatial data diversity provided by the system clearly reveals the spatial relationship between different types of marine area uses, as shown in Figure 1.

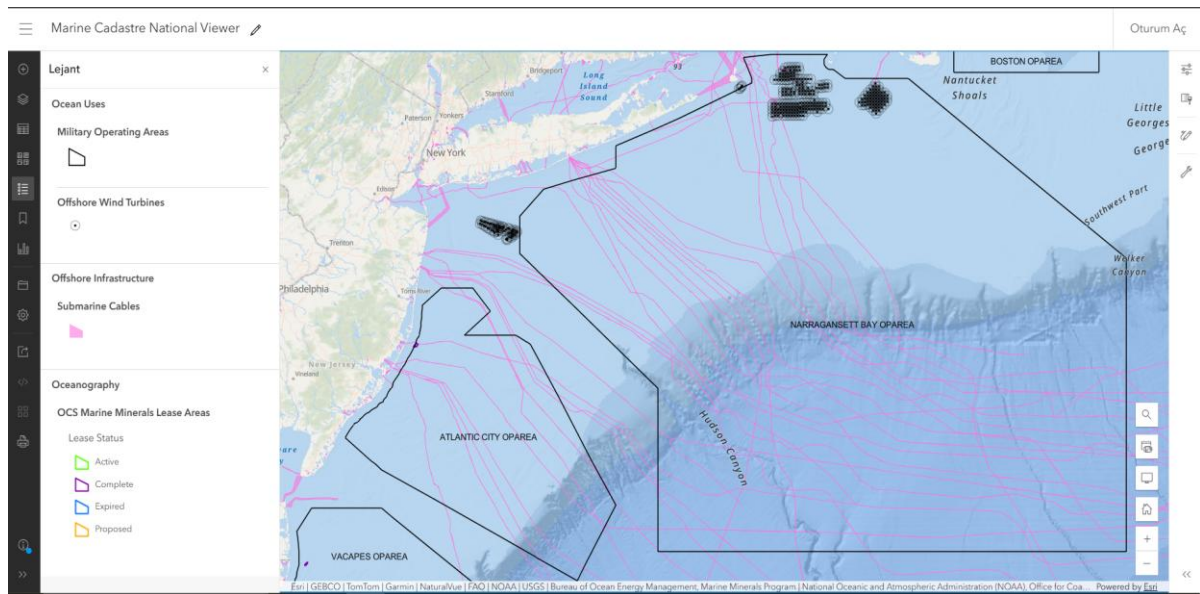


Figure 1. The NOAA Marine Cadastre information system interface and data types

The 'Australian Marine Spatial Information System (AMSIS)' is an advanced decision-support system managed by Geoscience Australia, which integrates complex and diverse data types across Australia's maritime jurisdiction areas. Structured in accordance with international standards (OGC) and the principles of the law of the sea, the system brings together a wide variety of significant data types, such as maritime jurisdiction areas, submarine cables and pipelines, natural gas and oil exploration sites, fisheries, and marine protected areas. The primary function of AMSIS is to support 'Blue Economy' strategies by associating the maritime jurisdictional boundaries determined in accordance with the United Nations Convention on the Law of the Sea (UNCLOS) with diverse spatial data. In scientific literature, AMSIS is characterized as an exemplary model in marine cadastre and marine information system studies, particularly for its multidisciplinary data management and user-friendly interface that allows various stakeholders (public, private sector, academia, citizens, etc.) to analyze conflicting or overlapping marine use demands on a spatial plane. It has been identified that this data integrity and spatial layer structure provided by the system clearly reveal the spatial relationship between different maritime jurisdiction areas and marine use types, as shown in Figure 2.

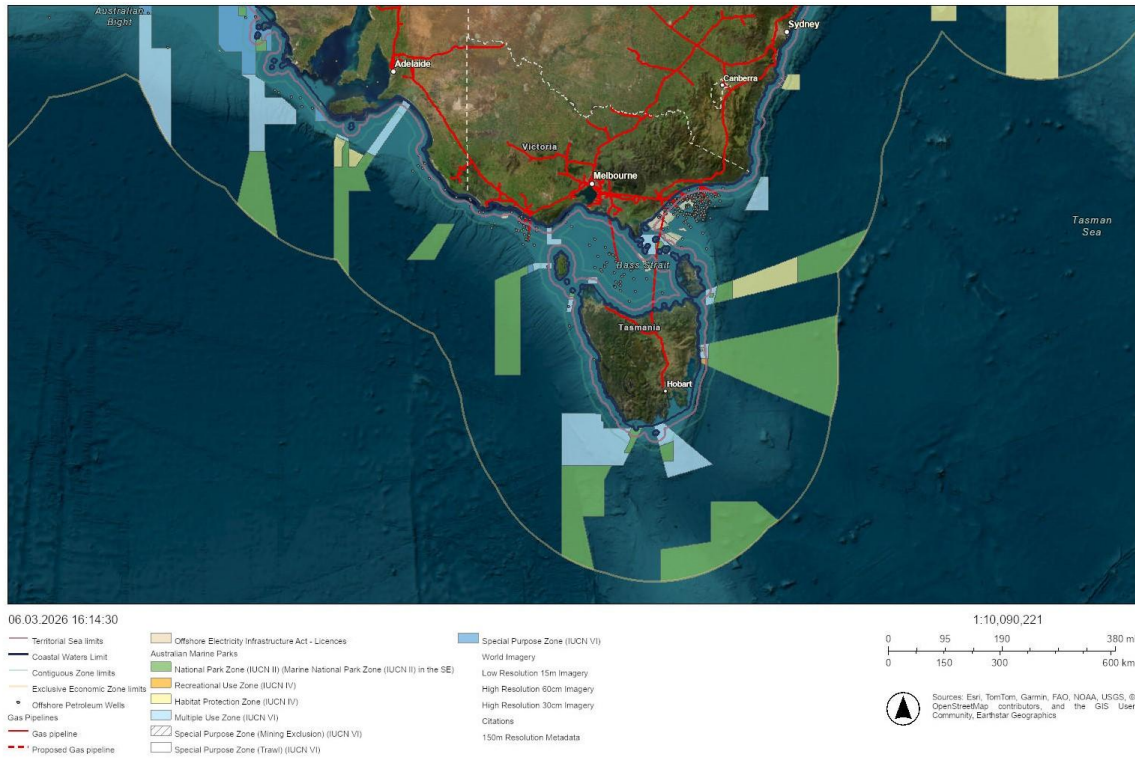


Figure 2. The AMSIS information system interface and data types

The 'Marine Data Infrastructure Germany (MDI-DE)' is a national spatial information system that supports the management of marine data related to Germany's sovereign waters in the North Sea and the Baltic Sea in a decentralized yet standardized manner. Established through the cooperation of eleven different institutions at both federal and state levels, this system specifically aims to comply with the European Union's Marine Strategy Framework Directive (MSFD) and INSPIRE directives. Technically utilizing international standards such as Web Map Services (WMS) and Web Feature Services (WFS), MDI-DE integrates diverse interdisciplinary data into a single system, including coastal protection, marine geology, oceanography, transportation, marine biodiversity, pollution monitoring, and offshore wind energy planning. The system provides a multi-stakeholder governance model for monitoring complex marine ecosystems. The data integration and interoperability structure provided by the system is illustrated in Figure 3, which includes a section of the portal interface and the types of spatial data provided.



Figure 3. The MDI-DE information system interface and data types

The 'Service Hydrographique et Océanographique de la Marine (SHOM)', France's national hydrographic service, is a well-established marine information system that manages the marine spatial data infrastructure and integrates a wide variety of data related to marine areas. Operating under the French Ministry of Defense, SHOM provides data that serve as a foundation not only for traditional nautical charting but also for the determination of maritime jurisdictional boundaries, monitoring of coastal dynamics, military activities, oceanography, and the planning of marine area use activities. The most notable feature of the system is its ability to provide high-resolution bathymetric data, tidal models, and oceanographic parameters in a format compliant with international S-100 standards. With its vision of a 'Pôle de Données Marine' (Marine Data Center), SHOM is considered a spatial management system with high reliability and data precision, making data of military sensitivity available for public benefit and scientific research. It has been determined that this data diversity in French maritime jurisdiction areas and the analysis tools provided by the platform can be followed through the portal interface and bathymetric data sets presented in Figure 4.

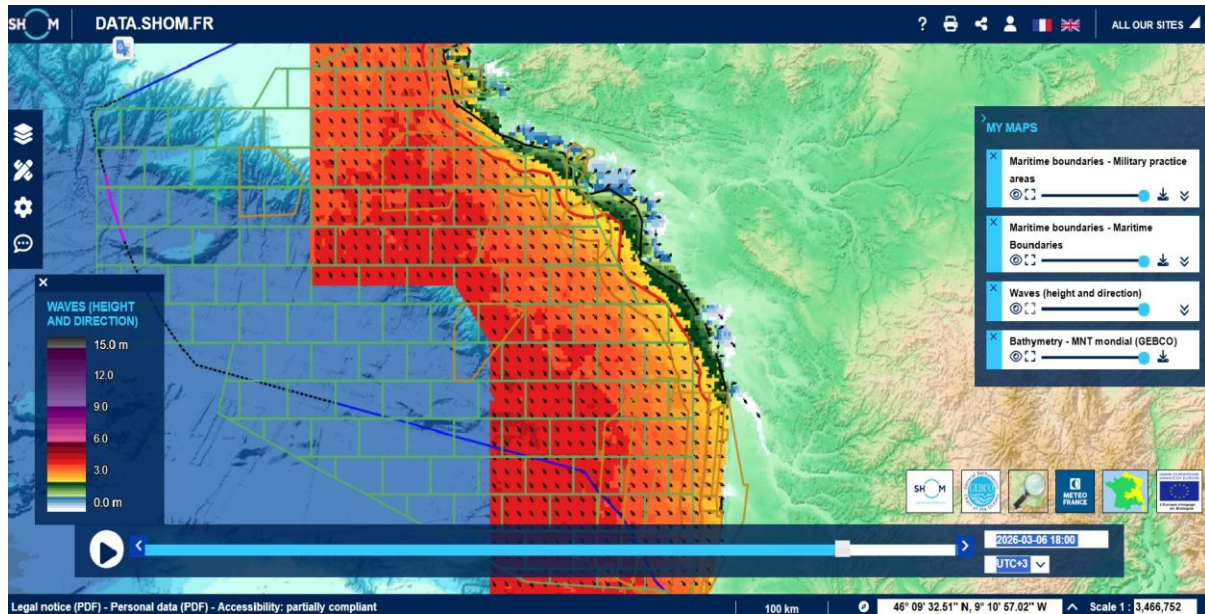


Figure 4. The SHOM information system interface and data types

The 'European Marine Observation and Data Network (EMODnet)' is one of the most comprehensive marine information systems that consolidates fragmented marine data across the continent into a single point of access, in line with the European Union's 'Blue Growth' and 'Marine Strategy Framework Directive' objectives. Operated through the collaboration of more than 120 institutions, this system establishes data-driven standards in eight main thematic classes: administrative units, bathymetry, biology, chemistry, geology, human activities, physics, and seabed habitats. EMODnet's core philosophy, the 'collect once, use many times' principle, is built upon INSPIRE standards and FAIR (Findable, Accessible, Interoperable, and Reusable) data principles to ensure the interoperability of data. It has been determined that the EMODnet information system is not merely a data repository, but also the most comprehensive open marine spatial data infrastructure on the path to a digital twin of European seas and one of the most prominent examples of cross-border marine spatial planning. A cross-sectional example for examining the ecosystem consisting of diverse spatial data types in European seas through the central portal interface is presented in Figure 5.

The 'Mid-Atlantic Ocean Data Portal' is a user-oriented and highly interactive regional marine information system established to facilitate marine spatial planning across a vast area extending from New York to Virginia. Managed by the Mid-Atlantic Regional Council on the Ocean (MARCO), this system was developed to resolve data discrepancies and gaps between state, federal, and local agencies. It has been determined that the system incorporates a wide variety of data types, such as marine biodiversity, commercial fishing and aquaculture areas, vessel traffic routes, and particularly potential offshore wind energy sites. It has been identified that this system can transparently manage the overlapping or conflicting interests of marine and ocean users (fishermen, energy companies, environmentalists) through spatial analysis tools. A cross-sectional example for examining the spatial interaction between regional marine area use and habitat protection areas through the central portal interface is presented in Figure 6.

Based on the comprehensive analysis of six marine information systems (NOAA Marine Cadastre, AMSIS, SHOM, MDI-DE, EMODnet, and Mid-Atlantic Ocean Data Portal), the structural similarities and differences between the data classes and types hosted by these systems were identified. These systems were categorized into three groups in terms of data classes as 'comprehensive', 'restricted', and 'not available'; the comparative findings obtained are presented in Figure 7.

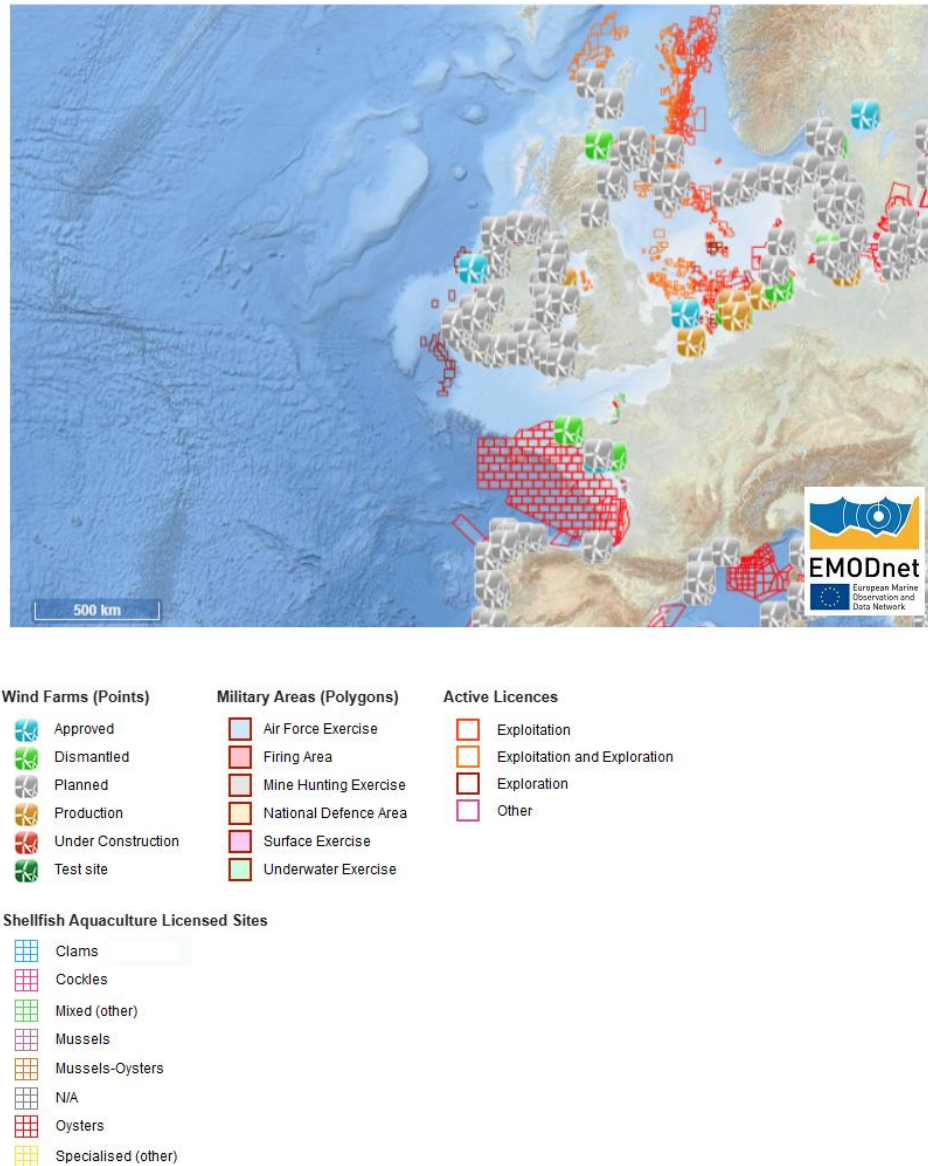


Figure 5. The EMODnet information system interface and data types

According to the results of the analysis, the results show that the EMODnet and Mid-Atlantic Ocean Data Portal systems possess the most comprehensive diversity of data types in 7 out of the 10 data classes examined. This can be explained by the fact that this situation stems from the fact that these systems are designed on a regional scale that transcends national boundaries (such as the European Union and Mid-Atlantic marine areas) and are structured through a multi-party inter-institutional cooperation model. In contrast, it has been determined that the SHOM marine information system regarding French marine areas has a narrower scope compared to other examples and predominantly focuses on hydrographic and oceanographic data sets. This highlights the direct impact of the systems' establishment purposes and target user groups on the data architecture.

It has been observed that identical or similar data contents are defined with different terminologies across various systems. For instance, it has been identified that maritime jurisdiction areas, which constitute the basis for a marine information system, are referred to as 'boundaries' in some information systems, while in others they are termed 'maritime boundaries' or 'administrative units'. This creates a need for international-level semantic interoperability and terminology.

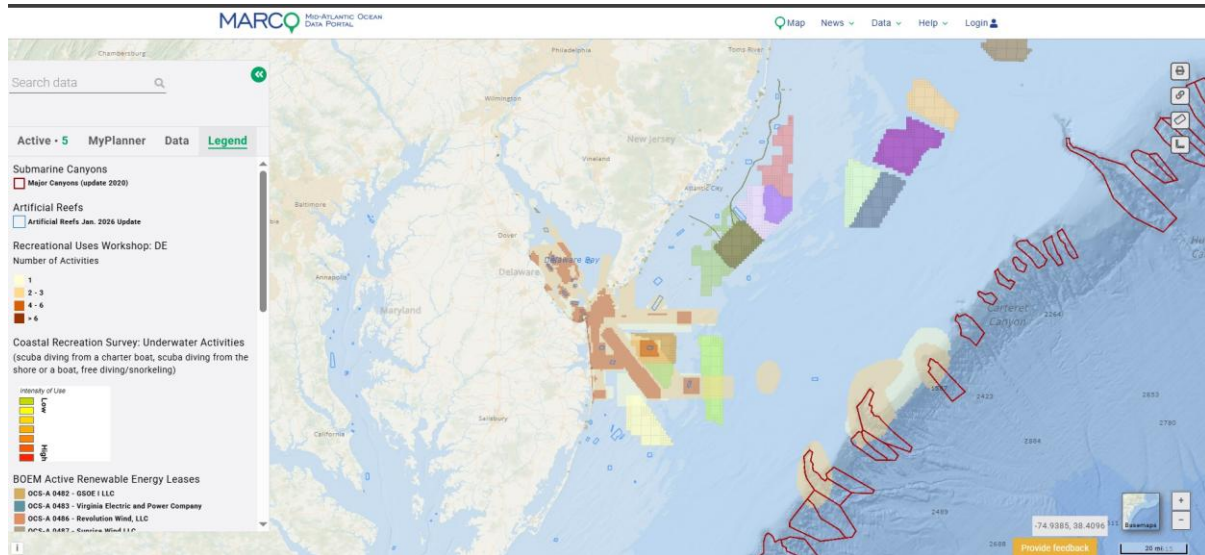


Figure 6. The Mid-Atlantic Ocean Data Portal information system interface and data types

It has been observed that European-scale systems such as EMODnet offer a data structure compliant with INSPIRE directives, whereas US-based systems like NOAA or the Mid-Atlantic portal prioritize their own national data standards (such as FGDC). This represents a key technical barrier to global-scale data integration. Comprehensive systems (e.g., EMODnet and Mid-Atlantic) are more advanced not only in terms of data diversity but also in terms of the variety of services offered for data access (WMS, WFS, API). It has been specifically observed that 'open data' policies are directly proportional to the scope of success of the system.

Data Classes	NOAA	AMIS	MDI-DE	SHOM	EMODnet	Mid-Atlantic
Boundaries	●	●	◐	●	◐	●
Marine Activities	●	●	●	◐	●	●
Infrastructure	●	●	●	◐	●	●
Marine Transportation	●	◐	◐	◐	●	●
Marine Habitats	●	●	●	○	●	●
Oceanography	◐	◐	●	●	●	◐
Nature, Environment	◐	◐	●	◐	●	●
Base Map / Plan	○	◐	○	○	○	●
Geology	◐	◐	●	◐	●	○
Recreation	○	○	○	○	○	●
● Comprehensive ◐ Restricted ○ Not Available						

Figure 7. A comparative analysis of marine information systems based on data classes

Identification of Data Classes and Types for the Turkish Marine Information System

This section is based on the synthesis of findings from the previous analyses with Türkiye's unique legal, technical, and administrative conditions. As a result of this evaluation, 88 fundamental marine spatial data types have been identified to be integrated into the system in the initial phase, forming the core structure of the developed Turkish Marine Information System (Figure 8). These data were grouped based on by considering the classification methods used in the information systems of developed countries and international organizations, alongside Türkiye's current conditions and requirements; thus, the fundamental data classes for the Turkish

Marine Information System have been established. Detailed explanations regarding these identified data classes and the marine spatial data types contained within them are presented below in a systematic manner.

The '*Marine Spatial Planning Base Data*' data class includes planning bases and fundamental reference datasets to be produced within the scope of the planned use and management of marine areas. Within this class, in addition to planning data such as marine spatial plans and integrated coastal zone plans, technical bases such as nautical charts, satellite imagery, aerial photographs, and orthophoto maps are included. This data class has been established to serve as the primary spatial reference framework for decision-support mechanisms in marine areas.

The '*Marine Cadastre*' data class is designed to encompass spatial and metadata to be produced within the scope of marine cadastre. This class includes coastal parcel polygon data, marine parcel polygon data, coastal parcel line data, marine parcel line data, and marine cadastral maps, as well as metadata specifying the rights, responsibilities, and restrictions (RRR) associated with these parcels (Figure 8).

Türkiye Marine Information System				
Marine Spatial Planning Base Data	Marine Cadastre	Marine and Coastal Boundaries	Marine Use	Coastal Use
Marine Spatial Planning	Coastal Parcel Polygon Data	Internal Waters Boundary	Weir and Fishery Areas	Coastal Erosion and Accumulation Data
Integrated Coastal Zone Planning	Marine Parcel Polygon Data	Coastal Waters Boundary	Military Training and Firing Zones	Reclamation Areas
Nautical Charts	Coastal Parcel Line Data	Territorial Waters Boundary	Military Forbidden Zones	Coastal Energy Facilities
Aerial Photographs and Orthophoto	Marine Parcel Line Data	Contiguous Zone Boundary	Shipwreck and Minefields	Fishing Ports
Satellite Imagery	Marine Cadastral Maps	Continental Shelf Boundary	Submarine Pipelines	Shipyards
		Exclusive Economic Zone Boundary	Submarine Cable Lines	Fish Markets
		Marine Administrative Boundary	Mineral and Oil Exploration-Operation Sites	Coastal Structures and Facilities
		Port Administrative Boundary	Dredging and Sand-Gravel Extraction Areas	Seawater Desalination
		Shoreline	Aquaculture Sites	Land Use and Land Cover
		Shore Edge Line	Fishing Sites	Coastal Settlement Population Data
		First 50-meter Shoreline	Offshore Renewable Energy Sites	River Flood Impact Areas
		Second 50-meter Shoreline	Marine Airport Areas	Lighthouses
		Coastal Guard Areas	Port Areas	
		Mania Line	Anchorage	
		River Boundary	Marine Highways	
			Deep-Sea Discharge Sites	
			Scientific Research Areas	
			Buoy and Warning Light	
Oceanography	Marine Transport	Nature and Environmental Protection	Marine Habitats	Tourism and Recreation
Bathymetry	Real-Time Marine Traffic Monitoring Data	Protected Areas	Marine Habitat Area	Tourist Activity and Sporting Areas
Bathymetry Contours	Spatial Marine Traffic Density	Artificial Reefs and Underwater Structures	Coastal Habitat Area	Diving-Restricted Zones
Seafloor Altimetry	Straits and Canals	Hazardous and Restricted Zones		Tourism Facilities
Marine Geology				
Submarine Fault Lines				
Marine Seismic Data				
Tidal Data				
Hurricane Data				
Marine Volcanism Data				
Seabed Landslide Data				
Tsunami Data				
Marine Mineral Data				
Marine Hydrocarbons and Hydrates Data				
Marine Water Quality Parameters				
Marine Pollution Data				
Swimming Water Quality Data				
Marine Meteorological Data				
Marine Noise Level Data				
Sea Level and Anomalies				
Seawater Salinity Data and Anomalies				
Seawater Temperature Data and Anomalies				
Seawater Chlorophyll Data				

Figure 8. A comparative analysis of marine information systems based on data classes

The '*Marine and Coastal Boundaries*' data class covers the maritime jurisdiction areas and administrative boundary data of Türkiye within the framework of international law and national legal regulations. Within this scope, the following spatial data types related to maritime jurisdiction boundaries have been identified: internal waters boundary, coastal waters boundary, territorial waters boundary, contiguous zone boundary, continental shelf boundary, and exclusive economic zone (EEZ) boundary. Furthermore, the class includes multi-administrative and managerial boundaries such as marine administrative boundaries, port administrative area boundaries, coastline, shore edge line, first 50-meter shoreline, second 50-meter shoreline, coast guard areas of responsibility, obstacle limitation surfaces (mania line), and river boundaries.

The '*Marine Use*' data class is designed to encompass spatial data related to usage activities occurring in marine areas. Within this class, the following spatial data types have been defined: weir and fishery areas (dalyan and voli), military training and firing zones, military forbidden zones, shipwreck and minefields, submarine pipelines, submarine cable lines, mineral and oil exploration-operation sites, dredging and sand-gravel extraction areas, aquaculture sites, fishing sites, offshore renewable energy sites, marine airport areas, port areas, anchorage areas, marine highways, deep-sea discharge sites, scientific research areas, and buoy and warning light locations (Figure 8).

The '*Coastal Use*' data class is designed to encompass spatial data related to usage activities occurring in coastal areas, as well as natural and artificial features in these zones. Within this class, the following spatial data types have been defined: fishing ports, shipyards, fish markets, reclamation areas, seawater desalination plants, land use and land cover, coastal settlement population data, river flood impact areas, lighthouses, coastal energy facilities, and coastal erosion and accumulation data.

The '*Oceanography*' data class is designed to encompass data related to the physical, chemical, biological, and geological characteristics of the seas. Within this class, the following spatial data types have been defined: bathymetry, bathymetric contours, seafloor altimetry, marine geology, submarine fault lines, marine seismic data, tidal data, hurricane data, marine volcanism data, tsunami data, marine mineral data, marine pollution data, seawater chlorophyll data, seawater temperature, and seawater salinity, among others (Figure 8).

The '*Marine Transport*' data class is designed to encompass data related to the management of maritime transport networks and improving navigation safety. Within this scope, the following spatial data types have been defined: real-time marine traffic monitoring data, spatial marine traffic density data, and straits and canals. The '*Nature and Environmental Protection*' data class is designed to encompass on ecological and biologically sensitive areas for the sustainable use of marine areas. These include hazardous and restricted zones, protected areas, artificial reefs, and underwater structures. The '*Marine Habitats*' data class is designed to define the distribution and characteristics of different habitat types within the marine ecosystem. This class facilitates the monitoring of ecosystem health by consolidating ecological characterization of habitats and biodiversity inventories into a structure compliant with international standards. Marine habitat area and coastal habitat area data are grouped under this class.

The '*Tourism and Recreation*' data class is designed to encompass data related to the use of coastal and marine areas for tourism purposes. This class is structured to model the spatial distribution of tourist activities, usage intensity, and recreational potential. Within this class, spatial data types related to tourist activity and sporting areas, diving-restricted zones, and tourism facilities have been defined (Figure 8). On the other hand, it is recommended that the system be designed with a flexible structure and the integration of new data types into the system whenever necessary.

Identification of the Responsible Institution for the Turkish Marine Information System

It has been determined that various institutions and organizations in Türkiye possess diverse duties, authorities, and responsibilities regarding the production, updating, security, sharing, and determination of usage standards for spatial and attribute data concerning coastal and marine areas. Issues such as the redundant production of spatial data by different institutions in varying standards, leading to losses in efficiency, time, and resources, remain a key issue in geographic information systems. Therefore, countries strive to establish and strengthen national geographic data infrastructures to produce accurate policies, strategies, and decisions, and to ensure interoperability as well as effective resource and technology management. To operate such spatial and verbal data for coastal and marine areas—produced or used by different institutions—through a single platform and to ensure the necessary coordination and cooperation between spatial data producers and users, the establishment of a marine and coastal information system is required. Identifying the institution responsible for managing such a system is one of the most critical steps in this process.

Declarations published by international organizations, such as the United Nations Integrated Geospatial Information Framework (UN-IGIF), emphasize the necessity of having a dedicated unit responsible for coastal and MSDI. In this context, an examination of systems in countries that have established or are developing marine information systems shows that institutions such as the Federal Department of Fisheries and Oceans (DFO) in Canada, the National Oceanic and Atmospheric Administration (NOAA) in the United States, the Department for Environment, Food and Rural Affairs (DEFRA) in the United Kingdom, the Geodata Agency in Denmark, Geoscience Australia in Australia, and the Ministry of Mining, Blue Economy and Maritime Affairs

in Kenya are identified as the authorities responsible for marine information systems. In Türkiye, some of the establishment, duties, and responsibilities of the General Directorate of Geo-Information System have been regulated in the Presidential Decree No. 1 on the Presidential Organization, dated 2018, as follows:

- To carry out the tasks and procedures related to the establishment, use, and development of the National Geographic Information System,
- To conduct studies and prepare the necessary legislation to ensure the determination of standards, as well as the fundamental policies and strategies regarding the national-level production, quality, and sharing of spatial data and information,
- To establish and operate the portal where spatial data produced by public and private sector institutions and organizations are presented.

Pursuant to Article 3 of Presidential Decree No. 49 on Geographic Information Systems, dated 2019, it has been identified that the General Directorate of Geo-Information System is held responsible for carrying out procedures related to the national geographic information system and infrastructure. Furthermore, one of the primary objectives in the 2024–2030 National Geographic Information Strategy and Action Plan of the General Directorate of Geo-Information System has been identified as strengthening the Directorate's guiding and regulatory role in the sector and establishing and disseminating standards by integrating contemporary technologies with geographic information systems.

In Presidential Decree No. 4 on the Organization of Affiliated, Related, and Associated Institutions and Organizations and Other Institutions and Organizations, dated 2018, some of the 'establishment, duties and responsibilities of the Office of Navigation, Hydrography, and Oceanography (SHOD)' have been regulated as follows:

- To carry out navigation, hydrography, and oceanography services and duties within the borders of national sovereignty, under the Turkish Naval Forces Command,
- To archive all kinds of data, plans, and maps belonging to hydrographic, oceanographic, and geophysical surveys and research to be conducted by public and private institutions and individuals through special means.

Pursuant to Article 14 of the Regulation on Navigation, Hydrography, and Oceanography Services (No. 30941), dated 2019, it has been identified that SHOD is held responsible for '*conducting all types of hydrography, oceanography, marine geophysics, and geology surveys, research, and works for military, scientific, and economic purposes within our maritime jurisdiction and interest areas under a specific plan and program,*' and for '*carrying out the duties of the national hydrography, oceanography, marine geology, and geophysics data and information center and archive.*'

A holistic evaluation of current legislation indicates that, in accordance with Presidential Decree No. 4 on the Organization of Affiliated, Related, and Associated Institutions and Organizations and Other Institutions and Organizations, duties for military and strategic purposes regarding marine areas have been entrusted to SHOD. In contrast, within the scope of the Presidential Decree No. 1 on the Presidential Organization, the General Directorate of Geo-Information Systems has been assigned tasks such as the collection and processing of spatial data in marine areas and associating them with data from different institutions.

Since marine areas are the intersection points of a wide variety of activities (transportation, tourism, fishing, energy, etc.), the information system to be developed in these areas must provide an infrastructure that not only stores data but also strengthens decision-support mechanisms, guides planning processes, and facilitates inter-institutional cooperation. Therefore, the institution to be responsible for the management and coordination of the system in question should:

- Possess a modern and technical infrastructure,
- Be experienced in data management and spatial information systems,
- Have legal and administrative competence to ensure inter-institutional coordination.

When evaluated within this framework, the General Directorate of Geo-Information Systems, which is currently responsible for the coordination of TUCBS, emerges as the most suitable institution to lead the marine information system. Given its technical capacity and experience in determining and managing national standards for geographic information systems, this institution is structured to collect and analyze data from various

institutions and sectors under a common umbrella, thereby providing reliable outputs for decision-making processes.

Conclusion

In this study, internationally recognized marine information systems were analyzed in detail, and 10 fundamental data classes and 88 data types that should be included in the initial phase of the Turkish Marine Information System were identified as essential for the initial phase. A strategic analysis was conducted to identify the institutional structure responsible for this system, taking Turkey's unique conditions into account. Within this scope, it has been evaluated that The General Directorate of Geo-Information System possesses the institutional capacity, knowledge, and experience required to implement and ensure the sustainability of the marine information system application.

The design and implementation of a marine information system enable users, practitioners, and managers with the opportunity to describe, analyze, and present spatial information in detail regarding different marine layers, such as the sea surface, water column, seabed, and subsoil (Uslu & Sesli, 2011). This comprehensive system enables a holistic approach to economic, socio-cultural, physical, and biological structures, as well as the rights, responsibilities, and restrictions (RRR) associated with public and private entities in marine areas. Such a system not only supports technical and administrative decision-making processes but also contributes to efforts aligned with the United Nations Sustainable Development Goals (Hafizh & Djunarsjah, 2024). Furthermore, it holds great importance in providing accessible, reliable, and up-to-date spatial data for policymakers, planners, technical staff, the private sector, coastal and marine authorities, academia, and the public.

The fundamental vision of the Turkish Marine Information System should be:

- To increase the understanding and use of spatial information related to marine areas,
- To enhance access, usability, integration, and sharing of spatial information concerning marine areas,
- To promote the efficient, effective, and economical use of digital marine spatial information and to enable its interoperability with relevant hardware and software systems,
- To contribute to sustainable development at local, regional, and global levels and to encourage the establishment of spatial data infrastructures.

The implementation of a marine information system in Türkiye will enable, 'integrated coastal zone management', 'marine spatial planning', and 'marine cadastre' efforts will have access to reliable, up-to-date, and accurate spatial data at international standards. This system will contribute to the decision-making, implementation, monitoring, and management processes of the relevant studies. Furthermore, by providing marine spatial data through an information system, it will enable answering key spatial questions such as "what," "where," "how much," and "how" regarding usage activities, thereby maximizing the value of marine spatial data. Consequently, more effective use and management of coastal and marine areas will be ensured, contributing to the protection of the ecosystem and forming a significant step toward achieving sustainable development goals.

Recommendations

Efforts should be initiated to establish a marine spatial data infrastructure in Türkiye that is compliant with international standards and interoperable with global systems. Following its establishment, 'marine information system' and 'marine cadastre' studies should be developed concurrently and implemented as a priority. Furthermore, further studies should focus on integrating the marine information system with TUCBS. This will represent a significant step toward achieving sustainable land management goals and ensuring the integrity of the national spatial data infrastructure. Additionally, a new umbrella organization (such as a Ministry of Maritime Affairs) responsible for these processes should be established to strengthen the technical infrastructure in the management of coastal and marine areas, institutionalize inter-institutional cooperation, and implement holistic policies.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

Conflict of Interest

* The authors declare that they have no conflicts of interest.

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Spatial Clustering and Determinants of Agricultural to Urban Land Conversion at the Neighborhood Level: Evidence from Konya, Türkiye

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Konya Technical University

Abstract: Urban expansion processes exert significant pressure on agricultural land, particularly in rapidly growing cities. This study analyzes the spatial distribution and determinants of agricultural-to-urban land conversion at the neighborhood level in Konya, Türkiye, for the period 1990–2018. First, the spatial pattern of agricultural land loss was examined using Global Moran's I and Local Moran's I (LISA) statistics. Global Moran's I result ($I = 0.2037$, $p < 0.01$) indicates that agricultural land conversion exhibits statistically significant positive spatial autocorrelation, suggesting that land-use transitions are spatially clustered rather than randomly distributed. LISA analysis further reveals that high conversion clusters are primarily concentrated along the southwestern urban growth corridor of the city. To identify the key determinants of agricultural land conversion, an Ordinary Least Squares (OLS) regression model was employed. The results demonstrate that the initial urban land ratio and distance to the city center are statistically significant predictors of conversion intensity. The negative coefficients of both variables indicate that agricultural land conversion increases in neighborhoods located closer to the city center and in areas with higher initial levels of urban development. Although the explanatory power of the model is moderate ($R^2 = 0.239$), the presence of spatial clustering highlights the importance of spatial interaction effects and neighborhood spillover dynamics in shaping land-use transitions. Overall, the findings suggest that urban growth in Konya follows a spatially structured and directional pattern. These results underline the critical role of spatial planning policies in guiding urban expansion and protecting agricultural land resources. The study demonstrates that neighborhood-level spatial analysis provides valuable insights for understanding urban land transformation processes and for developing sustainable land management strategies.

Keywords: Agricultural land conversion, Spatial econometrics, Urban expansion, Spatial autocorrelation

Introduction

Urban expansion has become one of the most influential drivers of land-use change worldwide, particularly in rapidly growing metropolitan regions. As cities expand to accommodate increasing population, economic development, and infrastructure demands, agricultural lands located at the urban fringe are often subjected to conversion pressures (Güneralp et al., 2020; Muchelo et al., 2024; Sheng & Huang, 2025). This transformation is not only a matter of spatial reallocation of land resources but also has broader implications for food security, environmental sustainability, ecosystem services, and regional socio-economic structures (Beckers et al., 2020; Kannan et al., 2021). Understanding the spatial dynamics of agricultural land loss has therefore become a critical research topic in urban and regional planning, land-use science, and spatial economics (Uyan & Ertunç, 2024).

Urban growth processes rarely occur randomly across space. Instead, they tend to exhibit directional and clustered patterns shaped by accessibility conditions, existing urban structure, and spatial interaction effects among neighboring areas (Gomes et al., 2018; Huang et al., 2020). Land located closer to urban cores typically experiences higher development pressure due to increased land value, better accessibility, and stronger agglomeration economies (Novotný et al., 2022). Similarly, areas with an already established urban structure

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often serve as nuclei for further expansion through processes of spatial diffusion and contiguous growth (Biłozor & Cieślak, 2021).

Ignoring the spatial dependence inherent in land-use change processes may lead to incomplete or biased interpretations (Qiu et al., 2007). Agricultural land conversion often demonstrates spatial spillover effects, meaning that land use decisions in one neighborhood can influence or be influenced by changes occurring in adjacent areas (Richards, 2018). This phenomenon highlights the importance of applying spatial statistical methods that explicitly account for spatial autocorrelation and local clustering patterns. Exploratory spatial data analysis (ESDA) techniques, including Global Moran's I and Local Indicators of Spatial Association (LISA), provide valuable tools for identifying whether land-use transitions follow systematic spatial structures (Salvati & Carlucci, 2014; Uyan & Ertunç, 2024). These methods allow researchers to detect clusters of high or low conversion intensity and to reveal potential spatial outliers, thereby offering insights into the underlying mechanisms of urban expansion (Abelairas-Etxebarria & Astorkiza, 2020).

Despite the growing body of literature on urban growth and land-use change, neighborhood level analyses remain relatively limited, particularly in medium sized cities undergoing accelerated transformation. Many existing studies focus on regional or metropolitan scales, potentially overlooking localized dynamics that are critical for effective spatial planning and policy development. Neighborhood-level investigations can capture spatial heterogeneity and provide a more detailed understanding of how urban expansion interacts with agricultural land systems (Follmann et al., 2021).

Konya represents a particularly relevant case for examining these processes. As one of Türkiye's major agricultural production centers, the city has experienced significant urban growth over recent decades. Rapid population increase, economic diversification, and infrastructure development have contributed to the outward expansion of urban land uses, often at the expense of productive agricultural areas. The spatial configuration of this expansion, however, remains insufficiently understood. Identifying whether agricultural land loss follows clustered spatial patterns and determining the key drivers of such transformations are essential for developing sustainable urban growth strategies and land protection policies.

In this context, the present study aims to analyze the spatial distribution and determinants of agricultural-to-urban land conversion at the neighborhood level in Konya between 1990 and 2018. Specifically, the study addresses two main research questions:

- (i) Does agricultural land conversion exhibit significant spatial clustering across neighborhoods?
- (ii) To what extent do initial urban land structure and accessibility to the city center explain the observed spatial variation in conversion intensity?

By integrating exploratory spatial statistical techniques with spatial econometric modeling approaches, this study seeks to reveal the spatial dependence structure underlying land-use transitions and to provide empirical evidence that can support more effective urban growth management and agricultural land protection policies.

Study Area

Konya, one of the largest provinces in Türkiye in terms of area, is located in the Central Anatolia Region. The city is notable for its extensive agricultural areas, semi-arid climate conditions, and increasing urban growth dynamics in recent years. Konya plays an important role in the national economy, especially in terms of grain production and irrigation-based agricultural activities. However, in recent years, population growth, industrialization, transportation investments, and urban development projects have created significant pressures for transformation on agricultural areas surrounding the city.

The study area selected is the Konya city center and the neighborhoods located around it. The main reason for choosing the neighborhood scale is to reveal the spatial heterogeneity of urban sprawl processes in more detail. When examining land use changes in the city between 1990 and 2018, it is observed that urban areas have expanded especially in regions close to the city center and along certain transportation axes. This situation leads to the fragmentation of agricultural areas and their transformation into urban uses.

Konya's topographical structure is largely flat and suitable for agriculture, and this feature facilitates the spatial continuity of urban sprawl. Furthermore, industrial areas and transportation infrastructure investments developing around the city center are among the important spatial factors accelerating land-use transformation. In this context, Konya offers a suitable study area for examining the spatial patterns of agricultural land loss and

analyzing the spatial determinants of urban sprawl processes. Although the study area initially consisted of 122 neighbourhoods, only 65 neighbourhoods were included in the analysis (Figure 1). Neighbourhoods that did not contain any agricultural land in 1990 were excluded from the study in order to ensure a meaningful evaluation of agricultural-to-urban land conversion dynamics.

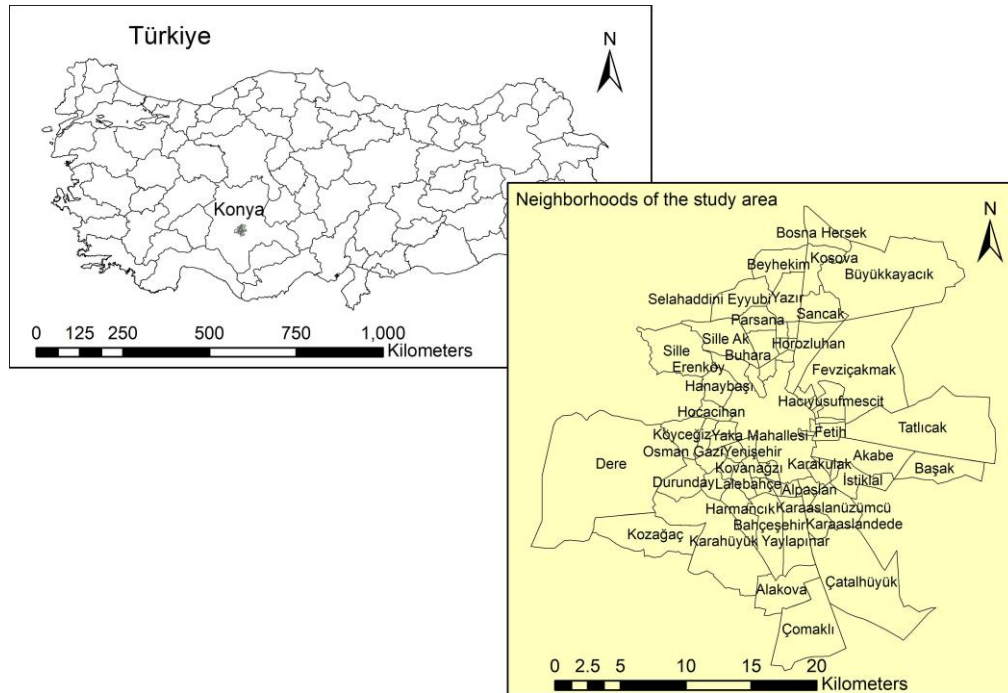


Figure 1. Location of the study area and neighborhood boundaries in Konya, Türkiye

Data Sources

This study utilized multiple spatial and statistical datasets to analyze agricultural-to-urban land conversion at the neighborhood level. Land-use data were derived from the CORINE Land Cover datasets for the years 1990 and 2018, which provide consistent and standardized information on land cover changes across Europe. Neighborhood boundary data were obtained from official administrative spatial datasets. Population statistics were collected from national statistical records corresponding to the study period. Distance-based accessibility variables, including distance to the city center and distance to major road networks, were calculated using geographic information system (GIS) techniques. All spatial data processing and spatial statistical analyses were conducted using ArcGIS Pro software. Exploratory spatial data analysis, including Global Moran's I and LISA, as well as regression modeling procedures, were performed within the GIS environment.

Method

Spatial Weights Matrix Construction

In spatial statistical analyses, defining spatial relationships among observational units is a fundamental step. In this study, spatial interaction among neighborhoods was represented by constructing a spatial weights matrix based on the Queen contiguity criterion. Under this approach, neighborhoods sharing either a common boundary or a vertex were considered spatial neighbors. This method is widely used in spatial econometric and spatial statistical applications due to its ability to capture both edge and corner adjacency relationships (Griffith & Paelinck, 2018). The spatial weights matrix was row-standardized to ensure comparability across neighborhoods with different numbers of neighbors. This standardization allows spatial lag effects to be interpreted as weighted averages of neighboring observations and helps prevent scale-related bias in spatial autocorrelation statistics (Elhorst, 2013).

Spatial Autocorrelation Analysis

To investigate whether agricultural to urban land conversion exhibits a systematic spatial pattern, Global Moran's I statistic was employed. Moran's I measures the degree of spatial autocorrelation by evaluating whether similar values of a variable tend to cluster in geographic space (Hussein et al., 2023; Zhang et al., 2023). A positive and statistically significant Moran's I value indicates spatial clustering, while a negative value suggests spatial dispersion (Majumder and Cajee, 2026).

$$Moran's Index = \frac{n \sum_i \sum_j w_{ij} (x_i - \bar{x})(x_j - \bar{x})}{S_0 \sum_i (x_i - \bar{x})^2}$$

Where n is the number of spatial units, x_i and x_j represent the rate of conversion of agricultural land to urban use in locations i and j , $\bar{x}$ is the mean conversion of agricultural land to urban use value, w_{ij} denotes the spatial weight between spatial units, and S_0 is the sum of all spatial weights ((Baek et al., 2022).

The statistical significance of Moran's I was evaluated using standardized z-scores and associated p-values (Kerry et al., 2024). In this study, the analysis aimed to determine whether neighborhoods experiencing high levels of agricultural land conversion were spatially concentrated rather than randomly distributed across the study area.

Local Spatial Autocorrelation (LISA)

While Global Moran's I provide an overall measure of spatial dependence, it does not reveal the specific locations of spatial clusters. Therefore, Local Indicators of Spatial Association (LISA) were used to identify localized spatial patterns of agricultural land conversion. LISA analysis enables the classification of neighborhoods into four categories: high-high clusters (areas with high conversion surrounded by similar areas), low-low clusters (areas with low conversion surrounded by similar areas), high-low outliers, and low-high outliers. These classifications provide valuable insights into spatial diffusion processes and help identify urban growth corridors as well as relatively stable agricultural zones (Anselin, 1995).

Regression Modeling

To examine the determinants of agricultural land conversion, an Ordinary Least Squares (OLS) regression model was employed. The dependent variable represents the proportion of agricultural land converted to urban use at the neighborhood level. Explanatory variables included the initial urban land ratio and distance to the city center, which are theoretically linked to urban growth dynamics and accessibility conditions. OLS is a non-spatial statistical method used to model the relationship between a dependent variable and one or more independent variables. It assumes that observations are spatially independent and randomly distributed, meaning the value at one location does not influence the value at another (Gao et al., 2020; Moindjié et al., 2022).

The regression model aims to quantify the extent to which spatial variation in agricultural land conversion can be explained by structural urban characteristics and location-based accessibility factors. The inclusion of these variables is consistent with urban economic theory, which suggests that land-use transitions are strongly influenced by proximity to urban cores and existing development patterns.

OLS regression provides a baseline analytical framework for evaluating the relationship between the intensity of agricultural land conversion and selected explanatory variables. In this study, the initial urban land ratio (URBAN1990_PCT) and distance to the city center (DIST_CENTER_KM) were included as independent predictors. The main dependent variable, AGRI_URBAN_PCT, measures the intensity of agricultural-to-urban land conversion by quantifying the percentage share of agricultural land transformed into urban use at the neighborhood level over the study period.

These variables were selected based on urban economic and land-use change theories, which emphasize the role of accessibility and existing urban structure in shaping urban expansion processes. Areas located closer to the city center generally experience stronger development pressure due to higher land values, better infrastructure availability, and increased economic activity. Similarly, neighborhoods with higher initial levels of urban development are more likely to undergo further land-use transformation through spatial diffusion and contiguous urban growth mechanisms.

The OLS model can be expressed as:

$$AGRI_URBAN_PCT = \beta_0 + \beta_1 URBAN1990_PCT + \beta_2 DIST_CENTER_KM + \varepsilon$$

where β represents the estimated regression coefficients and ε denotes the random error term.

Model Diagnostics

Several diagnostic tests were conducted to evaluate the validity and robustness of the regression model. Multicollinearity among explanatory variables was assessed using Variance Inflation Factor (VIF) values. Model stability and heteroskedasticity were evaluated through the Koenker (Breusch–Pagan) statistic, while the Jarque–Bera test was used to examine the normality of residuals.

Additionally, spatial autocorrelation in model residuals was examined using Moran's I statistic to determine whether spatial dependence remained after controlling for explanatory variables. This step is crucial in spatial econometric analysis, as significant residual spatial autocorrelation may indicate model misspecification or omitted spatial processes. Through these diagnostic procedures, the study ensures that the regression results provide reliable insights into the spatial determinants of agricultural land conversion.

Results

Figure 2 illustrates the spatial distribution of agricultural land converted to urban use between 1990 and 2018 together with the extent of urban areas in 1990. The map clearly demonstrates that urban expansion in Konya has occurred outward from the existing urban core toward peripheral agricultural zones.

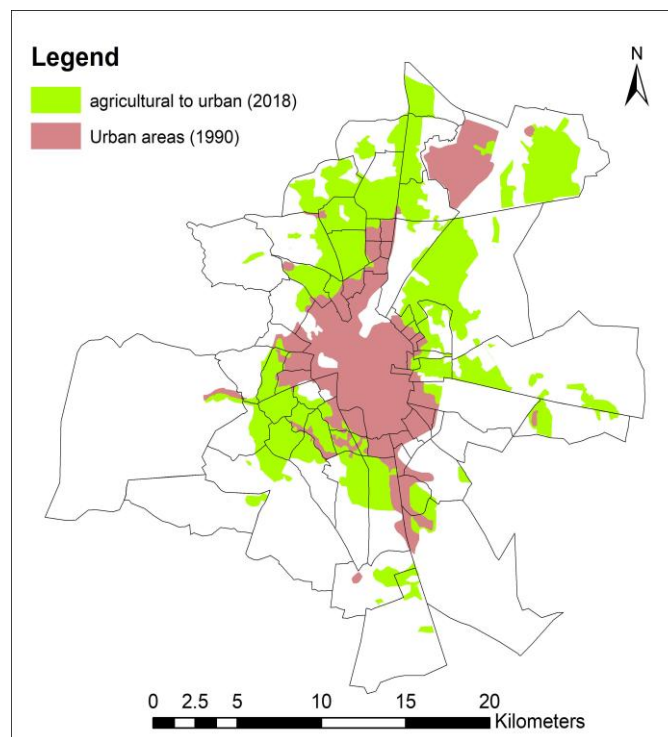


Figure 2. Spatial distribution of agricultural-to-urban land conversion between 1990 and 2018 and the extent of urban areas in 1990

Global Spatial Autocorrelation of Agricultural Land Conversion

The spatial distribution of agricultural-to-urban land conversion was first examined using the Global Moran's I statistic. The analysis results indicate a statistically significant positive spatial autocorrelation (Moran's I = 0.2037, $z = 2.72$, $p < 0.01$), suggesting that neighborhoods with similar levels of agricultural land conversion tend to be spatially clustered rather than randomly distributed across the study area (Table 1).

Table 1. Global Moran's I statistics for agricultural-to-urban land conversion

Moran's I	Expected Index	Variance	z-score	p-value
0.2037	-0.0154	0.0064	2.72	0.0065

This finding implies that agricultural land loss in Konya follows a spatially structured pattern influenced by urban expansion dynamics. High conversion intensity is not evenly distributed but instead concentrates in specific parts of the city, reflecting directional urban growth processes.

Local Spatial Clustering Patterns (LISA)

To further explore the spatial configuration of agricultural land conversion, LISA analysis was conducted. The LISA cluster map (Figure 3) reveals the presence of significant high-high spatial clusters, indicating neighborhoods with high conversion rates surrounded by similar high-conversion neighborhoods. These clusters are primarily concentrated along the southwestern urban growth corridor, highlighting the directional nature of urban expansion in Konya. The emergence of low-low clusters in peripheral areas suggests relatively stable agricultural zones where urban development pressure remains limited. The identification of spatial clusters demonstrates that agricultural land conversion is influenced not only by local characteristics but also by spatial interaction effects among neighboring neighborhoods. This pattern is consistent with spatial diffusion mechanisms commonly observed in urban growth processes.

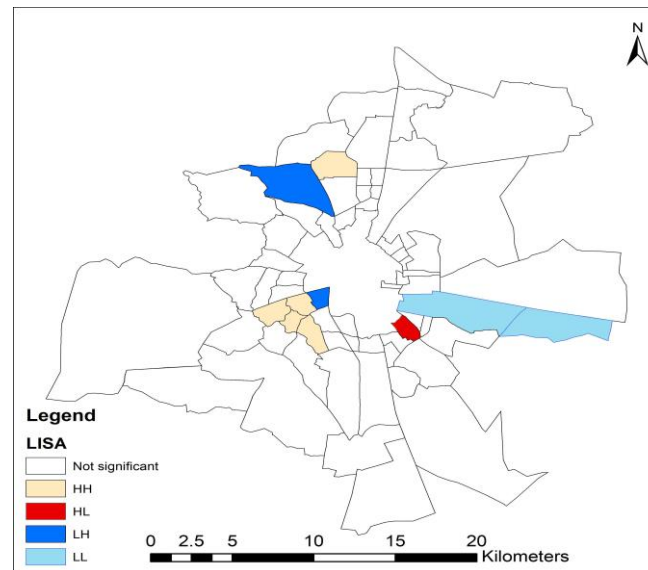


Figure 3. LISA cluster map illustrating spatial clusters of agricultural-to-urban land conversion intensity

Determinants of Agricultural Land Conversion: OLS Results

Table 2. OLS regression results for agricultural-to-urban land conversion

Variable	Coefficient	Std. Error	t-Statistic	p-value	VIF
Constant	112.31	15.51	7.24	0.047	-
URBAN1990_PCT	-0.566	0.157	-3.61	0.001	1.48
DIST_CENTER_KM	-6.84	1.63	-4.19	0.000	1.48
Model Diagnostics					
Indicator	Value				
Number of observations	65				
R ²	0.239				
Adjusted R ²	0.215				
AICc	671.96				
Koenker (BP) p-value	0.10				
Jarque-Bera p-value	0.22				

*Dependent variable: AGRI_URBAN_PCT

To quantify the factors influencing agricultural land conversion intensity, an Ordinary Least Squares (OLS) regression model was estimated. The model results (Table 2) indicate that both the initial urban land ratio (URBAN1990_PCT) and distance to the city center (DIST_CENTER_KM) are statistically significant predictors of agricultural-to-urban land conversion. The negative coefficient of the distance variable suggests that neighborhoods located closer to the urban core experience higher conversion intensity. Similarly, the negative relationship between the initial urban land ratio and agricultural land conversion implies that areas with a more developed urban structure tend to undergo further land transformation due to contiguous urban growth and spatial spillover effects. Although the model explains a moderate proportion of the spatial variation in agricultural land conversion ($R^2 = 0.239$), the regression results highlight the importance of accessibility conditions and existing urban development patterns in shaping land-use transitions.

Spatial Interpretation of Regression Findings

The combined results of spatial autocorrelation analysis and regression modeling indicate that agricultural land conversion in Konya is driven by spatially structured urban expansion processes. The presence of clustered high-conversion neighborhoods near the urban core supports the argument that land-use transformation follows accessibility gradients and development corridors. Furthermore, the spatial clustering identified through LISA analysis suggests that neighborhood-level land-use change is influenced by spillover dynamics, where urban expansion in one area increases the likelihood of conversion in adjacent areas. These findings emphasize the need to interpret regression results within a spatial econometric framework, acknowledging that land-use transitions are embedded in broader spatial interaction systems.

Discussion

The results of this study provide strong evidence that agricultural land conversion in Konya follows a spatially structured urban expansion pattern rather than occurring randomly across neighborhoods. The statistically significant positive Global Moran's I value confirms that neighborhoods experiencing high levels of agricultural-to-urban land conversion tend to be located near similar neighborhoods. This finding supports the argument that land-use transitions are influenced by spatial interaction mechanisms and neighborhood spillover effects.

The presence of high-high clusters identified through LISA analysis further indicates that urban growth in Konya is directional and corridor-based, particularly along the southwestern development axis. Such spatial clustering patterns are consistent with the concept of contiguous urban growth, where expansion occurs outward from existing urban cores through processes of spatial diffusion. Similar spatial dynamics have been reported in urban growth studies emphasizing the role of accessibility gradients and infrastructure-led development (Aljoufie et al., 2013; Anees et al., 2020).

Regression analysis results highlight the importance of initial urban structure and accessibility conditions in shaping agricultural land conversion. The negative relationship between distance to the city center and conversion intensity suggests that agricultural lands located closer to urban cores are more vulnerable to transformation (Liu et al., 2011; Huang et al., 2014). This finding aligns with classical urban economic theories, including bid-rent theory, which predicts that land values and development pressure decrease with increasing distance from the central business district (Chidi, 2019; Wang & Qiu, 2017).

The significance of the initial urban land ratio variable indicates that neighborhoods with an already established urban fabric are more likely to experience further land-use transformation. This pattern reflects path dependency in urban growth processes, where previous development creates structural conditions that facilitate additional expansion. The observed spatial clustering also suggests that land conversion decisions are not purely local but are influenced by development trends in adjacent neighborhoods (Krisztin et al., 2022).

Although the explanatory power of the regression model is moderate, the results underline the importance of incorporating spatial perspectives in land-use change analysis. Agricultural land loss is shaped not only by economic and accessibility factors but also by spatial dependence effects, which may arise from shared infrastructure, planning decisions, and market dynamics operating at broader spatial scales. From a planning perspective, the concentration of agricultural land conversion near urban growth corridors raises concerns regarding urban sprawl and the fragmentation of productive agricultural landscapes. If left unmanaged, such spatially clustered expansion may lead to irreversible land degradation, reduced agricultural productivity, and

increased pressure on natural resources. Therefore, spatially informed urban growth management strategies are essential for balancing development needs with agricultural land protection objectives.

Conclusion

This study examined the spatial distribution and determinants of agricultural-to-urban land conversion at the neighborhood level in Konya between 1990 and 2018. The findings demonstrate that agricultural land loss exhibits significant spatial clustering, indicating that urban expansion follows a structured and directional pattern rather than a random process. Global and local spatial autocorrelation analyses revealed that high conversion intensity is concentrated in neighborhoods located near the urban core and along major development corridors. Regression modeling further confirmed that initial urban land structure and proximity to the city center are key drivers of agricultural land transformation. These results highlight the critical role of accessibility and existing development patterns in shaping land-use change dynamics.

The study contributes to the growing body of literature emphasizing the importance of spatial analytical approaches in understanding urban growth processes. By integrating exploratory spatial statistics with regression-based analysis, the research provides empirical evidence that agricultural land conversion is influenced by spatial interaction mechanisms and neighborhood spillover effects. From a policy perspective, the results underline the necessity of proactive spatial planning and agricultural land conservation strategies. Identifying high-risk conversion zones through spatial analysis can support decision-makers in guiding urban expansion toward more sustainable development pathways. Future research may benefit from incorporating full spatial econometric models and additional socio-economic variables to further explore the complex drivers of land use transitions.

Scientific Ethics Declaration

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Determining the Impact of Urbanization on Green Spaces Using NDVI Analysis: The Case of Konya City

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Abstract: Rapid population growth and urbanization processes lead to significant changes in land use/cover, particularly in developing cities, often resulting in the reduction of green spaces. In this study, the impact of urbanization on vegetation Dynamics in Konya, located in the Central Anatolia Region of Türkiye, was analyzed using the Normalized Difference Vegetation Index (NDVI). Landsat satellite images from 2015 and 2025 were utilized to reveal the temporal and spatial variations in vegetation density. The analysis of NDVI values indicates that vegetation cover has notably decreased, particularly in areas experiencing urban expansion. Considering the development pattern of Konya over the last decade, it is evident that urban growth has predominantly occurred over agricultural lands and natural vegetation areas, leading to an overall declining trend in NDVI values. However, localized increases in NDVI were also observed in green spaces such as parks and recreational areas. This finding suggests that urbanization does not produce exclusively negative impacts; rather, its effects are strongly dependent on the nature of urban development and planning approaches. In conclusion, the case of Konya demonstrates that urbanization generally leads to a reduction in vegetation density. NDVI based analyses prove to be an effective tool for evaluating the ecological impacts of urban growth. The integration of such analyses into sustainable urban planning processes contributes to the protection and enhancement of green spaces and supports the development of scientifically informed strategies aimed at mitigating ecological impacts.

Keywords: NDVI, Urbanization, Vegetation change, Urban growth

Introduction

The rapid increase in global population and urbanization processes are leading to significant changes in land use and land cover, especially in developing countries. The most prominent feature of these changes is the decrease in vegetation. Cities, as centers where economic and social activities are concentrated, tend to experience higher spatial growth compared to surrounding areas (Rosyadi & Azahra, 2020). The limited availability of land in city centers is causing people to expand their settlements towards the periphery or outskirts of the city (Suprayogi & Rochani, 2021; Hafiz et al., 2025). In developing regions, the outward expansion of urban areas from the center leads to the depletion of natural areas and agricultural lands, the transformation of vegetation into an impermeable surface, and environmental problems such as transportation costs and traffic congestion, negatively impacting sustainability and urban development (Li et al., 2015; Magina et al., 2024; Hafiz et al., 2025).

Significant changes in land cover/use occur in urban areas as a result of population growth and economic development due to intense human activity and impact (Li et al., 2011). Increased construction leads to a decrease in green areas and disruption of the ecological balance. Therefore, quantitatively determining the effects of urbanization on vegetation is of great importance for sustainable urban planning. While remote sensing techniques and Geographic Information Systems (GIS) software are reliable, economical, and consistently effective tools for monitoring environmental changes in urban and rural areas (Selçuk et al., 2021;

Polat & Yalçın, 2020), the Normalized Difference Vegetation Index (NDVI) stands out as a method with high potential, particularly in determining land loss and monitoring vegetation (Yasin et al., 2022).

Indexes like NDVI provide reliable results in the analysis of vegetation and impermeable surfaces (Ullah et al., 2020). NDVI is analyzed through the normalized difference between near-infrared (NIR) and red bands obtained from satellites. High NDVI values indicate vegetation density, while low or negative values indicate settlement areas, bare surfaces, and water bodies (Hasyim et al., 2025).

This study aims to reveal the effect of urbanization on vegetation in Konya city over a 10-year period between 2015 and 2025 using NDVI analysis. Konya province was chosen as the study area due to its rapid urban development in recent years (Figure 1). In the study, NDVI values were calculated from satellite images of two different periods and increases and decreases in vegetation amount were revealed through temporal change analyses. In this respect, the study aims to evaluate the spatial and quantitative aspects of changes in urban growth and vegetation density.

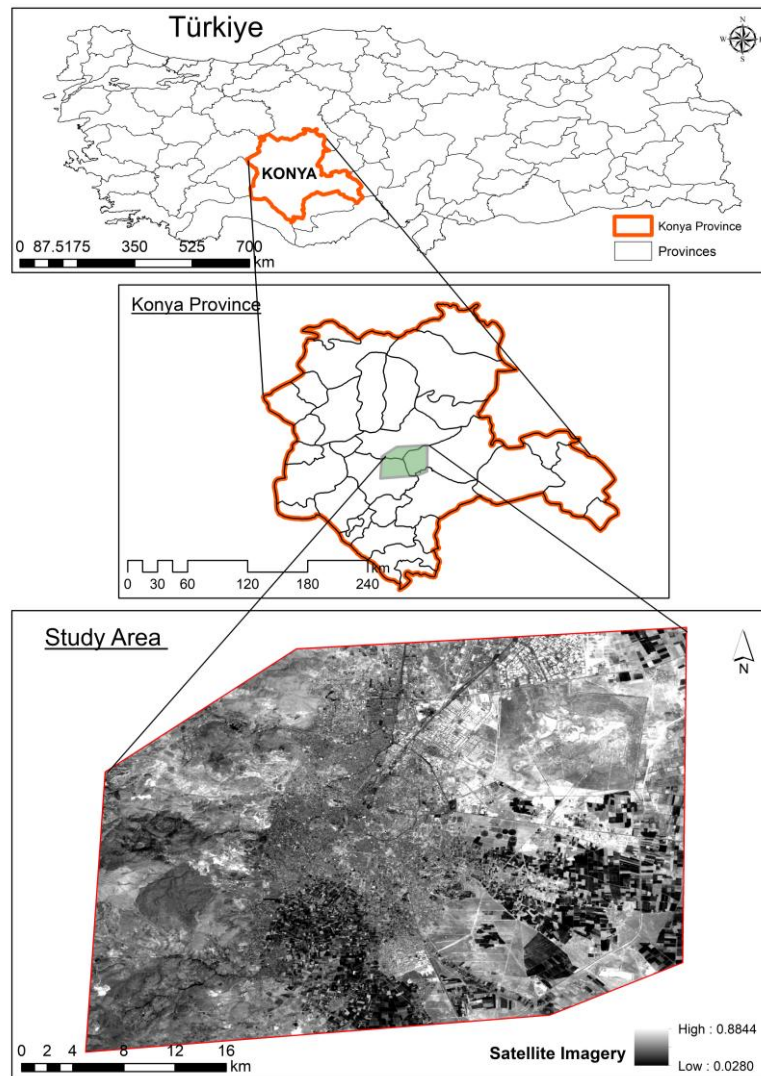


Figure 1. Konya location map.

Field of Study and Methodology

Located in the Central Anatolia Region, Konya province, situated at 37.87 degrees North latitude and 32.50 degrees East longitude, comprises the central districts of Selçuklu, Karatay, and Meram, and possesses the potential for continuous development and growth. The region is surrounded by extensive agricultural areas with semi-arid climate characteristics and exhibits changes in land use/cover due to urbanization pressures. With

these characteristics, the study area constitutes a suitable example for analyzing the relationship between urbanization and vegetation.

Landsat 8 satellite images from 2015-2025 were used in the study area, which was determined to represent Konya's urban fabric and its immediate development periphery. Atmospherically corrected visible and near-infrared bands were obtained via the Google Earth Engine (GEE) platform. Cloud filtering was applied to determine vegetation change, and median composites were created from images from the summer period (June-September). NDVI values were calculated to determine vegetation density.

Based on the findings, a Δ NDVI difference analysis was created between two periods using NDVI images. Δ NDVI values were divided into three classes: vegetation decrease, stable, and vegetation increase. The classification results were transferred from the GEE platform to ArcGIS software for visualization and spatial analysis. Finally, the area sizes corresponding to the classifications were calculated to reveal the quantitative dimension of the change. The data were organized according to the boundaries of the study area, and the classification results were visualized using appropriate symbology.

Results

According to the NDVI change analysis results for the period 2015-2025, vegetation decreased in an area of 187.61 km² (18.30%) in and around Konya city center, increased in an area of 79.91 km² (7.79%), and remained stable in an area of 757.93 km² (73.91%) (Table 1). The fact that the areas showing vegetation decrease are significantly greater than the areas showing increase indicates that the vegetation change in the study area is generally on a downward trend.

Table 1. Area distribution of NDVI change classes in the Konya area for the period 2015-2025

Class	Area (km ²)	Percentage (%)
Vegetation Decrease	187.61	18.30
Stable	757.93	73.91
Vegetation Increase	79.91	7.79
Total	1025.45	100.00

When the NDVI change map is examined, it is seen that vegetation reduction is spatially concentrated in areas where rapid construction has taken place in recent years, especially in the city center and its immediate surroundings (Figure 2). This situation indicates that urban growth is putting pressure on natural vegetation areas, especially along new development axes.

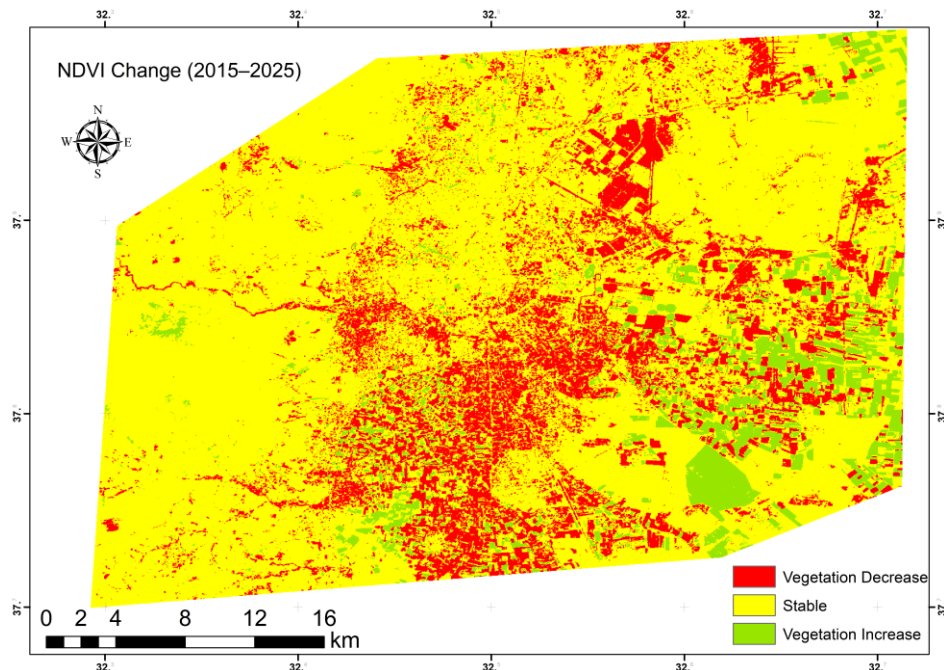


Figure 2. NDVI change distribution in Konya (2015-2025)

Comparing the NDVI maps for the years 2015-2025 reveals significant changes in the spatial distribution of vegetation (Figure 3). While the 2015 NDVI map shows that vegetation density is more distinctly preserved in areas close to the city center, the 2025 NDVI map shows a decrease in this density in and around the city center. This indicates that the impact of urban growth on vegetation is also a determining factor in spatial structure.

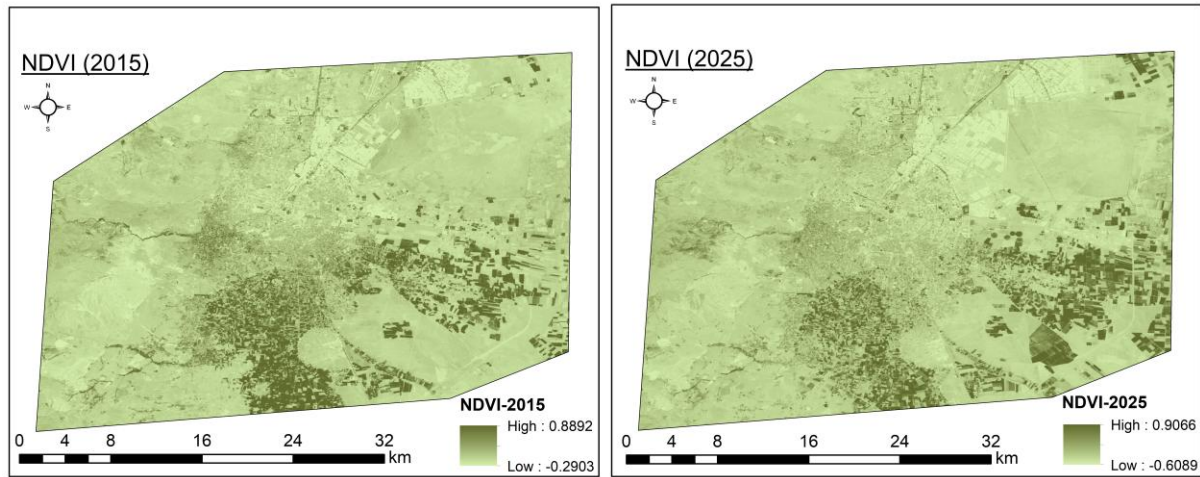


Figure 3. Comparative spatial distribution of NDVI 2015-2025

Discussion and Conclusions

The findings of this study are important in terms of comparing them with literature studies addressing the relationship between urbanization and vegetation and evaluating their results. The effects of urbanization on vegetation have been expressed with similar results in many studies conducted in different geographies. For example, in the study conducted by Weng & Lo (2001) in the Zhujiang Delta, they detected approximately a 30% decrease in NDVI values in urban areas. Similarly, Yasin et al. (2022), in their long-term evaluations, stated that urbanization increased fourfold and that there were significant losses in vegetation during this process. Bhomi et al. (2024) determined a loss of approximately 23.5 km² of forest cover between different periods. Magidi et al. (2022) revealed that vegetation density decreased in various land cover classes during the urbanization process. These findings show that the reducing effect of urbanization on vegetation yields similar results in different geographies. This study similarly shows that the NDVI value decreased by 18.3%, revealing the impact of urbanization on natural and semi-natural areas. A study by Hwang et al. (2022) in Seoul indicated a 39% greening trend in the pixel-based area, with only 7% showing brown discoloration. This suggests that afforestation and green space creation practices in urban areas can have positive effects on NDVI values. Indeed, this study also demonstrates that vegetation change exhibits regional differences and that the impact of urban growth varies spatially.

In conclusion, a decrease of 18.3% and an increase of 7.8% in vegetation were detected in the study area. The decrease in vegetation is largely attributed to the transformation of agricultural areas and natural vegetation into urbanized areas, transportation infrastructure, and other impermeable surfaces. The increases in vegetation observed in some regions are thought to reflect the impact of newly formed afforestation areas, parks, recreation areas, and irrigated areas.

NDVI-based analyses are used as an important tool in evaluating the ecological impacts of urban growth. Integrating such analyses into urban planning processes significantly contributes to the creation of sustainable cities, the protection and development of green spaces, and the adoption of scientifically based decisions aimed at maintaining ecological balance.

Recommendations

The use of NDVI-based monitoring approaches in urban planning processes is important for sustainable land management. Priority should be given to protecting and increasing green spaces, especially in rapidly urbanizing areas. Furthermore, promoting practices such as urban greening and afforestation will contribute to reducing the ecological impacts of urbanization.

Scientific Ethics Declaration

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* This study utilized the Google Earth Engine (GEE) platform for data processing and analysis.

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