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# An Alternative Automated Image Processing Tool for the Detection and Quantification of DNA Damage Using Comet Assay

Yakup Kutlu[1], Funda Turan[2], Ayşegül Ergenler[2*]

[1]Department of Computer Engineering Iskenderun Technical University, 31220 Iskenderun, Hatay, Türkiye. [2]Faculty of Marine Science and Technology, Iskenderun Technical University, 31220 Iskenderun, Hatay, Türkiye.

**Research Article**

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## Abstract

The comet test, also known as single cell gel electrophoresis (SCGE), is a fast, highly sensitive, and very simple technique used to identify DNA damage at the cellular level. This methodology integrates the simplicity of biochemical techniques for detecting DNA single strand breaks, soluble base labile sites, and connecting, in addition to the conventional cell-by-cell method used in cytogenetic assays. The Comet test, often used in Geno toxicological and biomonitoring investigations, typically involves the examination of prepared utilizing a fluorescent microscope (indicated by green light) and subsequent visible scoring. The fundamental goal of this study was to use image processing methods to automatically classify and measure DNA damage by analyzing comet test pictures, rather than relying on vision assessment of comet photographs.

**Keywords:** *Comet assay, DNA damage, Image processing technique*

## Introduction

One of the most used genotoxicity tests nowadays, Comet Analysis, differs from many others, such as sister chromatid exchange and micronucleus tests, in that it does not need cells at the division stage (Kleinjans and Van Schooten, 2002). This method involves using DNA helixes that have been damaged in some way, whether by heavy metals, pesticides, polychlorobiphenyls, pharmaceuticals, cosmetics, or UV radiation. Studying it is possible. Research on DNA damage and repair disorders caused by different substances in many mammalian cells often use the comet method, which is also known as the single cell gel electrophoresis technique. Furthermore, many aquatic creatures including crustaceans, fish, amphibians, and algae use this method (Parlak et al., 2011; Güner and Muranlı, 2013; Kaçar, 2015).



***Corrospondence:** Ayşegül Ergenler
aergenler@gmail.com

At an alkaline pH, the comet approach relies on the electrical field to facilitate the movement of DNA molecules with varying molecular weights and electrical charges. The cells or nucleoli are first immersed in agarose, lysed, and then stained with fluorescent dye using this technique. The alkaline electrophoresis buffer is used to carry out and neutralized the staining process. Figure 2 shows that while undamaged DNA does not create a comet in the fluorescence microscope-examined preparations, fragments of damaged DNA molecules with different molecular weights and electrical charges will move at different speeds in the electric field, giving the appearance of a comet. This method is dubbed "Comet" because to its outward look. (Frenzili et al., 2009; Şekeroğlu and Şekeroğlu, 2011; Parlak et al., 2011). The comet test is used to quantitatively assess DNA damage using metrics including tail moment, percentage of tail DNA, and tail length. In fish, the comet assay works well with a wide variety of tissues. Nevertheless, it is essential that the chosen tissue contains viable cells that are not actively mitotic (Da Rocha et al., 2009). Many research have used Single Cell Gel Electrophoresis (Comet assay) in fish liver, sperm cell suspensions, and gill epithelium (Zhou et al., 2006; Dietrich et al., 2007; Bony et al., 2010; Güner and Muranlı, 2013).

The comet assay technique is based on a simple idea. Electrophoresis follows lysis of cells immersed in agarose. A comet-shaped structure is formed by these relaxed loops of damaged DNA that extend to the anode. The next step is to use fluorescence microscopy to see comets stained with a DNA-binding dye. Measuring the size, shape, and quantity of DNA inside the comet is necessary for assessing the severity of DNA damage. One advantage of the comet assay is that it can be applied to any tissue of focus, recognizes multiple classes of DNA damage, and generates data at the single cell level. It also detects DNA strand breaks and alkali-labile sites by measuring the migration of DNA from immobilized nuclear DNA (Singh et al., 1988). Hartmann et al. (2003) also noted that the comet assay is advantageous. While most studies have focused on human cells, this method has shown promise in studying aquatic species. One may visually evaluate comets or use image analysis algorithms to rate them (Jha, 2008). A straightforward qualitative indicator of DNA damage is provided by visual scoring. All the same, it's very debatable (Konca et al., 2003). On the other hand, comet analysis software allows for very precise and repeatable readings. You may roughly categorize the comet analysis tools that are presently accessible as either automatic or manual. According to Konca et al. (2003), in manual analysis, a comet's head must be marked, the nucleus must be chosen, and threshold brightness values must be determined to separate the background. Here, we will build a computer-aided image processing approach to guarantee automated classification utilizing imaging processing methods, rather than manually analyzing the comet micrographs that were acquired by considering visually score.

## Material and Methods

A ten golden grey mullet, *Chelon auratus* specimens were collected Iskenderun Bay. Freshly dead fish samples were transported in a closely tight ice box filled with ice. The sampling process was carried out very quickly and sensitively with the least exposure to stress. Comet assay was performed on liver and gill tissues of mullet. The single cell gel electrophoresis was executed under alkaline conditions. The slides were neutralized with ice cold 0.4 M Tris buffer (pH 7.5) and stained with 80 ml ethidium bromide (20 mg/ml) and imaged with attachment of Leica fluorescent microscope integrated CC camera. Images of 100 cells (gill and liver) were visually scored from each sample as proposed by Collins (2004) by classifying the nucleoids. A sample images showing DNA cells in

Figure 1. In comet micrographs, images in 5 different categories are obtained as shown in Figure 2. Considering the visual scoring, the head part of the 0-degree image is clearly visible and the tail part is not formed, while as the Comet degree increases, the tail length in the image increases and the visibility of the head part decreases. Visual scoring technique or image processing software is used to classify and evaluate comet analysis images.

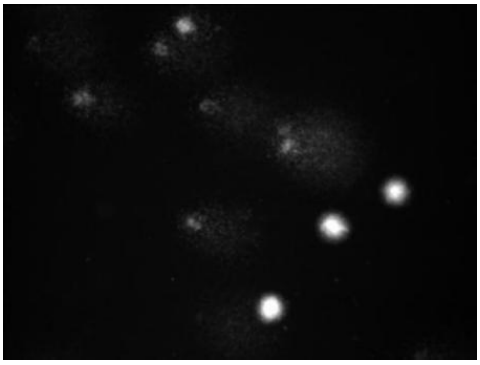


Figure 1. A sample images showing DNA cells.

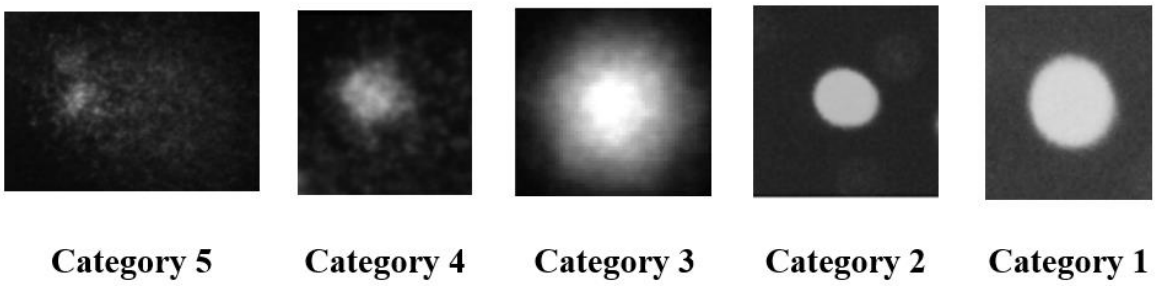



Figure 2. ROI images of a damaged DNA cell with a different label in the original labeled image.

***RGB and HSV Color Space***

The RGB color space is commonly used to represent colors in digital settings, consisting of three-element arrays with values of red, green, and blue. It can exhibit variations due to factors like reflection, viewing angle, object form, and the camera used. The HSV color space categorizes colors based on hue, saturation, and value, offering a framework more closely aligned with the human visual system. It is often used for segregating items based on color, with only the V (value) component affecting brightness. The HSV color space is derived from the transformation of RGB color space components and is used in various image processing tasks.Image processing methods can be tailored to address specific problems, requiring the use of multiple methodologies, specific criteria, and enhanced processing rates. Techniques for different challenges include picture modeling, image data extraction, and image comparison. The definition of operations is determined based on the problem statement, and the outcomes are analyzed. The identification of edges and corners is a significant field of study in image processing and image recognition systems, used in various domains such as segmentation and image recognition procedures(Saravanan et al., 2016).

### *Histogram*

A photo histogram, commonly referred to as a grayscale histogram, is a visual depiction of pixel values inside an image. The histogram of an image displays the pixel values at each data point within the picture, providing information on the quantity of pixels present. This enables the extraction of diverse information pertaining to the picture from the histogram. The precise positioning of pixels inside the picture remains indeterminable. Nevertheless, the brightness-darkness region values of the picture might provide broad information about it (Ekstrom, 2012; Roy et al., 2024). An image's gray level histogram visually represents the distribution of tones, including highlights, shadows, and the whole spectrum. Every picture has a unique histogram that can be seen via both the camera and the majority of post-processing tools. Figure 3 displays a histogram graph. The graph depicts a spectrum of 256 hues along the x-axis, ranging from darker shades on the left to brighter shades on the right. The vertical dimension of the curve on the y-axis represents the quantity of pixels linked to each tone.

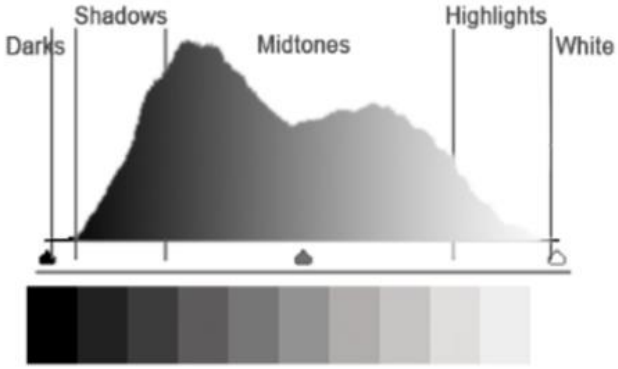



Figure 3. Histogram representation of an image (Glenys and Morgan, 2024).

### *Image Enhancement Methods*

The method of image enhancement encompasses several techniques that are designed to improve the visual representation of digital pictures and facilitate their use in sophisticated image processing analysis. The anticipated results of image enhancement procedures include the elimination of noise within the picture, the augmentation or concealment of borders, brightness, and designated characteristics. Prior to computing the parameters found in comet analysis, photographs must undergo certain procedures to render them suitable for analysis. The use of mathematical morphology is a well recognized methodology employed in the fields of image processing and computer vision. The study and processing of geometric structures based on set theory include the use of fundamental morphological operations, including dilatation, erosion, opening, and closure. During the process of dilation, the structural element undergoes expansion until it reaches the size of the object, at which point the center of the structural element aligns with the object as it moves across the picture. The morphological procedure known as erosion is used to reduce the size or thickness of an item that is shown in a binary picture (Gonzalez and Woods, 2002; Ekstrom, 2012).

Rule-based machine learning is a method of learning that identifies patterns in data by using a predefined set of rules or logic, and then employs these rules to make predictions about future occurrences.The aforementioned approach is a fundamental algorithm inside conventional statistical

and knowledge-based systems. One notable benefit of this technique is in the clarity and comprehensibility of the regulations. This practice guarantees the traceability and interpretability of the decision-making process. Moreover, training rule-based systems does not need substantial quantities of data (Weiss and Indurkhya, 1995; Li and Liu, 2014; Liu et al., 2022). Rule-based machine learning often depends on the use of expert information acquired inside the framework of a particular challenge. Specialists examine accessible data and establish the regulations of a certain domain. Typically, these rules are presented in a "If-This, Then That" structure and frequently depict significant connections.Numerous research have been conducted to build rule-based algorithms that include numerous effective approaches in numerical image processing and object extraction. These algorithms are expressed in the form of rules. This research contributes to the advancement of many methodologies based on these principles (Blaschke, 2010; Rotteinstener et al., 2005). The most favored approaches for automated object extraction, which is the essential structure of rule-based categorization, are object-based, computer-assisted, and expert systems that can function like the human brain. These methods are known for their speed and simplicity. According to Uzar (2012), rule-based classification approaches include the reduction of generic rules for object separation and grouping into particular classes. This process facilitates automated object extraction.

**Results and Discussion**

ZEISS Microscopy and BioMorph (Kutlu and Turan, 2018) softwares were used to obtain and analyze comet images. First of all, after obtaining the images, image processing analysis must be performed and a decision must be made in order to obtain meaningful results from these images. In these approaches, which are generally defined as computer-aided analysis, it is expected to create a generally to using data, ımage format conversion, filtering, segmentation, analyse, ROI, classification. Geometric transformation refers to mathematical operations applied to alter the position, size, orientation, or shape of an image or object. It is particularly necessary for fixing orientations, determining aspect ratios, and defining Regions of Interest (ROI). In this study, as shown in Figure 1, each DNA cell in the image is a region of interest and all of them must be determined and evaluated separately. For this reason, different image color spaces were examined to optimally identify these DNA cells in the image. In Figure 4, the HSV color space component images are provided. Using the V component in the HSV color space, edge detection with morphological enhancement has been performed. After that some image processing algorithm are evaluated such as filtering operation, morphological operation etc. to decrease noise and obtaine Ragion of interest (ROI). Median and Gaussian filters are applied to remove noise, and then the image is converted into a black and white (BW) image and image morphological improvement is performed on BW image after morphological enhancement, segmentation and labeling operation are evaluated on image. The results of these algorithms is shown in Figure 4 and Figure 5.

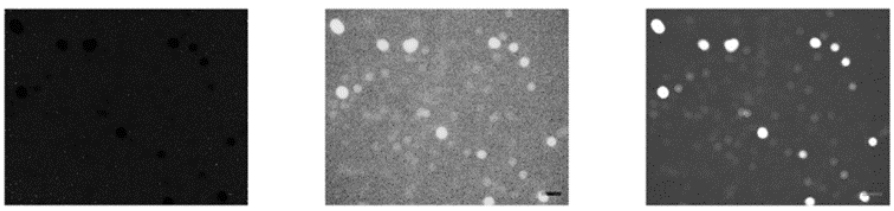


Figure 4. H, S, V components of the HSV format image are shown respectively.

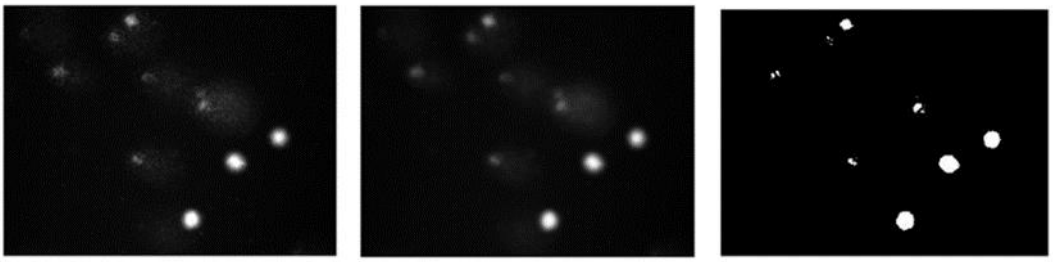


Figure 5. Sample images, median and gaussian filter, black and white conversion and morphological improvement algorithm results respectively.

After these processes, the distinct DNA cells are separated from the background, but it is necessary to evaluate each DNA cell individually. Therefore, the image obtained is subjected to a segmentation process, which aims to find each object in the image and to label them numerically. In Figure 6, after filtering, grayscale transformation, morphological enhancement, and segmentation, each DNA cell is labeled with different numbers. Thus, to emphasize that each DNA cell is separate from each other, they are visualized with different colors using image processing algorithms (Figure 7) and other areas have been designated as the background, with only the desired regions retained.

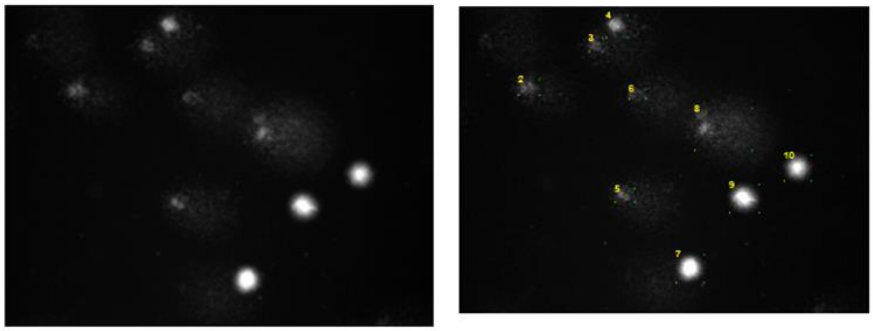


Figure 6. The original image and the labeled image of each possible damaged DNA cell after image processing, respectively.

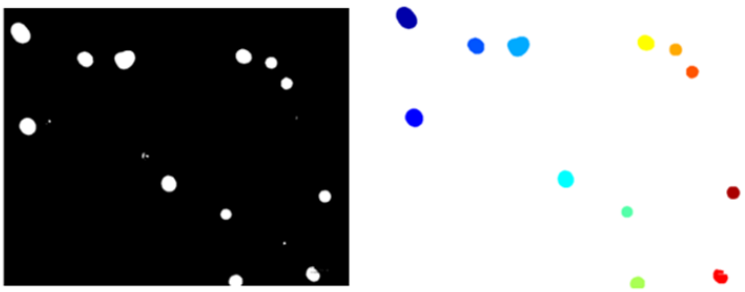


Figure 7. The result of segmentation and labeling algorithm.

In this step, each DNA cell is identified and separated as a ROI in the original image. As seen in Figure 8, each DNA cell is evaluated in its pure state and thus a new ROI is determined, which is

considered as a damaged DNA cell. Using this image, a new approach is presented to categorize comets based on their analysis scores. In this method, which we call the histogram-based analysis approach, a histogram graph is obtained for each ROI and difference of categories are determined by analyzing the histograms.

For a rule-based classification model, first, input output and the rules need to be established. The outputs of the models are categories as classes. The input of the models are histogram based features and morphological features. The length-width ratio of each DNA cell obtained through morphological operations is calculated. Our first rule is the length-width relationship; for class 1, this ratio is 1, whereas for class 2, this ratio is not exactly 1 but close to 1. For class 3, this ratio is smaller, and thus, classification can be done based on these rules.

Another rule is that, as shown in Figure 9, there is variation in histograms for each class. When all class histograms are analyzed, the values representing black color and white color are 0 and 255 respectively, and these regions are determined to be neglected. Therefore, values such as 5, 130, and 180 can be defined as boundary values within the range of 5-250. By examining the average of gray level color values between these boundaries, four different features have been identified as high, medium, low, and 0 (zero) none. For structures belonging to class 4, there are no values between 150 and 250, for class 3, there are no values beyond 180. While class 2 has values in every color range, for class 1, values below 50 are seen to be negligible (Figure 8 provides an example representation for class 2).

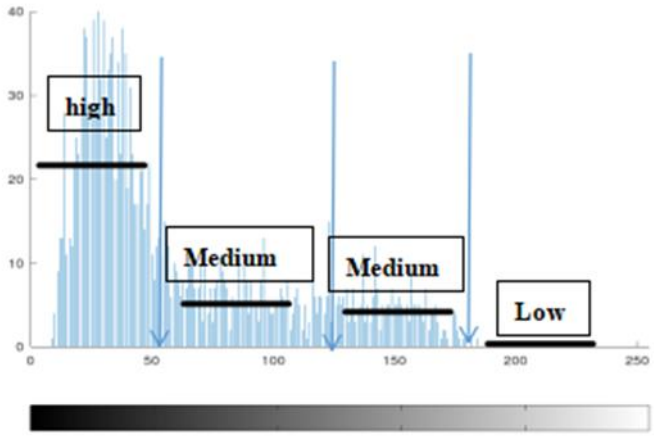
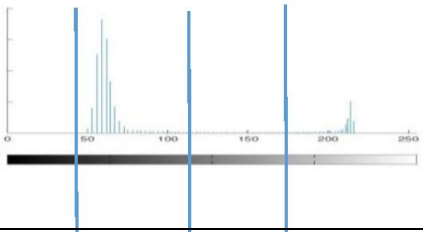
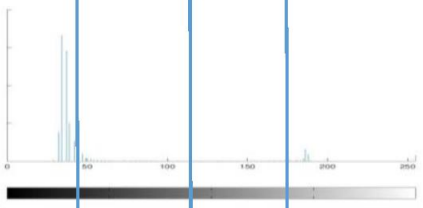
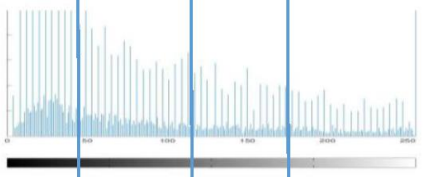
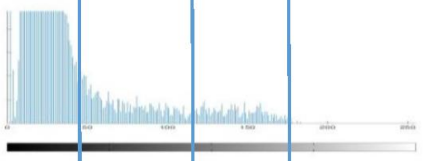
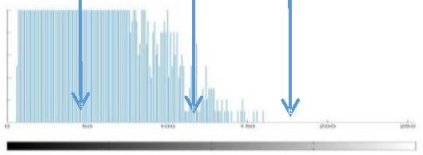



Figure 8. The example histogram has been divided into 4 regions, and the average values in these regions are evaluated as high, medium, low and zero.

Therefore, these 4 values obtained from the histogram graph are used as additional features for rule-based classification to distinguish these classes. Table 1, the boundary values obtained from the histogram and the evaluation of average density values as high, medium, low, and none according to these values are shown in detail.

Table 1. Visual representations of histogram graphs according to classes. Rules are formed based on four different features extracted from the values.

| | Range | Average value | |
|---|---|---|---|
| **Class1** | 5-50 | 0 |  |
| | 50-130 | **Medium** | |
| | 130-180 | **Medium** | |
| | 180-250 | **High** | |
| | **Range** | **Average value** | |
| **Class2** | 5-50 | **High** |  |
| | 50-130 | **Medium** | |
| | 130-180 | **Low** | |
| | 180-250 | **Low** | |
| | **Range** | **Average value** | |
| **Class3** | 5-50 | **High** |  |
| | 50-130 | **Medium** | |
| | 130-180 | **Medium** | |
| | 180-250 | 0 | |
| | **Range** | **Average value** | |
| **Class4** | 5-50 | **High** |  |
| | 50-130 | **Medium** | |
| | 130-180 | **Medium** | |
| | 180-250 | 0 | |
| | **Range** | **Average value** | |
| **Class5** | 5-50 | **High** |  |
| | 50-130 | **Medium** | |
| | 130-180 | **0** | |
| | 180-250 | 0 | |

This study presents the development and implementation of a completely automated tool for analyzing DNA damage in clinical applications. The program utilizes both fluorescence stained and silver stained comet assay pictures.The software under consideration comprises three classifiers that utilize Support Vector Machines (SVM) to classify the input images into four distinct categories. These categories include silver stained images exhibiting mild or moderate cell damage, silver stained images displaying severe cell destruction, luminous stained images exhibiting mild or moderate cell damage, and fluorescent stained images exhibiting severe cell damage.The algorithm will autonomously transition to the most appropriate segmentation technique depending on the image's class, ultimately resulting in improved measurement of DNA damage.

In summary, the efficacy of the suggested methodology is evaluated by a clinical expert, revealing its significant use in the realm of clinical research pertaining to DNA damage analysis and repair. The suggested approach is applicable in several fields like toxicology, pharmacogenomics, cancer, human epidemiology, and biomonitoring. It uses comet test pictures to analyze DNA damage.

**Acknowledgment**

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**Conflict of Interest**

The author declared that she have no competing interests.

**Ethical Approval Statements**

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study.

**Data Availability Statement**

The data used in the present study are available upon request from the corresponding author.

**References**

Blaschke, T. (2010). Object based image analysis for remote sensing. *ISPRS Journal of Photogrammetry and Remote Sensing*, 65(1), 2-16.

Cotelle, S., & Férard, J. F. (1999). Comet assay in genetic ecotoxicology: a review. *Environmental and Molecular Mutagenesis*, 34(4), 246-255.

Da Rocha, C. A. M., De Lima, P. D. L., Santos, R. A. D., & Burbano, R. M. R. (2009). Evaluation of genotoxic effects of xenobiotics in fishes using comet assay—a review. *Reviews in Fisheries Science*, 17(2), 170-173.

Ekstrom, M. P. (2012). Digital image processing techniques (Vol. 2). Academic Press.

Fatima, M., Usmani, N., Firdaus, F., Zafeer, M. F., Ahmad, S., Akhtar, K., Hossain, M. M. (2015). In vivo induction of antioxidant response and oxidative stress associated with genotoxicity and histopathological alteration in two commercial fish species due to heavy metals exposure in northern India (Kali) river. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 176, 17-30.

Frenzili, G., Nigro, M., Lyons, B. P. (2009). A review: Use of the comet assay in aquatic environmental impact assessments. *Mutation Research*, 681, 80-92.

Glenys Morgan, histogram image 17 Mart 2024, https://www.shootpetals.com/histogram.html#gallery[pageGallery]/2/

Güner, U., & Muranlı, F. D. G. (2013). Balıklarda tek hücre jel elektroforezi (comet assay). *Karadeniz Fen Bilimleri Dergisi*, 3(9), 103-114.

Hartmann, S., & Lucius, R. (2003). Modulation of host immune responses by nematode cystatins. International *Journal for Parasitology*, 33(11), 1291-1302.

Jha, A. N. (2008). Ecotoxicological applications and significance of the comet assay. *Mutagenesis*, 23(3), 207-221.

Kacar, A., Pazi, I., Gonul, T., Kucuksezgin, F. (2016). Marine pollution risk in a coastal city: use of an eco-genotoxic tool as a stress indicator in mussels from the Eastern Aegean Sea. *Environmental Science and Pollution Research*, 23(16), 16067-16078.

Kleinjans, J. C., van Schooten, F. J. (2002). Ecogenotoxicology: the evolving field. *Environmental Toxicology and Pharmacology*, 11(3-4), 173-179.

Klobučar, G. I., Štambuk, A., Pavlica, M., Perić, M. S., Hackenberger, B. K., Hylland, K. (2010). Genotoxicity monitoring of freshwater environments using caged carp (*Cyprinus carpio*). *Ecotoxicology*, 19(1), 77.

Końca, K., Lankoff, A., Banasik, A., Lisowska, H., Kuszewski, T., Góźdź, S., Wojcik, A. (2003). A cross-platform public domain PC image-analysis program for the comet assay. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 534(1-2), 15-20.

Lee, R. F., Steinert, S. (2003). Use of the single cell gel electrophoresis/comet assay for detecting DNA damage in aquatic (marine and freshwater) animals. *Mutation Research/Reviews in Mutation Research*, 544(1), 43-64.

Li, Xiaoli, and Bing Liu (2014): Data Classification: Algorithms and Applications "Rule-Based Classification." , 121-156. ISBN 9780367659141, Chapman & Hall

Liu, Y., Browne, W.N. & Xue, B. (2022). Visualizations for rule-based machine learning. *Natural Computing*, 21, 243–264 (2022).

Mitchelmore, C. L., Chipman, J. K. (1998). DNA strand breakage in aquatic organisms and the potential value of the comet assay in environmental monitoring. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 399(2), 135-147.

Rottensteiner, F., Trinder, J., Clode, S., Kubik, K. (2005). Using the Dempster–Shafer method for the fusion of LIDAR data and multi-spectral images for building detection. *Information Fusion*, 6(4), 283-300.

Singh, R. J., Hymowitz, T. (1988). The genomic relationship between Glycine max (L.) Merr. and G. soja Sieb. and Zucc. as revealed by pachytene chromosome analysis. *Theoretical and Applied Genetics*, 76(5), 705-711.

Şekeroğlu, Z. A., Şekeroğlu, V. (2011). Genetik toksisite testleri. *TÜBAV Bilim Dergisi*, 4(3), 221-229.

Uzar Dinlemek, M. (2012). Otomatik bina çıkarımı uygulamalarında çoklu algılama sistemi verilerinin kullanım olanaklarının analizi.

Kutlu, Y., Turan, C. (2018). An Intelligent Software for Measurements of Biological Materials: BioMorph. Natural and Engineering Sciences, 3(2), 225-232.

Weiss, S. M., Indurkhya, N. (1995). Rule-based machine learning methods for functional prediction. *Journal of Artificial Intelligence Research*, 3, 383-403.

Zhou, Y., Yang, H., Liu, S., Yuan, X., Mao, Y., Liu, Y., Zhang, F. (2006). Feeding and growth on bivalve biodeposits by the deposit feeder *Stichopus japonicus* Selenka (Echinodermata: Holothuroidea) co-cultured in lantern nets. *Aquaculture*, 256(1-4), 510-520.