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The assessment of bactericidal efficacy of seaside water samples and automated detection of sulfate-reducing bacteria using computer vision models

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A B S T R A C T

This study examines the presence of sulfate-reducing bacteria, specifically Desulfovibrio desulfuricans, in seawater samples from four coastal sites of the Caspian Sea: Neftchala, Bilgah, Sumgayit, and Pirallahi. The analysis aims to assess microbial dynamics and environmental factors across these locations. sulfate-reducing bacteria concentrations were evaluated using microbiological techniques, revealing significant contamination in the Pirallahi region, likely due to industrial activities. A computer vision model based on the “You only Look Once” algorithm was developed to enhance detection accuracy, automating the identification of Sulfate-Reducing Bacteria infected ampoules. The model demonstrated high accuracy with a mean Average Precision of 99.5 %, precision of 91.6 %, and recall of 98.7 %. This study highlights the potential of combining microbiological assessments with automated detection techniques to improve environmental monitoring. It offers insights into the relationship between industrial pollution and microbial contamination in sensitive marine ecosystems.

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I. Introduction

Sulfate-reducing bacteria (SRB) are a diverse group of anaerobic microorganisms that play a crucial role in sulfur and carbon cycling in marine environments. Among them, Desulfovibrio desulfuricans is one of the most studied due to its ability to reduce sulfate to sulfide, influencing the geochemical balance of marine sediments and contributing to biogeochemical processes such as organic matter degradation and nitrogen cycling [1]. The presence and activity of SRB in marine ecosystems are particularly significant because they can lead to the formation of corrosive hydrogen sulfide, posing environmental and industrial challenges, such as biofouling and infrastructure degradation [2]. Understanding the distribution and concentration of SRB, particularly in ecologically sensitive regions like the Caspian Sea, is essential for assessing their impact on both natural ecosystems and human activities.

The Caspian Sea, the world's largest inland body of water, is characterised by unique ecological conditions and diverse coastal environments. Previous studies have shown that microbial types, including SRB, are widely distributed in the sediments and waters of the Caspian Sea, where they participate in essential biogeochemical processes [1, 3]. However, the spatial distribution and concentration of SRB in different coastal locations of the Caspian Sea remain underexplored. This study focuses on analysing the concentration of Desulfovibrio desulfuricans in seawater samples collected from four coastal locations—Neftchala, Bilgah, Sumgayit, and Pirallahi. By examining the SRB distribution across these sites, we aim to provide insights into the microbial dynamics and environmental factors influencing their presence in this unique marine environment.

In recent years, the use of advanced technologies such as machine learning and computer vision has significantly enhanced our ability to monitor and analyse microbial communities in environmental samples. Object detection models, particularly those based on convolutional neural networks (CNNs), have demonstrated high accuracy in identifying microbial contamination in various contexts [4, 5]. In this study, we developed a computer vision (CV) model to automate the detection of SRB-infected ampoules, offering a novel approach to enhancing the efficiency and accuracy of SRB monitoring. This integration of microbiological analysis with automated detection techniques presents a comprehensive framework for managing microbial contamination in marine environments.

This study hypothesises that the concentration of sulfate-reducing bacteria (SRB), specifically Desulfovibrio desulfuricans, will vary significantly across the four coastal locations of the Caspian Sea — Neftchala, Bilgah, Sumgayit, and Pirallahi — due to differences in industrial activity and environmental conditions. It is expected that sites with higher industrial pollution, particularly Pirallahi, will exhibit higher SRB concentrations, as these bacteria thrive in environments where sulfate is abundant, a condition often exacerbated by industrial discharge. Furthermore, we hypothesise that the integration of computer vision models for SRB detection will demonstrate high accuracy, enabling more efficient and scalable environmental monitoring of microbial contamination in marine ecosystems. This approach is anticipated to streamline the identification process, reducing manual error and improving detection rates in comparison to traditional microbiological techniques.

II. Materials and methods

A. Sample Collection and Preparation

Water samples were collected from four coastal locations along the Caspian Sea in Azerbaijan: Neftchala, Bilgah, Pirallahi, and Sumgayit. Each location has distinct environmental and economic characteristics. Neftchala, a coastal city where Azerbaijan's most significant river flows into the Caspian Sea, is primarily influenced by fishing activities. Bilgah, a coastal region of Baku, is known for its tourism activities, while Pirallahi, another coastal area near Baku, is characterised by extensive oil drilling and processing facilities. Sumgayit, the second-largest city in Azerbaijan, is an industrial hub with numerous factories.

Samples were collected manually by driving to each location and drawing water directly from the seaside using vacuum-sealed buckets (Cordial Company, China) to ensure the preservation of the sample integrity during transport. The collected water samples were transported to the laboratory under controlled conditions to prevent contamination and maintain the samples' original state.

B. Quantification of Sulfate-Reducing Bacteria (SRB)

The concentration of sulfate-reducing bacteria (SRB) in seawater samples collected from the Caspian Sea coastal locations was analysed using a microbiological method adapted from our previous study [6-8]. Desulfovibrio desulfuricans are anaerobic bacteria known for reducing sulfates to sulfides and hydrogen, playing a pivotal role in biogeochemical cycles in marine environments [8-10]. Recent research continues to validate the use of Postgate B nutrient medium as optimal for the cultivation of SRB, where the pH is maintained between 7.0 and 7.5 to create suitable growth conditions [10-12].

To enhance SRB growth, the following additives were included in the Postgate B medium:

- Iron sulfate ($FeSO_4 \cdot 7H_2O$): 0.5-2 mL of a 5 % solution in 2 % hydrochloric acid.
- Sodium bicarbonate ($NaHCO_3$): 1 mL of a 5 % aqueous solution.
- Crystalline sodium sulfide ($Na_2S \cdot 9H_2O$): 1 mL of a solution prepared in 1% sodium carbonate (Na_2CO_3).

The experiments were conducted in 10 mL pre-sterilized test tubes (Cordial Company, China), each containing a water sample inoculated with SRB. The inoculated samples were maintained under anaerobic conditions in a thermostat at a controlled temperature of 30-32°C for an incubation period of 14 days, which allowed sufficient time for the SRB to grow and proliferate in the medium [6,11,12].

The concentration of bacteria in each sample was determined using the serial dilution technique, which involves diluting the sample stepwise and inoculating each dilution onto a growth medium to count colony-forming units (CFUs). The optical density measurements were also taken to estimate bacterial growth quantitatively. The results from different locations—Neftchala, Bilgah, Sumgayit, and Pirallahi—were compared to understand the spatial distribution of SRB concentrations across the Caspian Sea coastline. These results provide valuable insights into the microbial dynamics of SRB in diverse coastal environments, potentially influenced by factors such as pollution, industrial activity, and natural ecological variations [8,9].

C. Development of the Computer Vision Model for Object Detection

A computer vision (CV) model was developed using the YOLO (You Only Look Once) object detection algorithm to enhance the detection and monitoring of SRB contamination. The images of glass ampoules containing SRB cultures were manually collected from the laboratory. The collected images were then annotated using Roboflow, an online data annotation tool licensed under BY-NC-SA 4.0, to label infected and uninfected ampoules.

The annotated dataset was split into three subsets: 70% for training, 20% for validation, and 10% for testing. The YOLO model was trained on these datasets to detect SRB-infected ampoules accurately. The training process involved adjusting hyperparameters such as learning rate, batch size, and the number of epochs to optimise the model's performance. The evaluation metrics used to assess the model included mean Average Precision (mAP), precision, recall, box loss, class loss, and object loss. These metrics provided a comprehensive understanding of the model's ability to detect SRB contamination with high accuracy and reliability [13-15].

III. Results

A. Sulfate-reducing bacteria (SRB) Concentration in Seaside Water Samples

The concentration of sulfate-reducing bacteria (SRB) was analysed in seawater samples collected from four coastal locations along the Caspian Sea: Neftchala, Bilgah, Sumgayit, and Pirallahi. The analysis revealed that SRB was not detected in Neftchala, Bilgah, and Sumgayit samples, with SRB concentrations measuring 0 colony-forming units per millilitre (CFU/mL). Sulfate-reducing bacteria typically live in colonies, forming clusters and spreading along the walls of the test tubes. Based on the results from tube number 3, a 1 mL water sample taken from the Pirallahi area revealed the presence of thousands of sulfate-reducing bacteria (SRB). Given that the metabolic byproduct of SRB is H_2S, the amount of H_2S was analysed after quantifying the SRB. The sample from test tube number 3 was titrated using the iodometric titration method, revealing an H_2S concentration of 256 mg/L. This high level of H_2S suggests that, due to the acidic nature of the environment, the corrosion process is proceeding at an accelerated rate.

Given the limited dataset and the binary nature of SRB detection (presence or absence), Fisher's exact test (n=40) was applied to determine whether there was a statistically significant difference in the presence of SRB between Pirallahi and the other three locations (Neftchala, Bilgah, and Sumgayit). Fisher's exact test was chosen due to its suitability for small sample sizes and categorical data, comparing SRB presence across the four locations. The 2x2 contingency table (Table 2) below summarises the SRB detection across locations:

Table 1. 2x2 Contingency Table Comparing SRB Presence in Pirallahi vs. Other Locations

Location	SRB Present	SRB Absent	Total
Pirallahi	3	7	10
Other Locations	0	30	30
Total	3	37	40

Fisher's exact test revealed a statistically significant difference in SRB presence between Pirallahi and the other locations, with a p-value of 0.012 ($p < 0.05$). This result suggests that the presence of SRB in Pirallahi is unlikely to be due to random variation and is more likely associated with specific local environmental or industrial factors. The odds ratio was calculated as "infinity" (inf), further indicating that the odds of detecting SRB in Pirallahi are infinitely higher compared to the other locations where SRB was not detected at all.

B. Performance of the Computer Vision Model for Object Detection

A computer vision (CV) model based on the YOLO (You Only Look Once) object detection algorithm was developed to automate the detection of SRB contamination in glass ampoules. The model demonstrated high accuracy and robustness in detecting SRB-infected ampoules, as evidenced by the following performance metrics mentioned in Table 2.

Table 2. Models Performance metrics

Mean Average Precision (mAP)	Precision	Recall
99.5%	91.6%	98.7%

The mAP metric, which measures the accuracy of object detection models, was exceptionally high at 99.5 %, indicating the model's excellent performance in correctly identifying both true positives and negatives. Precision, which measures the proportion of accurate positive detections among all detections, was 91.6 %, while recall, the proportion of accurate positive detections among all actual positives, reached 98.7 %. These results demonstrate the model's effectiveness in minimising false positives and false negatives.

Figures 1-4 illustrate the training process and model performance:

- Figure 1: mAP scores over 300 epochs showing stable performance after initial training fluctuations.
- Figure 2: Box Loss over 300 epochs, indicating a steady decrease as the model learned to localise the detected objects better.
- Figure 3: Class Loss over 300 epochs, showing a continuous decrease, demonstrating the model's improved ability to classify detected objects correctly.
- Figure 4: Object Loss over 300 epochs, highlighting co nsistent learning in detecting objects against the background.

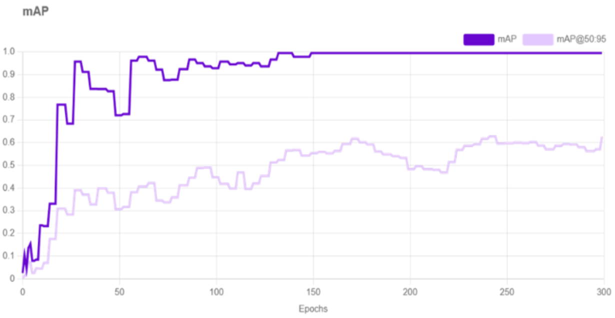

Fig 1. mAP Performace over 300 training epochs

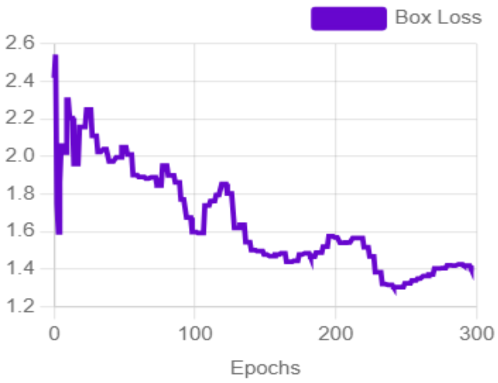

Fig 2. Box loss trend over 300 training epochs

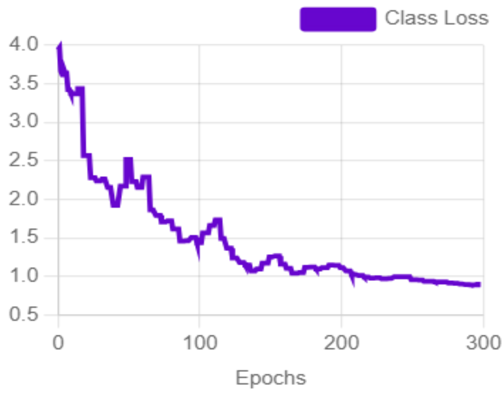

Fig 3. Class loss trend over 300 training epochs

Fig 4. Object loss trend over 300 training epochs

C. Visualization of Detection Results

Fig. 5 depicts the successful detection of SRB-infected ampoules using the trained computer vision model. The model accurately identifies the infected ampoules, demonstrating its utility in real-world applications for monitoring SRB contamination. The detection is visualised with bounding boxes, indicating the model's confidence in identifying the contamination.

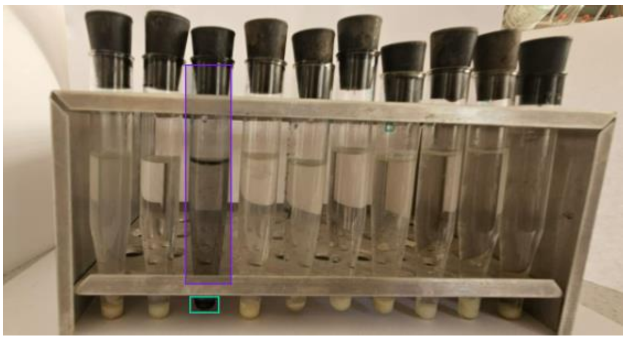

Fig. 5. Detection of SRB-infected ampoule

IV. Conclusion

This study highlights the localised presence of sulfate-reducing bacteria (SRB), specifically Desulfovibrio desulfuricans, in the coastal waters of the Caspian Sea, with a significant concentration detected only in Pirallahi, a region characterised by extensive oil drilling activities. This finding suggests a potential link between industrial pollution and microbial contamination in marine environments. Additionally, developing and successfully applying a computer vision model based on the YOLO algorithm for automated detection of SRB-infected ampoules demonstrates a powerful tool for environmental monitoring. The model achieved high accuracy, precision, and recall, underscoring its potential to enhance traditional microbiological assessments with rapid, automated analysis. Integrating advanced AI techniques with microbiological studies can provide a more efficient and scalable approach to managing microbial risks in marine and other sensitive ecosystems.

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