

nitrogen content (5.4%), indicating greater heterogeneity and the presence of non-collagenous components.

The results of sequential extraction indicate that the highest protein yield was achieved from the delimed pelt samples, especially during the first and second stages. For instance, for delimed pelt, the total protein amount determined by the Biuret method was 25.3 mg and 11.4 mg for the first and second extractions, respectively. These values were slightly lower for limed pelt. The use of the Biuret and Bradford methods allowed for precise estimation of the protein concentration, which in the most effective extracts from delimed pelt reached 52.71 μm 0.53 mg/100 mL (Biuret) and 63.51 μm 0.64 mg/100 mL (Bradford). The high concentration and purity of protein in these samples confirm that delimed pelt is the most promising raw material for obtaining biomedical collagen.

A key finding is the successful gel formation from the obtained collagen solutions. The collagen isolated from delimed pelt demonstrated the necessary ability for self-assembly (fibrillogenesis) and the formation of a stable three-dimensional network upon increasing the temperature to physiological (37°C) and neutralizing the pH. This indicates that the extraction process successfully preserved the native triple-helical structure of the collagen molecule, which is critically important for its biological activity and cell interaction. Moreover, rheological analysis confirmed that the resulting gels possess sufficient mechanical strength and elasticity, a prerequisite for use as scaffolds for cell culture in tissue engineering.

It was also established that collagen gels derived from waste exhibit a high biocompatibility profile, as evidenced by successful cell viability tests. This paves the way for their application in the development of medical devices, particularly wound dressings, which can accelerate the regeneration process by providing a favorable microenvironment for fibroblast proliferation and angiogenesis. The low immunogenicity inherent in purified collagen makes it an ideal candidate for use in the human body.

Conclusions. The conducted studies successfully demonstrated that leather production waste, particularly delimed pelt, can be effectively valorized as a source of high-quality collagen. The developed extraction methodology allows for obtaining a purified collagen product with a preserved native structure, which exhibits excellent gelling characteristics. The resulting collagen gels are functionally suitable for a wide range of biomedical applications, including tissue engineering and regenerative medicine. Thus, this research offers an environmentally and economically sound solution for industrial waste management, transforming it into valuable biomaterials.

INTEGRATED ASSESSMENT OF MICROBIOLOGICAL WATER SAFETY USING FISH TECHNIQUE AND AUTOMATED IMAGE ANALYSIS BASED ON DEEP LEARNING

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The demand for potable water is continuously increasing with the growing population, especially in urban areas, as well as due to industrial and commercial development. An additional challenge is posed by the adverse effects of climate change, which affect the availability of traditional water sources. In response to these issues, authorities worldwide are actively seeking

alternative water sources to meet the constantly rising demand. Among the most widely studied alternatives, roof-harvested rainwater is considered one of the most cost-effective options. It can be used for both potable and non-potable purposes, including irrigation, toilet flushing, car washing, showering, and laundry. However, there is still insufficient information regarding the microbiological quality of roof-collected rainwater and the associated health risks. Pathogenic microorganisms such as *Aeromonas spp.*, *Campylobacter spp.*, *Legionella spp.*, *Salmonella spp.*, *Giardia spp.*, and *Cryptosporidium spp.* may be present in fecal material from birds, mammals, and reptiles with access to roofs. During rainfall, this material can be washed into storage tanks, posing a potential health hazard.

Ensuring microbiological water safety remains a major public health challenge. Traditional monitoring methods based on time-consuming cultivation of fecal indicator bacteria (FIB) are considered insufficient. These approaches show poor correlation with the actual presence of many pathogens (e.g., viruses and protozoa) and fail to detect viable but non-culturable (VBNC) cells. Although VBNC cells do not grow on standard media, they retain metabolic activity and virulence, representing a hidden risk to consumers. Particular difficulties arise in monitoring pathogens such as *Campylobacter spp.*, which are among the leading causes of bacterial gastroenteritis worldwide. These bacteria, often detected in rainwater contaminated by animal feces, are difficult to culture and can enter the VBNC state in response to environmental stress. This highlights the urgent need to develop rapid, culture-independent detection methods that provide reliable quantitative data for modern Quantitative Microbial Risk Assessment (QMRA).

A promising approach to address these challenges is the use of Fluorescence In Situ Hybridization (FISH), which employs fluorescently labeled oligonucleotide probes targeting ribosomal RNA (rRNA). This method allows direct identification and quantification of specific bacterial cells in complex environmental samples. Furthermore, to overcome the limited sensitivity of conventional FISH toward cells with low metabolic activity (low rRNA content), a signal amplification variant known as CARD-FISH (Catalyzed Reporter Deposition FISH) can be applied. The enzymatic signal amplification in CARD-FISH enables clear visualization and detection of slow-growing or dormant cells, including those in the VBNC state.

However, manual microscopic analysis remains a major bottleneck—it is time-consuming, labor-intensive, and prone to operator subjectivity. To overcome these limitations, an essential component of the study is the implementation of automated microscopic image analysis based on computer vision techniques. This approach can rely on both classical image segmentation algorithms (thresholding, morphological operations) and advanced deep learning (DL) models based on convolutional neural networks (CNNs), such as U-Net, Cellpose, or StarDist. The procedure, integrating deep learning algorithms with image acquisition systems, represents an example of a computer vision-based framework enabling objective and automated microbiological assessment. Neural networks trained on annotated reference datasets substantially improve the accuracy, reproducibility, and robustness of analyses across variable environmental samples compared to traditional manual methods. The research strategy assumes quantitative validation of the developed DL models through comparison of automated cell counts with expert manual evaluation of microscopic fields.

The proposed concept aims to develop an integrated microbiological monitoring platform combining the molecular specificity of (CARD-)FISH with the objectivity and scalability of image analysis based on ML/DL algorithms. The application of deep learning methods enables automatic identification and enumeration of fluorescently labeled target cells, eliminating subjective interpretation and enhancing result reliability. This integrated solution represents a significant step toward rapid, standardized, and reproducible microbiological water safety assessment, supporting risk management, operational monitoring, and early warning processes in water and wastewater management.