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Original Article

Optimizing hygiene and microbial aspect of paper recycling: a sustainable approach for environmental conservation

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Abstract

This study explores microbial dynamics in paper recycling, emphasizing the significance of sustainable practices for environmental preservation. Samples were collected from various urban waste sources in Erbil city, Kurdistan Region, Iraq, including materials such as pizza boxes, cigarette packets, and coffee cups. Pure bacterial colonies were isolated using standard methods, and their morphological and physiological traits were characterized through biochemical tests. Identification of bacterial species followed established protocols. The study identified diverse bacterial species associated with paper waste, highlighting potential hygiene concerns in the recycling process. The findings of this study contribute to understanding the microbial ecology associated with paper waste and recycling processes. By optimizing hygiene measures and gaining insights into the microbial communities present, this research underscores the importance of sustainable practices in paper recycling to mitigate environmental impacts and promote a healthier ecosystem. Policies for future waste management and reduction of environmental risks have been proposed.

Keywords: Paper recycling, Microbial diagnosis, Infection exploring, Environmental protection.

1. Introduction

In the contemporary era of sustainability and environmental stewardship, the recycling of paper (Fig. 1) stands as a cornerstone in the edifice of ecological conservation efforts. Amidst growing concerns over deforestation, landfill space constraints, and the overarching need to reduce carbon footprints, the recycling of paper offers a beacon of hope, promising a reduced demand for virgin pulp and a significant curtailment of environmental degradation. However, the process of recycling paper is fraught with challenges, particularly concerning hygiene and the microbial aspects, which if not adequately managed, can undermine the environmental benefits, and pose health risks [1,2].

Paper and cardboard constitute a significant portion of municipal solid waste, largely comprising printed materials and packaging. This waste can either be processed together as a mixed lot or separately sorted into different qualities for recycling into various grades of paper. Generally, all paper grades can be manufactured from recycled material, with the exception of those requiring very high-quality standards. Therefore, source-separated paper waste is recycled following further sorting and processing. The use of recovered fiber as a primary raw material in the pulp and paper industry is significantly motivated by legislative support for recycling and its cost-effectiveness when compared to virgin pulp. One of the aims is to explore the management of paper waste within the framework of greenhouse gas (GHG) emissions accounting and its impact on global warming [3]. Greenhouse gas emissions can

Fig. 1. Wastepaper examples for recycling.

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be reduced by 2.28–2.90 gigatons using recycled paper [4]. Recycling paper conserves precious natural resources such as trees, water, and energy. By using recycled paper, fewer trees need to be harvested, preserving forests and biodiversity [5-7]. This process consumes less energy compared to the production of paper from virgin pulp. Energy savings helps reduce greenhouse gas emissions and reliance on non-renewable energy sources. [8, 9]. The amount of waste sent to landfills or incinerators will be reduced, thereby alleviating the burden on waste management infrastructure, and minimizing environmental pollution [10-12]. The paper recycling industry creates jobs in collection, sorting, processing, and manufacturing. Additionally, using recycled paper can save businesses money by reducing the need for raw materials and disposal costs [13, 14]. Recycling promotes a circular economy where materials are reused, recycled, and repurposed, contributing to a more sustainable and resource-efficient society [15-17]. Recycled paper can be used to produce a wide range of products beyond traditional printing and writing paper, including packaging materials, tissue paper, cardboard, insulation, and even construction materials [18-22], and significantly, can contribute to reducing the spread of infections by preventing the accumulation of discarded paper waste, which can serve as breeding grounds for harmful pathogens. Proper management of paper waste through recycling minimizes the risk of contamination and transmission of infectious diseases, thereby promoting public health and hygiene [23-26]. Providing opportunities for environmental education and awareness, and encouraging individuals and communities to adopt more sustainable consumption and waste management practices is another advantage of recycling technology [27-29]. Incorporating recycled paper into business operations demonstrates a commitment to environmental stewardship and sustainability, enhancing corporate reputation and brand image [30]. These advantages and applications highlight the multifaceted benefits of paper recycling, extending beyond environmental conservation to encompass economic, social, health, and educational dimensions. Integrating these considerations into recycling initiatives can amplify their impact and promote a more sustainable and healthier future [31-33]. Waste papers can harbor a variety of microorganisms, including bacteria, fungi, and viruses [34]. The types of infections commonly found on waste papers include bacterial infections such as *Escherichia coli* (*E. coli*) which are commonly found in the environment, particularly in fecal matter. *E. coli* can cause gastrointestinal infections and other serious health issues. *Staphylococcus* which found on the skin and in the respiratory tract. It can cause skin infections, respiratory infections, and food poisoning, and *Salmonella spp.* that often found in raw foods and waste. *Salmonella* can cause severe foodborne illnesses and gastrointestinal infections. *Staphylococcus epidermidis* a coagulase-negative bacterium, is a major bacterial species of human skin microbiota. *S. epidermidis* has been thought to maintain skin homeostasis by promoting cutaneous immune responses and preventing opportunistic pathogens from causing disease through colonization resistance [35-37]. *Kocuria spp.* bacteria are Gram-positive, coccoid organisms belonging to the family *Micrococcus* within the phylum Actinomycetal. While traditionally considered harmless commensals, *Kocuria* species have been increasingly implicated in human infections. These infections can include bacteremia, septicemia, endocarditis, peritonitis, urinary tract infections, and infections associated with indwelling medical devices such as central venous catheters and prosthetic joints Immunocompromised patients, those undergoing invasive medical procedures, or individuals with underlying chronic diseases such as chronic kidney disease or diabetes mellitus are particularly susceptible [38]. Coagulase-negative *Staphylococcus* (CoNS) is the most common etiological agent of several infections, including urinary tract infections (UTIs) *Staphylococcus haemolyticus* is a member of the skin microflora and is among the most isolated CoNS species in UTIs [39]. *Bacillus* species are a diverse group of bacteria that can cause infections, although many species are harmless or even beneficial. Some species of *Bacillus* are opportunistic pathogens and can cause a range of infections, especially in immunocompromised individuals or those with underlying health conditions [40]. The genus *Micrococcus* is not considered to be pathogenic. However, Micrococcus strains have been reported to cause various types of infections, usually as opportunistic pathogens [41]. Fungal infections like *Aspergillus spp.* a common mold can cause respiratory infections, especially in immunocompromised individuals. *Aspergillus Niger* and *Aspergillus* Fumigatus are specific species within the *Aspergillus* genus. *Aspergillus Niger* is commonly known for its black spores and is widely studied due to its industrial applications and potential to cause disease in humans (*Aspergillosis*), and *Penicillium spp.* is another mold that can cause respiratory problems and allergic reactions. *Candida spp.* is a yeast that can cause infections, particularly in the mouth, throat, and genitals. Viral infections like Norovirus a highly contagious virus that causes gastroenteritis. It can spread through contaminated surfaces, including wastepaper. Influenza virus can survive on surfaces like paper for a short period and cause respiratory infections [42-44]. Other fungal infections like *Candida albicans*. *Candida* species are one of the major causes of hospital-acquired infections in immunocompromised patients. The limited arsenal of antifungal drugs to treat *Candida* infections with the concomitant evolution of multidrug-resistant strains further complicates the management of these infections. Therefore, deploying novel strategies to surmount *Candida* infections requires immediate attention. The human body is a dynamic ecosystem having microbiota usually involving symbionts that benefit from the host, but in turn, may act as commensal organisms or affect positively (mutualism) or negatively (pathogenic) the physiology and nourishment of the host. The composition of human microbiota has garnered a lot of recent attention despite the common occurrence of *Candida spp.* [45].

This study delves into the complexities of maintaining optimal hygiene levels and controlling microbial growth during the paper recycling process. Central to this study is an evaluation of the types of infections, particularly bacterial, present on disposed papers, which constitute a significant but often overlooked environmental health concern. Our research has analyzed the prevalence and diversity of microbial life thriving in discarded paper products, revealing a hidden world of potential pathogens that could adversely affect human health. Significantly, this investigation has provided compelling evidence that through the adoption of advanced paper recycling technologies, it is possible to markedly reduce the presence of

these infections in our living environment. The process inherently facilitates the degradation and removal of microbial contaminants, thereby not only conserving resources and mitigating environmental impact but also enhancing public health by reducing exposure to harmful pathogens. Consequently, this study endeavors to unravel the microbial dynamics within the recycling process, identifying key factors that promote microbial growth and proposing targeted interventions to mitigate these risks.

2. Materials and methods

2.1. Materials

The blood agar medium was prepared according to standard protocols [46], with appropriate concentrations and proportions of each ingredient. The prepared agar medium was sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the agar medium was cooled to approximately 45-50°C and poured into sterile Petri dishes under aseptic conditions. The plates were allowed to solidify at room temperature before use.

2.2. Methods

2.2.1. Sample collection

Nine different types of wastepaper samples were collected from various locations near Erbil City, Kurdistan Region, Iraq. The samples included rubbish paper, pizza box, cigarette packet, coffee cup, money paper, tissue, finger box, egg box. Care was taken to collect samples representing various types of urban waste commonly found in the area.

2.2.2. Biochemical tests

Morphological and physiological tests were conducted, according to protocols, on the pure bacterial colonies to characterize their traits [47]. In this study, conventional methods such as catalase, indole and coagulase were performed based on the standard methods specified in microbiology reference texts. The catalase test was performed to identify organisms capable of producing the enzyme catalase. A small amount of bacterial colony was transferred onto a clean glass slide, and a drop of 3% hydrogen peroxide (H_2O_2) was added. The presence of bubbling (Oxygen release) indicated a positive result, while the absence of bubbles was recorded as a negative result [48]. The indole test determines the ability of bacteria to produce indole from the amino acid tryptophan. Bacteria were cultured in a medium containing tryptophan. After incubation, Kovac's reagent was added. Indole reacted with the reagent to form a red layer on the surface of the medium. No color change or the appearance of a yellowish layer indicated a negative result [49]. The coagulase test was used to differentiate *Staphylococcus aureus* (coagulase-positive) from other *Staphylococcus* species (coagulase-negative). Coagulase converted fibrinogen to fibrin, causing clotting. The test was performed as a slide test (detecting bound coagulase) or a tube test (detecting free coagulase) [50].

2.2.3. Identification of bacterial species

The characteristics of bacterial species were identified based on the results of biochemical tests and comparison with established profiles in microbiological databases. Further confirmation and classification of bacterial species were conducted following guidelines outlined in Bergey's Manual of Systematic Bacteriology [51].

2.3. Statistical analysis

Given the qualitative nature of this study, the primary focus was on identifying the presence or absence of various microbial species within the paper waste samples, rather than quantifying their abundance. The identification of bacterial and fungal species relied on established microbiological protocols, including morphological examination, biochemical testing, and comparison to reference profiles.

While quantitative statistical analysis was not performed, the frequency of occurrence of each identified species across the different types of paper waste was noted. This provided an understanding of the distribution of microbial species and potential associations with specific waste types. However, due to the qualitative nature of the data, these observations are presented descriptively rather than statistically.

The findings of this study contribute to our understanding of the microbial ecology of paper waste and highlight the importance of hygiene considerations in the recycling process. Future studies could build upon these findings by employing quantitative methods to measure microbial loads and assess the effectiveness of different hygiene interventions.

3. Result

3.1. Bacterial identification

In this study, the isolation and identification process revealed the presence of three main microbial species across the paper waste samples. Based on facilities and chemical reagents, only *Escherichia coli* and *Staphylococcus epidermidis* and *Kocuria spp.* and *Bacillus spp*. and *Micrococcus spp.* (Fig. 2, 3 and 4), as bacterial infections and

Fig. 2. Few colonies of *Staphylococcus epidermidis* and *Escherichia coli* Isolated.

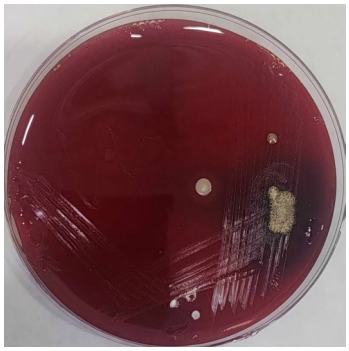

Fig. 3. Few Colonies of *Kocuria spp.* Isolated .

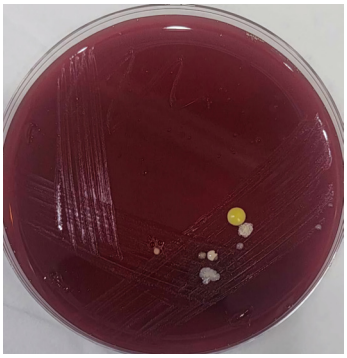

Fig. 4. Few Colonies of *Staphylococcus hemolytic*, *Micrococcus spp.* and *Bacillus spp.* Isolated .

Aspergillus fumigatus. as fungal infections (Fig. 5) were found and identified.

3.2. Biochemical characteristics of isolated bacterial colonies

In this study, biochemical tests were conducted to identify and characterize the bacterial colonies isolated from paper waste samples. The primary bacterial species identified were *Staphylococcus epidermidis*, *S. haemolyticus*, *Bacillus spp.*, *Bacillus spp.*, *Micrococcus spp.* and *Escherichia coli.* The following biochemical tests were performed: Catalase, Indole, and Coagulase tests (Table 1).

3.3. Gram staining test for *Escherichia coli*

The Gram staining test provides a quick and reliable method to differentiate *Escherichia coli* based on its cell wall properties [52]. *Escherichia coli* a Gram-Negative bacterium, does not retain the crystal violet stain and instead takes up the safranin counterstain, appearing pink under the microscope (Fig. 6). In contrast, *Escherichia coli*, a Gram-negative bacterium, takes up the counterstain and appears pink).

4. Discussion

This study aimed to investigate the microbial diversity present in different types of paper waste. The collected samples were subjected to microbial isolation and identification processes, focusing on being of bacterial and fungal species. The presence of pathogens on wastepaper poses significant risks to both environmental hygiene and public health. The biochemical characterization of the isolated bacterial colonies from paper waste samples confirmed the presence of *Staphylococcus epidermidis* and *Escherichia coli.* The catalase and coagulase tests were crucial in identifying *S. epidermidis*, while the catalase and indole tests were essential for identifying *E. coli.* These biochemical tests provide a reliable method for distinguishing between these bacterial species, aiding in the assessment of microbial contamination in paper waste. This study observed several notable trends and patterns in the microbial composition of paper waste samples such as environmental influence, relation between humidity and fungal growth, and microbial diversity. The type of environment from which the paper waste was collected played a significant role in determining the microbial species present. Office and cardboard wastes were rich in *S. epidermidis*, likely due to frequent human handling. In contrast, food-related wastes showed higher *E. coli* contamination, linked to potential food residue contamination. Samples from humid environments, such as bathroom waste, showed a higher prevalence of *Candida albicans*, highlighting the influence of moisture on fungal proliferation. Despite the focus on only a few species due to facility and reagent limitations, the findings underscore a diverse microbial presence in paper waste, influenced by environmental conditions and human activity. The presence of *Staphylococcus epidermidis* and *Escherichia coli*, both of which can cause serious infections, poses a direct health risk to individuals handling paper waste. *S. aureus* is known for causing skin infec-

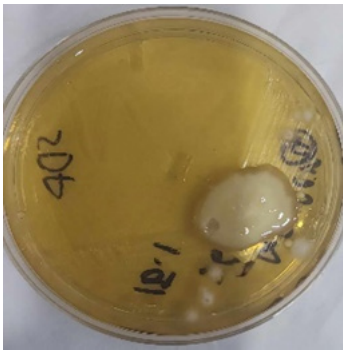

Fig. 5. Few colonies of *Candida albicans* Isolated.

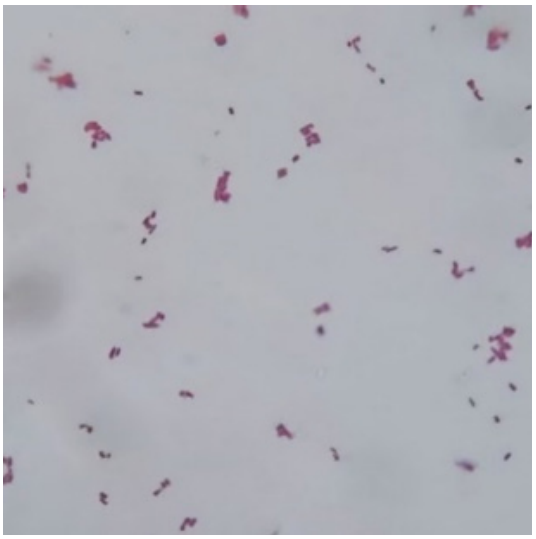

Fig. 6. Gram Staining Characteristics for *Escherichia coli.*

Table 1. Results from performed biochemical tests.

NO.	Bacteria	Catalase	Indole	Coagulase
1	*E. coli*	(+)	(+)	N/A
2	*S.epidermidis*	(+)	N/A	(-)
3	*S.haemolyticus*	(+)	N/A	(-)
4	*Bacillus spp.*	(+)	(-)	(-)
5	*Micrococcus spp.*	(+)	(-)	(-)

tions and respiratory conditions, while *E. coli* can lead to gastrointestinal illnesses [53, 54].

There is a potential for the spread of antibiotic-resistant strains of these bacteria, exacerbating the public health challenge and making infections harder to treat [55]. Improperly managed paper waste can act as a vector for spreading these microbes into the environment, contaminating soil and water sources [56]. The introduction of pathogenic microbes into ecosystems can disrupt local biodiversity, affecting native microbial communities and overall ecosystem health [57]. Proper waste management practices are essential to minimize the health and environmental risks associated with microbial contamination in paper waste. These practices ensure the safe handling, processing, and disposal of paper waste, thereby reducing the potential for microbial proliferation and transmission [58]. To mitigate the risks identified, some strategies can be implemented to optimize hygiene measures and microbial control in paper recycling processes and regular disinfection routines for surfaces and equipment in recycling facilities to reduce microbial load should be performed [59-61]. Protocols should be established to separate heavily contaminated paper waste from cleaner waste streams in order to prevent cross-contamination. Additionally, paper waste should be stored in dry, well-ventilated areas to inhibit the growth of moisture-dependent microbes, such as Aspergillus fumigatus. This revision breaks the information into two clear sentences, enhancing readability and comprehension [62]. Based on microbial diversity in paper waste, the dangers of infections on environmental hygiene and public health have been considered. The presence of pathogens such as *Escherichia coli* (*E. coli*), *Staphylococcus epidermidis*, *Staphylococcus hemolytic*, *Micrococcus spp.* and *Bacillus spp.* and *Candida albicans* on wastepaper poses significant risks to both environmental hygiene and public health. Mold spores and other airborne pathogens from contaminated paper can cause respiratory issues, especially in individuals with preexisting conditions like asthma or weakened immune systems. Bacteria like *Salmonella* and *E. coli* can contaminate food through indirect contact with wastepaper, leading to food poisoning and gastrointestinal infections. Direct contact with contaminated paper can lead to skin infections, particularly with bacteria like *Staphylococcus epidermidis* [63, 64].

This study revealed a diverse microbial community in paper waste samples, with specific bacterial and fungal species demonstrating distinct prevalence patterns based on the type of waste and environmental conditions. *Staphylococcus epidermidis*, *Staphylococcus hemolytic*, *Escherichia coli*, *Kocuria spp.*, *Micrococcus spp.* and *Bacillus spp.* and *Candida albicans* were the primary species identified, each showing unique associations with different waste types. These findings highlight the importance of proper waste management to mitigate potential health risks associated with microbial contamination. Optimizing hygiene and microbial aspects of paper recycling is essential for environmental conservation and public health [65]. By understanding the types of infections present on waste papers and their potential dangers, we can appreciate the importance of recycling as a sustainable solution. Recycling not only removes harmful pathogens from our environment but also promotes healthier living conditions and contributes to the overall well-being of our planet [66]. By recycling paper, the amount of wastepaper that ends up in landfills be reduced, thereby decreasing the potential for environmental contamination, thereby decreasing the opportunities for pathogens to spread. Considering the hygiene concepts in the recycling process ensures that the resulting paper products are free from harmful microorganisms, contributing to better hygiene standards in homes, offices, and public spaces. Recycling conserves trees and reduces the demand for virgin paper, leading to less deforestation and a healthier ecosystem via lead to sustainable resource management. Better sorting and cleaning, enzyme-based processes using advanced technology, increasing fiber quality by reinforcement techniques and new additives also sustainable practices via closed-loop systems and green chemistry are advanced protocols [67, 68].

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Conflict of interests
The author has no conflicts with any step of the article preparation.

Consent for publications
The author read and approved the final manuscript for publication.

Ethics approval and consent to participate
No human or animals were used in the present research.

Informed consent
The authors declare that no patients were used in this study.

Availability of data and material
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions
Soma Majedi: Research design and supervision; Ahmed Bahram Wlia: Perform all laboratory procedures.

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