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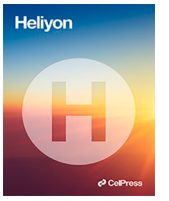

Research article

Characterization of the microbiological effects of pomegranate, banana, and mandarin peels on water under laboratory conditions

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ABSTRACT

The protection of natural resources, especially water resources, is attracting international attention by researchers in order to achieve sustainable development. Inadequate treatment of waste from the food industry leads to pollution of ground and surface water through leachate or direct discharge of waste. To understand the impact of inappropriate discharge of these wastes, the microbial groups (bacteria, yeasts, and moulds) of pomegranate peel (PP), banana peel (BP), mandarin peel (MP) and the water in which each waste is decomposed were studied. The microbial groups were isolated, quantified, and purified by elective media at 30 °C. The fungal microbial isolates were identified by their macro and microscopic characteristics. The findings show that the highest density of bacteria ($3.95 \pm 0.48 \times 10^5$ CFU/ml) was obtained in the water in which the BP is decomposed, the highest density of yeasts ($4.59 \pm 0.52 \times 10^5$ CFU/ml) and moulds ($4.10 \pm 0.34 \times 10^5$ CFU/ml) was recorded in the water in which the PP is decomposed compared to the microbial density of the initial and the final control water. The fungal microbial groups were more diverse between the decomposition waters; the waters in which PP and BP are decomposed showed a higher diversity with 9 and 8 species respectively, compared to the water in which MP is decomposed with 7 species, and compared to the initial and the final control water with 3 and 5 species respectively. Conclusively, direct dumping or landfilling of food waste in general, PP, BP, and MP in particular can cause pollution of surface and groundwater by microorganisms that can be harmful.

1. . Introduction

Over the last decade, the world has been facing a continuous increase in the amount of food wastes generally, and pomegranate, banana, and mandarin peels particularly [1]. This increase is due to population growth, and changes in the production and consumption patterns of people [1]. The mismanagement of food wastes leads to the degradation of the living and natural environment, as well as having negative effects on human health [2]. Landfilling is the main food wastes disposal practice worldwide especially in developing countries like Morocco [3]. Most Moroccan landfills are uncontrolled and open dumps [4]. The unavailability of an adequate treatment system for organic wastes can lead to groundwater pollution by leachate and contamination of the marine environment and surface waters by direct disposal of wastes [5,6].

Several studies have focused on this topic, such as the study by Chofqi [7] which reported that the physicochemical assessment of

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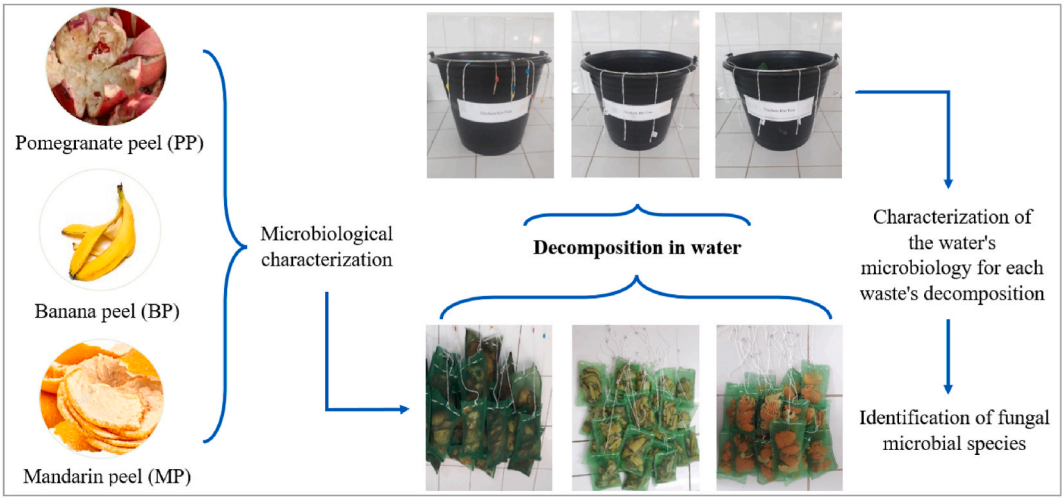


Fig. 1. The experimental design explaining the experiment performed.

well water in the region of El Jadida reveals a significant degradation of this water following its contamination by percolating water generated by the landfill of the city of El Jadida, Morocco. Landfills release many pollutants through landfill leachates and landfill gases consisting of CO_2, CH_4, CO, H_2S [8,9]. Additionally, landfill leachate, mainly those that are uncontrolled, interact with soil, surface water and groundwater and negatively affect their quality [10], also negatively affect populated biodiversity [11]. Numerous other studies have reported that the main groundwater pollutants from landfills include chloride, sodium, ammonium, total hardness, total dissolved solids, organic matter, as well as chemical oxygen demand, heavy metals and phosphate [8,12]. Indeed, several studies on the impact of landfills on human health have revealed intoxications caused by: soil, air, and polluted surface and groundwater [9, 13]. Consumption of plant products from soil surrounding landfills [8] and fish from contaminated waters [14]. Transmission of microbial infections by insects that thrive in landfills in contact with rats and other disease vectors [14,15]. Low birth weight; birth defects; neurological diseases; nausea and vomiting; increased cancer cases [16,17], and respiratory diseases [18,19] have also been attributed to landfills.

It is very clear that food wastes present several adverse effects on the environment and public health, however, there is a need for more in-depth studies on the impact of these wastes, especially the impact on water, whereas in most of the published scientific studies, researchers have mainly focused on the physicochemical study of food wastes and their impacts on water, while microbiological studies of the impact of food wastes on water remain unsatisfactory until the present study. For the previously mentioned reasons, the objective of the current research is the study of the microbiological impact of PP, BP and MP after decomposition in water, through a microbiological characterization and identification of the fungal microbial species of the water in which each waste is decomposed. In order to understand the microbiological impact of food waste on water resources, both through direct discharge and through leachate from landfills and/or poor treatment of food waste.

2. Materials and methods

2.1. Biological material

In the current study, the microbiological effects of three different types of food waste were studied. PP, BP, and MP were collected in their fresh state by households, festival organizers, and juice sellers in the city of Fez, Morocco.

The three wastes were first cut into pieces of 3–5 cm^2 and put in mesh bags in their fresh form (15 cm long and 6 cm wide). 45 mesh bags for each type of waste were placed in 20 L polythene buckets. For each type of waste, 3 buckets were used, and 15 mesh bags filled with the waste and tied with 3 mm diameter nylon threads marked with tickets (name and initial weight of waste) were randomly introduced into a bucket filled with 15 L of spring water (Fig. 1). The controls were prepared in the same way as the test, but did not contain any peels.

2.2. Quantification of microbial groups

Microbial group characterization was carried out before decomposition and after 6 months of PP, BP, and MP decomposition in the spring water. Before decomposition, microbiological characterization of PP, BP, MP, and the initial control water were performed. After 6 months, samples of the water in which each waste was decomposed were taken according to known microbiological standards (asepsis, homogeneity, standard condition, etc.). The quantification of microbial groups was conducted by plating 1 ml of the dilutions (10^{-1} to 10^{-5}) in the mass, according to El Barnossi et al. [20]. Enriched elective media were used: nutrient agar, YPG (Yeast, Peptone, Glucose), and malt agar, which were enriched by the addition of 7% (v/v) peel extract for the characterization of bacteria, yeast, and

Table 1
Microbial group density of MP, BP, PP and initial control water.

	PP	BP	MP	Initial control water
Bacteria CFU/gDw (x10^6)	30.4 ± 1.97 ^{c}	21.1 ± 4.79 ^{b}	2.7 ± 2.4 ^{a}	0.00185 ± 0.00138 ^{a}
Yeasts CFU/gDw (x10^6)	54.6 ± 2.22 ^{a}	1.27 ± 0.47 ^{b}	48.6 ± 6.9 ^{a}	0.037 ± 0.0144 ^{b}
Moulds CFU/gDw (x10^6)	49.2 ± 6.1 ^{c}	4.00 ± 1.2 ^{b}	11.4 ± 1.9 ^{a}	0.052 ± 0.033 ^{b}

Mean values (±SD, n = 3) followed by different letters in the same row are significantly different according to Tukey's multiple range tests at $p < 0.05$.

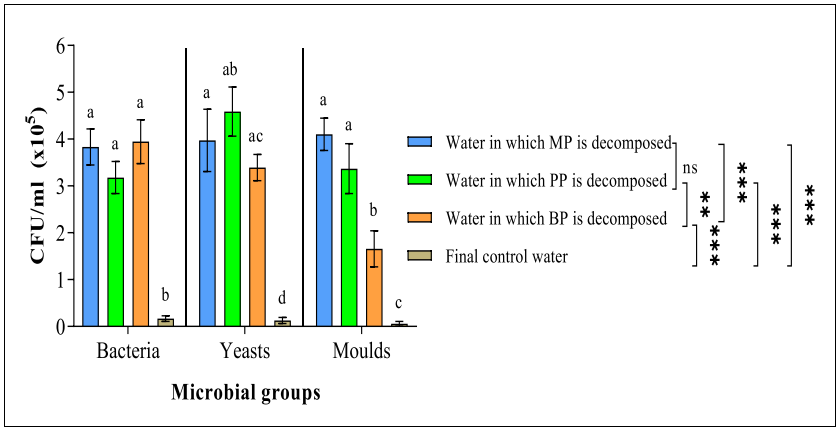


Fig. 2. Microbial group density of the water in which each waste is decomposed (PP, BP, and MP) compared to the final control water. Means (±SD, n = 3) marked with the same letter do not indicate a significant difference according to Tow-way ANOVA and Tukey's multiple interval tests at $p < 0.05$.

moulds, respectively. After incubation at 30 °C during 24 h for bacteria, 48 h for yeasts and 7 days for moulds, microbial colonies were enumerated, and the results were expressed as CFU/gDw of PP, BP, MP, and CFU/ml of water in which each waste is decomposed and of the initial and final control water [21,22].

2.3. Fungal biodiversity

The identification of each isolated and purified fungal colony was carried out. Macroscopic and microscopic characteristics were used as standard methods.

2.3.1. Macroscopic examinations

We have based on the following characteristics: On the front; colour, consistency (hairless, downy, powdery, plastery, silky, etc.), surface (flat, domed, wrinkled, cerebriform, etc.), presence of fine or broad rays. On the reverse side; colour, depth of arborization, pigment. And growth is expressed in cm per day or the number of days it took to invade the Petri dish.

2.3.2. Microscopic examinations

Microscopic examinations of moulds can be performed by several techniques, including: the flag technique: scotch tape between lactophenol blue, slide/lamellar examination; the scotch tape technique: idem; the agar slide culture technique and the agar square technique (two slides). The microscopic characters of each isolate were photographed, using a camera-equipped light microscope, from the fresh and dried smears and then stained with a lactophenol blue solution [23,24]. For the microscopic examinations, we based on: the description of the mycelium; diameter of filaments, presence or absence of septum, pigmentation of hyphae (granulations), branching and its mode, particular formations. The spore apparatus; sporangiophore with a sporangium, conidiophore with branched or unbranched spore columns. Spores; internal (sporangiosphores in sporangium), external (conidia or conidiospores on conidiophore).

2.3.3. Classification

For the determination of the genus of each fungal colony obtained, we adopted the Saccardo classification system [25,26]. The identification of species was carried out by reference to different identification keys [23,27–30]. The system adopted is that described by Kirk et al. [31].

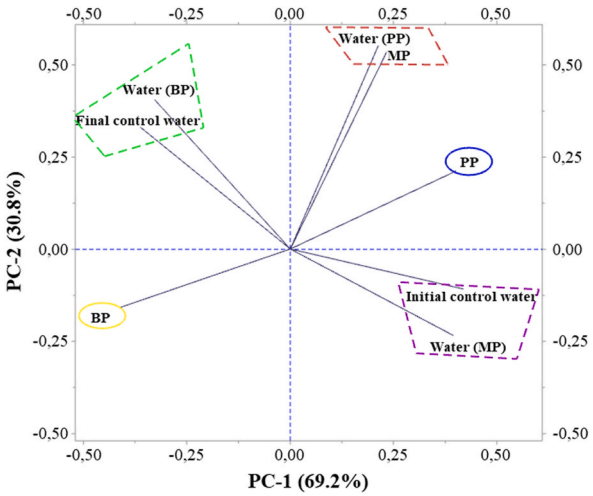


Fig. 3. Principal component analysis of the microbial density of different types of peels and different types of water in which each waste is decomposed in comparison with the initial and final control water. Water (PP): Water in which PP is decomposed. Water (BP): Water in which BP is decomposed. Water (MP): Water in which MP is decomposed.

2.4. Statistical analyses

The numerical results were presented as averages of three experiences ± SD (standard deviation). The significance of the difference between the means was checked by two-way ANOVA. For the multivariable test, Tukey multiple range tests at $p < 0.05$ were performed using GraphPad Prism 9. Also, principal component analyses were carried out using Minitab 19.1.1.

3. Results and discussion

3.1. Microbial groups of PP, BP, MP and water in which each peel is decomposed

The results of the microbiological characterization of PP, BP, MP and the initial control water show varying microbial densities from one substrate to another. For bacteria and moulds, PP contains the highest microbial density ($3.04 \pm 1.97 \times 10^7$ CFU/gDw), and for yeasts, PP and MP contain the highest density ($5.46 \pm 2.22 \times 10^7$ and $4.86 \pm 6.9 \times 10^7$ CFU/gDw respectively) compared to the other peels and to the initial control water (Table 1). Whereas, the results of the microbiological characterization of the water in which each waste is decomposed and the control water after 6 months of decomposition (Fig. 2) indicate that the microbial level related to the water in which each waste is decomposed was high compared to the final control water for all three microbial groups ($p < 0.05$). For bacteria, there was no significant difference between the waters in which the three wastes are decomposed ($p > 0.05$). For yeasts, the water in which PP is decomposed showed the highest density ($4.59 \pm 0.52 \times 10^5$ CFU/ml) compared to the water in which BP and MP are decomposed ($p < 0.05$). For moulds, the results obtained indicate that the water in which MP is decomposed had the highest density ($4.10 \pm 0.34 \times 10^5$ CFU/ml) compared to the water in which PP and BP are decomposed ant to the final control water ($p < 0.05$).

Regarding the principal component analysis, the results revealed that the density of the microbial groups varied according to the substrate. But the microbial groups of certain substrates vary in the same direction (Fig. 3).

The current research was able to prove that the density of microorganisms increased after the decomposition of the waste in water, and also demonstrated a high variability in the results obtained. The high variability in the results of the microbial groups associated with the water in which each waste (PP, BP and MP) is decomposed proves to be varied depending on the environmental conditions and also on the nature of the substrate decomposed in the water. The explanation for this variability is commonly found in microbiological studies of natural environments and was confirmed by the study of Iraqi [32]. Generally, the quantitative differences between the findings of our study and the literature led us to believe that a food waste represents a fungal group composed of species with given biochemical activities. Various scientists have mentioned that filamentous fungi are inactivated at high temperatures [33]. Fungi are usually known to be the most efficient decomposers of plant substrates due to their mycelial structure [34]. The results of the moulds densities are comparable with those obtained by Diaz-Raviña et al. [35] who demonstrated the variable number of fungi found in the compost examined (10^5–10^8 CFU/gDw). Furthermore, Anastasi et al. [36] showed a fungal load ranging from 5×10^4 to 8.20×10^5 CFU/gDw in compost and from 5.30×10^4 to 4×10^5 CFU/gDw in vermicompost. The quantitative differences between the findings of our study and those of the research literature indicates that a particular ecological group of microflora correlates with a particular plant substrate, and to a particular decomposition period. This approach has been confirmed by several researchers, including Mehta et al. [37] who reported that mesophilic fungi, such as *Aspergillus*, *Fusarium* and *Mucor*, dominate the later stages of composting, Larbi [38], who showed that some fresh composts contain about 10^6 CFU/gDw of fungal population, and also Riachi [39]

who showed that in green wastes compost, the fungal densities ranging between 10^5 and 10^6 CFU/gDw. In their studies, Chroni et al. [33] indicated that yeasts were present throughout the composting process of organic wastes, their density increased from an initial value of 3.7×10^7 CFU/gDw to 7.8×10^4 CFU/gDw after 33 days and stabilised at a slightly lower level thereafter. The results of the bacterial quantification are consistent with the study of Pepe et al. [40] who reported that the bacterial values were around 10^7 CFU/g of organic wastes, but at the end of the composting process of the agro-industrial wastes the density of the bacteria increased to a value of 10^8 CFU/g.

3.2. The fungal species isolated from the water in which each waste (PP, BP and MP) is decomposed compared to the initial and final control water

The fungal biodiversity results associated with the waters in which each waste (PP, BP and MP) is decomposed and the initial and final control water, as well as the macro and microscopic characteristics of each fungal species isolated are summarized in Fig. 4 and Tables 2 and 3 respectively. Throughout the microbiological characterization of the water in which each waste is decomposed, 66 filamentous fungi were isolated and identified (Fig. 4). The highest number of fungal isolates was obtained from the water in which the BP is decomposed, and the lowest number was obtained from the control water. The highest fungal biodiversity was obtained from the water in which the PP and BP were decomposed, compared to the water in which the MP was decomposed and the initial and final control water (Table 2). The fungal sequences show qualitative diversities (Table 3) that allow the identification in the present study of 14 fungal species belonging to different systematic groups (Phycomycetes and Ascomycetes), and the results obtained indicate that isolates belonging to the genus *Penicillium* were the most dominant compared to the other fungal genus.

The present result demonstrates that waters in which PP, BP and MP are decomposed have high and variable fungal biodiversity from one substrate to another, this biodiversity is mainly reflected by the presence of filamentous fungi which can be harmful, either to the environment and/or to human health. The variation obtained in the fungal biodiversity is likely to be caused by biotic and abiotic factors related to the decaying waste. The study by Anastasi et al. [41] reported that the total mycoflora was composed of 48 mitosporic genus, 17 ascomycetes and 4 zygomycetes genus, in addition to several basidiomycetes. The variability of the results obtained during our characterization is a common occurrence in studies of fungal microorganisms from environmental settings [42]. Generally, the qualitative differences found between our results and those in the literature led us to believe that a given ecological group of microflora corresponds to a given substrate. This idea has been supported by various authors, among them Awasthi et al. [43] who demonstrated that *Alternaria, Fusarium, Aspergillus* and *Penicillium* were recognized as general purpose saprophytic fungi on different types of organic wastes. Decomposer species can replace other species in fungal sequences when changes in the substrate interact with changes in saprophytic competitiveness and inoculation capability, giving them a decisive advantage [26,44], as a result, micro-organisms develop particular ecological survival strategies. The variation in the fungal biodiversity obtained may also be due to the different types of intraspecific and interspecific relationships between species, especially saprophytic competitiveness.

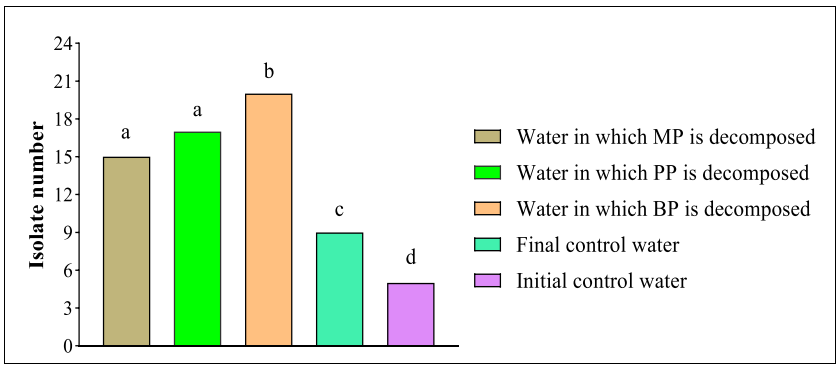
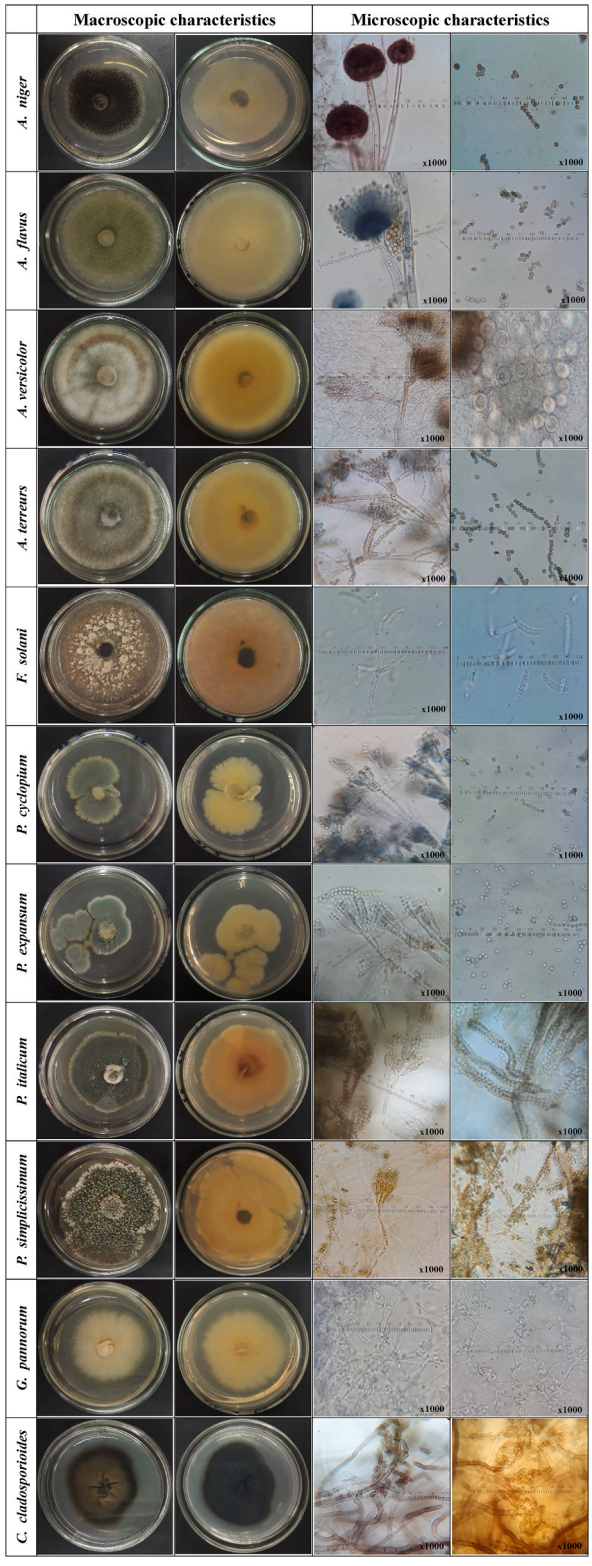


Fig. 4. Number of fungal isolates obtained from the water in which each waste is decomposed compared to the initial and final control water. Histograms denoted by the same letters indicate no significant difference according to Tukey's tests at $p < 0.05$.

Table 2
The fungal species isolated from the water in which each waste (PP, BP and MP) is decomposed compared to the initial and final control water.

	Fungal species
Water in which PP is decomposed	*Aspergillus flavus*; *Aspergillus versicolor*; *Penicillium cyclopium*; *Penicillium expansum*; *Penicillium italicum*; *Penicillium thomii*; *Moniliella actetoabutens*; *Geomyces pannorum*.
Water in which BP is decomposed	*Aspergillus flavus*; *Aspergillus versicolor*; *Aspergillus niger*; *Cladosporium cladosporioides*; *Fusarium oxysporum*; *Penicillium cyclopium*; *Penicillium simplicissimum*; *Penicillium thomii*; *Moniliella actetoabutens*.
Water in which MP is decomposed	*Aspergillus niger*; *Aspergillus terreurs*; *Fusarium oxysporum*; *Penicillium cyclopium*; *Penicillium expansum*; *Penicillium italicum*; *Penicillium thomii*.
Final control water	*Aspergillus niger*; *Aspergillus flavus*; *Fusarium solani*; *Penicillium cyclopium*; *Penicillium italicum*.
Initial control water	*Aspergillus niger*; *Penicillium italicum*; *Penicillium thomii*.

Table 3
Macro and microscopic characteristics of fungal species isolated from the water in which each waste (PP, BP, and MP) is decomposed compared to the initial and final control water.



The findings of this study are of considerable importance. Firstly, from an informational point of view, we have provided remarkable information on the fungal diversity related to the water in which three food wastes (PP, BP and MP) are decomposed, which was unknown until the present study. Secondly, the fungal biodiversity obtained can be considered to isolate, through selective screening, fungal species that can be used in biotechnological applications. Thirdly, water resources require protection against direct and/or indirect pollution by discharges to maintain sustainable development.

4. . Conclusion

The current research characterized the bacteria, yeasts and moulds of the water in which the PP, BP and MP are decomposed compared to the control water. Also, the fungal biodiversity of the water in which each waste is decomposed and of the initial and final control water was determined. The results of this research led us to conclude on the microbiological properties of the water in which each waste is decomposed, and also on the microbial biodiversity associated with these waters. Based on the results obtained, it is very clear that direct or indirect discharges of these wastes could cause negative effects on the environment, especially on water resources. This would require the recommendation for the protection of water resources in order to maintain sustainable development. In this context, further studies on the impact of these wastes are considered necessary, including the impact on air and the transmission of pathogenic microbes due to environmental pollution, as well as the impact of the water in which food waste is decomposed on crops, especially market gardening.

Author contribution statement

Azeddin El Barnossi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Abdelilah Iraqi Housseini: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

No data was used for the research described in the article.

Additional information

No additional information is available for this paper.

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