Investigating the Relationship between Physical Contamination and the Water Content of a Surface Containing Physical Contaminants

Group 1-04

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1 Introduction

1.1 Abstract

This study aims to investigate the relationship between physical contamination and the water content of a surface containing physical contaminants, filling in knowledge gaps in the process. Plasticine was placed into pepper suspensions of different water content, then the darkness of the plasticine was measured. The amount of pepper picked up by the plasticine decreases as the amount of water content of the petri dish with the pepper increases. The findings imply that contamination will occur upon contact of objects on a surface. As the moisture of the surface containing physical contaminants increase, the extent of physical contamination increases.

1.2 Background Info

Contamination in food is a major cause for concern in both the personal and industrial level. There are different types of food contamination: biological, chemical, physical and cross-contamination. Biological contamination refers to pathogenic bacteria such as *E. Coli., Salmonella enteritidis, Listeria monocytogenes, C. botulinum, Campylobacter* introduced into food prepared in unhygienic conditions. Chemical contamination refers to synthetic substances. Physical contamination refers to hair, dust or dirt, and may result in food becoming unhygienic and hence unsuitable for consumption.

The experiment aims to investigate how the moisture content of the surface affects the amount of physical contaminants transferred. Research findings in this experiment are also applicable to cooking, which is a concern in terms of hygiene and quality control, since physical contamination of food can occur virtually in any kitchen environment. Kitchen environments are usually damp and hence, by exploring the nature of physical contamination in relation to the moisture content of a surface with such physical contaminants, the research done in this experiment can shed light on how kitchen environments can be modified so as to reduce the chances of physical contamination.

1.3 Rationale

Sand, dust, dirt and hair are common physical contaminants of food and they are easily transferred to fresh food and vegetables during food preparation. This experiment aims to uncover the relationship of such physical contamination to another variable that affects kitchen environments, moisture. The relationship between the moisture of a surface with the rate of physical contamination has not received in-depth research and this experiment is aimed at filling this knowledge gap.

Another common habit of ours is touching our faces subconsciously. Although people may not realise it, they touch their faces very often, such that they are likely to fall sick if they accidentally ingest dangerous contaminants on our hands. Like plasticine, our skin has a hydrophobic nature. Hence, the members of this study aim to find out the amount of physical contamination transferred from our hands to face, and hence the danger of this action.

1.4 Literature Review

In Study 1 conducted by Clarke, Julian it was found that 70% of women and 56% of men were familiar with the 5-second rule; and alarmingly, most experimental participants used it to make decisions on food dropped on the floor. In addition, food dropped on a floor containing microorganisms can be contaminated in 5 seconds or less. The study provided us with a model for our original experiment through inoculating tiles with *E. Coli* bacteria.

In Study 2, conducted by Anthony Hilton it was found that the type and moisture of surface inoculated with bacteria affects the extent of bacterial transfer across surfaces. Hence, when carrying out our experiment, these two factors had to be kept constant. However, the study did not explicitly specify the method in which they quantified the amount of bacteria transferred.

In Study 3, conducted by Dawson, Paul, et. al., the research found that bacterial transfer rate to food decreased as the bacterial residence time on the surface increased from 2 hours, 4 hours, 8 hours to 24 hours with transfers of 6·5, 4·8, 4·6 and 3·9 log CFU ml^{-1} in the rinse solutions, respectively. The effect of time on bacterial transfer was the original independent variable for this present study.

In Study 4, conducted by Mafu et. al., it was discussed how biological residue in food processing plants resulted in bacterial contamination and how the extent of contamination was affected by hydrophobic and hydrophilic surfaces in cultures with different pHs (6, 7, and 8). Bacteria were found to contaminate polystyrene and glass surfaces to the same extent, regardless of their hydrophilic or hydrophobic nature. Hence, a hydrophobic object was used in this study as it will have no effect on the occurrence of bacterial contamination.

Study 5 conducted by Mustafa Akbulut et. al., researched on a coating that prevented cross-contamination of bacteria between fresh produce. It was also found that bacteria can survive for a longer period of time in aqueous environments, facilitating the transfer of bacteria between surfaces. Although there was no research on how the amount of water affects bacterial transfer. The study implied that the water content of the solution containing bacteria can affect the extent of bacterial contamination.

In Study 6 conducted by Ivanova, Elena, the texture of a surface was found to affect bacterial contamination. Rougher surfaces, especially those with tiny spikes, such as that of Cicada insect wings, prevent bacteria from settling on the surface. It was therefore important that the surface texture of the object used was standardised to be as smooth as possible in order to ensure a fair experiment and facilitate bacteria transfer.

1.5 Contribution to Field of Study

The relationship between the moisture of a surface with physical contaminants with the extent of physical contamination has not received in-depth research. Hence, this experiment is aimed at filling this knowledge gap.

1.6 Objective

This study aims to investigate how the moisture of a surface containing physical contaminants affects the extent of bacterial contamination of an object in contact with the surface.

1.7 Hypothesis

The members carrying out this study hypothesise that as the moisture content of the surface containing physical contaminants increases, the physical contamination of an object will increase proportionally. The object used for this experiment is plasticine and comprises about 65% of bulking agents, which are principally Gypsum, 10% petroleum jelly, 5% lime, 10% lanolin and 10% stearic acid. Gypsum is calcium sulfate which is an ionic compound. It is made of a lattice of oppositely-charged calcium and sulfate ions.

1. Adhesive forces exist between water and Gypsum. This is due to the adhesive nature of water resulting in it adhering to other objects, such as Gypsum, and facilitating the transfer of physical contaminants. Water's adhesive properties are observed in its forming of a concave meniscus when water is placed in a cylinder or a tube, where water tends to stick to the walls of the cylinder partly due to water's high surface tension. It is also observed in the phenomenon of capillary action where water is attracted to the charged walls of the capillary tubes and hence adheres to it. This allows water to travel up the tube, which is vital in processes such as the movement of water up the xylem of plants. Such adhesive properties of water are in contrast with other more cohesive substances such as mercury. When mercury is placed in a cylinder or tube, mercury would form a convex meniscus instead.
2. There are ion-dipole interactions between water molecules and the calcium and sulfate ions. Water molecules are polar because oxygen atoms are much more electronegative than hydrogen, and the molecule shape is bent like a "V". So bonding electrons are pulled towards oxygen, giving it a partial negative charge (delta-negative), while hydrogen atoms are left with partial positive (delta-positive). The delta-negative oxygen atoms of water are attracted to the calcium ions, while the delta-positive hydrogen atoms of water are attracted to the sulfate ions, all by ion-dipole forces.

2 Materials & Methods

2.1 Materials

2.1.1 Original Experiment Materials

The rounded apparatus that were originally planned were: an incubator, a stopwatch, a biological safety cabinet, an autoclave and a wooden tile (surface to be inoculated with *E. Coli.* bacteria). The synthetic object to pick up contaminants was to be made with flour, salt and water, so as to standardise moisture, surface area, salinity and other fixed conditions as controlled variables. An overnight bacterial culture of *E. Coli.* was planned. A normal saline solution was initially planned to be used in serial dilution. A pipette, 4 petri dishes and a stopwatch were also used.

2.1.2 Revised Experiment Materials

After making adjustments to our procedure due to resource and time limitations in resources and time, the revised materials used were: fine grounded black pepper (*Piper nigrum*) purchased from local supermarkets to represent physical contaminants of food. Plasticine which had a suitable moisture content replaced a synthetic object made with the materials mentioned above which was too dry to increase moisture content or keep the exposed surface area constant. A box of plasticine provided by the lab had a suitable moisture content and was used instead. A 100ml beaker was used to contain tap water. A 3ml dropper, 4 petri dishes and a stopwatch were also used.

2.2 Methods

2.2.1 Original experiment with bacterial transfer of E. Coli.

The materials stated above would be prepared while taking into account the independent, dependent and controlled variables of the experiment. Then, the synthetic object would be created using flour and water. After that, the specific amount of bacteria required for the experiment would be prepared by mixing 0.1 g of bacteria sample with 10 ml of sterile normal saline. Serial dilution would be carried out with the sterile normal saline. 0.1 ml of the dilution would be spread on Muller Hinton Agar plates, then the plates would be incubated at 36°C overnight. The synthetic object would be sterilised using autoclave, then 10 ml, 20 ml and 30 ml of water would be added using a pipette on four different synthetic objects respectively using a pipette while the fifth synthetic object would be left dry for our control setup. After that, a colony counter would be used to count the bacteria colonies on each different wooden tile, then the findings would be recorded and a graph of the dependent variable, amount of E.Coli. left on the wooden tile, against the independent variable, moisture content of the surface inoculated with bacteria would be drawn. The experiment would then be repeated another 2 times.

2.2.2 Revised experimental procedure on Physical Contamination

The grounded black pepper was evenly placed onto each Petri dish. About 10g of plasticine was moulded into a circular object with a flat surface with diameter of 7cm, measured with a ruler, then was cut into 4 equal quadrants. A stopwatch was used to time the experiment for 2.5, 5.0 and 7.5 seconds. 2.5 ml, 5.0 ml and 7.5 ml of water was added dropwise into the petri dish containing pepper using a dropper while the fourth dish was left dry. (As shown in Fig.1) This allowed the independent variable, the amount of water to be dropped into each petri dish with pepper, to be varied. The pepper and water suspension was gently shaken to mix and form a homogenous pepper suspension. A flat plasticine quadrant was then placed gently into the pepper suspension for up to 20 seconds, using the stopwatch to time the duration. The object was then removed. An image of the contaminated surface was captured and analysed in ImageJ (National Institute of Health, Bethesda, MD) to measure the average intensity of the darkness of the side of the object stained with pepper, which was the dependent variable of our experiment. The images were taken with approximately the same camera angle. A 1cm by 1cm square of the surface of the plasticine with the most pepper picked up was used to calculate the average intensity through ‘measure’ function on the software. The experiment was repeated to form triplicates. Finally, the average of the 3 readings was calculated, then used to plot a graph of y against x.

Figure 1: Petri dish with black pepper after water is added

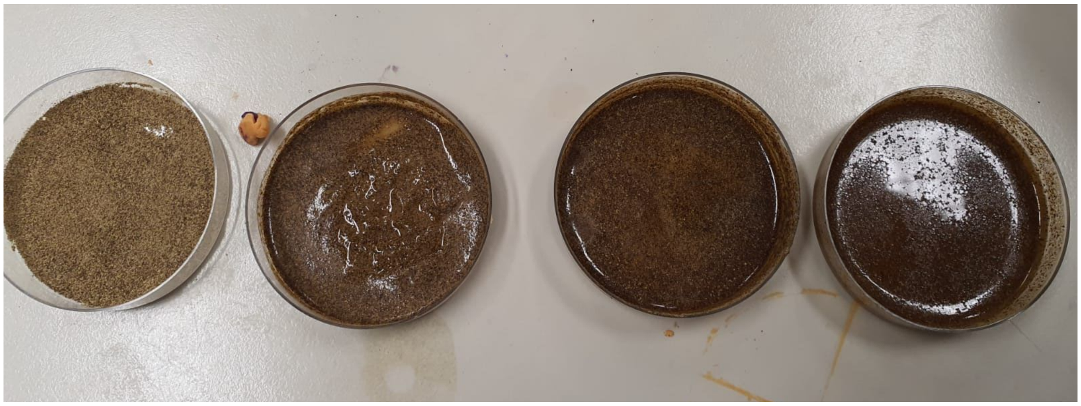
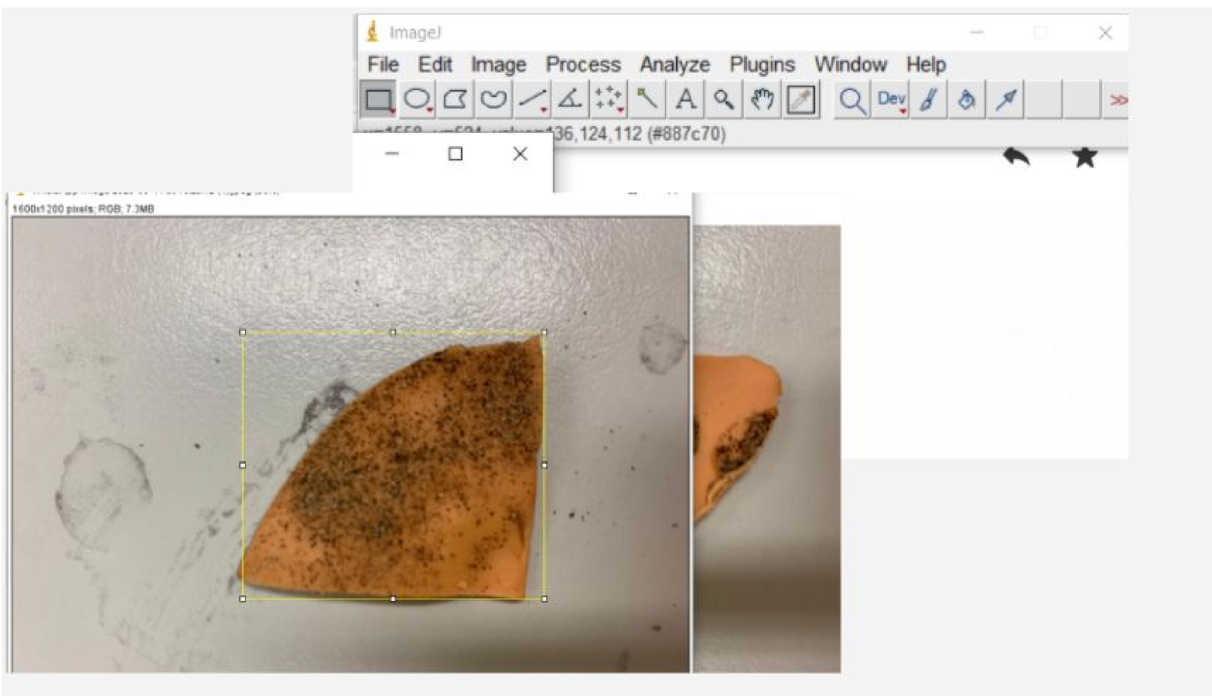


Figure 2: Using ImageJ software



3 Results

3.1 Observations

In Fig. 1 (below), there are plasticine-pepper samples varied in the amount of pepper picked up after being dipped for the same amount of time (20s) in pepper of different moisture contents. The control setup i.e. the plasticine - pepper sample with no moisture appears dark-greyish in color and has picked up pepper over the largest surface area compared to other setups. When the concentration of pepper solution decreases, the dark color of the plasticine-pepper sample lightens up and the amount of pepper residing on the surface of the plasticine decreases consistently. These can be observed across all 3 repetitions of the set-up. This could be due to the fact that plasticine is hydrophobic, so less pepper is picked up when there is more water inside the pepper suspension, leading to more pepper suspension being repelled by the plasticine.

Figure 2: Compilation of all set-ups of plasticine dipped in pepper suspension
From left to right: Set-Up 1, Set-Up 2, Set-Up 3 and Set-Up 4 with 0.0ml, 2.5ml, 5.0ml, 7.5ml of water added respectively

Figure 3: Graph of trend of brightness of plasticine sample

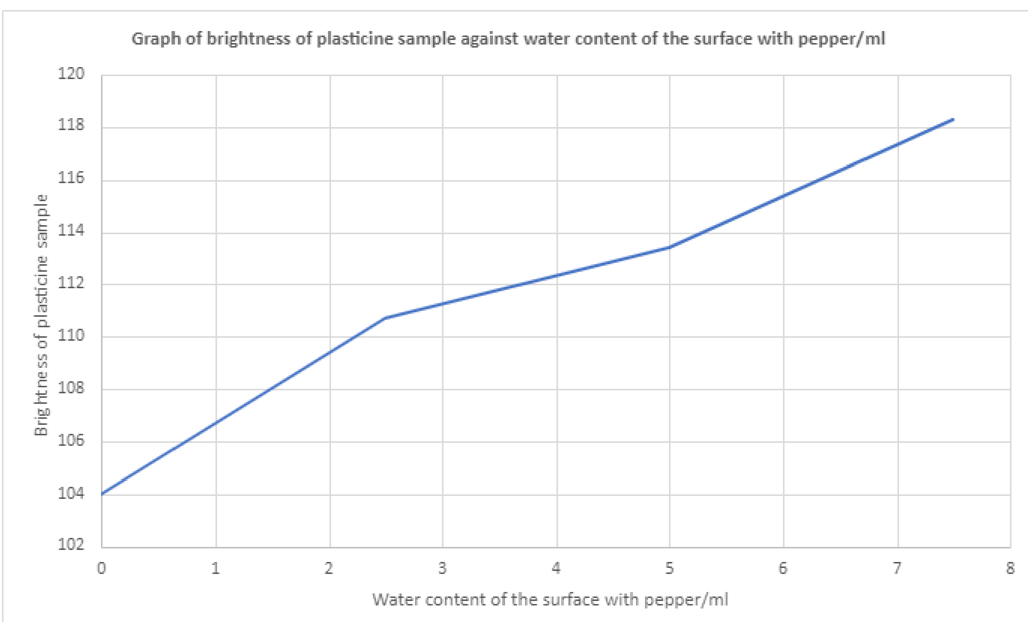
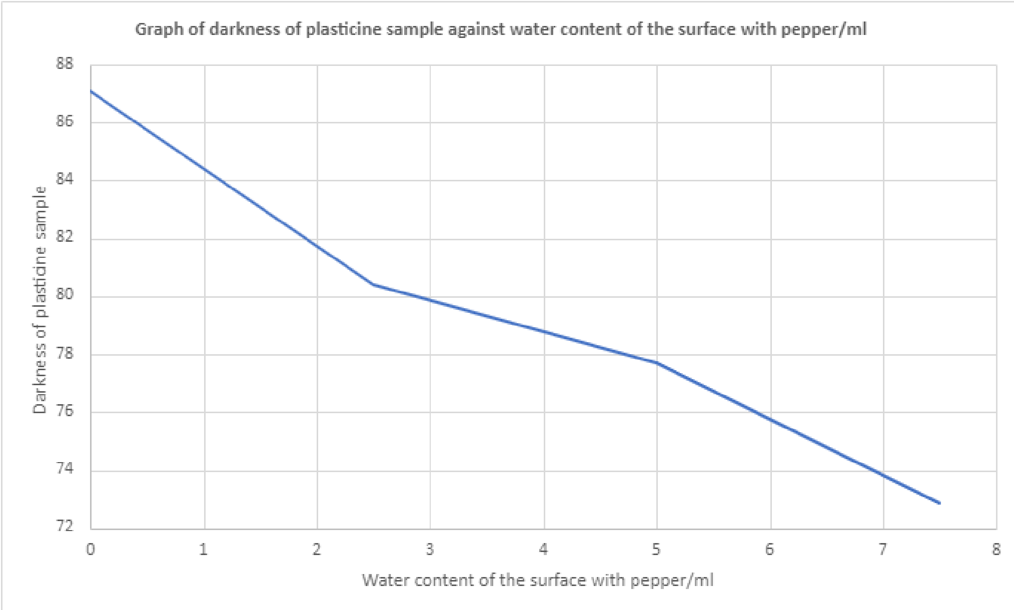


Figure 4: Graph of trend of darkness of plasticine sample



As shown in Fig. 5 (below), values for brightness of the samples ranged from 103.499 to 108.748 pixel intensity. The highest pixel intensity was 118.748 and the lowest pixel intensity was 103.499. As shown in Fig. 6 (below), values for darkness of the samples ranged from 72.376 to 87.625 pixel intensity. The highest pixel intensity was 87.625 while the lowest unit was 72.376.

Figure 5: Table of results found for brightness of sample, with pixel intensity measured from black (0) to white (255)

Pixel intensity showing brightness of sample				
Water content of pepper/ml	1	2	3	Mean (3d.p.)
0.0	104.843	103.499	103.732	104.024
2.5	108.978	111.103	112.015	110.699
5.0	113.789	112.215	114.283	113.429
7.5	117.423	118.748	118.633	118.268

Figure 6: Table of results found for darkness of sample, with pixel intensity measured from black (0) to white (255)

Pixel intensity showing darkness of sample				
Water Content of Pepper/ml	Darkness of sample after image analysis (1st Result)	Darkness of sample after image analysis (2nd Result)	Darkness of sample after image analysis (3rd Result)	Mean Average (3dp)
0.0	86.281	87.625	87.392	87.100
2.5	82.146	80.021	79.109	80.425
5.0	77.335	78.909	76.841	77.695
7.5	73.701	72.376	72.491	72.856

3.2 Trends

A high brightness of the plasticine-pepper sample represents a low amount of pepper picked up by the plasticine as there is a high surface area exposed to light, and vice versa. Across the 3 experiments, mean values of the average brightness of the samples increased relatively consistently as the water content of the pepper in the petri dish increased with each new setup, as shown by the clear upwards trend in the graph. Similarly, across the 3 experiments, mean values of the average darkness of the samples decreased relatively consistently as the water content of the pepper in the dish increased with each new setup, as shown by the clear downwards trend in the graph. This shows that the amount of pepper picked up by the plasticine decreases as the amount of water content of the petri dish with the pepper increases.

3.3 Analysis of Results

People have to be careful of dry surfaces that seem to be free of contaminants, such as dust, dirt and bacteria, as they will contaminate objects more easily. Our results have also disproved our hypothesis.

Although the environment of kitchens is usually damp, it does not affect bacterial contamination of food dropped on surfaces. Instead, it reduces the amount of dust and dirt picked up. However, this poses another danger as there is less physical indication of the food being contaminated.

4 Discussion & Conclusion

4.1 Interpretation & Explanation of data

Our conclusion drawn from the results disproves our hypothesis. Firstly, the major component of black pepper is piperine, and is a large molecule with a solubility in water of 40 mg per litre, and be can infer from this it is slightly polar. Hence, it can be argued that piperine is hydrophobic and hence justifying our lab observations of the pepper not dissolving in water and forming a suspension instead. A large component in plasticine is gypsum, calcium sulfate, which is an ionic compound. The water molecules can interact with the calcium ions and sulfate ions by ion-dipole forces. Hence, since the concentration of pepper in the suspension decreases as the amount of water increases, there will be less pepper particles existing between the water molecules interacting with the gypsum.

4.2 Inferences

Thus, it can infer that objects in contact with a damp or wet surface will have less bacteria transferred from the surface to the object, objects in contact with any surface will be contaminated to some extent, regardless of water content.

4.3 Comparison to Other Studies

The study has confirmed that a certain amount of *Piper nigrum* is transferred immediately upon contact with the surface. It has also shown that water does not aid in physical contamination, and instead reduces physical contamination. However, the members of this study were unable to look into biological contamination, which may be affected by the water content differently.

4.4 Potential Impact in Science

Our research has shown that there is increased physical contamination between surfaces with less water content. We have also shown that objects will contain contaminants upon contact with a surface, especially dry surfaces. Hence, we should be wary of food that is dropped on any surface, regardless of water content, as it will be contaminated.

4.5 Possible Improvements

Piper Nigrum consists of abiotic particles and hence cannot provide an accurate representation of bacterial transfer since there may be biotic factors affecting bacterial transfer. Furthermore, since the mass of *Piper nigrum* is negligible, the experimental results are taken by using colour analysis, which is a form of quantitative analysis. Hence, with additional time, the experiment would have been conducted with *E. Coli* instead of *Piper nigrum*, to better simulate the bacterial transfer because of the moisture of the surface inoculated with *E. Coli*. As for the possible improvements to our current experiment, the different set-ups could be weighed and their mass compared to find the different amounts of *Piper nigrum.* This would be more accurate as a quantitative analysis was carried out instead of a qualitative analysis. With more time, other factors affecting bacterial transfer, such as time and temperature, could also be investigated.

4.6 Conclusion

In conclusion, as the amount of *Piper nigrum* (pepper) picked up by the plasticine will decrease proportionately with the increase in moisture of the petri dish containing the *Piper nigrum.*

Adhesive forces are unable to exist between water and Gypsum due to the surface tension of water. When the plasticine is dipped in the suspension, the surface tension is not broken, and hence water molecules do not adhere to the Gypsum. As such, the transfer of contaminants is not facilitated, and the adhesive forces between water and *Piper nigrum* reduce the amount of contaminants transferred, hence disproving the hypothesis.

5 Bibliography

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