University of Glasgow | College of Medical, Veterinary & Life Sciences

Head of College Scholars List Scheme

Summer Studentship

Report Form

This report should be completed by the student with his/her project supervisor. It should summarise the work undertaken during the project and, once completed, should be sent by email to: jill.morrison@glasgow.ac.uk within four weeks of the end of the studentship

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Testing and Evaluating the SODIS Method of Water Disinfection on Samples from the River Kelvin

Lay Summary

Techniques for the disinfection of drinking water used in industrialised nations – filtration and chlorination – are poorly suited to application in the developing world as they rely upon infrastructure that is rarely available. In solar methods of disinfection, plastic bottles are filled with water and exposure to natural sunlight kills bacteria. This project tested the principles of solar disinfection and identified plastics best suited to the disinfection process. Although many bacteria in river water were found to be sensitive to ultraviolet light, some were identified that were resistant. This and the presence of some bacteria able to produce UV-resistant spores present challenges to the method.

Start Date**: 1st July**

Finish Date**: 29th August**

Aims and Objectives

1. Investigate the range of UV doses required to kill a standard organism (*Escherichia coli*) and test for the emergence of UV resistance.

2. Investigate whether an anti-oxidant (Vitamin C) offers bacteria protection from UV sterilisation.

3. Compare the ability of 3 different plastic containers to transmit UV and allow SoDis to take place.

4. Investigate the degree to which UV sterilisation has a bactericidal effect on both primary Kelvin river colonists and spore formers from the same source and how the two groups of data compare.

Methodology

1. The main apparatus involved throughout the project was the UV cross-linker, a chamber with UV lamps where an agar plate streaked with microbes could be subjected to UV light of a given energy and duration. *E.Coli* (NCIMB 8742) was streaked onto agar plates and subjected to various doses of UV in the chamber, left to incubate overnight in 37c^{o}, and the number of surviving colonies counted the next day. In all later experiments an energy intensity of 1,000 μJ/cm^{2} was used with only duration of exposure varying.

2. Vitamin C effervescent tablets where used to create aqueous solutions of anti-oxidant(5gL^{-1}), their purpose being to curtail the emergence of reactive-oxygen-species to determine whether UV light itself or the RoS it produces have the dominant bactericidal effect. The solution was applied in two ways: A) *E.Coli* was mixed in the solution in a sterile universal before being streaked on agar and B) 1ml VC solution was added to the molten agar and left to set, after which *E.Coli* was applied to agar.

3. Three different plastic sheets acquired from containers (clear and green Polyethylene-terephthalate and clear High Density Polyethylene) were held over agar plates inside the cross-linker during radiation to see if the bactericidal effect is altered by the UV passing through the plastic.

4. Using a plastic filtering unit, a sufficient concentration of microbes was extracted from a sample of River Kelvin water to grow primary colonists on agar. Three single colonies of such organisms where streaked onto agar plates to provide pure cultures for testing. An environmental sample was also heated to 70c^{o} for 20mins to kill primary colonists and allow spore-formers to be filtered out and tested in the same manner.

Results

1. *E.Coli* proved to be susceptible to UV radiation as exposure time of 10sec left 18 colonies surviving while only 1 survived exposure of 40sec, this correlation suggested UV was killing a large number of the cells(control plates exposed to no UV grew as a single dense layer). However, when a new plate was streaked with *E.Coli* from the single surviving colony and exposed again to UV they showed extensive resistance with as many as 50 colonies surviving 10sec and 32 surviving 40sec exposure.

Duration of 1000µJcm^{-2} UV exposure (seconds)	Number of surviving colonies	
	Original 8742 strain	Survivors from previous test
0	Single Layer	Single Layer
10	18	50
20	4	24
30	0	30
40	1	32

2. The anti-oxidant experiment was carried out twice to get an average value, the results showed no correlation between the presence of Vitamin C and the survival of the *E.Coli* 8742 and thus suggested that UV radiation itself has the dominant bactericidal effect.

Duration (seconds)	Average number of surviving *E.Coli* colonies	
	E.Coli without anti-oxidant (control)	*E.Coli* in presence of anti-oxidant
10	33.5	25
20	18.5	12.5
30	6	10.5
40	11	10

3. In the case of all the plastics, transparent or not, the bacteria were apparently protected from the effect of UV to a certain degree by the intervening material. The results seem to suggest that the HDPE taken from a standard milk carton transmitted UV light more effectively than the PET sheets taken from soft drink bottles. PET was tested with and without colour tinting, this did not appear to have altered it's effectiveness.

Duration (seconds)	Number of surviving *E.Coli* colonies		
	Clear PET	Green PET	HDPE
10	Unaffected Growth	Unaffected Growth	Approx. 250
30	Unaffected Growth	Unaffected Growth	52
60	Approx. 200	Approx. 200	12

4. Four organisms taken from the Kelvin River-water were used in this experiment: two Primary colonists as controls(Figures 1 and 2) and two spore formers, a Methylene-blue stain was used to confirm that the organism produces spores(Figures 3 and 4).

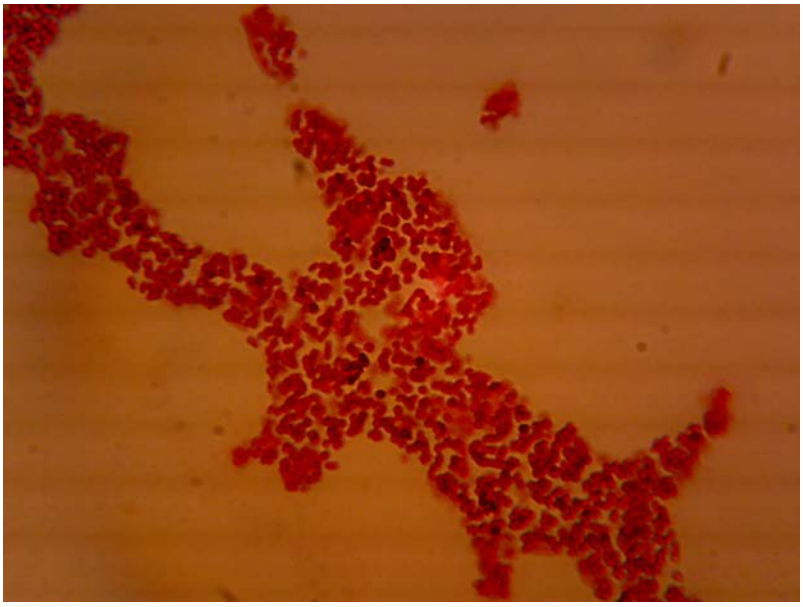


Figure 1. Primary Colonist 1 Gram Stain

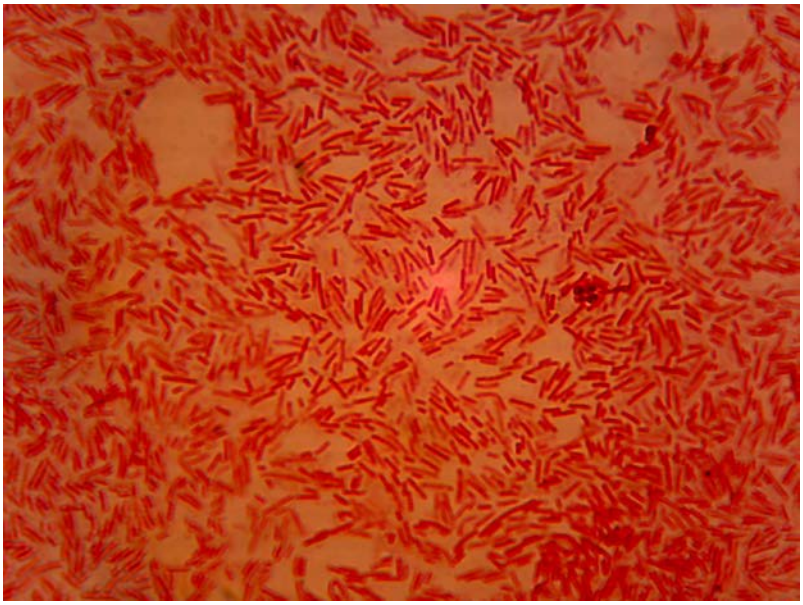


Figure 2. Primary Colonist 2 Gram Stain

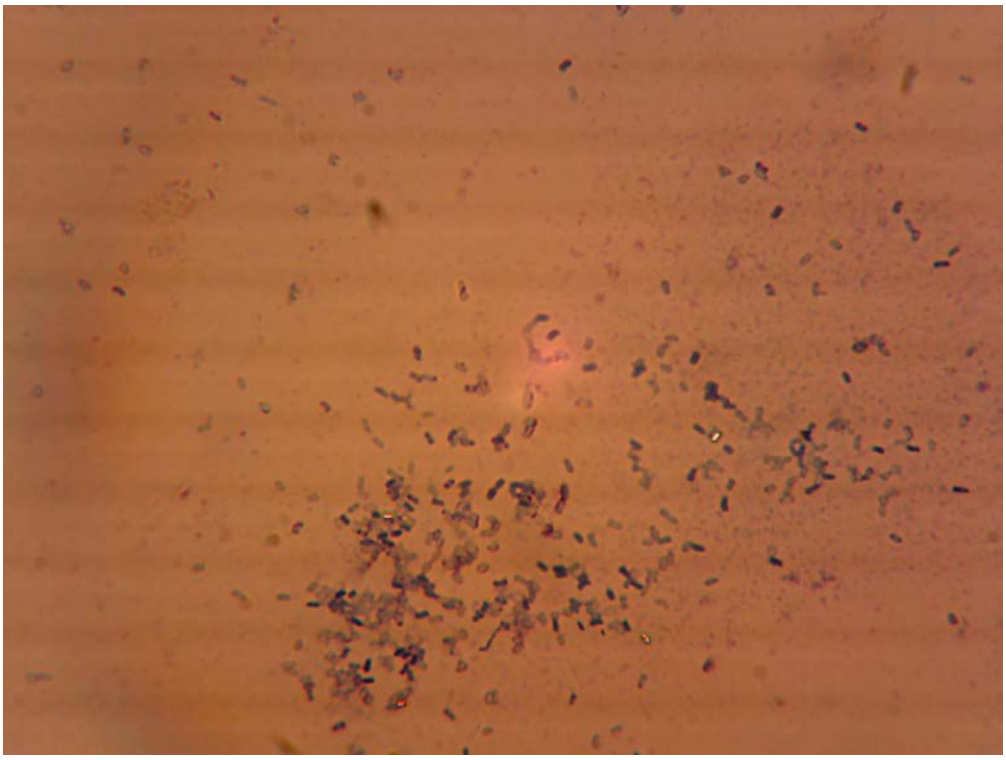


Figure 3. Spore-former 1 Meth-Blue (few vegetative cells suggests only spores survive irradiation)

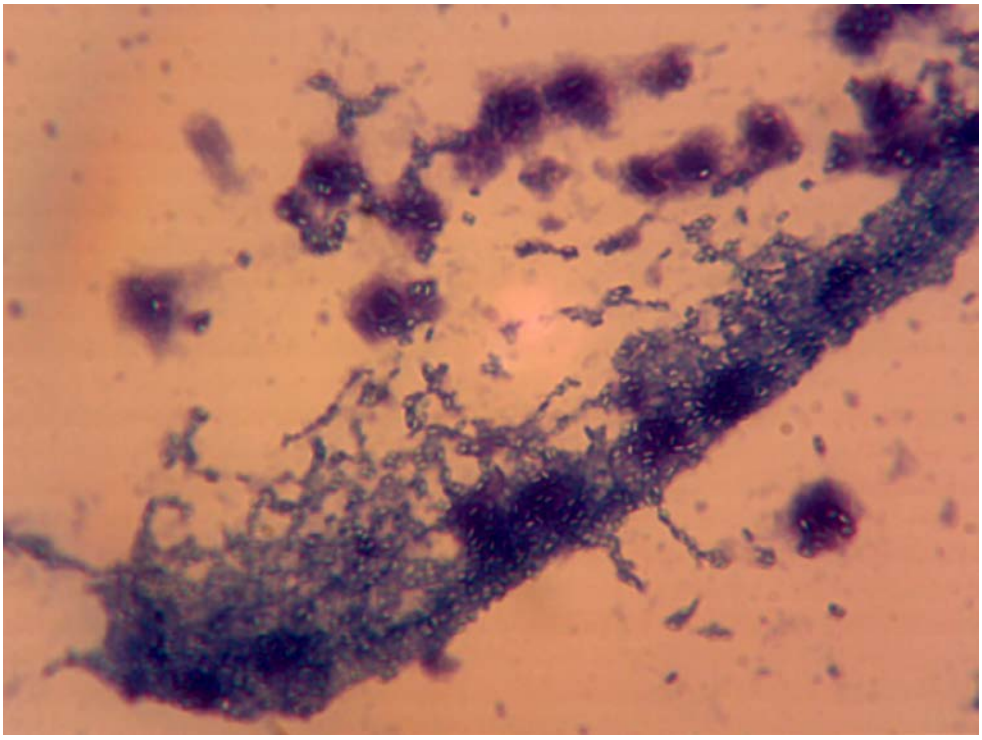


Figure 4. Spore-former 2 Meth-Blue (spores appear in stain as bright white spots)

The results show that the Primary Colonists are as susceptible to UV as *E.Coli*, the spore-formers, however, show a significant resistance to the radiation treatment with as many as ten times more colonies emerging from spore-plates than from the control group(Figure5).

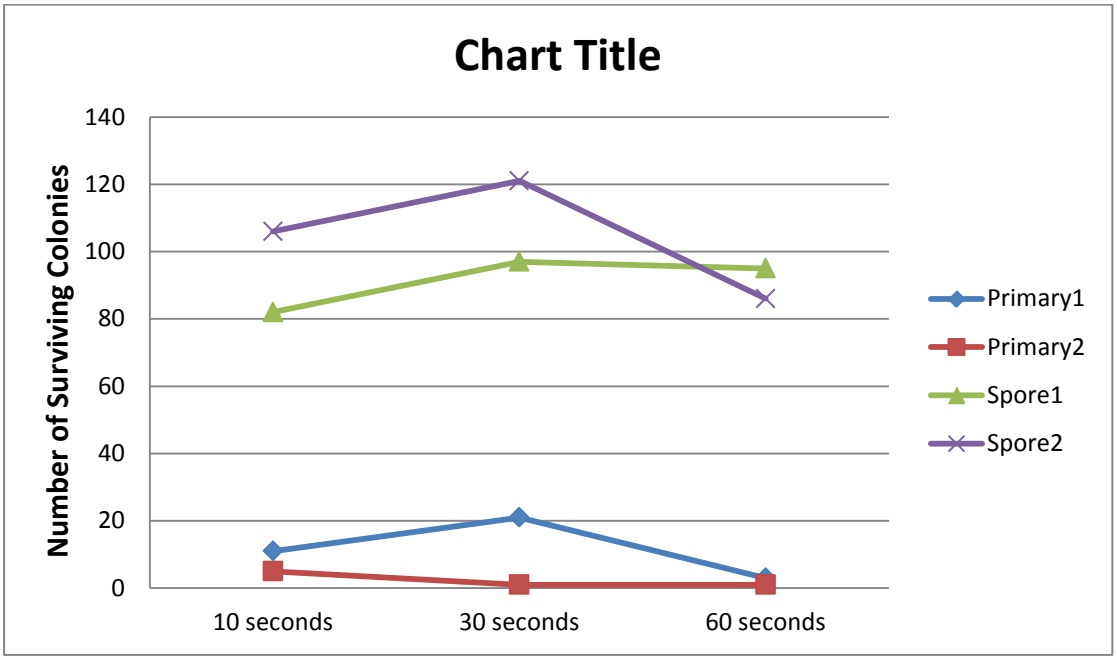


Figure 5. Shows the greater resistance of the spore-forming bacteria compared to Primary Colonists

Duration (seconds)	Number of Surviving Colonies			
	Kelvin Primary Colonist 1	Kelvin Primary Colonist 2	Kelvin Spore-former 1	Kelvin Spore-former 2
10	11	5	82	106
30	21	1	97	121
60	3	1	95	86

Discussion

1. The original *E.Coli* strain and Kelvin Primary Colonists showed similar vulnerability to UV treatment but after just one such treatment the cells from the few surviving colonies showed great resilience in the face of further, even stronger doses of radiation. This can be an important factor to bear in mind concerning the application of SoDis in the field: if resistance can arise so easily and quickly, it could impair the effectiveness of the method. A study by Goldman & Travisano(2011) into *E.Coli* has suggested that the organism may be predisposed to the development of UV resistance, and that resistant strains were twice as resilient to desiccation and starvation as ordinary cells but very sensitive to salinity. This suggests that naturally occurring protective mechanisms against starvation and drought coincide with protection against UV as well and thus all the traits are selected for when the population is exposed to UV treatment. One will also observe a trade-off with mechanisms offering protection from other stresses such as salinity.

2. The experiments with Vitamin C appear to show that UV radiation and its mutagenic effect on bacterial DNA is the dominant cause of cell death as the anti-oxidant would have been expected to suppress the emergence of Reactive Oxygen Species. Alternatively, Vitamin C may have been an unsuitable compound for use in this experiment and future studies may try different anti-oxidants to investigate whether they offer bacteria some protection from UV treatment.

3. Throughout the developing world, health charities recommend PET bottles for use in SoDis, however, the results strongly suggest that the HDPE taken from a milk carton is more effective than PET at allowing UV to pass through and to kill bacteria. Possible explanations for this difference in transmission may be linked to the comparative density of the two plastics as the PET sheets were twice as thick as the HDPE, additionally, the angle of the sheet to the agar may have been a factor as the HDPE sheet was flat and could lie parallel to the agar substrate while the PET sheets were curved and could not be laid flat over the plate. Future experiments into this phenomenon could use wider range of plastics and attempt to account for factors such as the curvature and thickness of the intervening plastic sheets.

4. The results show a significant gulf between the effect UV irradiation has on bacterial species that can and cannot produce endospores. This is unsurprising as endospores have evolved as a method of

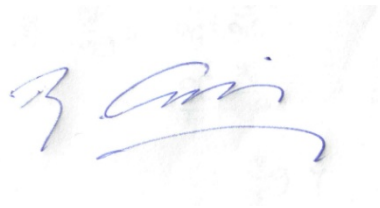
coping with a wide range of stresses such as heat, desiccation and UV exposure. As the methylene-blue stains show, the spores are the only discernible life-form left on the plate after exposure, left in the debris of the generation that created them, and are ready to germinate into the vegetative form of their life-cycle when environmental conditions become favourable. They do not appear to be competitive Primary colonist species, instead they most likely fill a high-stress niche in the Kelvin ecology where the endospores allow them to survive, for example, on rocks close to the water surface exposed to the mutagenic and desiccating effects of the sun. Future experiments should investigate what, levels of UV dose are required to kill these organisms and possible alternative methods of disinfection to aid SoDis.

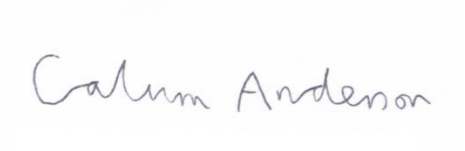
Reflection

I found the undertaking of this studentship to be a very insightful and useful experience of a working lab environment. Looking back on how I first started out on the project I am quite surprised at how at home with the research I felt by the time I had finished. Under the patient guidance of Dr Aitken I learned many of the basic skills used in biological research and went from requiring constant help and advice about the laboratory tools, resources and setups to being almost completely self-sufficient in my studies and experiments as the studentship wore on. This visible improvement in my analytic skills has given me tremendous confidence for going into my third year at the university as well as a taste for lab work. I have found the studentship to have been an invaluable source of both theoretical knowledge and useful, real world, experience, both of which , I am certain, will serve me well as I progress in my academic career.

I would also like to reiterate my gratitude to my Project Supervisor Dr Aitken for the patient and diligent assistance he provided me with during this challenging project. I would also like to extend my thanks to those who ran the Head of College Scholars Scheme and made this rewarding experience possible.

Signatures

Supervisor  Date **15/09/2013**



Student Date **10/09/2013**