Team Clearwater-Aid
Thursday October 12th, 2006
Team-Bio Laboratory Procedure

Reduction of *Escherichia coli* in Contaminated Water Systems by Flocculation with *Moringa Oleifera*

Abstract:

Moringa Oleifera is a native African tree in the genus Moringa and family Moringaceae. Much research has been done on the seeds of the Moringa tree, analyzing their use as a natural bio-flocculant; however, no experiments have been done to test the Moringa seeds ability to reduce microbial levels in contaminated waters. This experiment focuses on quantifying numerically the amount of *E. coli* that would be reduced in a contaminated sample by flocculation with *Moringa Oleifera*.

Objective:

The objective is to test several quantities of Moringa seeds at various time intervals in an attempt to calculate if, and how much *E. coli* can be flocculated out of a contaminated solution by flocculation with *Moringa Oleifera.*

Materials:

1. 1000 µl pipette + tips
2. 200 µl pipette + tips
3. 20 µl pipette + tips
4. Shaking Incubator
5. 40 L.B. plates
6. Autoclave
7. 37°C incubator
8. 5 250 ml beakers
9. 1 1.5 L beaker
10. 5 Magnetic stir-rods
11. Moringa Seeds
12. *E. Coli* sample
13. magnetic stir plate
14. 500 ml peptone buffer
15. 25 g agar media

Procedure:

1. Mix 1 L deionized water and 25 g agar media and autoclave
2. Inoculate 1 L agar media aseptically with *E. coli* sample.
3. Place on shaking incubator for 12-16 hours
4. Divide 1 L *E. coli* solution into 5 250 ml beakers
5. Add .5g, 1g, 2g, and 4g crushed Moringa seeds into 4 of 5 beakers, 1 control.
6. Place magnetic stir rod into each beaker and stir for 5 minutes on magnetic stir plate.
7. Dilutions at desired times.
    i. Remove 20 µl solution with micropipette
    ii. Inoculate aseptically into 1980 µl buffer; (10^{-2}) dilution; (#1)
    iii. Remove 20 µl from dilution 1 and place into 1980 µl buffer; (10^{-4}); dilution (#2)
    iv. Remove 20 µl from dilution 2 and place into 1980 µl buffer; (10^{-6}); dilution (#3)
    v. Remove 100 µl from dilution 3 and place into 900 µl buffer; (10^{-7}); dilution. Plate on L.B.
    vi. Remove 100 µl from dilution 4 and place into 900 µl buffer; (10^{-8}); dilution. Plate on L.B.
1. Count colonies formed on each plate at each time and concentration.

Results:

10^{7} Dilutions

Time	**Control**	**.5g**	**1g**	**2g**	**4g**
	Innumerous growth with few contaminations	Innumerous growth with few isolated colonies	Plate Damaged	Innumerous growth with few isolated colonies	Innumerous growth
	Innumerous growth with few isolated colonies	Innumerous growth	Innumerous growth with few isolated colonies	Innumerous growth with few isolated colonies	Innumerous growth with few isolated colonies
	Innumerous growth with few isolated colonies	Innumerous growth	Innumerous growth	Innumerous growth	Innumerous growth with contaminations
	Zero *E. coli* colonies	Innumerous growth	Innumerous growth	Innumerous growth	Innumerous growth

0hr

4hr

8 hr

20hr

10^8 Dilutions

Time	Control	.5g	1g	2g	4g
0hr	Contaminated	Innumerous growth	Innumerous growth	Innumerous growth with few isolated colonies	Innumerous growth with contaminations
4hr	Innumerous growth with contamination	Innumerous growth	Innumerous growth with few isolated colonies	Innumerous growth with few isolated colonies	Innumerous growth
8 hr	Innumerous growth	Innumerous growth with contaminations	Less than 30 isolated colonies	Innumerous growth with few isolated colonies & contamination	Innumerous growth
20hr	Zero *E. coli* colonies	Innumerous growth	Innumerous growth	Innumerous growth	Innumerous growth

Conclusion:

Observations of L.B. plates show *Moringa Oleifera* was unable to reduce *Escherichia coli* levels in solution. Observation of the solutions containing various amounts of *Moringa Oleifera,* gives evidence that the experiment must be refined and retested. After viewing the solutions containing *E. coli* and *Moringa Oleifera* it was seen that the *Moringa Oleifera* seeds floated to the top of the solution; in essence doing exactly the opposite of what was hypothesized. This however gives knowledge on the properties of the seeds. The density of *Moringa Oleifera* is lighter than that of the *E. coli* solution. Perhaps *E. coli* in the solution did flocculate to *Moringa Oleifera*, or perhaps it didn't. Regardless of whether or not it did, settlement did not occur. It is known that in a turbid water sample, flocculation and settlement will occur when *Moringa Oleifera* is added. The presence of the soil is the backbone for flocculation, and simple flocculation with *E. coli* is not possible.

Revision Plans:

It is proven that *Moringa Oleifera* works as a natural flocculent in a turbid water sample. This will be the background for the next experiment. *Moringa Oleifera* will be added to a turbid water sample containing microbes, and microbial levels will be examined before and after flocculation. It can be hypothesized that as soil particulates within the solution flocculate and settle, microbial levels above the settlement zone will decrease. This hypothesis is based on the idea that microbes exist in conjunction with soil particulates. As these particulates flocculate and settle, the microbes that are associated with those soil particulates will also flocculate and settle. As samples are drawn, diluted and plated from the zone above settlement, we will see a reduction in the microbial levels within that zone.

Areas of Focus:

1. Aseptic techniques
    a. Dilutions
    b. Plating

    c. Autoclaving

2. Speed of experiment
    a. Making plates
    b. Autoclaving
    c. Dilutions

3. Quantity/Size of experiment
    a. Quantity of plates
    b. Dilutions