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BIODEGRADATION AND RECYCLING OF CIGARETTE BUTTS AS USEFUL INSECTICIDES

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ABSTRACT

Cigarette butts, one of the most ubiquitous forms of garbage in the world, need to be recycled because their toxicity can kill saltwater and freshwater fish. They have consistently been one of the most common items found by Clean Up volunteers. Cigarette butts may seem small, but with an estimated 4.5 trillion butts (worldwide) littered every year is undoubtedly a menace. Cigarette butt litter on public beaches, sidewalks, and parks is disgusting. From smokers tossing used filters on the ground to a lack of disposal bins in public areas, the refuse of cigarette smoking can be found everywhere, especially in cities. This littering problem is an environmental issue. Cigarette filters are designed to capture smoke particles; this tar accumulates in the filters. The litter often ends up in waterways via storm drains. Chemicals absorbed in filters then leech into fresh water systems killing organisms and polluting the environment. Thus it's high time we recycle this enormous waste and make it as some useful product for the society.

KEYWORDS: Cigarette butts, Toxicity, Environmental Issue, Pollution and Recycle.

INTRODUCTION

Cigarette butts represent 30 percent of the waste found on American shorelines and waterways. Cigarette butts contain hazardous chemicals such as cadmium, arsenic and lead that are partially filtered out during smoking. But when the butt is discarded, these chemicals leach into the environment contaminating our waterways and land. When butts are discarded, wind and rain carry them into the water supply. The toxic chemicals they contain are then leached into aquatic ecosystems, threatening the quality of the water and marine life. Most cigarette filters are made from cellulose acetate. Depending on conditions, estimates for the time taken for them to degrade ranges for British American Tobacco from 10 months - 3 years. This resistance to biodegrading is a factor in littering, environmental damage and suggested lung damage. In the 2006 International Coastal Cleanup, the number of individual cigarettes and cigarette butts collected amounted to 24.7% of the total number of garbage items collected, over twice as many items as any other category. The *New York Times* (May 28, 2009) reports that, "dozens of municipalities across the nation have had enough. Weary of the butts' unsightliness and the costs of sweeping them up, cities have passed bans on smoking on beaches and playgrounds." The newspaper goes on to point out that such toxins as nicotine, benzene, and cadmium leach out of discarded filters long after they have been thrown away. Meanwhile, the people at *ButtsOut.net* are offering a partial solution – the personal, portable ash tray. It's a small box that can be clipped onto "belt, bag, ski suit, or clothing." Drop in the finished smoke, close the lid, and the butt is taken out of the waste stream for safe disposal. "No man who smokes daily can be said to be at any time in perfect health." And now: "Over 37 million people (one of every six Americans alive today) will die from cigarette smoking. It seems that smoking can be more useful than it seems to be. Butts that are thrown everywhere can be of great help for making insecticide using simple method. That is very useful for the people. With a few simple steps you are able to harness these chemicals and transform them into an insecticide for home [1].

An insecticide is a pesticide used against insects. They include ovicides and larvicides used against the eggs and larvae of insects respectively. The use of insecticides is believed to be one of the major factors behind the increase in agricultural productivity in the 20th century. Insecticides mostly affect insect nervous systems in the same way [2]. Nerve cells in all animals (including insects and humans) carry impulses from the point of stimulus (for example, touching a hot stove) to the nerve center (the brain) or from the nerve center back to the muscles, to signal a response (move the finger off the hot stove).

Nerve cells do not actually touch each other. When an impulse (a tiny electrical current) gets to the end of one nerve cell, it must jump across a gap, the synapse, which separates that cell from the next. The gap contains acetylcholine (ACh). Molecules of ACh carry the impulse across the gap to the receiving cell, to which they attach themselves. When this happens, the receiving cell sends out a new impulse, continuing the process [3]. After the ACh crosses the synapse and reaches the receiving cell, molecules of another compound, cholinesterase (ChE), attach themselves to the ACh and remove it from the membrane of the receiving cell. This leaves the cell in its original state, able to receive another impulse [4]. All of this happens in a tiny fraction of a second, but a nerve impulse must pass through hundreds or thousands of cells and gaps before it reaches its final destination. Organophosphates and carbamates interfere with this process by tying up the ChE, and so we call them cholinesterase inhibitors. When ChE is unavailable to pull the ACh off the receiving cell, the ACh stays attached, providing a constant signal for the cell to keep sending an impulse. This muddles the message the nerve cells send. When this happens, the nerve system is unable to distinguish between real and "imagined" impulses [5].

Insects may encounter insecticides in several ways. Perhaps the most common way is by direct contact. In this case, insecticide residues remain on the surface of the plant you have treated. The insect comes in contact with the material as it walks across the treated surface. The insecticide enters the insect through its feet and then makes its way to the site of action (for example, nerve cells or hormone sites).

If the insect is present at the time you apply the insecticide, the spray also may cover the insect and penetrate its body directly [6]. One common turf and ornamental insecticide, acephate (Valent's Orthene), has systemic characteristics. These qualities make this product particularly effective for controlling insects, such as aphids, which suck plant juices. If the plant tissues contain acephate, the aphids will ingest the insecticide directly when they feed. Nicotine is effective against ground and soil pests, especially root aphids and fungus gnats, and on many leaf-chewing insects, such as aphids, immature scales, leafhoppers, thrips, and leafminers, pear psylla, and asparagus beetle larvae [7].

There is also a possibility of making fashion items using old cigarette butts. It seems to be more of a fabric reclamation project – which makes it considerably less grim, since fibre is fibre. This doesn't make any harm to the environment and for the person's health – if everyone reduced their consumption of them but in the meantime, there is many things can be done with them. However it is a money making business for the Cigarette Company. Malicious nonsmoking bosses know about this arithmetic and even pay in addition sometimes when an employer quits smoking, to torture them. According to the statistics, nonsmokers have to suffer at average 10-15 years longer than smokers. And their death is saddened by the lack of illnesses; the happiness on the occasion of death approaching is practically missed. We have nothing to do just to feel sorry for them. You can also get rid of unnecessary energy with the help of cigarettes. Mexican Indians say that smoking takes away more than 80% of the whole energy. In other words directly during smoking a person feels, experiences, wants something, in general, he lives approximately 1/5 more than usual. Poor nonsmoker with all his energy has to do something. Those, who quit smoking, it's especially difficult task for want of habit [1].

This study was undertaken to know the effectiveness of cigarette butts in killing insects and to degrade the cigarette butts extract or insecticide using micro organisms. The cigarette butts have a high potential to kill the various kinds of insects including mosquitoes. It can be used to discover a new type of mosquito repellent also in the days to come.

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MATERIALS AND METHODS

Materials for Making Insecticide

The first step is to collect used cigarette butts, keep them in a lidded jar or bottle sealed, as these tend to smell quite strongly.

1. 80 used cigarette filters
2. Water, 1 liter
3. 1/8 of a bar of non-scented soap
4. 50 ml of alcohol

Method

1. The filters are put in a pot or a container that can be heated.
2. Alcohol is added and the filters are soaked in the alcohol.
3. Water is added and the pot is covered and left undisturbed for 6 hours.
   From this step onwards this process tends to emit a terrible tar-like smell. We should make sure that we have enough ventilation with open windows or a kitchen fan.
4. After 6 hours add finely chopped or grated soap and boil the mixture.
5. Once it starts boiling bring the heat to a minimum and allow to simmer for half an hour and allow to cool.
6. Finally filter the liquid from the solids. (paper and filters)

Subculturing

1. Preparation of Minimal Broth

Berg's mineral salt media

a. Sodium nitrate -2 g/l
b. Dipotassium hydrogen phosphate – 0.5 g/l
c. Magnesium sulphate – 0.2 g/l
d. Manganese sulphate – 0.2 g/l
e. Ferrous sulphate – 0.2 g/l
f. Calcium chloride – 0.2 g/l
g. Glucose – 5 g/l

The components are added in a 100 ml conical flask and completely dissolved. The pH was checked using pH stripes and adjusted using NaOH/HCl. The conical flask was plugged with cotton and sterilized. The media is sterilized at 121°C at 15 psi for 20 minutes.

2. Dispersion of The Sample

a. The working place was first wiped with ethanol and two burners were lit on either side to create a sterile environment.
b. The autoclaved media was allowed to cool.
c. Various concentration of cigarette butt viz. 0.01%, 0.1%, 1% and 5 % was added to the respective flask.
d. The inoculated flask was then kept in rotating shaker for 5 days.

3. Spread Plating

a. The nutrient agar was prepared in 250 ml flask and was sterilized by autoclaving at 121°C at 15 psi for 20 minutes.
b. 20 ml of the media was poured in the petriplates before getting solidified.
c. The media was allowed to solidify.
d. 1 ml of the above inoculated sample was pipette out using sterile pipettes.
e. An L- bend rod was sterilized by dipping in ethanol and used for spreading the sample.
f. The sample was spread using the L- bend rod by rotating the petriplates on either side.
g. The petriplates were incubated in an inverted position at 37°C for 24 hours.
h. The plates were then observed for the presence of isolated colonies.

4. Subculture of Isolated Colonies

The next step is the development of a pure culture to transfer the organisms from the petriplate to a tube containing the nutrient agar slant. After this, the subculture was incubated for 24 hours and a stained slide of the culture was made to determine if a pure culture has been achieved.

Materials

1. Nutrient media composition
2. Agar
3. Sterile conical flask
4. Sterile tubes.
5. Inoculating loop.

Methods

a. Nutrient media was prepared and sterilized autoclaving was done at 121°C at 15 psi for 20 minutes.
b. Slants were prepared using nutrient media by placing the tubes horizontally in slanting position.
c. The isolated organisms were inoculated in the slants by streaking.
d. All the tubes were plugged with cotton.
e. The tubes were incubated at 28°C for 24 hours.
f. Once the organisms attained its full growth it was stored in refrigerator (4°C) for further analysis.

Identification of Insecticide Degrading Bacteria

1. Gram staining
2. Spore staining
3. Motility test

Morphological characterization of isolated organism

Gram's staining

Gram staining is an empirical method of differentiating bacterial species into two large groups – gram positive and gram negative based on the chemical and physical properties of their cell walls. The procedure is based in the ability of microorganisms to retain the purple colour of the crystal violet during decolourization with alcohol. Gram negative bacteria are decolourized by the alcohol, losing the purple colour of crystal violet. Gram positive bacteria are not decolorized and remain purple. After decolourization, safranin, a red counter stain, is used to impact a pink colour to the decolourized gram negative organisms. Spore staining technique was used to determine the occurrence of endospore in the given bacteria. Manitol Motility Test Agar is a semisolid medium used for the detection of motility of *Enterobacteriaceae*. Bacterial motility can be observed directly from examination of the tubes following incubation. Growth spreads out from the line of inoculation if the organism is motile. Highly motile organisms provide growth throughout the tube. Growth of non-motile organisms only occurs along the stab line. Various standard Biochemical tests for the isolated microorganisms such as Catalase test, Oxidase test, Indole test, Methyl red – Voges Proskaeur test, Citrate Utilization test, Urease test, Triple Sugar Iron Agar test were carried out. Degradation on cigarette butt extract/insecticide was found out using estimation of arsenic, lead, cadmium, sulphur, acetone and ammonia.

Estimation of Acetone

Reagents

1. Sodium bisulfate – 5% in water
2. Nessler's solution (Koch and Mckonky's reagent)
3. Sulphuric acid 1:1 dilution by volume
4. Standard acetone solution

The acetone is first absorbed into solution of sodium bisulfate then treated with Nessler's solution, precipitate forms, varying in intensity from a faint haze to definite turbidities depending upon the amount of acetone present.

Technique

0.5ml of 5% sodium bisulfate is spreaded over bottom of the Erlenmeyer flask and the cork is carefully inserted so as to allow the sample to suspend about 1 cm. The flask is heated in boiling water bath for 15 minutes and then cooled. The cork and cotton roll is removed. 1 ml of water is added, and then 1 ml of Nessler's bringing total volume of 2.5 ml. The solution is poured into a test tube and the amount of turbidity is compared with standard set. Standard are prepared at the same time. Similar test tubes from 0, 0.002, 0.004, … 0.0010 mg of acetone with addition of water 1ml and 0.5ml of 5% sodium bisulfate is added along with 1 ml of Nessler's solution The full development of turbidity is witnessed after 15 minutes

Estimation of ammonia

Reagents

1. Alkaline phenol reagent

2. Hypochlorite solution
3. Manganese salt

Technique

Place the calibrated test tubes/colorimeter tube and add 1.5ml of sample containing 0.5 to 6 gamma of ammonium nitrate, 1 drop (about 0.05ml) of 0.003M Manganous salt solution, 1ml of alkaline phenol reagent, and 0.5 ml of hypochlorite solution. The two latter solutions should be cold, when added to sample. Loss of ammonia is reduced by keeping the sample tubes in ice bath during their preparation. Mix the content of tubes by gentle rotation and place them immediately in briskly boiling water bath for about 5 mins. Cool and make to convenient volume (example- 6 or 10ml) and read in a photoelectric colorimeter with absorption of near 625nm.

Estimation of Sulphur

Piria and Schif's Method – Procedure

In a small nickel crucible a thin layer of potassium nitrate and sodium carbonate mixture is placed. Then a known weight of sample is placed over this layer and rest of crucible is filled with potassium nitrate and sodium carbonate mixture. This filled crucible is then inverted on the base of bigger nickel crucible so that its content remains intact. The bigger crucible is then filled up with potassium nitrate and sodium carbonate mixture so as to cover small crucible completely.

It is then heated strongly on blowpipe to fuse the mixture for about 3-4 hours. After this it is cooled and then placed in a big beaker to which dilute hydrochloric acid is added gradually to dissolve completely the contents of crucible. The crucibles are taken out of beaker and washed thoroughly with water into the beaker. The resultant solution is then precipitated with 10% barium sulphate solution till the precipitate is complete. The precipitate of barium sulphate is digested on water bath filtered in a preweighed sintered glass crucible, washed dried and weighed. Estimation of Arsenic, Cadmium and lead was done by Atomic Absorption Technique.

Calculation

Percentage of sulphur

Weight of sample = W mg (1ml)

Weight of barium sulphate = w mg

Percentage of sulphur = $\frac{w \times 32 \times 100}{W \times 233}$

RESULT AND DISCUSSION

More than 700 Cigarette butts/ samples were collected from different areas mostly from road sides and near to lakes. After 48 hours of immersing the cigarette butts in water the chemicals present in the butt leached out. The nicotine easily dissolved in water. The prepared insecticide exactly looked as green tea. If the concentration is more, it can be diluted by using water. After extracting all the chemicals from the butt, the used cigarette butt was filtered out. Now the cigarette butt insecticide is ready for use.

By adding non-scented soap, it helps to disperse well on insect's body. It can be kept for more than 3 weeks, at 37 ºC. However it should be kept away from sunlight, to prevent the denaturation of insecticides. Freshly prepared insecticide will be more effective, if it is more than 3 weeks. Thus these kinds of insecticides are very effective, easily available and cost effective.

The insecticide was tested by spraying on mosquitoes and spiders. After spraying the insecticides, mosquitoes died within 5 seconds and spiders within 10 seconds. Mosquitoes were killed by direct contact of insecticide. The mode of action of insecticides in mosquito and spiders was by nerve impulse blockage. When used on Cockroaches and Frog it leads to irritation. The irritation is due to the chemicals present in insecticide especially the nicotine.

By spread plate techniques, colonies were isolated from cigarette butt extract and were subcultured in nutrient agar slants in order to obtain pure culture from these colonies. Sub culturing is done to maintain the pure culture of the isolated microorganisms. A pure culture contains single bacterial colonies.

Bacterial smear was prepared for two colonies and gram staining was carried out. Yellow colonies were indentified which were of purple colour cocci in bunches and Green colonies of the pink rods. Thus Staining helped in identification of the organism's morphology and cell arrangement (Figure 1 & 2).

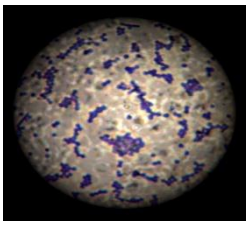


Figure 1. Gram staining of *Staphylococcus spp.*

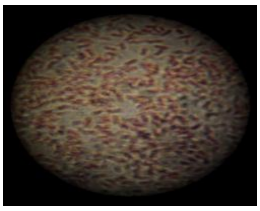


Figure 2. Gram staining of *Pseudomonas spp.*

The Biochemical tests conducted confirmed the presence of *Pseudomonas* and *Staphylococcus sps* (Tables 1 & 2).

Table 1. Biochemical Tests for the Isolated Organisms

Biochemical test	Green colonies
Catalase	+
Oxidase	+
Indole	–
Methyl red	–
Voges-praeuker	–
Citrate	+
Mannitol motility media	+
Urease	–
Tsi	Alk. /Alk.

Table 2. Biochemical Tests for the Isolated Organisms

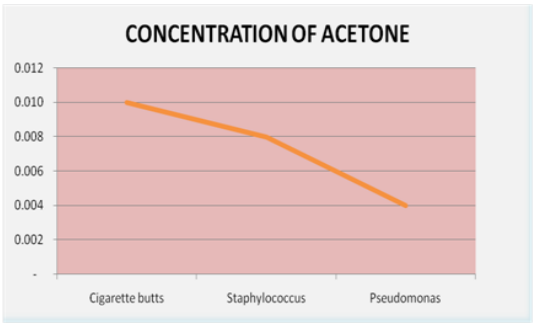
Biochemical test	Yellow colonies
Catalase	+
Oxidase	–
Indole	–
Methyl red	+
Voges-praeuker	+
Citrate	+
Mannitol motility media	Non motile ferments Mannitol
Urease	+
Tsi	Acid/acid

Test for Degradation of Insecticide from Cigarette Butts

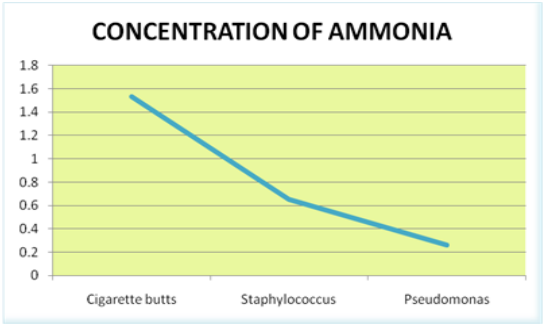
It was observed that aerobic growth by the isolate of cigarette butt extract on minimal media using *Staphylococcus* and *Pseudomonas* resulted in the degradation of insecticide after 45 days of incubation at 37°C (Figure 3).

Figure 3. Degradation of Cigarette Butt Using *Staphylococcus and Pseudomonas*

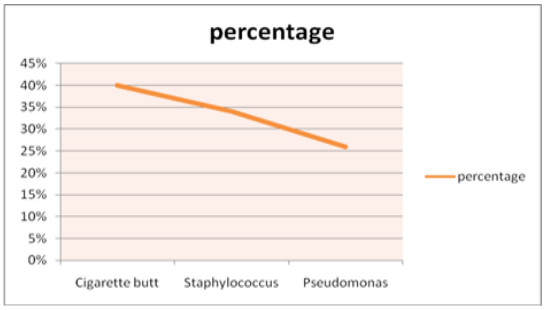
From the estimation of acetone and Ammonia, it was clearly seen that cigarette butts has highest acetone and ammonia content, where the *Staphylococcus* and *Pseudomonas* has degraded the content of acetone and ammonia after the incubation of 45 days whereas amount of sulphur conent used by the organism was relatively low (Graphs 1 -3).



Graph 1. Estimation of Acetone

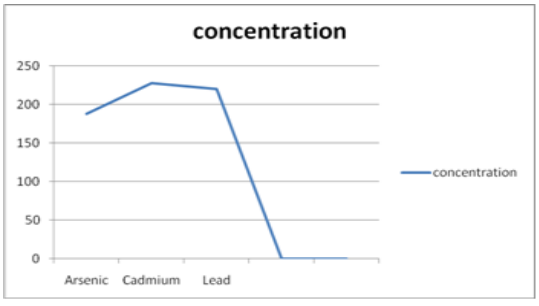


Graph 2. Estimation of Ammonia

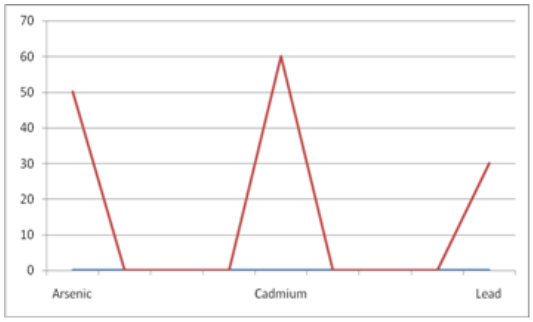


Graph 3. Estimation of Sulphur

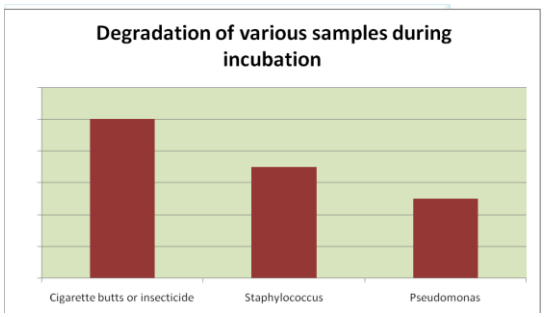
The amount of arsenic, cadmium and lead in the degradation was found to be low. The organism has utilized the metals present in it. This technique was done by using Atomic absorption spectroscopy. Thus ***Staphylococcus sp*** and ***Pseudomonas sp*** showed the degradative property on cigarette butts insecticide. When compared to *Staphylococcus*, *Pseudomonas* showed good amount of degradation, by estimating the chemicals from insecticide. The organism thus has the ability to degrade the amount of chemicals and toxins present in it (Graphs 4 – 6).



Graph 4. Estimation of Arsenic Lead and Cadmium in Insecticide



Graph 5. Estimation of Arsenic Lead and Cadmium in Degradation



Graph 6. Degradation Level of Insecticide

SUMMARY AND CONCLUSION

Cigarette butts are one of the most pollutable or daily littered waste in the world. By recycling this cigarette butt into an effective product, we can make a solution. Making insecticide from cigarette butt extract is the unique way of reusing the cigarette and effective killing of insects. It will be very effective, economical and easily available. Thus Recycling is an effective way of using the wasted cigarette butt as a useful insecticide. Cigarette butt is easily available and hence by collecting, we are cleaning our environment and making useful insecticide for garden purpose. Mosquitoes are the major insect vector in the world. Since it is easy to prepare this insecticide it can be used as a mosquito repellent for domestic purpose. Hence let's start using these biodegradable and eco-friendly insecticide made from a environmental waste such as cigarette butt and thus save our degenerating environment.

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