ANTI-SMOKING EDUKIT 2

CIGARETTE BUTTS

An EDUCATIVE KIT with simple experiments to determine the effects of CIGARETTE BUTTS on life in soils and water

USER MANUAL

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INTRODUCTION

The harmful effects of smoking on humans are well-known and are shown on cigarette packages by explicit photos. Yet, today, millions of people are nevertheless still "voluntary slaves" of this "silent killer".

Scientific research has shown that cigarette smoke contains over 4500 chemicals and that many of these are very toxic and some even carcinogenic.

Most types of cigarettes are now provided with a filter which – at least according to the producers – adsorbs the "dangerous" compounds present in cigarette smoke, as shown by the yellow-brownish color of smoked cigarette filters.

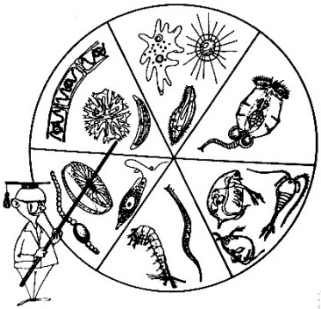
After smoking, cigarette butts should "in principle" be disposed of in an ashtray, but by far the largest part of cigarette butts is thrown away and hence wind up in the environment !

In fact, scientific literature clearly shows that the number of cigarette butts which are (in)directly discharged to the environment is staggering. This should be no surprise as information on smoking indicates that more than **5000 billion (5000.000.000.000) cigarettes are smoked yearly worldwide !**

Besides the deleterious effects of smoking on humans, a seldom asked question is whether the cigarette butts released in the environment don't also a negative effect on the plants and the small animals living in the soil and in water. This is a crucial question to answer as these organisms collectively play a key role to ensure the ecological health of soil and water.

Objective of the ANTI-SMOKING EDUKIT 2

CIGARETTE BUTTS



The objective of the ANTI-SMOKING EDUKIT seeks to demonstrate with the aid of simple and practical tests that the chemicals which leach out of cigarette butts (<u>which actually occurs each time it rains</u>) have harmful effects on the plants and the tiny animals in soil and water.

Plants are well-known for their production of seeds which "hibernate" (= overwinter) in soil. When environmental conditions (e.g. temperature and light) become again favorable, seeds germinate and give rise to new plants.

Likewise a number of tiny **animals** also produce "(over)wintering" forms (= resting stages) to overcome unfavorable environmental conditions.

De experiments that can be performed with the ANTI-SMOKING EDUKIT consist in exposing "resting stages" of tiny animals and plants to the chemicals which leach out from cigarette butts. Afterwards analysis of the results will reveal if these chemicals have had an influence on the "survival" of the plants and tiny animals which are representative of the organisms living in soil and water.

To mimic what happens in the environment when cigarette butts are thrown away from which chemicals leach out when it rains, butts are simply inserted in a tube with water. Resting stages of tiny animals and plants are then exposed to the leachates from the cigarette butts.

The ANTI-SMOKING EDUKIT contains all the materials to perform tests on : a) a small freshwater animal (which also occurs in moist soil), b) a small marine animal, c) seeds of plants

Because the tiny organisms used for the experiments are very small in size, a microscope is hence needed for the observations.

NB : this should not be considered as a problem since "pocket" microscopes are now available and marketed at a very low price (e.g. the Mini-Microscope from Pfiffikus sells at 10-12 €). Pocket microscopes are well-suited suited for the ANTI-SMOKING EDUKIT experiments and they are additionally useful for many other interesting observations.

COLLECTION OF CIGARETTE BUTTS AND SEPARATION OF THE FILTER FROM THE BUTT

The experiments of the ANTI-SMOKING EDUKIT are performed on cigarette butts that are collected locally.

A cigarette butt is composed of 2 parts : a cylinder in thin paper which contains the (black) burnt tobacco and the unburnt tobacco, and the filter which contains the chemicals which are produced by the smoking of the cigarette.

The length of the part of the cigarette butt which contains tobacco varies from one butt to the other. For reasons of uniformity the experiments will be performed only on the filter which concentrates the cigarette smoke.

1. Collect about 10 cigarette butts (preferably from an ashtray) and place them in the empty zipper bag included in the Anti-Smoking Edukit.
   NB : only "dry" cigarette butts shall be collected, i.e. butts on which no rain has fallen !
2. Separate the filter butt from the tobacco leftovers by breaking the butt at the place where the filter touches the tobacco.
3. Take the zipper bag containing 5 empty tubes; place one filter butt in each tube and close the tubes with their stoppers.

4. Place one tube with a filter butt in the zipper bag labeled 1, one in zipper bag labeled 2, and 3 in zipper bag labeled 3.
5. Wash your hands thoroughly to eliminate the stinking smell of the cigarette butts.

EXPERIMENT 1

Test with a small freshwater animal

This is a "rapid" test with observations already after 30 minutes

The test organism is a very small (< 1mm) "rotifer". Rotifers are a group of organisms which occur worldwide in surface waters (puddles, pools, ponds, rivers), but also in moist soils.

The rotifer species used for the experiment is one of the few aquatic organisms which, when the water in which it lives "dries out" (which can occur during a period of summer drought) literally "rolls up" and can completely dry out without losing its viability.

When the dried out rotifers are rehydrated, they "reactivate" very rapidly (in less than 1 hour) and crawl and swim around actively.

Dry rotifer *Reactivated swimming rotifer*

The experiment consists in putting dry rotifers in a tube with tapwater and in a second tube with tapwater in which a cigarette butt filter has leached out.

One then examines the impact of the chemicals that have leached from the filter into the tapwater, on the "viability" of the rotifers after their "reactivation" from their resting state by hydration.

PERFORMANCE

All the materials for this experiment are included in the zipper bag labeled 1.

Control test

1. Take one of the two plastic (unbreakable) glass slides.
2. Open one of the two conical tubes which contain a small (2 mm) paper filter disc with a number of dry rotifers on the disc.
3. Turn the conical tube upside down on the glass slide and let the paper disc fall out of the tube, onto the middle of the glass slide.
   NB : in case the disc sticks to the bottom of the tube, it can be set free with the aid of a needle.
4. Take the pipette (with the small bulb) and fill it with tapwater.
5. Keep the pipette vertical and drop exactly **5 drops** on top of the paper filter disc on the glass slide.

6. Open the small zipper bag and take out 1 of the 2 (transparent unbreakable) round coverslips.
7. Put the coverslip on top of the wet filter disc to cover both the filter and the water layer.
8. Label the glass slide as “C” (= Control).

Leaching test

1. Fill the pipette again with tapwater.
2. Take the tube containing the filter butt and insert the pipette in the tube until the tip touches the filter butt.
3. Squirt the water from the pipette into the filter butt.
4. Fill the pipette a second time with tapwater and squirt the contents again on the filter butt.
5. Repeat the same operation again until the tube is half filled with water.
6. Close the tube with the stopper and shake it for a few minutes to allow the chemicals in the filter to dissolve in the water.
   NB : Open the tube and sniff the contents. The foul-smelling odor is clear proof that chemicals have leached from the filter butt in the water.
7. Insert the (empty) pipette in the tube until the tip touches the filter butt.
8. Suck the leachate water from the filter butt in the pipette.
9. Next, perform the same operations as those indicated starting from step 5 in the control test.
10. Label the glass slide as “L” (= Leachate).

Reactivation of the rotifers and observations

1. Keep the 2 glass slides at room temperature (at least 20°C !) for about half an hour.
2. Place the “control” slide under the microscope and look for living (= actively moving) rotifers at the edge of the filter disc, and also swimming near the filter disc.

NB : If no actively moving rotifers are observed, remove the slide from the microscope and perform a second analysis half an hour later.

3. Perform the same analysis with the leachate glass slide.

Conclusions

In the "control" slide there will be a number of actively moving rotifers under the coverslip. In contrast, the "leachate" slide will not display any living rotifers.

This simple and rapid test clearly shows that the chemicals which leach out of cigarette butts have an undeniable negative effect on the rotifers, and totally inhibit their "reactivation and revival".

Scientific experiments conducted with other water-based organisms also demonstrate the "killing" impact that cigarette butt leachates have on aquatic life and, as already indicated previously, these organisms play an important role in the health of the aquatic environment.

EXPERIMENT 2

Test with a small marine animal

This is a test with observations made after 24 hours

In all seashore areas of the world, visitors cannot help but to notice the large number of cigarette butts discarded on beaches.

The question can then be asked as to whether chemicals in cigarette butts (*which leach out in seawater when beaches are flooded at high tide*) are causing deleterious effects on "marine" organisms.

To test this hypothesis, an experiment can be undertaken with a small marine crustacean (the brine shrimp *Artemia*). This shrimp produces eggs which can dry out without losing their viability.

When dry brine shrimp eggs (*the size of which is < 1 mm*) are hydrated in seawater, they develop in one day and set free tiny larvae (which are also < 1 mm in size) that actively move and swim.

Dry brine shrimp eggs *Swimming brine shrimp larva*

The experiment consists in analyzing the impact of the chemicals that leach out from a cigarette filter butt placed in seawater, on the "hatching" of the shrimp eggs and on the activity of the larvae.

PERFORMANCE

All the materials needed for this experiment are included in the zipper bag labeled 2.

Control test

1. Take the large tube filled with seawater and fill the pipette with seawater.

2. Squirt the seawater in the small tube labeled with the letter "C" (= Control) till the tube is almost completely filled.
   NB : this small tube at first sight seems to be empty but in fact it contains a small number of dry brine shrimp eggs.
   These eggs are, however, so small that one can barely see them with the naked eye !
3. Close the small tube with its stopper.

Leaching test

1. Fill the pipette again with seawater.
2. Open the large tube containing a filter butt.
3. Insert the pipette in this tube and lower it until the tip touches the filter butt.
4. Squirt all of the seawater into the filter butt.
5. Repeat this operation twice until the tube containing the filter butt is almost full of seawater.
6. Close the tube with the stopper and shake it for a few minutes to allow the chemicals in the filter butt to dissolve in the seawater.
7. Insert the (empty) pipette in the tube until the tip touches the filter butt.
8. Suck the leachate seawater from the filter butt in the pipette.
9. Take the small tube labeled with the letter "L" (= Leachate) and squirt the leachate from the pipette in this tube until it is almost completely filled.
   NB : this tube also contains a number of dry brine shrimp eggs.

Incubation

The time required to insure the hatching of larvae from brine shrimp egge takes about 24 hours at a temperature of 25°C, and under appropriate illumination. These two conditions are needed to trigger the formation of larvae from the eggs.

Suitable temperature and light conditions can easily be obtained by placing both the control and the leachate tubes under a desk lamp.

A thermometer will simply determine the distance at which the 2 tubes should be placed in order to obtain a temperature close to 25°C in the tubes.

NB : the lamp should not be a LED lamp because LED's give virtually no heating !

Observations

Since both the brine shrimp eggs and the larvae are very small, a microscope is also needed here for the observations.

1. After an incubation of (at least) 24 hours at 25°C and with light, take the "control" tube and shake it gently to homogenize the contents over the total volume of the tube.
2. Take the pipette and withdraw about half of the tube contents.
3. Take a glass slide and dispense **5 drops** from the pipette in the middle of the slide.
4. Eject the remaining seawater in the pipette back into the small tube and rinse the pipette a few times with tapwater.
5. Remove 1 of the 2 round coverslips from the small zipper bag and cover the large drop on the slide with the coverslip.
6. Place the slide under the microscope.
   Under the coverslip one should see actively swimming brine shrimp larvae and brine shrimp eggs which have not (or not yet) hatched.
   NB : in case no swimming larvae are observed, continue the incubation for a few more hours, and proceed with an additional analysis.
7. Perform the same steps and observations with the "leachate" tube.

Conclusions

In contrast to the control tube slide where actively swimming larvae will be observed, the "leachate" tube will show either no larvae or only a few larvae. These larvae will also be much less active than those in the control tube.

To visualize even greater differences between the control tube and the leachate tube, a second observation can be made after a few additional hours.
NB : this, however, requires that one must then first wash and dry the 2 slides and the 2 coverslips.
This second analysis will show that the majority of the larvae from the "leachate" tube are dead (literally poisoned and killed by the chemicals that have leached from the cigarette butt filter), whereas the larvae from the "control" tube are still alive and actively swimming.

Again, this experiment conducted with a small marine organism also shows that the chemicals which leach from cigarette butts thrown on beaches have an undeniable deleterious effect on the small marine organisms living in wet sand of sea coasts.

EXPERIMENT 3

Test with seeds of plant

This is a test with observations made after 2 days

Most cigarette butts end up in the environment and it is therefore also needed to find out if chemicals leaching out of the butts during rainfall events have an impact on plants.

The following experiment will inform if leachates of filter butts have an influence on the germination of plant seeds, and on the growth of roots and shoots of germinated seeds.

Seeds of cress are used for the experiment since these seeds germinate very rapidly and already produce roots after 2 days, and shoots within 2-3 days.

PROCEDURE

All the materials for this experiment are included in the zipper bag labeled 3.

Control test

1. Fill the pipette with tapwater.
2. Open one of the 2 zipper bags containing a rectangular piece of thick cardboard filter and squirt the contents of the pipette on the cardboard filter.
3. Fill the pipette again with tapwater and empty it on the cardboard filter.
4. Repeat this operation until the cardboard filter is totally wet.
   NB : this can be achieved and verified by closing the zipper bag and spreading with the fingers the water over the total surface of the cardboard filter.
5. Open the zipper bag and pour out the excess water.
6. Slide the top half of the wet cardboard filter out of the zipper bag and put the bag with the cardboard filter on a flat surface.

7. Take 1 of the 2 small tubes containing a (small) number of cress seeds and spread the seeds on the free part of the cardboard filter.
8. Spread (with the aid of a needle or a small tweezer) the seeds, at equal distance from each other, on the upper side of the cardboard filter and over the full width.
9. Push the cardboard filter back into the zipper bag, taking care that the seeds stay in place.
10. Press all the seeds with your finger so that they stay firmly in place.
11. Close the zipper bag tightly in order to keep the filters moist for several days.

Leaching test

1. Fill the pipette with tapwater.
2. Open one of the 3 tubes containing a filter butt.
3. Insert the pipette in the tube till its opening touches the filter butt and squirt all the contents of the pipette into the filter butt.
4. Fill the pipette again with tapwater and repeat this operation till the tube is almost full of water.
5. Repeat these operations with the 2 other tubes containing a filter butt.
6. Close the 3 tubes with their stopper and shake them for a few minutes to allow the chemicals in the filter butt to leach out.
7. Open the first tube and insert the pipette in the tube until its opening touches the filter butt.
8. Suck up the leachate from the filter butt.
9. Open the second zipper bag with a cardboard filter and squirt the leachate on the cardboard filter.
10. Repeat this operation till no more leachate can be sucked out from the filter butt.
11. Repeat these operations with the second and third tube containing a filter butt, to transfer all the leachate on the cardboard filter.

12. Close the zipper bag and spread with the fingers the leachate over the total surface of the cardboard filter to wet the cardboard filter totally.
13. Open the zipper bag and pour out the excess leachate.
14. Then perform the same operations as for the control test, starting with step 6.

Incubation

1. Put 2 thick books back to back flat on a horizontal surface (e.g. a table), and insert the 2 zipper bags with the filters and the seeds "in vertical position" between the 2 books, with the seeds on the top side of the zipper bags.
2. Allow the seeds to germinate for 2 days at room temperature.

Observations

1. Analyze both zipper bags after 2 days and note if the seeds have germinated and have started to develop roots.
2. Repeat the observations again 1 and 2 days later and note the further growth of the roots and the development of shoots.

Conclusions

In the zipper bag with only tapwater on the cardboard filters, the majority of seeds will have germinated and roots and shoots will have developed. In the zipper bag with "leachate" from the filter butts there will be no (or barely any) germination of the seeds and no (or very little) growth of the roots and no development of shoots.

This experiment with plant seeds is a clear proof that the chemicals which leach out from cigarette butts during rainfall events, and seep into the soil, have an undeniable deleterious effect on the germination of plant seeds and the growth of the plants.

GENERAL CONCLUSION

All 3 experiments clearly show that chemicals leaching out from cigarette butts during rainfall events are toxic and have deleterious effects on animals and plants in water and soil.

Smoking and its consequences, insofar as **millions of cigarette butts end up daily in the environment**, is not only directly harmful for humans, but also indirectly harmful to the environment by damaging the health of organisms living in water and soil.

The "well-being" of the organisms in the environment is as important as the health of humans because animals and plants collectively play a crucial role in keeping waters and soils healthy !

The key message conveyed by all ANTI-SMOKING EDUKIT 2 – *CIGARETTE BUTTS* experiments is unambiguously that :

PREVENTION IS BETTER THAN CURE

and whoever does not smoke will not only protect his/her own health, but also that of the environment

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